Fixing the model for transcription
The DNA moves, not the polymerase

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The traditional model for transcription sees active polymerases tracking along their templates. An alternative (controversial) model has active enzymes immobilized in “factories.” Recent evidence supports the idea that the DNA moves, not the polymerase, and points to alternative explanations of how regulatory motifs like enhancers and silencers work.

Although the vital processes of replication and transcription that occur within eukaryotic nuclei depend upon stochastic interactions between individual molecules, the relevant molecular machines and their templates are nonetheless highly ordered. For example, replication occurs in sub-nuclear hot-spots or “factories,” and we have also suggested that transcription does so too. We define such a “transcription factory” as a cluster of at least two RNAPs active on different templates (a typical nucleoplasmic factory in a HeLa cell contains ~8 enzymes engaged on ~8 templates). The raison d’être of all factories is the same: to enhance production by concentrating relevant machines, resources and expertise in one place. For example, HeLa nuclei contain a 1 μM pool of diffusing RNA polymerase II (RNAP II), but essentially all nascent RNA is made in nucleoplasmic factories where the concentration is 1,000-fold higher.

This heterodox idea is controversial (reviewed in ref. 8) as it presupposes acceptance of some principles not found in our textbooks: (1) factories represent critical architectural motifs to which RNAPs and transcription factors (TFs) tether chromatin in loops, (2) active RNAPs are transiently immobilized in factories and work by reeling in their templates as they extrude their transcripts, (3) individual complexes housed in one factory carry out most (if not all) processes involved in producing a mature transcript (including RNA synthesis, processing and proof-reading) and (4) different factories specialize in transcribing different sub-sets of genes. Here, we describe recent data supporting the idea that active polymerases are immobilized while they are active. Of course, movements are relative and the polymerase might be fixed to a factory, but both might be moving together through the nucleus.

Distinguishing Between Tracking and Fixed RNAPs

According to the traditional model, active RNAPs track like locomotives down their templates. As with so many received ideas, there seems to be little (if any) evidence supporting such tracking in vivo. In contrast, early experiments suggested that active polymerases were attached to the nuclear substructure, and so immobilized; most of a loop could be detached using nucleases without removing nascent RNA or transcribed templates. We also now know that fixed polymerases are powerful molecular motors able to reel in their templates in the required way, with many single-molecule analyses relying upon enzyme immobilization.

We recently showed (albeit indirectly) that active RNAPs are immobile. For the experiment, we needed two genes that could be switched on rapidly—one to act as a reference point, while the other had to
be long enough to provide sufficient spatial resolution. We stimulated human umbilical endothelial vein cells (HUVECs) by treating them with tumor necrosis factor α (TNFα); this cytokine signals through nuclear factor κB (NFκB) to activate and repress many genes and TNFAIP2 and SAMD4A—which both encode regulators of this cascade—are amongst the first to respond. The two lie -50 Mbp apart on the genetic map. TNFAIP2, a short 10 kbp gene, is turned on within ~10 min and is then transcribed repeatedly over the next few hours. SAMD4A is 221 kbp, and although the pioneering polymerase also initiates within ~10 min, it only terminates after another ~75 min (as the gene is so long). We sought to monitor by chromosome conformation capture (3C; reviewed in ref. 13) how close together different parts of the two genes were at different times after stimulation.

If the conventional model for transcription applies, we would not expect the short gene to lie near enough to the long gene to give a 3C product at any time after stimulation. If, for whatever reason, the two happened to lie together (for example, before stimulation), then they would soon separate as the pioneering polymerase tracked down the long gene. But if both responding genes were transcribed by polymerases that were transiently immobilized in the same “NFκB-factory” that specialized in transcribing TNFα-responding genes, the short gene, which would repeatedly attach to (and detach from) the factory as it initiates and terminates, should always lie close to just the part of the long gene being transcribed at that particular moment (Fig. 1). Then, we would not expect to see any 3C products before stimulation. But after 10 min (when both initiate), the short gene should lie next to the promoter of the long gene (but no other part). Then, as the polymerase replays the long gene, introns 1, 2, 3, etc. should successively be brought into the factory to lie transiently next to the short gene. And after 85 min, when the pioneering polymerase is about to terminate, the transcribed region now lies next to the short gene. As a result, the short gene lies next to just that segment of the long gene that is being transcribed at that particular moment. This is the result obtained, indicating that the DNA moves (and not the polymerase).

**Specialized Transcription Factories**

The results described above imply that these TNFα-responsive genes are being transcribed in dedicated “NFκB-factories”; indeed, they all have NFκB bound to their promoters. There is now excellent evidence for the specialization of factories in such a manner, and the nucleolus provides the prototypic example. Simply put, it is a “mega-factory” where RNAPI transcribes ribosomal DNA to produce the ribosomal RNA that is then assembled into ribosomes. Active RNAPII and III are also each concentrated in distinct nucleoplasmonic factories. Moreover, different RNAPII factories specialize in transcribing intron-less and intron-containing genes. In other examples, transcription units encoding factors involved in globin production (e.g., Hbb-b1, its locus control region or LCR, Eraf) are often co-transcribed in dedicated “EκLFA-factories”; and genes regulated by estrogen receptor α (ERα) appear to co-associate (presumably in “ERα-factories”).

**Polymerases Fixed in Factories: Some Implications**

The model illustrated in Figure 1 has various implications, not only on how we...
perceive polymerases work, but also on the way related processes are arranged and executed. For example, it has been difficult to explain how regulatory motifs like enhancers, silencers, barriers and insulators all work. But if transcription only occurs in factories, it becomes immediately obvious that tethering a promoter more or less closely to a factory will determine (to a significant extent) how often that promoter will be transcribed; promoters tethered close to a factory (e.g., those in the “hot” halo in Fig. 2) are much more likely than others lying further away to diffuse (randomly) and collide with RNAPs concentrated in the factory.

As a result, the position of a promoter in a loop relative to a factory is one critical determinant of initiation frequency. Then, we suggest that an enhancer acts by bringing its target promoter closer to the relevant factory containing the appropriate TFs—and it could do so if it first attached to a factory and was itself transcribed (Fig. 2). Conversely, transcription of a silencer element might tether its target promoter close to a factory containing the “wrong” kind of TFs. In both cases, the regulatory motifs are transcription units, and their activity depends upon them being transcribed (Fig. 2). Consistent with this, old studies showed that canonical enhancers/silencers were transcribed, and recent genome-wide ones confirm that most are and that they bear activation-related chromatin marks. We also now know that a large number of tightly-regulated genes have RNAPs on their promoters before they are “turned on”—and this ensures a prompt transcriptional response. For example, the promoter of the uPA gene is “poised” by attachment to a factory, looping the adjacent chromatin to organize the genome locally.

We now also know that transcripts initiate not only from classical promoters, but from many other points on one or other strand. Therefore, we imagine that the average rate of production of any transcript (whether sense or antisense, genic or non-genic) will depend on how closely the template is tethered to a factory. Of course, other factors like the underlying DNA sequence, histone modifications and chromatin compaction will play important roles. Note also that where overlapping sense and antisense transcripts are seen, they must be produced sequentially, as complementary bases in a template cannot be transcribed simultaneously by either tracking or fixed polymerases.

Finally, trans-splicing—a regulated process that leads to the formation of chimeric transcripts encoded by distant genomic regions—is another phenomenon that has been difficult to explain using the conventional model. Although rare in mammals, it is seen more frequently in the protein-coding transcripts of various metazoans. We expect the splicing machinery acting on two nascent transcripts in one factory to occasionally (mis-)splice one transcript to another and, as factories specialize, we would also predict that trans-splicing should mainly occur between transcripts generated from promoters that bind the same transcription factors.

Figure 2. A parsimonious model for transcriptional regulation. (i) Gene a is being transcribed by a polymerase in a factory (pink) and, as a result, genes b and c are tethered close to the factory. (ii) Intuition suggests (and computer simulations support; see ref. 7) the idea that genes tethered close to the pink factory are more likely to be transcribed (i.e., those in the “hot” halo around the pink factory) especially if bound by the “right” TFs (in this case red). (iii) Gene b has attached to the factory and is now being transcribed; this has brought c into the “hot” zone (which makes it more likely to be transcribed). In other words, b acts as an enhancer of c. Another factory (purple) is also shown. (iv) The structure is the same as in (iii) but we are at a different stage during development. Now, different transcription factors have bound to c (purple), enhancing its affinity for a different transcription factory (also purple). Even though c is in the “hot” zone around the pink factory, it remains unlikely to be transcribed there. In this case, b has silenced c by distancing it from its favored (purple) factory.

Outstanding Questions

Obviously, fixing active polymerases begs many questions. For example, how many factories are “dedicated” to transcribing TNFα-responsive genes, do other signaling pathways adopt similar strategies and act through analogous specialized factories, how many types of such specialized factories might there be, and how rapidly can one be converted into another? Fortunately, the techniques for answering these questions are now at hand.

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References