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Facilitated diffusion of a DNA binding protein on chromatin

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Facilitated diffusion accounts for the rapid rate of association of many bacterial DNA binding proteins with specific DNA sequences *in vitro*. In this mechanism the proteins bind at random to non-specific sites on the DNA and diffuse (by 'sliding' or 'hopping') along the DNA chain until they arrive at their specific functional sites. We have investigated whether such a mechanism can operate in chromatin by using a bacterial DNA binding protein, *Escherichia coli* RNA polymerase, that depends on linear diffusion to locate initiation sites on DNA. We have measured the competition between chromatin and its free DNA for the formation of initiation complexes. Only the short linker segments exposed by the removal of histone H1 are available for interaction with the polymerase, but the sparsely distributed promoter sites on the linker DNA of such a polynucleosome chain are located at the same rate as those on DNA. We conclude that the polymerase is free to migrate between the separate linker DNA segments of a polynucleosome chain to reach a promoter site. This chain thus permits the 'hopping' of proteins between neighboring linker segments in their search for a target site on the accessible DNA.

Key words: facilitated diffusion/chromatin/RNA polymerase

Introduction

Bacterial DNA binding proteins as diverse as the *lac* repressor (Berg *et al.*, 1981; Winter *et al.*, 1981; Winter and Von Hippel, 1981; Von Hippel *et al.*, 1982), RNA polymerase (Chamberlin *et al.*, 1982; Belintsev *et al.*, 1980; Park *et al.*, 1982) and the restriction endonuclease *Eco*RI (Jack *et al.*, 1982), locate specific DNA sequences *in vitro* by facilitated diffusion, whereby the search is confined within the molecular domain rather than throughout the bulk solution. We have used *Escherichia coli* RNA polymerase as a test protein to discover whether a similar mechanism can operate in chromatin and whether there are permissive and non-permissive states of chromatin structure with respect to such migration. This is of particular interest in view of the suggestion (Wasylyk *et al.*, 1983) that linear diffusion of putative RNA chain initiation factors might be implicated in the function of enhancer sequences in eukaryotes. The long range action of enhancers (Moreau *et al.*, 1981) would require diffusion of the proteins through chromatin structure.

Our approach to studying facilitated diffusion in chromatin has been to measure the competition between chromatin and naked DNA for formation of initiation complexes with the enzyme. *E. coli* RNA polymerase has been used for these studies, rather than the cognate enzyme, because of the abundant evidence that its search for initiation sites is governed by facilitated diffusion. No such evidence exists for the eukaryotic polymerase. The initiation of RNA chains is confined to sites in the linker regions of chromatin (Williamson and Felsenfeld, 1978; Wasylyk *et al.*, 1979; Hannon *et al.*, 1984), which are short relative both to the separation between promoter sites (Cedar and Felsenfeld, 1973; Hannon *et al.*, 1984) and the mean free diffusional path of the enzyme on DNA (Belintsev *et al.*, 1980) in the conditions of initiation complex formation *in vitro*. The enzyme must therefore on average explore a considerable number of linker segments before finding a promoter site. We demonstrate that restricted diffusion of RNA polymerase on chromatin would be expected to lead to a greatly reduced efficiency of initiation on the linker segments by comparison with the situation on free DNA. Since we observe no such difference, we must infer that the diffusional path length is not dissimilar in chromatin to that on naked DNA and that the enzyme can move freely between linker segments without hindrance from the nucleosome cores.

Results

Kinetics of initiation complex formation and polymerase inactivation in vitro

The kinetics of initiation complex formation on chromatins of differing structure and on the corresponding free DNA are illustrated in Figure 1a. For these experiments we used chicken erythrocyte chromatin fragments in the native state and depleted of histones H1 and H5; in the depleted chromatin the spacing of the nucleosome cores is preserved, but the solenoid structure is lost (Allan *et al.*, 1980a, 1981). This material was also recombined with histone H1 at ratios of one or two molecules of H1 per nucleosome, which regenerates the chromatin solenoid (Allan *et al.*, 1981). Chromatin or DNA samples were incubated for varying times with *E. coli* RNA polymerase in conditions that allow the formation of initiation complexes but not the elongation of nascent chains (Hannon *et al.*, 1984). Promoter sites were in excess over enzyme molecules, except in the case of the H1-saturated complex containing two molecules of H1 per nucleosome, for which an excess would require unattainable chromatin concentration (Hannon *et al.*, 1984). It is an important condition for the interpretation of the results that the incubations of H1-depleted chromatin do not lead to extensive sliding of the nucleosome cores and hence to exposure of long stretches of free DNA. We have established that this does not occur by examination of the fragments resulting from digestion with micrococcal nuclease (Hannon, 1986).

The kinetics of initiation on the erythrocyte DNA are not dissimilar to those on typical weak bacterial promoters (McClure, 1980). An apparent second order rate constant of $\sim 10^6$ M^{-1} s^{-1} is calculated from the data in Figure 1a. The rate of initiation on the linker histone-depleted chromatin is seen to be scarcely lower than on bare DNA. For complexes containing one molecule of H1 per nucleosome, the rate was reduced by $<50\%$ of that in the total absence of H1. As expected from our previous observations (Hannon *et al.*, 1984), the complex containing two molecules per nucleosome of H1 was inert in this assay; all initiation

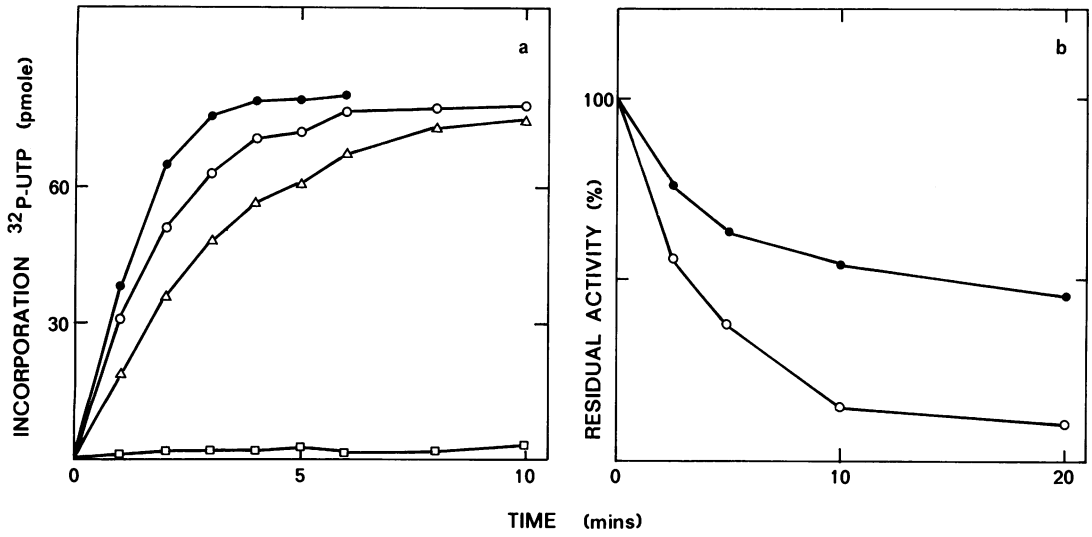

Fig. 1. Kinetics of initiation complex formation and RNA polymerase inactivation *in vitro*. **(a)** Initiation complex formation. Initiation complexes were set up, as described in the text and Materials and methods. 20 μg DNA (●), 100 μg H1- and H5-depleted chicken erythrocyte chromatin, average chain length 56 nucleosomes (○) and the depleted chromatin, recombined with histone H1 at ratios of one (△) or two (□) molecules per nucleosome, were incubated with 8 units of *E. coli* RNA polymerase holoenzyme in 1.6 ml of the initiation mixture. At the times indicated, 100 μl were withdrawn and the extent of initiation was assayed by elongation of the nascent RNA chains at 0.8 M NaCl, as described in Materials and methods and in Hannon *et al.* (1984). **(b)** RNA polymerase inactivation. *E. coli* RNA polymerase holoenzyme (8 units) was incubated at 37°C in 1 ml of the initiation mixture with (●) or without (○) 100 μg/ml transcriptionally inert chromatin (the H1- and H5-depleted chicken erythrocyte chromatin) recombined with bovine liver H1° at a ratio of two molecules per nucleosome (Hannon *et al.*, 1984). At the times indicated 200 μl aliquots were withdrawn, 10 μg DNA was added and residual polymerase allowed to initiate on the DNA for a further 15 min at 37°C. The extent of initiation was determined by elongation of nascent RNA chains at 0.8 M NaCl, as before.

sites are occluded, and the rate of complex formation is consequently immeasurably small.

To evaluate the consequences of enzyme inactivation in our incubation conditions, we incubated the polymerase alone and in the presence of either bovine serum albumin at 10 μg/ml or the inert, H1-saturated, chromatin complex for varying times and then measured the enzyme activity after introduction of a DNA template. The results (Figure 1b) reveal that the enzyme becomes inactivated at a significant rate, but that the polymerase is protected by the presence of chromatin and bovine serum albumin (not shown). Comparable results were obtained by other workers with bovine serum albumin (Kadesch *et al.*, 1982). When chromatin was present, some 50% of the activity remained at the end of the incubation. Thus in the reactions shown in Figure 1a, a high proportion of the polymerase molecules find initiation sites on all the templates, except for that containing two H1 molecules per nucleosome. The inactivation of enzyme could account for only a minor part of the difference in apparent rates of initiation on the different templates, but these in any case were small.

Kinetics of promoter site location

Effect of nucleosome structure and polynucleosome chain length. Direct measurements of initiation kinetics, as in the experiment described above, could reveal differences in the rates of location of promoter sites only if this is the rate-limiting step in initiation. The small differences between the different templates may signify that a subsequent step is rate limiting. To isolate for study the process by which the enzyme searches for its initiation sites, we have employed a competition assay. This approach depends for its validity on the following assumptions. (i) The facilitated diffusion mechanism, that has been shown to operate for the enzyme on bacterial DNA (Zavriev and Belintsev, 1979; Belintsev *et al.*, 1980; Park *et al.*, 1982), owing to its nature (Von Hippel *et al.*, 1982), must operate equally on the eukaryotic duplex. (ii) The heterogeneous initiation sites on the linker DNA fraction of chromatin do not differ in character from those of the total population present in naked DNA, so that the probability of forming a stable complex on encountering an initiation site does not differ between the two templates.

Pairwise competition assays were set up between H1- and H5-depleted chicken erythrocyte chromatin and an equimolar amount (in total nucleotide content) of the extracted DNA. Since our procedure involved separation of chromatin and DNA by sucrose gradient centrifugation before the initiation assays, it was necessary to use relatively homogeneous populations of chromatin fragments. These were obtained by sucrose gradient sedimentation of the total population of chromatin fragments released from cell nuclei by micrococcal nuclease digestion before removal of H1 and H5 (Allan *et al.*, 1980a, 1981).

The effect of nucleosome structure and of polynucleosome chain length was examined, using chromatin fragments with an average chain length of 12, 30 and 67 nucleosomes. Competition experiments were set up between the chromatin and the corresponding extracted DNA and also between the two chromatins with chain lengths of 12 and 67 nucleosomes. The distributions of initiation complexes in the sucrose gradients used to separate these mixtures are shown in Figure 2a–d. In the chromatin plus DNA mixtures a ratio of initiation complexes of 1:3.3 ± 0.4 in favor of DNA was found. In the case of the polynucleosome chains of differing lengths (Figure 2c), the ratio was 1:0.9, consistent with the results of competition between DNA and each of the two chromatin samples. Since the proportion of initiation sites in chromatin, reflecting the fraction of the DNA in the linker regions, is 0.16 (Hannon *et al.*, 1984), it follows that all templates form initiation complexes in proportion to the total amount of available DNA in each chain, notwithstanding that the linkers are separated into short segments of DNA by the nucleosome structure. There is no detectable effect of polynucleosome chain

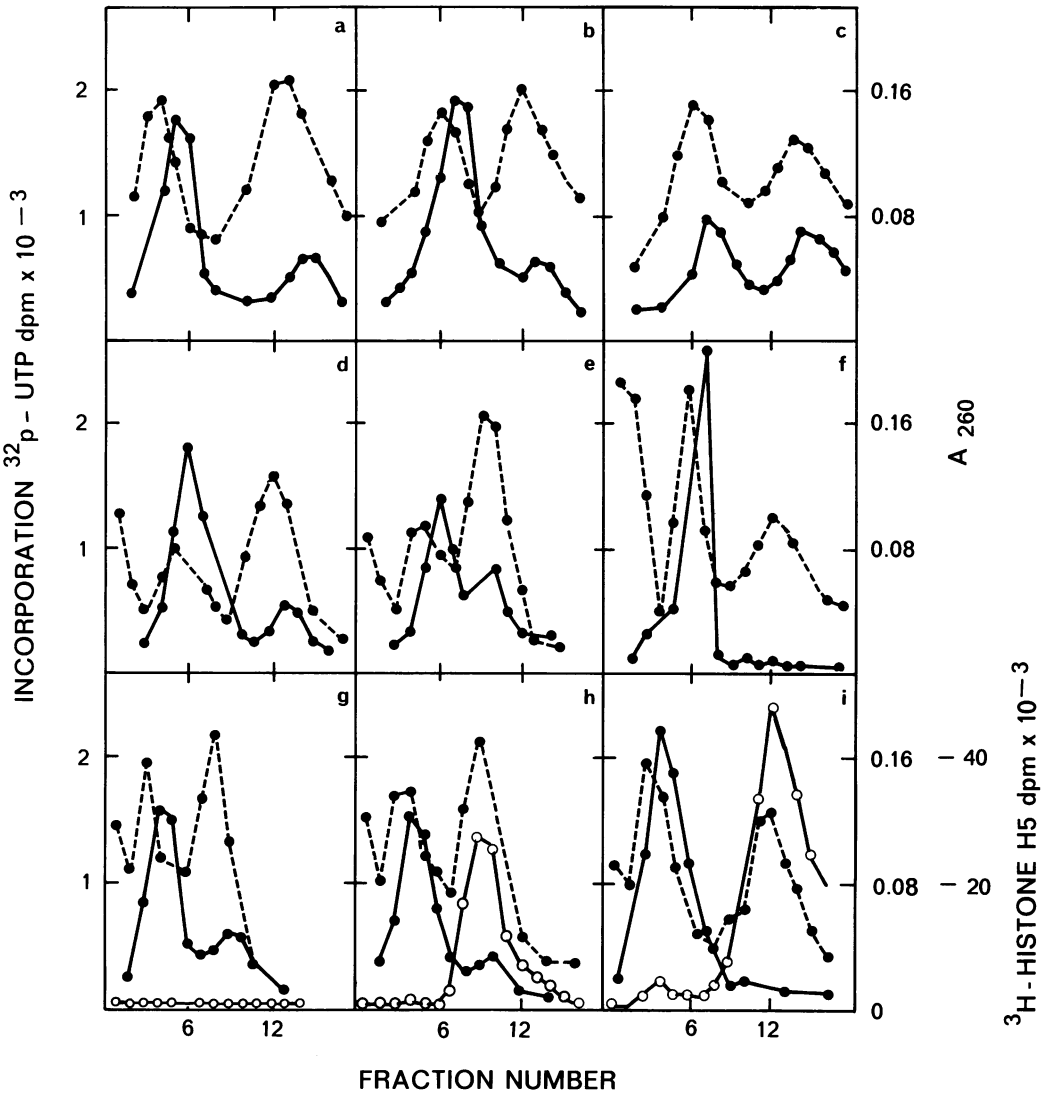

Fig. 2. Competition between chromatin and DNA for formation of chromatin complexes with *E. coli* RNA polymerase holoenzyme. The distribution of initiation complexes on competing templates was assayed after sucrose gradient sedimentation (from left to right): **(a−d)** effect of nucleosome structure and polynucleosome chain length; **(d−f)** effect of variation in linker length; **(g−i)** effect of histone H5 content. **(a)** 25 μg of H1- and H5-depleted chicken erythrocyte chromatin of 67 nucleosomes average chain length with 20 μg of the extracted DNA. Sedimentation for 90 min. **(b)** 25 μg of H1- and H5-depleted chicken erythrocyte chromatin of 12 nucleosomes average chain length with 20 μg of the extracted DNA. Sedimentation for 210 min. **(c)** 25 μg each of the H1- and H5-depleted chicken erythrocyte chromatin samples with average chain lengths of 12 and 67 nucleosomes. Sedimentation for 110 min. **(d)** 20 μg of histone H1- and H5-depleted chicken erythrocyte chromatin of 30 nucleosomes average chain length and 10 μg of the extracted DNA. Sedimentation for 150 min. **(e)** 25 μg of the same material after removal of the core histone tails by treatment with trypsin and 10 μg of the extracted DNA. Sedimentation for 150 min. **(f)** 15 μg of H1-depleted bovine cerebral cortex chromatin of average chain length 12 nucleosomes, with 20 μg of erythrocyte DNA from chromatin of the same length. Sedimentation for 180 min. **(g)** 20 μg of H1- and H5-depleted chicken erythrocyte chromatin of 30 nucleosomes average chain length and 20 μg of the extracted DNA. Sedimentation for 90 min. **(h)** 20 μg of the same chromatin, recombined with ^{3}H-labelled histone H5 at a ratio of one molecule per nucleosome, with 20 μg of the extracted DNA. Sedimentation for 90 min. **(i)** 20 μg of the same chromatin, recombined with the ^{3}H-labelled H5 ratio of two molecules per nucleosome, with 20 μg of the extracted DNA. Sedimentation for 90 min. In all cases the direction of sedimentation is from left to right. Absorbance at 260 nm (..), [^{32}P]UTP incorporation (—●—); labelled histone H5 (O).

length in the range tested. The nature of our assay precludes the examination of shorter chains.

Effect of variation in linker length. The natural range of variations in linker length is 0−72 bp, calculated by subtracting the constant core DNA length (168 bp in the ionic conditions of our assay — Weischet *et al.*, 1979) from the reported nucleosome periodicities, which fall in the range 168−240 bp (Compton *et al.*, 1976). Linker histone-depleted chromatins from different sources were prepared to cover this range of linker lengths. In Figure 2d−f it is seen that the ratio of initiation complexes formed on chromatin to those on DNA increases with linker length. The probability of formation of an initiation complex, expressed as the ratio of complexes formed by each chromatin, relative to free DNA, is plotted against linker length in Figure 3a. A linear relation is obtained. These results show that the efficiency of location of promoter sites is independent of linker length down to a very low limit.

Effect of linker histone content. Linker histone may be able to redistribute between the DNA and chromatin chains on the timescale and conditions of the experiment. Accordingly, we labelled the histone, before recombining it in varying ratios with the depleted chromatin. In agreement with earlier data (Thomas and Rees, 1983), we found that it was not transferred to DNA to a detectable extent (Figure 2g−i). The ratio of initiation com-

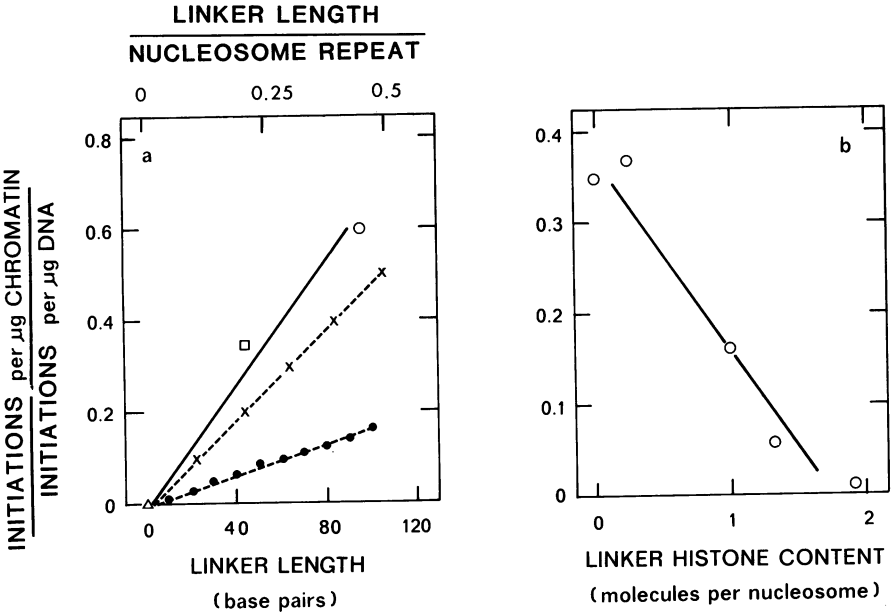

Fig. 3. Effects of variation in linker length and histone H5 content on the kinetics of RNA polymerase location onto promoter sites in chromatin. **(a)** Variation in linker length. The experimental results obtained in Figure 2 (d–f) are shown by the open symbols: (△) cerebral cortex chromatin of linker length < 5 bp, (□) chicken erythrocyte chromatin of linker length 44 bp, (○) the latter chromatin lacking core histone tails, with a mean interparticle 'linker' of 90 bp. The dashed curves represent the predicted results based on the simulated random walks on chromatin with two different boundary conditions. Curve (x) represents the ratio of initiation complexes on chromatin competing with DNA if linear diffusion on the linker DNA is not limited by the nucleosome cores and curve (●) if it is. **(b)** Variation in histone H5 content. The experimental results obtained in Figure 2 (g–i) are summarized.

plexes on the chromatin relative to those on DNA is seen to decrease with increasing concentration of histone H5. There is, however, a roughly parallel decrease in the number of available initiation sites, so that the correction for masked DNA accounts for the observed decrease in the competition ratios. This suggests that the location of the remaining sites is not appreciably impeded by the higher-order structure locally induced by histone H5. We exclude that the polymerase may select a sub-population of the polynucleosome chains from the form of the sedimenting boundaries of the initiation complexes, which are identical to those of the bulk chromatin. Sedimentation rate is a sensitive measure of H5 content and the accompanying condensation of chromatin (Hannon *et al.*, 1984).

Discussion

The starting point for the present study was the suggestion by Chambon and co-workers (Wasylyk *et al.*, 1983) that enhancer sequences in eukaryotes may act as bi-directional entry sites for rate-limiting transcription factors in chromatin. The mechanism of linear diffusion, by which bacterial DNA binding proteins locate specific sites on DNA, was invoked by these authors to explain how eukaryotic transcription factors migrate from their entry sites to the relevant promoters in chromatin. No other current model for the mechanism of enhancers so simply explains the behavior of enhancers, in particular, their action over distances of up to several kb on the nearest promoter site, regardless of the direction along the DNA or the orientation of the promoter sequence relative to that of the enhancer. These properties do not fit an alternative model, for example, in which transcription factor(s), bound at the enhancer, are brought into direct contact with a promoter site as a result of DNA (or chromatin) looping (Dynan and Tjian, 1985). We note that in the published models for the enhancer mechanism, the DNA between the enhancer and promoter is represented as being free of histones. This is probably because the chromatin structure, that is known to be present, is an embarrassment in the 'sliding' model and an encumbrance in the 'bending' model.

The most striking conclusion that emerges from our observations is the wholly unexpected efficiency with which the enzyme finds its initiation sites on chromatin. The kinetics are essentially identical to those on DNA, taking the substrate chain length as the sum of the inter-nucleosomal linkers. Accepting the evidence in favor of facilitated diffusion of the enzyme on DNA, it can scarcely be doubted that the enzyme operates according to the same mechanism on the polynucleosome chain. It should be stressed that this conclusion is independent of any heterogeneity (such as must be expected) in the affinity of different promoter sites for the enzyme, so long as the population uncovered in the chromatin is not unrepresentative of the total.

It is noteworthy that initiation sites on the linker DNA, regardless of their positions relative to the nucleosome cores, are available to the enzyme, even though both non-specific (Revzin and Woychik, 1981) and specific (Simpson, 1982) complexes occupy some 40 bp, i.e. a stretch of DNA that is in general longer than the linker itself. Considering the relative dimensions of the enzyme and of the nucleosome core (illustrated in Figure 4) and the close proximity of adjoining cores, steric crowding might be expected, but there is no indication of such an effect.

We have now to consider whether the diffusional search for a promoter site could be confined within single linker segments, or whether the enzyme can move freely over more than one linker in the course of a single 'sliding' or 'hopping' excursion.

The competition assay makes no assumptions about the nature of the rate-limiting step in initiation. Consider the extreme case of competition between a very long and a very short DNA chain. In the limit of a very short chain, assuming effectively random distribution of promoter sites in the nucleosome sequence, nearly

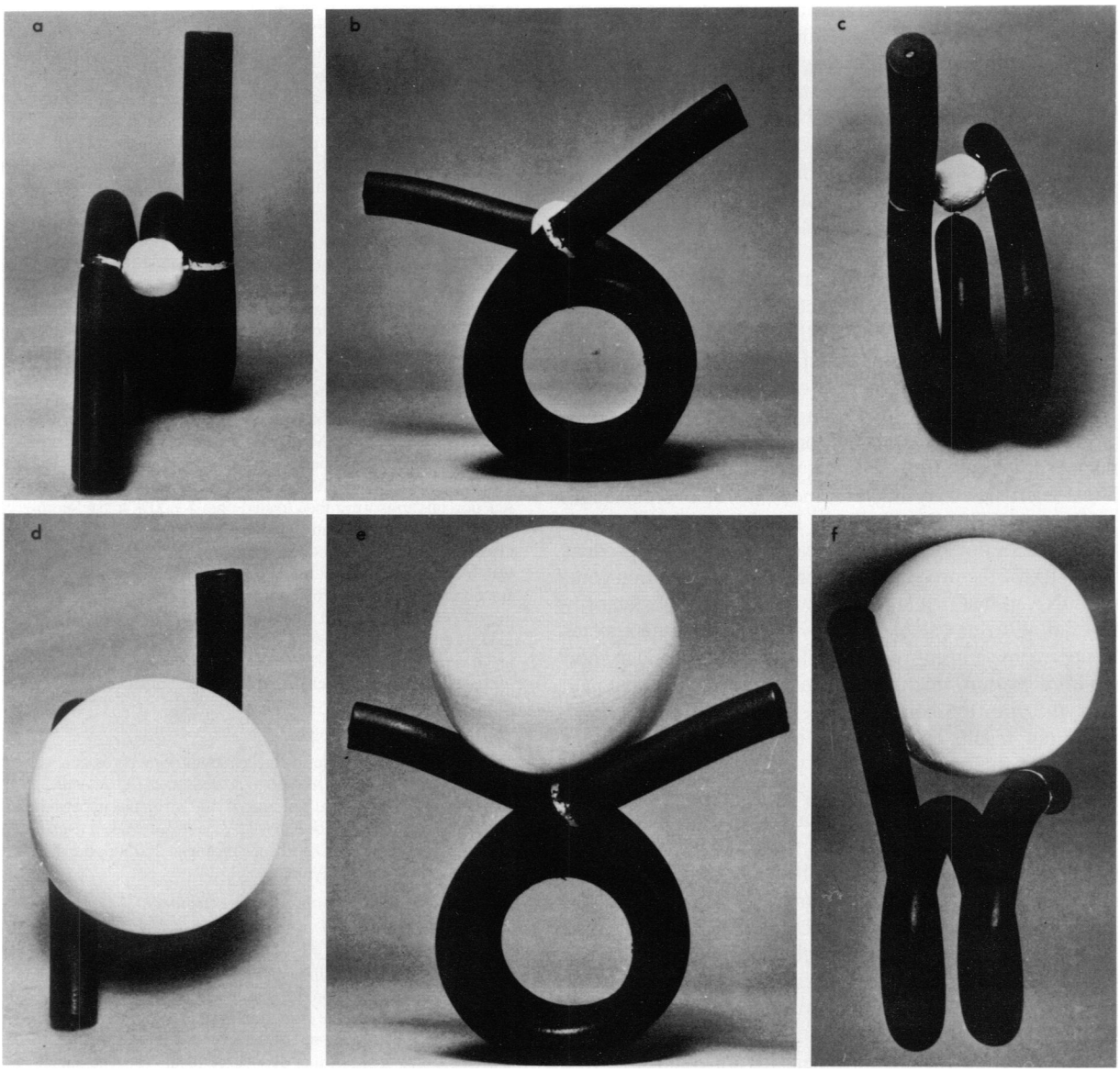

Fig. 4. Location of histone H1 and RNA polymerase in chromatin. **(a–c)** Three orthogonal views of the location of H1 in chromatin adapted from a previous study (Allan *et al.*, 1980b). **(d–f)** RNA polymerase replaces H1 in (a–c). The enzyme is represented as a sphere of the same volume as the suggested ellipsoid (Pilz *et al.*, 1972). This represents a highly schematic view of how the polymerase may 'hop' between neighboring linker segments in chromatin.

all collisions would occur between the enzyme and pieces of DNA containing no promoter sites. In searching for a promoter site the enzyme would therefore need to dissociate and rebind on another fragment and the non-specific interaction, which retains the enzyme within the nucleic acid domain and is the basis of the facilitated diffusion mechanism, would therefore cease to be advantageous and would indeed become deleterious. The competition between long and short DNA chains could be envisaged in the following terms: if they are present in equimolar proportions on a nucleotide basis and promoter sites are in very large excess over enzyme molecules, the initial weak interactions will ensure a rapid and equal partition of enzyme between the two populations with a finite concentration of unbound enzyme, determined by the dissociation constant. As the enzyme explores the DNA sequence and locates promoter sites to form tight complexes, it is removed from the equilibrium; this will occur more rapidly on the longer DNA. In consequence, the binding equilibrium of the weakly associating molecules will be re-established, the sequestered enzyme being replaced by weakly bound molecules, drawn from the solution. When the final state, in which all the enzyme molecules are bound at initiation sites, is reached, the distribution will thus favor the longer DNA molecules.

Quantitatively, if the length of a DNA is N base pairs, and there is one molecule site for every p base pairs, then for a mean free path of linear diffusion of l base pairs, the probability that a polymerase molecule, alighting on the DNA, will locate a promoter site before it dissociates will be l/p, if the DNA is long ($N_L > l$) and if the promoter sites are far apart ($p > l$). If the DNA is short ($N_S < l$), as is the case for a linker, all polymerase molecules attaching to a chain that contains a promoter will locate that promoter; the probability that an enzyme will find a promoter site is therefore merely the fraction of linkers that contain such a site; i.e. N_S/p. If the probability of finding a promoter indeed determines the partition of the enzyme between short and long molecules, present in equal proportions, the long DNA will be favored over the short in the ratio l/N_S if $p > l > N_S$. The argu-

ment is somewhat different and leads to p/N_S if $l > p > N_S$.

These relations allow us to predict the ratio of initiation complexes on chromatin relative to DNA, given that the linker length $N_S = 44$ (chicken erythrocyte chromatin) and $l = 500$ bp on free DNA (Zavriev and Belintsev, 1979; Belintzev *et al.*, 1980). The predicted ratio is thus 12, whereas the experimentally determined ratio is the observed ratio, 0.25, divided by the ratio of available sites, 0.16, hence 1.2. The calculated value is only notional, because the probability that an RNA polymerase molecule will find a promoter site does not vary linearly with the distance between the point of its initial attachment and the promoter site. The probability per unit distance in fact decreases with distance, because of the random walk statistics that govern facilitated diffusion. A quantitative computer simulation of this process has been carried out for the polymerase on DNA and chromatin.

The results are shown in Figure 3a for a random walk on a linker bounded by nucleosomes and one on the linker DNA of a polynucleosome chain treated as if it were a continuous length of DNA, uninterrupted by nucleosomes (but of overall length equal to the sum of linker lengths). The experimental results clearly obey the second model. Moreover the simulation predicts a ratio of ~10 for the probability of formation of initiation complexes on DNA to that on chromatin, which agrees satisfactorily with the limiting value calculated above. This agreement stems from the approximate linearity of the relation in Figure 3a at short distances of migration, relative to the mean free diffusional path of the enzyme, i.e. the conditions $N_S < l$ is fulfilled.

Although our results establish that the location of promoter sites is facilitated to the same extent on the ensemble of separated linker segments as on the naked DNA, they cannot discriminate between the various possible mechanisms of facilitated diffusion. In the original general formulation of the problem, four hypothetical mechanisms for facilitated diffusion were considered (Berg *et al.*, 1981). Both the 'sliding' and 'hopping' mechanisms entail a one-dimensional search for promoter sites on the DNA. These may be contrasted with two mechanisms that entail a random three-dimensional search for specific sites, restricted to the domain of a single DNA molecule, involving either dissociation and rebinding or collision-transfer.

Only the 'sliding' or 'hopping' mechanisms are compatible with the sequence correlation observed in the location of promoter sites by the enzyme on DNA (Park *et al.*, 1982). The likelihood would thus seem to be that the mechanism of facilitated diffusion on chromatin, as on DNA, is either 'sliding' or 'hopping', or a combination of these two. We suggest that the close proximity of linker segments flanking the nucleosome core may allow proteins to bind simultaneously to both segments. The earlier model of H1 binding to chromatin is a precedent for this scheme (Allan *et al.*, 1980b). The view that two very different proteins, *E. coli* RNA polymerase and histone H1, may bind in a similar manner to the nucleosome core is illustrated in Figure 4. An advantage of this model is that it would overcome the difficulties of envisaging how the enzyme might be able to form non-specific complexes with promoter sites throughout the linker region.

Materials and methods

Preparation of materials

Chromatin was prepared by micrococcal nuclease digestion of chicken erythrocyte (Allan *et al.*, 1980a) or bovine cerebral cortex (Allan *et al.*, 1984) nuclei, fractionated according to size by sucrose gradient centrifugation, and depleted of histones H1 (and H5) by chromatography on DNA cellulose (Allan *et al.*, 1980a). The depleted chicken erythrocyte chromatin was treated with trypsin to cleave off the core histone tails, and the extent of digestion was monitored by polyacrylamide gel electrophoresis of the resulting fragments (Allan *et al.*, 1982).

DNA was extracted from the chromatin by overnight digestion with protease K (10 μg/ml) in 0.1% SDS, 10 mM EDTA, at 37°C, extracted twice with 1:1 phenol:chloroform, precipitated with ethanol and dried *in vacuo*.

Chicken erythrocyte histones H1 and H5 were prepared as previously described (Allan *et al.*, 1981) and labelled with *N*-succinimydyl [^{3}H]propionate for 1 h at 4°C in 0.1 M borate buffer, pH 8.8 (Hannon *et al.*, 1984).

RNA polymerase was prepared from *E. coli* strain MRE 600 by the method of Burgess and Jendrisak (1975) and the holoenzyme was purified by chromatography on phosphocellulose (Gonsalez *et al.*, 1977).

Assays of RNA chain initiation

The assays for RNA chain initiation were performed under the conditions described in detail previously (Hannon *et al.*, 1984). This assay was modified to examine the competition between two templates. For each chromatin sample, *E. coli* RNA polymerase (7 units) was allowed to form initiation complexes for 15 min at 37°C on ~25 μg of the chromatin template in the presence of an equal weight (in nucleotide units) of competitor; either naked DNA or other chromatin. The reaction mixtures contained 0.5 mM EGTA, 80 mM NaCl, 1 mM $MnCl_2$, 50 mM Tris–HCl, pH 7.9, and 0.4 mM each of ATP, GTP and CTP in 500 μl. At the end of the incubations, re-initiation was prevented by the addition of 15 μl rifampicin at 500 μg/ml and 250 mM EDTA. Free nucleotides were removed by overnight dialysis against buffer containing 10 mM Tris–HCl, pH 7.5, 0.1 mM EDTA, 1 mM phenylmethyl sulfonyl fluoride and 80 mM NaCl (80TEP buffer). Dialysed reaction mixtures were then loaded onto 5–20% linear sucrose gradients made in 80TEP buffer and centrifuged at 40 000 r.p.m. in the Beckman SW40 rotor. Gradient fractions were collected and the absorbance at 260 nm of each fraction was measured. To assay the initiation complexes, 0.25 ml from each gradient fraction was incubated for 30 min at 37°C in a mixture containing the above ingredients plus 800 mM NaCl, 6 mM $MgCl_2$, 0.05 mM UTP and 1 μCi [^{32}P]-UTP. Templates and labelled transcripts were co-precipitated by the addition of an equal volume of cold 25% TCA, collected on Whatman GF/C filters, treated with 0.5 ml Soluene (Packard) at 55°C for 1 h and counted in 3.5 ml toluene–Permablend scintillant.

Experiments with ^{3}H-labelled H5

Histone H1- and H5-depleted chicken erythrocyte chromatin was recombined with labelled H5 in the specified ratio to nucleosomes (Allan *et al.*, 1981). Chromatins were incubated in a final volume of 500 μl of the initiation mixture, dialysed and fractionated as described above. Aliquots were taken from the sucrose gradient fractions to monitor the absorbance profile at 260 nm, the distribution of H5 and the activity of RNA polymerase.

Simulation of random walks on the computer

The ratio of the number of initiation complexes per unit mass of naked DNA to that per unit mass of free DNA in the linker fraction of a polynucleosome chain was estimated by simulating the random walks with a computer (DEC PDP 11/44); the simulation program was written in FORTRAN 77. It was assumed that an enzyme molecule could bind randomly to any available site and, having bound, could execute a one-dimensional random walk until it either encountered a promoter site (a success) or dissociated (a failure). The random walk is described by a diffusion coefficient D, and the dissociation by a rate constant, k. The binding of an enzyme molecule and its eventual fate we term a trial.

In simulating the random walk, a step of length x nucleotides was taken with equal probability either to the right or to the left; this direction was chosen with the aid of a random-number generator. If this step took the enzyme past the predetermined position of a promoter site, a success was registered and the trial terminated. If not, a failure was registered at random with a probability of $1/kt$; the step at which dissociation took place was also chosen using the random-number generator. The random walk was continued until either a success or a failure occurred. The values of x, the step length and t, the time interval between steps, were chosen so that the trial ended in a reasonable time and such that $x^2/t = D$, expressed in units of $(bp)^2/s$.

In the case of naked DNA, end effects were ignored by assuming that the initial binding site in a trial was between two promoters. A promoter site was taken to begin at a particular base pair and the probability that any given base pair is at the beginning of a promoter site was taken as p. It follows that the probability that the number of binding sites between two consecutive promoters is n is given by $p(1-p)^n$. Thus for each trial with naked DNA, the size of region containing n binding sites was first chosen using the random-number generator according to this probability distribution. The position at which the enzyme bound within this region was then chosen at random and the random walk started from this point. The proportion P_n of a number, N, of trials that culminated in a success was determined.

In the case of chromatin, the number of base pairs between two nucleosomes was taken as L. It then follows that the probability that a linker contains at least one promoter is $Q = 1 - (1-p)^L$. For values of L which are small compared with the average distance between promoters, this approximates to the probability that a linker contains only one promoter. In simulating the binding of an enzyme

to a linker containing a promoter, the initial binding position of the enzyme was chosen randomly to be in the range $1-L$. The random walk was then started at this point and continued as before until a success or failure occurred. During the random walk, the enzyme was reflected back the way it had come if it encountered a nucleosome core. The proportion, P_c, of a number, N, of trials that culminated in a success was determined. The proportion of all binding events irrespective of whether the enzyme bound to a linker containing a promoter or not was then taken to be QP_c. It is recognized that this probability will be in error when L is large and the number of linkers containing two or more promoters becomes significant. It then follows that the number of binding events with naked DNA which lead to a success, relative to the number with chromatin, is simply P_n/QP_c.

The number, N, of trials simulated was taken to be sufficiently large that P_n or P_c was essentially independent of N.

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