The Organization of Replication and Transcription

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Models for replication and transcription often display polymerases that track like locomotives along their DNA templates. However, recent evidence supports an alternative model in which DNA and RNA polymerases are immobilized by attachment to larger structures, where they reel in their templates and extrude newly made nucleic acids. These polymerases do not act independently; they are concentrated in discrete "factories," where they work together on many different templates. Evidence for models involving tracking and immobile polymerases is reviewed.

The idea that polymerases track like locomotives along their templates pervades our thinking; textbook models for replication and transcription (*1*) show a polymerase complex binding to an origin or promoter in DNA, before moving off as it makes a nascent chain (Fig. 1, A and B). This idea stems not from any experimental evidence, but from a perception of relative size; the smallest object—the polymerase—should move. However, it is now known that polymerizing machines can be enormous, dwarfing the template (*2*–*4*). I will review the evidence for tracking and discuss the alternative model in which fixed polymerases reel in their templates as they extrude newly made nucleic acids (Fig. 1, C and D).

Several interrelated factors make the analysis of polymerase action difficult. Most polymerase complexes are inactive; for example, a rapidly dividing bacterium contains ~40 DNA polymerases but only two to six replication forks (*2*). The inactive population is easily extracted, so biochemists have concentrated on it (*2*–*4*). However, the active fraction is tightly bound to DNA and to the substructure (*5*); attempts to release it invariably break the template, and the broken DNA strands usually become entangled in an intractable gel. As a result, polymerases are often assayed in unphysiological buffers to minimize the formation of such gels, but this can result in artifacts. I begin by discussing DNA polymerases (as the evidence that they are immobilized is convincing), but because other polymerases have extensive structural homology, they probably work similarly (*6*).

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Immobile DNA Polymerases

In 1963, Jacob *et al.* (*7*) suggested that DNA polymerases might be attached to the bacterial membrane to facilitate control over the initiation of replication and the distribution of duplicated templates to daughter cells. They imagined that the specific growth of membrane between two attached progeny chromosomes would ensure that the chromosomes segregated correctly to daughter cells. We now know that a bacterial counterpart of the eukaryotic spindle probably segregates the chromosomes (*8*), but this suggestion prompted biochemists to see if polymerases and nascent DNA were associated with a cellular substructure. Cells were exposed briefly to a labeled DNA precursor, broken open, and treated with a nuclease to detach most DNA from the substructure; labeled (newly made) DNA remained bound to the substructure—the cell wall and membrane in bacteria (*9*) or nuclear remnants like "matrices" or "nucleoid cages" in eukaryotes (*10*).

Despite these results, few accepted the conclusion that DNA polymerases were attached to a substructure, primarily for two reasons. First, nascent DNA could have stuck artifactually during isolation in the unphysiological buffers used to minimize the formation of intractable gels. For example, nuclei were prepared in hypotonic buffers containing high concentrations of magnesium ions to suppress aggregation, before matrices were isolated by an additional treatment with 2 M NaCl. However, these are the conditions that might aggre-

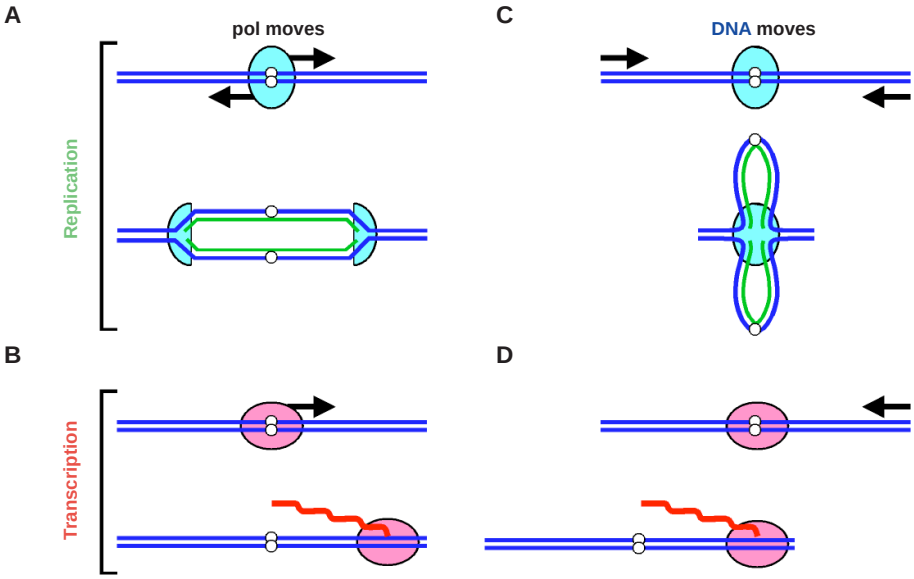

Fig. 1. Models for replication and transcription by (**A** and **B**) tracking and (**C** and **D**) immobile polymerases (ovals). Small circles mark origins or promoters and arrows show movement of the polymerase (pol) or template. Blue lines denote parental strands, green lines denote daughter strands, and red lines denote transcripts. (A) A tetramer containing four DNA polymerases splits, and the two halves (each with a polymerase on a leading and lagging strand) move apart. (B) An RNA polymerase tracks along the template as it makes a transcript. (C) A fixed complex contains four DNA polymerases. Daughter strands are extruded in loops as the parental duplex slides in from the sides through the fixed sites. The origin is shown here and in Fig. 2D as detaching from the complex after initiation, but it may remain attached throughout. (D) The template slides past the fixed polymerase as a transcript is extruded.

gate nascent nucleic acids (*11*). The second reason seemed more compelling: Replication reactions can be reconstructed in vitro from pure ingredients, without adding any known immobilizing components. However, this evidence is compromised by the following experiment (*12*). During replication, a helicase unwinds the duplex to provide single strands for a polymerase. When this reaction was performed with a pure helicase (T antigen of simian virus 40), template, and adenosine 5′-triphosphate, it was anticipated that the dodecameric helicase would bind to an origin and split into two hexamers that would track away from each other as they unwound the duplexes (as in Fig. 1A, but without the DNA synthesis). However, intact dodecamers associated with two single-stranded loops were seen in the electron microscope (as in Fig. 1C, but without the DNA synthesis). Like other helicases (*13*), each half of the protein complex remained attached to (and so immobilized by) its partner as it pumped in duplex DNA from each side and extruded two single-stranded loops. Because the helicase dictates the geometry of the two replication forks, the four polymerases acting there must adopt the same geometry (and immobility).

Other evidence suggests that DNA polymerases are immobilized. Adjacent origins of replication in a mammalian chromosome often fire simultaneously (*14, 15*). After growth in [^{3}H]thymidine, protein removal, naked DNA spreading, and DNA-fiber autoradiography (or immunolabeling), stretches of newly made DNA are seen scattered along a duplex. These stretches initiate and elongate synchronously, presumably through the coordinate action of adjacent polymerases. In addition, nascent DNA resists detachment, even when physiological conditions are used during cell lysis and analysis (*16*). The formation of intractable gels was avoided by encapsulating cells in agarose to protect the fragile template, allow entry of nucleases, and the electrophoretic removal of long chromatin fragments containing up to 150 kb of DNA. If polymerases tracked along the template, most polymerizing activity and newly made DNA should be removed with the electroeluted chromatin; however, most remained, suggesting that the newly made DNA was held by polymerases attached to the substructure.

Many DNA Polymerases Are Immobilized in Replication Factories

Seeing is believing, and the direct visualization of replication sites provides convincing evidence that DNA polymerases are immobilized. In one experiment, rat fibroblasts in S phase (the synthesis phase of the cell cycle when DNA is replicated) were incubated with bromodeoxyuridine (BrdU), and sites of incorporation were visualized with fluorescently labeled antibodies directed against the analog; these sites were not diffusely spread throughout nuclei but concentrated in ~150 foci (*17*). If polymerases track, something must corral them into a very small region. Similar foci (Fig. 2A) have now been seen using a wide range of cells and precursors. Early during S phase, foci are small and discrete, but later, when heterochromatin is replicated, they become larger and less numerous (*18, 19*). Double immunolabeling shows that these foci contain the necessary replication factors like DNA polymerase α, proliferating cell nuclear antigen, cyclin A, cdk2, and RPA70 (*19*). The foci are not fixation artifacts because they are seen after the incorporation of fluorescein–deoxyuridine triphosphate (dUTP) by permeabilized (but unfixed) cells or of Cy5-dUTP by living cells (*20*). Moreover, they remain when most chromatin is removed (*18, 21*), implying that newly made DNA is attached to an underlying substructure. Finally, electron microscopy of chromatin-depleted nuclei shows that newly made DNA is initially associated with electron-dense bodies (diameters of 100 to 1000 nm) strung along a "nucleoskeleton"; with time, this DNA is extruded from these structures into adjacent regions (*21*).

Many forks must be active in each focus. Indeed, calculations based on the number of foci, rate of fork progression, spacing between forks, size of the genome, and length of S phase show that ~40 forks must be active in each early S phase focus in a human cell. This resulted in the notion that each focus was a "factory" containing many polymerizing machines working on different templates (Fig. 2, B through E) (*21*). These factories are probably the in situ counterparts of isolated "megacomplexes" that contain many polymerases (*22*). When DNA is stripped from the factory and spread to give linear fibers, newly made DNA can be revealed as fluorescent tracts strung along a fiber (Fig. 2, F and G).

Recently, polymerases in living *Bacillus subtilis* were visualized with a construct in which the catalytic subunit of the enzyme, PolC, was fused with the green fluorescent protein (*23*). If the two forks moved independently (as in Fig. 1A), two fluorescent spots should be seen in the area occupied by DNA. However, one discrete spot was generally seen in the middle of the cell. As it was again unlikely that the method was sensitive enough to detect only four polymerases (one on each arm of the two forks),

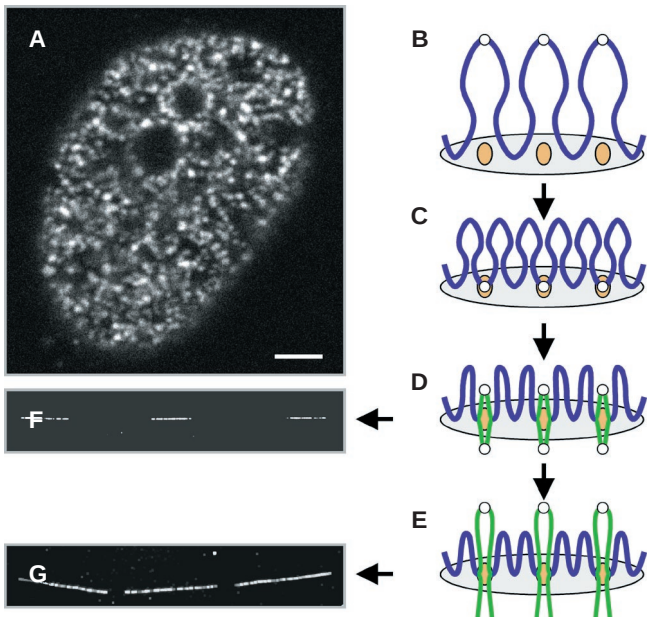

Fig. 2. Visualizing newly made DNA in HeLa cells. (**A**) Replication foci. A cell in mid–S phase was grown for 5 min in 150 μM BrdU and fixed; then, Br-DNA was indirectly immunolabeled with a fluorochrome (Cy3), and a fluorescent image of the center of the cell was collected with a confocal microscope. Newly made DNA appears as discrete white foci in the nucleus (black "holes" are nucleoli). Scale bar, 2.5 μm. Image provided by A. Pombo. (**B** through **E**) Model showing the organization of the DNA duplex (shown as a single blue line) in a replication focus. Origins (small circles) in three chromatin loops attach to polymerizing sites (small ovals) in the factory (large oval). Replication occurs as daughter duplexes (single green lines), containing one parental strand and one newly made strand, are extruded in loops as the parental duplex slides through the fixed sites; during this process, parental loops shrink, and daughter loops grow. Stripping the looped DNA from the factory and spreading it as a linear fiber produces a structure like that seen in (**F**) or (**G**), where unreplicated regions are invisible. (F and G) Newly made DNA in individual DNA fibers. Cells were grown for 15 or 30 min in BrdU and DNA fibers were spread; Br-DNA was indirectly immunolabeled with Cy3 and photographed in a conventional fluorescence microscope (*15*). Each panel contains three regions of newly replicated DNA strung along one fiber of ~125 μm (~375 kbp). The three regions probably initiated together [as in (C) and (D)], as they have equal lengths. Images supplied by D. A. Jackson.

the four active polymerases and many of the ~40 inactive ones were probably concentrated in one factory.

Immobilized RNA Polymerases

The evidence described above has changed the way we think about DNA polymerases. A similar change may soon occur with transcription, as the evidence is so similar. What is the evidence for the traditional model (Fig. 1B)? Once again, biochemists have successfully reconstructed transcription reactions in vitro with pure components, without knowingly adding immobilizing components, but this evidence may also be compromised. Lengthy preincubations in high protein concentrations are usually required, resulting in the assembly of large complexes; some of these are large enough to pellet during a 5-min spin in a microcentrifuge (*24*). Therefore, RNA polymerases may become immobilized during these in vitro reactions.

The beautiful photographs of "Miller" spreads (*25*) are often cited as proof that polymerases track. These spreads are prepared by dropping nuclei in a solution that is little more than distilled water; the nuclei burst, revealing extended transcription complexes that look like Christmas trees. RNA polymerases appear frozen in the act of transcription, trailing their transcripts behind them. But, these images are static ones, and polymerases may have been torn away from larger structures. Moreover, the analogous experiment in which DNA is spread after extraction in a hypertonic (rather than a hypotonic) solution reveals all nascent RNA to be associated with the substructure (the nucleoid cage), implying that it is made there (*26*). There seem to be no rational grounds for selecting one set of conclusions over the other.

Evidence for the immobilization of RNA polymerases is circumstantial, but taken together, this evidence becomes stronger. First, nascent transcripts and their templates are bound to the substructure; when HeLa cells were grown for 2.5 min in [^{3}H]uridine and lysed in 2 M NaCl, we might expect tracking polymerases and their transcripts to be stripped from templates, but no [^{3}H]RNA is lost (*26*). Moreover, treatment with Eco RI detached most DNA from the substructure, leaving transcribed sequences and [^{3}H]RNA. These experiments were also criticized because nascent transcripts might have precipitated in the hypertonic conditions, but the same result was obtained with a "physiological" buffer and cells that were encapsulated in agarose: Engaged polymerases, their transcripts, and transcribed regions all resisted nucleolytic detachment (*27, 28*).

Two topological problems occur during transcription of helical templates (*29*). One problem, the generation of torsional stress, has been widely discussed and is solved by topoisomerase action (*30*). The second problem arises when a polymerase tracks along a helical template: The transcript becomes entwined about the template, once for every 10 base pairs (bp) transcribed (Fig. 3A). In a eukaryotic transcription unit of 10^6 bp, the transcript would become entwined 10^5 times. The untwining mechanism must work perfectly, because leaving even one entwinement would prevent escape to the cytoplasm. Fortunately, this untwining problem does not occur if the polymerase is fixed, and DNA rotates instead (Fig. 3D).

Discrete transcription sites can be visualized after allowing mammalian cells to extend nascent transcripts in bromo–uridine 5′-triphosphate (Br-UTP) and immunolabeling the resulting Br-RNA; it is not diffusely spread throughout euchromatin but is concentrated in discrete "foci" (Fig. 4A) (*28, 31–33*). Actinomycin D inhibits incorporation into nucleolar foci (so they result from transcription by RNA polymerase I), whereas α-amanitin (2 and 250 μg/ml) prevent labeling of different types of nucleoplasmic foci (so they result from polymerases II and III, respectively) (*33*). Antibody-blocking experiments indicate that polymerase II sites are distinct from polymerase III sites (*33*), but both kinds of site have roughly the same size (diameters 40 to 80 nm) (*32, 33*).

We now come to a crucial question: Does each focus represent one transcription unit, or are many different units packed into one focus to form a transcription factory? This is difficult to answer for nucleoplasmic foci because they are so small and numerous. However, nucleolar foci provide

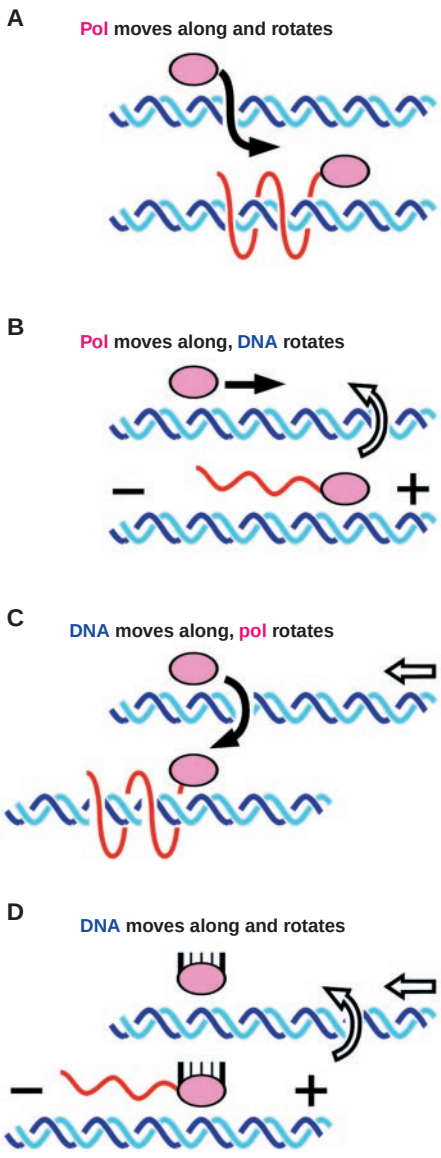

Fig. 3. Possible movements of an RNA polymerase (oval) and a template. Solid and open arrows show movements of the polymerase and the template, respectively; the transcript is shown as a wavy red line. (**A**) If the polymerase moves both laterally and rotationally, a (trailing) transcript becomes entwined about the template. It could be freed by (i) pulling on the transcript (but this would probably break it), (ii) rotating one end of the transcript around the helical axis to free the transcript (but the number of rotations would have to be exact because one too many, or one too few, would not suffice), or (iii) a topoisomerase-like cutting, passing the transcript through a double-strand break in DNA (or passing the duplex through a single-strand break in the transcript), and ligation. Alternatively, the transcript might not trail behind but ride piggyback on the polymerase and so would not become entwined (not shown). Then, that polymerase would have to carry engaged ribosomes and nascent proteins in bacteria (where translation occurs cotranscriptionally) or carry ribonucleoprotein complexes with transcripts of more than 10^5 nucleotides in eukaryotes. (**B**) If the polymerase moves along without rotating, the transcript does not become entwined about the template. Supercoils (+ and – indicate their sense) generated ahead of and behind the moving enzyme can be removed by topoisomerases. However, it is difficult to imagine any mechanism that might prevent the polymerase from rotating while allowing translocation; even one accidental rotation, which is especially likely when the transcript is short and frictional drag is limited, would yield an entwined transcript. (**C**) If the DNA moves along as the polymerase rotates, a trailing transcript becomes entwined [as in (A)]. Alternatively, it could ride piggyback on the polymerase (as discussed above). (**D**) If the enzyme is attached to the substructure, the untwining problem does not occur. However, supercoils are generated on each side and must be removed [as in (B)].

a clear precedent for the organization of many different transcription units into one factory.

Transcription Factories

The nucleolus is dedicated to the production of 45*S* ribosomal RNA (rRNA) and ribosomes (*34*). It contains three zones that are distinguishable in the electron microscope: a "fibrillar center" and associated "dense fibrillar component," which are embedded in the "granular component," a region where ribosomes mature. A (triploid) HeLa cell contains ~540 45*S* rRNA genes arranged in tandem repeats on different chromosomes; during interphase, only ~120 genes (and some chromosomes) come together to form ~30 fibrillar centers (*35*). Each fibrillar center contains a store of polymerase I and about four active genes at or close to its surface (*35, 36*). Each gene is associated with ~125 engaged polymerases, and their transcripts can be visualized after extension in Br-UTP as crescent-shaped structures that are equivalent to the dense fibrillar component (Fig. 4A, lower box). As a result, a nucleolar factory with a fibrillar center at its core contains ~500 active polymerases and about four transcription units (*37*). It is difficult to imagine how polymerases and their transcripts could possibly track along a template through the dense nucleolar interior; in contrast, a template could easily snake end-on along the path of least resistance over the surface of the fibrillar center (Fig. 4C). Presumably, such templates are stripped off fibrillar centers during the preparation of Miller spreads to give the "Christmas trees" with their ~125 "branches" (Fig. 4E).

Although little is known about the microarchitecture of nucleoplasmic transcription sites, recent evidence suggests that several different transcription units and polymerases are organized into one site, much as in the nucleolus. This evidence depends on how accurately polymerases, sites, and transcription units per site can be estimated—a daunting technical problem that has been addressed repeatedly over the years (*35*). Thus, early estimates indicated that 20,000 to 100,000 polymerases were active within the nucleoplasm of a mammalian cell. Two recent estimates give similar values, with a typical HeLa nucleus containing ~90,000 nascent transcripts, with ~15,000, ~65,000, and ~10,000 being made by polymerases I, II, and III, respectively (*33, 35*).

Are these polymerases active on different transcription units, or do many polymerases simultaneously transcribe one unit? Whereas polymerase III units are only ~100 bp long and so are unlikely to be associated with more than one polymerase, it is widely thought that polymerase II units are often covered by many. However, the evidence shows that only a few units, such as activated heat-shock and actin genes, are that busy (*38*); most units seen in spreads are associated with only one polymerase (*35, 39*). Even the adenoviral unit has only one polymerase every 7.5 kb, the length of a typical unit in the host (*40*).

Estimates for the number of sites in the nucleoplasm range from 500 to 10,000 (*28,*

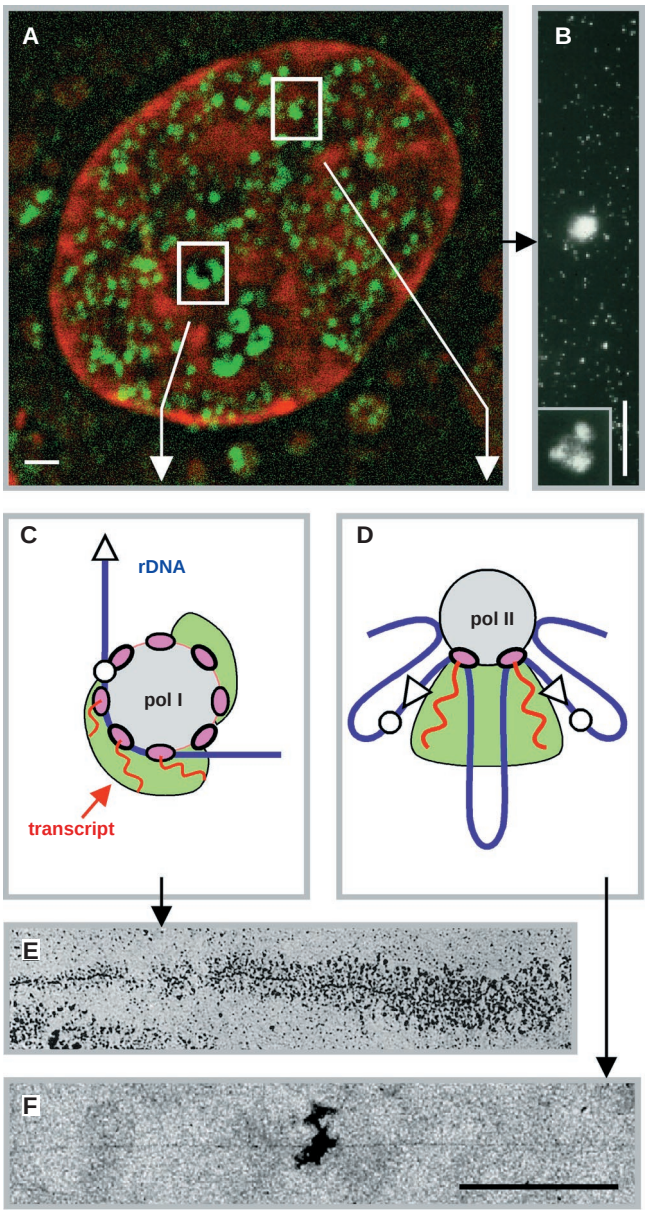

Fig. 4. Visualizing newly made RNA in HeLa cells. (**A**) Transcription foci. Cells were permeabilized, nascent transcripts were extended in Br-UTP, and cryosections (100 nm) were prepared; then, Br-RNA was immunolabeled with fluorescein isothiocyanate (green), nucleic acids were counterstained with TO-TO-3 (red), and a fluorescence image was collected on a confocal microscope. Newly made RNA (green) is concentrated in discrete foci in the cytoplasm (made by the mitochondrial polymerase), nucleoplasm, and nucleoli. Image provided by A. Pombo. Scale bar, 1 μm. (**B**) Transcripts associated with individual transcription units. Cells [labeled as in (A)] were lysed with sarkosyl to strip all proteins except engaged RNA polymerases (and their transcripts) from DNA; after spreading DNA, Br-RNA was indirectly immunolabeled and imaged in a fluorescence microscope. Many small faint foci and one large focus, which contains three subfoci (inset, 1/32 exposure and ×5 magnification) are seen. About 55,000 faint foci (each containing about one transcript made by polymerase II or III) and ~120 bright subfoci (each containing ~125 polymerase I transcripts) were seen per cell, equivalent to the numbers of active polymerases [from (*35*), with permission]. Scale bar, 10 μm. (**C**) Model for a nucleolar factory. The lower box in (A) contains two crescent-shaped foci, each with nascent transcripts from one transcription unit. The two crescents are reproduced in (C), but the transcripts and template in only one are shown for clarity. rDNA slides (open arrowhead) through polymerases (ovals) on the surface of a core (the fibrillar center) containing polymerase I, as nascent transcripts are extruded to form the (crescent-shaped) dense fibrillar component. (**D**) Analogous model for a nucleoplasmic factory. Only two transcripts in the green focus in the upper box in (A) are shown in the corresponding green region in (D). Transcripts are extruded from the surface of a core containing polymerase II. (**E**) Electron micrograph of a Miller spread, showing an rRNA transcription unit with ~125 transcripts [from (*25*), with permission]. This is equivalent to one crescent-shaped structure in (A) and (C) and a subfocus in the inset in (B); it is obtained by stripping a transcription unit off the surface of the core in (C). (**F**) Electron micrograph of a polymerase II transcription unit with one transcript [from (*35*), with permission]. This is equivalent to part of one nucleoplasmic focus in (A) or one small focus in (B); it is obtained by stripping a transcription unit off the surface of the core shown in (D). Scale bar in (F) [for (E) and (F)], 1 μm (~2.9 kb DNA).

31, 32, 41). The most detailed estimate has a HeLa nucleus containing 5000 to 8000 polymerase II sites and ~2000 polymerase III sites (*32, 33, 35, 42*). Because all estimates indicate that there are more nascent transcripts than sites and because most transcription units are associated with only one polymerase, it follows that each site must contain more than one active transcription unit (*43*). Then, active RNA polymerases, like active DNA polymerases, will be concentrated in factories. Moreover, most polymerase II factories would also be multifunctional because they contain "holoenzymes" that are able to cap, polyadenylate, and splice messages (*3*).

The Pulling Power of a Polymerase

In conventional models (Fig. 1, A and B), energy released during hydrolysis of triphosphates drives polymerase movement; in alternative models (Fig. 1, C and D), the same energy drives template movement. But, can an immobilized polymerase work? Two approaches show that it can. One approach involved adding a binding domain to the RNA polymerase of phage T7 and then attaching the hybrid protein to a large plastic bead; it made RNA just as well as its unbound counterpart that was released by proteolytic cutting between the two domains (*29*). A second approach involved adsorbing the RNA polymerase of *Escherichia coli* onto a glass slide (*44*). After adding a template with a promoter at one end and a gold particle at the other, two kinds of particles could be seen in the light microscope. One particle moved with Brownian motion, and the other was restricted to a small hemisphere about a point on the slide; presumably, some templates were free, and others were tethered through the promoter to a bound enzyme. When transcription was initiated, tethered particles became even more restricted in their movement as they were reeled in by the attached polymerase. The elongation rate (deduced from the rate at which the tether decreased in length) was similar to that found with a free enzyme. Clearly, the polymerase has sufficient power to reel in the template and extrude the transcript.

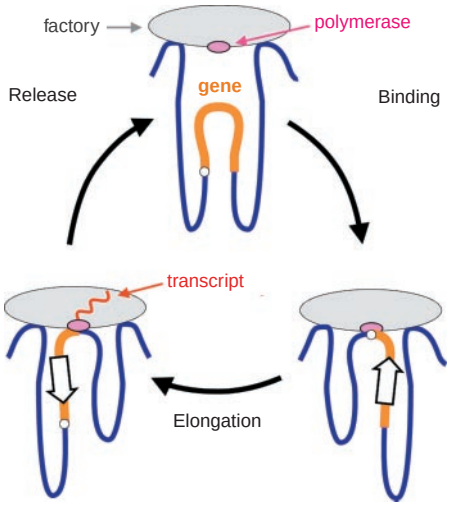

Fig. 5. A transcription cycle. A chromatin fiber is tied in loops (only one is shown) to a factory. The promoter (small circle) binds to one of the polymerases in the factory, and the transcript is generated as the template slides (open arrows) through the polymerase; at termination, the template detaches so the cycle can repeat. This model can be extended to explain how an inactive gene at the tip of a long heterochromatic loop could be activated (not shown). First, a transcription unit near the factory attaches, creating subloops. Then, the resulting transcription reels in the loop, "remodeling" and "opening" its chromatin. Now, other enhancers and transcription units attach, creating successively smaller loops until the inactive gene is brought sufficiently close to the factory to bind. Such transcription cycles can be incorporated into dynamic models for the way genomes are organized in prokaryotes and eukaryotes and for how chromosomes might pair during meiosis (*51, 52*).

This approach was adapted to measure the pulling power of a polymerase (*45*). The gold particle was replaced by a polystyrene bead (diameter of 0.5 μm) so it could be held in an optical tweezer. At saturating nucleoside triphosphate (NTP) concentrations, polymerases stalled reversibly when a force of ~14 pN opposed the pull of the enzyme. This is about one-thirtieth of the force needed to break bonds in the duplex (*46*). This force is larger than that generated by kinesin and myosin and makes the polymerase the most effective of all known motors. It is so effective because of its low gearing, moving DNA ~0.34 nm for each NTP hydrolyzed, which is one-tenth of the step length of kinesin.

Conclusions

For both DNA and RNA polymerases, I have discussed two distinct issues: Do active enzymes track or remain static because they are attached to a larger structure, and if they do remain static, are they grouped together in factories? The evidence for DNA polymerases being fixed is indirect and of four general types: (i) There is theoretical evidence, because it is easy to imagine how attached DNA polymerases might be coordinately controlled (Fig. 2, B through E). (ii) Some soluble activities may become immobilized during reactions in vitro (a helicase at one replication fork immobilizes its partner at the other and vice versa). (iii) Active DNA polymerases and nascent DNA resist detachment from the substructure. (iv) Newly made DNA is concentrated in discrete foci, implying that the polymerases are not free to track (Fig. 2A). The same evidence supports the idea that active polymerases are grouped together. As a result, many now accept that DNA polymerases are fixed in factories. In eukaryotes, these factories are not permanent structures; most small factories that are active at the beginning of S phase must be disassembled once they have replicated neighboring DNA, and their machinery is incorporated into the newer and larger factories found later.

The idea that RNA polymerases are also fixed is less widely accepted, even though the evidence is of the same four types listed above: (i) Logic suggests that polymerases must be fixed (Fig. 3D), as no satisfactory solution to the entwinement problem has been found (Fig. 3, A through C). (ii) All three nuclear RNA polymerases of eukaryotes assemble during in vitro reactions into large complexes that pellet in a microcentrifuge. (iii) Active RNA polymerases, nascent transcripts, and active transcription units are associated with the substructure. (iv) Newly made RNA is concentrated in discrete foci, again implying that the polymerases are fixed (Fig. 4A). But, are those polymerases and their transcription units concentrated in factories? In eukaryotes, the answer for polymerase I is clear, as the nucleolus is the prototypic multifunctional factory that makes rRNA and assembles it into ribosomes. For polymerases II and III, all estimates of enzyme and site number are consistent with more than one polymerase per site, and because only one polymerase is found on a typical transcription unit, it follows that there are many units per site. Again, these factories are not permanent structures; for example, a few large nucleolar factories break up into many smaller ones when transcription increases.

If the RNA and DNA polymerases discussed above are fixed, it seems that other types of polymerases will be also (*47*). Immobilization could be achieved by fixing the two partners in a dimeric complex to each other [as in helicases (*13*)] or by attaching the polymerase to the wall of a viral capsid (*48*), cell membrane (*23*), or internal skeleton (*21*). Detailed models involving fixed polymerases have been drawn for replication, transcription, and reverse transcription (*49*). These necessarily involve template movement, and one model is illustrated in Fig. 5. Instead of a polymerase attaching at a promoter, tracking along, and then detaching at termination, a transcription cycle involves an initial DNA binding, passage through the fixed polymerization site, and subsequent detachment. Accessibility of the promoter to the bound polymerase will determine the rate of initiation, and this will mainly be affected by proximity to a factory.

Life-forms concentrate molecules in their environment so that those molecules can react together. By extension, we might

expect that the polymerases responsible for the vital processes of replication and transcription would be concentrated within the cell in specific locations. The organization of polymerases into factories raises many questions. How ordered are the polymerases within a factory? What signals enable many DNA polymerases in one factory to fire synchronously? Do some transcription factories specialize in the transcription of particular genes (*33, 50*)? What other functions does each kind of factory carry out (*3*)? Transcription and translation are closely coupled in bacteria, so does a fixed RNA polymerase organize the ribosomes that translate its transcript (*51*)? Fortunately, techniques are now available to answer these questions.

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