



Actinomycin and DNA transcription

(actinomycin mechanism of action/transcriptional complex/melton/premelton)

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ABSTRACT Recent advances in understanding how actinomycin binds to DNA have suggested its mechanism of action. Actinomycin binds to a premelted DNA conformation present within the transcriptional complex. This immobilizes the complex, interfering with the elongation of growing RNA chains. The model has a number of implications for understanding RNA synthesis.

Understanding the mechanism of action of actinomycin D has long been a major goal in cancer research and in molecular biology. Actinomycin is a cyclic polypeptide-containing antibiotic that binds to DNA and inhibits RNA synthesis (1–4). It does this by interfering with the elongation of growing RNA chains by the RNA polymerase enzyme (5). Nucleolar (ribosomal) RNA synthesis is particularly sensitive to the presence of actinomycin, and this probably accounts for its pharmacological activity as well as its extreme toxicity to mammalian cells (6, 7).

Several years ago, we determined the three-dimensional structure of an actinomycin–deoxyguanosine complex by x-ray crystallography. The information obtained from this study suggested a model to understand the general features of how actinomycin binds to DNA (8–11). According to this model, the phenoxazone ring system on actinomycin intercalates between adjacent base pairs, while pentapeptide chains lie in the narrow groove of the B helix and form hydrogen bonds [in the case of d(pGpC) sequences] with guanine residues on opposite chains. Implicit in this model was the assumption that actinomycin binds to B-DNA or to a distorted form of B-DNA. The possibility that actinomycin might bind to some other discretely different DNA conformational state was not envisioned at that time.

Here, I propose a modification to our actinomycin–DNA binding model that allows one to understand its mechanism of action. I propose that actinomycin binds to a premelted DNA conformation—called β -DNA—found within the transcriptional complex. This acts to immobilize (or pin) the complex, preventing the elongation of growing RNA chains. The model has implications for understanding the early melting behavior of DNA and, along with this, suggests a mechanism to understand the formation of the RNA polymerase–promoter tight binding complex. Other possible implications are discussed.

Actinomycin–DNA Binding Model. Fig. 1*a* summarizes key structural features of my model to understand how actinomycin binds to DNA.

As in our earlier model, the phenoxazone ring system on actinomycin intercalates between (base paired) dinucleotide sequences of the type d(pGpX) (where X = C, T, A, or G), while pentapeptide chains lie in the narrow groove of the structure, hydrogen bonding [in the case of d(pGpC) sequences] to guanine residues on opposite chains. Although

the complex is approximately 2-fold symmetric, it is not *exactly* 2-fold symmetric; for this reason, actinomycin binds more tightly to one guanine residue (and, therefore, more tightly to one chain) than to the other.

The modification proposed here is that actinomycin binds to β -DNA, *not* to B-DNA. β -DNA is a discretely different DNA structural form inferred by our studies of drug intercalation. The structure is composed of repeating units called β structural elements. These are a family of hyperflexible base-paired dinucleotide structures, each possessing the same mixed sugar puckering pattern (i.e., C3' *endo* (3'–5') C2' *endo*) and having similar backbone conformational angles, but varying in the degree of base unstacking. Lower energy forms contain base pairs partially unstacked, while higher energy forms contain base pairs completely unstacked. Direct evidence for the existence of the β structural element—pinned by a series of simple intercalators—has been provided by our extensive crystallographic studies of model drug–nucleic acid complexes (see refs. 12 and 13 and refs. cited therein).

Fig. 1*b* shows the β -DNA structure stabilized by ethidium. The complex is an organized right-handed helical structure in which the β structural element plus the intercalator form the asymmetric unit of the helix (the coordinates and the helical parameters for this model complex can be found in ref. 13). This maximally elongated and unwound DNA duplex structure—pinned by ethidium at saturating concentrations—gives rise to neighbor-exclusion intercalative drug binding (14, 15).

Mechanism of Action of Actinomycin D. I next propose that β -DNA is a metastable obligatory structural intermediate in DNA melting. This concept readily leads to understanding the mechanism of action of actinomycin D.

Fig. 2*a* and *b* shows an electron micrograph of nucleolar genes undergoing very active transcription (16) and my interpretation of this process—indicating the mechanism of action of actinomycin.

Actinomycin binds to β -DNA found within the boundaries connecting double-stranded DNA with single-stranded DNA in the transcriptional complex. This immobilizes the complex, interfering with the elongation of growing RNA chains.

In extremely active genes such as these, RNA polymerases tend to lie in a close-packed arrangement along DNA. Interference with the movement of one polymerase by actinomycin is expected to affect the movement of other polymerases. This could explain why nucleolar RNA synthesis is so sensitive to actinomycin.

Complex Structural Nature of DNA Phase Boundaries. Why is β -DNA an obligatory structural intermediate in DNA melting? This reflects the complex nonlinear nature of the boundaries that connect double-stranded B-DNA (or A-DNA) with single-stranded DNA (12, 13). Two distinctly different sources of nonlinearity appear as DNA chains unwind, and these determine the sequence of conformational changes that occur along this boundary (see Fig. 3).

The first source of nonlinearity stems from changes in the sugar-pucker conformations (i.e., C2' *endo* \rightleftharpoons C3' *endo*).

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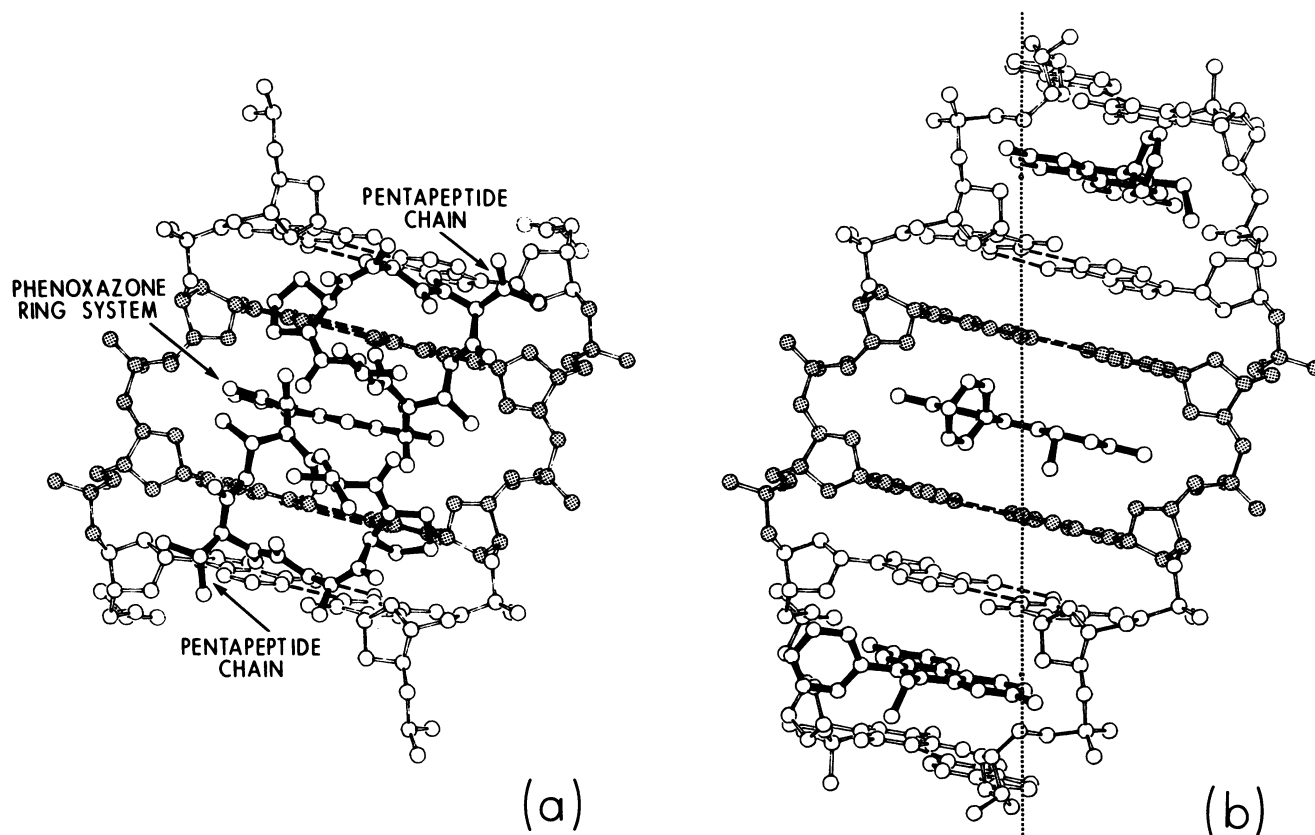


FIG. 1. (a) The actinomycin- β -DNA model complex. (b) β -DNA, "pinned" by ethidium. Heavy dashed lines indicate hydrogen bonds connecting base pairs. Stippled balls indicate atoms within a β structural element. For simplicity, only small fragments of the β -DNA structure are shown.

These changes require small energies (i.e., $\sim kT$) and appear as part of the initial structural distortions accompanying

DNA unwinding. Starting with B-DNA (or A-DNA), the effect of unwinding DNA is to counterbalance this with an

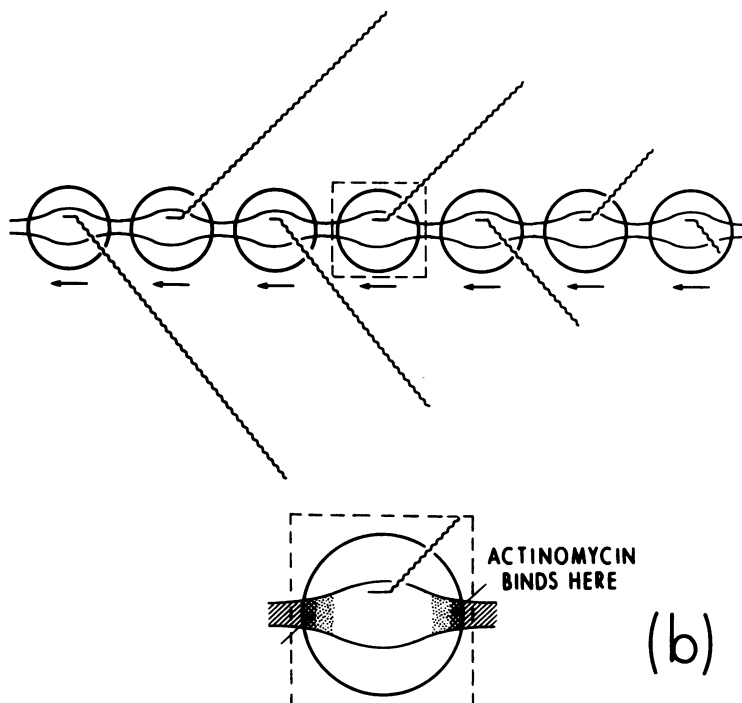
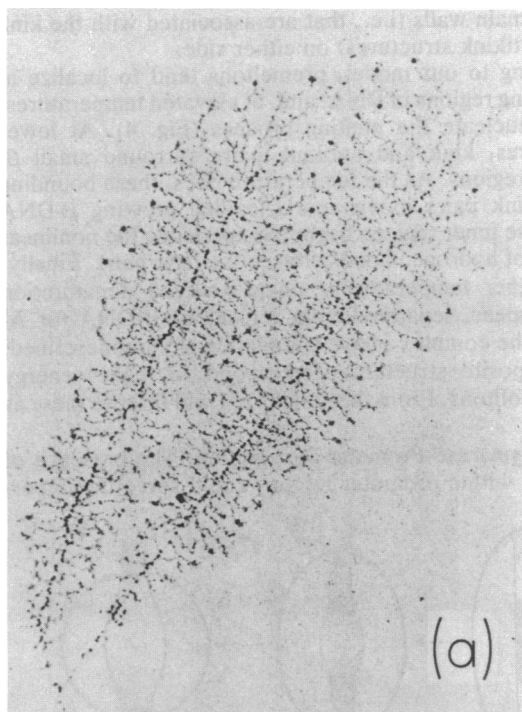


FIG. 2. (a) Electron micrograph of nucleolar genes undergoing transcription (16). (b) Interpretation of the micrograph in *a*, showing the proposed mechanism of action of actinomycin. Actinomycin binds to β -DNA, a conformational intermediate that exists within the boundaries connecting double-stranded B-DNA (or A-DNA) with single-stranded DNA in the transcriptional complex. This immobilizes the complex, interfering with the elongation of growing RNA chains.

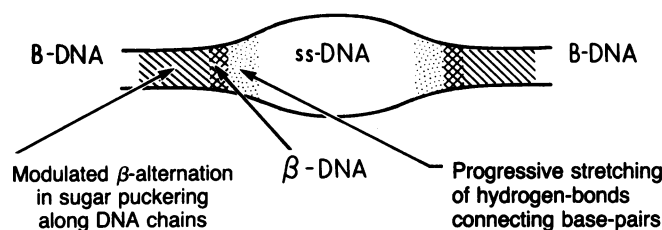


FIG. 3. Schematic illustration to show the complex nonlinear nature of the DNA phase boundaries within a transcriptional complex. Starting with B-DNA, the effect of unwinding DNA is to counterbalance this with an equal but opposite right-handed superhelical writhe. This gives rise to a modulated β alternation in sugar pucker in both chains, accompanied by a gradual partial unstacking of alternate base pairs (herringbone region). β -DNA arises as the end result of this series of conformational changes (cross-hatched region). Further unwinding causes the progressive stretching of hydrogen bonds between base pairs (stippled region). Eventually, this results in the disruption of base pairing and the appearance of single-stranded (ss) DNA.

equal but opposite right-handed superhelical writhe. This is achieved through a modulated β alternation in sugar pucker along the chains, combined with a gradual partial unstacking of alternate base pairs. β -DNA forms along this minimal energy pathway as an end result. This defines the first part of the complex phase boundary.

The second source of nonlinearity arises from the stretching and the eventual rupture of hydrogen bonds connecting base pairs. At first, the β -DNA structure is able to accommodate further unwinding through the gradual loss of superhelical writhe. This reflects the appearance of β structural elements having increasingly higher energy—these have base pairs further unstacked and unwound. Eventually, however, a limit is reached and further unwinding begins to cause the nonlinear stretching of hydrogen bonds connecting base pairs. Continued unwinding results in the disruption of these hydrogen bonds and in the appearance of single-stranded DNA. This series of conformational changes—involving the stretching and the disruption of hydrogen bonds connecting base pairs—corresponds to the second part of the complex phase boundary.

How Actinomycin Intercalates into Naked DNA Molecules. How does actinomycin intercalate into naked DNA molecules—DNA molecules not undergoing transcription?

Elsewhere, we have proposed that actinomycin intercalates into the centers of premeltons—these being examples of kink-antikink bound states in DNA structure (12, 13). [This entity is closely related to the “breather solitons” of the sine-Gordon equation, which is playing a significant role in superconducting device technology, nonlinear optics, and the theory of elementary particles (17, 18).] Such structural solitons arise spontaneously and contain a modulated β alternation in sugar pucker about the central β -DNA core region, which gradually merges into B-DNA (or A-DNA) on either side. We are uncertain, as yet, whether the centers of premeltons are open enough to accommodate an intercalator directly; however, structures such as these are known to have an intrinsic ability to undergo (concerted) low frequency breather motions. It is possible that such motions facilitate intercalation and other subsequent structural rearrangements that accompany actinomycin binding to DNA.

Relationship to DNA Melting. The stability of a premelton is expected to be sensitive to local base composition, temperature, pH, ionic strength, the presence of organic solvents, the extent of negative superhelicity, and other factors. It would also reflect the collective properties of nucleotide base sequences in extended DNA regions. Since the ease with which β structural elements form is expected to be

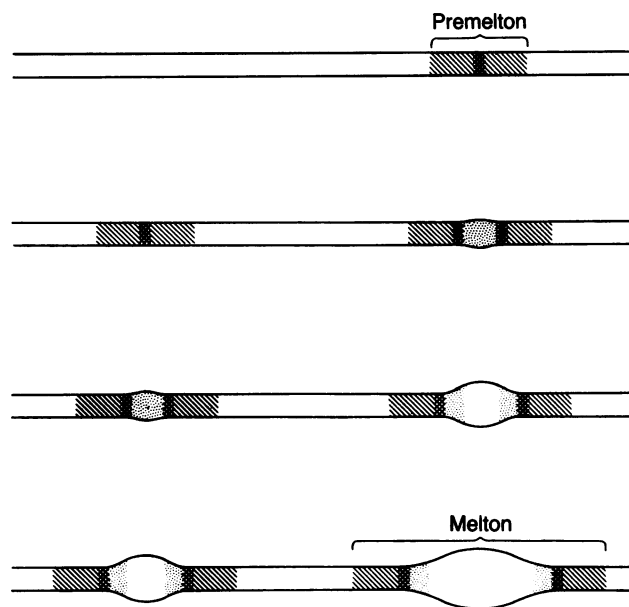


FIG. 4. Schematic illustration of DNA melting to show how premeltons become meltons with increasing temperature. The presence of premeltons in DNA structure at lower (premelting) temperatures permits actinomycin to intercalate into double-stranded DNA. Both the premelton and the melton are examples of structural solitons in DNA.

correlated with the magnitude of localized base-stacking energies, base sequences with minimal overlap (i.e., as occur, for example, in alternating purine-pyrimidine sequences) may be favored along with sequences that contain high A·T/G·C base ratios. The energetics in the kink and the antikink regions are another important factor. The tendency of a premelton to localize within a given DNA region would depend on the depth of the energy minimum in the central core region coupled with the height and separation of the energy domain walls (i.e., that are associated with the kink and the antikink structures) on either side.

According to our model, premeltons tend to localize at early melting regions in DNA, and, at elevated temperatures, serve to nucleate the melting process (Fig. 4). At lower temperatures, kink and antikink pairs surround small β -DNA core regions. As the temperature rises, these bounding kink-antikink pairs move apart, leaving growing β -DNA cores whose inner regions begin to experience the nonlinear stretching of hydrogen bonds connecting base pairs. Finally, at still higher temperatures, single-stranded denaturation bubbles appear, separated from regions of B-DNA (or A-DNA) by the complex phase boundaries already described. Such composite structures correspond to higher energy structural solitons. From this point on, I will refer to these as *meltons*.

RNA Polymerase–Promoter Recognition. The presence of premeltons within promoter regions could serve the impor-

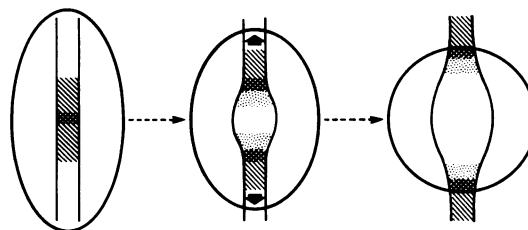


FIG. 5. A mechanism to understand the formation of the RNA polymerase–promoter tight binding complex.

tant purpose of providing nucleation centers for site-specific DNA melting by the RNA polymerase enzyme. For example, one can envision the formation of the transcriptionally competent (tight binding) complex to involve attachment by the polymerase to a premelton located at (or near) the start site for transcription, followed by a cascade of conformational changes within this complex that lead to the formation of a melton (Fig. 5). Such a process could be facilitated by the presence of negative superhelicity in DNA (19–22).

Recent experimental evidence indicates the presence of micrococcal nuclease hypersensitive sites in naked relaxed eukaryotic DNA molecules, many of these located at the 5' ends of genes (23, 24). These same sites are sensitive to cleavage by 1,10-phenanthroline-copper(I), a known intercalating agent. Addition of *Escherichia coli* single-strand-specific DNA binding protein to these DNA molecules made negatively superhelical melts DNA at (or very near to) these hypersensitive sites (25). These data suggest the presence of premeltons in these regions.

Pausing by the RNA Polymerase During Transcription. The ability of actinomycin to interfere with the elongation of growing RNA chains without causing premature chain termination suggests a possible relationship to the phenomenon of pausing by the RNA polymerase enzyme during transcription (26–28).

Since the ease with which a melton moves along DNA reflects the detailed isoenergetic behavior of coupled (melting and reannealing) processes occurring at the (leading and trailing) phase boundaries, transient departures from isoenergetics could interfere with movement. Such an effect could be sequence specific and give rise to pausing by the RNA polymerase during transcription.

Although it is conceivable that similar effects contribute to attenuation and termination during RNA synthesis, these are known to be more complex phenomena that involve RNA secondary structure and/or other protein factors (29, 30). For these reasons, I will not comment further about them.

Some Experimental Predictions. My model makes a number of testable experimental predictions. It predicts that actinomycin binds to β -DNA (i.e., not to B-DNA), and this can be checked by determining the structure of a suitable actinomycin-deoxyribonucleotide model complex by x-ray crystallography. It predicts that actively transcribed nucleolar genes contain large numbers of tight binding sites for intercalators; the number and distribution of these sites can be investigated with radioactively labeled photochemical probes (i.e., such as psoralen). The model further predicts these same sites to be sensitive to cleavage by 1,10-phenanthroline-copper(I). This can be studied in the presence of other intercalators. Ethidium, for example, could bind competitively to these sites and, therefore, protect DNA against cleavage by this agent. Such an effect would be readily detectable.

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