COMMENTARY

How mobile are active RNA polymerases?

P. R. COOK

Sir William Dunn School of Pathology, University of Oxford, South Parks Road, Oxford OX1 3RE, UK

The 'text-book' model for transcription

The photographs of 'genes in action' taken by Miller *et al.* (1970) are some of the most evocative in molecular biology. When bacteria are burst osmotically to spread their DNA, RNA polymerases can be seen frozen in the act of transcription as they processed along the template; the resulting nascent transcripts are covered with ribosomes, which are also caught in the synthetic act. These beautiful images adorn standard texts and we are all familiar with the model for transcription that they apparently confirm so powerfully. The model underlies our terminology – the 'processivity' of polymerases, nuclear 'run-ons', 'upstream' and 'downstream'. But this model is usually a two-dimensional one. I will argue that recent data on the three-dimensional organisation of DNA ill-fit this accepted model and can be accommodated by an alternative model derived from studies on eukaryotes. The essential element of the alternative is the *immobilisation of the polymerase* so that the DNA moves past the fixed polymerase, rather than *vice versa*.

There seem to be only two kinds of evidence for the accepted model. 'Miller' spreads provide one kind. But these are only obtained by violently bursting cells by diluting them 1 in 50 in distilled water; any attachments might well be lost. The second kind of evidence is superficially even more convincing: pure prokaryotic polymerases will transcribe pure templates *in vitro* in the *absence* of any additional immobilising elements. (Evidence from eukaryotic polymerases is less decisive. When pure, they are often inefficient and do not initiate correctly; when impure, they may contain immobilising elements (Cook, 1989).) I will discuss recent observations aimed at testing an extension of the traditional model – the 'twin-domain' model – which compromise this second kind of evidence.

The twin-domain model

Our intuition that RNA polymerase tracks along the DNA stems from our perception of relative size; it is the smaller of the two that moves. For example, the simplest and best-studied bacteriophage polymerases have a mass of about $0.1\times10^6 M_r$, about 1/30 of the mass of a 5 kb (1 kb=10^3 base pair) plasmid. But the active polymerase is part of a much larger complex, which includes nascent RNA, ribosomes and nascent polypeptide. As the mass of one ribosome roughly equals that of plasmid DNA, this complex can easily dwarf the template, even if it is associated with an equal mass of protein. (In eukaryotes, the complex includes ribosome-sized spliceosomes and so is also large relative to the transcribed part of the template.)

It has long been recognised that transcription of circular DNA poses topological problems (Maaloe and Kjeldgaard, 1966; Gamper and Hearst, 1982). Imagine a bacterial polymerase tracking along the transcribed strand of a plasmid template 1 kb long (Cook and Jackson, 1988). On transcription of *each* helical turn (i.e. every ~10 base-pairs), the polymerase (radius 7.5 nm), plus nascent transcript, attached ribosomes (radii 15 nm) and nascent protein must all thread through the centre of the circular template (Fig. 1A and B). If condensed by the type of supercoiling found in nucleosomes, this has a radius of only 9 nm. Even if such threading were possible, perhaps because the DNA was not so highly condensed, the resulting transcript must still be untwined from the template (Fig. 1B). As these threading and untwining problems seem insuperable, such rotation of polymerase about the template axis is unlikely. The alternative is that the template rotates about its helical axis. In a circular template this means that compensatory supercoils must necessarily accumulate on each side of the polymerase. This led Liu and Wang (1987) to suggest a 'twin-supercoiled-domain' model for transcription: translocation of the RNA polymerase complex along a right-handed double-helical DNA generates positive supercoiling 'waves' ahead of, and negative supercoiling waves behind, the moving RNA polymerase (Fig. 1C).

Testing the twin-domain model

Liu and colleagues directly tested their model (Tsao *et al.* 1989). Pure bacterial (or bacteriophage) polymerase was allowed to generate the twin domains by transcribing relaxed DNA *in vitro* (Fig. 1C); then topoisomerase I, which is able to remove negative supercoils (but not positive supercoils), was added and, after deproteinisation, the resulting template was found to be positively supercoiled (Fig. 1D). Positive supercoils only appeared once transcripts longer than 5000 nucleotides had been synthesised and cutting these with even low concentrations of RNase prevented their appearance.

The results of this elegant experiment strongly support the twin-domain model. Nonetheless, the result was surprising: positive and negative supercoils would be expected to diffuse around the circle and annihilate each other before the topoisomerase could act. Tsao *et al.* (1989) suggest that the diffusion rate must be sufficiently slow

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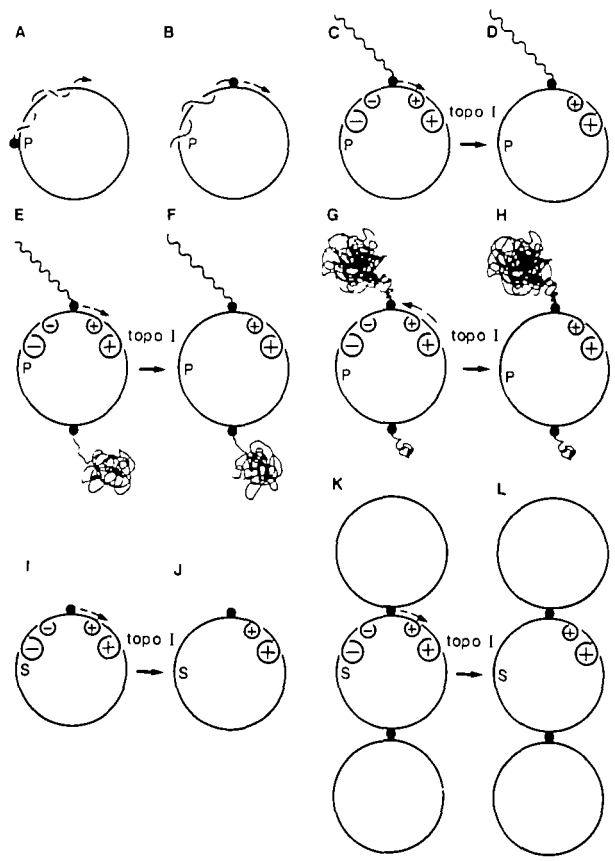

Fig. 1. How polymerase (A-H) and T antigen (I-L) might affect supercoiling. The broken arrows indicate the movement of protein (A,B,C,E,I,K) or DNA (G). A. RNA polymerase (filled circle) binds to a promoter (P) on a relaxed circular template and tracks along the helix. A tracking SV40 T antigen follows a similar path. B. The polymerase has transcribed a quarter of the circle and the resulting transcript (wavy line) is wound around the template, once for every helical turn transcribed. C,D. If frictional resistance on the polymerase/transcript complex prevents it from rotating around the template axis as it tracks, then the template must rotate instead. This leads to a compensatory accumulation of right-handed (+) supercoils ahead of the moving polymerase and left-handed (−) supercoils behind it. Removal of negative supercoils by topoisomerase I (topo I) then leaves a positively supercoiled template if the topoisomerase acts before the supercoils diffuse around the circle and annihilate each other. E,F. Supercoils might be prevented from annihilating each other if a second polymerase/transcript complex on the same molecule aggregates with other complexes/templates; an inactive complex at the bottom forms a barrier that prevents supercoils generated by the polymerase at the top from diffusing and destroying each other. As before, topoisomerase treatment leaves positive supercoils. G,H. The promoter (P) binds to an RNA polymerase (filled circle at the top), immobilised in an aggregate. The transcript is generated as the DNA moves laterally (broken arrow) past the polymerase, rotating as it does so, leading to compensatory supercoiling. Twin domains are stably segregated by a second inactive aggregate at the bottom. Topoisomerase treatment then leaves positive supercoils. I,J. T antigen (filled circle) binds at S, tracks a quarter of the way around a relaxed template to generate the twin domains and then topoisomerase treatment leaves positive supercoils. It is difficult to see how the T antigen moves laterally without rotating about the helical axis. K,L. The top T antigen bound at S and tracked a quarter of the way around the middle circle. It also bound to another template and the frictional drag of the complex prevents it from rotating so that twin domains of supercoiling are generated. These are segregated by an immobile T antigen/template complex at the bottom. Topoisomerase treatment leaves positive supercoils.

that the topoisomerase acts before the supercoils destroy each other. Unfortunately we have no data on such rates, but we do know that gamma rays release supercoils in nucleoid 'halos' within seconds (Cook *et al.* 1976).

An alternative view

Alternatively, supercoils might not annihilate each other because the twin domains were stably segregated from each other, perhaps because another transcription complex had stalled on the opposite side of the template or had aggregated with other complexes on the same or different templates. In Fig. 1E, nascent RNA synthesised by the polymerase at the bottom has aggregated with other transcripts and templates; this now inactive complex stably segregates the positive and negative supercoils generated by the active polymerase at the top. Addition of topoisomerase releases the negative supercoils to leave the positive coils (Fig. 1F).

A number of observations support the idea that aggregation is involved. First, high concentrations of polymerase ($7\,\mu g\,ml^{-1}$) and template ($20\,\mu g\,ml^{-1}$) were used so there were an average of 10 polymerases per template. Second, the kinetics suggested that only a fraction of the templates were active, so that this fraction was probably covered with many more than 10 polymerases (Tsao *et al.* 1989). Third, bacterial polymerase is known to aggregate at the low ionic strength used (Chamberlin, 1974). (Note, however, that the ionic strength in the vicinity of DNA may be higher.) Fourth, gentle lysis of bacteria releases aggregates of polymerase and nascent RNA; these aggregates maintain the looped domains in the folded chromosome (Stonington and Pettijohn, 1971). Fifth, aggregation explains why positive supercoiling is generated only after the synthesis of very long molecules of RNA – long enough to form aggregates.

The results of an additional test of the twin-domain model also suggest that aggregation segregates domains. Liu and colleagues argued that any protein, not just a polymerase, that tracked along a DNA strand should generate twin domains. Therefore they performed an analogous experiment to the one described above in which the polymerase was replaced by SV40 T antigen, a protein that tracks along the helix using ATP (Yang *et al.* 1989). As before, incubation of relaxed plasmid circles with T antigen and ATP, followed by addition of topoisomerase I to remove negative supercoils, left a positively supercoiled template (Fig. 1I,J).

This result is even more surprising than that obtained with the polymerase. We have an additional problem to that of preventing the twin domains from annihilating each other: T antigen, even if tetrameric, is so much smaller than the polymerase plus transcript that it might be expected to be able to track along the helix, rotating about the helical axis without causing any supercoils to accumulate as in Fig. 1A (i.e. the threading problem is not nearly so acute). In addition, there is no long transcript to provide the frictional drag that might prevent rotation of the T antigen. As supercoils *do* result, the DNA must rotate and not the T antigen. Yang *et al.* suggest that both problems are solved if a tracking T antigen binds to others,

which might be on the same or different templates; aggregation both immobilises the T antigen and segregates the domains (Fig. 1K,L). Such aggregation is again likely in view of the high concentrations of T antigen used (i.e. 30 $\mu g\,ml^{-1}$ relative to DNA at 2.5 $\mu g\,ml^{-1}$), the known propensity of T antigen to aggregate, the requirement of aggregation for DNA-binding and enzymic activity (Bradley *et al.* 1982) and the stimulatory effects of polyethylene glycol on the reaction (Yang *et al.* 1989).

These results, involving either polymerase or T antigen, are most simply explained if aggregation segregates the two domains in *both* cases. Whatever the precise explanation, it is clearly possible that, in these apparently simple experiments using pure soluble reagents, complex structures might be generated. Therefore, the second kind of evidence for the 'text-book' model of transcription, that pure polymerases transcribe pure templates *in vitro* in the assumed *absence* of immobilising elements, is compromised.

An immobile polymerase

Before discussing one particular alternative model it is worth considering the formal possibilities. The polymerase and DNA move relative to each other both rotationally and laterally. One possibility, the one implicit in the 'textbook' model, involves the polymerase moving laterally along the static template as well as rotationally about its axis (Fig. 1A). But then, as discussed previously, the threading and untwining problems seem insuperable. In the second, the twin-domain model, the enzyme translocates laterally but does not rotate: instead the DNA rotates (Fig. 1C). But how can the enzyme be prevented from rotating as it translocates? Even if it rotated once accidentally, transcript and template would become intertwined, as in Fig. 1B. (Of course, it is possible that an occasional accidental rotation, and consequent entwining, can be tolerated in bacteria: in eukaryotes the message *must* be untwined before it can be passed to the cytoplasm. Even so, some eukaryotic transcripts may accidentally become entwined and their destruction might account for part of the phenomenon of nuclear RNA turnover.) The third possibility is that the enzyme rotates and the DNA moves laterally. This again requires enzyme movement in only one dimension and leaves unsolved the threading and untwining problems.

The fourth possibility is that the enzyme is completely static and DNA both rotates and translocates (Fig. 2). This sidesteps threading/untwining problems and those of moving a bulky transcript and associated ribosomes (or spliceosomes). It appeals because only one of the players moves. It is also the model used to explain how transcription occurs in higher cells where the polymerase is apparently fixed to a skeleton (Jackson *et al.* 1981; Jackson and Cook, 1985; Cook, 1989). The mode of action of the restriction enzyme *Eco*K provides a precedent for both DNA translocation and rotation past an immobile enzyme (Yuan *et al.* 1980).

This fourth possibility is also consistent with the results of the experiment of Tsao *et al.* (1989). I have argued that aggregation might explain how twin domains are segregated and how the T antigen – and, by extension, the polymerase – might be prevented from rotating. But this same aggregation would necessarily prevent the polymerase from moving along the DNA; if the polymerase is immobile, the DNA must both rotate and translocate

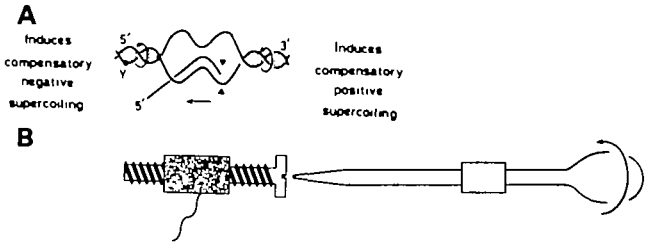

Fig. 2. A model for transcription; the polymerase is immobile and DNA moves past it, rotating as it does so. A. Strands of a DNA duplex are shown separated on each side of the fixed polymerisation site (filled triangles). RNA synthesis was initiated when Y lay between the triangles. DNA then moved to the left (arrow): as the duplex enters the fixed polymerase complex strands separate and move to the left, RNA is synthesised and extruded downwards to the left (its 5' end is marked) and then strands rejoin. The transcribed base always retains the same stereochemical relationship to the site between the triangles. The duplexes on each side of the polymerisation site move to the left and spin (arrows), inducing compensatory supercoils that are removed by topoisomerases (not shown). B. Analogous movements of a bolt (DNA) driven through a fixed nut (polymerase) using a ratchet screw-driver. The nut 'sees' the thread (transcribed strand) as it passes through, whilst RNA (wavy line) is synthesised. As the screwdriver twists (arrow), the bolt is driven to the left and torsional strain develops in the wrist, unless relieved by spinning the ratchet (topoisomerase). Another ratchet to the left of the nut (not shown) also relieves strain. Extra nuts can be added to the unit and as long as they lie between the ratchets no strain develops between them. In this way highly active transcription units containing many polymerases (e.g. rDNA units) can be built.

(Fig. 1G). Rotation leads to compensatory supercoiling on either side of the polymerase, just as in the twin-domain model; then the topoisomerase removes the negative supercoiling (Fig. 1H).

If the polymerase is fixed *in vivo*, it becomes attractive to suppose that it is flanked by topoisomerases, which are also fixed so that supercoils are removed automatically (see Rose *et al.* 1988; Stewart *et al.* 1990). In bacteria, gyrase relaxes positive supercoils and topoisomerase I negative ones, so these two enzymes might bracket each transcription unit. If there is more than one polymerase in a transcription unit, there is no need for topoisomerases to bracket each polymerase in the unit; torsional strain need only develop at the ends of the unit (Cook, 1989). Inhibition of one or other of the topoisomerases would lead to accumulation of supercoils of appropriate sign (Lockshon and Morris, 1983; Pruss and Drlica, 1986; Wu *et al.* 1988). Because bacterial and eukaryotic DNA are segregated into domains of about 100 kb (Pettijohn, 1988; Jackson *et al.* 1990), template movements resulting from one gene's transcription would be restricted to one domain.

Immobilisation of the polymerase *in vivo* begs the questions: what is the mechanism of immobilisation? Can RNA polymerase, a bivalent protein, anchor itself to one piece of DNA whilst transcribing another, much as in Fig. 1G? (This is consistent with what is known of the structure of the bacterial 'nucleoid' (Stonington and Pettijohn, 1971; Pettijohn, 1988).) Or – a more extreme possibility – is there a skeleton in bacteria like the nucleoskeleton of higher cells to which polymerases are attached? Is a fixed polymerase dedicated to transcribing only the gene that is close enough to attach? Most importantly, the idea of an immobilised polymerase leads naturally to an attachment hypothesis for gene activation; genes can be-

come active only after they have attached to the fixed polymerase (Cook, 1989).

What might happen *in vitro* when a pure template is transcribed by a pure polymerase? The answer is probably that each of the four formal possibilities applies at some stage during the transcription cycle. Initially, the polymerase might both translocate and rotate. As the lengthening transcript becomes intertwined with the template, its increasing frictional drag would progressively curtail polymerase movement, until it ceases and DNA movement takes over. High polymerase:template ratios – by inducing aggregation – would curtail polymerase movement sooner. The four possible modes of polymerase action might differ in efficiency. Efficiency is high with >10 polymerases per template but falls precipitously when the enzyme is diluted (Chamberlin *et al.* 1983); this is usually attributed to 'denaturation' (Chamberlin *et al.* 1979, 1983) but could result from dilution below a critical concentration needed for aggregation and, so, efficient synthesis.

Conclusion

Efficient transcription by a pure enzyme of a pure template provides superficially decisive evidence in favour of the traditional model for transcription involving a *mobile* polymerase. A modification of this traditional model – the twin-domain model – suggests that the enzyme tracks along DNA as template rotation generates twin domains of supercoiling. Any suggested aggregate is then an artifact that has little relevance to what occurs *in vivo*. According to this view we would be wrong to (over) interpret results in terms of an organisation of plasmid DNA into looped domains (i.e. the twin domains) by attachment to an RNA-containing 'scaffold' to which the polymerase was attached and at which transcription occurred. It is also of little value to map which sequences are attached to such a scaffold or to argue the toss with others who obtain different results with aggregates prepared in slightly different ways. But from the viewpoint of most molecular biologists, this seems to be what some eukaryotic cell biologists are doing.

However, the twin-domain model requires that the enzyme tracks along the DNA without rotating about it. It is difficult to imagine on theoretical grounds how its motion could be so restricted, especially initially when the nascent RNA is short. Furthermore, a direct test of the twin-domain model gave surprising results, which can be interpreted in terms of either a mobile polymerase and slow diffusion of supercoils or a polymerase immobilised by aggregation. As a result, this kind of evidence is not as decisive as it initially appears. (By analogy, evidence for a mobile *DNA* polymerase based on *in vitro* experiments involving pure template and T antigen (Ishimi *et al.* 1988) is also compromised.)

Independent studies on eukaryotes have led to an alternative model for transcription that also involves an immobile polymerase. It is easy to imagine why eukaryotic polymerases must be immobile; immobilisation prevents (or at least minimises) entwining of the template with a transcript that must eventually be translocated to the cytoplasm. Bacteria may tolerate some entwining as transcription and translation sites are not so spatially segregated; consequently the need for an immobilised polymerase may not be so pressing. But a common mechanism in both eukaryotes and prokaryotes has its attractions. Perhaps, then, it is time to re-examine our assumptions and determine how mobile active RNA polymerases really are.

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