



The Sins of Our Kin: From Genomic Imprinting to Ancient Signaling Systems

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The Sins of Our Kin: from Genomic Imprinting to Ancient Signaling Systems

ABSTRACT

The bond between parents and their offspring is often regarded as something loving and supportive. But, this bond is more of a battle. Genomic imprinting is the result of the evolutionary struggle between mothers and fathers for reproductive success. The first part of the thesis describes the 3'- untranslated region (3'-UTR) of the insulin-like growth factor type 1 receptor (IGF1R) as an ancient long noncoding RNA. The 3'-UTR of the mouse *Igf1r* mRNA is targeted by miR-675-3p derived from the imprinted *H19* long noncoding RNA. We undertook a comparative analysis of vertebrate *IGF1R* 3'-UTRs to determine the evolutionary history of miR-675 target sequences and to identify conserved features that are likely to be involved in post-transcriptional regulation of IGF1R translation. The conserved structures we identify emphasize the central importance of IGF signaling pathways in the mediation of intragenomic conflicts over embryonic growth.

The second part is aimed at investigating the *Retrotransposon Gag-like 1 (RTL1)* and the molecular evolution of self-targeting imprinted microRNAs. Transcription of the sense strand of the *RTL1* gene produces a protein-coding mRNA that is targeted for degradation by microRNAs transcribed from the antisense strand of the protein-coding sequence. The sense and antisense transcripts are oppositely imprinted. We present an evolutionary model for the establishment of a new self-targeting microRNA derived from within a tandem repeat that inhibits production of RTL1 protein when maternally-derived in heterozygotes but not when paternally-derived.

Lastly, oxytocin has commonly been viewed as a molecule that can melt away malevolence and increase bonding. Its effects, however, are involved in the process of smooth muscle contraction. Oxytocin is a member of a nonapeptide family constituting an evolutionarily conserved signaling system found in vertebrates and invertebrates. We postulate that there is an evolutionary connection among the various functions attributed to these peptides. We believe that the acute anorexic effects of oxytocin, and its orthologs, released during egg-laying inhibits the consumption of a female's own eggs and that the first evolutionary use of oxytocin peptides in social bonding was in the promotion of nurturing feelings towards clutches of fertilized eggs.

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TO MY PARENTS

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A doctoral dissertation is written in the first person: *I* did this and *I* conclude that. But If I have learned anything during my 6 years of graduate school it is that science at its best is a wonderfully collective enterprise. I want to thank Kern Reeve, who shepherded my first experience working in research as an undergraduate and Dan Hartl, Robin Hopkins, and John Wakeley for believing in me throughout it all.

There is an old saying, ‘Sons branch out, but one woman leads to another.’ Perhaps its author was cognizant of sex-specific parental effects. But in any case, this thesis owes its existence to the women of 410 and their unfailing support. To Alex, the wisest choice this female ever made. Most importantly, to my mom and dad. Parents, of course, are what humans are all about. I am inordinately grateful for mine.

1

Introduction

Hanging in the Museo del Prado in Madrid are two paintings by Spanish baroque artist Juan Carreno de Miranda of a child entitled ‘La Monstrua vestida’ and ‘La Monstrua desnuda’: the monster clothed and the monster naked. The girl was 6 when she was brought to the court of Charles II of Spain as an object to be seen as she was excessively obese with a small triangular mouth, small hands and feet, and almond-shaped eyes. While her exact medical diagnosis is unknown, Dr. Andrea Prader immediately recognized her features consistent with Prader-Willi

Syndrome (PWS), in which children feed poorly in infancy followed by hyperplasia, have underdeveloped sex organs, severe learning disabilities, as well as a short stature [33]. PWS was first identified as a rare genetic disease by Swiss doctors in 1956, but years later doctors noticed that the same cytological deletion was observed in a complementary disease; Angelman's syndrome (AS).

Dr. Harry Angelman was a pediatrician who treated three handicapped children in the 1960s who showed similar forms of mental retardation, behavioral features, and physical abnormalities. After visiting the Castelvecchio museum in Verona and seeing the oil painting 'Boy with a Puppet' he was inspired to term these patients "Puppet Children," and later the name was changed to Angelman's syndrome. In the case of the painting, the subject appears to have a cheerful disposition with a fixed smile. An unusually happy demeanor with frequent laughter and smiling is one of the notable characteristics of AS, which occurs along with developmental disabilities, seizures, and truncal hypotonia [5].

In both genetic disorders, the exact portion on the long arm of chromosome 15 at region q11-q13 is missing [130]. However, PWS is due to the absence of a normally active set of paternally derived genes at this locus, while AS is due to the absence of the maternally derived alleles. Transmitted through the mother, the disease exhibits itself as AS and transmitted through the father the disease exhibits itself as PWS. The two syndromes are examples of genomic imprinting, a phenomena in which genes are mono-allelically expressed in a parent-of-origin specific manner.

This manner of heritability appears to belie the nature of the law of equivalence and imply that alleles carried on maternal and paternal chromosomes of diploid organisms behave differently. An advantage of diploidy is the ability to hide recessive mutations in one of the two copies of a gene. Complex organisms with a high

number of cell types are more likely to withstand mutations associated with DNA replications that occur during development because of diploidy [184]. As such, almost all mammalian genes are equally expressed from both their parental alleles.

However, the genomes of eutherian mammals contain a set of genes that are preferentially expressed depending on whether they are inherited via an egg or sperm—meaning either from the maternal or the paternal copy of the gene. These genes carry parental imprints—most often in every cell where the gene is expressed, the imprinted allele is active while the other allele is silent [192]. However, sometimes imprinting is seen only in certain tissues or a bias in gene expression depending on the parent-of-origin. Distinct from mere mono-allelic expression, genomic imprinting is an epigenetically predetermined arrangement to express either parental allele in a particular cell-lineage. Accordingly, these genes are an evolutionary paradox as their form of regulation forgoes all advantages of diploidy. The cost of imprinting is mirrored in its association with human disorders, including PWS, AS, Beckwith-Wiedemann, and Silver-Russel syndromes [188]. However, the endurance of genomic imprinting for over 160 million years of eutherian evolution suggests that despite the disadvantages, imprinting must bestow some evolutionary advantages. Comparative genomics allows us to explore the origins of genomic imprinting in mammals and the selective forces important for its pervasiveness.

The first indication that the contribution of the maternal and paternal genomes is not equal during mammal development came from a set of fundamental mouse experiments in the early 1980s [166] [230]. Using refined nuclear transplantation techniques, maternal or paternal pronuclei were extracted from and then reintroduced into zygotes, creating embryos with either maternal or paternal genomes. Neither gynogenetic nor androgenetic zygotes were viable, but both differed both

morphologically and developmentally. Parthenogenetic embryos initially developed normally but failed to live to term because of an absence of placental tissues. Androgenetic embryos were developmentally delayed and died early, but did contain an abundance of placental tissues. The groups determined that successful embryogenesis only arises with a maternal and paternal pronucleus, but not when either two maternal or two paternal pronuclei were transplanted. Thus, they hypothesized that parental genomes bear different imprints created during gametogenesis, consequently requiring both the maternal and paternal genomes for normal development.

An example of naturally occurring genomic imprinting in humans is seen in the complete hydatidiform mole [137]. This abnormal conceptus lacks fetal tissue and is made up completely of trophoblast tissue with an entirely paternal genotype. Though the fetus does not survive, it still makes extra-embryonic tissue and is reminiscent of mouse androgenotes. Similarly, triploid conceptuses with an extra paternally derived chromosome set have large overgrown placenta and fetal growth retardation, whereas those with two maternal chromosome sets fail to develop a normal placenta [165].

1.1 EVOLUTIONARY ORIGINS OF GENOMIC IMPRINTING

Genomic imprinting is extensive among eutherian mammals and the vast majority of these genes participate in the regulation of fetal growth and are expressed in both fetal and placental tissues. In mammals, the ancestral form of placentation evolved over 160 million years ago after the divergence of therians from monotremes and about the same time that the first genes acquired imprinting [172]. Imprinting has also been detected in some of the same gene clusters in marsupials, but a few have different spatial and temporal expression patterns between the two groups. No

imprinted genes have been discovered in monotremes to date.

Early mammals evolved from a reptile-like ancestor around 300 million years ago and Mammalia is divided into three extant lineages: eutherians, marsupials, and monotremes. Eutherians shared a common ancestor with marsupials around 166 million years ago and monotremes 180 million years ago (Morison et al 2005). The three groups differ in their modes of reproduction. Monotremes lay eggs after a short intrauterine growth period supported by secretions transferred across the yolk sac. Marsupials have brief gestations, a short-lived fully functional placenta, are born altricial, but have developed a long lactation that supports young until they are fully developed. Eutherians carry their young in-utero for an extended period of time and deliver highly developed young at birth. Of the three, monotremes and marsupials have an elaborate lactation whereas eutherians have elaborated placentation. Thus, the mass of monotreme and marsupial neonates relative to their mothers is smaller than that of eutherian neonates (less than 1% versus 15% respectively) [101].

Haig and Westoby[96] were the first to postulate that imprinting evolved because what is beneficial for a gene inherited via one kind of gamete may be different from what is beneficial for that gene when inherited via the other gamete. Paternally and maternally derived alleles in offspring, referred to as padummal and madummal respectively (in contradistinction to paternal and maternal alleles present in females and males), have opposing interests during pregnancy. From a male's selfish fitness perspective, any padummal genes in his offspring should be expressed in a way as to benefit the health and survival of his progeny, even if this endangers the health of the mother and her future off-spring, who might have different fathers. Conversely, madummal genes should be expressed as to limit the current offspring's command over her resources to a degree that is more suitable for her continued health and

reproduction. The kinship theory of genomic imprinting affirms that paternal genes boost the withdrawal of the mother's resources for the benefit of the focal father's offspring but at the cost of offspring in future pregnancies. In comparison, the maternal genome reduces its support for each embryo regardless of the paternal genome. This ingenious reasoning also predicts that kin selection and genomic imprinting are tightly linked [99].

During pregnancy, each female mammal alone is tasked with the strain of gestation and nursing, whereas the male may contribute little or no parental care beyond his original genetic contribution. As such, generally paternal genes are under selection to maximize extraction of maternal resources while maternal genes are under selection to give fewer resources. These quarrels over resources happen most often in the placenta and endosperm—physical locations where females transfer nutrients to the young.

The result of such evolutionary 'tug-of-war' is a compromise in which each offspring receives fewer resources than it might want and a female gives up more resources than she ideally might wish [99]. But, any compromise between a mother and her fetus during pregnancy is less of an amicable mutualism than an outcome of conflict mediation [85]. It was Haig who saw the placenta as a fetal organ devised to leech the maternal uterus instead of just an organ created to provide nutrients to the fetus. As the placenta is of fetal origin, paternal genes have extensive possibilities to withdraw maternal nutrients. Trophoblast cells penetrate deep into the mother's vessels, forcing them to dilate, and the placenta becomes directly responsible for bringing maternal and fetal blood into contact, aiding nutrient exchange and determining resource allocation. In many eutherian species, including humans and mice, the remodeling of the mother's blood vessels is so severe that the mother

is unable to regulate the volume of blood reaching the placenta versus other tissues.

1.2 TRANSFER OF NUTRIENTS

To date, at least 130 mammalian genes show differential parent-specific expression. Those that have been characterized are disproportionately expressed in the placenta and vital for fetal growth. This was first described with genetic experiments using uniparental duplication of specific chromosomal segments which led to the discovery of imprinted genes in the mouse genome, such as *insulin-like growth factor 2 receptor* (*Igf2r*), *insulin-like growth factor 2* (*Igf2*) and *H19* [12] [15] [55]. The latter two genes reside in an imprinted domain that includes *INS*, *KCNQ1*, *KCNQ1OT1*, *CDKN1C*, *TSSC5*, and *TSSC3*.

IGF2 is paternally expressed in therians and is a vital fetal and postnatal growth factor that is highly conserved in all vertebrates. It is not expressed in almost all adult tissues, save the choroid plexus and leptomeninges where it is biallelically expressed. In contrast, in utero it is expressed in the placenta and embryo; but only the paternal allele is expressed while the maternal allele is transcriptionally silent. It is closely involved in growth of the fetus in eutherian mammals and has been involved in numerous aspects of placental development, including blood vessel formation, trophoblast invasion, and nutrient transfer.

The *Igf2r* gene encodes *IGF2R* and a mannose-6-phosphate receptor. *Igf2r* is expressed from the maternally inherited chromosome in the mouse [12], but imprinting in humans is polymorphic as it occurs only in a small proportion of the population. It lies within an imprinted cluster expressed in the placenta and plays an integral role in IGF2 signaling. IGF2R antagonistically controls IGF2 by binding to it and carrying it to a lysosome for degradation. In mice, *Igf2r* imprinting is

governed by the reciprocally imprinted, paternally expressed lncRNA Airn, which is an antisense transcript to *Igf2* [222]. In marsupials, IGF2R is also imprinted and maternally expressed despite missing distinct crucial characteristics that regulate its imprinting in mice, like differential methylation and antisense IGF2R RNA production [128]. *IGF2R* is biallelically expressed in the monotreme, but orthologs lack the IGF2 binding domain and thus create proteins that only have mannose-6-phosphate binding properties, perhaps nullifying the need to be imprinted in these species [128]. Imprinting in this region most probably occurred in the therian ancestor, but is preserved by different imprinting marks and mechanisms in various mammalian lineages.

Lying 100 kb downstream of the *IGF2* gene on chromosome 11 in humans is the *H19* gene, which is expressed from the maternal allele. *H19* encodes a lncRNA that contains a microRNA precursor that is involved in the post-transcriptional down regulation, acts in cis to down-regulate IGF2 expression via shared insulator and enhancer sequences, and may act as a miRNA scaffold. Deletion of the paternally expressed *IGF2* results in intrauterine growth restriction and small placenta development whereas deletion of the maternally expressed *H19* gene results in fetal overgrowth [55].

These two genes compete for enhancer sequences downstream of the *H19* locus which regulate expression of both genes. A paternal region upstream of *H19* is methylated, surrounding a CTCF insulator binding site and spreads somatically into the *H19* gene. On the unmethylated maternal chromosome, the imprinting control region (ICR) binds the insulator protein CTCF, blocking access of *Igf2* to shared downstream enhancers. CTCF is unable to bind the methylated insulator elements on the paternal chromosome, allowing enhancers to drive the expression of

Igf2 gene [19]. *H19* silencing on the paternal chromosome occurs by the methylation of its promoter, which is established by a silencing element located in the *H19* DMR downstream of the boundary element [58].

Though the marsupial placenta has less intrauterine growth and an abridged placental attachment in comparison to eutherian placentas, *IGF2* is both paternally imprinted in marsupial species and demonstrates an equivalent biological function stimulating vascular endothelial growth factor production and yolk sac vasculogenesis [3]. Marsupial *H19* orthologs are conserved in the miRNA precursor structure and exon structure, indicating functional selection on both features. Furthermore, the mechanism controlling imprinting of *IGF2-H19* is also conserved in therians, which suggests a pivotal regulated role for these genes in mammalian development. It also indicates that the intricacy of imprinting mechanisms is conserved across mammalia and may have evolved in response to evolutionary pressures in the common therian ancestor. Imprinting is more common in eutherians— which is probably due to increased selective pressure of parental conflict during the extended eutherian gestation.

The expression pattern at the imprinted *Dlk-Dio3* domain is also consistent with the kinship theory of genomic imprinting’s assumption of conflict between parentally inherited genomes. In eutherians, this domain contains three paternally expressed protein coding genes: *Delta-like homolog 1 (Dlk1)*, *Retrotransposon-like 1(Rtl1)*, and *Type III iodothyronine deiodinase (Dio3)*. It also produces maternally expressed RNA transcripts including an antisense *Rtl1* transcript and *Meg3*. The *Rtl1* gene is linked with a fully complementary maternally-expressed antisense *Rtl1*, which serves as the primary transcript for six miRNAs: mir-127, mir-136, miR-431, miR-433, miR-434, and miR-432. Due to their antisense genomic organization, they are

able to cleave *Rtl1* mRNA through RNA interference [52]. During gestation, the domain is expressed in fetal and placental tissues and is vital for successful prenatal growth, placentation, muscular and skeletal development, and postnatal metabolism [200].

Ablation of *Dlk1* in mice results in significant growth retardation and, later in life, increased adiposity associated with adipocyte hypertrophy [171]. Conversely, overexpression of the gene leads to a reciprocal phenotype; reduced adiposity, hyperglycemia, and insulin resistance [146]. Accordingly, *Dlk1* is predicted to play a role in controlling adipocyte differentiation and acts as a paternally expressed growth enhancer.

A deletion of the paternally expressed *Rtl1* gene results in pre- and post-natal growth retardation of the embryo and placenta and late fetal and neonatal lethality. Reciprocally, deletion of the maternally-expressed anti-*Rtl1* too results in neonatal lethality, but with offspring displaying a 156% increase in placental weight [213]. Exploration of the parental deletions show reciprocal abnormalities, mostly seen in the labyrinth zone of the placenta, where maternal-fetal interactions occur: maternal deletions reveal increased fetal capillaries and labyrinthine trophoblasts and paternal deletions show an opposite phenotype. These data indicate a vital role of the paternally expressed *Rtl1* gene in the growth of fetal capillaries at the maternal-fetal interface, whereas the maternally expressed antisense *Rtl1* antagonizes these growth promoting effects [213].

1.3 IMPRINTING IN THERIAN LINEAGE

Genomic imprints have evolved independently at least three times: in insects [127], endosperms [73], and mammals [194]. Most imprinted genes are clustered together

in a handful of domains dispersed throughout the genome; often comprised of both maternally and paternally expressed imprinted genes, non-coding RNAs (ncRNAs), and also non-imprinted genes. From the many protein-coding genes and ncRNAs regulated by genomic imprints in eutherians, only eight of those are also imprinted in marsupials and no imprinted genes have been identified in monotremes[128].

The epigenetic mechanisms controlling mono-allelic expression of imprinted genes involve differential DNA methylation marks. Within an imprinted cluster, the expression of imprinted genes is coordinately established by a cis-regulatory element, the ICR. De novo DNA methylation is initiated at the ICR by members of the DNA methyltransferase 3 family in a parent-specific manner. ICRs are CpG-rich and differential DNA methylation is seen between the two—this means one parental allele is methylated on the majority of CpG island regions of DNA with a high frequency of CpG dinucleotides relative to the bulk of the genome [72], while the other allele is either not methylated or methylated on a fewer number of CpG islands.

Epigenetic marks are stable in somatic cells, however, the genome undergoes extensive reprogramming during gametogenesis and early embryogenesis[192]. As the fetus develops its own germ cells, the imprint that was inherited from the parental germ cell is first removed and then DNA is methylated de novo relevant to the sex of the individual [173]. Upon fertilization, the egg cytoplasm contains two pronuclei that are epigenetically distinct. Both the maternal and paternal pronuclei undergo a genome-wide demethylation. However, the parent-specific DNA methylation imprints are retained, in spite of total demethylation. Around the time of implantation, a genome-wide re-methylation takes place [192] where imprint marks are retained and persist throughout development and adulthood.

ICRs, which contain the paramount epigenetic differences between parental alle-

les, are the hallmarks of imprinted genes. However, the exact factors that recruit the epigenetic machineries to create differential marks is still elusive. Intriguingly, retrotransposed elements and tandem repeats located within or close to ICRs are good candidates for this role. These sequences are similar to transposable elements, mobile stretches of DNA that have had the ability to colonize new chromosomal locations by hopping from one genomic position to another within a lineage of cells. During the process, a copy of the element is usually maintained at the original site. Thus, a family of transposable elements can build up inside a genome, often to staggering numbers [164]. Retrotransposition produces new genes by reverse transcribing mRNA and incorporating the resulting cDNA into the genome at a new site. This process is mediated by long terminal repeats (LTRs) that flank transposable elements and are recognized by retroviral transposases. Essentially, these gene duplication events enabled the evolution of novel functions and expression profiles.

Transposons are responsible for many mammalian-specific traits and have played a key role in therian evolution, including genomic imprinting of the placenta. Some of the first imprinted genes are believed to have evolved from the domestication of retrotransposons[121] including Peg10, Mcts2, Nnat, Rtl1, and Dlk1. The DLK1-DIO3 domain, which contains the Rtl1 protein coding gene and antiRtl1 non-coding RNA transcript, is only imprinted in eutherian mammals. The paternally expressed Rtl1 gene derives from Ty3/Gypsy type retrotransposon that has evolved a conserved open reading frame missing LTRs[121]. Interestingly, a comprehensive analysis of this domain shows that Rtl1 retrotransposed into the domain region prior to the eutherian-marsupial split, but it only became imprinted in eutherians [120]. In marsupials, the domain has amassed a number of LINE repeats— the marsupial locus is double that of eutherians. Therefore, this suggests that eutherians have

resisted expansion by repetitive elements in this region while gaining noncoding miRNAs[59].

Orthologs of every imprinted gene found in eutherians and marsupials have been examined in monotremes. The majority of imprinted gene cluster orthologs map to similar clusters with similar gene arrangements in the platypus. Comparisons of the imprinted cluster sequences, the collection of neighboring genes whose expression is controlled from an ICR, show an accumulation of LTRs and repetitive elements concurring with the gain of genomic imprinting. Thus, it appears that imprinting arose coincident with repeat invasion and expansion in the eutherian genome. This is consistent with the host defense hypothesis that postulates that imprinting evolved from mechanisms within the cell that silenced foreign invading DNA sequences[10]. Host defense-mediated silencing may have occurred all over the genome to hinder the accelerated augmentation of repeat elements in therians. However, silencing was retained where it led to a selective advantage.

Each imprinted gene cluster expresses at least one lncRNA gene that is reciprocally imprinted relative to the neighboring protein-coding genes. Moreover, some of these ncRNA genes are transcribed in an antisense orientation relative to the protein coding gene. These clusters also contain many small RNAs belonging to families of small regulatory RNA genes which mainly act through sequence-specific recognition of other cellular RNAs; the C/D small RNAs and miRNAs. miRNAs are 19-21 nt long ncRNAs that are processed from stem-loop structures nested within larger primary transcripts. They function as antisense RNAs by forming short base-pairing to partially complementary sequences, most often within the 3'-untranslated region (UTR) of mRNAs [13]. The binding of the complex formed between a miRNA and its correlated Argonaute proteins on the 3'-UTR elicits translation repression of

mRNA degradation through RNA interference mechanisms. Though we do not fully grasp all the features that control the specificity of the short interactions between a miRNA and its target, perfect base-pairing of the seed sequence (nt 2-7 from the 5'-end of the miRNA) is necessary for post-transcriptional gene silencing [143]).

Mammalian miRNAs wield regulatory roles during placental development, from trophoblast invasion to angiogenesis[57]. The vast majority of imprinted miRNA genes are produced from 3 chromosomal domains: C19MC, C2MC, and C14MC. The first two are paternally expressed while the latter is maternally expressed. The three clusters also represent evolutionary novelties as C14MC is found in all eutherians, while C19MC is confined to primates and C2MC is restricted to rodents. antiRtl1 resides within the C14MC domain and contributes significantly in the silencing of Rtl1 mRNA. Imprinted miRNA clusters may have assisted in the phenotypic variability of the placenta by probably improving nutrient exchange between the mother and fetus within each species. The maternally expressed trans-inhibition of Rtl1 by miRNAs processed from antiRtl1 bears a resemblance with the trans-inhibition of Igf2 by the maternally expressed Igf2r. It implies that perhaps the same evolutionary forces at the root of the kinship theory of genomic imprinting have enlisted RNA interference to regulate fetal and placental growth by selecting for mutations that create strand specific miRNAs in antiRtl1 while leaving Rtl1 unchanged.

1.4 CONCLUSIONS

Genomic imprinting in mammals is primarily a conserved process, but has expanded in eutherian mammals. Both imprinting and viviparity first emerged after the divergence of therians and monotremes. In therians, paternally expressed genes are

growth enhancing, whereas maternally expressed genes are growth limiting, suggesting that conflict and kinship are leading drivers of imprinting evolution. The imprinted genes observed in mammals today are the result of evolutionary pressures that drove the selection of this mechanism in a common ancestor, and this process is continuing to evolve. Remarkably, imprinted genes are stable and display high levels of conservation in both their coding and non-coding sequences across different therian species.

2

Lost in translation: The 3'-UTR of IGF1R as an ancient long noncoding RNA

2.1 ABSTRACT

The insulin-like growth factor (IGF) signaling system is a major arena of intragenomic conflict over embryonic growth between imprinted genes of maternal and paternal origin and the IGF type 1 receptor (IGF1R) promotes proliferation of many human cancers. The 3'-untranslated region (3'-UTR) of the mouse *Igf1r* mRNA is

targeted by miR-675-3p derived from the imprinted H19 long noncoding RNA. We undertook a comparative sequence analysis of vertebrate *IGF1R* 3'-UTRs to determine the evolutionary history of miR-675 target sequences and to identify conserved features that are likely to be involved in post-transcriptional regulation of IGF1R translation. Sequences of *IGF1R* 3'-UTRs were obtained from public databases and analyzed using publicly available algorithms. A very long 3'-UTR is a conserved feature of vertebrate *IGF1R* mRNAs. We found that some ancient microRNAs, such as let-7 and mir-182, have predicted binding sites that are conserved between cartilaginous fish and mammals. One very conserved region is targeted by multiple, maternally expressed imprinted microRNAs that appear to have evolved more recently than the targeted sequences. The conserved structures we identify in the *IGF1R* 3'-UTR are strong candidates for regulating cell proliferation during development and carcinogenesis. These conserved structures are now targeted by multiple imprinted microRNAs. These observations emphasize the central importance of IGF signaling pathways in the mediation of intragenomic conflicts over embryonic growth and identify possible targets for therapeutic interventions in cancer.

2.2 INTRODUCTION

Parent-specific gene expression (genomic imprinting) is proposed to be an outcome of evolutionary conflict between genes of maternal and paternal origin [95]. Soon after the first presentation of this hypothesis, three imprinted loci were identified in mice: *Igf2* was a paternally expressed gene (PEG) that encoded insulin-like growth factor II (IGF-II) [54]; *Igf2r* was a maternally expressed gene (MEG) that encoded a receptor for IGF-II [11]; and H19 was a MEG that encoded a long noncoding RNA [14, 26]. *Igf2* promoted fetal growth whereas *Igf2r* inhibited fetal growth [198].

These phenotypic effects were broadly consistent with the theoretical prediction that PEGs should enhance prenatal growth and MEGs inhibit prenatal growth [93] with unimprinted genes favoring an intermediate level of growth [84].

H19 and *Igf2* were each other’s closest chromosomal neighbors and shared common regulatory elements [139, 261]. Therefore, the intuitive prediction was that *H19* (a MEG) should act antagonistically to *Igf2* (a PEG) and function as a fetal growth inhibitor. However, despite more than two decades of study, the functions of *H19* remain poorly understood. Comparisons among *H19* genes from several eutherian species identified conserved secondary structures [115]. One conserved hairpin was shown to be processed as the pre-miRNA for miR-675 [34]. The 3’-untranslated region (3’-UTR) of the murine *Insulin-like growth factor 1 receptor (Igf1r)* gene was subsequently found to possess two target sites for miR-675-3p [2]. IGF1R mediates the fetal growth-promoting effects of IGF-II [198] and binding of miR-675-3p to the 3’-UTR of *Igf1r* mRNA inhibits translation of the receptor [125]. Therefore, miR-675-3p is predicted to function as a maternally expressed inhibitor of fetal growth.

The initial impetus for the present study was to examine the evolutionary history of the miR-675-3p target sequences reported for mouse *Igf1r* mRNA [125]. We soon found that one of these targets was absent from the *IGF1R* genes of rats and humans, but the second target was conserved in the *IGF1R* gene of a cartilaginous fish (*Callorhinchus milii*) where it was located in one of the most highly conserved regions of the entire 7-kb 3’-UTR. This discovery prompted us to undertake an evolutionary analysis of the full length of the *IGF1R* 3’-UTR to complement an earlier evolutionary analysis of the *IGF1R* coding sequence [103].

Our principal objectives were 2-fold. First, we wished to understand better the evolution of genomic imprinting in mammalian development and the interplay be-

tween PEGs, MEGs and unimprinted genes in the IGF signaling system. Second, we wished to contribute to an understanding of the control of IGF1R expression because of the receptor’s important role in cancer biology. IGF1R mediates growth-promoting effects of both IGF-I and IGF-II [198] and is highly expressed in many cancers. Furthermore, cells with inactivated *IGF1R* are resistant to oncogenic transformation [214]. For these reasons, IGF1R was promoted as the Achilles’ heel of most, if not all, cancers [17]. Such high hopes were dashed by disappointing results of clinical trials of therapies targeting IGF1R [16] [189]. These expensive failures may partly reflect imperfect understanding of the regulation of *IGF1R* expression. Highly conserved structures within the 3’-UTR deserve consideration as targets for more effective therapeutic interventions.

2.3 METHODS

Sequences were aligned using BLAST with default settings. miRanda [19], TargetScan [20] and reports in the literature were used to identify putative target sequences of microRNAs. Polyadenylation sites of human *IGF1R* mRNAs were identified in APASdb [21] and APAdb [22] databases. Potential secondary structures of *IGF1R* 3’-UTRs were explored using mFold [23].

2.4 RESULTS

The most abundant human *IGF1R* transcript exceeds 12 kb in length of which 1 kb is 5’-UTR, 4 kb is coding sequence and 7 kb is 3’-UTR. We will call this the ‘long transcript’. A number of shorter transcripts with alternative polyadenylation sites are reported in APASdb and APAdb databases. The second most common transcript in these databases is 6.4 kb in length with a 1.3 kb 3’-UTR. We will call

this the ‘short transcript’. The short transcript possesses an atypical polyadenylation site (upstream sequences do not contain canonical polyadenylation signals).

Putative 3'-UTRs of the long transcript were obtained from genomic sequences of *Homo sapiens* (human), *Monodelphis domestica* (opossum), *Pelodiscus sinensis* (turtle) and *C. milii* (ghostshark). The stop codon of *IGF1R* mRNAs was easy to identify. Significant similarity was also apparent between the 3' end of a human *IGF1R* cDNA and sequences from the other species (Fig 2.4.1). All nucleotides from the end of the stop codon to the end of UCUGUAUGCA were considered to constitute 3'-UTRs of lengths 7087 (*Homo*), 7517 (*Monodelphis*), 7135 (*Pelodiscus*) and 9136 (*Callorhinchus*) nucleotides.

Comparisons among the four focal sequences allowed ‘excavation’ of the deep history of the *IGF1R* 3'-UTR. Features shared by *Homo* and *Callorhinchus* can be inferred to have been present in the last common ancestor of extant jawed vertebrates (stratum 1); features shared by *Homo* and *Pelodiscus* to have been present in the last common ancestor of extant amniotes (stratum 2); and features shared by *Homo* and *Monodelphis* to have been present in the last common ancestor of therian mammals (stratum 3). Stratum 3 also corresponds to the conjectured origin of genomic imprinting and microRNA-675 (Fig 2.4.2a). If a feature is not shared between two sequences, it may have been present in the ancestor but lost in one of the descendent lineages or have been absent in the ancestor but gained in one of the descendent lineages. Data from *IGF1R* 3'-UTRs of other species will be reported where these provide more precise timing for particular evolutionary events.

In comparisons of *Homo* and *Callorhinchus* 3'-UTRs, BLAST detected a few small islands of conservation in a sea of otherwise unalignable sequence. The roughly 10% of alignable sequence constitutes stratum 1. By contrast, BLAST aligned the *Homo*

and *Pelodiscus* sequences for roughly half their length (stratum 2) and the *Homo* and *Monodelphis* sequences for most of their length (stratum 3). Our analysis will focus on the deeply conserved structures of strata 1 and 2. Figure 2.4.2b provides landmarks for orientation in the following discussions of the *IGF1R* 3'-UTR.

2.4.1 STRATUM 1

Stratum 1 consists of features shared by *Homo* and *Callorhinchus* 3'-UTRs. BLAST aligned two segments longer than 100 nucleotides. The longest alignment was a stretch of 300 nucleotides (78% identity) at the 3' end of the long (12 kb) human *IGF1R* mRNA. In addition to the polyadenylation site, this region contains a miR-7-3p target site that has been lost from the *Pelodiscus* sequence (Fig.2.4.1b), a conserved let-7-3p target site (Fig.2.4.1c) and a conserved miR-186 target site (Fig.2.4.1d). All these sites were predicted for the human 3'-UTR by miRanda.

The second longest alignment was a stretch of 175 nucleotides (75% identity) a kilobase from the start of the 3'-UTR that we will call the 'very conserved region' (VCR; Fig.2.4.1e). The VCR includes the polyadenylation site of the short (6.4 kb) human *IGF1R* mRNA. The VCR contains a target site for miR-675 [125], a target site for the miR-16 family of microRNAs [41] [82] and a target site for miR-376c [260]. It is notable that miR-675 and miR-376 are both imprinted and maternally expressed [34] [77]. The miR-675 and miR-16 target sites are proximal to the polyadenylation site and thus included within the short transcript. The miR-376 binding site occurs distal to the end of the short transcript.

Shorter alignments included other predicted target sequences for microRNAs. The 3'-UTR of human *IGF1R* possesses three target sequences for let-7-5p microRNAs [262]. The proximal and central target sequences are conserved in the ghost-

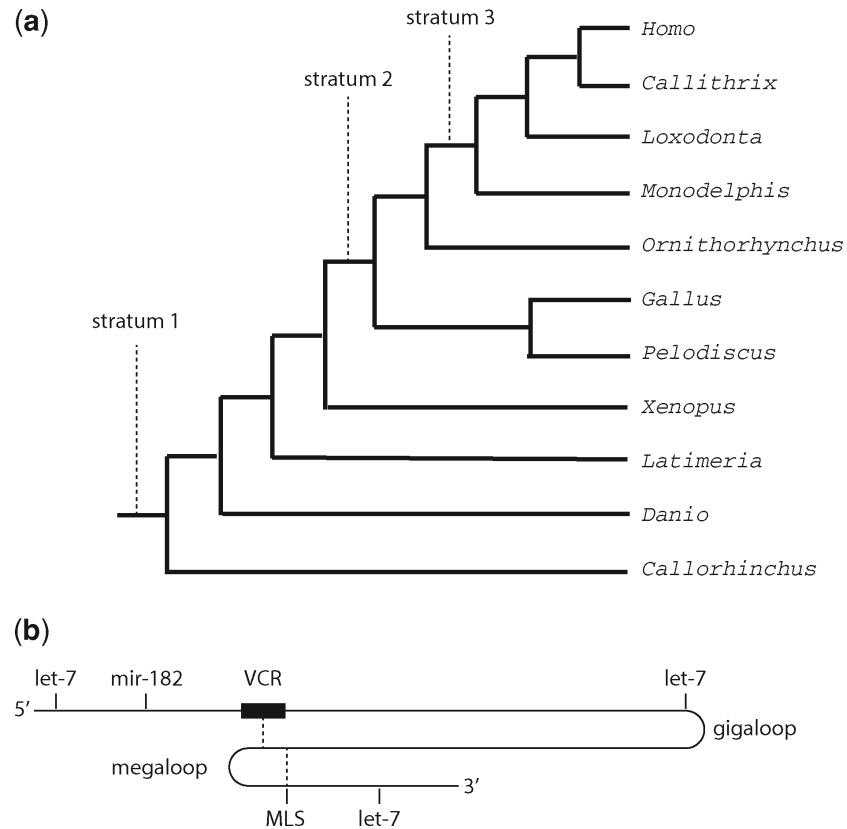


Figure 2.4.2: (a) Phylogenetic relationships of species whose IGF1R 3'-UTRs are used in this study. The origin of genomic imprinting and the H19 long noncoding RNA is thought to coincide with stratum 3 (in an ancestor of marsupial and eutherian mammals). (b) Landmarks on the 7-kb 3'-UTR of the human 'long' IGF1R mRNA including conserved *let-7*-5p and *miR-182* target sites. The 1.3 kb 3'-UTR of the 'short' transcript terminates within the VCR, which also includes a conserved *miR-675*-3p-binding site. The 0.8-kb megaloop is formed by pairing of the megaloop stems (MLS). The 4.8-kb gigaloop is a putative structure formed by pairing of VCR and a complementary sequence (cVCR)

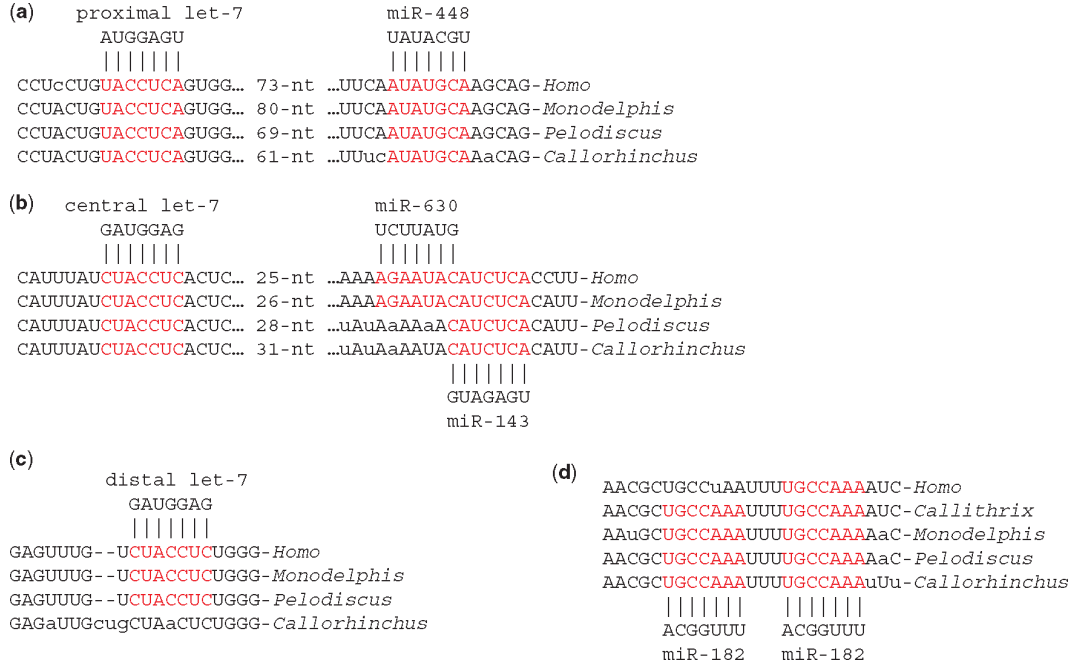


Figure 2.4.3: (a–c) let-7 target sequences; (d) tandem target sequences for miR-182. mi-croRNAs are represented by their seed sequence. Nucleotides of candidate target sequences are shown in red. Nucleotides that differ from the consensus in lower case.

shark sequence whereas the distal target sequence is recognizable, but imperfect, in the ghostshark sequence (Fig.2.4.3a–c). The proximal let-7-5p target sequence is close to a conserved miR-448 target sequence and the central let-7-5p target sequence close to a conserved miR-143 target sequence (Fig.2.4.3a–b) [29, 30]. Another short region of conservation contains tandem target sequences for miR-182 that are present in *Callorhinchus*, *Pelodiscus*, *Monodelphis* and *Callithrix* (marmoset) *IGF1R* genes. Only one of these tandem targets is conserved in the human *IGF1R* gene (Fig.2.4.3d) [248].

Sequence conservation over such large evolutionary distances suggests that the conserved sequences are deeply constrained by function. Postranscriptional regulation of *IGF1R* by let-7, miR-7 and miR-182 are plausible candidates for such ancient functions because these microRNA families are themselves ancient, conserved between protostomes and deuterostomes [39] [202] [51]. By contrast, miR-675 is known only from marsupial and eutherian mammals [223] [56] and miR-376 only from eutherian mammals [77]. Either the miR-675 and miR-376 binding sites were targets for unidentified ancient microRNAs, perhaps still present in *Pelodiscus* and *Callorhinchus*, or the more recent imprinted microRNAs evolved to target sequences that were conserved for functions unrelated to binding by microRNAs. If the latter scenario is correct, then the VCR is likely to have been an original target of miR-675 and miR-376 which evolved to target its sequence. By contrast to the deeply conserved target site for miR-675 in the VCR, the second reported target site for miR-675 [125] is found only in the *Igf1r* 3'-UTRs of house mice and their close relatives.

The two longest regions of sequence conservation between ghostshark and human *IGF1R* in our study corresponded to the 3' ends of the human 'short' (6.4 kb) and 'long' (12 kb) *IGF1R* transcripts. This hints that alternative polyadenylation at these sites is anciently conserved. Other short regions of conservation between *Homo* and *Callorhinchus* sequences corresponded to polyadenylation sites of less common human *IGF1R* transcripts in APASdb and APAdb (data not shown). An earlier comparison of the 3'-UTRs of eight human genes with orthologous sequences in *Squalus acanthias*, another cartilaginous fish detected conservation of the 3' end of the 3'-UTR for six of eight genes [65].

2.4.2 STRATUM 2

Stratum 2 consists of features shared by *Homo* and *Pelodiscus* IGF1R 3'-UTRs. The strongest conservation was a 388-nt alignment that includes the VCR (85% identity with 3% gaps) and a 497-nt alignment at the 3' terminus (82% identity with 8% gaps). This degree of conservation is similar, if not greater, than found for the IGF1R coding sequence (79% identity with 1% gap).

Although the facile alignment of *Homo* and *Callorhinchus* 3'-UTRs ends shortly after the miR-16 binding site of the VCR at the location of the polyadenylation site for the short human transcript (Fig.2.4.1e), strong similarity continues beyond this point for *Homo*, *Monodelphis*, *Pelodiscus* and *Xenopus* sequences. This 'extended VCR' contains a remarkable concentration of predicted binding sites for microRNAs, including miR-335-3p [104], miR-376a [260] and miR-493 [180]. The *Monodelphis* and *Xenopus* sequences also have a predicted binding site for miR-379-5p adjacent to the miR-493 binding site (Fig.2.4.4a). miR-376a, miR-379 and miR-493 are encoded in a large cluster of maternally expressed imprinted microRNAs found only in eutherian mammals [77]. miR-335 is a paternally expressed imprinted microRNA located in the second intron of its MEST host gene [203] [105]. miR-335 is well conserved in armadillo and elephant *MEST* genes but absent from the *MEST* genes of opossum and platypus. Therefore, conservation of the extended VCR appears to be more ancient than the imprinted miRNAs that currently target its sequence. The simplest interpretation is that the extended VCR has important functions independent of binding of imprinted microRNAs and that these microRNAs evolved to target the conserved sequence.

A notable feature of stratum 2 is a pair of complementary sequences, 800 nu-

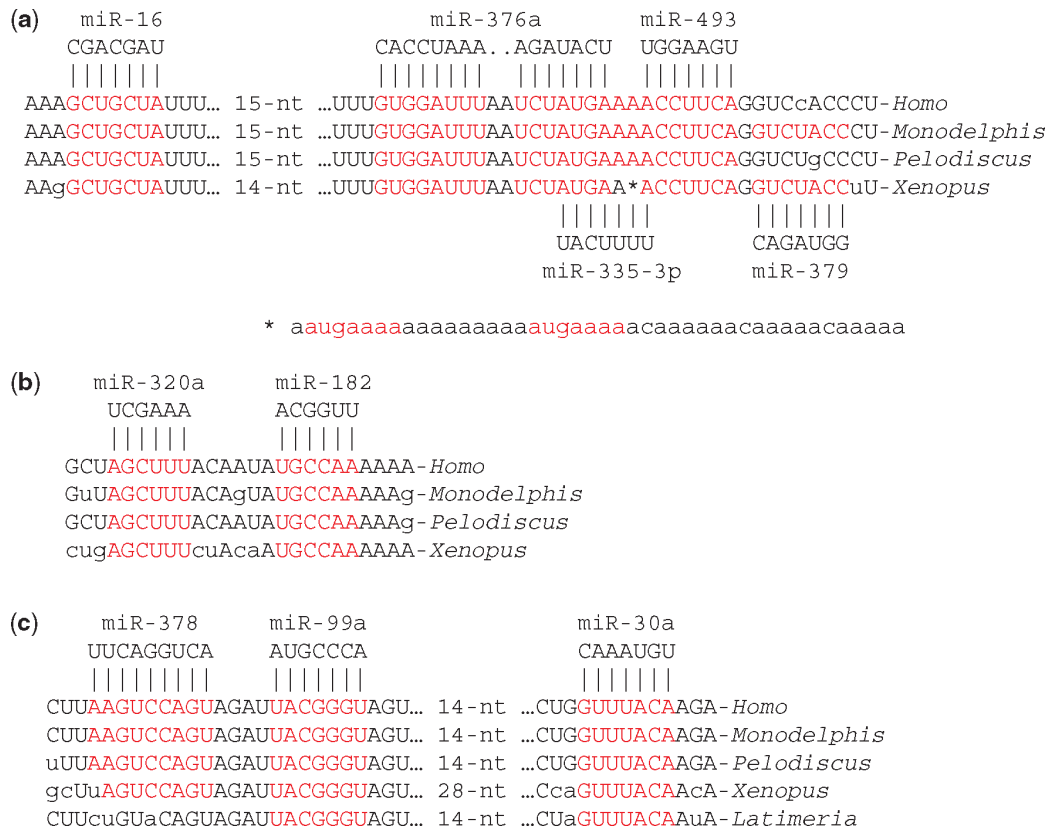


Figure 2.4.4: (a) miR-16 target site (also shown in Fig. 2e) and nearby target sites for miR-376a, miR-335-3p, miR-493 and miR-379 (the *Xenopus* sequence contains a 44-bp insertion at the site of the asterisk that includes two target sites for miR-335-3p are shown in red); (b) conserved pair of target sites for miR-320a and miR-182; (c) conserved triplet of target sites for miR-378, miR-99a and miR-30a

cleotides apart, that are predicted to form the stems of a strong double helix (18 bp, -32.3 kcal/mol). Similar complementary sequences are found in *Xenopus* and *Latimeria* (coelacanth) 3'-UTRs (Fig.2.4.5a), but neither in *Danio* (zebrafish) nor *Callorhinchus* 3'-UTRs. The region between the 5' and 3' stems will be called the 'megaloop'. The megaloop contains, among other features, a conserved pair of target sites for miR-320a and miR-182 (Fig.2.4.4b) and a conserved triplet of target sites for miR-378, miR-99a and miR-30a (Fig.2.4.4c). All of these features are also found in the 3'-UTR of *Xenopus silurana* and are therefore predicted to have been present in the ancestor of all extant tetrapods. The miR-99a and miR-30a target sites are found in the *Latimeria* sequence and can therefore be inferred to have been present in the last common ancestor of tetrapods and lobe-finned fishes.

2.4.3 ALTERNATIVE SECONDARY STRUCTURES

IGF1R mRNAs were longer than the maximum size that could be submitted to mFold. Therefore, we explored folding of the 7-kb 3'-UTR of the long human transcript without the 5'-UTR and coding sequence. The mFold algorithm makes many simplifying assumptions and the predicted structures of such a long transcript are unlikely to be accurate in all details. For example, the algorithm does not consider the possibility of pseudo-knots, triplexes, G-quadruplexes and other complex structures, nor does it take account of interactions between the RNA sequence and RNA-binding proteins. Our purpose in this exploratory analysis was to search the predicted structures for repeated stable features involving sequences that were already of interest from our evolutionary analysis.

The 5' and 3' stems of the megaloop (see above) formed a strong double helix in many secondary structures predicted by mFold. By contrast, the miR-675 binding

(a)

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5' -GCCAGAGAUGCUGAAGAUAACAGACCUUGG... 823-nt ...AAAAAGUAGUUCUGUAUCUUCAGUAUCUUGGU-3' Homo
5' -GCCAGAGAUGCUGAAGAUAACAGACCUAG... 810-nt ...AAGAAGUAGUUCUGUAUCUUCAGUAUCUUGGU-3' Monodelphis
5' -GCCAGAGGUGCUGAAGAUAACAGAACUGUC... 794-nt ...GAGAAACUGUUCUGUAUCUUCAGUAUCUUGGU-3' Pelodiscus
5' -GCCAGAGAUGAUAAGAUAACAGAACUGAA... 969-nt ...AAGUUAAGUUCUGUAUCUUCAGUAUCCUGGU-3' Latimeria

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(b)

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5' -GAAACAUUUGCAUACACAUCGUC ... 4540 bp ... CUCUGUGUGUGCAAAUGUGUG-3' Homo
5' -GAAACAUUUGCAUACACAUCGUC ... 4534 bp ... UUCUCUGUGUGCAAAUGUGUG-3' Loxodonta
5' -GGAUCAUUUGCAUACACAUCGUC ... 4882 bp ... CUCUGUGUGUGCAAAUGUGUG-3' Monodelphis
5' -GGAUCAUUUGCAUACACAUCGUC ... 4770 bp ... GUGUGUGUAUGUAAAGUAUGU-3' Ornithorhynchus
5' -GGAUCAUUUGCAUACACAUCGUC ... 4777 bp ... GUGUGUGUGUGUGUAUGAUCA-3' Pelodiscus
5' -GGAUCAUUUGCAUACACAUCGUC ... 4814 bp ... GUCUGUGUGUGUGUGUGUCUG-3' Gallus

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Figure 2.4.5: Nucleotides predicted to form the stems of (a) megaloop and (b) giga-loop are shaded

site of the VCR was often located within a long single-stranded bulge or in weakly bonded secondary structures. Thus, the VCR exhibits the intriguing combination of highly conserved primary sequence (suggesting important conserved function) and lack of clear secondary structure. Similarly, regions of high sequence conservation between human and mouse 3'-UTRs of the *MeCP2* gene do not form stable secondary structures [45]. We suggest that the VCR is analogous to 'intrinsically disordered' domains of proteins that have important regulatory functions [251]. A flat energy landscape in the vicinity of the VCR means that this region is intrinsically mobile and potentially able to interact with proteins, microRNAs and other regions within the *IGF1R* 3'-UTR.

Although the VCR was 'disordered' in many predicted structures, the miR-675 target site was sometimes recovered as part of a strong double helix (15 bp, -25.8 kcal/mol) formed with a complementary sequence (cVCR), 4.5 kb distant located between the stems of the megaloop (Fig. 2.4.5b). We will refer to this putative pairing of VCR and cVCR as the gigaloop. From the temporal perspective of transcription, the 5'-stem of the gigaloop (VCR) is transcribed first, then the 5'-stem of the megaloop, then the 3'-stem of the gigaloop (cVCR), then the 3'-stem of the megaloop (Fig. 2.4.2b). Therefore, the stems of the gigaloop have an opportunity to pair before the 3'-stem of the megaloop is transcribed. One intriguing scenario is that the VCR initially adopts an inactive conformation paired to the cVCR but acquires its active, open conformation by formation of the megaloop. We are unable to tell whether the megaloop and gigaloop could both exist in the same folded structure because pseudo-knots (part of a loop forming a stem with another sequence outside of its loop) are precluded by the mFold algorithm.

The age of the VCR-cVCR double helix is unclear. A strong double helix is

predicted for the 3'-UTRs of *Loxodonta* (elephant, 13 bp, -21.5 kcal/mol) and *Monodelphis* (opossum, 12 bp, -19.4 kcal/mol), but the cVCR is poorly conserved in *Ornithorhynchus*, *Pelodiscus* and *Gallus*. The homologous sequences of the latter species consist mostly of simple UG repeats, but when the VCR and repetitive sequences are induced to pair, mFold predicts a 12 bp double helix in *Ornithorhynchus* (-18.9 kcal/mol), a 11 bp double helix in *Pelodiscus* (-16.7 kcal/mol) and a 10 bp double helix in *Gallus* (-17.9 kcal/mol). Because of the simplicity of the UG repeats and the general 'stickiness' of Us and Gs (G and U can form 'wobble' pairs with each other as well as the standard Watson-Crick pairing of G with C and U with A), we consider the evidence for functional pairing of VCR and cVCR to be equivocal in *Pelodiscus* and *Gallus*. A sequence complementary to the VCR within the megaloop was found in neither the *Xenopus* nor *Latimeria* sequences.

2.4.4 3'-UTRs AS BOOLEAN DEVICES

Individual microRNAs (in complex with Argonaute proteins) have been reported to both inhibit and promote translation of mRNAs [44, 45]. The assemblage of microRNAs within a cell can be considered a 'news report' of cellular conditions that is 'read' by the 3'-UTRs of genes that have 'subscriptions' (target sites) to the news service. As a result of adaptive natural selection, each 3'-UTR is expected to respond adaptively to some microRNAs but not others. A notable feature of our comparative analysis is the clustering of microRNA-binding sites (Figs 1-4). Close proximity of binding sites could facilitate antagonistic or synergistic interactions among Argonaute-microRNA complexes (for example by steric hindrance). This could enable the 3'-UTR to perform the equivalent of simple logical calculations. If an effect Z was achieved by the binding of both miR-X and miR-Y, then the

interaction of the microRNAs would be formally equivalent to the Boolean operation $X \text{ AND } Y = Z$. If instead, miR-X and miR-Y had redundant effects, this would be equivalent to $X \text{ OR } Y = Z$. If binding of miR-Y prevented miR-X causing Z, this would be equivalent to $X \text{ NOT } Y = Z$. In a similar manner, combinations of riboswitches have been proposed to perform Boolean computations [228] [169].

The long 3'-UTR of IGF1R (> 7000 nucleotides) may function as a 'computational device' that responds to information about cellular conditions provided by the binding of miRNAs (among other factors). miRDB lists 185 microRNAs binding human IGF1R 3'-UTR, many of them with multiple target sites [48]. A comparison to other long transcripts with predicted target sites for miR-675-3p (*KCNN3*, *TAOK1*, *PYGO1*, *MRPL19*, *ANKH* and *ADAM22*) suggests that 185 is a 'middling' number of predicted microRNAs for a transcript of this length. Even if many of these predicted sites are nonfunctional, the potential for allosteric interactions within and among clusters of binding sites could enable the 3'-UTR to perform complex, hierarchically organized computations to determine if and when the mRNA is translated. Most research on the effects of microRNAs on expression of IGF1R investigates effects of individual microRNAs on protein or mRNA levels. If the 'computational model' of 3'-UTR function is correct, the investigation of one microRNA at a time may give inconsistent results because the meaning of a microRNA 'word' must be understood in the context of the cellular 'sentence' of co-occurring microRNAs.

2.4.5 IMPRINTED MICRORNAs AND THE REGULATION OF IGF1R

Our comparative analysis of the *IGF1R* 3'-UTR has uncovered deeply conserved sequences that are targeted by multiple maternally expressed imprinted microRNAs. An evolutionary interpretation of this finding requires a digression about the co-

evolution of microRNA seeds and their mRNA targets. A typical microRNA binds to the 3'-UTRs of many genes [49]. Therefore, mutations to the seed sequence of ancient microRNAs are strongly selected against because each mutation affects the expression of many genes. By contrast, a change to an mRNA's target sequence directly affects the expression of only that gene. For this reason, the mRNAs that a microRNA targets are more evolutionarily labile than the seed sequence that recognizes these targets [40].

Additional evolutionary complexities arise when an imprinted microRNA targets an unimprinted mRNA because mutations in the seed sequence are subject to selection only when the microRNA is inherited from parents of one sex but mutations in the target sequence are subject to selection when inherited from parents of both sexes. Therefore, changes that promote the fitness of the targeted sequence need not promote the fitness of the targeting sequence. For example, PEGs are predicted to favor more fetal growth than unimprinted genes (and MEGs favor less fetal growth). A novel maternally expressed microRNA that targeted the biallelically expressed *IGF1R* 3'-UTR would be favored by natural selection if it inhibited expression of the protein because signaling via IGF1R promotes fetal growth. Such targeting would, in turn, favor mutations of the target site that eliminated binding by the microRNA. For this reason, many interactions between imprinted microRNAs and unimprinted targets are expected to be evolutionarily short-lived. But the simple expedient of eliminating the target is foreclosed if the targeted sequence performs other essential functions.

These considerations suggest an evolutionary model for post-transcriptional regulation of unimprinted mRNAs by imprinted microRNAs. Maternally expressed microRNAs target conserved regions of the *IGF1R* 3'-UTR both because these re-

gions are functionally important and because natural selection favors maintenance of the target sequence for reasons unconnected to targeting by the microRNAs. By this process, multiple imprinted microRNAs have come to target the highly conserved sequences of the extended VCR. Our analysis suggests that the VCR may have been the original functional target of the imprinted microRNAs because the targeted sequences are evolutionarily older than the imprinted microRNAs that bind them (the microRNA seed sequences evolved to match the target sequences rather than the other way round).

Because of the distinct selective forces acting on imprinted and unimprinted loci, the unimprinted *IGF1R* 3'-UTR is predicted to undergo evolutionary change at other sites to compensate for inhibitory effects of imprinted microRNAs. Such changes are predicted to stabilize the relationship between the 3'-UTR and maternally expressed microRNAs because mutations to a microRNA that eliminated its interaction with *IGF1R* would be selected against because these would increase expression of IGF1R and increase fetal growth. Once the seed sequence of an imprinted microRNA is 'evolutionarily tethered' in this manner, other unimprinted mRNAs will evolve binding sites for the microRNA if such binding enhances the fitness of the genomic sequence encoding the unimprinted mRNA. As a result, some of the interactions between an imprinted microRNA (or long noncoding RNA) and unimprinted RNAs will promote the fitness of the 'targeting' sequence and others the fitness of the 'targeted' sequences. These complexities may partly explain the difficulty of assigning an unambiguous function to *H19* and miR-675 in the regulation of cell proliferation. *H19* inhibits cell proliferation in some systems[247] but promotes proliferation in other systems [151] [144] and has been interpreted as both a tumor suppressor[256] [138]and oncogene [152].

IGF1R protein mediates the growth-promoting effects of IGF2. Therefore, evolutionary theory would predict *IGF1R* to be paternally expressed if it were imprinted. However, inactivating mutations of *Igf1r* in mice provide no evidence of imprinting[150] and effects of *IGF1R* mutations on human growth appear independent of parental origin [241]. The absence of imprinting of *IGF1R*, despite its role in promoting fetal growth, could be explained if the gene’s effects are not dosage sensitive, because selection for imprinted expression is weak or nonexistent if one active copy is functionally equivalent to two [88]. Data on haplosufficiency versus haploinsufficiency of effects of *IGF1R* on prenatal growth are ambiguous and differ between mice and humans. Mice with one inactivated copy of *Igf1r* do not exhibit growth deficits at birth or before weaning[150] [106]. In one of these knockouts, inactivation of one copy of *Igf1r* did not reduce steady-state levels of mRNA[150]whereas, in another knockout, inactivation of one allele was associated with a 50% reduction in the cell-surface expression of *Igf1r* protein [106]. On the other hand, some children with heterozygous mutations of *IGF1R* exhibit intrauterine growth retardation although this phenotype has variable penetrance even for children with the same mutation[244].

Although *IGF1R* transcripts are not known to exhibit parent-specific expression, the *IGF1R* genomic locus contains a sequence within intron 2 that is more heavily methylated when inherited from mothers than from fathers [216]. Moreover, the first intron of human *IGF1R* contains the promoter of a long noncoding RNA *IRAIN* that is antisense to the 5'-UTR of *IGF1R*, interacts with the *IGF1R* promoter [229], and is exclusively transcribed from the paternally derived allele [122]. If *IRAIN* acts in cis, then a functional interaction with the *IGF1R* promoter could result in differential expression of the maternal and paternal alleles of *IGF1R*. Clearly, there

are complexities in the regulation of *IGF1R* expression yet to be explicated.

2.5 CONCLUSIONS AND IMPLICATIONS

Evolutionary sequence analysis is not a substitute for experimental investigation of mechanisms but evolutionary analysis can guide experiment by identifying structures of interest. The 7 kb 3'-UTR of the major *IGF1R* transcript compares to a median length of human 3'-UTRs of about 1.2 kb [162]. Our analysis suggests that a very long 3'-UTR is an ancient feature of *IGF1R* genes and that the 3'-UTR contains deeply conserved sequences. The sequence we call the VCR stands out as deserving detailed investigation for three reasons. First, its primary sequence is deeply conserved and must therefore be strongly constrained by function. Second, the VCR is located immediately adjacent to the polyadenylation site of the short (6.4 kb) human *IGF1R* mRNA. Third, the VCR is targeted by multiple imprinted microRNAs (including miR-675-3p derived from the *H19* RNA). IGF signaling has been a focus of evolutionary conflict between genes of maternal and paternal origin over relative proliferation of particular cell types as witnessed by the opposite imprinting of *IGF2* and *IGF2R* [89] [92]. For this reason, regions of the *IGF1R* mRNA targeted by imprinted microRNAs are strong candidates for effects on the control of cell proliferation.

3'-UTRs play many important roles in the control of protein translation and function [163]. The 3'-UTRs of *IGF1R* mRNAs have been little studied, beyond the identification of microRNA target sites, perhaps because of the transcripts' length and complexity. Remarkably, we have found no articles that discuss polyadenylation of mammalian *IGF1R* mRNAs, even though polyadenylation is considered a requirement for efficient translation. The only papers we found on *IGF1R* polyadenylation

came from teleost fish. One study in turbot found that a 13-kb *IGF1R* transcript was polyadenylated in oocytes and early embryos but not in larvae or adult somatic tissues [25]. Our study found that sequences near the polyadenylation sites of the human 6.4 and 12 kb mRNAs are conserved between mammals and cartilaginous fish (and other small regions of ancient conservation included additional polyadenylation sites). The polyadenylation of human *IGF1R* transcripts and the dynamics of alternative polyadenylation deserve future study.

Our study investigated the 3'-UTR of a single gene (*IGF1R*) with a focus on its interaction with a single imprinted microRNA (miR-675-3p). We have undoubtedly explored only the tip of the iceberg in understanding the role of noncoding RNAs in intragenomic conflicts. The largest clusters of microRNAs in the human and mouse genomes are imprinted. These include a cluster of maternally expressed microRNAs (C14MC) present in all eutherian mammals [77] and the paternally expressed C19MC cluster of primates [25] [175] and *Sfmbt2* cluster of muroid rodents [110]. The maternally expressed H19 long noncoding RNA is not only the substrate for the production of miR-675 but also acts as a competing endogenous RNA (ceRNA) or 'molecular sponge' for absorbing other microRNAs [42] [187]. Similarly, *Maternally expressed gene 3* (*MEG3*), a close genomic neighbor of the C14MC microRNAs, is an imprinted long noncoding RNA with ceRNA functions [78]. Conflict breeds complexity. Untangling the web of interactions is a task for future experimental and evolutionary studies.

3

Retrotransposon Gag-like 1 (RTL1) and the molecular evolution of self-targeting imprinted microRNAs

3.1 ABSTRACT

Transcription of the sense strand of the *RTL1* gene produces a protein-coding mRNA that is targeted for degradation by microRNAs transcribed from the antisense strand

of the protein-coding sequence. The sense and antisense transcripts are oppositely imprinted: sense mRNAs are expressed from the paternally-derived chromosome, antisense microRNAs from the maternally-derived chromosome. Two microRNAs at the *RTL1* locus, miR-431 and the rodent-specific miR-434, are derived from within tandem repeats. We present an evolutionary model for the establishment of a new self-targeting microRNA derived from within a tandem repeat that inhibits production of RTL1 protein when maternally-derived in heterozygotes but not when paternally-derived. The interaction of sense and antisense transcripts can be interpreted as a form of communication between maternally-derived and paternally-derived *RTL1* alleles that possesses many of the features of a greenbeard effect. This interaction is evolutionary stable, unlike a typical greenbeard effect, because of the necessary complementarity between microRNAs and mRNA transcribed from opposite strands of the same double helix. We conjecture that microRNAs and mRNA cooperate to reduce demands on mothers when an allele is paired with itself in homozygous offspring.

3.2 BACKGROUND

From his experiments on hybridization in peas, Gregor Mendel concluded that “it is perfectly immaterial whether the dominant character belong to the seed-bearer or to the pollen-bearer; the form of the hybrid remains identical in both cases” [1]. This identity of reciprocal heterozygotes was a largely unquestioned assumption of Mendelian genetics until the discovery of ‘imprinted’ loci at which alleles were differentially expressed depending on whether they were inherited via an egg or a sperm [188]. The *callipyge* (*CLPG*) phenotype of sheep (hypertrophied muscles of the ovine rump) violates basic Mendelian presumptions. Sheep-breeders were un-

able to fix this desirable trait within a herd (*callipyge* comes from the Greek for beautiful buttocks) because the only animals to express the trait inherited the underlying mutation from their father but not their mother (+/*CLPG*). The reciprocal heterozygotes who inherited the mutation from their mother (*CLPG*/+) had rumps of normal proportions as did sheep who inherited the mutation from both parents (*CLPG/CLPG*) or neither parent (+/+) [44]. The difference between the heterozygous genotypes could be explained by genomic imprinting but the normal phenotype of both homozygous genotypes implied some unknown interaction between maternal and paternal alleles.

The *callipyge* region of ovine chromosome 18 and orthologous regions of human chromosome 14 and mouse chromosome 12 were subsequently found to be the home of a major cluster of imprinted genes [231] [209] [253] including many non-coding RNAs [170] [37] [212]. One gene from this cluster, *Retrotransposon Gag like 1* (*RTL1*), is derived from a *sushi-ishi*-like retroelement [153] [32] that has retained a protein-coding sequence in eutherian but not marsupial genomes [120]. RTL1 protein is encoded by a single exon, with conservation of the capsid and protease domains of the ancestral retroelement, but with loss of critical residues in polymerase, RNaseH and integrase domains [153]. The paternal copy of RTL1 is transcribed from the antisense strand of the DNA double helix to produce a protein-coding ‘sense’ mRNA whereas the maternal copy is transcribed from the sense strand to produce multiple ‘antisense’ microRNAs [38] [211]. In some cells, these microRNAs are coordinately transcribed as part of a long antisense transcript [211]. In other cells, individual microRNAs are transcribed from unique promoters [205][225]. These microRNAs are complementary to the *RTL1* coding sequence and promote degradation of *RTL1* mRNA [112] [52] [224]. The *RTL1* sequence is thus constrained by dual

roles as the progenitor of a paternally-expressed mRNA and of maternally-expressed microRNAs.

Rtl1 mRNA is expressed in endothelial cells of the labyrinthine zone of the murine placenta. Deletion of the coding sequence results in complete suppression of *Rtl1* mRNA when inherited from fathers (paternal-knockout) but three-fold increased *Rtl1* mRNA when inherited from mothers (maternal-knockout). The latter effect is a consequence of the absence of the maternally-expressed antisense microRNAs that target paternally-expressed *Rtl1* mRNA [213]. Both knockouts are associated with abnormal placental morphology. Mice with the paternal knockout suffer pre-natal growth retardation associated with detachment of endothelial cells from the basement membrane of placental capillaries [129]. Mice with the maternal knockout have normal birth weight but suffer post-weaning growth retardation.

Overexpression of *Rtl1* causes muscular hypertrophy in mice, suggesting a role of the gene in the callipyge phenotype of sheep [254], induces hepatocellular carcinomas in mice [197], and promotes cellular proliferation in human melanoma [63]. Non-expression of *Rtl1* is the major cause of post-implantation failure of cloned pigs [258]. A critical evaluation of all the reported effects of *Rtl1*-associated miRNAs, both pro-oncogenic and anti-oncogenic, is beyond the scope of this paper.

3.3 GENOMIC IMPRINTING AND INTRAGENOMIC CONFLICT

The kinship theory of genomic imprinting was developed to explain the origin and maintenance of imprinted gene expression both in prenatal and postnatal life [87] [89]. Simple models predicted that incremental decreases in the expression of paternally-expressed genes (PEGs) would reduce patrilineal fitness whereas incremental decreases in the expression of maternally-expressed genes (MEGs) would

reduce matrilineal fitness [87]. In these models, fitnesses were a function of the level of gene product $X = xm + xp$, where xm was the level of expression of the maternally-derived allele and xp the level of expression of the paternally-derived allele, with an allele's 'strategy' represented by the two-element vector xm, xp . The models found the unbeatable strategy to be maternal silence 0, X if natural selection favored higher values of X for paternally-derived alleles and paternal silence X , 0 if natural selection favored higher values of X for maternally-derived alleles.

In the context of maternal–fetal relations and placental development, the kinship theory predicts that PEGs will increase fetal demands on mothers and MEGs will have opposing effects [97]. The classic example are the opposite effects on embryonic growth in mice of *Insulin-like growth factor 2 (Igf2)*, a paternally-expressed growth-enhancer, and *Insulin-like growth factor 2 receptor (Igf2r)*, a maternally-expressed growth-inhibitor that targets IGF-II to lysosomes for degradation [94]. A single amino-acid substitution in the IGF2-binding site of the IGF2R protein resulted in embryonic and placental overgrowth in mice because it abolished clearance of IGF2 by the receptor [108]. Similar antagonistic effects on fetal growth are observed in humans. Fetal overgrowth can be caused either by the mutational inactivation of the maternal copy of *CDKN1C*, a paternally-silent growth-inhibitor [29] whereas intrauterine growth-restriction can be caused by inactivating mutations of the paternal copy of *IGF2*, the aforementioned maternally-silent growth-enhancer [18].

The opposite effects on levels of RTL1 protein of paternally-expressed *RTL1* mRNA and maternally-expressed microRNAs have been interpreted as consistent with the kinship theory [142]. Moreover, the prenatal growth retardation observed in paternal knockouts of *Rtl1* is compatible with RTL1 protein functioning as a fetal demand enhancer and the increase of *Rtl1* mRNA when the imprinted microR-

NAs are knocked-out is compatible with the microRNAs acting as demand inhibitors [213][129]. These hypotheses remain to be tested in experimental systems that cause less severe perturbation in the level of *Rtl1* mRNA.

The conclusion that the phenotypic effects of *RTL1* support the kinship theory is premature because the details of this system violate a key assumption of early models of the theory. This was the assumption that xm and xp were fixed properties of an allele and therefore independent of the identity of the other allele of a diploid individual. An *RTL1* allele is transcribed both as a PEG (mRNA) and as MEGs (microRNAs) that inhibit the PEG. Therefore, the level of expression of the paternal allele depends on the identity of the maternal allele. At such a locus, an allele's strategy could be thought of as a three-element vector xm, xp, xq where xm and xp are the levels of expression when maternally- and paternally-derived in heterozygotes, and xq is the level of expression in homozygotes.

Haig [90][98] made a first attempt to address this theoretical puzzle in the context of self-targeting microRNAs expressed during seed development. He argued that imprinted noncoding RNAs are subject to distinctive selective forces when they regulate transcripts of the allele inherited from the other parent. If an mRNA possesses a sequence complementary to the imprinted noncoding RNA, and the complementary sequences pair, then the mRNA can be considered to 'recognize' its similarity to the allele from which the microRNA was transcribed and can evolve to respond strategically to this information. By this means, the maternal and paternal alleles at a locus could coordinate their activities with the noncoding RNA functioning as a signal by which one allele 'communicates' its presence to the other. Such strategic coordination between alleles would possess the formal properties of a 'greenbeard effect' with possibilities for 'altruistic' behavior in homozygotes but 'selfish' behavior

in heterozygotes [98].

Classic formulations of greenbeards invoked a triad of trait, recognition, and response: an actor with the trait recognizes the trait in another individual and responds altruistically [196] [21]. Such effects were dismissed as implausible because it was considered unlikely that the three aspects of the triad could be instantiated at a single locus. Interest in greenbeards has revived because of the realization that recognition of identity at the molecular level is simpler than recognition of relatedness [86] and because theoretical models find that tight linkage of trait, recognition, and response is not necessary to facilitate the evolution of cooperation [114].

A microRNA can be considered a *trait* that is *recognized* by the microRNA's targets and that *informs* the targeted sequences of the identity of the targeting sequence. Regulation of the targeted sequence by the microRNA can be considered the *response* of the target to this information. If the microRNA acts on an mRNA transcribed from the other allele at its locus, then regulation of the mRNA by the microRNA can be conceptualized as a response to self-recognition. 'Self-targeting' microRNAs thus combine all three aspects of the greenbeard triad in a single nucleic acid sequence.

The mammalian *RTL1* locus meets the above criteria for 'self-recognition' and thus provides a test-case for disentangling the evolutionary complexities of such systems. The current paper investigates the evolutionary history of microRNAs at the *RTL1* locus and then develops an evolutionary model of how this complex system could have evolved.

3.4 RESULTS

3.4.1 MRNAS PROCESSED FROM RTL1-ANTISENSE TRANSCRIPTS

Our sequence analysis builds upon prior analyses of the *RTL1* sequence [153][52]. We obtained *RTL1* sequences from the reference genomes of representative primates (*Homo*, *Callithrix*), rodents (*Mus*, *Rattus*, *Nannospalax*, *Heterocephalus*, *Cavia*), afrotherians (*Elephantulus*, *Orycteropus*, *Trichechus*) and xenarthrans (*Dasypus*, *Choloepus*). Fig 3.4.1 shows relative locations of features we will discuss in the human and mouse *RTL1* genes and provides a phylogenetic overview of when particular features arose.

Sequences highly similar to pre-miR-136, pre-miR-127, and pre-miR-433 were found in all species. Sequences highly similar to pre-miR-431 were found in all species except xenarthrans. We do not know whether this is a real or artifactual absence because of gaps in xenarthran genome assemblies. We also found sequences similar to pre-miR-432 in all species although these sequences had undergone substantial divergence in rats and mice with loss of functional miR-432. pre-miR-432 can be inferred to have been present in an ancestor of all extant eutherian mammals but to have been lost within the rodent lineage where it has been replaced by miR-434. Alignments of these sequences are provided in Fig S1 of Additional File 1.

Our analysis will distinguish between ‘autotargets’ and ‘allotargets’. An autotarget is the sequence that is directly complementary to a microRNA because it is transcribed from the opposite strand of the DNA double helix. Allotargets are sequences that are complementary to the microRNA’s seed but are not the microRNA’s autotarget. The distinction between autotargets and allotargets is evo-

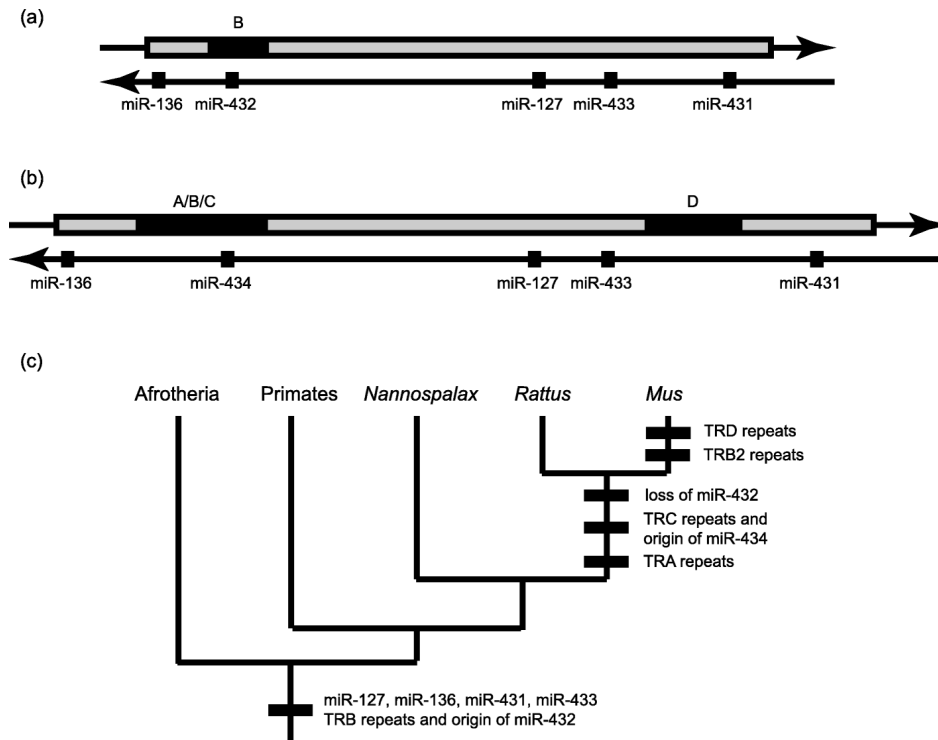


Figure 3.4.1: The location of tandem repeats (A–D) within the protein-coding region of the sense strand (above) and of microRNAs on the antisense strand (below) of (a) human RTL1 and (b) mouse Rtl1. (c) Phylogenetic 'road-map' of when particular features arose.

lutionarily significant because changes to seed sequences are necessarily associated with changes to autotargets (microRNA and autotarget are transcribed from opposite strands of the *RTL1* DNA double helix). By contrast, changes to seed sequences are not associated with complementary changes to allotargets. (The seed is the 7-nucleotide sequence principally responsible for functional binding of microRNAs to their targets.)

Three of the antisense microRNAs have predicted allotargets on the sense *RTL1* mRNA (Fig 3.4.2): miR-136-3p has a highly-conserved allotarget that overlaps the autotarget of miR-127-3p; miR-431-5p has a conserved allotarget overlapping the autotarget of miR-431-3p; miR-432-5p has a conserved allotarget overlapping the autotarget of miR-432-3p. In addition to targets on the sense mRNA, at least four microRNAs (miR-127-3p, miR-431-5p, miR-432-5p, miR-433-3p) have predicted allotargets on the antisense pre-miRNA sequences from which they are processed. Nucleotides 2–9 of miR-127-3p (CGGAUCCG) are self-complementary as are nucleotides 1–8 of miR-433-3p (AUCAUGAU). These microRNAs possibly bind to their own seed sequences (Fig S2 of Additional File 1).

Our *in silico* predictions of intralocus allotargets hint at hidden complexities of RNA–RNA interactions (and possibly RNA–DNA interactions) at the *RTL1* locus. We acknowledge the limitations of non-experimental analyses but hope that our analyses can provide some guidance for experiment. Experimental data on the interaction between mRNA and microRNAs at the *RTL1* locus are limited. The microRNAs are known to initiate cutting of *RTL1* mRNA but the specific sequences targeted by each microRNA are unknown. We identified potential targets by the criterion of perfect matches to seed sequences, but complementarity is not proof that an interaction occurs *in vivo* and may miss targets that lack perfect comple-

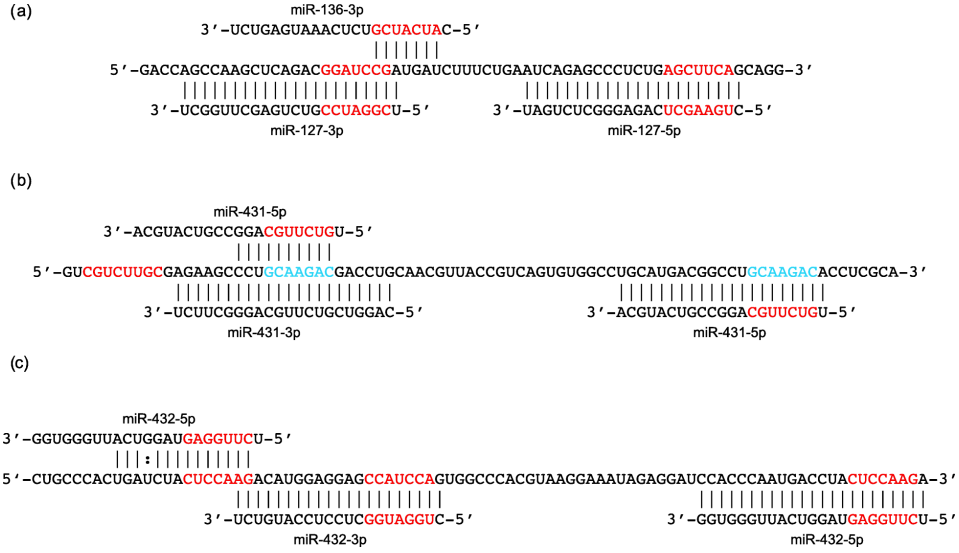


Figure 3.4.2: (a) an allotarget of miR-136-3p overlaps the autotarget of miR-127-3p; (b) an allotarget of miR-431-5p overlaps the autotarget of miR-431-3p; (c) an allotarget of miR-432-5p overlaps the autotarget of miR-432-3p.

mentarity to the seed sequence (so-called non-canonical targets). Canonical targets of microRNAs are typically complementary to the seed sequence of a microRNA, not to the entire length of the microRNA as occurs for autotargets.

3.4.2 TANDEM REPEATS AT THE RTL1 LOCUS

A notable feature of prior analyses of the RTL1 sequence [153] [52] was the presence of tandem repeats within the protein-coding sequence. Davis et al. [52] recognized four classes of tandem repeats which they named TRA, TRB, TRC, and TRD.

Thirty-three nucleotides separate the initial nucleotides of miR-432-5p's allotarget

and autotarget in the RTL1 mRNA. This 33-nucleotide periodicity corresponds to one TRB unit. miR-432 is processed from within the TRB array. We detected seven degenerate copies of TRB in the RTL1 genes of afrotherians, xenarthrans, humans and non-muroid rodents (Fig S3 in Additional File 1). We therefore conclude that these repeats originated before the divergence of all extant eutherian mammals and that at least three TRBs were present at the origin of miR-432 because pre-miR-432 extends across more than two repeats. TRB repeats are older than the other repeats discussed below and the individual units have undergone greater evolutionary divergence.

The rat and mouse Rtl1 genes contain 12-nucleotide TRA repeats located immediately adjacent to the ancestral TRB repeats. We count ten TRA units in mouse Rtl1 and at least twenty in rat Rtl1. The rat and mouse Rtl1 genes also contain 24-nucleotide TRC repeats that expanded from within the TRB repeats. These repeats are located immediately 5' of the sequence complementary to pre-miR-432 on the sense strand (Fig S4 in Additional File 1). We count twelve TRC units in mouse Rtl1 and twenty-three TRC units in rat Rtl1. The TRA and TRC repeats are absent from the Rtl1 genes of non-muroid rodents. miR-434 is processed from within the TRC arrays of rats and mice. Because of the duplication of TRC units, mouse Rtl1 possesses four perfect matches (GGUUCAA) to the miR-434-3p seed, three of them allotargets, and three perfect matches (AGUCGAG) to the miR-434-5p seed, two of them allotargets (Fig S5 in Additional File 1). miR-434-5p and miR-434-3p both degrade Rtl1 mRNA in mice [52].

A 66-nucleotide sequence within the ancestral pre-miR-432 sequence has undergone six-fold tandem amplification in the Rtl1 gene of mice (we will call these TRB2 units because each contains two TRB units). As a consequence, the mouse Rtl1

mRNA (sense) contains seven perfect matches (CCAUCCA) to the seed of miR-432-3p and two perfect matches (CUCCAAG) to the seed of miR-432-3p. However, the TRB2 repeats disrupt formation of the pre-miR-432 hairpin preventing formation of the microRNA. The rat *Rtl1* gene also cannot produce pre-miR-432 but, in this case, because of deletions within the ancestral pre-miR-432 sequence. Deep-sequencing reported in miRBase detects miR-432 in neither rats nor mice [40]. We have not resolved the relative timing of the loss of miR-432 and gain of miR-434.

TRB and TRC repeats exhibit interesting parallels (Fig S6 in Additional File 1). TRB and TRC units are ‘partial palindromes’ with internal self-complementarity. Tandem duplications of the repeat units created larger palindromes that folded back upon themselves to form the hairpins from which pre-mir-432 and pre-miR-434 are processed. Both arrays contain more units than are required to generate the embedded pre-miRNAs. One model of the origin of pre-miRNA hairpins derives them from inverted duplications [4]. The tandem duplications that generated pre-mir-432 and pre-miR-434 do not fit this model but in some respects resemble the alternative model in which partial self-complementarity of the ascending and descending arms of pre-miRNA hairpins arises by ‘chance’ [64]. However, in the case of pre-mir-432 and pre-miR-434, the length of self-complementarity that had to be generated by chance was greatly reduced because of tandem duplication of already self-complementary units.

Finally, the mouse *Rtl1* sequence contains an AG-rich region of 33-nucleotide repeats (TRDs) that is remarkable for a stretch of 442 nucleotides without a cytosine (227 guanines, 192 adenines, 23 uracils). At the amino acid level, the AG-rich region encodes 143 amino acid residues: 115 glutamic acids, 14 aspartic acids, and 14 glycines. TRD repeats are absent from the *Rtl1* genes of rats and non-rodents.

The lengths of the various repeat-units are all multiples of three—TRA (12), TRB (33), TRB2 (66), TRC (24), TRD (33)—as required to preserve the open reading frame of *RTL1* mRNA. An intriguing question is whether the principal selective forces in the fixation of these repeats acted on properties of the RNA sequence or properties of the encoded amino acids. Many proteins contain internal repeats with a variety of functions [116] but we conjecture that the complex repeated structures associated with miR-432 and miR-434 (TRA, TRB, TRB2, TRC) were primarily shaped by interactions between sense and antisense RNAs rather than by functional properties of the RTL1 amino-acid sequence. The TRD repeats of mouse *Rtl1* are not associated with a microRNA. It is possible that these repeats evolved because of their contribution to the amino acid sequence of Rtl1 protein. Another possibility is that TRD repeats are associated with formation of an R-loop, between the C-rich template DNA and a G-rich RNA transcript, that blocks transcription of sense *Rtl1* mRNA. In this configuration, the G-rich non-template DNA would be single-stranded but probably fold back on itself to form G-quartet structures [220].

3.4.3 *cis* AND *trans* EFFECTS

The establishment of imprints in mammalian genomes involves interactions between *trans*-acting and *cis*-acting factors in parental germlines: an imprinting control region (ICR) is differentially marked in male and female germlines by *trans*-acting factors, then the epigenetically-inherited state of the ICR determines expression of imprinted genes in *cis* in the next generation [188]. The distinction between *trans*-acting and *cis*-acting factors is crucial for understanding the evolution of genomic imprinting in mammals. If there is heterozygosity at a *trans*-acting locus that acts in the mother’s germline, an allele can influence gene expression in all of a mother’s

offspring, not just the 50% that receive the *trans*-acting allele. By contrast, if there is heterozygosity at a *cis*-acting locus, then the effect is experienced only by those offspring that inherit the *cis*-acting allele. Therefore, a gene that acts in *trans* to imprint an ICR is subject to different selective forces than the ICR that acts in *cis* to determine gene expression in the next generation. This is a source of evolutionary conflict between maternal genes (in mothers) and maternally-derived genes (in offspring) [84] and has been conceptualized as a conflict between imprinting genes and imprinted genes [31].

Conflict between imprinting genes and imprinted genes occurs because of their independent segregation at meiosis. Conflict is not predicted when *trans*-acting loci modify *cis*-acting loci in the haploid phase because *trans*-acting and *cis*-acting alleles are necessarily inherited together in products of syngamy. The establishment of epigenetic marks in gametophytes (haploid phase of plants) rather than sporophytes (diploid phase of plants) may explain why ‘imprinting’ genes are imprinted in plants, but not mammals, and why clusters of imprinted genes under *cis* control of an ICR have not been described in plants [98]. Conflict is also not predicted between *trans*-acting and *cis*-acting loci whose effects are restricted to the diploid generation in which the loci act.

3.4.4 A MODEL OF THE ORIGIN OF SELF-TARGETING MICRORNAS

The kinds of effects reported at the *RTL1* locus, in which one allele influences the expression of its homolog have been labelled *cum* effects [98] or *trans*-homolog interactions [112]. Such effects do not map neatly onto the *cis* versus *trans* distinction: they occur at the same locus but not on the same chromosome. In this section, we develop a model in which self-targeting microRNAs are derived from tandem

repeats. The model illustrates how *cum* effects are subject to distinctive selective forces from *cis* and *trans* effects. The model is based on the observation that miR-432 and miR-434 are derived from tandemly-repeated sequences in the *RTL1* gene.

Consider an ancestral allele (A) of *RTL1* that contains a single copy of a partially palindromic sequence. In AA homozygotes, the maternal allele is expressed as an antisense non-coding RNA and the paternal allele is expressed as a sense *RTL1* mRNA (Fig a 3.4.3). Now consider the introduction into a population fixed for A of a new allele A^* with a tandem duplication of the palindromic sequence that forms a hairpin from which an antisense microRNA is processed. In an outbred population, A^* is initially present only in A^*A heterozygotes who inherit A^* from their mother or AA^* heterozygotes who inherit A^* from their father. In A^*A heterozygotes, the maternally-expressed microRNA targets the paternally-expressed mRNA (Fig b 3.4.3). In AA^* heterozygotes, the maternally-expressed antisense transcript does not produce a microRNA and therefore does not inhibit translation of *RTL1* protein (Fig c 3.4.3). Thus, A^* reduces production of *RTL1* protein when maternally-inherited, via a *cum* effect, but not when paternally-inherited. If *RTL1* protein reduces fetal demand, then the microRNA acts as a maternally-expressed demand inhibitor.

Once A^* becomes frequent in the gene pool, it encounters itself in A^*A^* homozygotes. Now, the maternally-expressed A^* microRNA targets the paternally-expressed A^* mRNA reducing translation of *RTL1* protein (Fig d 3.4.3). Thus, paternal A^* possesses the ‘neat trick’ of decreasing *RTL1* protein, and demands on the mother, when the maternal allele is A^* but not when it is A . A^* thereby exploits A in AA^* heterozygotes but exhibits ‘self-restraint’ in A^*A^* homozygotes. If A^* mRNA detects A^* microRNA, then there is an ‘altruistic’ reduction in *RTL1* translation. If the A^* mRNA does not detect A^* microRNA, then ‘selfish’ levels of

RTL1 are produced.

Green-beard effects are generally considered to be evolutionary unstable because of the threat of ‘false beards’: variants that enjoy the benefits of others’ altruism but do not reciprocate. In terms of our model, a population fixed for A^* could be invaded by a variant A^{**} that continues to inhibit paternal expression of A^* in $A^{**}A^*$ heterozygotes but evades restraint (or partially evades restraint) in A^*A^{**} heterozygotes. Thus, maternal A^{**} would enjoy the benefit of ‘altruistic’ restraint by paternal A^* , but paternal A^{**} would act ‘selfishly’ and not reciprocate (or incompletely reciprocate) when the roles were reversed.

If one assumes that increased transcription increases the number of mRNAs that escape degradation, then the simplest example of A^{**} would be an allele with a variant sense promoter that increased transcription of the paternally-expressed mRNA. This would favor an allele (A^{***}) with a variant antisense promoter that increased transcription of the maternally-expressed microRNA. Such considerations predict an escalation in the strength of antagonistic promoters. Another possibility would be the acquisition, by the mRNA, of ‘decoy’ target sites for the microRNA that absorb the microRNA but are resistant to cleavage.

The model presented above for the introduction of a single microRNA could potentially explain the origin of multiple self-targeting microRNAs encoded by the *RTL1* sequence because the model is iterable. One could think of this process as successive introductions of new traits for self-recognition: a green beard, followed by a blue mustache, followed by red whiskers. Once a trait is introduced, it should tend to be maintained by natural selection because changes to the target site on paternally-expressed mRNA to evade repression would be associated with changes to the maternally-expressed microRNAs which would thereby be less effective in-

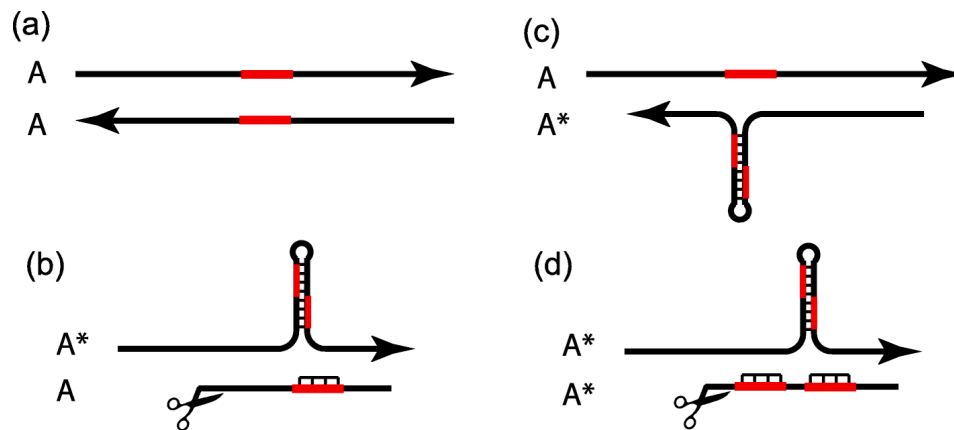


Figure 3.4.3: At the RTL1 locus an ancestral A allele has a single copy of a partially palindromic sequence (indicated in red) and the derived A* allele has a tandem duplication of this sequence which is processed as a microRNA from the maternal antisense transcript. a In AA homozygotes the maternal allele (above) is expressed as RTL1-antisense noncoding RNA and the paternal allele (below) is expressed as sense RTL mRNA. No microRNAs inhibit translation of RTL1 mRNA. b In A*A heterozygotes, the maternal A* allele produces a microRNA that binds to the mRNA transcribed from the paternal A allele which possesses a single-copy of the partially palindromic sequence. Translation of RTL1 protein is inhibited. c In AA* heterozygotes, the A maternal allele does not produce the microRNA and the paternal A* mRNA is translated. d In A*A* homozygotes, the maternal A* allele is processed as a microRNA that binds to both copies of the palindromic sequence of the mRNA transcribed from the paternal A* allele. Translation of RTL1 protein is inhibited.

hibitors of established alleles. This is a simple consequence of the complementarity of targeting and targeted sequences transcribed from opposite strands of the same double helix.

Maternal resources are predicted to be least efficiently distributed when allocation is controlled by paternally-expressed genes in offspring, more efficiently distributed when controlled by maternally-expressed genes in offspring, and most efficiently distributed when controlled by genes expressed in mothers [84]. The level of RTL1 protein is jointly determined by products of the maternal and paternal allele at the *RTL1* locus. We conjecture that coordination between *RTL1* alleles allows greater reproductive efficiency relative to control by a simple paternally-expressed demand enhancer. The cooperation posited here has some resemblances to the ‘coadaptation’ theory of genomic imprinting that posits an advantage from ‘matching’ of expressed alleles between parents and offspring [250] [91], although in our model the relevant ‘matching’ is between the maternal and paternal alleles of offspring. We acknowledge the limitations of this model. An ideal population-genetic model would describe the dynamics of the system for all allele frequencies. We have found such models, of fitness trade-offs within families mediated by offspring demand, to be analytically intractable. Most previous models have avoided these complications by seeking an evolutionary stable strategy (ESS) that cannot be invaded by alternative strategies when an allele is near fixation in the population. We did not use an ESS approach because greenbeard effects are not predicted to be evolutionarily stable.

3.4.5 SEQUENCE CONSERVATION AT THE RTL1 LOCUS

Greenbeard altruism has been predicted to be vulnerable to exploitation by mutations that maintain the trait, and thus receive the benefits, but do not respond in

kind (so-called ‘false beards’). As a consequence, evolutionary models of greenbeard effects often predict periods of cooperation interspersed with periods of exploitation, accompanied by turnover in the tags used in self-recognition [236]. Despite this prediction, the antisense microRNAs associated with the *RTL1* genomic sequence are, for the most part, highly conserved: pre-miR-136, pre-miR-127, and pre-miR-433 are highly similar in all eutherian *RTL1* genes, as is pre-miR-432 (except in muroid rodents) and pre-miR-433 (except in xenarthrans where the sequence has not been found).

A number of factors may have stabilized *cum* interactions at the *RTL1* locus. First, the greenbeard triad of trait, recognition and response are all instantiated by the same short sequence and its antisense complement. ‘False beards’ may be difficult to evolve because mutational changes to autotargets that allow the paternally-expressed mRNA to evade inhibition by unmutated maternally-expressed microRNAs would come at the cost of a failure of maternally-expressed microRNAs with the mutated seed to inhibit paternally-expressed mRNAs with the unmutated target. Second, escalation in the strength of sense and antisense promoters increases the cost of loss of inhibition. Third, the accumulation of *trans* allotargets creates a selective force that opposes loss of the microRNA. Such allotargets can be considered to have ‘eavesdropped’ on the conversation between *RTL1* sense and antisense transcripts.

Despite evolutionary conservation of the *RTL1* antisense microRNAs in most eutherian mammals, we find evidence of two episodes of rapid evolutionary change in the *RTL1* sequence. First, we infer an early epoch of rapid change before the divergence of the major clades of extant eutherian mammals. During this period, the *RTL1* gene originated from a retroelement, became subject to imprinted expression

and antisense transcription, and gave rise to at least four self-targeting microRNAs. We are unable to tell whether these changes comprised a single episode of rapid change or multiple episodes. The details are lost in the fog of evolutionary time.

Second, we infer a relatively recent episode of rapid change in the *Rtl1* genes of muroid rodents. The neighborhood of the ancestral pre-miR-432 has undergone an expansion of TRA and TRC repeats in an ancestor of rats and mice and of TRB2 repeats in the mouse lineage. Moreover, the number of TRA and TRC repeats differs between the mouse and rat *Rtl1* genes suggesting changes in one or both lineages have continued after rats and mice diverged. A novel microRNA (miR-434) is present in both rats and mice and miR-432 has been lost in both species. We hypothesize that miR-434 functionally replaced miR-432 in self-targeting of *Rtl1* mRNA. This replacement involved the loss of the miR-432 seed sequence and gain of the miR-434 seed. Our analysis has not resolved whether the gain of miR-434 and loss of miR-432 were evolutionarily contemporaneous or whether there was an extended period in which both microRNAs were produced. Thus, we do not know at what stage in the process miR-432-related sequences were deleted in the rat lineage and TRB2 repeats amplified in the mouse lineage. Some of these questions might be resolved by comparative studies of *Rtl1* genes across rodent phylogeny.

The origins of miR-432 and miR-434 were both associated with tandem duplications of short repeats and, in both cases, more repeats are present than the number required to generate the pre-miRNA hairpin. Furthermore, differences in the number of repeats between mouse and rat *Rtl1* genes show change in the number of TRC repeats continued after the origin of miR-434. One consequence of the amplification of TRC repeats has been the generation of additional allotargets for miR-434-5p and miR-434-3p. An unanswered question is whether these extra targets increase or

decrease degradation of *Rtl1* mRNA. Does the possession of multiple binding sites increase the probability that an mRNA will be degraded, or do the extra allotargets function as ‘sponges’ [76] that increase the proportion of *Rtl1* mRNAs that escape degradation?

Our model of the origin of self-targeting miRNAs from tandem repeats applies directly to the origins of miR-432 and miR-434, the two microRNAs at the *RTL1* locus that are associated with obvious repeats. miR-127, miR-136, miR-431 and miR-433 are not associated with obvious repeats. Future work could explore whether these microRNAs also originated from tandem repeats, now highly degenerate, or originated in some other way.

3.5 CONCLUSIONS

Studies of molecular evolution have focused, for the most part, on the coevolution of DNA and proteins. For example, whether a DNA sequence is under selection is inferred from the ratio of synonymous to nonsynonymous changes in protein-coding sequences. Much of cellular biology has similarly focused on DNA and proteins. Under the traditional model of cellular control, DNA is transcribed as messenger RNAs that are translated as proteins which are the effective actors within cells. Some of these proteins function as transcription factors that bind to DNA promoters and determine where and when genes are expressed as protein-coding mRNAs.

Noncoding RNAs are increasingly recognized as performing important roles in cellular control. Imprinted chromosomal regions, in particular, are the source of many noncoding RNAs, including long noncoding RNAs (lncRNAs), microRNAs (miRNAs), and small nucleolar RNAs (snoRNAs) [76]. The one-to-one nature of pairing between complementary nucleic acid sequences gives these interactions both

dosage-sensitivity and sequence-specificity. Imprinted gene products are predicted to be dosage-sensitive in their effects because inactivation of one allele by imprinting would have little effect on fitness at a dosage-insensitive locus [89]. Non-coding RNAs may therefore be particularly predisposed to the origin and evolutionary maintenance of imprinted expression.

The sequence-specificity of interactions between non-coding RNAs and their nucleic acid targets promises a strategic richness of ‘clever tricks’ at imprinted loci. These include opportunities for ‘self-recognition’ in which an RNA recognizes the DNA sequence from which it was transcribed or recognizes RNAs transcribed from the complementary strand of the DNA double helix. *cum* effects have been reported at multiple imprinted loci in addition to *RTL1* [112] [66] [237] [28] [27] but the mechanisms have not been determined. This paper has investigated the evolution of self-targeting microRNAs at the *RTL1* locus. The neighboring protein coding locus to *RTL1* is *DLK1*. *DLK1* is a paternally-expressed gene (PEG) that, in mice, is targeted in *cum* by multiple maternally-expressed microRNAs from the callipyge region [71]. Mammalian genomes contain many other imprinted miRNAs but, to our knowledge, none have been shown to act in *cum*. Other examples of antisense miRNAs that target sense mRNAs in *cum* have been found in human and mouse genomes [246] but none are known to be imprinted. An evolutionary understanding of *cum* effects at imprinted loci is in its infancy.

*Yet mark'd I where the bolt of Cupid fell: It fell upon
a little western flower, Before, milk-white, now purple
with love's wound, And maidens call it love in idleness*

A Midsummer Night's Dream

4

What's love got to do with it?

4.1 ABSTRACT

Oxytocin has commonly been viewed as a molecule that can melt away malevolence and increase bonding. Its effects, however, are crucially involved in the process of smooth muscle contraction. Synthesized almost exclusively in mammals, oxytocin is a member of a nonapeptide family constituting one of the most ancient and evolutionarily conserved signaling systems found in vertebrates and invertebrates. We postulate that there is an evolutionary connection among the various functions

attributed to these peptides and examine the molecules as examples of the fundamental evolutionary processes by which existing gene products are co-opted into new functions. Oxytocin and its orthologs, which we are proposing be named oxytoken, are involved in the birth process, including oviposition in non-mammalian vertebrates and invertebrates while simultaneously reducing feeding behavior. We believe that the acute anorexic effects of oxytoken released during egg-laying inhibits the consumption of a female's own eggs and that the first evolutionary use of oxytoken peptides in social bonding was in the promotion of nurturing feelings towards clutches of fertilized eggs.

4.2 INTRODUCTION

Our bodies respond to endogenous 'potions' that evoke strong feelings toward offspring, and sexual partners, that sometimes subvert our rational self-interest. In recent years, oxytocin has gained notoriety as the 'love hormone', 'trust hormone', or 'cuddle hormone' [259]. A related peptide, vasopressin, promotes affiliative behavior in rodents [111]. However, for more than a century, these peptides were associated with diverse physiological functions not directly related to emotional bonding. Henry Dale[50]detected an activity in pituitary extracts that caused contraction of involuntary muscles. Similar extracts induced milk ejection from mammary glands [207] and uterine contractions during labor[81]. All these effects are now ascribed to oxytocin. A related pituitary extract, later named vasopressin, caused elevation of blood pressure by the contraction of arteriolar smooth muscle [182][50]. What's love and trust got to do with the contraction of involuntary muscles? Is there an evolutionary connection among the varied functions attributed to oxytocin and vasopressin, or, do we simply observe the repurposing of ancient signaling systems for

diverse and unrelated functions? A discussion of these interesting evolutionary questions requires a preliminary diversion into the less captivating subject of chemical names.

4.3 A NOMENCLATURAL PROPOSAL FOR OXYTOCIN-RELATED NEUROPEPTIDES

Oxytocin (“quick birth”) was the trade name given of a hypophyseal extract (-hypophamine) that caused contraction of uterine smooth muscle. Vasopressin (“vessel constrictor”) was the trade name of a distinct hypophyseal extract (-hypophamine) that possessed both pressor and antidiuretic activities [118]. At the time of naming, little was known about the active principles of these extracts. Subsequent chemical analysis and synthesis revealed oxytocin and vasopressin to be cyclic nonapeptides that differed at two amino-acid residues [239] [242]. Because of their structural simplicity and analysis by chemical methods, the names oxytocin and vasopressin were used to refer to specific chemical structures. When a related peptide was found that differed at a single amino-acid residue, the peptide was given a new chemical name (vasotocin, mesotocin, isotocin, conopressin, etc.). Such names represent structural identity (chemical kinds) rather than orthology (historical kinds: [168]). This use of chemical names can be contrasted with the evolutionary (or historical) names of gonadotropin-releasing hormones (GnRHs). Whereas oxytocin-related nonapeptides acquire a new name with a change of a single amino acid residue, GnRH-related decapeptides retain the same name despite differing for up to five out of ten amino acid residues [123].

The chemical names of oxytocin-related peptides obscure evolutionary relationships because readers need to remember that vasotocin is orthologous to vasopressin

but mesotocin and isotocin are orthologous to oxytocin. A system of evolutionary names for oxytocin-related peptides, similar to that employed for GnRH peptides, would facilitate evolutionary discussion. Larhammar et al. [136]) proposed that all members of the evolutionary lineage that contains mammalian oxytocin be called oxytocin and all members of the lineage that contains mammalian vasopressin be called vasopressin. This proposal unfortunately creates ambiguity about whether ‘oxytocin’ or ‘vasopressin’ is being used to refer to chemical or historical kinds. To avoid this ambiguity, we will name peptides orthologous to human oxytocin as ‘oxytokins’ and peptides orthologous to human vasopressin as ‘vasotokins’, with a hard ‘k’ indicating the evolutionary kind and reserving the soft ‘c’ of oxytocin (and related names) for chemical kinds. We will often use the older chemical names when discussing the older literature but will use the new nomenclature when this simplifies evolutionary arguments. The last common ancestor of jawless vertebrates (cyclostomes) and jawed vertebrates (gnathostomes) produced a single peptide that was ancestral to both oxytokin and vasotokin [83]. We will call this peptide ‘oxyvasotokin’. Oxytokins, vasotokins, oxyvasotokins, and homologous peptides of invertebrates (anetocin, octopressin, conopressin, cephalotocin, etc.) will be collectively labelled ‘tokins’.

This nomenclatural proposal presupposes the existence of distinct lineages of oxytokin and vasotokin genes since their origin by tandem duplication of an ancestral oxyvasotokin gene. This presupposition is valid for the sequences encoding the nonapeptides themselves but not for all parts of their precursor sequences. Oxytokin and vasotokin genes are closely-linked and share a three-exon structure: the signal sequence, nonapeptide hormone, and start of the neurophysin domain are encoded by exon 1; the ‘core’ of the neurophysin domain is encoded by exon 2; and the

remainder of the neurophysin domain is encoded by exon 3. Although exons 1 and 3 of oxytokin and vasotokin genes evolve independently, exons 2 evolve in concert such that exon 2 sequences of oxytokin and vasotokin genes from the same species are more similar to each other than to exon 2 sequences of either gene in distantly-related species. For this central exon, vasotokin and oxytokin genes comprise a single evolutionary lineage, most plausibly explained by an evolutionary history of frequent gene conversions. This means that distinct evolutionary lineages can be recognized for exons 1 and 3, but not for exon 2 [245].

Oxytokin and vasotokin nonapeptides (encoded by exon 1) can thus be recognized as distinct historical kinds. These lineages are now distinguished by the presence of a neutral amino acid at position 8 of oxytokin but a basic amino acid at position 8 of vasotokin. This residue may partly explain differential effects of oxytokin and vasotokin when interacting with shared receptors. The oxyvasotokin peptide of lampreys has a basic amino acid at position 8 and thus resembles a vasotokin. After tandem duplication of the oxyvasotokin gene in an ancestral gnathostome, oxytokin and vasotokin peptides evolved divergent functions in their interactions with a shared set of ancestral receptors.

Physiological responses to vertebrate tokins are mediated by a family of cell-surface receptors with high sequence similarity, especially in the extracellular binding domain [75]. Distinct V1 and V2 token receptors existed before the whole-genome duplications in early vertebrate ancestry that gave rise to three lineages of V1 receptors (OTR, V1a, V1b) and three lineages of V2 (V2a, V2b, V2c) [240]. Signaling via these receptors stimulates an increase in intracellular calcium ions except for signaling via V2a receptors which signals via cAMP [161]. V2b and V2c receptors have been lost from mammalian genomes [135]. Mammalian genomes thus possess three

receptors of the V1 lineage—V1a, V1b and OTR—encoded by *AVPR1A*, *AVPR1B*, *OXTR* genes respectively and a V2a receptor encoded by *AVPR2*. Our discussion will focus on the V1-lineage receptors that are implicated in reproductive and social functions. V1a and V1b receptors are known as vasopressin receptors and the OTR receptor is known as the oxytocin receptor, but all three receptors can interact with both peptides.

4.3.1 SYNTHESIS AND RELEASE OF TOKIN PEPTIDES

Oxytokin and vasotokin are synthesized in magnocellular neurons of the supraoptic and paraventricular nuclei of the hypothalamus of tetrapod brains and secreted into the bloodstream via the posterior pituitary. In vertebrates, oxytokin is also synthesized peripherally in the corpus luteum, intrauterine tissues, ovary, and gastrointestinal tract. In amphibians and fish, these peptides are synthesized in the preoptic nucleus of the hypothalamus and subsequently released to the periphery. Neurosecretory brain centers containing tokins are also present in tunicates, worms[243], gastropods, and cephalopods [240].

Tokins have unique qualities that facilitate global coordination of complex behaviors. Unlike classic neurotransmitters which mediate rapid neuron-to-neuron communication, oxytokin is dispersed throughout the brain to act on its receptors as well as being released into the circulatory system to effect remote tissues. This global, as opposed to local, coordinates whole brain networks in the control of behavior, rather than distinct individual areas of the brain. Also, compared to classic neurotransmitters with half-lives of seconds, tokins have half-lives of minutes. This longer time-frame allows tokins to coordinate sequences of actions rather than single acts.

The hypothalamus contains many neuronal subpopulations — differentiated by the signals they use to transmit and receive information. A few of these populations are neuroendocrine neurons that release peptides which regulate the secretion of hormones from the pituitary gland, which in turn, control the secretion of other hormones released from the ovaries, testes, and adrenal glands [141]. Hypothalamic neuropeptides regulate metabolism and are released during parturition and sexual stimulation of genitals where they act not only on the body itself, but also on the brain. These actions, like when to eat or mate, are governed by the location and density of the peptides’ receptors in the hypothalamus.

4.4 SPASMODIC CONTRACTIONS: GAMETE RELEASE AND PARTURITION

Tokins have important roles in the reproductive physiology and behavior of invertebrates. Token signaling in ascidians (sea squirts) regulates tissue contraction for salt regulation. Though they lack sexual steroidogenesis, tokins directly act on its receptor expressed in oocytes to induce oocyte maturation and ovulation [160]. Token peptides injected into leeches induce the stereotypic sequence of twisting behaviors associated with courtship and copulation[243]. Similar phenomena are seen in nematodes and hermaphroditic gastropods with tokins involved in ejaculation of sperm or egg-laying [240]. Octopressin, a token of octopuses, induces muscular contractions of the penis and oviduct [234] [119]. These patterns suggest that tokins play a vital biological role in invertebrate reproduction, though with diversification of the molecular mechanisms in different evolutionary lineages.

We conjecture that an ancient function of vertebrate tokins was the coordination of mating behaviors and gamete release in both sexes (similarly to effects reported in invertebrates). In many taxa with external fertilization, tokins stimulate con-

tractions of the male and female reproductive tracts at spawning (with males often spawning soon after the release of eggs). In taxa with internal fertilization, spermatozoa are released into the female reproductive tract whose contractions extrude zygotes or young embryos (males ‘spawn’ before females). Viviparity evolves via the prolonged retention of embryos within the oviduct rather than the rapid extrusion of the fertilized egg to the outside. This requires the suppression of contractions of the oviduct or uterus until the time of birth. Akin to a delayed oviposition, contractions that at one time caused spawning and the release of eggs, are now involved in the birth process.

The process of female gamete release can be broken down into ovulation (release of oocytes from the ovarian follicle) and oviposition (expulsion of oocytes from the oviduct at spawning). Oxytocin has been reported to have roles in both processes. In vertebrates, ovulation is induced by a surge of luteinizing hormone (LH), released from the pituitary, that triggers the production of ovarian prostaglandins. The direct release of a mature oocyte is a consequence of confined proteolysis at the apical area of the follicle; a process mediated by prostaglandins, which degrade extracellular matrices in mammals [178] and rearrange actin cytoskeleton in follicle cells in teleost fishes ultimately leading to the release of oocytes [177]. In all species, oocytes must first mature and be released from the ovary prior to being fertilized by a male’s sperm. The ovary, which releases oocytes and synthesizes steroid hormones, limits the speed of these cycles. Some of these cycles only last a few days, while others span a whole year, often relying on seasonal or periodic maturation of the gonads.

The exact timing of ovulation varies in vertebrates. Ovulation in cats and camels is triggered by coitus whereas ovulation in rats and humans is governed by cyclical behavior of the suprachiasmatic nucleus of the hypothalamus. Other species

are seasonal breeders where a long period of infertility is interrupted by environmental cues, like day-length and temperature, so that young are born in favorable conditions.

In oviparous female teleosts, spawning coincides with ovulation because oviducts have been lost and eggs are released to the outside directly via a duct of the hollow ovary. During the reproductive season of teleost fish, oxytocin is expressed in both the brain and follicular envelope of the ovary and is the catalyst for smooth muscle tissue contraction in the ovary [9]. Female catfish, goldfish, pupfish, masu salmon, three-spined sticklebacks, and half-spotted goby show changes in oxytocin levels in the preoptic area in relation to their reproductive phase, with a peak just before ovulation and a significant decrease after [218][179] [140] [134] [158]. More specifically, in vitro studies of catfish have suggested that oxytocin stimulates oocyte maturation, ovulation, and prostaglandin secretion [218]. The peptide also indirectly affects ovulation— peripheral injections of oxytocin in female goldfish increases circulating levels of estradiol (a hormone that regulates folliculogenesis and peaks right before ovulation)[167]. In the viviparous guppy, fertilization and pregnancy take place within the ovarian follicle. Oxytocin is involved in the birth process which can be considered delayed ovulation [133].

Actions of oxytocin on the ovulation and oviposition have been described in birds. Oocyte maturation and ovulation are mediated primarily via vasotocin receptors on follicular cells and following sexual steroidogenesis [219]. Oxytocin increases in the paraventricular nucleus right before oviposition in hens [43]. Oviposition involves relaxation of the abdominal muscles and sphincter between the shell gland and vagina and the contraction of smooth muscles of the shell gland. Exogenous oxytocin binds to receptors in the uterus and can induce premature oviposition in

laying hens. Oxytokin plasma levels, rapidly released from the posterior pituitary, and V1a receptor expression in the uterus increase to reach a peak at oviposition and drop rapidly afterwards [232]. The avian uterus also preferentially expresses the oxytokin receptor, which is potently activated by both oxytokin and vasotokin.

Oxytokin has been implicated in events associated with mammalian ovulation. Many species show some evidence of gonadal expression of oxytokin; in women, the peptide and its receptors are expressed in cumulus cells around the oocytes [70]) and oxytokin is expressed in Sertoli cells of the bovine testis [181]. While, there are considerable interspecies differences in the regulation of luteolysis, studies in the mouse point to the peptide's role in gonadal function. Here, oxytokin has a capacity to stimulate ovulation and can advance the luteinizing hormone preovulatory surge in the rat[199]. In humans, there are cyclic changes in oxytokin levels: oxytokin peaks at the time of ovulation and is significantly elevated in the follicular phase as compared to the luteal phase [22]. Substantial evidence suggests that oxytokin plays a role in regulating the preovulatory LH surge and stimulating sexual behavior around ovulation [215]. In mammals, oxytokin peaks at ovulation and can stimulate LH release both in vivo and in vitro and induces an earlier onset of the preovulatory LH surge in women [61][109]. Though the precise mechanisms remain unknown, oxytokin may increase stores of releasable LH in cells which would then be available to be subsequently secreted. The appearance of oxytokin receptor mRNA in granulosa cells before ovulation suggests that the peptide is involved in follicular development [181]and progesterone secretion [20].

Oxytokin has well-known effects of promoting uterine contractions during labor and is used for this purpose in human obstetrics. However, mammals that lack oxytokin still deliver their offspring normally. James Ferguson was the first to re-

port that stretching of the uterus and cervix of a pregnant rabbit induces oxytokin secretion— a reflex that initiates a positive feedback loop as oxytokin stimulates smooth muscle contraction of the uterus, which then stimulates more oxytokin secretion [24]. However, without the peptide, other mechanisms allow for parturition, which suggests that additional pathways to birth exist and may be compensating for the functional loss of oxytokin. Pregnant guinea pigs give birth despite lesions to the neural stalk that prevent secretion of oxytokin [208]oxytokin-deficient mice have no obvious defects in parturition [233], and in humans circulating oxytokin is not essential for the initiation and maintenance of labor [174].

The density of oxytokin receptors rise during pregnancy and just before the onset of labor, their concentrations surge, reaching a peak during early labor. Consequently, the uterus can be stimulated by low concentrations of maternal oxytokin levels that previously had no effect. In humans, there is a 100-fold increase of oxytokin receptors from the start to end of gestation [67]. The same heightened receptor density and uterine responsiveness is seen in the rat [69], guinea pig [208], rabbit [154], quokka [102], wallaby [193], and during oviposition in the chicken [131].

In general, the oxytocin and its receptor within the pregnancy uterus serves two distinct physiological functions: stimulation of contractions and production of prostaglandins. At some stage of the labor process, elevated plasma oxytokin is seen in all placental mammals studied, including human, pig, cow, sheep, and rabbit. Oxytokin binding stimulates prostaglandin secretion, particularly PGF2a, in mammals which causes the increased contractility of uterine smooth muscle tissue [145] [235]. Contractions during labor increase in frequency and strength due to a parallel rise in the density and sensitivity of oxytokin receptors. During parturition, its secretion is pulsatile and peaks during fetal expulsion. For example in the rabbit

[68], oxytokin peaks during delivery of the first kit, and large pulses of oxytokin coincide with parturition in bats [48].

Vaginal examination in late pregnancy stimulates the Ferguson reflex so oxytokin is released from the posterior pituitary, which stimulates uterine prostaglandin production. Earlier in pregnancy, this response does not occur, presumably because there are inadequate oxytokin receptors. Women who go into preterm labor seem to have an increased expression of oxytokin receptors and higher myometrial sensitivity to oxytokin [249]. Furthermore, failed induction of labor is associated with a reduced number of oxytokin receptors. Thus, the initiation of labor appears to depend on mechanisms that induce the expression of oxytokin receptors in the myometrium rather affect the oxytokin level itself.

During the expulsive stage of labor, oxytokin levels in the blood increase, and a greater fraction of oxytokin receptor sites are occupied. Myometrial contractions are enhanced and delivery is facilitated. Following parturition, the concentration of uterine oxytokin receptors falls off rapidly, dampening the response of the uterus to elevated levels of oxytokin in the blood during lactation.

Activation of oxytokin and vasotokin (V1a) receptors located on smooth muscle cells triggers the massive increase in Ca^{2+} needed for myometrium contraction during labor. While oxytokin receptor antagonists only delay parturition in mice, total vasotokin receptor deficiencies result in mice with fewer pups, delayed labor initiation, and increased post-delivery hemorrhage [238]. Though vasotokin receptor's distribution pattern is different from the oxytokin receptor, it too is expressed in the uterus and is vital for maximum contractile forces in the uterus.

4.4.1 LACTATION

Oxytokin's only fundamental role in mammals is to act as the mediator of the milk let-down response by causing cells of the mammary gland to contract and secrete milk into ducts. Although different species vary widely in their suckling habits, mammals appear to be characterized by a common physiological mechanism of milk letdown. In response to suckling, oxytokin cells release intermittently in brief synchronized bursts that cause contraction of the myoepithelial cells around the alveoli in the mammary glands. Mammals that lack oxytokin mate, conceive, and give birth; the peptide is important, but not essential, for these phenomena.

The conspicuous lack of any substitute pathways for lactation is curious, given the presence of an alternative mechanism for uterine contractions at parturition. The two events are important for reproductive success in mammals. Contractions of smooth muscle tissue related to parturition is an ancient phenomenon— although evidence that lactation is an ancient phenomenon, parturition and oviposition, as well as maternal behaviors are considerably older.

4.5 RECEPTORS

How can oxytokin, one hormone, be involved simultaneously in so many different things? It is not hard to conceive that different sub-populations of oxytokin neurons do different things, if they project to very different places that are far apart. Nor is it hard to conceive how oxytokin produced at peripheral sites has specific, local actions on adjacent cells— those cells might be indifferent to the oxytokin concentrations in the plasma but might receive enough oxytokin from adjacent cells to be activated. However, it is to understand how the magnocellular oxytokin neurons can do several apparently different things simultaneously without conflict— they can regulate milk

ejection, parturition, feeding, social and sexual behavior, and sodium excretion.

Even milk letdown isn't simple. It is bizarre that in marsupials oxytokin secreted from the pituitary can provide an appropriate supply of milk to newborns of different ages, whose patterns of suckling and whose needs are different. The marsupial mammary gland that is being suckled by the newborn is sensitive to oxytokin, and this suckling leads to intermittent pulses of oxytokin which will trigger milk at this gland, but not at the other gland, suckled by the older sibling, which is less sensitive to oxytokin [147]. The older sibling sucks more vigorously, which leads to occasional larger pulses of oxytokin— they cause milk ejection at both glands— but the glands suckled by the younger offspring respond similarly to large pulses and small pulses.

In the case of sodium excretion through the kidneys— the kidney is very sensitive to oxytokin, and at the kidney, this excretion is regulated by a graded, continual release of oxytokin governed by the continual background activity of oxytokin neurons. Sodium excretion is a slow process and pulses of oxytokin pulses are too brief and intermittent. By contrast, the mammary glands are relatively insensitive to oxytokin, all that matters is those large pulses. So by regulating their background activity and generating intermittent pulses, magnocellular oxytokin neurons can fulfill both functions without conflict.

These differences are probably achieved by changes in how oxytokin is interpreted at the receptor or post-receptor level. The response of a tissue to oxytokin is defined by the level of expression of oxytokin receptors in that tissue. Oxytokin can regulate both uterine contractions and milk letdown because the uterus is only fully sensitive to oxytokin at the time of birth, and the mammary gland, only during lactation.

In ascidians, the token receptor (CiVPR) mRNA was detected in various tissues: the neural complex, alimentary tract, endostyle, heart, and ovary[124]. The distri-

bution of token receptors suggests that the token peptide serves as a multifunctional neuropeptide and was transferred from the brain ganglion to target peripheral tissues and the receptor evokes an intracellular calcium elevation in response to the token[124].

The distribution pattern of the oxytokin receptor in teleost fish in the brain is widespread with a pattern similar to those of oxytokin receptors in other taxa [107], suggesting that the functions in teleost fish may be conserved as compared to those of the oxytokin receptor. Peripherally in African cichlid fish, the V1a2 receptor mRNA expressed in ovary, intestine, liver and oxytokin receptor in gill, mouth epithelium, gall, and gas bladder [157]. Oxytocin receptor detected in testis, ovary, intestine, and kidney of ricefield eels and teleost white sucker [255] [100].

These examinations in species separated by several hundred million years suggest that there has been strong conservation not only of the genes but also of the main functions of oxytokin and vasotokin. The duplication of the oxyvasotokin gene in the jawless fish allowed subsequent neuroendocrine systems to diverge into refined roles.

4.6 APPETITE

Time spent feeding is time not spent on reproduction, but an animal must feed to reproduce. Thus, these fitness-enhancing behaviors are expected to be regulated coordinately and often to be mutually exclusive activities: feeding is the first priority of a starving animal but reproduction becomes a stronger motivation in an animal replete with food. An early indication that feeding and mating are coordinated was found in *C. elegans* where mate seeking behavior is responsive to nutritional status and regulated by an insulin-like signaling pathway [148]. There is extensive evidence

that token peptides reduce appetite at the same time as they enhance reproductive activities.

Oxytokin neurons in the paraventricular nucleus extend into the spinal cord and affect thermogenesis, pain processing, and erectile function. Other neurons innervate the dorsal vagal complex in the brainstem, which affects the regulation of digestive functions of the stomach and esophagus, and sends a message back to the hypothalamus to stop eating when the stomach is full. Oxytokin neurons also project to the nucleus accumbens where the peptide halts reward-driven food consumption while heightening the reward linked with social interactions. Physiology is at its finest here because oxytokin's varying actions can be explained by different neuronal subpopulations projecting to different brain sites: oxytokin release can act locally at specialized brain sites. But much more interesting is how the magnocellular oxytokin neurons, involved in milk let-down and parturition in mammals, also have a role in appetite.

Oxytokin's importance in appetite regulation as a satiety peptide was recognized first by Olson and her colleagues[183]who found that ingestion of food and administration of cholecystokinin (CCK) elevated plasma oxytokin levels. This led them to propose that satiety might involve stimulation of hypothalamic oxytokinergic pathways that control the inhibition of ingestion. Later, Arletti et al. [8]noted that central administration of oxytocin reduces both food intake and time spent eating in a dose-dependent manner, and increases the latency to the first meal in fasted rats[8].

Food in the stomach stimulates the release of gastric juices and digestive hormones, including insulin, leptin and CCK. As food is digested, leptin and CCK signal magnocellular neurons in the hypothalamus. Not only do these neurons re-

lease oxytokin into plasma, but also release the peptide locally near the amygdala and ventromedial nucleus, helping to produce that feeling of satiety. The satiety effect may be the result of oxytokin sensitizing the brain to CCK's effects[23], as well as to oxytokin causing the smooth muscles of the digestive tract to contract[149]. Both of these sites are dense with oxytokin receptors and are important in regulating glucose homeostasis, sexual behavior, and feeding.

Oxytocin's ability to reduce food intake was first reported in 1989 in rats[8] and since then a plethora of studies support the involvement of oxytocin in reducing feeding. The peptide reduces consumption of food, regardless of whether the subject is offered sugar or tasteless laboratory chow[252]. All the evidence so far has linked oxytokin with stopping feeding due to increased satiation or in response to uncomfortable physiological changes, like stomach distention.

In rodents, central oxytokin administration inhibits eating in fasted rats and suppresses voluntary intake of carbohydrates[183]. In mice, a mutated oxytokin receptor increases meal sizes [233]and if given at high doses, inhibits food intake[113]. Moreover, while oxytokin suppresses feeding in rodents, it concurrently intensifies sexual behaviors, such as facilitating lordosis[8]. V1a receptor deficient mice eat 20% less a day as compared to wild-type mice[6]. In non-human primates, administration of oxytokin reduces intake of sweet beverages. In humans, oxytokin secretion is stimulated by gastric distention or administration of CCK. Intravenous and intranasal oxytokin in dogs and goats increases their glucagon and insulin secretion, both of which reduce the feeling of hunger. In humans, oxytokin administration reduces food intake and improves gastric motility[23], an effect that is most likely driven by oxytokin receptors on gut smooth muscle[190]. In mammals, both oxytokin and its receptor are expressed in the gut where they suppress intestinal motility, reg-

ulate the permeability of the mucosal lining of the intestine, and protect against inflammation.

Oxytokin functions in the regulation of energy intake in non-mammalian vertebrates. In birds, goldfish, chicks, and gilthead sea breams, central injections of oxytokin has an anorexigenic effect[159] [167][221]. Injections of oxytokin caused a significant reduction in food intake by 43% in goldfish, while simultaneously prompting ovulation (Mennigen et al 2017). In goldfish, administration of vasotokin too significantly reduces food consumption and this action is attenuated by treatment with a V1a receptor antagonist[7]. This suggests that the anorexigenic actions of oxytokin and vasotokin are partially mediated via V1a receptors.

Tokin peptides have half-lives of a few minutes. Therefore, their effects on appetite are predicted to be acute, but short-lived. Eggs contain a rich source of nutrients for the support of offspring development and are an attractive food source for adults of many species, but consuming your own eggs seems an exercise in reproductive futility. Therefore, a mechanism that inhibited the consumption of one's own offspring would be highly adaptive. I conjecture that the surge of token peptides associated with the contraction of involuntary muscles during spawning in both sexes was coupled, early in animal evolution, to an immediate inhibition of appetite to prevent the consumption of an animal's own offspring.

Most marine teleosts release large numbers of small, buoyant eggs that contain relatively small amounts of yolk. Some sturgeons lay more than 5 million eggs[30]and mackerel may deposit as many as 6 million eggs during each yearly breeding cycle[60]. The eggs are fertilized externally and develop and hatch while floating in the water. This mode of reproduction, which provides for wide dispersal of the juveniles, may have evolved as a way of reducing predation and adverse effects

of local environmental changes[156]. Freshwater fishes lay fewer eggs than marine species—these eggs are significantly larger than those of marine species because of a greater amount of yolk. Eggs hatch into juveniles with adult-like body forms. In addition, nest-building and parental care are more common in freshwater species than in marine species. Clutch size in both freshwater and marine fishes is correlated positively with fish length[60]: larger fishes tend to lay a greater number of relatively small eggs.

Filial cannibalism has often been reported in teleost fish[155]. Rohwer[201] was the first to recognize the prospect that filial cannibalism might be an adaptive strategy to maximize a parent's lifetime reproductive success. His assertion is based on the assumption that it pays to kill your offspring if the costs associated with care exceed the benefits, and that parents can use their young as an alternative food source. Males and females should display differences in the frequency of filial cannibalism because they invest differently in gamete production; a female cannot fully recoup the energy she invested in a clutch by eating her eggs, but a male can gain energy by eating his partner's eggs. Filial cannibalism is notably well-documented in fishes with male parental care and rarely seen in females. Many fish will eat eggs as a rich source of nutrients, but males and females do not eat their own recently fertilized eggs immediately after spawning. Moreover, the female medaka fish carries her brood in her pelvic fin for several hours after giving birth. This time also coincides with when she has maximal oxytocin receptors in the preoptic area of her hypothalamus, beginning right before laying her eggs[179].

Captive loggerhead turtles have decreased appetite before and during their breeding period, compared to the rest of the year[117]. Cannibalism, of either related or unrelated clutches, in female poison frogs is suppressed after oviposition[226] and

in female cichlid fish after spawning[47], emphasizing the prominent role of oviposition in mediating aggressive and affiliative behaviors. The disposition for filial cannibalism ends suddenly when oviposition occurs, initiating parental care. The same relation between filial cannibalism and oviposition is also found in microhylid frogs— females only cannibalize clutches when they are not spawning[217]. Similarly in mammals, female rodents are aggressive towards unrelated pups, but shift to affiliative behaviors after mating [185].

It is reasonable that oxytokin’s inhibitory effects on appetite are directly linked to its stimulatory effects on sexual behavior and this link reflects an evolutionarily conserved regulation of the two motivational drives. The peptides are created and released just before oviposition and ovulation, while also inhibiting feeding in the female. Oxytokin’s primary effect originated as inducing muscular contraction of reproductive ducts and regulating oviposition and spawning behaviors, all accompanied with a decrease in appetite. This suggests that the complex mammalian caregiving system may have grown out of the avoidance of filial cannibalism. Though oxytokin is central in egg-laying and avoiding noxious behavior, the peptide has evolved to have other functions for nurturance.

4.6.1 WHAT’S LOVE GOT TO DO WITH IT?

In the previous section, we argued that the acute anorexic effects of token peptides released during spawning inhibits the consumption of a female’s own eggs (and in males inhibits the consumption of eggs fertilized by a male’s sperm). This is perhaps the simplest form of parental care and is all that is required in species in which parents and offspring do not maintain contact after spawning. However, in species in which one or both parents remains with the eggs, the parental guardian needs

to become ‘attached’ to the eggs as things to be nurtured rather than eaten. (In many egg-guarding species, appetite continues to be suppressed and the caretaking parent does not feed during the extended period of brood care.) We conjecture that the first evolutionary use of token peptides in social bonding was in the promotion of nurturing feelings towards clutches of fertilized eggs.

We are not postulating that the world is full of hedonistic amphibians or ecstatic sponges, as a lizard never trusts other lizards. Oxytokin in an iguana is only released during mating and egg laying and cobras bolt the moment they are born. Instead of relying on maternal care, a young lizard sprints away from the nest the moment they hatch. Most fish mothers do not even wait for their offspring to hatch— she swims away the moment her eggs are fertilized.

Though parental care is widespread, our understanding of its underlying mechanisms remains rudimentary. Though evidence is sparse, oxytokin may affect maternal behavior in some avian species, like promoting nest-building in zebra finches and rearing chicks in Thai hens[79] [43]. After oxytokin injection, virgin female rats display all essential aspects of maternal behavior, including nest building and assuming a nursing posture[62] [186]. Similar to rats, sheep show maternal behavior only after parturition, or, alternatively, with experimentally increased oxytokin levels[126].

In the highly social world of moles, voles, and prairie dogs, oxytokin can regulate all forms of attachment, including bonding to a mate, tolerance of neighbors in the cage or colony, even tolerance of one’s own offspring. By inhibiting oxytokin, researchers have induced mothers to shun their offspring; with administration, it causes mothers to nurture offspring not their own.

Though a large amount of experimental evidence substantiates oxytokin’s role in

regulating the onset of maternal behavior, we still do not fully understand what bolsters nurturance in females. Pedersen and Prange[186] were the first to discover that oxytocin has a central effect on motherhood, with central (intracerebroventricular) infusion of oxytocin into both gonadally intact or ovariectomized (but estrogen-primed) female rats showing maternal behavior hours after treatment. Oxytocin is only one of many maternal chemicals, but perhaps is not even that critically important. A current broad view posits that the peptide advances positive bonding behavior, but this view is challenged by a growing number of studies; the link between hormones and behaviors cannot be accurately summarized by basic proximate mechanisms. Two groups in the 1990's concurrently concluded that female mice with an oxytocin knockout were able to reproduce, give birth, and care for their offspring[174] [257], though not able to lactate. In addition, a recent reconsideration of oxytocin's involvement suggests that once maternal behaviors have been established, the peptide is not critical for maternal behavior. If oxytocin's receptors are blocked in a mother rat's brain three days after birth, her maternal care is not affected[195]—oxytocin has a greater relevance for the onset rather than maintenance of care.

Rats display an intricate collection of maternal behaviors immediately after giving birth. She will find soft materials to build a nest and groom her pups. Before she was pregnant, she would have avoided newborn pups but now as a mother she collects her offspring into a nest, allows them to suckle, and keeps a watchful eye so they don't wander away. She even forcefully attacks and defends her nest from outsiders. These behaviors are all seen after a vaginal birth, but are absent if her pups are birthed by a caesarean section or her oxytocin release is impaired. Her maternal behaviors can be rescued with a central injection of oxytocin.

Mother sheep are more discerning than rats. During birth, the ewe's vagina and cervix is stretched, which excites neurons and triggers oxytokin release from the hypothalamus. Some of the peptide reaches the olfactory bulb, which immediately alters her behavior—fixed to her lamb's smell[49]. Without oxytokin in the olfactory bulb, dams are disgusted by the smell of amniotic fluid.

Moreover, the peptide might not even be necessary for cooperative breeding in non-mammalian vertebrates. Adam Reddon and his colleagues[191] showed recently that there is a negative correlation between brain oxytokin expression and cooperative breeding in cichlid fishes, which suggests that the peptide may reduce affiliative nature. But other groups have argued that the peptide is vital for parental care in teleost fish as an antagonist inhibits behaviors like digging nest sites or fanning oxygen-rich water over growing brood[176].

Sexual pleasure in humans is connected to eating because it signals the brain via the vagus nerve. Leptin and CCK produced during digestion signal the brain via the vagus nerve. In response, the hypothalamus releases oxytokin. Barry Komisaruk and Beverly Whipple[132] were the first to discover that women with spinal cord injuries experience sexual stimulation via the vagus nerve. Bypassing the spinal cord, nerves from the clitoris, mammary glands, uterus, and skin connect to the vagus nerve, causing the release of oxytokin. Evidently, the vagus is the conduit for sensations that accompany arousal as well as for satiety.

When it comes to behavior, oxytokin is released two ways in the brain: direct transport from oxytokin-producing cells to specific regions and broad diffusion throughout the brain for action on its receptors. The location of these receptors are associated with mental states that can be broadly categorized as anticipatory, appetitive, and aversive.

Consider the lobster— outside of mating both sexes are highly aggressive, territorial, and often tend to be cannibalistic. But during their courtship ritual, a female must exit her protective exoskeleton, leaving herself vulnerable, before mating. Centrally released token prompts her to suspend all wariness just long enough for the tryst. Oxytoken has been called the love hormone[259]. It is a wishful phrase, so patently reductionist that it hardly deserves being challenged. Still, the peptide is a player in the sensation of love. It shows up in circumstances where affiliative behaviors are called for. During childbirth, it is released from the brain and into the bloodstream. It stimulates the milk letdown reflex and stimulates smooth muscle tissue contractions. It gets the baby born, it gets the baby fed, and it might as well help get the baby loved. Vasotoken is also a good candidate for bonding as it, like oxytoken, is needed for lactation. It enables the body to retain water, and if you cannot retain fluids, lactation is impossible. Vasotoken also enhances memory[206], and it is a good idea to remember individuals who are significant— children for example.

We still lack conclusive evidence whether oxytoken systems reliably evolve in connection with bonding behavior. Though the prevalent thought is that these peptides promote positive behavior, this relationship may not be ancestrally rooted as parental care is not pervasive in the animal kingdom.

In mammals, oxytoken is a key mediator of bonding of mother to offspring as well as, perhaps, attachment of offspring to mother, forged during parturition[36], lactation, and emotionally ‘warm’ touch [210]. One possible evolutionary scenario is that, given its role in parturition, oviposition, and lactation, oxytoken was co-opted to possess specific neuromodulatory effects that support mothering in an early mammal[36] [46]. Then again, as oxytoken supports mothering in some avian species and even promotes elaborate nesting behavior in turtles[35], perhaps a more

plausible scenario is that oxytokin's physiological role in reproduction set the stage for its neuromodulatory influence on maternal behavior in vertebrates — it may have a deeper evolutionary origin predating the evolution of mammals. Once oxytokin also came to play a critical physiological role in lactation in mammals, its regulation of maternal behavior may have evolved to become more elaborate. Have oxytokin's social effects evolved to support energy regulation, which includes the most efficient feeding strategy for the given environment?

4.7 SYNTHESIS

Evolution does not take large leaps. Rather than create new systems, most changes use the differentiation of a pre-existing characteristic to garner a new outcome. These new systems are not exaptations in the sense that they do not replace existing systems, but expand as overlapping systems. The advent of each new function increases the complexity of the organism[53]. The oxytokin behavioral system differentiated out of an old system that still exists even though it added newer capabilities. Therefore, the oxytokin network evolved as a continuity of systems.

The expression of token in early invertebrate development points to the use of peptide signaling in the early ontogenesis of this ancient group of organisms. The central nervous system of sea squirt larvae, which is made up of around 100 neurons express token genes[204]. Tokens are also expressed in roundworm larvae[74] and red flour beetles[227]. Oxytokin neuron developed is conserved in vertebrates as they are created early in embryonic development in both mammals and non-mammals[80]. In humans, oxytokin is first generated prenatally, which indicates that the peptide plays a role in fetal physiology because unlike receptor expression in the brain (which vary across lifespan) the number of oxytokin neurons is similar between gestation

to adulthood [80].

Examinations into when this system made its appearance in evolutionary history and how it evolved over time can offer valuable constraints on functional interpretation. A peptide system that exists today most likely did not originally appear with strictly the same suite of organized effects or activating circumstances. The oxytokin system has been shaped over time among lineages in ways sensitive to new demands on species in the lineage. Oxytokin, in mammals, now has functions that are redundant. While natural selection is efficient at removing behaviors that are counter adaptive, oxytokin's function on the uterus during parturition is not essential but also not deleterious. Any mechanism that favors a successful birth will be preserved as long as maintaining the repetitive function is not costly.

References

- [1] Mendel G. Experiments in plant hybridisation, 1865. (translation in W Bateson Mendel's principles of heredity. Cambridge University Press, 1909.). 2006.
- [2] V. Agarwal, G. W. Bell, J.-W. Nam, and D. P. Bartel. Predicting effective microrna target sites in mammalian mrnas. *elife*, 4:e05005, 2015.
- [3] E. I. Ager, A. J. Pask, G. Shaw, and M. B. Renfree. Expression and protein localisation of IGF2 in the marsupial placenta. *BMC Developmental Biology*, 8(1):17, 2008. ISSN 1471-213X. doi: 10.1186/1471-213X-8-17. URL <http://bmcddevbiol.biomedcentral.com/articles/10.1186/1471-213X-8-17>.
- [4] E. Allen, Z. Xie, A. M. Gustafson, G. H. Sung, J. W. Spatafora, and J. C. Carrington. Evolution of microRNA genes by inverted duplication of target gene sequences in Arabidopsis thaliana. *Nat Genet*, 36, 2004. doi: 10.1038/ng1478. URL <https://doi.org/10.1038/ng1478>.
- [5] H. Angelman. 'Puppet' Children A Report on Three Cases. *Developmental Medicine & Child Neurology*, 7(6):681–688, Nov. 2008. ISSN 00121622, 14698749. doi: 10.1111/j.1469-8749.1965.tb07844.x. URL <http://doi.wiley.com/10.1111/j.1469-8749.1965.tb07844.x>.
- [6] T. Aoyagi, J.-i. Birumachi, M. Hiroyama, Y. Fujiwara, A. Sanbe, J. Yamauchi, and A. Tanoue. Alteration of Glucose Homeostasis in V1a Vasopressin Receptor-Deficient Mice. *Endocrinology*, 148(5):2075–2084, May 2007. ISSN 0013-7227, 1945-7170. doi: 10.1210/en.2006-1315. URL <https://academic.oup.com/endo/article-lookup/doi/10.1210/en.2006-1315>.
- [7] K. Araishi, K. Watanabe, T. Yamazaki, T. Nakamachi, and K. Matsuda. Intracerebroventricular administration of arginine vasotocin (AVT) induces anorexigenesis and anxiety-like behavior in goldfish. *Peptides*, 119:170118, Sept. 2019. ISSN 01969781. doi: 10.1016/j.peptides.2019.170118. URL <https://linkinghub.elsevier.com/retrieve/pii/S0196978119300968>.
- [8] R. Arletti, A. Benelli, and A. Bertolini. Influence of oxytocin on feeding behavior in the rat. *Peptides*, 10(1):89–93, Jan. 1989. ISSN 01969781. doi:

- 10.1016/0196-9781(89)90082-X. URL <https://linkinghub.elsevier.com/retrieve/pii/019697818990082X>.
- [9] P. Banerjee, R. Chaube, and K. P. Joy. Molecular cloning, sequencing and tissue expression of vasotocin and isotocin precursor genes from Ostariophysian catfishes: phylogeny and evolutionary considerations in teleosts. *Frontiers in Neuroscience*, 9, May 2015. ISSN 1662-453X. doi: 10.3389/fnins.2015.00166. URL http://www.frontiersin.org/Neuroendocrine_Science/10.3389/fnins.2015.00166/abstract.
 - [10] D. Barlow. Methylation and imprinting: from host defense to gene regulation? *Science*, 260(5106):309–310, Apr. 1993. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.8469984. URL <https://www.sciencemag.org/lookup/doi/10.1126/science.8469984>.
 - [11] D. Barlow, R. Stöger, B. Herrmann, K. Saito, and N. Schweifer. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the tme locus. *Nature*, 349(6304):84–87, 1991.
 - [12] D. P. Barlow, R. Stöger, B. G. Herrmann, K. Saito, and N. Schweifer. The mouse insulin-like growth factor type-2 receptor is imprinted and closely linked to the Tme locus. *Nature*, 349(6304):84–87, Jan. 1991. ISSN 0028-0836, 1476-4687. doi: 10.1038/349084a0. URL <http://www.nature.com/articles/349084a0>.
 - [13] D. P. Bartel. Metazoan MicroRNAs. *Cell*, 173(1):20–51, Mar. 2018. ISSN 00928674. doi: 10.1016/j.cell.2018.03.006. URL <https://linkinghub.elsevier.com/retrieve/pii/S0092867418302861>.
 - [14] M. S. Bartolomei, S. Zemel, and S. M. Tilghman. Parental imprinting of the mouse h19 gene. *Nature*, 351(6322):153–155, 1991.
 - [15] M. S. Bartolomei, S. Zemel, and S. M. Tilghman. Parental imprinting of the mouse H19 gene. *Nature*, 351(6322):153–155, May 1991. ISSN 0028-0836, 1476-4687. doi: 10.1038/351153a0. URL <http://www.nature.com/articles/351153a0>.
 - [16] R. Baserga. The decline and fall of the igf-i receptor. *Journal of cellular physiology*, 228(4):675–679, 2013.
 - [17] R. Baserga, F. Peruzzi, and K. Reiss. The igf-1 receptor in cancer biology. *International journal of cancer*, 107(6):873–877, 2003.

- [18] M. Begemann, B. Zirner, G. Santen, E. Wirthgen, L. Soellner, H. M. Büttel, R. Schweize, W. Workum, G. Binder, and T. Eggermann. Paternally inherited IGF2 mutation and growth restriction. *New Engl J Med*, 373, 2015. doi: 10.1056/NEJMoa1415227. URL <https://doi.org/10.1056/NEJMoa1415227>.
- [19] A. C. Bell and G. Felsenfeld. Methylation of a CTCF-dependent boundary controls imprinted expression of the Igf2 gene. *Nature*, 405(6785):482–485, May 2000. ISSN 0028-0836, 1476-4687. doi: 10.1038/35013100. URL <http://www.nature.com/articles/35013100>.
- [20] Bennegard-Eden, B., Hahlin, M., and Kindahl, H. Interaction between oxytocin and prostaglandin F2a in human corpus luteum? *Human Reproduction*, 10(9):2320–2324, 1995.
- [21] J. M. Biernaskie, S. A. West, and A. Gardner. Are greenbeards intragenomic outlaws? *Evolution.*, 65, 2011. doi: 10.1111/j.1558-5646.2011.01355.x. URL <https://doi.org/10.1111/j.1558-5646.2011.01355.x>.
- [22] W. Blaicher, D. Gruber, C. Bieglmayer, A. M. Blaicher, W. Knogler, and J. C. Huber. The Role of Oxytocin in Relation to Female Sexual Arousal. *Gynecologic and Obstetric Investigation*, 47(2):125–126, 1999. ISSN 0378-7346, 1423-002X. doi: 10.1159/000010075. URL <https://www.karger.com/Article/FullText/10075>.
- [23] J. E. Blevins and D. G. Baskin. Translational and therapeutic potential of oxytocin as an anti-obesity strategy: Insights from rodents, nonhuman primates and humans. *Physiology & Behavior*, 152:438–449, Dec. 2015. ISSN 00319384. doi: 10.1016/j.physbeh.2015.05.023. URL <https://linkinghub.elsevier.com/retrieve/pii/S0031938415003066>.
- [24] D. Bonnycastle and J. Ferguson. The action of pitocin and adrenalin on different segments of the rabbit uterus. *Journal of Pharmacology and Experimental Therapeutics*, 72(1):90–98, 1941.
- [25] M.-L. Bortolin-Cavaillé, M. Dance, M. Weber, and J. Cavaillé. C19mc micrornas are processed from introns of large pol-ii, non-protein-coding transcripts. In *Nucleic acids research*, 2009.
- [26] C. I. Brannan, E. C. Dees, R. S. Ingram, and S. M. Tilghman. The product of the h19 gene may function as an rna. *Molecular and cellular biology*, 10(1): 28–36, 1990.
- [27] C. L. Bretz and J. Kim. Trans-allelic mutational effects at the Peg3 locus. *PLoS One*, 13, 2018. doi: 10.1371/journal.pone.0206112. URL <https://doi.org/10.1371/journal.pone.0206112>.

- [28] C. M. Brideau, K. P. Kauppinen, R. Holmes, and P. D. Soloway. A non-coding RNA within the *Rasgrf1* locus in mouse is imprinted and regulated by its homologous chromosome in trans. *PLoS One*, 5, 2010. doi: 10.1371/journal.pone.0013784. URL <https://doi.org/10.1371/journal.pone.0013784>.
- [29] F. Brioude, I. Netchine, F. Praz, M. Jule, C. Calmel, D. Lacombe, P. Edery, M. Catala, S. Odent, B. Isidor, and S. Lyonnet. Mutations of the imprinted *CDKN1C* gene as a cause of the overgrowth Beckwith-Wiedemann syndrome: clinical spectrum and functional characterization. *Hum Mutat*, 36, 2015. doi: 10.1002/humu.22824. URL <https://doi.org/10.1002/humu.22824>.
- [30] C. Bulman and J. Koslow. Development and depth distribution of the eggs of orange roughy, *Hoplostethus atlanticus* (Pisces: Trachichthyidae). *Marine and Freshwater Research*, 46(4):697, 1995. ISSN 1323-1650. doi: 10.1071/MF9950697. URL <http://www.publish.csiro.au/?paper=MF9950697>.
- [31] A. Burt and R. Trivers. Genetic conflicts in genomic imprinting. *Proc R Soc B*, 265, 1998. doi: 10.1098/rspb.1998.0589. URL <https://doi.org/10.1098/rspb.1998.0589>.
- [32] M. Butler, T. Goodwin, M. Simpson, and M. Singh. Poulter R (2001) vertebrate LTR retrotransposons of the Tf1/sushi group. *J Mol Evol*, 52, 2001. doi: 10.1007/s002390010154. URL <https://doi.org/10.1007/s002390010154>.
- [33] M. G. Butler, P. D. K. Lee, B. Y. Whitman, and P.-W. S. Association, editors. *Management of Prader-Willi syndrome*. Springer-Verlag, New York, 3rd ed edition, 2006. ISBN 978-0-387-25397-8.
- [34] X. Cai and B. R. Cullen. The imprinted h19 noncoding rna is a primary microrna precursor. *Rna*, 13(3):313–316, 2007.
- [35] J. L. Carr, M. A. Messinger, and G. M. Patton. Nesting Behavior in Three-Toed Box Turtles (*Terrapene carolina triunguis*) Following Oxytocin-Induced Oviposition. *Chelonian Conservation and Biology*, 7(1):124–128, Aug. 2008. ISSN 1071-8443. doi: 10.2744/CCB-0667.1. URL <http://www.bioone.org/doi/abs/10.2744/CCB-0667.1>.
- [36] C. S. Carter. Oxytocin pathways and the evolution of human behavior. *Annual review of psychology*, 65:17–39, 2014.
- [37] J. Cavallé, H. Seitz, M. Paulsen, A. C. Ferguson-Smith, and J. P. Bachellerie. Identification of tandemly-repeated C/D snoRNA genes at the imprinted human 14q32 domain reminiscent of those at the Prader-Willi/Angelman syndrome region. *Hum Mol Genet*, 11, 2002. doi: 10.1093/hmg/11.13.1527. URL <https://doi.org/10.1093/hmg/11.13.1527>.

- [38] C. Charlier, K. Segers, D. Wagenaar, L. Karim, S. Berghmans, O. Jaillon, T. Shay, J. Weissenbach, N. Cockett, G. Gyapay, and M. Georges. Human-ovine comparative sequencing of a 250-kb imprinted domain encompassing the callipyge (clpg) locus and identification of six imprinted transcripts: DLK1, DAT, GTL2, PEG11, antiPEG11, and MEG8. *Genome Res*, 11, 2001. doi: 10.1101/gr.172701. URL <https://doi.org/10.1101/gr.172701>.
- [39] K. Chen and N. Rajewsky. Deep conservation of microRNA-target relationships and 3'utr motifs in vertebrates, flies, and nematodes. In *Cold Spring Harbor symposia on quantitative biology*, volume 71, pages 149–156. Cold Spring Harbor Laboratory Press, 2006.
- [40] K. Y. Chen and N. Rajewsky. The evolution of gene regulation by transcription factors and microRNAs. *Nature Reviews Genetics*, 8:93–103, 2007.
- [41] L. Chen, Q. Wang, G.-d. Wang, H.-s. Wang, Y. Huang, X.-m. Liu, and X.-h. Cai. mir-16 inhibits cell proliferation by targeting igf1r and the raf1–mek1/2–erk1/2 pathway in osteosarcoma. *FEBS letters*, 587(9):1366–1372, 2013.
- [42] W. cheng Liang, W. M. Fu, Y.-B. Wang, Y.-X. Sun, L. liang Xu, and C.-W. Wong. H19 activates wnt signaling and promotes osteoblast differentiation by functioning as a competing endogenous rna. In *Scientific reports*, 2016.
- [43] D. Chokchaloemwong, N. Prakobsaeng, N. Sartsoongnoen, S. Kosonsiriluk, M. El Halawani, and Y. Chaiseha. Mesotocin and maternal care of chicks in native Thai hens (*Gallus domesticus*). *Hormones and Behavior*, 64(1): 53–69, June 2013. ISSN 0018506X. doi: 10.1016/j.yhbeh.2013.04.010. URL <https://linkinghub.elsevier.com/retrieve/pii/S0018506X13000986>.
- [44] N. E. Cockett, S. P. Jackson, T. L. Shay, F. Farnir, S. Berghmans, G. D. Snowder, D. M. Nielsen, and M. Georges. Polar overdominance at the ovine callipyge locus. *Science*, 273, 1996. doi: 10.1126/science.273.5272.236. URL <https://doi.org/10.1126/science.273.5272.236>.
- [45] J. F. Coy, Z. Sedlavcek, D. Bachner, H. Delius, and A. Poustka. A complex pattern of evolutionary conservation and alternative polyadenylation within the long 3'-untranslated region of the methyl-cpg-binding protein 2 gene (mcp2) suggests a regulatory role in gene expression. *Human molecular genetics*, 8 7:1253–62, 1999.
- [46] B. J. Crespi. Oxytocin, testosterone, and human social cognition: Oxytocin and social behavior. *Biological Reviews*, 91(2):390–408, May 2016. ISSN 14647931. doi: 10.1111/brv.12175. URL <http://doi.wiley.com/10.1111/brv.12175>.

- [47] F. Cunha-Saraiva, S. Balshine, R. H. Wagner, and F. C. Schaedelin. From cannibal to caregiver: tracking the transition in a cichlid fish. *Animal Behaviour*, 139:9–17, May 2018. ISSN 00033472. doi: 10.1016/j.anbehav.2018.03.003. URL <https://linkinghub.elsevier.com/retrieve/pii/S0003347218300824>.
- [48] W. B. Currie, R. C. Gorewit, and F. J. Michel. Endocrine changes, with special emphasis on oestradiol-17 β , prolactin and oxytocin, before and during labour and delivery in goats. *Reproduction*, 82(1):299–308, Jan. 1988. ISSN 1470-1626, 1741-7899. doi: 10.1530/jrf.0.0820299. URL <https://rep.bioscientifica.com/doi/10.1530/jrf.0.0820299>.
- [49] A. P. C. Da Costa, R. G. Guevara-Guzman, S. Ohkura, J. A. Goode, and K. M. Kendrick. The Role of Oxytocin Release in the Paraventricular Nucleus in the Control of Maternal Behaviour in the Sheep. *Journal of Neuroendocrinology*, 8(3):163–177, Mar. 1996. ISSN 0953-8194, 1365-2826. doi: 10.1046/j.1365-2826.1996.04411.x. URL <https://onlinelibrary.wiley.com/doi/abs/10.1046/j.1365-2826.1996.04411.x>.
- [50] H. H. Dale. The Action of Extracts of the Pituitary Body. *Biochemical Journal*, 4(9):427–447, Jan. 1909. ISSN 0306-3283. doi: 10.1042/bj0040427. URL <https://portlandpress.com/biochemj/article/4/9/427/37972/The-Action-of-Extracts-of-the-Pituitary-Body>.
- [51] S. Dambal, M. Shah, B. L. Mihelich, and L. Nonn. The microrna-183 cluster: the family that plays together stays together. In *Nucleic acids research*, 2015.
- [52] E. Davis, F. Caiment, X. Tordoir, J. Cavaillé, A. Ferguson-Smith, N. Cockett, M. Georges, and C. Charlier. RNAi-Mediated Allelic trans-Interaction at the Imprinted Rtl1/Peg11 Locus. *Current Biology*, 15(8):743–749, Apr. 2005. ISSN 09609822. doi: 10.1016/j.cub.2005.02.060. URL <https://linkinghub.elsevier.com/retrieve/pii/S0960982205002332>.
- [53] T. W. Deacon. Rethinking Mammalian Brain Evolution. *American Zoologist*, 30(3):629–705, Aug. 1990. ISSN 0003-1569. doi: 10.1093/icb/30.3.629. URL <https://academic.oup.com/icb/article-lookup/doi/10.1093/icb/30.3.629>.
- [54] T. M. DeChiara, E. J. Robertson, and A. Efstratiadis. Parental imprinting of the mouse insulin-like growth factor ii gene. *Cell*, 64(4):849–859, 1991.
- [55] T. M. DeChiara, E. J. Robertson, and A. Efstratiadis. Parental imprinting of the mouse insulin-like growth factor II gene. *Cell*, 64(4):849–859, Feb.

1991. ISSN 00928674. doi: 10.1016/0092-8674(91)90513-X. URL <https://linkinghub.elsevier.com/retrieve/pii/009286749190513X>.
- [56] E. J. Devor, J. N. D. Mik, and B. M. Schickling. Conserved sequences and secondary rna structures confirm that h19 functions as a pri-mirna and as an rna-binding protein platform. 2013.
 - [57] L. Doridot, F. Miralles, S. Barbaux, and D. Vaiman. Trophoblasts, invasion, and microRNA. *Frontiers in Genetics*, 4, 2013. ISSN 1664-8021. doi: 10.3389/fgene.2013.00248. URL <http://journal.frontiersin.org/article/10.3389/fgene.2013.00248/abstract>.
 - [58] R. A. Drewell. H19 imprinting and DNA methylation epigenetic switch. page 10, 2000.
 - [59] C. A. Edwards, W. Rens, O. Clarke, A. J. Mungall, T. Hore, J. Graves, I. Dunham, A. C. Ferguson-Smith, and M. A. Ferguson-Smith. The evolution of imprinting: chromosomal mapping of orthologues of mammalian imprinted domains in monotreme and marsupial mammals. *BMC Evolutionary Biology*, 7(1):157, 2007. ISSN 14712148. doi: 10.1186/1471-2148-7-157. URL <http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-7-157>.
 - [60] M. A. Elgar. Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos*, pages 283–287, 1990.
 - [61] J. J. Evans, F. L. Pragg, and D. R. Mason. Release of Luteinizing Hormone from the Anterior Pituitary Gland in vitro Can Be Concurrently Regulated by at Least Three Peptides: Gonadotropin-Releasing Hormone, Oxytocin and Neuropeptide Y. *Neuroendocrinology*, 73(6):408–416, 2001. ISSN 0028-3835, 1423-0194. doi: 10.1159/000054659. URL <https://www.karger.com/Article/FullText/54659>.
 - [62] S. Fahrbach. Oxytocin induction of short-latency maternal behavior in nulliparous, estrogen-primed female rats. *Hormones and Behavior*, 18(3):267–286, Sept. 1984. ISSN 0018506X. doi: 10.1016/0018-506X(84)90016-3. URL <https://linkinghub.elsevier.com/retrieve/pii/0018506X84900163>.
 - [63] G. Fan, D. Ye, S. Zhu, J. Xi, X. Guo, J. Qiao, Y. Wu, W. Jia, G. Wang, and G. Fan. Kang J (2017) RTL1 promotes melanoma proliferation by regulating Wnt/ -catenin signalling. *Oncotarget*, 8, 2017.
 - [64] F. Fenselau de Felippes, K. Schneeberger, T. Dezulian, D. H. Huson, and D. Weigel. Evolution of Arabidopsis thaliana microRNAs from random sequences. *RNA*, 14, 2008. doi: 10.1261/rna.1149408. URL <https://doi.org/10.1261/rna.1149408>.

- [65] D. Forest, R. Nishikawa, H. Kobayashi, A. Parton, C. J. Bayne, and D. W. Barnes. Rna expression in a cartilaginous fish cell line reveals ancient 3' noncoding regions highly conserved in vertebrates. *Proceedings of the National Academy of Sciences of the United States of America*, 104 4:1224–9, 2007.
- [66] T. Forné, J. Oswald, W. Dean, J. R. Saam, B. Bailleul, L. Dandolo, S. M. Tilghman, J. Walter, and W. Reik. Loss of the maternal H19 gene induces changes in Igf2 methylation in both cis and trans. *Proc Natl Acad Sci U S A*, 94, 1997. doi: 10.1073/pnas.94.19.10243. URL <https://doi.org/10.1073/pnas.94.19.10243>.
- [67] A.-R. Fuchs. The Role of Oxytocin in Parturition. In *Current Topics in Experimental Endocrinology*, volume 4, pages 231–265. Elsevier, 1983. ISBN 978-0-12-153204-8. doi: 10.1016/B978-0-12-153204-8.50014-1. URL <https://linkinghub.elsevier.com/retrieve/pii/B9780121532048500141>.
- [68] A.-R. FUCHS and Y. M. DAWOODf. Oxytocin Release and Uterine Activation during Parturition in Rabbits*. *Endocrinology*, 107(4):1117–1126, 10 1980. ISSN 0013-7227. doi: 10.1210/endo-107-4-1117. URL <https://doi.org/10.1210/endo-107-4-1117>.
- [69] A.-R. Fuchs, S. Periyasamy, and M. S. Soloff. Systemic and local regulation of oxytocin receptors in the rat uterus, and their functional significance. *Canadian Journal of Biochemistry and Cell Biology*, 61(7):615–624, July 1983. ISSN 0714-7511. doi: 10.1139/o83-077. URL <http://www.nrcresearchpress.com/doi/10.1139/o83-077>.
- [70] K. Furuya, N. Makimura, C. Mitsui, N. Ishikawa, S. Tokuoka, E. Imaizumi, Y. Mizumoto, K. Seki, I. Nagata, and R. Ivell. A Novel Biological Aspect of Ovarian Oxytocin: Oxytocin Gene Expression in Cumulus/Luteal Cells and the Effect of Oxytocin on Embryogenesis in Fertilized Oocytes. *Hormone Research*, 41(1):41–47, 1994. ISSN 1423-0046, 0301-0163. doi: 10.1159/000183942. URL <https://www.karger.com/Article/FullText/183942>.
- [71] Y. Q. Gao, X. Chen, P. Wang, L. Lu, W. Zhao, C. Chen, C. P. Chen, T. Tao, J. Sun, Y. Y. Zheng, J. Du, C. J. Li, Z. J. Gan, X. Gao, H. Q. Chen, and M. S. Zhu. Regulation of DLK1 by the maternally expressed miR-379/miR-544 cluster may underlie callipyge polar overdominance inheritance. *Proc Natl Acad Sci U S A*, 112, 2012. doi: 10.1073/pnas.1511448112. URL <https://doi.org/10.1073/pnas.1511448112>.
- [72] M. Gardiner-Garden and M. Frommer. CpG Islands in vertebrate genomes. *Journal of Molecular Biology*, 196(2):261–282, July 1987. ISSN 00222836. doi:

- 10.1016/0022-2836(87)90689-9. URL <https://linkinghub.elsevier.com/retrieve/pii/0022283687906899>.
- [73] O. Garnier, S. Laouéillé-Duprat, and C. Spillane. Genomic imprinting in plants. *Epigenetics*, 3(1):14–20, Jan. 2008. ISSN 1559-2294, 1559-2308. doi: 10.4161/epi.3.1.5554. URL <http://www.tandfonline.com/doi/abs/10.4161/epi.3.1.5554>.
 - [74] J. L. Garrison, E. Z. Macosko, S. Bernstein, N. Pokala, D. R. Albrecht, and C. I. Bargmann. Oxytocin/Vasopressin-Related Peptides Have an Ancient Role in Reproductive Behavior. *Science*, 338(6106):540–543, Oct. 2012. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.1226201. URL <http://www.sciencemag.org/cgi/doi/10.1126/science.1226201>.
 - [75] G. Gimpl and F. Fahrenholz. The Oxytocin Receptor System: Structure, Function, and Regulation. *Physiological Reviews*, 81(2):629–683, Apr. 2001. ISSN 0031-9333, 1522-1210. doi: 10.1152/physrev.2001.81.2.629. URL <https://www.physiology.org/doi/10.1152/physrev.2001.81.2.629>.
 - [76] M. Girardot, J. Cavaillé, and R. Feil. Small regulatory RNAs controlled by genomic imprinting and their contribution to human disease. *Epigenetics*, 7, 2012. doi: 10.4161/epi.22884. URL <https://doi.org/10.4161/epi.22884>.
 - [77] E. A. Glazov, S. McWilliam, W. C. Barris, and B. P. Dalrymple. Origin, evolution, and biological role of mirna cluster in dlk-dio3 genomic region in placental mammals. *Molecular biology and evolution*, 25(5):939–948, 2008.
 - [78] L. Gong, H. Xu, H. Chang, Y. Tong, T. Zhang, and G. Guo. Knockdown of long non-coding rna meg3 protects h9c2 cells from hypoxia-induced injury by targeting microrna-183. *Journal of cellular biochemistry*, 119 2:1429–1440, 2018.
 - [79] J. Goodson, L. Lindberg, and P. Johnson. Effects of central vasotocin and mesotocin manipulations on social behavior in male and female zebra finches. *Hormones and behavior*, 45:136–43, 03 2004. doi: 10.1016/j.yhbeh.2003.08.006.
 - [80] V. Grinevich, M. G. Desarménien, B. Chini, M. Tauber, and F. Muscatelli. Ontogenesis of oxytocin pathways in the mammalian brain: late maturation and psychosocial disorders. *Frontiers in Neuroanatomy*, 8, Jan. 2015. ISSN 1662-5129. doi: 10.3389/fnana.2014.00164. URL <http://journal.frontiersin.org/article/10.3389/fnana.2014.00164/abstract>.

- [81] M. Gunther. The Posterior Pituitary and Labour. *BMJ*, 1(4550):567–567, Mar. 1948. ISSN 0959-8138, 1468-5833. doi: 10.1136/bmj.1.4550.567-b. URL <http://www.bmj.com/cgi/doi/10.1136/bmj.1.4550.567-b>.
- [82] S. Guo, C. Jiang, G. Wang, Y. Li, C. Wang, X. Guo, R. Yang, Y. Feng, F. Wang, H. Tseng, et al. Microrna-497 targets insulin-like growth factor 1 receptor and has a tumour suppressive role in human colorectal cancer. *Oncogene*, 32(15):1910–1920, 2013.
- [83] P.-C. Gwee, B.-H. Tay, S. Brenner, and B. Venkatesh. Characterization of the neurohypophysial hormone gene loci in elephant shark and the Japanese lamprey: origin of the vertebrate neurohypophysial hormone genes. *BMC Evolutionary Biology*, 9(1):47, 2009. ISSN 1471-2148. doi: 10.1186/1471-2148-9-47. URL <http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-9-47>.
- [84] D. Haig. Genomic imprinting and the theory of parent-offspring conflict. *Semin Devel Biol*, 3, 1992.
- [85] D. Haig. Genetic Conflicts in Human Pregnancy. *The Quarterly Review of Biology*, 68(4):495–532, Dec. 1993. ISSN 0033-5770, 1539-7718. doi: 10.1086/418300. URL <https://www.journals.uchicago.edu/doi/10.1086/418300>.
- [86] D. Haig. Gestational drive and the green-bearded placenta. *Proc Natl Acad Sci U S A*, 93, 1996. doi: 10.1073/pnas.93.13.6547. URL <https://doi.org/10.1073/pnas.93.13.6547>.
- [87] D. Haig. Parental antagonism, relatedness asymmetries, and genomic imprinting. *Proc R Soc B*, 264, 1997. doi: 10.1098/rspb.1997.0230. URL <https://doi.org/10.1098/rspb.1997.0230>.
- [88] D. Haig. The kinship theory of genomic imprinting. 2000.
- [89] D. Haig. Genomic imprinting and kinship: how good is the evidence? *Annu Rev Genet*, 38, 2004. doi: 10.1146/annurev.genet.37.110801.142741. URL <https://doi.org/10.1146/annurev.genet.37.110801.142741>.
- [90] D. Haig. Kin conflict in seed development: an interdependent but fractious collective. *Annu Rev Cell Devel Biol*, 29, 2013. doi: 10.1146/annurev-cellbio-101512-122324. URL <https://doi.org/10.1146/annurev-cellbio-101512-122324>.
- [91] D. Haig. Coadaptation and conflict, misconception and muddle, in the evolution of genomic imprinting. *Heredity*, 113, 2014. doi: 10.1038/hdy.2013.97. URL <https://doi.org/10.1038/hdy.2013.97>.

- [92] D. Haig. Maternal–fetal conflict, genomic imprinting and mammalian vulnerabilities to cancer. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370, 2015.
- [93] D. Haig and C. Graham. Genomic imprinting and the strange case of the insulin-like growth factor ii receptor. *Cell (Cambridge)*, 64(6):1045–1046, 1991.
- [94] D. Haig and C. Graham. Genomic imprinting and the strange case of the insulin-like growth factor-II receptor. *Cell.*, 64, 1991. doi: 10.1016/0092-8674(91)90256-X. URL [https://doi.org/10.1016/0092-8674\(91\)90256-X](https://doi.org/10.1016/0092-8674(91)90256-X).
- [95] D. Haig and M. Westoby. Parent-specific gene expression and the triploid endosperm. *The American Naturalist*, 134(1):147–155, 1989.
- [96] D. Haig and M. Westoby. Parent-Specific Gene Expression and the Triploid Endosperm. *The American Naturalist*, 134(1):147–155, July 1989. ISSN 0003-0147, 1537-5323. doi: 10.1086/284971. URL <https://www.journals.uchicago.edu/doi/10.1086/284971>.
- [97] D. Haig and J. F. Wilkins. Genomic imprinting, sibling solidarity, and the logic of collective action. *Phil Trans R Soc B*, 355, 2000. doi: 10.1098/rstb.2000.0720. URL <https://doi.org/10.1098/rstb.2000.0720>.
- [98] D. a. v. i. d. Haig. Imprinted green beards: a little less than kin and more than kind. *Biology Letters*, 9, 2013. doi: 10.1098/rsbl.2013.0199. URL <https://doi.org/10.1098/rsbl.2013.0199>.
- [99] T. M. D. Haig. Gen0mic imprinting in mammalian development: a parental tug-of-war. page 5, 1991.
- [100] H. Hausmann, W. Meyerhof, H. Zwiers, and Lederis. Teleost isotocin receptor: structure, functional expression, mRNA distribution and phylogeny. *FEBS Letters*, 370(3):227–230, 1995. ISSN 00145793. doi: 10.1016/0014-5793(95)00832-T.
- [101] V. Hayssen and R. C. Lacy. Basal metabolic rates in mammals: Taxonomic differences in the allometry of BMR and body mass. *Comparative Biochemistry and Physiology Part A: Physiology*, 81(4):741–754, Jan. 1985. ISSN 03009629. doi: 10.1016/0300-9629(85)90904-1. URL <https://linkinghub.elsevier.com/retrieve/pii/0300962985909041>.
- [102] H. Heller. THE EFFECTS OF OXYTOCIN AND VASOPRESSIN DURING THE OESTROUS CYCLE AND PREGNANCY ON THE UTERUS OF

- A MARSUPIAL SPECIES, THE QUOKKA (SETONIX BRACHYURUS). *Journal of Endocrinology*, 58(3):657–671, Sept. 1973. ISSN 0022-0795, 1479-6805. doi: 10.1677/joe.0.0580657. URL https://joe.bioscientifica.com/view/journals/joe/58/3/joe_58_3_029.xml.
- [103] C. Hernández-Sánchez, A. Mansilla, F. de Pablo, and R. Zardoya. Evolution of the insulin receptor family and receptor isoform expression in vertebrates. *Molecular biology and evolution*, 25(6):1043–1053, 2008.
 - [104] H. Heyn, M. Engelmann, S. Schreek, P. Ahrens, U. Lehmann, H. Kreipe, B. Schlegelberger, and C. Beger. Microrna mir-335 is crucial for the brca1 regulatory cascade in breast cancer development. *International journal of cancer*, 129 12:2797–806, 2011.
 - [105] Y. Hiramuki, T. Sato, Y. Furuta, M. A. Surani, A. Sehara-Fujisawa, and V. Mouly. Mest but not mir-335 affects skeletal muscle growth and regeneration. In *PloS one*, 2015.
 - [106] M. Holzenberger, J. Dupont, B. Ducos, and P. Leneuve. Igf-1 receptor regulates lifespan and resistance to oxidative stress in mice. *Nature*, 421:182–187, 2003.
 - [107] L. S. Huffman, L. A. O’Connell, C. D. Kenkel, R. J. Kline, I. A. Khan, and H. A. Hofmann. Distribution of nonapeptide systems in the forebrain of an African cichlid fish, *Astatotilapia burtoni*. *Journal of Chemical Neuroanatomy*, 44(2):86–97, July 2012. ISSN 08910618. doi: 10.1016/j.jchemneu.2012.05.002. URL <https://linkinghub.elsevier.com/retrieve/pii/S0891061812000312>.
 - [108] J. Hughes, M. Surakhy, S. Can, M. Ducker, N. Davies, F. Szele, C. Bühnemann, E. Carter, R. Trikin, M. P. Crump, S. Frago, and A. B. Hassan. Maternal transmission of an Igf2r domain 11: IGF2 binding mutant allele (Igf2rI1565A) results in partial lethality, overgrowth and intestinal adenoma progression. *Sci Rep*, 9, 2019. doi: 10.1038/s41598-019-47827-9. URL <https://doi.org/10.1038/s41598-019-47827-9>.
 - [109] M. L. Hull, R. A. Reid, J. J. Evans, P. S. Benny, and D. R. Aickin. Endocrinology: Pre-ovulatory oxytocin administration promotes the onset of the luteinizing hormone surge in human females. *Human Reproduction*, 10(9):2266–2269, Sept. 1995. ISSN 0268-1161, 1460-2350. doi: 10.1093/oxfordjournals.humrep.a136282. URL <https://academic.oup.com/humrep/article-lookup/doi/10.1093/oxfordjournals.humrep.a136282>.

- [110] K. Inoue, M. Hirose, H. Inoue, Y. Hatanaka, A. Honda, A. Hasegawa, and K. Mochida. The rodent-specific microRNA cluster within the *sfmbt2* gene is imprinted and essential for placental development. *Cell reports*, 19 5:949–956, 2017.
- [111] T. R. Insel. The Challenge of Translation in Social Neuroscience: A Review of Oxytocin, Vasopressin, and Affiliative Behavior. *Neuron*, 65(6):768–779, Mar. 2010. ISSN 08966273. doi: 10.1016/j.neuron.2010.03.005. URL <https://linkinghub.elsevier.com/retrieve/pii/S0896627310001765>.
- [112] M. Ito, A. N. Sferruzzi-Perri, C. A. Edwards, B. T. Adalsteinsson, S. E. Allen, T. H. Loo, M. Kitazawa, T. Kaneko-Ishino, F. Ishino, C. L. Stewart, and A. C. Ferguson-Smith. A trans-homologue interaction between reciprocally imprinted miR-127 and *Rtl1* regulates placental development. *Development.*, 142, 2015. doi: 10.1242/dev.121996. URL <https://doi.org/10.1242/dev.121996>.
- [113] Y. Iwasaki, Y. Maejima, S. Suyama, M. Yoshida, T. Arai, K. Katsurada, P. Kumari, H. Nakabayashi, M. Kakei, and T. Yada. Peripheral oxytocin activates vagal afferent neurons to suppress feeding in normal and leptin-resistant mice: a route for ameliorating hyperphagia and obesity. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology*, 308(5):R360–R369, Mar. 2015. ISSN 0363-6119, 1522-1490. doi: 10.1152/ajpregu.00344.2014. URL <http://www.physiology.org/doi/10.1152/ajpregu.00344.2014>.
- [114] V. A. A. Jansen and M. Baalen. Altruism through beard chromodynamics. *Nature.*, 440, 2006. doi: 10.1038/nature04387. URL <https://doi.org/10.1038/nature04387>.
- [115] V. Juan, C. Crain, and C. Wilson. Evidence for evolutionarily conserved secondary structure in the h19 tumor suppressor rna. *Nucleic acids research*, 28(5):1221–1227, 2000.
- [116] A. V. Kajava. Tandem repeats in proteins: from sequence to structure. *J Struct Biol*, 179, 2012. doi: 10.1016/j.jsb.2011.08.009. URL <https://doi.org/10.1016/j.jsb.2011.08.009>.
- [117] Y. Kakizoe, K. Sakaoka, Y. Akune, Y. Kanou, T. Saito, and I. Uchida. Change of Plasma Chemistry Values in Captive Breeding Loggerhead Turtles (*Caretta caretta*). *ISRN Zoology*, 2013:1–7, 2013. ISSN 2090-5238. doi: 10.1155/2013/514576. URL <https://www.hindawi.com/archive/2013/514576/>.

- [118] O. Kamm, T. B. Aldrich, I. W. Grote, L. W. Rowe, and E. P. Bugbee. THE ACTIVE PRINCIPLES OF THE POSTERIOR LOBE OF THE PITUITARY GLAND. ¹ I. THE DEMONSTRATION OF THE PRESENCE OF TWO ACTIVE PRINCIPLES. II. THE SEPARATION OF THE TWO PRINCIPLES AND THEIR CONCENTRATION IN THE FORM OF POTENT SOLID PREPARATIONS. *Journal of the American Chemical Society*, 50(2):573–601, Feb. 1928. ISSN 0002-7863, 1520-5126. doi: 10.1021/ja01389a050. URL <https://pubs.acs.org/doi/abs/10.1021/ja01389a050>.
- [119] A. Kanda, K. Takuwa-Kuroda, E. Iwakoshi-Ukena, Y. Furukawa, O. Matsushima, and H. Minakata. Cloning of Octopus cephalotocin receptor, a member of the oxytocin/vasopressin superfamily. *Journal of Endocrinology*, 179(2):281–291, Nov. 2003. ISSN 0022-0795, 1479-6805. doi: 10.1677/joe.0.1790281. URL <https://joe.bioscientifica.com/view/journals/joe/179/2/281.xml>.
- [120] T. Kaneko-Ishino and F. Ishino. Retrotransposon silencing by DNA methylation contributed to the evolution of placentation and genomic imprinting in mammals: Evolution of placenta and imprinting. *Development, Growth & Differentiation*, 52(6):533–543, July 2010. ISSN 00121592, 1440169X. doi: 10.1111/j.1440-169X.2010.01194.x. URL <http://doi.wiley.com/10.1111/j.1440-169X.2010.01194.x>.
- [121] T. Kaneko-Ishino and F. Ishino. The role of genes domesticated from LTR retrotransposons and retroviruses in mammals. *Frontiers in Microbiology*, 3, 2012. ISSN 1664-302X. doi: 10.3389/fmicb.2012.00262. URL <http://journal.frontiersin.org/article/10.3389/fmicb.2012.00262/abstract>.
- [122] L. Kang, J. Sun, X. Wen, J. Cui, G. Wang, A. R. Hoffman, J.-F. Hu, and W. Li. Aberrant allele-switch imprinting of a novel igflr intragenic antisense non-coding rna in breast cancers. *European journal of cancer*, 51 2:260–70, 2015.
- [123] T. L. Kasten, S. A. White, T. T. Norton, C. T. Bond, J. P. Adelman, and R. D. Fernald. Characterization of Two New preproGnRH mRNAs in the Tree Shrew: First Direct Evidence for Mesencephalic GnRH Gene Expression in a Placental Mammal. *General and Comparative Endocrinology*, 104(1):7–19, Oct. 1996. ISSN 00166480. doi: 10.1006/gcen.1996.0135. URL <http://linkinghub.elsevier.com/retrieve/pii/S0016648096901355>.
- [124] T. Kawada, M. Aoyama, I. Okada, T. Sakai, T. Sekiguchi, M. Ogasawara, and H. Satake. A novel inhibitory gonadotropin-releasing hormone-related

- neuropeptide in the ascidian, *ciona intestinalis*. *Peptides*, 30(12):2200–2205, 2009.
- [125] A. Keniry, D. Oxley, P. Monnier, M. Kyba, L. Dandolo, G. Smits, and W. Reik. The h19 lincRNA is a developmental reservoir of mir-675 that suppresses growth and igf1r. *Nature cell biology*, 14(7):659–665, 2012.
 - [126] E. B. Keverne and K. M. Kendrick. Oxytocin Facilitation of Maternal Behavior in Sheepa. *Annals of the New York Academy of Sciences*, 652(1):83–101, June 1992. ISSN 00778923. doi: 10.1111/j.1749-6632.1992.tb34348.x. URL <http://doi.wiley.com/10.1111/j.1749-6632.1992.tb34348.x>.
 - [127] S. Khosla, G. Mendiratta, and V. Brahmachari. Genomic imprinting in the mealybugs. *Cytogenetic and Genome Research*, 113(1-4):41–52, 2006. ISSN 1424-8581, 1424-859X. doi: 10.1159/000090814. URL <https://www.karger.com/Article/FullText/90814>.
 - [128] J. Killian, J. C. Byrd, J. V. Jirtle, B. L. Munday, M. K. Stoskopf, R. G. MacDonald, and R. L. Jirtle. M6P/IGF2R Imprinting Evolution in Mammals. *Molecular Cell*, 5(4):707–716, Apr. 2000. ISSN 10972765. doi: 10.1016/S1097-2765(00)80249-X. URL <https://linkinghub.elsevier.com/retrieve/pii/S109727650080249X>.
 - [129] M. Kitazawa, M. Tamura, T. Kaneko-Ishino, and F. Ishino. Severe damage to the placental fetal capillary network causes mid- to late fetal lethality and reduction in placental size in Peg11/Rtl1 KO mice. *Genes Cells*, 22, 2017. doi: 10.1111/gtc.12465. URL <https://doi.org/10.1111/gtc.12465>.
 - [130] J. H. M. Knoll, R. D. Nicholls, R. E. Magenis, J. M. Graham, M. Lalonde, S. A. Latt, J. M. Opitz, and J. F. Reynolds. Angelman and Prader-Willi syndromes share a common chromosome 15 deletion but differ in parental origin of the deletion. *American Journal of Medical Genetics*, 32(2):285–290, Feb. 1989. ISSN 0148-7299, 1096-8628. doi: 10.1002/ajmg.1320320235. URL <http://doi.wiley.com/10.1002/ajmg.1320320235>.
 - [131] T. Koike, K. Shimada, and L. Cornett. Plasma levels of immunoreactive mesotocin and vasotocin during oviposition in chickens: Relationship to oxytocic action of the peptides in vitro and peptide interaction with myometrial membrane binding sites. *General and Comparative Endocrinology*, 70(1):119–126, Apr. 1988. ISSN 00166480. doi: 10.1016/0016-6480(88)90100-1. URL <https://linkinghub.elsevier.com/retrieve/pii/0016648088901001>.
 - [132] B. R. Komisaruk, B. Whipple, A. Crawford, S. Grimes, W.-C. Liu, A. Kalnin, and K. Mosier. Brain activation during vaginocervical self-stimulation and

- orgasm in women with complete spinal cord injury: fMRI evidence of mediation by the Vagus nerves. *Brain Research*, 1024(1-2):77–88, Oct. 2004. ISSN 00068993. doi: 10.1016/j.brainres.2004.07.029. URL <https://linkinghub.elsevier.com/retrieve/pii/S0006899304011461>.
- [133] G. A. Kujala. Corticosteroid and neurohypophyseal hormone control of parturition in the guppy, *Poecilia reticulata*. *General and Comparative Endocrinology*, 36(2):286–296, Oct. 1978. ISSN 00166480. doi: 10.1016/0016-6480(78)90035-7. URL <https://linkinghub.elsevier.com/retrieve/pii/0016648078900357>.
- [134] E. Kulczykowska and A. Kleszczyńska. Brain arginine vasotocin and isotocin in breeding female three-spined sticklebacks (*Gasterosteus aculeatus*): The presence of male and egg deposition. *General and Comparative Endocrinology*, 204:8–12, Aug. 2014. ISSN 00166480. doi: 10.1016/j.ygcen.2014.04.039. URL <https://linkinghub.elsevier.com/retrieve/pii/S0016648014001932>.
- [135] D. Lagman, D. Ocampo Daza, J. Widmark, X. M. Abalo, G. Sundström, and D. Larhammar. The vertebrate ancestral repertoire of visual opsins, transducin alpha subunits and oxytocin/vasopressin receptors was established by duplication of their shared genomic region in the two rounds of early vertebrate genome duplications. *BMC Evolutionary Biology*, 13(1):238, 2013. ISSN 1471-2148. doi: 10.1186/1471-2148-13-238. URL <http://bmcevolbiol.biomedcentral.com/articles/10.1186/1471-2148-13-238>.
- [136] D. Larhammar, G. Sundström, S. Dreborg, D. O. Daza, and T. A. Larsson. Major Genomic Events and Their Consequences for Vertebrate Evolution and Endocrinology. *Annals of the New York Academy of Sciences*, 1163(1):201–208, Apr. 2009. ISSN 00778923. doi: 10.1111/j.1749-6632.2008.03659.x. URL <http://doi.wiley.com/10.1111/j.1749-6632.2008.03659.x>.
- [137] S. D. Lawler, S. Povey, R. A. Fisher, and V. J. Pickthall. Genetic studies on hydatidiform moles II. The origin of complete moles. *Annals of Human Genetics*, 46(3):209–222, July 1982. ISSN 0003-4800, 1469-1809. doi: 10.1111/j.1469-1809.1982.tb00713.x. URL <http://doi.wiley.com/10.1111/j.1469-1809.1982.tb00713.x>.
- [138] D.-F. Lee, J. Su, H. S. Kim, B. Y. Chang, and Dmitri. Modeling familial cancer with induced pluripotent stem cells. *Cell*, 161:240–254, 2015.
- [139] P. A. Leighton, J. R. Saam, R. S. Ingram, C. L. Stewart, and S. M. Tilghman. An enhancer deletion affects both h19 and igf2 expression. *Genes & development*, 9(17):2079–2089, 1995.

- [140] S. C. Lema, L. J. Wagstaff, and N. M. Gardner. Diurnal rhythms of behavior and brain mRNA expression for arginine vasotocin, isotocin, and their receptors in wild Amargosa pupfish (*Cyprinodon nevadensis amargosae*). *Marine and Freshwater Behaviour and Physiology*, 43(4):257–281, July 2010. ISSN 1023-6244, 1029-0362. doi: 10.1080/10236244.2010.498632. URL <http://www.tandfonline.com/doi/abs/10.1080/10236244.2010.498632>.
- [141] G. Leng. The endocrinology of the brain. *Endocrine Connections*, pages R275–R285, Dec. 2018. ISSN 2049-3614. doi: 10.1530/EC-18-0367. URL <https://ec.bioscientifica.com/view/journals/ec/7/12/EC-18-0367.xml>.
- [142] A. Lewis and L. Redrup. Genetic imprinting: conflict at the callipyge locus. *Curr Biol*, 15, 2005. doi: 10.1016/j.cub.2005.04.003. URL <https://doi.org/10.1016/j.cub.2005.04.003>.
- [143] B. P. Lewis, C. B. Burge, and D. P. Bartel. Conserved Seed Pairing, Often Flanked by Adenosines, Indicates that Thousands of Human Genes are MicroRNA Targets. *Cell*, 120(1):15–20, Jan. 2005. ISSN 00928674. doi: 10.1016/j.cell.2004.12.035. URL <https://linkinghub.elsevier.com/retrieve/pii/S0092867404012607>.
- [144] H. Li, B. Yu, J. Li, L. Su, M. Yan, Z. Zhu, and B. Liu. Overexpression of lncrna h19 enhances carcinogenesis and metastasis of gastric cancer. In *Oncotarget*, 2014.
- [145] G. C. Liggins, C. S. Forster, S. A. Grieves, and A. L. Schwartz. Control of Parturition in Man. *Biology of Reproduction*, 16(1):39–56, Feb. 1977. ISSN 0006-3363, 1529-7268. doi: 10.1095/biolreprod16.1.39. URL <https://academic.oup.com/biolreprod/article/2768197/Control>.
- [146] S.-P. Lin, N. Youngson, S. Takada, H. Seitz, W. Reik, M. Paulsen, J. Cavaille, and A. C. Ferguson-Smith. Asymmetric regulation of imprinting on the maternal and paternal chromosomes at the Dlk1-Gtl2 imprinted cluster on mouse chromosome 12. *Nature Genetics*, 35(1):97–102, Sept. 2003. ISSN 1061-4036, 1546-1718. doi: 10.1038/ng1233. URL <http://www.nature.com/articles/ng1233>.
- [147] D. Lincoln and M. Renfree. Mammary gland growth and milk ejection in the agile wallaby, *macropus agilis*, displaying concurrent asynchronous lactation. *Journal of reproduction and fertility*, 63:193–203, 10 1981. doi: 10.1530/jrf.0.0630193.
- [148] J. Lipton. Mate Searching in *Caenorhabditis elegans*: A Genetic Model for Sex Drive in a Simple Invertebrate. *Journal of Neuroscience*, 24

- (34):7427–7434, Aug. 2004. ISSN 0270-6474, 1529-2401. doi: 10.1523/JNEUROSCI.1746-04.2004. URL <http://www.jneurosci.org/cgi/doi/10.1523/JNEUROSCI.1746-04.2004>.
- [149] C.-Y. Liu, J.-Z. Liu, D.-P. Xie, P.-Y. Liu, and P. S. Wang. Endogenous oxytocin excites phasic contraction of gallbladder in rabbits through oxytocin receptor. page 7, 2003.
 - [150] J.-P. Liu, J. B. Baker, A. S. Perkins, E. J. Robertson, and A. Efstratiadis. Mice carrying null mutations of the genes encoding insulin-like growth factor i (igf-1) and type 1 igf receptor (igf1r). *Cell*, 75:59–72, 1993.
 - [151] W. long Gao, M. Liu, Y. Yang, H. Yang, and Q. Liao. The imprinted h19 gene regulates human placental trophoblast cell proliferation via encoding mir-675 that targets nodal modulator 1 (nomo1). *RNA biology*, 9 7:1002–10, 2012.
 - [152] S. Lottin, E. Adriaenssens, and T. Dupressoir. Overexpression of an ectopic h19 gene enhances the tumorigenic properties of breast cancer cells. *Carcinogenesis*, 23 11:1885–95, 2002.
 - [153] C. Lynch and M. Tristem. A co-opted gypsy-type LTR-retrotransposon is conserved in the genomes of humans, sheep, mice, and rats. *Curr Biol*, 13, 2003. doi: 10.1016/S0960-9822(03)00618-3. URL [https://doi.org/10.1016/S0960-9822\(03\)00618-3](https://doi.org/10.1016/S0960-9822(03)00618-3).
 - [154] M. Maggi, A. Peri, S. Giannini, G. Fantoni, V. Guardabasso, and M. Serio. Oxytocin and V1 vasopressin receptors in rabbit endometrium during pregnancy. *Reproduction*, 91(2):575–581, Mar. 1991. ISSN 1470-1626, 1741-7899. doi: 10.1530/jrf.0.0910575. URL <https://rep.bioscientifica.com/doi/10.1530/jrf.0.0910575>.
 - [155] A. Manica. Filial cannibalism in teleost fish. *Biological Reviews of the Cambridge Philosophical Society*, 77(2):261–277, May 2002. ISSN 14647931, 1469185X. doi: 10.1017/S1464793101005905. URL <http://doi.wiley.com/10.1017/S1464793101005905>.
 - [156] M. P. Marchetti, P. B. Moyle, and R. Levine. Alien fishes in california watersheds: characteristics of successful and failed invaders. *Ecological Applications*, 14(2):587–596, 2004.
 - [157] J. Martos-Sitcha, J. Fuentes, J. Mancera, and G. Martínez-Rodríguez. Variations in the expression of vasotocin and isotocin receptor genes in the gilt-head sea bream *Sparus aurata* during different osmotic challenges. *General and Comparative Endocrinology*, 197:5–17, Feb. 2014. ISSN 00166480.

- p>doi: 10.1016/j.ygcen.2013.11.026. URL
- <https://linkinghub.elsevier.com/retrieve/pii/S001664801300467X>
- .
- [158] K. P. Maruska, M. H. Mizobe, and T. C. Tricas. Sex and seasonal covariation of arginine vasotocin (AVT) and gonadotropin-releasing hormone (GnRH) neurons in the brain of the halfspotted goby. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology*, 147(1): 129–144, May 2007. ISSN 10956433. doi: 10.1016/j.cbpa.2006.12.019. URL <https://linkinghub.elsevier.com/retrieve/pii/S1095643306005216>.
 - [159] K. Masunari, M. S. I. Khan, M. A. Cline, and T. Tachibana. Central administration of mesotocin inhibits feeding behavior in chicks. *Regulatory Peptides*, 187:1–5, Nov. 2013. ISSN 01670115. doi: 10.1016/j.regpep.2013.10.004. URL <https://linkinghub.elsevier.com/retrieve/pii/S0167011513001390>.
 - [160] S. Matsubara, A. Shiraishi, T. Osugi, T. Kawada, and H. Satake. The regulation of oocyte maturation and ovulation in the closest sister group of vertebrates. *eLife*, 8:e49062, Oct. 2019. ISSN 2050-084X. doi: 10.7554/eLife.49062. URL <https://elifesciences.org/articles/49062>.
 - [161] S. A. Mayasich and B. L. Clarke. The emergence of the vasopressin and oxytocin hormone receptor gene family lineage: Clues from the characterization of vasotocin receptors in the sea lamprey (*Petromyzon marinus*). *General and Comparative Endocrinology*, 226:88–101, Jan. 2016. ISSN 00166480. doi: 10.1016/j.ygcen.2016.01.001. URL <https://linkinghub.elsevier.com/retrieve/pii/S0016648016300028>.
 - [162] C. Mayr. Evolution and biological roles of alternative 3'utrs. *Trends in cell biology*, 26 3:227–237, 2016.
 - [163] C. Mayr. Regulation by 3'-untranslated regions. *Annual review of genetics*, 51:171–194, 2017.
 - [164] B. McCLINTOCK. MECHANISMS THAT RAPIDLY REORGANIZE THE GENOME. page 24, 1978.
 - [165] D. E. McFadden and D. K. Kalousek. Two different phenotypes of fetuses with chromosomal triploidy: Correlation with parental origin of the extra haploid set. *American Journal of Medical Genetics*, 38(4):535–538, Mar. 1991. ISSN 0148-7299, 1096-8628. doi: 10.1002/ajmg.1320380407. URL <http://doi.wiley.com/10.1002/ajmg.1320380407>.
 - [166] J. McGrath and D. Solter. Completion of mouse embryogenesis requires both the maternal and paternal genomes. *Cell*, 37(1):179–183, May 1984. ISSN

00928674. doi: 10.1016/0092-8674(84)90313-1. URL <https://linkinghub.elsevier.com/retrieve/pii/0092867484903131>.
- [167] J. A. Mennigen, H. Volkoff, J. P. Chang, and V. L. Trudeau. The non-peptide isotocin in goldfish: Evidence for serotonergic regulation and functional roles in the control of food intake and pituitary hormone release. *General and Comparative Endocrinology*, 254:38–49, Dec. 2017. ISSN 00166480. doi: 10.1016/j.ygcen.2017.09.008. URL <https://linkinghub.elsevier.com/retrieve/pii/S0016648017305014>.
 - [168] R. G. Millikan. In Defense of Proper Functions. *Philosophy of Science*, 56(2): 288–302, June 1989. ISSN 0031-8248, 1539-767X. doi: 10.1086/289488. URL <https://www.journals.uchicago.edu/doi/10.1086/289488>.
 - [169] T. Miyamoto, S. Razavi, R. DeRose, and T. Inoue. Synthesizing biomolecule-based boolean logic gates. *ACS synthetic biology*, 2 2:72–82, 2013.
 - [170] N. Miyoshi, H. Wagatsuma, S. Wakana, T. Shiroishi, M. Nomura, K. Aisaka, T. Kohda, M. A. Surani, T. Kaneko-Ishino, and F. Ishino. Identification of an imprinted gene, Meg3/Gtl2 and its human homologue MEG3, first mapped on mouse distal chromosome 12 and human chromosome 14q. *Genes Cells*, 5, 2000. doi: 10.1046/j.1365-2443.2000.00320.x. URL <https://doi.org/10.1046/j.1365-2443.2000.00320.x>.
 - [171] Y. S. Moon, C. M. Smas, K. Lee, J. A. Villena, K.-H. Kim, E. J. Yun, and H. S. Sul. Mice Lacking Paternally Expressed Pref-1/Dlk1 Display Growth Retardation and Accelerated Adiposity. *Molecular and Cellular Biology*, 22(15):5585–5592, Aug. 2002. ISSN 0270-7306. doi: 10.1128/MCB.22.15.5585-5592.2002. URL <http://mcb.asm.org/cgi/doi/10.1128/MCB.22.15.5585-5592.2002>.
 - [172] I. M. Morison, J. P. Ramsay, and H. G. Spencer. A census of mammalian imprinting. *Trends in Genetics*, 21(8):457–465, Aug. 2005. ISSN 01689525. doi: 10.1016/j.tig.2005.06.008. URL <https://linkinghub.elsevier.com/retrieve/pii/S0168952505001666>.
 - [173] S. K. Murphy and R. L. Jirtle. Imprinting evolution and the price of silence. *BioEssays*, 25(6):577–588, June 2003. ISSN 0265-9247, 1521-1878. doi: 10.1002/bies.10277. URL <http://doi.wiley.com/10.1002/bies.10277>.
 - [174] K. Nishimori, L. J. Young, Q. Guo, Z. Wang, T. R. Insel, and M. M. Matzuk. Oxytocin is required for nursing but is not essential for parturition or reproductive behavior. *Proceedings of the National Academy of Sciences*, 93(21):11699–11704, Oct. 1996. ISSN 0027-8424, 1091-6490. doi: 10.1073/pnas.93.21.11699. URL <http://www.pnas.org/cgi/doi/10.1073/pnas.93.21.11699>.

- [175] M. Noguer-Dance, S. Abu-Amero, M. Al-Khtib, A. Lefèvre, P. Coullin, G. E. Moore, and J. Cavaillé. The primate-specific microrna gene cluster (c19mc) is imprinted in the placenta. *Human molecular genetics*, 19 18:3566–82, 2010.
- [176] L. A. O’Connell, B. J. Matthews, and H. A. Hofmann. Isotocin regulates paternal care in a monogamous cichlid fish. *Hormones and Behavior*, 61(5): 725–733, May 2012. ISSN 0018506X. doi: 10.1016/j.yhbeh.2012.03.009. URL <https://linkinghub.elsevier.com/retrieve/pii/S0018506X12000980>.
- [177] K. Ogiwara and T. Takahashi. A Dual Role for Melatonin in Medaka Ovulation: Ensuring Prostaglandin Synthesis and Actin Cytoskeleton Rearrangement in Follicular Cells1. *Biology of Reproduction*, 94(3), Mar. 2016. ISSN 0006-3363, 1529-7268. doi: 10.1095/biolreprod.115.133827. URL <https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod.115.133827>.
- [178] J. Ohnishi, E. Ohnishi, H. Shibuya, and T. Takahashi. Functions for proteinases in the ovulatory process. *Biochimica et Biophysica Acta (BBA) - Proteins and Proteomics*, 1751(1):95–109, Aug. 2005. ISSN 15709639. doi: 10.1016/j.bbapap.2005.05.002. URL <https://linkinghub.elsevier.com/retrieve/pii/S1570963905001469>.
- [179] T. Ohya and S. Hayashi. Vasotocin isotocin immunoreactive neurons in the medaka fish brain are sexually dimorphic and their numbers decrease after spawning in the female. *Zoological Science*, 23(1):23–29, Jan. 2006. ISSN 0289-0003. doi: 10.2108/zsj.23.23. URL <http://www.bioone.org/doi/abs/10.2108/zsj.23.23>.
- [180] K. Okamoto, T. Ishiguro, Y. Midorikawa, H. Ohata, M. Izumiya, N. Tsuchiya, A. Sato, H. Sakai, and H. Nakagama. mir-493 induction during carcinogenesis blocks metastatic settlement of colon cancer cells in liver. *The EMBO journal*, 31 7:1752–63, 2012.
- [181] K. Okuda, Y. Uenoyama, Y. Fujita, K. Iga, K. Sakamoto, and T. Kimura. Functional Oxytocin Receptors in Bovine Granulosa Cells1. *Biology of Reproduction*, 56(3):625–631, Mar. 1997. ISSN 0006-3363, 1529-7268. doi: 10.1095/biolreprod56.3.625. URL <https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod56.3.625>.
- [182] G. Oliver and E. A. Schäfer. On the Physiological Action of Extracts of Pituitary Body and certain other Glandular Organs: Preliminary Communication. *The Journal of Physiology*, 18(3):277–279, July 1895. ISSN 00223751. doi: 10.1113/jphysiol.1895.sp000565. URL <http://doi.wiley.com/10.1113/jphysiol.1895.sp000565>.

- [183] B. R. Olson, M. D. Drutarosky, M.-S. Chow, V. J. Hruby, E. M. Stricker, and J. G. Verbalis. Oxytocin and an oxytocin agonist administered centrally decrease food intake in rats. *Peptides*, 12(1):113–118, Jan. 1991. ISSN 01969781. doi: 10.1016/0196-9781(91)90176-P. URL <http://linkinghub.elsevier.com/retrieve/pii/019697819190176P>.
- [184] S. P. Otto and A. C. Gerstein. The evolution of haploidy and diploidy. *Current Biology*, 18(24):R1121–R1124, Dec. 2008. ISSN 09609822. doi: 10.1016/j.cub.2008.09.039. URL <https://linkinghub.elsevier.com/retrieve/pii/S0960982208012682>.
- [185] S. Parmigiani and F. S. Vom Saal, editors. *Infanticide and parental care*. Number v. 13 in Ettore Majorana international life sciences series. Harwood Academic Publishers, [Chur, Switzerland] ; Langhorne, Pa., USA, 1994. ISBN 978-3-7186-5505-2.
- [186] C. Pedersen, J. Ascher, Y. Monroe, and A. Prange. Oxytocin induces maternal behavior in virgin female rats. *Science*, 216(4546):648–650, May 1982. ISSN 0036-8075, 1095-9203. doi: 10.1126/science.7071605. URL <http://www.sciencemag.org/cgi/doi/10.1126/science.7071605>.
- [187] F. Peng, T. Li, K.-L. Wang, G.-Q. Xiao, and J. Wang. H19/let-7/lin28 reciprocal negative regulatory circuit promotes breast cancer stem cell maintenance. In *Cell death and disease*, 2017.
- [188] J. Peters. The role of genomic imprinting in biology and disease: an expanding view. *Nature Reviews Genetics*, 15(8):517–530, Aug. 2014. ISSN 1471-0056, 1471-0064. doi: 10.1038/nrg3766. URL <http://www.nature.com/articles/nrg3766>.
- [189] M. Pollak. The insulin and insulin-like growth factor receptor family in neoplasia: an update. *Nature Reviews Cancer*, 12(3):159–169, 2012.
- [190] j. qin, m. feng, c. wang, y. ye, p. s. wang, and c. liu. Oxytocin receptor expressed on the smooth muscle mediates the excitatory effect of oxytocin on gastric motility in rats. *Neurogastroenterology & Motility*, 21(4):430–438, Apr. 2009. ISSN 13501925, 13652982. doi: 10.1111/j.1365-2982.2009.01282.x. URL <http://doi.wiley.com/10.1111/j.1365-2982.2009.01282.x>.
- [191] A. R. Reddon, C. M. O’Connor, E. Nesjan, J. Cameron, J. K. Hellmann, I. Y. Ligocki, S. E. Marsh-Rollo, I. M. Hamilton, D. R. Wylie, P. L. Hurd, and S. Balshine. Isotocin neuronal phenotypes differ among social systems in cichlid fishes. *Royal Society Open Science*, 4(5):170350, May

2017. ISSN 2054-5703, 2054-5703. doi: 10.1098/rsos.170350. URL <https://royalsocietypublishing.org/doi/10.1098/rsos.170350>.
- [192] W. Reik and J. Walter. Genomic imprinting: parental influence on the genome. *Nature Reviews Genetics*, 2(1):21–32, Jan. 2001. ISSN 1471-0056, 1471-0064. doi: 10.1038/35047554. URL <http://www.nature.com/articles/35047554>.
- [193] M. B. Renfree and D. R. Blanden. Progesterone and oestrogen receptors in the female genital tract throughout pregnancy in tammar wallabies. page 8.
- [194] M. B. Renfree, T. A. Hore, G. Shaw, J. A. Marshall Graves, and A. J. Pask. Evolution of Genomic Imprinting: Insights from Marsupials and Monotremes. *Annual Review of Genomics and Human Genetics*, 10(1):241–262, Sept. 2009. ISSN 1527-8204, 1545-293X. doi: 10.1146/annurev-genom-082908-150026. URL <http://www.annualreviews.org/doi/10.1146/annurev-genom-082908-150026>.
- [195] M. E. Rich, E. J. deCárdenas, H.-J. Lee, and H. K. Caldwell. Impairments in the Initiation of Maternal Behavior in Oxytocin Receptor Knockout Mice. *PLoS ONE*, 9(6):e98839, June 2014. ISSN 1932-6203. doi: 10.1371/journal.pone.0098839. URL <http://dx.plos.org/10.1371/journal.pone.0098839>.
- [196] M. Ridley and A. Grafen. Are green beard genes outlaws? *Animal Behav*, 29, 1981. doi: 10.1016/S0003-3472(81)80034-6. URL [https://doi.org/10.1016/S0003-3472\(81\)80034-6](https://doi.org/10.1016/S0003-3472(81)80034-6).
- [197] J. D. Riordan, V. W. Keng, B. R. Tschida, T. E. Scheetz, J. B. Bell, K. M. Podetz-Pedersen, C. D. Moser, N. G. Copeland, N. A. Jenkins, L. R. Roberts, D. A. Largaespada, and A. J. Dupuy. Identification of Rtl1, a retrotransposon-derived imprinted gene, as a novel driver of hepatocarcinogenesis. *PLoS Genet*, 9, 2013. doi: 10.1371/journal.pgen.1003441. URL <https://doi.org/10.1371/journal.pgen.1003441>.
- [198] E. J. Robertson. Insulin-like growth factors, imprinting and embryonic growth control. In *seminars in DEVELOPMENTAL BIOLOGY*, volume 6, pages 293–299. Elsevier, 1995.
- [199] G. Robinson and J. J. Evans. Oxytocin has a role in gonadotrophin regulation in rats. *Journal of Endocrinology*, 125(3):425–432, June 1990. ISSN 0022-0795, 1479-6805. doi: 10.1677/joe.0.1250425. URL https://joe.bioscientifica.com/view/journals/joe/125/3/joe_125_3_012.xml.
- [200] S. T. d. Rocha, C. A. Edwards, M. Ito, T. Ogata, and A. C. Ferguson-Smith. Genomic imprinting at the mammalian Dlk1-Dio3 domain. *Trends*

- in Genetics*, 24(6):306–316, June 2008. ISSN 01689525. doi: 10.1016/j.tig.2008.03.011. URL <https://linkinghub.elsevier.com/retrieve/pii/S0168952508001315>.
- [201] S. Rohwer. Parent Cannibalism of Offspring and Egg Raiding as a Courtship Strategy. *The American Naturalist*, 112(984):429–440, Mar. 1978. ISSN 0003-0147, 1537-5323. doi: 10.1086/283284. URL <https://www.journals.uchicago.edu/doi/10.1086/283284>.
 - [202] S. Roush and F. J. Slack. The let-7 family of microRNAs. *Trends in cell biology*, 18(10):505–516, 2008.
 - [203] H. Royo, M.-L. Bortolin, H. Seitz, and J. Cavaillé. Small non-coding rnas and genomic imprinting. *Cytogenetic and Genome Research*, 113:108 – 99, 2006.
 - [204] K. Ryan, Z. Lu, and I. A. Meinertzhagen. The cns connectome of a tadpole larva of *Ciona intestinalis* (l.) highlights sidedness in the brain of a chordate sibling. *eLife*, 5:e16962, dec 2016. ISSN 2050-084X. doi: 10.7554/eLife.16962. URL <https://doi.org/10.7554/eLife.16962>.
 - [205] Y. Saito, G. Liang, G. Egger, J. M. Friedman, J. C. Chuang, G. A. Coetzee, and P. A. Jones. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell*, 9, 2006. doi: 10.1016/j.ccr.2006.04.020. URL <https://doi.org/10.1016/j.ccr.2006.04.020>.
 - [206] E. Savaskan, R. Ehrhardt, A. Schulz, M. Walter, and H. Schächinger. Post-learning intranasal oxytocin modulates human memory for facial identity. *Psychoneuroendocrinology*, 33(3):368–374, Apr. 2008. ISSN 03064530. doi: 10.1016/j.psyneuen.2007.12.004. URL <https://linkinghub.elsevier.com/retrieve/pii/S0306453007002818>.
 - [207] E. A. Schafer and K. Mackenzie. The Action of Animal Extracts on Milk Secretion. *Proceedings of the Royal Society B: Biological Sciences*, 84 (568):16–22, July 1911. ISSN 0962-8452, 1471-2954. doi: 10.1098/rspb.1911.0042. URL <http://rspb.royalsocietypublishing.org/cgi/doi/10.1098/rspb.1911.0042>.
 - [208] J.-C. Schellenberg, V. Pliška, and R. A. Lutz. Oxytocin Receptors in Guinea Pig Myometrium Near Term and During Labor1. *Biology of Reproduction*, 62(2):427–431, Feb. 2000. ISSN 0006-3363, 1529-7268. doi: 10.1095/biolreprod62.2.427. URL <https://academic.oup.com/biolreprod/article-lookup/doi/10.1095/biolreprod62.2.427>.

- [209] J. V. Schmidt, P. G. Matteson, B. K. Jones, X. J. Guan, and S. M. Tilghman. The Dlk1 and Gtl2 genes are linked and reciprocally imprinted. *Genes Dev*, 14, 2000.
- [210] I. Schneiderman, O. Zagoory-Sharon, J. F. Leckman, and R. Feldman. Oxytocin during the initial stages of romantic attachment: Relations to couples' interactive reciprocity. page 20, 2014.
- [211] H. Seitz, N. Youngson, S. P. Lin, S. Dalbert, M. Paulsen, J. P. Bachelierie, A. C. Ferguson-Smith, and J. Cavaillé. Imprinted microRNA genes transcribed antisense to a reciprocally imprinted retrotransposon-like gene. *Nat Genet*, 34, 2003. doi: 10.1038/ng1171. URL <https://doi.org/10.1038/ng1171>.
- [212] H. Seitz, H. Royo, M. L. Bortolin, S. P. Lin, A. C. Ferguson-Smith, and J. Cavaillé. A large imprinted microRNA gene cluster at the mouse Dlk1-Gtl2 domain. *Genome Res*, 14, 2004. doi: 10.1101/gr.2743304. URL <https://doi.org/10.1101/gr.2743304>.
- [213] Y. Sekita, H. Wagatsuma, K. Nakamura, R. Ono, M. Kagami, N. Wakisaka, T. Hino, R. Suzuki-Migishima, T. Kohda, A. Ogura, T. Ogata, M. Yokoyama, T. Kaneko-Ishino, and F. Ishino. Role of retrotransposon-derived imprinted gene, Rtl1, in the feto-maternal interface of mouse placenta. *Nature Genetics*, 40(2):243–248, Feb. 2008. ISSN 1061-4036, 1546-1718. doi: 10.1038/ng.2007.51. URL <http://www.nature.com/articles/ng.2007.51>.
- [214] C. Sell, G. Dumenil, C. Deveaud, M. Miura, D. Coppola, T. DeAngelis, R. Rubin, A. Efstratiadis, and R. Baserga. Effect of a null mutation of the insulin-like growth factor i receptor gene on growth and transformation of mouse embryo fibroblasts. *Molecular and cellular biology*, 14(6):3604–3612, 1994.
- [215] D. Selvage and C. A. Johnston. Central Stimulatory Influence of Oxytocin on Preovulatory Gonadotropin-Releasing Hormone Requires More than the Median Eminence. *Neuroendocrinology*, 74(2):129–134, 2001. ISSN 0028-3835, 1423-0194. doi: 10.1159/000054678. URL <https://www.karger.com/Article/FullText/54678>.
- [216] A. J. Sharp, E. Migliavacca, Y. Dupré, E. Stathaki, and M. R. Sailani. Methylation profiling in individuals with uniparental disomy identifies novel differentially methylated regions on chromosome 15. *Genome research*, 20 9:1271–8, 2010.
- [217] M. P. Simon. The ecology of parental care in a terrestrial breeding frog from New Guinea. *Behavioral Ecology and Sociobiology*, 14(1):61–67, Dec. 1983.

- ISSN 0340-5443, 1432-0762. doi: 10.1007/BF00366657. URL <http://link.springer.com/10.1007/BF00366657>.
- [218] V. Singh and K. Joy. An involvement of vasotocin in oocyte hydration in the catfish *Heteropneustes fossilis*: A comparison with effects of isotocin and hCG. *General and Comparative Endocrinology*, 166(3):504–512, May 2010. ISSN 00166480. doi: 10.1016/j.ygcen.2010.02.020. URL <https://linkinghub.elsevier.com/retrieve/pii/S001664801000081X>.
 - [219] V. Singh and K. Joy. Vasotocin induces final oocyte maturation and ovulation through the production of a maturation-inducing steroid in the catfish *Heteropneustes fossilis*. *General and Comparative Endocrinology*, 174(1): 15–21, Oct. 2011. ISSN 00166480. doi: 10.1016/j.ygcen.2011.07.009. URL <https://linkinghub.elsevier.com/retrieve/pii/S0016648011002905>.
 - [220] K. Skourti-Stathaki and N. J. Proudfoot. A double-edged sword: R loops as threats to genome integrity and powerful regulators of gene expression. *Genes Dev*, 28, 2014. doi: 10.1101/gad.242990.114. URL <https://doi.org/10.1101/gad.242990.114>.
 - [221] A. K. Skrzynska, M. Gozdowska, E. Kulczykowska, G. Martínez-Rodríguez, J. M. Mancera, and J. A. Martos-Sitcha. The effect of starvation and re-feeding on vasotocinergic and isotocinergic pathways in immature gilthead sea bream (*Sparus aurata*). *Journal of Comparative Physiology B*, 187(7):945–958, Oct. 2017. ISSN 0174-1578, 1432-136X. doi: 10.1007/s00360-017-1064-y. URL <http://link.springer.com/10.1007/s00360-017-1064-y>.
 - [222] F. Sleutels, R. Zwart, and D. P. Barlow. The non-coding Air RNA is required for silencing autosomal imprinted genes. *Nature*, 415(6873):810–813, Feb. 2002. ISSN 0028-0836, 1476-4687. doi: 10.1038/415810a. URL <http://www.nature.com/articles/415810a>.
 - [223] G. Smits, A. J. Mungall, S. Griffiths-Jones, P. Smith, D. Beury, L. Matthews, J. Rogers, A. J. Pask, G. Shaw, J. L. Vandeberg, J. R. McCarrey, M. B. Renfree, W. Reik, and I. Dunham. Conservation of the h19 noncoding rna and h19-igf2 imprinting mechanism in therians. *Nature Genetics*, 40:971–976, 2008.
 - [224] G. Song and L. Wang. A conserved gene structure and expression regulation of miR-433 and miR-127 in mammals. *PLoS One*, 4, 2009. doi: 10.1371/journal.pone.0007829. URL <https://doi.org/10.1371/journal.pone.0007829>.
 - [225] G. u. i. s. h. e. n. g. Song and L. i. Wang. MiR-433 and miR-127 Arise from Independent Overlapping Primary Transcripts Encoded by the miR-433-

- 127 Locus. *PLoS ONE*, 3, 2008. doi: 10.1371/journal.pone.0003574. URL <https://doi.org/10.1371/journal.pone.0003574>.
- [226] S. Spring, M. Lehner, L. Huber, and E. Ringler. Oviposition and father presence reduce clutch cannibalism by female poison frogs. *Frontiers in Zoology*, 16(1):8, Dec. 2019. ISSN 1742-9994. doi: 10.1186/s12983-019-0304-2. URL <https://frontiersinzoology.biomedcentral.com/articles/10.1186/s12983-019-0304-2>.
- [227] E. Stafflinger, K. K. Hansen, F. Hauser, and Schneider. Cloning and identification of an oxytocin/vasopressin-like receptor and its ligand from insects. *Proceedings of the National Academy of Sciences*, 105(9):3262–3267, 2008. ISSN 0027-8424. doi: 10.1073/pnas.0710897105. URL <https://www.pnas.org/content/105/9/3262>.
- [228] N. Sudarsan, M. C. Hammond, K. F. Block, R. Welz, J. E. Barrick, A. Roth, and R. R. Breaker. Tandem riboswitch architectures exhibit complex gene control functions. *Science*, 314 5797:300–4, 2006.
- [229] J. Sun, W. Li, Y. Sun, D. Yu, X. Wen, H. W. Wang, and J. Cui. A novel antisense long noncoding rna within the igflr gene locus is imprinted in hematopoietic malignancies. In *Nucleic acids research*, 2014.
- [230] M. A. H. Surani, S. C. Barton, and M. L. Norris. Development of reconstituted mouse eggs suggests imprinting of the genome during gametogenesis. *Nature*, 308(5959):548–550, Apr. 1984. ISSN 0028-0836, 1476-4687. doi: 10.1038/308548a0. URL <http://www.nature.com/articles/308548a0>.
- [231] S. Takada, M. Tevendale, J. Baker, P. Georgiades, E. Campbell, T. Freeman, M. H. Johnson, M. Paulsen, and A. C. Ferguson-Smith. Delta-like and Gtl2 are reciprocally expressed differentially methylated linked imprinted genes on mouse chromosome 12. *Curr Biol*, 10, 2000. doi: 10.1016/S0960-9822(00)00704-1. URL [https://doi.org/10.1016/S0960-9822\(00\)00704-1](https://doi.org/10.1016/S0960-9822(00)00704-1).
- [232] T. Takahashi and M. Kawashima. Mesotocin Receptor Binding in Oviduct Uterus of the Hen Before and After Oviposition. *Poultry Science*, 87 (3):546–550, Mar. 2008. ISSN 0032-5791, 1525-3171. doi: 10.3382/ps.2007-00284. URL <https://academic.oup.com/ps/article-lookup/doi/10.3382/ps.2007-00284>.
- [233] Y. Takayanagi, Y. Kasahara, T. Onaka, and Takahashi. Oxytocin receptor-deficient mice developed late-onset obesity. page 5, 2005, file

- = Takayanagi et al. - Oxytocin receptor-deficient mice developed late-ons.pdf:/Users/avantikamainieri/Zotero/storage/8LRLZXJ4/Takayanagi et al. - Oxytocin receptor-deficient mice developed late-ons.pdf:application/pdf.
- [234] K. Takuwa-Kuroda, E. Iwakoshi-Ukena, A. Kanda, and H. Minakata. Octopus, which owns the most advanced brain in invertebrates, has two members of vasopressin/oxytocin superfamily as in vertebrates. *Regulatory Peptides*, 115(2):139–149, Sept. 2003. ISSN 01670115. doi: 10.1016/S0167-0115(03)00151-4. URL <https://linkinghub.elsevier.com/retrieve/pii/S0167011503001514>.
 - [235] G. D. Thorburn and J. R. Challis. Endocrine control of parturition. 59:56, 1979.
 - [236] A. Traulsen and M. A. Nowak. Chromodynamics of cooperation in finite populations. *PLoS One*, 2, 2007. doi: 10.1371/journal.pone.0000270. URL <https://doi.org/10.1371/journal.pone.0000270>.
 - [237] T. F. Tsai, J. Bressler, Y. Jiang, and A. L. Beaudet. Disruption of the genomic imprint in trans with homologous recombination at Snrpn in ES cells. *Genesis*, 37, 2003. doi: 10.1002/gene.10237. URL <https://doi.org/10.1002/gene.10237>.
 - [238] H. Tsuchiya, S. Fujimura, Y. Fujiwara, and T.-a. Koshimizu. Critical role of V1a vasopressin receptor in murine parturition†. *Biology of Reproduction*, 12 2019. ISSN 0006-3363. doi: 10.1093/biolre/iox220.
 - [239] H. Tuppy. The amino-acid sequence in oxytocin. *Biochimica et Biophysica Acta*, 11:449–450, Jan. 1953. ISSN 00063002. doi: 10.1016/0006-3002(53)90071-7. URL <https://linkinghub.elsevier.com/retrieve/pii/0006300253900717>.
 - [240] R. Van Kesteren, A. Smit, R. De Lange, K. Kits, F. Van Golen, R. Van Der Schors, N. De With, J. Burke, and W. Geraerts. Structural and functional evolution of the vasopressin/oxytocin superfamily: vasopressin-related conopressin is the only member present in Lymnaea, and is involved in the control of sexual behavior. *The Journal of Neuroscience*, 15(9):5989–5998, Sept. 1995. ISSN 0270-6474, 1529-2401. doi: 10.1523/JNEUROSCI.15-09-05989.1995. URL <http://www.jneurosci.org/lookup/doi/10.1523/JNEUROSCI.15-09-05989.1995>.
 - [241] D. C. M. Veenma, H. Eussen, L. C. P. Govaerts, S. W. K. de Kort, and R. J. Odink. Phenotype-genotype correlation in a familial igf1r microdeletion case. *Journal of medical genetics*, 47 7:492–8, 2009.

- [242] V. d. Vigneaud, H. C. Lawler, and E. A. Popenoe. ENZYMATIC CLEAVAGE OF GLYCINAMIDE FROM VASOPRESSIN AND A PROPOSED STRUCTURE FOR THIS PRESSOR-ANTIDIURETIC HORMONE OF THE POSTERIOR PITUITARY. *Journal of the American Chemical Society*, 75(19): 4880–4881, Oct. 1953. ISSN 0002-7863, 1520-5126. doi: 10.1021/ja01115a554. URL <https://pubs.acs.org/doi/abs/10.1021/ja01115a554>.
- [243] D. A. Wagenaar, M. S. Hamilton, T. Huang, W. B. Kristan, and K. A. French. A Hormone-Activated Central Pattern Generator for Courtship. *Current Biology*, 20(6):487–495, Mar. 2010. ISSN 09609822. doi: 10.1016/j.cub.2010.02.027. URL <https://linkinghub.elsevier.com/retrieve/pii/S0960982210002113>.
- [244] M. J. E. Walenkamp, H. J. van der Kamp, A. M. Pereira, and S. G. Kantt. A variable degree of intrauterine and postnatal growth retardation in a family with a missense mutation in the insulin-like growth factor i receptor. *The Journal of clinical endocrinology and metabolism*, 91 8:3062–70, 2006.
- [245] M. Wallis. Molecular evolution of the neurohypophysial hormone precursors in mammals: Comparative genomics reveals novel mammalian oxytocin and vasopressin analogues. *General and Comparative Endocrinology*, 179(2):313–318, Nov. 2012. ISSN 00166480. doi: 10.1016/j.ygcen.2012.07.030. URL <https://linkinghub.elsevier.com/retrieve/pii/S0016648012003681>.
- [246] J. Wang, Z. Li, B. Liu, G. Chen, N. Shao, X. Ying, and Y. Wang. Systematic study of cis-antisense miRNAs in animal species reveals miR-3661 to target PPP2CA in human cells. *RNA*, 22, 2015.
- [247] S. Wang, X. Wu, Y. Liu, J. Yuan, F. Yang, J. Huang, Q. Meng, and C. Zhou. Long noncoding rna h19 inhibits the proliferation of fetal liver cells and the wnt signaling pathway. *FEBS letters*, 590 4:559–70, 2016.
- [248] X. Wang, H. Li, L. Cui, J. Feng, and Q. Fan. Microrna-182 suppresses clear cell renal cell carcinoma migration and invasion by targeting igflr. *Neoplasma*, 63(5):717–725, 2016.
- [249] D. Wathes, S. Borwick, P. Timmons, S. Leung, and S. Thornton. Oxytocin receptor expression in human term and preterm gestational tissues prior to and following the onset of labour. *Journal of Endocrinology*, 161(1):143–151, Apr. 1999. ISSN 0022-0795, 1479-6805. doi: 10.1677/joe.0.1610143. URL <https://joe.bioscientifica.com/view/journals/joe/161/1/143.xml>.

- [250] J. B. Wolf and R. Hager. A maternal-offspring coadaptation theory for the evolution of genomic imprinting. *PLoS Biol*, 4, 2006. doi: 10.1371/journal.pbio.0040380. URL <https://doi.org/10.1371/journal.pbio.0040380>.
- [251] P. E. Wright and H. J. Dyson. Intrinsically unstructured proteins: re-assessing the protein structure-function paradigm. *Journal of molecular biology*, 293 2: 321–31, 1999.
- [252] Z. Wu, Y. Xu, Y. Zhu, A. K. Sutton, R. Zhao, B. B. Lowell, D. P. Olson, and Q. Tong. An Obligate Role of Oxytocin Neurons in Diet Induced Energy Expenditure. *PLoS ONE*, 7(9):e45167, Sept. 2012. ISSN 1932-6203. doi: 10.1371/journal.pone.0045167. URL <https://dx.plos.org/10.1371/journal.pone.0045167>.
- [253] A. A. Wylie, S. K. Murphy, T. C. Orton, and R. L. Jirtle. Novel imprinted DLK1/GTL2 domain on human chromosome 14 contains motifs that mimic those implicated in IGF2/H19 regulation. *Genome Res*, 10, 2000. doi: 10.1101/gr.161600. URL <https://doi.org/10.1101/gr.161600>.
- [254] X. Xu, F. Ectors, E. E. Davis, D. Pirottin, H. Cheng, F. Farnir, T. Hadfield, N. Cockett, C. Charlier, M. Georges, and H. Takeda. Ectopic expression of retrotransposon-derived PEG11/RTL1 contributes to the callipyge muscular hypertrophy. *PLoS One*, 10, 2015. doi: 10.1371/journal.pone.0140594. URL <https://doi.org/10.1371/journal.pone.0140594>.
- [255] W. Yang, N. Zhang, B. Shi, S. Zhang, L. Zhang, and W. Zhang. Isotocin Regulates Growth Hormone but Not Prolactin Release From the Pituitary of Ricefield Eels. *Frontiers in Endocrinology*, 9:166, Apr. 2018. ISSN 1664-2392. doi: 10.3389/fendo.2018.00166. URL <http://journal.frontiersin.org/article/10.3389/fendo.2018.00166/full>.
- [256] T. Yoshimizu, A. Miroglio, M.-A. Ripoche, and A. Gabory. The h19 locus acts in vivo as a tumor suppressor. *Proceedings of the National Academy of Sciences of the United States of America*, 105 34:12417–22, 2008.
- [257] W. S. Young III, E. Shepard, J. Amico, L. Hennighausen, K.-U. Wagner, M. E. LaMarca, C. McKinney, and E. I. Ginns. Deficiency in Mouse Oxytocin Prevents Milk Ejection, but not Fertility or Parturition. *Journal of Neuroendocrinology*, 8(11):847–853, Nov. 1996. ISSN 09538194. doi: 10.1046/j.1365-2826.1996.05266.x. URL <http://doi.wiley.com/10.1046/j.1365-2826.1996.05266.x>.
- [258] D. Yu, J. Wang, H. Zou, T. Feng, L. Chen, J. Li, and X. Qi. Silencing of retrotransposon-derived imprinted gene RTL1 is the main cause for postim-

- plantation failures in mammalian cloning. *Proc Natl Acad Sci U S A*, 115, 2018. doi: 10.1073/pnas.1814514115. URL <https://doi.org/10.1073/pnas.1814514115>.
- [259] P. J. Zak, R. Kurzban, and W. T. Matzner. Oxytocin is associated with human trustworthiness. *Hormones and behavior*, 48(5):522–527, 2005.
 - [260] L. Zehavi, R. Avraham, A. Barzilai, D. Bar-Ilan, R. Navon, Y. Sidi, D. Avni, and R. Leibowitz-Amit. Silencing of a large microrna cluster on human chromosome 14q32 in melanoma: biological effects of mir-376a and mir-376c on insulin growth factor 1 receptor. *Molecular cancer*, 11(1):44, 2012.
 - [261] S. Zemel, M. S. Bartolomei, and S. M. Tilghman. Physical linkage of two mammalian imprinted genes, h19 and insulin-like growth factor 2. *Nature genetics*, 2(1):61–65, 1992.
 - [262] H. Zhu, N. Shyh-Chang, A. V. Segrè, G. Shinoda, S. P. Shah, W. S. Einhorn, A. Takeuchi, J. M. Engreitz, J. P. Hagan, M. G. Kharas, et al. The lin28/let-7 axis regulates glucose metabolism. *Cell*, 147(1):81–94, 2011.