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CONFORMATIONAL CHANGES INDUCED BY SALT IN COMPLEXES OF HISTONES AND SUPERHELICAL NUCLEAR DNA

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SUMMARY

When HeLa cells are lysed in solutions containing a non-ionic detergent and 0·75 M-NaCl, structures are released that retain many of the morphological features of nuclei. These nucleoids contain all the nuclear DNA, RNA and the 'core' histones, but few other proteins characteristic of chromatin. Their DNA is intact. The core histones dissociate on raising the salt concentration. We have probed the structure of nucleoid–histone complexes using the intercalating dye, ethidium, or the RNA polymerase of *Escherichia coli*. Both have a higher affinity for superhelical DNA than they do for relaxed DNA. The binding of ethidium is measured fluorometrically, and using this probe we find that the DNA of nucleoids containing all the core histones behaves as if it were supercoiled slightly positively. As the salt concentration is increased, free energy characteristic of negative supercoiling appears between 0·92 M and 0·95 M-NaCl. This transition, which is reversible in the presence of the arginine-rich histones, occurs without dissociation of these histones from the DNA and so must reflect a conformational change in the complex. In contrast to the results with ethidium, we find that RNA polymerase can detect the presence of some negative free energy of supercoiling in nucleoids containing the core histones. The transformations of the free energy that can assist the binding of ethidium and RNA polymerase are discussed.

INTRODUCTION

The nuclear DNA of higher cells can now be prepared free of most cytoplasmic and nuclear material without being broken (Cook & Brazell, 1975, 1978; Cook, Brazell & Jost, 1976; McCready, Akrigg & Cook, 1979). Living HeLa cells are lysed in solutions containing a non-ionic detergent and 0·75 M-NaCl to release structures that resemble nuclei. These nucleoids contain all the nuclear RNA and DNA packaged within a cage of fibrous protein. The cage protects the fragile DNA so that nucleoids can be pipetted without breaking their DNA. They contain the 'core' histones (i.e. H2A, H2B, H3 and H4) but few other proteins characteristic of chromatin. When the NaCl concentration is increased from 0·75 M to 2·0 M these histones dissociate from the DNA. We describe here sensitive and rapid methods for monitoring the integrity and conformation of the DNA in nucleoids containing different types and amounts of histones. We are currently trying to reconstruct nuclei using nucleoids, and preliminary results using these novel methods are described in the accompanying paper (Levin & Cook, 1981).

We have probed the structure of nucleoid–histone complexes using the intercalating dye, ethidium, or the RNA polymerase of *Escherichia coli*. Both agents are

known to have a higher affinity for superhelical DNA than they do for relaxed DNA (Bauer & Vinograd, 1974; Wang, Jacobsen & Saucier, 1977). The binding of ethidium was measured fluorometrically; the fluorescence of the dye is enhanced when it binds to DNA so that the amount of fluorescence gives a convenient measure of the binding (Paoletti, Le Pecq & Lehmaṅ, 1971). However, ethidium binds to the RNA, as well as the DNA, in nucleoids and this binding is very sensitive to the salt concentration (for a review see Waring, 1970). Therefore any *complete* analysis of the effects of salt on the binding of ethidium to nucleoids would be very complicated. Nevertheless, a comparative approach permits us to study 2 aspects of histone binding in some detail. Bound proteins mask ethidium-binding sites so that we can measure the amount of bound histone by comparing nucleoids containing histones with those without histones (Angerer & Moudrianakis, 1972; Stratling & Seidel, 1976). In addition, any histone binding that alters the superhelical status of the DNA can be detected by a comparison of unirradiated and γ-irradiated nucleoids containing broken and so relaxed DNA (see Bauer & Vinograd, 1974, for a review of the interactions of ethidium with superhelical DNA). Both comparisons were made at the *same* salt concentration and this considerably simplifies the analysis.

MATERIALS AND METHODS

Nucleoids and nuclei

Techniques for isolating nucleoids from HeLa cells and for staining, counting, photographing and manipulating them have been described (Cook, Brazell & Jost, 1976).

Nuclei were prepared from HeLa cells after swelling them in 1·5 mM-$MgCl_2$, 0·1 mM-phenylmethylsulphonylfluoride, 1·0 mM-Tris (pH 8·0) at 4 °C for 15 min, adding Triton X-100 to a final concentration of 0·25 % (v/v) and then breaking them by homogenization. Sucrose was added to a final concentration of 0·25 M and the nuclei harvested and washed by centrifugation (1000 g; r_{av} = 10 cm; 10 min; 4 °C).

γ-irradiation

Supercoiling was removed from nucleoid DNA by breaking the DNA with γ-rays from a caesium 137 source (Cook & Brazell, 1976). Since high doses of radiation reduce the fluorescence of free dye, ethidium was always added to nucleoids after irradiation. A dose of 9·6 J kg^{-1} (delivered over a period of about 100 s) removes nearly all the supercoiling from the DNA of histone-free nucleoids (Cook & Brazell, 1975, 1978). The histones cannot protect the DNA from the effects of the radiation for 2 reasons. First, the fluorescence in 0·75 M-NaCl of mixtures of ethidium and histone-containing nucleoids (isolated in 0·75 M-NaCl) that have been irradiated with 9·6 or 96 J kg^{-1} are similar. Second, when the salt concentration surrounding these nucleoids irradiated in the presence of histones is raised to 2 M-NaCl, their fluorescence is indistinguishable from that of the same number of histone-free nucleoids irradiated in 2 M-NaCl (see also Table 2). In both cases the 10-fold higher dose has no additional effect, showing that the lower dose is sufficient to release all supercoils. In any case, these doses are orders of magnitude lower than those required to have direct effects on protein or RNA. Therefore a dose of 9·6 J kg^{-1} was used for all fluorometric experiments.

Spectrofluorometry

Nucleoid suspensions were diluted with 10 mM-Tris (pH 8·0), 0·1 mM-phenylmethylsulphonylfluoride and various concentrations of NaCl to $0{\cdot}2 \times 10^6$ nucleoids ml^{-1}. After the addition of ethidium (0·2 μg ml^{-1}) the fluorescence of ice-cold samples was determined (Cook

& Brazell, 1978). Control experiments showed that at 0·2 μg ml^{-1} the dye was in excess and that DNA–histone interaction was undisturbed.

Analysis of proteins

Proteins were separated by electrophoresis on polyacrylamide gel slabs containing sodium dodecyl sulphate (Kornberg & Thomas, 1975) and analysed by planimetry (Levin, Jost & Cook, 1978).

Transcription

Procedures for transcribing nucleoids with the RNA polymerase of *E. coli* (EC 2.7.7.6) have been described in fig. 3 and table 2 of Colman & Cook (1977).

RESULTS

Nucleoids containing different histones may be isolated by lysing living HeLa cells in various concentrations of salt and then freeing the released nucleoids from the dissociated proteins by sedimentation (Table 1). Nucleoids isolated in 1·95 M-NaCl contain no histones and few other proteins characteristic of chromatin (Levin, Jost & Cook, 1978). We used these as reference nucleoids. On the other hand, nucleoids isolated in 0·75 M-NaCl contain all the core histones.

Table 1. *The amount of histone in nucleoids isolated at different salt concentrations*

NaCl concn used during isolation (M)	Percentage of each histone in nucleoids			
	H1	H2A + H2B	H3	H4
Nuclei	100	100	100	100
0·4	20	100	100	100
0·75	0	95	96	95
1·00	0	22	57	60
1·20	0	12	41	40
1·95	0	0	0	0

Nucleoids were prepared from cells grown in [*methyl*-^{3}H]thymidine (56000 Ci mol^{-1}; 0·02 μCi ml^{-1}) for 24 h. Equivalent amounts of radioactivity (i.e. equal numbers of nucleoids) were applied to a polyacrylamide gel containing sodium dodecyl sulphate and the amount of a particular protein was estimated after electrophoresis as described in Materials and methods. The results are expressed as a percentage of the amount of that protein found in an equal number of nuclei.

We used ethidium to probe the structure of nucleoid–histone complexes by fluorometry. The binding of ethidium can be described in 2 ways. The relative fluorescence is the fluorescence of ethidium bound at a given salt concentration expressed as a percentage of that bound by the same number of unirradiated reference nucleoids in 2·0 M-NaCl; it is a direct measure of the amount of bound dye. Since histone-binding prevents the binding of the dye the relative fluorescence inversely reflects the amount of histone bound. The ratio is the relative fluorescence obtained with unirradiated nucleoids divided by that obtained with nucleoids that have been γ-irradiated and so

contain broken DNA; it reflects the sense and degree of supercoiling in the DNA of unirradiated nucleoids since only intercalative binding is affected by γ-irradiation. Ratios greater than unity indicate the presence of free energy of supercoiling associated with negative supercoiling (i.e. $\tau < 0$, using the nomenclature of Bauer & Vinograd (1974)).

The effects of salt on histone-free nucleoids

We first measured the effect of reducing the salt concentration on the reference nucleoids (i.e. histone-free nucleoids isolated in 1·95 M-NaCl). As the salt concentration is reduced the relative fluorescence increases as electrostatic binding of ethidium increases (Fig. 1A). More ethidium binds to unirradiated nucleoids than to their irradiated counterparts; the ratio is > 1 indicative of positive free energy of supercoiling in unirradiated DNA, which is able to assist intercalative dye-binding (Fig. 1B). This difference in binding diminishes as the salt concentration is reduced, reflecting both a salt-induced unwinding of the DNA, with a concomittant reduction in the amount of free energy of supercoiling available to assist dye-binding, and a decrease in the proportion of intercalative binding. In some of the experiments described in the accompanying paper (Levin & Cook, 1981) buffers contained guanidine thiocyanate. Therefore the effects of this chaotrope on the ratio are included here for comparison.

The effects of salt on nucleoids containing the core histones

Nucleoids isolated in 0·75 M-NaCl contain at least 90% of the core histones found in the nucleus (Table 1). Fig. 2A illustrates the ethidium-binding capacity of these nucleoids as the salt concentration is raised from 0·75 M to 2 M. The relative fluorescence of irradiated nucleoids decreases as the high concentrations suppress both electrostatic and intercalative binding.

In contrast to histone-free nucleoids, unirradiated nucleoids containing the core histones bind less ethidium than their irradiated counterparts (Fig. 2A); in 0·75 M-NaCl the ratio is slightly less than one (Fig. 2B). The ratio remains at or below unity as the salt concentration is increased, until between 0·92 M and 0·95 M it increases sharply reflecting an increase in dye-binding to unirradiated nucleoids (Fig. 2A). This transition occurs over a very narrow range – much narrower than the range in which the core histones dissociate from the DNA (i.e. 0·8–1·2 M) – suggesting that the histone–DNA complex is disrupted cooperatively with release of free energy without dissociation of the histones from the DNA.

The effects of salt on nucleoids containing histones H_3 and H_4

In 0·75 M-NaCl, the histone-free reference nucleoids possess free energy of supercoiling, which can assist intercalative dye-binding; in contrast, the nucleoids containing the histones possess none. Which, then, of the four core histones, removes this free energy? We can answer this question by using nucleoids isolated in 1·0 M-NaCl. These contain about 60% of the H_3 and H_4 found in the nucleus but only 20% of

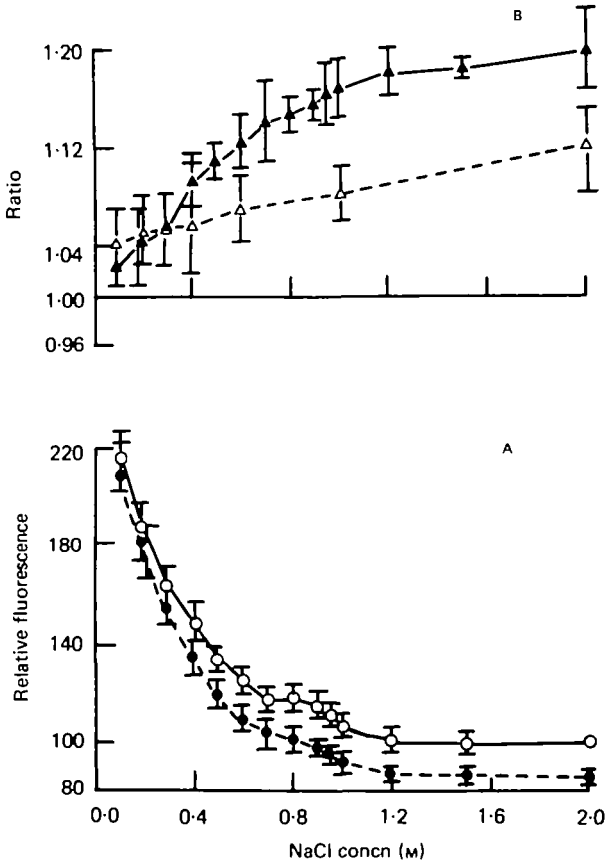

Fig. 1. The effect of salt on the binding of ethidium to irradiated and unirradiated nucleoids isolated in 1·95 M-NaCl. Nucleoids, isolated from step gradients containing 1·95 M-NaCl were diluted to $0{\cdot}2 \times 10^6$ ml^{-1} and various salt concentrations using 10 mM-Tris (pH 8·0) and the appropriate NaCl concn. Some nucleoids were irradiated, ethidium was added and the fluorescence measured. A. The fluorescence of unirradiated (○) or irradiated (●) nucleoids in the absence of histones is expressed as a percentage relative to that of unirradiated reference nucleoids in 2·0 M-NaCl. B. The ratio, obtained in the presence (△ or absence (▲) of 0·075 M-guanidine thiocyanate, is the relative fluorescence of unirradiated nucleoids divided by that of irradiated nucleoids. Error bars give the standard deviation of the means obtained from at least 6 different experiments like that described in A.

the H2A and H2B (Table 1). The ratio of such freshly isolated nucleoids is greater than one (Fig. 2C), and as the salt concentration is increased from 1 M-NaCl, the ratio increases slightly just as it does with the histone-free nucleoids. However it decreases sharply as the salt concentration is reduced. Salt affects these H2A- and H2B-depleted nucleoids in much the same way as it affects nucleoids containing all the core histones (cf. Fig. 2B), implying that it is H3 and H4 that remove the free energy of supercoiling, presumably by folding the DNA into nucleosomes.

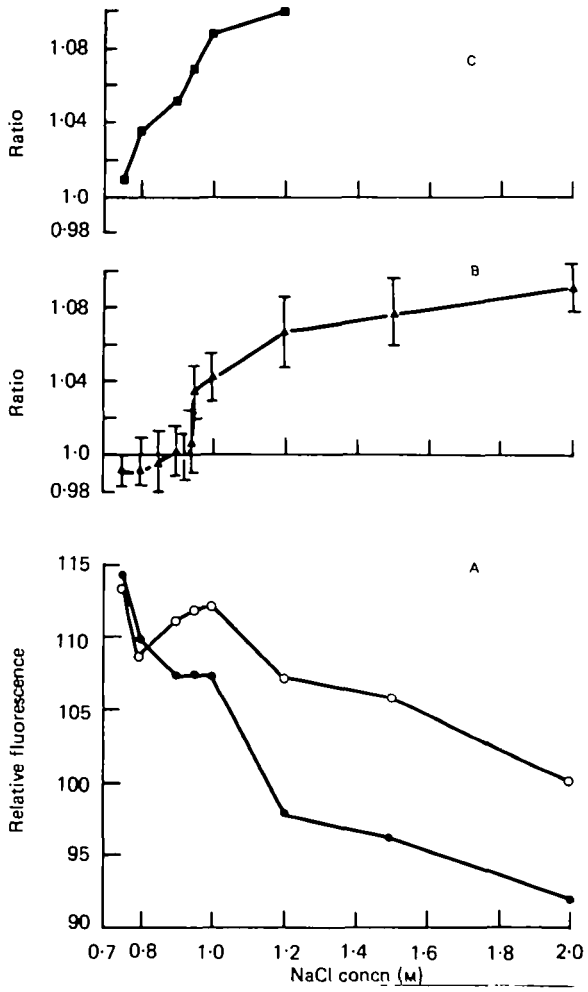

Fig. 2. The effects of salt on the amount of ethidium bound by unirradiated and irradiated nucleoids isolated in 0·75 M and 1·0 M-NaCl. A. The effects of increasing the salt concentration on the amount of ethidium bound by nucleoids isolated in 0·75 M-NaCl. 1 vol. of HeLa cells (80×10^6 ml^{-1}) in phosphate-buffered saline was added to 3 vol. of lysis mixture (0·75 M-NaCl). After 5 min the lysed cells were diluted 100-fold with 10 mM-Tris (pH 8·0), 0·1 mM-phenylmethylsulphonylfluoride and various concentrations of NaCl. Some nucleoids were irradiated, ethidium was added and the fluorescence measured. The fluorescence of unirradiated (○) and irradiated (●) nucleoids in 1 typical experiment is expressed as a percentage relative to the fluorescence of unirradiated reference nucleoids in 2·0 M-NaCl. B. Mean ratios (▲), obtained from at least 6 different experiments like that in A, are the relative fluorescences at the various salt concentrations of unirradiated nucleoids divided by that of irradiated nucleoids. Error bars give the standard deviation of the means. C. The effect of increasing or reducing the salt concentration on the amount of ethidium bound by nucleoids isolated in 1·0 M-NaCl. The ratio (■) is the relative fluorescence at the various salt concentrations of unirradiated nucleoids divided by that of irradiated nucleoids. Nucleoids isolated from step gradients containing 1 M-NaCl were diluted to $2{\cdot}4 \times 10^6$ ml^{-1} using 15 % sucrose, 1·0 M-NaCl and 10 mM-Tris (pH 8·0). Some nucleoids were irradiated. The mixture was diluted to $0{\cdot}2 \times 10^6$ nucleoids ml^{-1} and various concentrations of salt using 10 mM-Tris (pH 8·0) and the appropriate salt concentrations. Ethidium was added and the fluorescence measured.

Internucleosomal supercoiling

Benyajati & Worcel (1976) suggest that the DNA in *Drosophila* chromatin, isolated in 0·9 M-NaCl, is negatively supercoiled (i.e. $\tau < 0$) and not apparently positively supercoiled as we find. They interpreted their results in terms of negative supercoiling in *inter*-nucleosomal DNA and based a model of chromatin structure on this interpretation (Worcel & Benyajati, 1977). If the inter-nucleosomal DNA is negatively supercoiled in nucleoids isolated in 0·75 M-NaCl, reducing the salt concentration should remove supercoiling from inter-nucleosomal DNA in much the same way as it does from nucleoid DNA freed of all histones. It does not (Table 2): the ratio obtained with histone-free nucleoids isolated in 1·95 M falls significantly as the salt

Table 2. *A comparison of the effects of salt concentration on the binding of ethidium to histone-free nucleoids and their counterparts containing core histone*

Salt concentration used during isolation (M)	Nuclear core histones (%)	Ratio of relative fluorescences of nucleoids in different salt concentrations		
		2·00 M	0·75 M	0·25 M
1·95	None	1·21	1·15	1·05
0·75	90	1·18	0·98	0·975

Nucleoids isolated from step gradients containing 1·95 M or 0·75 M-NaCl were diluted to $0{\cdot}2 \times 10^6$ ml^{-1} and the appropriate salt concentrations. Some samples were irradiated before ethidium was added and the fluorescence measured. The ratio is the relative fluorescence of unirradiated nucleoids divided by that of irradiated nucleoids.

concentration is reduced from 0·75 M to 0·25 M, whereas the ratio obtained with the nucleoids isolated in 0·75 M-NaCl remains unchanged. At salt concentrations below 0·75 M the free energy of supercoiling seems immune to salt effects suggesting that the core histones lock *inter*- and *intra*-nucleosomal DNA in a stable configuration. The results of Benyajati & Worcel can be explained as easily by the disruption during preparation of a few nucleosomes to release negative supercoiling (see Fig. 2B); this released intra-nucleosomal supercoiling then appears to be internucleosomal.

Probing conformation with RNA polymerase

We have shown that the core histones remove free energy of supercoiling leaving none to assist dye-binding; do they also leave none to assist binding of RNA polymerase, which – like ethidium – has a higher affinity for superhelical DNA (Wang, Jacobsen & Saucier, 1977).

The DNA to which the RNA polymerase binds exists in two inter-convertible states, 'closed' and 'open' (Chamberlin, 1974). Supercoiling facilitates 'opening'. RNA polymerase can therefore be used to probe the question of whether or not there is any free energy of supercoiling available to open the sites. Only polymerase in open sites can initiate and escape inactivation by the inhibitor, rifampicin, and we used this

observation to measure the proportion of open sites. Unirradiated nucleoids isolated in 1·95 M-NaCl were pre-incubated with polymerase and synthesis started by the simultaneous addition of triphosphates and rifampicin (Table 3). These nucleoids direct RNA synthesis at 73% of the rate of their counterparts in the absence of rifampicin. A total of 73% of the sites are open. In contrast only 44% of the sites in unirradiated nucleoids isolated in 0·75 M-NaCl are open; the core histones remove free energy of supercoiling reducing the proportion of open complexes. Irradiation reduces the proportion of open sites in histone-free nucleoids from 73% to 38%. It also slightly reduces the proportion in histone-complexed nucleoids (from 44% to 32%); some free energy of supercoiling in the histone-complexed nucleoids, which is not available to assist dye-binding, can nevertheless open sites for the polymerase.

Table 3. *Irradiation* (163 $J\,kg^{-1}$) *affects RNA synthesis directed by histone-free and histone-complexed nucleoids*

Nucleoids	Irradiation	Relative synthesis (%)
Histone-free	−	73
Histone-free	+	38
Histone-complexed	−	44
Histone-complexed	+	32

The RNA synthesis directed by histone-free and histone-complexed nucleoids (isolated in 1·95 M and 0·75 M-NaCl, respectively) in the presence or absence of rifampicin was determined; the maximum rate of synthesis in the presence of the drug is expressed as a percentage of the maximum rate in its absence.

DISCUSSION

Base-pairs unpair when DNA functions, i.e. during transcription, replication and recombination. Supercoiling favours such an unpairing since the sense of the naturally occurring supercoils opposes that of the double-helix (Bauer & Vinograd, 1974). Therefore our template should prove useful for functional studies since it contains intact superhelical DNA. The fluorometric approach described here permits us to detect agents that alter free energy of supercoiling.

We have used 2 probes, ethidium and the RNA polymerase of *E. coli*, to monitor the superhelical status of nucleoid DNA. Both detect positive free energy of supercoiling associated with negative supercoils (i.e. $\tau < 0$) in the DNA of histone-free nucleoids. However these probes behave differently when the core histones are present: ethidium detects no negative supercoiling (i.e. $\tau \geqslant 0$) whereas RNA polymerase, although it detects that the majority of supercoiling has disappeared, can nevertheless detect that some remains. The experiments using H2A- and H2B-depleted nucleoids (Fig. 2c) implicate H3 and H4 as the histones that remove the negative supercoiling, presumably by folding the DNA into nucleosomes. This conclusion, which is in accord with studies by others using much simpler viral templates (Kornberg, 1977; Felsenfeld, 1978), is confirmed directly using our template, in the accompanying paper (Levin & Cook, 1981).

Free energy associated with negative supercoiling appears in nucleoid DNA over a very restricted range of salt concentrations (0·92–0·95 M) suggesting a highly co-operative transition. In principle, such a transition might result as H_3 and H_4 dissociate to leave their imprint, the supercoil and its associated energy, in the DNA. This clearly cannot be the case, since the DNA of nucleoids isolated in 1·0 M-NaCl is negatively supercoiled, even though H_3 and H_4 remain undissociated (Fig. 2c). The negative supercoiling disappears only when the salt concentration is reduced, suggesting that the reduction induces a conformational change in the DNA–histone complex together with the disappearance of the available free energy (Fig. 3). The complex of DNA with H_3 and H_4 must therefore exist in 2 conformations, one existing above 0·95 M-NaCl and the other below 0·92 M-NaCl (Fig. 3). Only the high-salt form contains energy that can be tapped to assist ethidium intercalation.

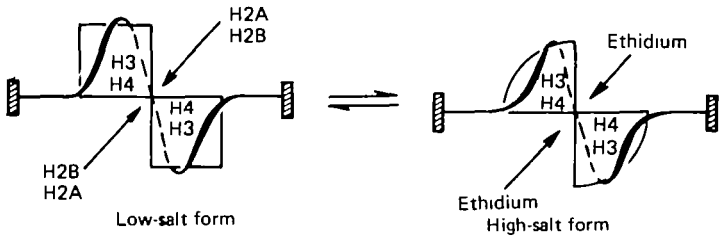

Fig. 3. Conformational states of complexes of the core histones and superhelical DNA. A DNA duplex containing 1 supercoil is constrained between the hatched rectangles. Complexes of histone H_3 and H_4 (the squares or quadrants), and the DNA can exist in 2 states, the low- and high-salt forms. Both complexes contain free energy additional to that found in their counterparts containing broken DNA. In the low-salt form that energy can be tapped by H2A and H2B to form a nucleosome; in the high-salt form it can be used to assist ethidium binding. The diagram is not drawn to scale and no precise disposition of the histones in the nucleosome is implied.

Such a conformational change might explain a puzzling observation made earlier. We found that H2A and H2B – but not H_3 and H_4 – dissociated more slowly from unirradiated nucleoids than irradiated nucleoids (Levin, Jost & Cook, 1978). If the conformational change and dissociation are separate events, any energy of folding by H_3 and H_4 need not necessarily influence their dissociation. Since it is the dissociation of H2A and H2B that is affected, their binding must be assisted by the latent free energy of supercoiling present in the low-salt form.

We have shown that the free energy of supercoiling can exist in a variety of guises each, in turn, assisting the binding of ethidium, H2A and H2B or RNA polymerase. Such transformations, which are so intimately involved with a kind of energy that can unpair bases, might determine the functional state of a gene (Cook, 1974; Akrigg & Cook, 1980). Either a relaxation of DNA, or the binding of an unwinding agent, could inactivate genes by removing the free energy of supercoiling required by an RNA polymerase. In this context it is interesting to note that the *lac* repressor unwinds the *lac* operator (Wang, Barkley & Bourgeois, 1974), and that nucleoid DNA

from inert hen erythrocytes and human sperm is relaxed (Cook & Brazell, 1976, and unpublished results).

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REFERENCES

AKRIGG, A. & COOK, P. R. (1980). DNA gyrase stimulates transcription. *Nucl. Acids Res.* **8**, 845–854.

ANGERER, L. M. & MOUDRIANAKIS, E. N. (1972). Interaction of ethidium bromide with whole and selectively deproteinized deoxynucleoproteins from calf thymus. *J. molec. Biol.* **63**, 505–521.

BAUER, W. & VINOGRAD, J. (1974). In *Basic Principles in Nucleic Acid Chemistry* (ed. P. O. P. Ts'o), vol. 2, pp. 265–303. New York and London: Academic Press.

BENYAJATI, C. & WORCEL, A. (1976). Isolation, characterization, and structure of the folded interphase genome of *Drosophila melanogaster*. *Cell* **9**, 393–407.

CHAMBERLIN, M. J. (1974). The selectivity of transcription. *A. Rev. Biochem.* **43**, 721–775.

COLMAN, A. & COOK, P. R. (1977). Transcription of superhelical DNA from cell nuclei. *Eur. J. Biochem.* **76**, 63–78.

COOK, P. R. (1974). On the inheritance of differentiated traits. *Biol. Rev.* **49**, 51–84.

COOK, P. R. & BRAZELL, I. A. (1975). Supercoils in human DNA. *J. Cell Sci.* **19**, 261–279.

COOK, P. R. & BRAZELL, I. A. (1976). Conformational constraints in nuclear DNA. *J. Cell Sci.* **22**, 287–302.

COOK, P. R. & BRAZELL, I. A. (1978). Spectrofluorometric measurement of the binding of ethidium to superhelical DNA from cell nuclei. *Eur. J. Biochem.* **84**, 465–477.

COOK, P. R., BRAZELL, I. A. & JOST, E. (1976). Characterization of nuclear structures containing superhelical DNA. *J. Cell Sci.* **22**, 303–324.

FELSENFELD, G. (1978). Chromatin. *Nature, Lond.* **271**, 115–122.

KORNBERG, R. D. (1977). Structure of chromatin. *A. Rev. Biochem.* **46**, 931–954.

KORNBERG, R. D. & THOMAS, J. O. (1975). An octamer of histones in chromatin and free in solution. *Proc. natn. Acad. Sci. U.S.A.* **72**, 2626–2630.

LEVIN, J. M. & COOK, P. R. (1981). Reconstruction of complexes of histone and superhelical nuclear DNA. *J. Cell Sci.* **50**, 209–224.

LEVIN, J. M., JOST, E. & COOK, P. R. (1978). The dissociation of nuclear proteins from superhelical DNA. *J. Cell Sci.* **29**, 103–116.

MCCREADY, S. J., AKRIGG, A. & COOK, P. R. (1979). Electron microscopy of intact nuclear DNA from human cells. *J. Cell Sci.* **39**, 53–62.

PAOLETTI, C., LE PECQ, J.-B. & LEHMAN, I. R. (1971). The use of ethidium bromide–circular DNA complexes for the fluorometric analysis of breakage and joining of DNA. *J. molec. Biol.* **55**, 75–100.

STRATLING, W. H. & SEIDEL, I. (1976). Relaxation of chromatin structure by ethidium bromide binding: determined by viscometry and histone dissociation studies. *Biochemistry* **15**, 4803–4809.

WANG, J. C., BARKLEY, M. D. & BOURGEOIS, S. (1974). Measurements of unwinding of *lac* operator by repressor. *Nature, Lond.* **251**, 247–249.

WANG, J. C., JACOBSEN, J. H. & SAUCIER, J. M. (1977). Physiochemical studies on interactions between DNA and RNA polymerase. Unwinding of the DNA helix by *Escherichia coli* RNA polymerase. *Nucl. Acids Res.* **4**, 1225–1241.

WARING, M. (1970). Variation of the supercoils in closed circular DNA by binding of antibiotic and drugs: evidence for molecular models involving intercalation. *J. molec. Biol.* **54**, 247–279.

WORCEL, A. & BENYAJATI, C. (1977). Higher order coiling of DNA in chromatin. *Cell* **12**, 83–100.

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