Looking for a promoter in 3D

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Direct time-resolved single-molecule observations of promoter search by *Escherichia coli* RNA polymerase indicate no evidence of facilitated diffusion, according to a new report.

Gene expression in bacteria critically depends on the highly regulated process of RNA polymerase (RNAP) recruitment to promoter sequences on DNA, which mark the transcription start sites[1]. This recruitment occurs in the highly crowded cellular environment where RNAP has to locate a few thousand promoters in the proverbial haystack of compacted genomic DNA obstructed by a multitude of DNA-bound proteins, including those belonging to the transcription, replication, recombination and repair machineries[2]. Although promoter searching in the cell still awaits a rigorous experimental interrogation, an article by Wang *et al.*[3], appearing in this issue, represents the latest foray into *in vitro* single-molecule investigation of this process, using *E. coli* RNAP holoenzyme and nanomanufactured DNA curtains featuring phage λ genomic DNA.

Most of the operons in the *E. coli* genome are transcribed by RNAP holoenzyme consisting of an enzymatic core ($\alpha_2\beta\beta'\omega$) and the major sigma subunit, σ^{70} (**Fig. 1**)[4]. By itself, the core has low nonspecific affinity toward double-stranded DNA and needs association with σ^{70} to be able to recognize promoters in a sequence-specific fashion. Archetypal promoters recognized by σ^{70}-containing holoenzyme feature two elements: so-called –35 (base pairs upstream of the transcription start site), TTGAAT, and –10, TATAAT[5]. RNAP can efficiently initiate transcription from such promoters without the aid of additional factors. The search for promoters conducted by the RNAP holoenzyme is similar to the process by which sequence-specific transcription factors locate their binding sites in the genome. Early work indicated that in some instances, such searching occurred more rapidly than the three-dimensional (3D) diffusion limit, prompting the submission of various acceleration mechanisms collectively known as facilitated diffusion. Much theoretical work has been undertaken to investigate this phenomenon[2,6–10], mostly drawing on von Smoluchowski's discourse on reaction rates in colloidal systems, but the influx of the experimental data needed to inform it has been scarce. Wang *et al.*[3], in their latest work, aimed at the direct visualization of promoter searching by RNAP holoenzyme on its native template, thereby challenging the pervasiveness of facilitated diffusion as the mechanism of target engagement by DNA-binding proteins.

Single-molecule-resolved visualization of RNAP holoenzymes was achieved by tagging them with quantum-dot fluorophores, whereas the DNA search space was approximated by >48,000-nucleotide-long λ coliphage linear genome (whose factor-independent early expression is carried out by its host, *E. coli* RNAP[11]). Phage DNA was functionalized by end labeling with biotin and digoxigenin, which allowed for its controlled orientation on the silica-based nanofabricated substrate. Using total internal reflection fluorescence microscopy, Wang *et al.*[3] observed in real time promoter engagement by individual RNAP molecules (and promoter clearance in the presence of NTPs), recording their positions at rates of up to 100 frames per second[3]. These experiments, involving hundreds of individual RNAP and DNA molecules, revealed the molecular mechanism of promoter searching to be dominated by random 3D diffusion with no occurrence of facilitated diffusion (that is, 3D excursions combined with one-dimensional (1D) sliding or hopping) at physiological concentrations of RNAP (although a modest acceleration of binding rate attributable to short-range sliding was observed at conditions of nonphysiological dilution)[3]. This is at variance with earlier findings of 1D sliding of RNAP along DNA[12,13], including recent work by Suzuki *et al.*[14], who employed atomic force microscopy (albeit at lower temporal resolution of 1–2 frames per second) to record instances of both 1D diffusion and hopping during the RNAP search for promoters on DNA.

In the context of decades-long research of target-search mechanisms by DNA-binding proteins, the observations reported by Wang *et al.*[3] do not mean that such proteins are incapable of facilitated diffusion along DNA, be it by sliding, hopping or intersegmental transfer. Rather, they indicate that such mechanisms do not make a significant contribution when such search occurs within a space represented by a native coliphage genome and is carried out by RNAP at concentrations elevated toward the physiologically relevant range (≥500 pM). These observations are complemented by in-depth theoretical analysis, again following von Smoluchowski's coagulation equation, which converges on increasing irrelevance of facilitated searches as the RNAP concentration tends toward *in vivo* estimates[3]. The authors also demonstrate that their conclusions are applicable to the search process by other DNA-binding proteins in the cell. Indeed, independent analysis by Sheinman *et al.*[7] indicates that in a typical cell volume, ten proteins can locate a single target on the physiologically relevant time scale by a simple 3D diffusion. Although all types of searches can be sped up through increased concentration (starting at

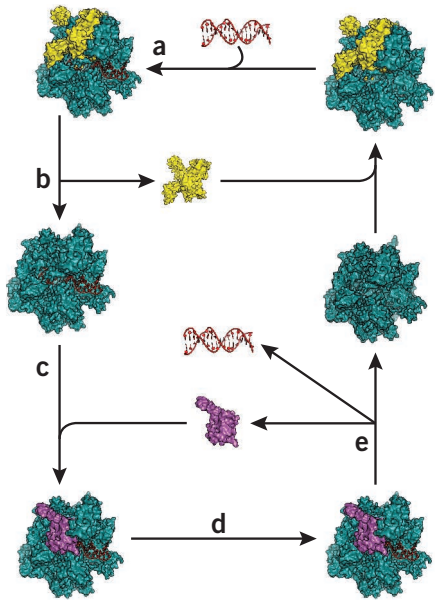

Figure 1 Simplified transcription cycle in bacteria. (**a**) Initiation: RNAP holoenzyme consisting of the core (deep teal) and σ^{70} (yellow) is bound to the promoter DNA (red). (**b**) Promoter clearance: σ^{70} dissociates from the DNA-bound core. (**c**) Elongation factor NusG (purple) binds to the core to form the minimal elongation complex. (**d**) Transcription elongation. (**e**) Termination: the elongation complex dissociates at the terminators, releasing free core, which then binds free σ^{70} to form the holoenzyme capable of engaging the promoter again.

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the extreme dilution of one protein per cell), 1D diffusion mechanisms eventually lose efficiency, owing to so-called 1D crowding, that is, the accumulation of roadblocks (proteins) on DNA that antagonize sliding. Moreover, emerging experimental data indicate that, at least in some instances (as in the case of the cAMP receptor), nonspecific interactions with DNA (the prerequisite for 1D sliding) can slow down target searching rather than accelerate it[15].

Together with the theoretical analysis of search mechanisms, observations of promoter engagement by *E. coli* RNAP by random 3D diffusion (and revisiting operator searching by the Lac operator, which has been crucial to the introduction of the 1D-sliding hypothesis) reported by Wang *et al.*[3] challenge the widely accepted notion of facilitated diffusion dominating the kinetic behavior of DNA-binding proteins in the cell. It must be noted, nonetheless, that this challenge does not firmly establish the 3D random excursions as the dominant mechanism of promoter searching *in vivo*. Several important distinctions between the experimental setup employing DNA curtains and the cellular environment exist that do not allow for straightforward extrapolation of these findings to the realities of gene expression. The most obvious of these distinctions is that promoter searching in the cell doesn't happen on linear, stretched DNA molecules but on compacted, coiled genome. Recent microfluidics experiments demonstrated diphasic ~6-fold expansion of *E. coli* genomic DNA upon cell lysis; the expansion force was estimated at the level of 100 pN, and the free energy of compaction at $10^5\ k_BT$ (ref. 16). The expansion was reversed by addition of the crowding agent PEG 20000 at a concentration of >12%, which indicates the importance of crowding (and entropic 'forces') in bacterial chromosome compaction *in vivo*. Not only was the chromosome substantially coiled in the cell but, compared to the linearly stretched DNA curtains, its behavior was consistent with topological cross-linking (63–284 'cross-links' of undetermined nature per molecule). These conditions would further disfavor 1D sliding, thus simultaneously increasing the probability of intersegmental transfer as another departure from the random 3D diffusion. Another important and unexpected observation from these experiments was a much slower (~10-fold) dissociation rate of DNA-bound proteins from chromosomes of exponentially growing cells compared to that of stationary-phase bacteria[16]. It underscores the tremendous yet poorly understood impact that the physiological state of the cell has on the kinetics of protein-DNA interactions, which cannot be anticipated from the first-principles theoretical analysis.

DNA-curtain experiments, carried out at 0.2 mg ml^{-1} protein concentration, didn't directly probe the role of molecular crowding in the cell, which is usually approximated by a protein content of 100–200 mg ml^{-1} (refs. 17,18). Various theoretical interrogations of the impact that crowding has on DNA-protein interactions differ in details, converging on a decrease in diffusion coefficient, a shift of the equilibrium toward association (which in the case of RNAP means that its displacement from the promoter would be dominated not by dissociation from DNA but by transcriptional promoter escape) and/or changes in association and dissociation constants[17–19]. Explicit experimental interrogation of crowding effects, both 3D and 1D, would have to include a physiologically relevant mix of proteins, including DNA-binding ones, instead of sucrose (meant to simulate increase in viscosity) or PEG (to simulate high-molecular-weight crowding).

The simplified task of promoter search by preformed RNAP holoenzyme (which follows the setup of most *in vitro* transcription experiments) also falls short of approximating the cellular context of gene expression. It is instructive to recall the turnover of RNAP in *E. coli*, starting (as opposed to ending) with promoter engagement: shortly after promoter escape, the majority of σ^{70} rapidly dissociates from the core RNAP[20,21], which continues transcription often for tens of thousands of nucleotides and eventually dissociates from DNA during termination. This separation of σ^{70} from the RNAP core necessitates that they search for each other before they can re-form the holoenzyme capable of productive promoter engagement. Their respective diffusion behaviors would be affected by the differences in their DNA-binding affinities: σ^{70} is reported to have a specific affinity toward –10 promoter elements, whereas the core exhibits low nonspecific DNA affinity[22,23]. Adding the complexities of competition with alternate sigma subunits[24–26], the role of anti-sigma and sigma-recycling factors[27–29] and the unexplored kinetics of dissociation of elongation factors, which compete with sigma factors for binding to core RNAP[30,31], underscores a need for further exploration, both experimental and theoretical, before assuming that the promoter search by the holoenzyme is necessarily the rate-limiting step for promoter engagement in the cell[32]. In fact, instead of closing the case on the promoter-search mechanism in bacteria, the work by Wang *et al.*[3] provides the solid foundation for and highlights the importance of further investigations of this process.

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1. Saecker, R.M., Record, M.T. Jr. & Dehaseth, P.L. *J. Mol. Biol.* **412**, 754–771 (2011).
2. Tafvizi, A., Mirny, L.A. & van Oijen, A.M. *ChemPhysChem* **12**, 1481–1489 (2011).
3. Wang, F. *et al. Nat. Struct. Mol. Biol.* **20**, 174–181 (2013).
4. Borukhov, S. & Nudler, E. *Curr. Opin. Microbiol.* **6**, 93–100 (2003).
5. Shultzaberger, R.K., Chen, Z., Lewis, K.A. & Schneider, T.D. *Nucleic Acids Res.* **35**, 771–788 (2007).
6. Koslover, E.F., Diaz de la Rosa, M.A. & Spakowitz, A.J. *Biophys. J.* **101**, 856–865 (2011).
7. Sheinman, M., Benichou, O., Kafri, Y. & Voituriez, R. *Rep. Prog. Phys.* **75**, 026601 (2012).
8. Benichou, O., Loverdo, C., Moreau, M. & Voituriez, R. *Rev. Mod. Phys.* **83**, 81–129 (2011).
9. Normanno, D., Dahan, M. & Darzacq, X. *Biochim. Biophys. Acta* **1819**, 482–493 (2012).
10. Bauer, M. & Metzler, R. *Biophys. J.* **102**, 2321–2330 (2012).
11. Court, D.L., Oppenheim, A.B. & Adhya, S.L. *J. Bacteriol.* **189**, 298–304 (2007).
12. Harada, Y. *et al. Biophys. J.* **76**, 709–715 (1999).
13. Kabata, H. *et al. Science* **262**, 1561–1563 (1993).
14. Suzuki, Y., Shin, M., Yoshida, A., Yoshimura, S.H. & Takeyasu, K. *FEBS Lett.* **586**, 3187–3192 (2012).
15. Porschke, D. *Eur. Biophys. J.* **41**, 415–424 (2012).
16. Pelletier, J. *et al. Proc. Natl. Acad. Sci. USA* **109**, E2649–E2656 (2012).
17. Morelli, M.J., Allen, R.J. & Wolde, P.R. *Biophys. J.* **101**, 2882–2891 (2011).
18. Lee, B., Leduc, P.R. & Schwartz, R. *Sci. Rep.* **1**, 97 (2011).
19. Qin, S., Cai, L. & Zhou, H.X. *Phys. Biol.* **9**, 066008 (2012).
20. Bar-Nahum, G. & Nudler, E. *Cell* **106**, 443–451 (2001).
21. Reppas, N.B., Wade, J.T., Church, G.M. & Struhl, K. *Mol. Cell* **24**, 747–757 (2006).
22. Feklistov, A. & Darst, S.A. *Cell* **147**, 1257–1269 (2011).
23. Mekler, V., Pavlova, O. & Severinov, K. *J. Biol. Chem.* **286**, 270–279 (2011).
24. Maeda, H., Fujita, N. & Ishihama, A. *Nucleic Acids Res.* **28**, 3497–3503 (2000).
25. Wade, J.T. *et al. Nat. Struct. Mol. Biol.* **13**, 806–814 (2006).
26. Österberg, S., del Peso-Santos, T. & Shingler, V. *Annu. Rev. Microbiol.* **65**, 37–55 (2011).
27. Gaal, T., Mandel, M.J., Silhavy, T.J. & Gourse, R.L. *J. Bacteriol.* **188**, 7966–7970 (2006).
28. Piper, S.E., Mitchell, J.E., Lee, D.J. & Busby, S.J. *Mol. Biosyst.* **5**, 1943–1947 (2009).
29. Hofmann, N., Wurm, R. & Wagner, R. *PLoS ONE* **6**, e19235 (2011).
30. Sevostyanova, A., Svetlov, V., Vassylyev, D.G. & Artsimovitch, I. *Proc. Natl. Acad. Sci. USA* **105**, 865–870 (2008).
31. Yang, X. & Lewis, P.J. *Transcription* **1**, 66–69 (2010).
32. Ishihama, A. *FEMS Microbiol. Rev.* **34**, 628–645 (2010).