

SPECIAL ARTICLE

The Topology of Transcription by Immobilized Polymerases

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Current models for RNA synthesis involve an RNA polymerase that tracks along a static template. However, research on chromatin loops suggests that the template slides past a polymerase immobilized in a large transcription factory. The evidence for immobilized polymerases is reviewed, and a model for transcription by such fixed enzymes is presented. According to the model, gene activation would involve reducing gene-factory distance and increasing the affinity of a promoter for a factory. Locus controlling regions and enhancers would attach to a factory and increase the chances that a promoter could bind to a polymerase; after transcriptional termination, the gene would detach from the factory. As some RNA processing occurs cotranscriptionally, processing sites are also likely to be associated with the factory. © 1996

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TWO MODELS FOR TRANSCRIPTION

The current model for RNA synthesis involves a polymerizing complex that tracks along the template—the enzyme moves while the DNA remains stationary (Fig. 1, model 1). However, a considerable body of evidence supports an alternative in which (i) the template moves rather than the polymerase, and (ii) many active polymerases and transcription units are housed in large structures—or transcription “factories”—in the eukaryotic nucleus. Then RNA would be made as the template is reeled through a fixed polymerization site within a factory (Fig. 1, model 4; [10]). (The term “polymerase” is used here to describe the cluster of ~80 different polypeptides that form an active complex in which the polymerizing subunits are present only as minor components [e.g., 2, 21].) Evidence for this model will be summarized and models for transcription by immobilized enzymes presented.

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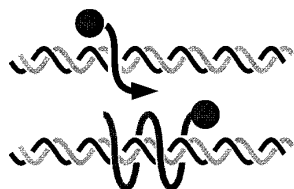
EVIDENCE FOR THE CONVENTIONAL MODEL

The polymerization site and template must move relative to each other, to allow each new template base to occupy the site in turn. Hitherto, it has been tacitly assumed that a (small) RNA polymerase tracks along the larger template (Fig. 1, model 1), but there is no direct evidence for such movement. [Note that any model requires a motor activity—either to move the template or to move the polymerase—and the energy required is derived from the hydrolysis of nucleotide triphosphates during elongation.] There seem to be only two kinds of evidence, both indirect, that suggest that polymerases might move.

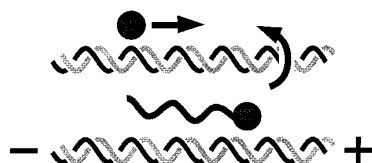
The first kind of evidence is provided by the beautiful images of “Miller” spreads [e.g., 19]. These photographs of “genes in action” are some of the most evocative in molecular biology. They are prepared by bursting bacteria or certain eukaryotic cells to spread their DNA. Then RNA polymerases can be seen frozen in the act of transcription, apparently tracking along the template, trailing the nascent transcripts. But these images are static ones and tell us nothing about movement. They are also highly selected; most transcripts cannot be distinguished from the tangled mass of DNA in the middle of the spread, and we are shown only the photogenic ones at the edge. Moreover, it is quite possible that polymerases were torn away from larger structures during the violent spreading procedure.

The second kind of evidence is even more indirect. It is based upon the following argument; modern biochemistry has proved very successful at dissecting the details of transcription, and as this success is based upon the conventional model, then—the argument runs—that model must be correct. This can be put another way: if the small, soluble, polymerases purified by biochemists work *in vitro*, there is little need to postulate the existence of larger, immobilizing, structures *in vivo*. Nevertheless, this argument is specious; whether polymerases work tells us nothing about whether or not they move. Moreover, we now know that we have misjudged the size of polymerases; they are contained in enormous structures that dwarf the tem-

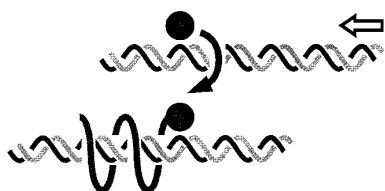
1. Pol translocates and rotates



2. Pol translocates, DNA rotates



3. DNA translocates, pol rotates



4. DNA translocates and rotates

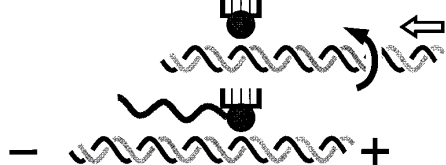


FIG. 1. Models for transcriptional elongation involving mobile or static polymerases (circles) and double-helical templates. The top figure in each model shows initial relative positions; subsequent movements are shown by arrows. Bottom figures show final positions after generation of transcripts (wavy lines attached to polymerases). + and - indicate the formation of domains of positive and negative supercoiling. In model 4, the hatched area immobilizes the polymerase. (Redrawn from Cook and Gove [5].)

plate. And although the small soluble isolates do work, they do so poorly.

TOPOLOGICAL CONSIDERATIONS

During transcription, the polymerase and DNA move relative to each other, both rotationally around the helix axis and laterally along it. As the two players (i.e., polymerase and DNA) can each perform two movements, there are four formal possibilities (Fig. 1).

In the first, the polymerase moves laterally along the static template and rotationally about its axis (i.e., the polymerase is fully mobile). But as each helical turn is transcribed, the polymerase, plus nascent transcript,

must rotate around the template. This results in the entwining of the transcript about the template, once for every 10 base pairs transcribed. In a eukaryotic transcription unit that is 10^6 bp long, this means that the transcript is entwined 10^5 times, and some mechanism must be found to untwine the tangle to allow the transcript to escape to the cytoplasm. The mechanism for untwining such a tangle must be precise; untwining once too few—or once too many—times would still leave an entangled transcript. As this problem seems insuperable, this—the conventional model—seems unlikely. Model 3—which also involves a rotating polymerase—faces the same intractable problem.

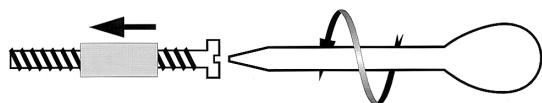
The untwining problem can be sidestepped if the DNA rotates instead of the polymerase. In model 2, the enzyme translocates laterally but its rotation is restricted, perhaps by the frictional drag of the transcript; instead DNA rotates. This modification of the conventional model is also known as the “twin-supercoiled-domain” model [17]. But how is the polymerase prevented from rotating as it translocates? Even one accidental rotation—which is especially likely when the transcript is short and frictional drag is limited—would yield an entwined transcript. Imagining any mechanism that might prevent such accidental rotation without immobilizing the polymerase is difficult.

In model 4, the untwining problem is eliminated because the enzyme is static, instead DNA both translocates and rotates. Domains of supercoiling are also generated by this model on each side of the fixed polymerase and must be removed [10].

Examination of the topological principles involved therefore suggests that the polymerase must be immobilized. However, rotation of a looped or circular template (as in models 2 and 4) introduces a new problem; supercoils are generated on each side of the polymerase, and the torsional strain associated with these supercoils will soon limit transcription unless removed by topoisomerases [10]. The template movements of model 4 are analogous to those of a bolt when it is driven through a fixed nut using a screwdriver (Fig. 2). As the template moves through, it rotates so that the transcribed base on the template strand maintains the same topological relationship to the fixed polymerization site. The nut “sees” the whole length of the thread as it passes through; the fixed active site sees the transcribed strand in the same way. As a right-handed twist on the screwdriver drives the bolt with a right-handed thread through the nut, a right-handed twist accompanies DNA translocation through the polymerase.

Continuously screwdriving with a conventional driver is impossible; if we maintain our grip on the driver, we can rotate it by only half a turn before our forearm becomes too strained to twist further (Fig. 2). Our rotating template becomes similarly supercoiled in a right-handed (i.e., +ve) sense, and this strain energy would soon stop transcription. However, we can drive

Remove supercoils in arm by loosening grip
and rotating wrist (or use a ratchet screwdriver)



Remove supercoils
in DNA
with topoisomerase

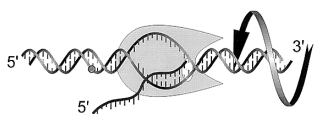


FIG. 2. DNA movements at the polymerization site are analogous to those of a screw through a fixed nut. (Top) The bolt (DNA) rotates as it is driven by the screwdriver through the fixed nut (polymerase). (Bottom) The double helix moves to the left and rotates (arrow) so the transcribed base in the polymerizing site (white oval) always retains the same stereochemical relationship to the page (i.e., the factory); this rotation induces compensatory supercoils to accumulate as RNA is extruded. These supercoils must be removed by topoisomerases. (Redrawn from Cook [4].)

the screw through successive half turns by relaxing our grip, rotating our wrist backward, regripping the driver tightly, and repeating the right-hand turn. A topoisomerase that breaks and rejoins template strands as they pass through would similarly allow continuous advancement.

Continuous driving is possible if we use a ratchet (e.g., a Yankee) screwdriver; now simply pushing the driver to the left spins the bolt continuously through the nut. As the thread advances turn by turn, the ratchet in the screwdriver automatically spins in step, and no strain energy accumulates. One ratchet/topoisomerase is required on the right side of the nut/polymerase. For similar reasons another is required to remove the left-handed (–ve) supercoils that arise to the left of the nut/polymerase (i.e., on the “downstream” side). Therefore, a helical template can advance smoothly—without accumulating strain energy or supercoils—by passing successively through a topoisomerase, a fixed polymerizing site, and another topoisomerase. This arrangement ensures that the newly made transcript does not become entangled with the template and that template rotations are confined to the small region between the two topoisomerases.

Such an arrangement is likely to be modified to allow the transcription of highly active genes like the ribosomal cistrons transcribed by many polymerases. The analogy then becomes one of a single bolt being driven through a series of fixed nuts; no topological problems arise within the bolt, only at its ends, so topoisomerases would only be needed there. According to this model, domains of supercoiling are not normally associated with ongoing transcription, but inhibition of topoisomerase action will lead to their appearance. All these movements are, of course, relative; although a template

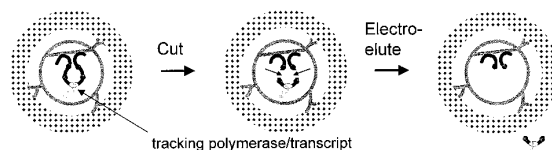
may move relative to a polymerase, both could move together relative to an external viewpoint.

POLYMERASES ARE ATTACHED TO AN UNDERLYING NUCLEOSKELETON

Models involving tracking RNA polymerases can be distinguished from those involving immobile enzymes using the approach illustrated in Fig. 3 [11]. Human cells are encapsulated in agarose microbeads to protect them during subsequent manipulations and suspended in a buffer containing a physiological concentration of salts, and then both cell and nuclear membranes are permeabilized with a detergent. When a restriction endonuclease is now added, it can diffuse into the nucleus to cut the chromatin loops into fragments containing ~10 kb of DNA. Any detached chromatin fragments are now removed from the beads by electrophoresis. If polymerases tracked around the loops, they should electroelute from the bead with the detached fragments. But if polymerases are attached to an underlying structure, they should remain in the agarose bead after elution. It was found that essentially all RNA polymerizing activity (measured by incorporation of [³²P]UTP into RNA) resisted elution, despite removal of >75% of the chromatin. As large chromatin fragments containing ~150 kb DNA can escape from the beads, the polymerizing activity must be too large to elute, probably because it is attached to the nucleoskeleton seen in this material [8, 12].

Nascent RNA—whether labeled by short incuba-

A. Mobile polymerase



B. Attached (immobile) polymerase

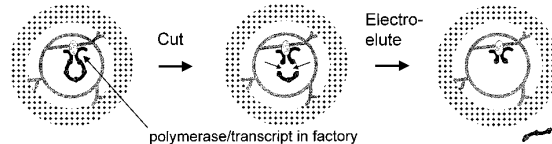


FIG. 3. Are polymerases free to track along the chromatin fiber? (A) HeLa cells are encapsulated in agarose (diamonds) to protect them and lysed. The cell interior—including the cytoskeleton, nuclear lamina (shaded circle), and a polymerase (plus transcript) tracking around a chromatin loop covered with nucleosomes—is now accessible to molecular probes. After cutting chromatin into ~10 kbp pieces with restriction enzymes (arrows), most chromatin and associated polymerases should electroelute from the agarose. (B) Active polymerases and associated transcripts are housed in a factory (oval) attached to an internal nucleoskeleton. Despite cutting and electroelution to remove 75% chromatin, all polymerizing activity and nascent RNA remains in the agarose.

tions with [^3H]uridine (*in vivo*) or [^{32}P]UTP (*in vitro*)—also resisted elution [11]. As expected, this nascent RNA could be degraded with RNase, but then the polymerizing activity still resisted elution. This means that the polymerase could not be attached through nascent RNA to the underlying structure. It was important to show in this experiment that the polymerizing activity studied was the major nuclear activity, and not some minor—residual—activity. Unfortunately, the overall rate of transcription *in vivo* is unknown, so it is impossible to calculate exactly how much activity survives lysis and elution. However, it is known that all the DNA polymerizing activity found *in vivo* is retained on lysis, and that the lysed cells can make $\sim 7\times$ more RNA than DNA *in vitro* [13]. Therefore, it seems likely that the major polymerizing activity was being studied. Moreover, immobilized polymerases have been shown to work efficiently *in vitro* [5, 22], with enough power to reel in templates that have the length of the chromatin loops found *in vivo* [27].

ACTIVE POLYMERASES ARE CONCENTRATED IN TRANSCRIPTION FACTORIES

Seeing is believing. Therefore, perhaps the most convincing evidence that polymerases are not free to track throughout chromatin is the demonstration that nascent transcripts are concentrated within discrete nuclear structures—transcription factories [15, 26].

In principle, visualizing the sites containing newly made RNA should be easy—for example, using autoradiography after incubation with [^3H]uridine—but it proves difficult to do so in practice. One problem stems from the rapid rate of elongation (i.e., ~ 1400 nucleotides/min) *in vivo*, coupled to the length of fully extended transcripts. Thus, a nucleotide in a still-growing transcript could move ~ 500 nm away from the site of polymerization in a minute! A second problem is associated with the rapidity with which completed transcripts then move away from the synthetic site, to accumulate at later bottlenecks in the processing pathway. Both problems can be overcome using shorter labeling periods, but then so little label is incorporated that detection becomes difficult. This problem is magnified because [^3H]uridine must first be transported through membranes, converted into immediate precursors, and equilibrated with internal pools before it can be incorporated into nascent RNA. Problems associated with internal pools can be overcome by permeabilizing the cells and then washing away the pools, but then the unphysiological conditions used could artifactually precipitate nascent transcripts onto underlying structures. Still another problem is associated with localizing precisely the incorporated labels; after autoradiography, silver grains may be hundreds of nanometers away from any incorporated ^3H . Therefore, imaging the synthetic sites has only been possible using new, sensi-

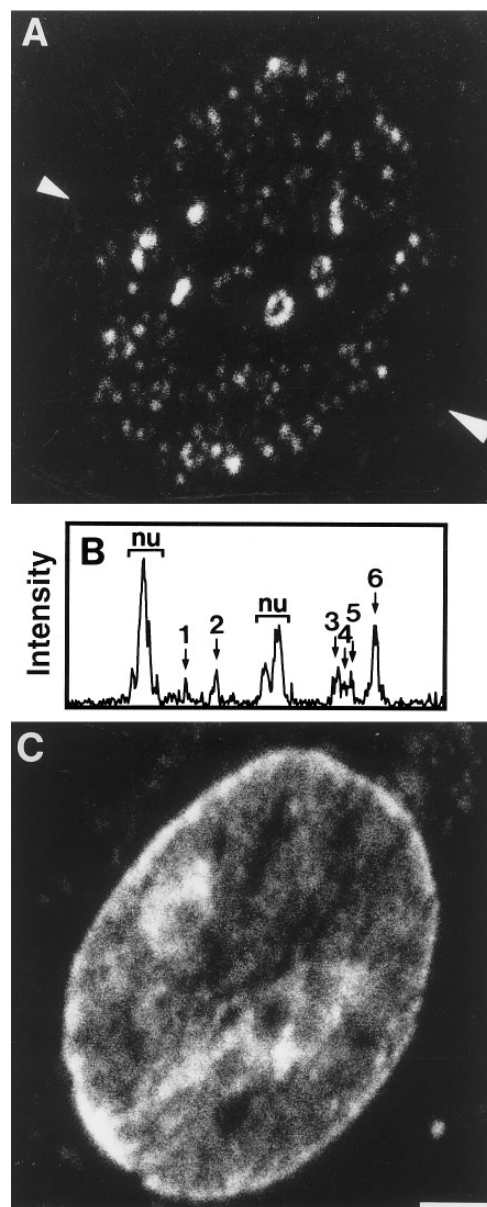


FIG. 4. Transcription factories imaged by light microscopy. Permeabilized cells were incubated with Br-UTP for 15 min, and cryosections (100 nm thick) were prepared and Br-RNA immunolabeled with fluorescein (nucleic acids were counterstained with TOTO-3). Green and far-red images of one section were collected sequentially and are shown without further background subtraction or digital processing. (A) Transcription sites (green channel). Three individuals counted (blind) 126, 146, and 148 extranucleolar foci in this image. Nucleolar foci are often crescent shaped (as here); mitochondrial foci in the cytoplasm are also occasionally seen. (B) The fluorescence intensity (scale 0–255) along a line—from small to large arrowhead in (A)—passing through six extranucleolar foci; all appear as discrete peaks above the background. nu, nucleolar foci. (C) Nucleic acid (far-red channel). Bar, 2.5 μm . (Reproduced, with permission of the Company of Biologists, from Iborra *et al.* [9].)

tive, immunodetection methods and short incubation periods.

A typical procedure is as follows [9]. Cells are permeabilized, internal NTP pools washed away, incubated

briefly with Br-UTP or biotin-CTP and the other NTPs to allow nascent RNA chains to be extended by only a few nucleotides, before sites containing the incorporated analogue are indirectly immunolabeled. Figure 4 illustrates the sites containing Br-RNA in a human cell. The nascent transcripts are not diffusely spread but concentrated in discrete sites or "foci." The foci within nucleoli are often larger, and more crescent-shaped, than the extranucleolar foci (Fig. 4). Because of the poor resolution of the light microscope, both types of sites appear larger than they really are. Such images clearly show that polymerases are not free to track throughout the nucleus.

The best-characterized transcription factories are contained within nucleoli, where RNA polymerase I makes rRNA [7]. They seem to have three zones: an area where inactive polymerases are stored (the fibrillar center), another area containing growing transcripts (the dense fibrillar component), and a processing center (the granular component) where the transcripts and associated proteins are converted into mature ribosomal subunits. Although extranucleolar factories are smaller and not so well characterized, they seem to be built according to the same general principles [9]. For Figure 5, the cell was permeabilized, nascent RNA chains were elongated by ~ 34 , ~ 170 , or ~ 370 nucleotides in biotin-CTP, and the incorporated biotin was indirectly immunolabeled with gold particles. The particles are clustered, marking a region (average diameter ~ 70 nm) equivalent to the dense fibrillar component. Some of these particles truly label nascent RNA at synthetic sites as the biotin-RNA is

sensitive to RNase H and so still H-bonded to the template. These particles overlap a region rich in RNA polymerase II (equivalent to the fibrillar center). These factories often lie around the interchromatin granule clusters (ICGCs) that contain many proteins involved in splicing. They are also attached to the underlying nucleoskeleton, as can be shown using the approach illustrated in Fig. 3 [9, 15].

The diameter of these clusters of gold particles marking nascent RNA remains constant whether nascent chains are extended by 34 or 2000 nucleotides. This is difficult to reconcile with a model for transcription involving a tracking polymerase; then the volume occupied by transcripts should increase roughly in proportion to the length of template transcribed. However, it is consistent with the extrusion of ~ 20 transcripts into a zone that occupies a constant volume in the factory. Standard stereological techniques allow the total number of factories in the three-dimensions of a nucleus to be calculated from the numbers and areas seen in two dimensions. It turns out that there are ~ 2100 in a typical HeLa nucleus. As each nucleus probably contains $\sim 40,000$ active RNA polymerases, each factory must contain ~ 20 active polymerases, each associated with one transcription unit.

The labeling experiments described above involved permeabilized cells, raising the possibility that diffusely spread transcripts had aggregated into clusters on lysis. Eliminating this without imaging transcripts (or active factories) in living cells is impossible. However, an important control experiment makes it unlikely. The polymerase in the factories can be immuno-

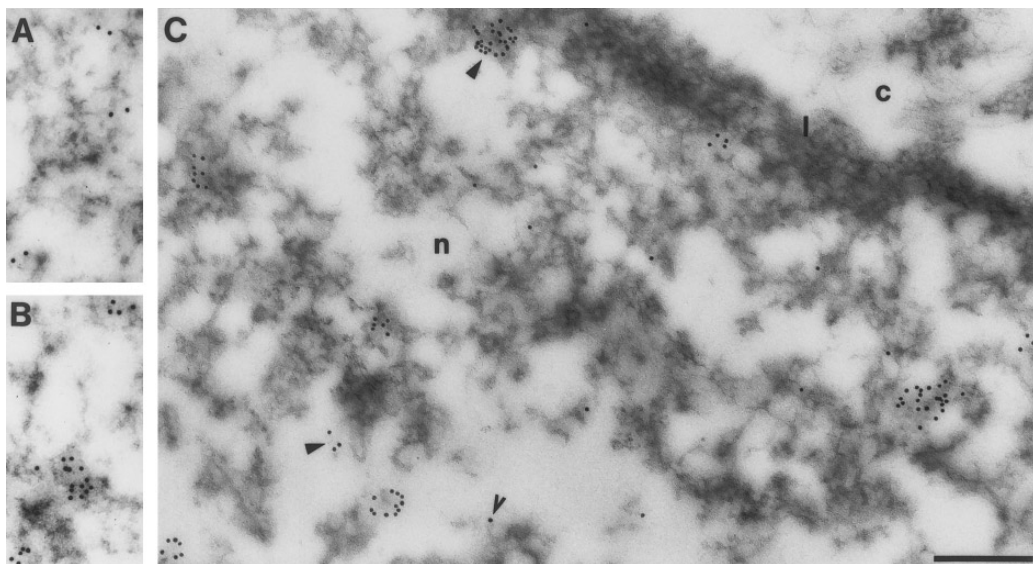


FIG. 5. Transcription factories imaged by electron microscopy. Nascent RNA was elongated in biotin-CTP for (A) 1, (B) 5, and (C) 15 min; after sections were cut, incorporated biotin on the surface was immunolabeled with 9-nm gold particles. There are 3, 3, and 9 clusters of gold particles in A, B, and C, respectively. c, cytoplasm. l, lamina. n, nucleus. Solid arrowheads, typical clusters. Open arrowhead, a lone particle. Bar, 250 nm. (Reproduced, with permission of the Company of Biologists, from Iborra *et al.* [9].)

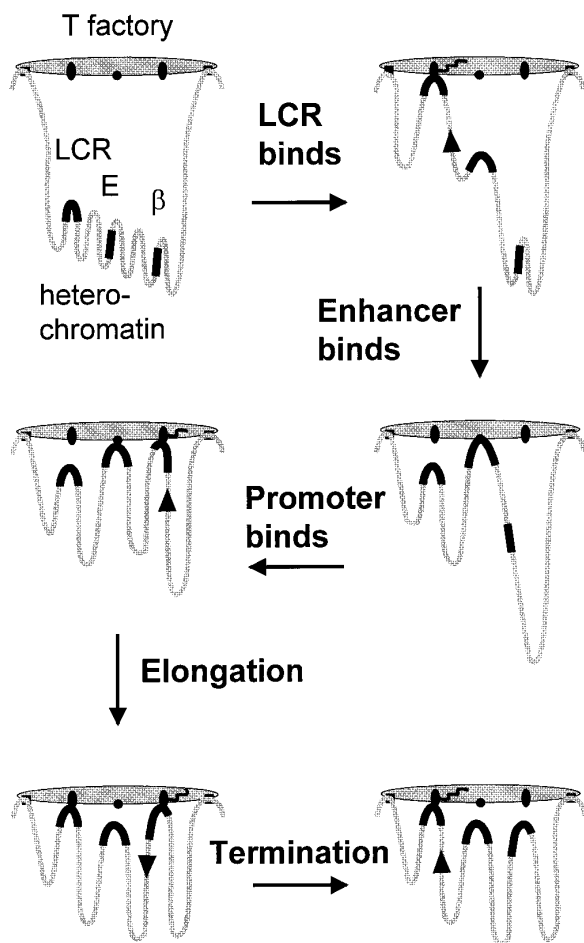


FIG. 6. How a β -globin gene might become active. Large oval, transcription (T) factory. Small ovals, polymerases. Wavy line, transcript. See text for details.

labeled in unpermeabilized cells; if transcripts were aggregating, then this polymerase would be expected to do so too. However, the number and distribution of sites containing the polymerase are the same in permeabilized and unpermeabilized cells.

MODELS FOR GENE ACTIVATION AND TRANSCRIPT PROCESSING

If active polymerases are concentrated in a few transcription factories, a gene out in a loop is obviously inactive; it can only become active by attachment to a factory. Gene activation would then involve increasing the chances that a promoter could attach by (i) "opening" chromatin to make the factory more accessible, and (ii) decreasing promoter-factory distance by decreasing the length of the tether that attaches the promoter to the factory.

Consider an inactive β -globin gene that is initially contained in a long loop (Fig. 6). The gene is static, so it is condensed as heterochromatin on to the lamina.

As the concentration of key activating protein(s) rises during hemopoiesis, the activators would bind to the locus controlling region [3], enhancing its chances of competing successfully for a polymerase in the factory. (Note that many LCRs are known to be transcription units [e.g., 3].) Once the LCR has initiated, the loop is reeled through the polymerase; this opens up the loop and pulls the enhancer (E) closer to the factory, increasing its chances of binding. After it has bound, the β -

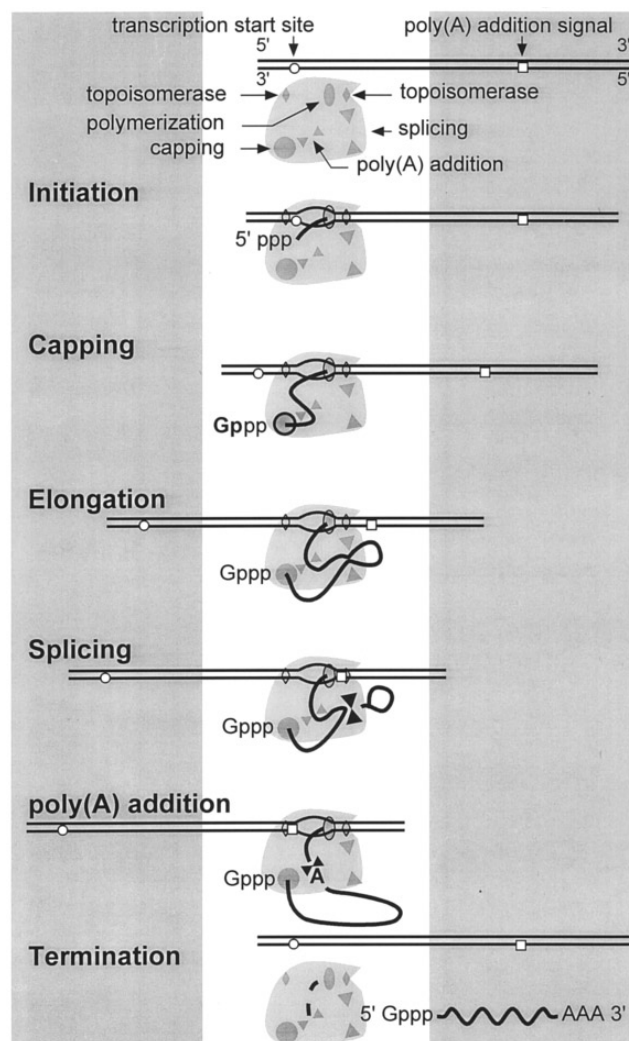


FIG. 7. A model for cotranscriptional RNA processing. Six sites (one each for polymerization, capping, splicing, poly(A) addition, plus two for topoisomerase action) are shown. The template binds to the polymerization site and then slides through it to initiate; flanking topoisomerases remove strain energy during this step and subsequent steps. The 5' end of the transcript is immediately captured by a capping site, and a cap (Gp) is added. As elongation continues, the nascent RNA remains attached at both ends as it loops through splicing and poly(A) addition sites. As splicing signals in the transcript pass through the splicing site, splicing occurs with release of a lariat; passage of poly(A) signals through the poly(A) site also stimulates transcript cleavage and poly(A) addition (indicated by A). At termination the template is released, the processed transcript moves away, and the extreme 3' end of the transcript is degraded.

globin promoter is closely tethered to the factory, so its chances of competing successfully for a polymerase are now high. The β -globin gene is then transcribed. At termination the gene detaches; however, as its promoter remains close to the factory, it has a high probability of reattaching. According to this model, LCRs, enhancers, and genes are continually attaching and detaching, and gene activation involves changing probabilities of attachment.

A model of cotranscriptional RNA processing is illustrated in Fig. 7. This model is consistent with (i) both ends of nascent RNA remaining attached during elongation [11], (ii) topoisomerase and polymerizing activities lying close together [24], (iii) activities involved in initiation and termination being contained in one protein and so also lying together (e.g., the La antigen is both an initiating and terminating factor for polymerase III [18], the vaccinia capping enzyme is a termination factor [6], and its cap-specific methyltransferase stimulates poly(A) polymerase activity [23], and (iv) some splicing occurring cotranscriptionally [1, 16, 20, 25].

CONCLUSION

We have argued that transcription occurs as the template moves through the fixed polymerization site, rather than vice versa, and that many active polymerases and transcription units are immobilized in factories that have diameters of ~ 70 nm. The transcription of a gene must then involve three basic steps: (i) the template first attaches to a polymerizing site in a factory, (ii) it then slides through the site as the transcript is extruded into the factory, and (iii) finally the template dissociates from the polymerization site, and the transcript is transported to other parts of the factory for further processing, before translocation to the cytoplasm.

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