# Control of Eukaryotic Transcription Elongation

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Minireview

Control of eukaryotic transcription elongation
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Abstract

The Spt4, Spt5, and Spt6 proteins are conserved eukaryotic transcription-elongation factors. Recent studies have provided the first evidence that they are generally required in multicellular eukaryotes, including during development and for viral gene expression.

It has been clear for over 20 years that the control of transcription elongation is a prominent mechanism of gene regulation in prokaryotes. In eukaryotes, although this level of regulation was clearly demonstrated in 1988 [1], it is mainly over the past five years that transcription elongation has blossomed into a broadly active area of investigation [2,3]. For example, several recent studies of the human immunodeficiency virus (HIV) Tat protein, a transcriptional activator that stimulates transcription past a pause site, have helped us to understand factors required for elongation [4,5].

Three transcription-elongation factors that have emerged as playing critical roles are the Spt4, Spt5, and Spt6 proteins. These three proteins, conserved among eukaryotes, were initially discovered by genetic studies in Saccharomyces cerevisiae [6]. Further studies in S. cerevisiae suggested that Spt4, Spt5, and Spt6 are essential for transcription via the modulation of chromatin structure [7,8]. Spt4 and Spt5 are tightly associated in a complex, while the physical association of the Spt4-Spt5 complex with Spt6 appears to be considerably weaker [9,10]. Evidence over the past two years has demonstrated that Spt4, Spt5, and Spt6 play roles in transcription elongation in both yeast and humans [9,10], including a role in activation by Tat [11]. The breadth of the requirement for these factors in multicellular eukaryotes was not known, however. Now, four papers [12-15] have illuminated the roles of these factors in vivo. These new studies, taken together with earlier work, portray Spt4, Spt5, and Spt6 as general transcription-elongation factors, controlling transcription both positively and negatively in important regulatory and developmental roles.

Spt4, Spt5, and Spt6 have characteristics of general transcription-elongation factors

Two recent studies [12,13] of Spt5 and Spt6 have provided the first view of their roles in vivo in a multicellular eukaryote, in this case Drosophila melanogaster. In both studies, specific antisera were used to determine the localization of Spt5 and Spt6 on polytene chromosomes. Both studies demonstrated that Spt5 and Spt6 colocalize to hundreds of sites on polytene chromosomes and that this localization strikingly coincides with that of actively transcribing RNA polymerase II (Pol II), Pol IIo (Figure 1). This correlation between the Spt proteins and active transcription was strengthened by the demonstration that the induction of heat shock (hsp) gene transcription causes massive recruitment of both Spt5 and Spt6 to hsp loci. These results, then, show that Spt5 and Spt6 are associated with virtually all active transcription, strongly suggesting a general positive role for these factors.

Evidence for a negative role, although less general, came from the colocalization between Spt5, Spt6 and the non-transcribing form of Pol II, Pol IIa, at a small subset of polytene positions [12]. Most of these positions correspond to the Salivary gland secreted (Sgs) loci, and the colocalization results suggest that the Sgs genes are regulated by Spt5, Spt6, and perhaps by transcriptional pause, similar to hsp genes [12]. In addition, Spt5, but not Spt6, is present at the uninduced hsp70 locus, where Pol IIa is paused approximately 25 nucleotides from the initiation site [12,13]. As well as suggesting a negative role for Spt5, this result indicates that Spt5 and Spt6 do not play identical roles in vivo. While
the other member of this triad of proteins, Spt4, was not directly examined in either of these studies [12,13], its strong physical association with Spt5 suggests that Spt4 is likely to be similarly localized. These results, suggesting negative as well as positive control by the Spt proteins in vitro, are consistent with earlier in vitro results [10,16].

In addition to the genome-wide view provided by the polytene chromosome studies, higher-resolution analysis has provided additional insights about Spt5 and Spt6 [13]. Chromatin immunoprecipitation analysis revealed that Spt5 and Spt6 are bound within the open reading frames of heat shock (hsp) genes after heat shock induction, providing strong evidence for a direct role in transcription elongation. These two papers [12,13], then, provided strong evidence for general roles for Spt4, Spt5, and Spt6 in elongation. In future studies, genetic analysis of Spt4, Spt5, and Spt6 in D. melanogaster should provide important insights into both their positive and negative roles in vivo.

A role for Spt5 in neuronal development in zebrafish
An intriguing result emerged from recent studies of foggy, a zebrafish mutant identified in a screen for mutations that cause neuronal development defects [14]. The foggy mutation causes a previously unidentified pattern of neuronal defect: a reduction of dopamine-containing neurons and a corresponding increase in serotonin-containing neurons in the hypothalamus. Positional cloning and sequence analysis revealed that foggy encodes the zebrafish Spt5 homolog. Thus, a mutation that impairs a transcription-elongation factor causes a novel neuronal developmental defect. Biochemical analysis of the mutant Fogg/Spt5 protein demonstrated a defect in its ability to negatively control elongation in vitro, suggesting that the foggy mutation allows increased expression of particular genes in vivo, thereby causing this specific neuronal defect [14]. Similarly, a developmental role for Spt6 had been previously suggested on the basis of the developmental defects of a Caenorhabditis elegans spt6 (emb-5) mutant during gastrulation [17].

Studies of the foggy mutant raise the issue of the roles of general factors in developmental control. Is the role of Fogg/Spt5 in zebrafish dedicated to specific aspects of neuronal development, or does the foggy mutant phenotype reflect a particular type of defect in a general factor? On the basis of other studies of Spt5, the latter possibility seems more likely. The foggy mutation also causes cardiovascular defects [18], indicating that this mutation is pleiotropic, albeit still highly specific in the neuronal defects that it causes. How might a mutation that alters a general elongation factor cause specific neuronal defects? One possibility is that particular genes have a stronger requirement for Fogg/Spt5 than do others: a mutation that mildly reduces Fogg/Spt5 activity might affect a small subset of genes, causing specific mutant phenotypes. Consistent with this idea, reduced levels of Pol II in S. cerevisiae cause specific mutant phenotypes [19]. A second possibility is that Fogg/Spt5 interacts with several gene-specific transcription factors and the foggy mutation impairs only an interaction between Spt5 and a neuronal-specific transcription factor. This idea is supported by the demonstration of allele-specific phenotypes among different spt5 mutants in S. cerevisiae [9]. Allele-specific developmental defects have been identified for mutants of general factors, including Pol II (for examples, see [20,21]). It would be of great interest to identify additional foggy alleles and study their effect on neuronal development. The identification of proteins that interact with Fogg/Spt5 might identify neuron-specific transcription-elongation factors.

Human cytomegalovirus and Spt6
The fourth recent study [15] concerns the human cytomegalovirus (CMV) activator pUL69. This regulator is required for transcription of several CMV genes [22] and is also responsible for arresting CMV-infected cells in G1 phase of the cell cycle [23]. To learn more about the function of pUL69, a two-hybrid protein-protein interaction screen was performed to identify pUL69-interacting proteins, and human Spt6 was identified. Additional experiments provide compelling evidence that pUL69 is physically associated with Spt6 in CMV-infected cells. The biological relevance of this interaction was established by the complete correlation between pUL69 mutations that impair interaction with Spt6 and those that impair pUL69-dependent transcriptional activation.

How might an interaction with Spt6 help explain transcriptional activation by pUL69? One model comes from experiments that addressed the interaction of human Spt6 with histones. Previous studies of yeast Spt6 had demonstrated
an Spt-histone interaction in vitro [8]. In the current study [15], the authors have demonstrated a human Spt6-histone H3 interaction and show that this interaction is antagonized by pUL69. These results suggest that activation by pUL69 occurs by overcoming the repression of transcription caused by an Spt6-histone interaction. Of interest with respect to the models discussed above for the effects of Foggy/Spt5 in the zebrafish, this work demonstrates that Spt6 specifically interacts with a particular transcriptional activator. Earlier work suggested that Spt6 also interacts with the estrogen receptor [24]. Spt6 may, therefore, interact with many gene-specific transcription factors. The pUL69 studies also raise interesting and related questions. First, does the pUL69-Spt6 interaction serve to control transcription initiation or elongation? Second, what directs the association of Spt6 with the CMV genome after infection? Third, does Spt6 play a role in all pUL69-dependent transcription? Finally, do Spt4 and Spt5 also play a role in pUL69 activation? The answers to these questions will undoubtedly teach us more about Spt4, Spt5, and Spt6.

The recent studies discussed in this article portray Spt4, Spt5, and Spt6 as general elongation factors that are likely to have many interactions with other factors in vivo, including Pol II, histones, and gene-specific transcription factors. Future studies will need to address the distinctions between their positive and negative roles, the individual roles of Spt4, Spt5, and Spt6, and their possible regulation during development.

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References