Directing traffic on DNA—How transcription factors relieve or induce transcriptional interference

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Directing traffic on DNA—How transcription factors relieve or induce transcriptional interference

Nan Hao a, Adam C. Palmer b, Ian B. Dodd a, and Keith E. Shearwin a

aDepartment of Molecular and Cellular Biology, School of Biological Sciences, University of Adelaide, Adelaide, SA, Australia; bDepartment of Systems Biology, Harvard Medical School, Boston, MA, USA

ABSTRACT

Transcriptional interference (TI) is increasingly recognized as a widespread mechanism of gene control, particularly given the pervasive nature of transcription, both sense and antisense, across all kingdoms of life. Here, we discuss how transcription factor binding kinetics strongly influence the ability of a transcription factor to relieve or induce TI.

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CONTACT Keith E. Shearwin keith.shearwin@adelaide.edu.au Department of Molecular and Cellular Biology, School of Biological Sciences, University of Adelaide, Adelaide, SA 5005, Australia.

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activator for a promoter upstream of the IME1 gene.\textsuperscript{10} TI is also responsible for the establishment of mosaic expression of homeobox gene Ubx during embryonic development in \textit{D. melanogaster},\textsuperscript{11} while in mammals, the promoter for the \textit{Airn} long non-coding RNA exerts \textit{cis}-acting repression on the downstream \textit{Igf2r}
promoter by transcriptional overlap on the paternal allele, but is relieved by DNA methylation-associated repression of the Airn promoter on the maternal allele.\textsuperscript{12} TI can also operate in a multi-layered manner. For example, the yeast FLO11 transcript is controlled by the upstream ICR1 promoter, which itself is regulated by the convergent PWRI promoter. Activation or repression of PWRI induces or relieves TI on ICR1, causing stimulation or inhibition of FLO11 transcription.\textsuperscript{13}

Using a TF to induce or relieve TI is relatively straightforward when \( P_{\text{interfering}} \) is upstream of \( P_{\text{target}} \). However, when the promoters are convergent, the situation becomes more complex (Fig. 1C). First, TI can be reciprocal, with the activity of the target promoter exerting TI on RNAP at the interfering promoter. Second, there are potential interactions between the elongating RNAPs from \( P_{\text{target}} \) and the TF in their path bound at \( P_{\text{interfering}} \). The elongating RNAP from \( P_{\text{target}} \) may dislodge the transcription factor, altering its regulation of \( P_{\text{interfering}} \). If the bound transcription factor is not dislodged, it may alternatively act as a roadblock to elongating RNAP, blocking downstream transcription,\textsuperscript{15,16} which may be important if the biologic function of \( P_{\text{target}} \) requires its transcription to pass \( P_{\text{interfering}} \) (e.g., to express a gene beyond \( P_{\text{interfering}} \), as in Fig. 1C).

In a recent study,\textsuperscript{5} we combined mathematical modeling with \textit{in vivo} experiments on the well-characterized bacteriophage \( \lambda \) to examine how repression of \( P_{\text{interfering}} \) (in this case, the \( \lambda \) PR promoter) can give relief of TI on convergent \( P_{\text{target}} \) (the \( \lambda \) PRE promoter).\textsuperscript{5} We began by applying stochastic simulations to explore the key parameters that drive this “relief of TI” mode of regulation, and showed that for each of the three major TI mechanisms mentioned above, the magnitude of relief of TI was strongly dependent on the properties of the transcription factor and its binding site. These properties determine whether the TF acts as a roadblock to transcription from \( P_{\text{target}} \) and whether its occupancy of its binding site is sensitive to dislodgement by RNAPs from \( P_{\text{target}} \) (Fig. 1C). Roadblocking by a TF requires that the TF–DNA complex presents a strong barrier to the elongating RNAP (i.e., it is not easily dislodged) \textit{and} that its spontaneous unbinding kinetics are slow relative to the RNAP elongation rate (Fig. 2A). The occupancy of the TF-binding site is most sensitive to elongating RNAP when it easily dislodged (weak barrier) and has a low rate of spontaneous unbinding, such that the increased removal due to the RNAP significantly perturbs its binding equilibrium (Fig. 2B). Modeling thus predicted that relief of TI is readily tunable, maximized by rapid repressor-binding kinetics, but can be compromised by repressors with slow-binding kinetics, as a result of roadblocking as well as loss of repression by repressor dislodgement (Fig. 2C).

We then validated our model predictions experimentally using the \( \lambda \) PR – PRE promoter pair. Repression of the strong lytic promoter \( P_R \) by the \( \lambda \) CI or Cro repressors very efficiently relieved TI on the convergent lysogenic promoter \( P_{\text{RE}} \). We showed that these repressors did not roadblock RNAP and that CI repression of \( P_R \) was not sensitive to \( P_{\text{RE}} \) transcription, implying fast DNA-binding kinetics for these repressors.

Conversely, repression of the same \( P_R \) promoter with a DNA-cleavage-defective Cas9 protein (dCas9) gave sub-optimal relief of TI, providing an important counter-example for TI regulation by a repressor with slow-binding kinetics.\textsuperscript{5} Interestingly, the binding orientation of dCas9, determined by the strand to which the guide RNA hybridizes,\textsuperscript{17,18} is known to influence its strength as a barrier to elongating RNAPs. This property allowed testing of a single repressor with the same binding kinetics but differing roadblock propensities. Consistent with the model prediction, our results clearly demonstrated that the effect of dCas9 on the relief of TI depends on its binding orientation: In the strong-barrier orientation, the barrier effect of dCas9 makes it incapable of relieving TI, whereas in the weak-barrier orientation, dCas9 does relieve TI but poorly, since its repression of \( P_R \) was reduced due to its dislodgement by RNAPs from \( P_{\text{RE}} \).\textsuperscript{5}

As well as demonstrating that relief of TI is dependent on TF-binding properties, the results demonstrate a novel method for estimating the \textit{in vivo} DNA-binding kinetics of a repressor, based on the sensitivity or insensitivity of repression to dislodgement of the repressor by elongating RNAPs. This approach should enhance our ability to study TF binding \textit{in vivo}, as current methodologies require the use of imaging techniques that rely on fluorescent tagging of proteins and do not measure specific, functional DNA binding.

As an extension of our published study,\textsuperscript{5} we used stochastic modeling of protein traffic on DNA to ask how different TI mechanisms and different TF properties
might combine to influence the activity of a target promoter, \( P_{\text{target}} \), when the TF is an activator, rather than a repressor, of the interfering promoter \( P_{\text{interfering}} \) (Fig. 1C). Again, we looked at four different categories of activators: activators that are either a strong barrier or a weak barrier to elongating RNAPs, and activators with either fast or slow DNA-binding kinetics.

Simulations were performed as previously reported,\(^5\) where promoter firing is simulated as a two-step process. We simulated promoter activator with the simple assumption that the presence of bound activator accelerates RNAP loading at \( P_{\text{interfering}} \) by 200-fold, and thereby \( P_{\text{interfering}} \) is effectively proportional to the occupancy of the activator-binding site.

As might be expected, activation of \( P_{\text{interfering}} \) with each of the classes of activator induces TI on the convergent \( P_{\text{target}} \) promoter and reduces its activity (Fig. 2D).

**Figure 2.** The kinetic properties of a TF determine its effect on modulating TI between convergent promoters. (A, B) TF-binding kinetics and the ability of the TF to resist dislodgement by an elongating RNAP both contribute to the overall roadblock effect (A) and to the overall occupation of the TF-binding site (B). The kinetic and resistance properties of several DNA-binding proteins (\( \lambda CI, \lambda Cro, \lambda LacI \) and dCas9 bound either to the template strand (t) or non-template strand (nt)) are classified, as determined in Hao et al.\(^5\) (C, D) TF-binding kinetics influence how gene expression from the target promoter responds to changes in TF concentration. (C) Schematic of the simulated change in target gene expression upon relief of TI by repressors with different properties (slow/fast kinetics, strong/weak barrier to elongating RNAPs).\(^5\) (D) Schematic of simulated induction of TI by activators with different properties. Higher concentrations of slow-binding TFs are needed to relieve or induce TI because their activity is reduced by elongating RNAPs from the target promoter. Slow-binding TFs, that are strong barriers, have a high roadblocking propensity, which can inhibit relief of TI by repressors or can aid induction of TI by activators.
Strong induction of TI was seen in all TI scenarios, but the response curves varied depending on the properties of the activators. An activator with fast-binding kinetics gave a strong induction of TI at lower activator concentrations than were required by an activator with slow kinetics (Fig. 2D). This is because occupation of its binding site by the slow-binding activator is reduced due to dislodgement by elongating RNAPs, which, in turn, suppresses $P_{\text{interfering}}$ activation. In contrast, an activator with fast kinetics naturally binds and unbinds more frequently, so that a dislodged activator is quickly replaced.

Induction of TI by slow-binding activators was dependent on their ability to resist dislodgement by RNAP. An activator with slow kinetics but strong barrier properties showed a more potent induction of TI, when compared with an activator that is a weak barrier but has otherwise identical kinetics (Fig. 2D). A strong barrier activity helps induce TI by two mechanisms: it provides some level of protection against dislodgement by RNAPs, making such a TF a more effective activator of the interfering promoter; and roadblocking by the activator contributes directly to inhibition of transcription from the target promoter.

Thus, while induction of TI by an activator and relief of TI by a repressor for convergent promoters are both made more responsive by fast TF kinetics, their responses to the transcription barrier properties of the TF are reversed. A repressor that acts as a roadblock inhibits relief of TI, while an activator that acts as a roadblock augments induction of TI.

These results underscore the importance of the kinetic properties of transcription factors in their interaction with genomic traffic, and may prove useful in the field of synthetic biology for the design of artificial circuits which exploit TI.19,20

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References


ORCID

Nan Hao http://orcid.org/0000-0001-5836-3507
Adam C. Palmer http://orcid.org/0000-0001-5028-7028
Ian B. Dodd http://orcid.org/0000-0003-2969-6841
Keith E. Shearwin http://orcid.org/0000-0002-7736-2742


