Cooperative relaxation of supercoils and periodic transcriptional initiation within polymerase batteries

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Summary

Transcription and DNA supercoiling are known to be linked by a cause-effect relationship that operates in both directions. It is proposed here that this two-way relationship may be exploited by the *E. coli* genome to facilitate constitutive transcription of supercoil-sensitive genes by polymerase batteries made up of uniformly spaced RNA polymerase elongation complexes. Specifically, it is argued that (1) polymerases transcribing DNA in tandem cooperate to relax each other's transcription-driven positive supercoils; and (2) negative supercoils driven upstream by elongation complexes tend to be 'harnessed' and used to cooperatively (and periodically) initiate fresh transcription from promoters. Harnessing of transcription-driven negative supercoils is thought to be achieved through the erection of protein barriers to the rotational upstream propagation of supercoils from transcription events. The possible relevance of such cooperation amongst polymerases to the activation of transcription by DNA-binding protein factors is emphasized. Some testable predictions are made and implications are discussed.

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Introduction

Several years ago, Liu and Wang[1] proposed that the translocation of an RNA polymerase elongation complex along double helical DNA leads to the overwinding (positive supercoiling) of the DNA ahead of the polymerase, and underwinding (negative supercoiling) of the DNA behind the polymerase. This proposal is now widely known as the 'twin supercoiled domain' model of transcription. One of the principal features of the model is that supercoils introduced into DNA by a processive polymerase are thought to be detected and relaxed by the cellular topoisomerases, gyrase and topoisomerase I, concurrently with transcription. It is thought that any imbalances in the relative rates of relaxation of positive and negative supercoils give rise to changes in the linking number of template DNA. Much evidence has been gathered in favour of such transcription-driven formation of supercoiled DNA domains[2-17].

The cause-effect relationship between transcription and DNA supercoiling is also known to operate in the reverse direction. Supercoiling of DNA can have a marked effect on the strength of transcription from bacterial promoters[18-31]. Negative supercoiling generally increases the frequency of initiation of transcription, while positive supercoiling inhibits transcription. Such effects are now reasonably well understood in terms of both promoter-polymerase interactions (occurring during the formation of the closed promoter complex[24,31]) and the energetics of melting of DNA during open complex formation and subsequent transcriptional elongation[22,25,27].

Transcription and supercoiling thus appear to be linked by a remarkable relationship; transcription is capable of affecting the state of supercoiling of DNA, which is, in turn, able to affect the occurrence of transcription. This two-way relationship has been the subject of several interesting discussions in the literature[32-36]. Among other things, we do not yet fully understand how the cell manages to keep this relationship from spiralling into a positive or negative 'feedback' loop. Several interesting issues concerning this aspect of the relationship are addressed in this paper, and the arguments described below yield potential insights into how constitutive transcription might occur.

Current concepts: a brief review

Supercoiling influences initiation of transcription

The degree to which template supercoiling affects the

initiation of transcription has been investigated in a large number of *E. coli* promoters[18-31]. Many have been found to show an increased frequency of transcriptional initiation, upon transition from relaxed to progressively negatively supercoiled states. It appears that negative supercoiling increases the rate constant (K_f) of formation of open promoter complexes by lowering the energy required to melt DNA[22,27]. At certain promoters, however, negative supercoiling can decrease K_f, for reasons which are not yet completely understood. The rate constant of formation of the closed promoter complex (K_B) is also known to be modulated by supercoiling. This might occur through the twist-induced (relative) relocation of the −10 and −35 regions of the promoter on the surface of double-helical DNA, leading to altered kinetics of promoter recognition by RNA polymerase[24], or through the effect of DNA writhe on the dissociation/association of RNA polymerase with promoters and upstream DNA[31].

DNA probably rotates around RNA polymerase

There are two ways in which RNA polymerase might conceivably effect transcription: (1) through rotation of the polymerase as it follows the template strand on its path around the other strand of the DNA double helix, or (2) through rotation of DNA, on its axis, about the polymerase that does not rotate as it effects a relative translocation between itself and the DNA. Although the two possibilities are not mutually exclusive and both DNA and RNA polymerase might actually rotate to different extents in different situations, now it appears that it might mainly be the DNA that performs the necessary relative rotation on topologically constrained templates. The relevant arguments are as follows. The RNA-DNA hybrid within the 17-base-pair transcription bubble has a constant length of about 12 base pairs (the equivalent of slightly over one turn of B-DNA) during transcriptional elongation, suggesting that the nascent RNA strand is able to unwind and separate from the template strand at the same rate at which it is synthesized[37]. If RNA polymerase were to follow the template strand on its path around the double helix [as suggested in (1) above], nascent RNA dissociating from the strand would tend to get wound around the re-forming DNA double helix, creating problems during translation. However, rotation of DNA [scenario (2)] would succeed concurrently in both unwinding the RNA-DNA hybrid and separating the transcript from the DNA duplex. Electron microscopic evidence indicates that an RNA polymerase associated with negatively supercoiled DNA is invariably located at the apex of a DNA loop, during both transcriptional initiation and elongation[38]. The polymerase appears somehow to shift the loop along the DNA during transcription, always remaining at the top of the loop (recent work indicates that a stationery RNA polymerase can effect transcription by making the DNA translocate with respect to the itself[39] by exerting a pull; this could be a useful way of keeping a polymerase at the top of a moving loop, with twist being constantly transformed into writhe and retransformed into twist, to facilitate loop translocation). It has been pointed out that the maintained presence of the polymerase at the apex of a loop during transcription can only be reconciled with rotation of DNA about the processive polymerase, and not with rotation of the polymerase[38].

Transcription creates twin supercoiled domains

The torsional effects of the rotation of DNA during transcription would be to overwind DNA ahead of the polymerase and leave it underwound behind the polymerase, creating two supercoiled domains[1]. If transcription were occurring on relatively short stretches of linear DNA, supercoils could be diffused away quickly through unrestrained rotation of the duplex. If transcription were occurring on a small, covalently closed, circular piece of DNA, such as a plasmid, all positive and negative supercoils could rush around the DNA circle in opposite directions and mutually annihilate each other, with no net changes arising in linking number[1]. The situation, however, is substantially different with genes present on the chromosome. The genome of *E. coli* is compacted into various domains of supercoiling (about fifty) that are kept topologically segregated from each other[40]. Within individual supercoiled domains, DNA-bound proteins are understood to create barriers to the rotational diffusion and propagation of supercoils in the following possible ways: (1) loops of DNA, stabilized by the binding of protein(s) to the base of the loop, are thought to be capable of impeding the propagation of supercoils[41]; (2) binding of large protein complexes to DNA could impede the propagation of twist if such complexes were not able to tumble (or rotate) fast enough with DNA; (3) Z-DNA binding proteins, binding to stabilize the Z-conformation (adopted as a result of negative supercoiling of upstream regions), could block further propagation of negative supercoils from a transcription event (note: negative supercoiling can drive the B-Z transition in DNA[10,42,43]); (4) nascent proteins and DNA-bound protein complexes anchored to the bacterial membrane would almost certainly block propagation of supercoils, since no DNA rotation could possibly propagate across a membrane anchor[1].

It is generally agreed that the most effective generation of transcription-driven supercoils would arise from the attachment of ribosome-associated nascent polypeptides to the membrane, for this would almost completely prevent RNA polymerase from following the template strand on its helical path[6-17] and force DNA to rotate. Notably, in the absence of membrane anchors, supercoils would still be expected to be generated as a consequence of transcription (for reasons 1-3 mentioned above), albeit with less efficiency[1-5,7,16,32-36]. It is believed that gradients of superhelical density would be found on both sides of the RNA polymerase, with the level of supercoiling falling away steadily from the polymerase on either side[1-4].

Transcription-driven supercoils must be relaxed

If transcription-driven supercoils were not relaxed at the

same rate at which they were being generated, (1) the accumulation of positive supercoils in the DNA ahead would make it increasingly difficult for the polymerase to further unwind DNA during elongation, and (2) the accumulation of negative supercoils in the DNA behind the polymerase could lower the energy required for DNA melting to such a point that promoter DNA might melt and not remain recognizable by the polymerase. It is worth noting that under extreme conditions of generation and 'quarantining' of supercoils, the 50 base pairs of DNA normally unwound[44] every second by an RNA polymerase could add five positive supercoils to the DNA immediately downstream, raising local positive superhelical density ahead of the polymerase at an alarming rate during transcription, and suggesting that if positive supercoils introduced were not relaxed concurrently, transcription could abort prematurely even if it were initiated.

Topoisomerases might relax supercoils

Wang and colleagues suggest that gyrase relaxes positive supercoils concurrently with transcription, and topoisomerase I (topo I) relaxes negative supercoils[1,2]. Further, they argue that net changes in the linking number of DNA must be observed when any one of these topoisomerases is allowed to relax supercoils more efficiently than the other. In many *in vitro* and *in vivo* studies since, changes in the linking number of DNA have been demonstrated to occur through drug- or mutation-induced inactivation of either topo I or gyrase, or through inclusion of only one topoisomerase in transcription reaction mixtures. Such experiments have now been performed with various plasmids, as well as with chromosomal DNA, and with genes encoding both membrane-associated and soluble cytoplasmic proteins[2-17]. Under normal *in vivo* conditions, rates of relaxation of positive and negative supercoils might be expected to depend upon[2]: (1) the relative cellular abundances of topo I and gyrase (as well as any other toposisomerases involved in supercoil relaxation); (2 the distribution of sites for the action of such topoisomerases in DNA, upstream and downstream of the transcribing polymerase; (3) the size, orientation and relative transcriptional activity of neighbouring transcription units; and (4) the freedom with which supercoils might diffuse away into regions neighbouring a transcriptionally active gene.

Of course, if all the above factors applied equally to DNA on both sides of every transcription complex in the *E. coli*, transcription would cause little net change in linking number. Given the variables involved in determining the relative rates of relaxation of supercoils, however, this is unlikely to be the case if, indeed, supercoils are relaxed mainly by topoisomerases. In this regard, it is interesting that current opinion holds that transcriptionally generated supercoils can (and do) actually exist, although to a much lesser extent than might have been anticipated, within cells expressing normal levels of topoisomerases[1,6,9]. Such supercoiling is believed to be involved in certain *cis*-interactions between transcription units[1,4,6,9,11-14]; a well-discussed case in this context is that of the interaction of the Leu 500 promoter with its neighbouring transcription units[6,9,11,14]. However, as argued below, every transcriptional elongation by a processive RNA polymerase may not be accompanied by topoisomerase action.

Matters arising from the review of concepts

Why doesn't feedback occur?

If transcription-driven changes in linking number can occur as a result of unequal topoisomerase action (as current views hold), any tendency to accumulate one form of supercoil could, through a process of feedback involving both transcription and supercoiling, end up inducing more and more (or less and less) transcription of the region responsible for the generation of the supercoils. A point could thus be reached at which DNA would become either excessively positively supercoiled, or negatively supercoiled, and be unable to support further transcription. Does this situation ever occur? If it does not, why not?

Is there enough gyrase to accompany all transcription?

Assuming rotation of DNA around its axis within the transcribed region, and very effective quarantining of supercoils in flanking regions, roughly 5 positive supercoils can be reckoned to be introduced into the DNA ahead of a polymerase synthesizing mRNA every second (50 bases/second[44]), while 8.5 supercoils might be introduced ahead of a polymerase synthesizing rRNA (85 bases/second[44]). In cells growing with a generation time of 24 minutes, there are estimated to be about 3,300 RNA polymerase complexes actually transcribing at any time[44], with 80% of these engaged in the synthesis of rRNA, suggesting that 25,000-26,000 positive supercoils might tend to be introduced into various sites around the genome (transiently raising local superhelical density to different extents at different sites, in a manner dependent on the actual respective extents of DNA and RNA polymerase rotation, and blocking of supercoil propagation).

The average *E. coli* cell contains about 500 molecules of gyrase[1,45]. Each molecule is capable of relaxing 0.5-1.0 positive supercoils per second[1,46-48], indicating that the gyrase population in the cell can relax only 250-500 positive supercoils per second. A second type II topoisomerase in *E. coli*, topoisomerase IV (topo IV), estimated to exist at 500-5,000 molecules per cell (Ullsperger and Cozzarelli, personal communication), functions mainly to decatenate chromosomes at termination of replication[49-51]. Topo IV has sequence homology with gyrase and is capable of relaxing positive supercoils, but it is able to do so only at a rate two orders of magnitude lower than that of gyrase (Ullsperger and Cozzarelli, unpublished observations[52]. No other type II topoisomerase is known to exist in *E. coli*. In any consideration of positive supercoiling arising from the unwinding of

DNA by a processive polymerase, it is necessary also to keep in mind that replication drives positive supercoils into the DNA ahead[52,53]. In cells growing with a generation time of 24 minutes, there are six processive replication complexes unwinding DNA at a rate of 750-1,000 base pairs per second[44], collectively introducing 450 to 600 positive supercoils at various sites. The available gyrase population of 500 molecules could thus be preoccupied with the relaxation of replication-driven positive supercoils. *Prima facie* it would seem that there may not be enough topoisomerase molecules in the *E. coli* to relax the positive supercoils driven ahead of every RNA polymerase elongation complex, even granting that the rate of introduction of supercoils described in the calculation above may be an overestimate. What is the alternative?

Inferences and possible insights

RNA polymerase complexes might relax each other's supercoils

A transcribing RNA polymerase introduces the same number of positive and negative supercoils per second, in the two domains it creates[1]. Thus, when several polymerases elongate in tandem (see Fig. 1), the positive supercoils driven ahead of each polymerase can be effectively annihilated by the negative supercoils being driven behind the polymerase immediately preceding it. Similarly, the negative supercoils behind each polymerase can be effectively annihilated by the positive supercoils driven in front of the polymerase following it. On the whole, therefore, the region of DNA between any two polymerases in a battery of transcribing polymerases ought to remain either completely relaxed or in some low, equilibrium state of supercoiling (just as the region of DNA between two codirectionally oriented transcription units is probably relaxed, as Liu and Wang[1] have pointed out).

In such a situation, the length of a transcription unit and the density of packing of polymerases within the battery, turn out to be of much less significance to the cell, from the point of view of the demand for supercoil-relaxing enzymes, than might have been anticipated if transcription by every RNA polymerase were to have been individually accompanied by gyrase action. In any transcription battery there would always be only one polymerase (the leading polymerase of the pack; see Fig. 1) at any given time, that would be engaged in introducing positive supercoils into downstream DNA, while only one polymerase (the immediate last polymerase to have successfully entered the elongation mode) would introduce negative supercoils into upstream DNA. This would occur relatively independently of the average spacing of polymerases or the initiation frequency (or strength) of the promoter involved, as long as there were always a minumum of two elongation complexes transcribing the unit. The supercoils generated by all other polymerases within such a battery would cancel each other. The low requirement of gyrase molecules thus achieved could be further minimized by organizing several similarly oriented transcription units in tandem, so that a supercluster of many operons (each operon containing 5 to 6 genes) could transcribe with the help of a mere 5 to 10 gyrase molecules acting downstream, relaxing the 5 (or fewer) positive supercoils being introduced into DNA by the leading RNA polymerase engaged in transcribing the most downstream gene in the cluster.

Accumulation of supercoils, through a process of feedback linked to unequal relaxation of positive and negative supercoils at the ends of batteries could, of course, still occur, but any such build up of supercoils would now occur at a much slower rate per (average) unit transcription event than was earlier envisaged. Further, the arrival of the replication fork would remodel the whole situation at any transcriptionally active region once in every cell generation, as discussed elsewhere[54]. Note that whereas the strength of transcription from individual genes/operons located within a supercluster could be easily altered without excessively influencing the need for gyrase action at the downstream end of the cluster, the positioning and organization of genes on the chromosome could probably not be easily altered. The low availability of gyrase molecules would effectively limit the number of sites at which gyrase could act, so that it would be in the cell's best interests to organize genes codirectionally, as well as in operons and operon clusters. This could partly explain the observed orientational preferences of genes[53] on the *E. coli* chromosome.

What the foregoing discussion shows is that RNA polymerase molecules can cooperate to reduce the need for gyrase action accompanying transcriptional elongation. Below, I describe how the negative supercoils generated by an RNA polymerase freshly launched into the elongation mode can be used to regulate cooperatively the initiation of transcription by a new polymerase at the freshly vacated promoter.

Elongation might regulate initiation

As argued above, the negative supercoils created behind an RNA polymerase (say polymerase n; see Fig. 1) are annihilated by the positive supercoils generated ahead of its immediate successor (polymerase n+1), from the moment the latter polymerase enters the elongation mode until the moment the former polymerase (i.e. polymerase n) terminates its own transcription. Let us consider, however, what happens to these negative supercoils, in the moments preceding elongation by polymerase n+1. In fact, going back even further in time, let us ask what happens to these negative supercoils, prior to open complex formation by polymerase n+1. From the arguments put forward above, it is clear that such supercoils would tend to accumulate in the region, at a rate determined by the extent and rate of topoisomerase I action on the region, and the net effect arising from barriers to rotation placed upstream. What effect would such accumulation have on the promoter?

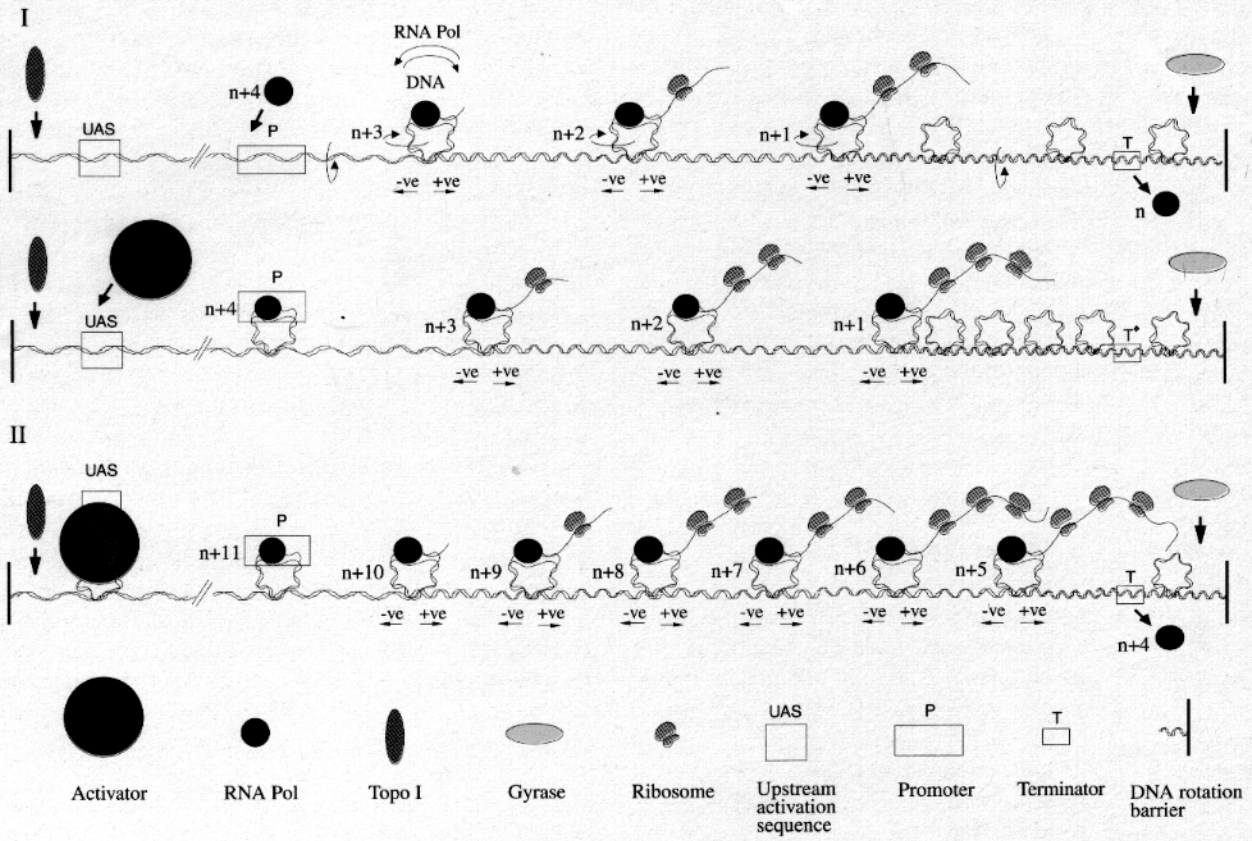

Fig. 1. The diagram shows ongoing transcription of a hypothetical transcription unit, which is bounded on both sides by physical blocks to the rotational diffusion of supercoils (the blocks represent the net impedance offered to the propagation of supercoils by all DNA-bound objects flanking the transcription unit). Gyrase and topo I are shown acting to relax positive and negative transcription-driven supercoils, respectively, at some definite rate, ahead of the transcription unit and behind it. RNA polymerases transcribing the unit are located at the apex of DNA loops; such looping forces DNA to rotate as the polymerase tracks along the duplex, effectively translocating the loop continuously along the transcription unit. As shown in (Ia), the DNA between polymerases constituting the transcription battery is either relaxed or in some equilibrium (low) level of supercoiling, because the supercoils generated in either direction by all but the first and last polymerases in the battery are being mutually annihilated within the battery. Each polymerase that functions as the leader of the pack at any given time introduces supercoils into downstream DNA only during its tenure as leading polymerase; as soon as the polymerase termintates transcription, it stops introducing supercoils into DNA and also stops relaxing the positive supercoils generated by its successor, which now becomes the leader. Thus, at any given time, gyrase molecules acting downstream are required only to relax the supercoils generated by one polymerase, regardless of the frequency at which the promoter initiates transcription (this is likely to be equally true of identically oriented genes organized in an operon or a cluster of operons, with no significant blocks to DNA rotation placed between constituent genes or operons). (Ib) The situation behind the last polymerase at any given time. The immediate last polymerase to have initiated transcription introduces negative supercoils into upstream DNA. Given some definite efficiency of quarantining of these supercoils in the region (by the blocks to rotational diffusion of torsion), and further, given some definite rate of relaxation of negative supercoils by topo I acting upstream of the promoter, each RNA polymerase would be required to perform only a definite length of transcriptional elongation in order to raise local superhelical density at the promoter to the level required for fresh initiation of transcription by the next polymerase. This leads to the periodic initiation of transcription and uniform spacing of polymerases along the transcription unit. (Ib and II) The effect of activator binding to an upstream activation sequence. The binding of the activator (after initiation of transcription by polymerase n+4) in (Ib) leads to a more efficient quarantining of negative supercoils in the promoter region and a quicker rise in local negative superhelical density accompanying elongation by polymerase n+4. This reduces the elongation required to be performed by each polymerase, resulting in an enhanced frequency of transcription by all polymerases following polymerase n+4, as shown in the new steady-state scenario in (II).

This situation is schematically represented in Fig. 1(Ia,b), depicting ongoing transcription with the aid of polymerases marked n+3 and n+4. As polymerase n+3 proceeds down the transcription unit, the promoter's topology changes continuously in response to accumulating supercoils. As soon as a particular topology is reached, a fresh holoenzyme, polymerase n+4, binds to the promoter to form a closed complex (in the Fig., changes in promoter topology are depicted by both the underwinding, or twist, of DNA and the formation of a negative supercoil, or writhe, in the DNA). If the energy required to melt promoter DNA at a particular moment is available, polymerase n+4 could quickly isomerize into the open complex. If not, it waits (with an 'off rate' of binding much lower than the 'on rate'), allowing more untwisting to accumulate. As soon as the required lowering of energy for DNA melting is achieved, the polymerase isomerizes into the open complex and makes the transition to the ternary complex and the elongation mode. The fresh generation of positive supercoiling accompanying this transition now neutralizes the negative supercoils driven upstream of polymerase

n+3, thus precluding any further influence of elongation by that polymerase on the promoter. The whole process then begins anew, with polymerase n+4 now aiding another polymerase, n+5, to initiate transcription; while, as described already, it has its own positive supercoils relaxed by the polymerase n+3. In this manner, the elongation step in transcription might regulate initiation. It is pertinent to note, however, that such a mode of cooperative initiation of transcription would be mainly relevant to promoters at which the rate of open complex formation is much slower than that of closed complex formation (suggesting a requirement for additional energy to melt DNA). Where the two rates are comparable, transcription-driven negative supercoils are unlikely to be required to initiate transcription, and each polymerase would probably initiate transcription quite independently of its predecessor. However, such supercoils would still be required for the relaxation of positive supercoils driven ahead of polymerases to follow.

The effect of changes in global supercoiling

Global changes in DNA supercoiling are thought to be wrought largely by growth transitions[55], as well as by the osmotically induced entry of ionic solutes into the cell[56]. These changes affect patterns of gene expression. Changes in the state of global supercoiling might shift the equilibrium of the isomerization reaction (characterizing open complex formation) upwards or downwards. Upon global increase of negative supercoiling, for instance, the amount of chain elongation required to be performed by polymerase n+3 to initiate transcription by polymerase n+4 would be reduced, because the energy (from transcription-driven negative supercoiling) now required for promoter melting by polymerase n+4 would have been effectively lowered due to increase in the basal level of negative supercoiling. This would result in the earlier initiation of transcription by polymerase n+4 (and every succeeding polymerase), because each predecessor polymerase would be required to perform a shorter length of elongation. Thus, the frequency of initiation at the promoter could be effectively increased.

The effect of protein factor binding to upstream sequences

A similar argument relating to an upshift of equilibrium supercoiling, may be used to explain the role that activator proteins play in initiating transcription 'at a distance'[57,58]. Activator proteins are thought to associate with other DNA-bound proteins in the neighbourhood of the promoter, to enhance the quantum of transcription occurring from the promoter. Let us consider, for the sake of simplicity, activators that bind upstream of a promoter at sequences known as upstream activation sequences (UAS; please see Fig. 1). The size of the activator protein, as well as the size, number and location of the other proteins with which it would tend to associate, would determine the ease with which DNA upstream of an active promoter would rotate in order to allow for the diffusion of waves of negative supercoiling propagating upstream. This is because association of an activator with another protein (which could be the polymerase itself) would result in the formation of a looped domain of DNA (not shown). Alternatively, the activator could cause DNA to loop around itself to form a looped domain (shown). Looped DNA domains are known to efficiently block rotational diffusion of torsion in DNA[41]. Blocking of the upstream propagation of supercoils would result in the more efficient accumulation of negative supercoils immediately upstream of a transcribing polymerase (given a steady rate of relaxation of negative supercoils by topo I). Since less of the supercoiling would be lost through rotational diffusion, and more of it used to raise local superhelical density, this could lower the length of DNA needed to be transcribed by polymerase n+4 to generate sufficient negative supercoiling, at the promoter, for polymerase n+5 to initiate transcription (Fig. 1,Ib and II). A really efficient way of achieving this would be to allow each 'RNA polymerase-in-waiting' (bound to the promoter as a closed complex) in turn to associate with the DNA-bound transcription factor, so as to form a DNA loop that would help such a polymerase to contain the very supercoils (originating from the immediate last polymerase to have initiated transcription) that would help it initiate transcription. In this regard, it is interesting that there is evidence now that transcription factors 'acting at a distance' do indeed interact with RNA polymerase[58].

Periodic initiation – revisiting unexplained observations

The model outlined above suggests that fresh initiation can occur at a strong promoter (with $K_f < K_B$) only when the previous polymerase has completed a certain length of transcriptional elongation. Thus, the model predicts that transcriptional initiation must display a definite rhythm or periodicity. Baker and Yanofsky[59] and Imamoto[60] discovered almost 25 years ago that transcriptional initiation from the *trp* operon in *E. coli* occurs in a distinctly periodic fashion, every 2.5 minutes. They concluded that each RNA polymerase probably must complete a certain length of transcription before fresh initiation could occur at the vacated promoter. A few years later, Contesse *et al.*[61] made similar observations with regard to the lac operon (periodicity of roughly 40 seconds). Baker and Yanofsky later indicated that their earlier estimates of periodicity were off by a factor of five, and that it was difficult to reproducibly observe periodic initiation, or to understand how it could possibly occur[62]. Miller and Hamkalo[63] examined spreads of bacterial DNA electron microscopically and could find no evidence for periodicity in the spacing of RNA polymerase molecules on transcriptionally active regions. However, one cannot be sure about whether they were observing individual structural genes or groups of genes. (In this connection, the 'christmas tree' structures observed in later studies of spread DNA might be considered to be particularly evocative of periodicity in RNA polymerase spacing).

Almost a decade after Baker and Yanofsky's first reports,

Maaloe[64] surmised that the notion of rhythm in the initiation of transcription had not found widespread acceptance, essentially because the experiments performed by Baker and Yanofsky were considered to have been very difficult to do, and the assumptions that had to be made to interpret the data were hard to accept. There was at that time no way of understanding how transcriptional elongation could possibly have any effect on initiation. Maaloe suggested, however, that the discussion of these old experiments be reopened, since the concepts they proposed had not ever actually been disproved. To the best of my knowledge, no such revisiting has ever been done since. Now, with the 'twin supercoiled domain' model helping to throw light on how the two-way cause-effect relationship between transcription and supercoiling might operate, the time may have come to examine new possibilities.

Implications and predictions

Several interesting predictions emerge from the issues discussed in this paper.

(1) The effect of transcriptional activity on the state of supercoiling of bacterial DNA must vary in direct proportion to the number of topologically segregated groups of transcriptionally active units contained within the bacterial genome, and not to (1) the absolute number of RNA polymerase elongation complexes performing transcription, (2) the absolute number of transcriptionally active genes and operons, or (3) the strength with which transcription occurs on any of the individual genes or operons.

(2) Increasing evidence of supraoperonic clustering of genes oriented in the same direction should be found. Such clustering (or sharing of resources) is crucial, if the various housekeeping genes of *E. coli* are to be transcribed at their requisite levels, in the face of what may be considered to be a paucity of (positive) supercoil-relaxing activity in the cell.

(3) Relocation of a gene from such a cluster of operons – to a relatively silent region of the genome containing no clusters or strong, neighbouring transcription units – could result in some reduction in the strength of transcription, under conditions that would normally have been expected to give rise to strong, constitutive transcription (i.e. strong positional effects should be observed). A relocated gene might expend too much effort in abortive initiations of transcription. Transcriptional silencing of relocated genes should increase with the increase in numbers of relocated genes, because this would spread out the available gyrase molecules left over from replication and 'cooperative' transcription even more thinly.

(4) Segments of DNA between RNA polymerases in a transcription battery, or between neighbouring active transcription units oriented in the same direction, are likely to be less topologically strained than silent regions of the genome. Silent regions are likely to undergo extensive supercoiling and compaction to form the structural core of the various supercoiled domains constituting the bacterial nucleoid[54]. Strongly transcribed regions of the genome are not likely to participate in the nearly 1,000-fold compaction of DNA that gives rise to most of the bacterial nucleoid; these regions are thus likely to remain at the surface of the nucleoid, extended into the cytoplasm in the form of large DNA superloops. There is evidence that this may be the case[65,66].

(5) If the mechanism of functioning of upstream activation sequences outlined in this paper is valid, the placing of two (or more) copies of such a sequence in tandem might result in much stronger activation of transcription. Since activators themselves are likely to be produced in small amounts[54], duplicating the genes coding for the activator proteins would also help to ensure that both copies of the upstream activation sequence in such an experiment are bound by activator protein.

(6) Initiation of transcription at a constitutively transcribing promoter must occur in a periodic fashion due to the positive influence of transcriptional elongation on initiation. Though we generally tend to describe the strength of a promoter in terms of the frequency at which it supports the initiation of transcription, we do not normally pay any attention to whether the frequency discussed is actually some average frequency at which initiation occurs stochastically, or whether transcriptional initiation actually occurs periodically with such a frequency. Experiments such as those performed by Baker and Yanofsky, Imamoto and Contesse *et al.* in the late 1960s and early 1970s[59-62] could be performed again with more sophisticated equipment and techniques. Miller-type spreads of transcriptionally active DNA[63] could be reexamined for periodicity in the spacing of polymerases.

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