Molecular mechanisms of chromatin assembly

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Preface

This lecture focuses on chromatin assembly and does not attempt to review the other subject cited with the award, namely the detection of radioisotopes by X-ray film. Methodological aspects of that subject have been reviewed recently (Laskey, 1980) and underlying principles of the methods have been discussed more fully elsewhere, first for fluorography of isotopes which emit weak \( \beta \)-particles (Laskey & Mills, 1975) and second for detection of \( \gamma \)-rays or high-energy \( \beta \)-particles by using intensifying screens (Laskey & Mills, 1977).

Introduction

The proteins of the eukaryotic nucleus perform the dual roles of physically packaging nucleic acids and of expressing the information that they encode. A hierarchy of packaged structures exists from the nucleosome, in which 166 base-pairs of DNA are coiled twice around the surface of a protein cylinder, to the highly condensed metaphase chromosome, in which the length of DNA is compacted approx. 10^4-fold.

This lecture is concerned primarily with the assembly of the nucleosome subunits of eukaryotic chromatin. Experimental systems for assembling chromatin from purified DNA may have an important role to play in providing an interface between two rapidly emerging fields. These are recombinant DNA techniques for cloning isolated segments of the genome and techniques for expressing cloned DNA by transcribing it either \textit{in vitro} or after micro-injection into intact cells. The ability to reconstruct chromatin from isolated segments of purified DNA extends the range of questions that can be asked about the mechanism and control of their expression in transcription systems.

Attempts to reconstruct chromatin \textit{in vitro} have been made for many years, but until recently they have required prolonged dialysis from unphysiological salt concentrations (>1 M-NaCl), usually in the presence of high concentrations of urea (approx. 5 M). Although this approach has been useful in understanding chromatin structure, attempts to study gene expression in this way have been repeatedly confused by artefacts (reviewed by Felsenfeld, 1978; Chambon, 1978; Lilley & Pardon, 1979).

Much of the work to be considered here will be concerned with the alternative approach, of assembling chromatin from purified DNA at physiological ionic strength. To achieve this we have exploited the cellular machinery for chromatin assembly by devising a cell-free system from eggs of the toad \textit{Xenopus laevis}; 1 ml of the cell-free system can assemble 10^{14} nucleosomes in 1 h at physiological ionic strength. We have fractionated this system to investigate possible mechanisms of nucleosome assembly. Recently several alternative methods for assembling nucleosomes at physiological ionic strength have been described. The methods available include self-assembly systems in which histones and DNA interact directly without 'assembly factors', factor-mediated systems in which assembly is mediated by purified assembly factors, and finally unfractionated cell extracts. Information derived from all three classes of assembly system will be considered here, but with some emphasis on systems derived from \textit{Xenopus} eggs. A more general review of other aspects of nucleosome assembly has been published elsewhere (Laskey & Earnshaw, 1980).

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DR. R. A. LASKEY

Chromatin structure

The structure of chromatin has been reviewed extensively (Kornberg, 1977; Chambon, 1978; Felsenfeld, 1978; Lilley & Pardon, 1979). The principal feature is a repeating chain of nucleosomes. The prevailing model for the structure of the nucleosome (Finch \textit{et al.}, 1977) is summarized diagrammatically in Fig. 1. Nuclease-digestion studies have defined two DNA domains within the nucleosome, a protected nucleosome core and an exposed linker (indicated by arrows in Fig. 1). The core consists of 146 base-pairs of DNA coiled around an octamer of histones (two each of histones H2A, H2B, H3 and H4). The nucleosome is completed by a variable length of linker DNA and by one molecule of histone H1, which occupies the
site where DNA enters and leaves the nucleosome (Thoma et al., 1979) and which protects a further 20 base pairs of DNA from nuclease digestion.

Superimposed on this repeating pattern are local variations in non-histone chromosomal proteins (Mayfield et al., 1978; Elgin & Weisbrod, 1975). The best-studied of these are the proteins of the 'high mobility group' (Goodwin et al., 1978), two of which, HMG 14 and HMG 17, are diagnostic of nucleosomes which contain potential transcription templates (Levy & Dixon, 1978; Weisbrod & Weintraub, 1979; Weisbrod et al., 1980).

A cell-free nucleosome assembly system from eggs of Xenopus laevis

Eggs of Xenopus laevis have several striking advantages for studies of chromosomal replication. First, they divide rapidly after fertilization. The first cell cycle is 1.5 h, and subsequent cell cycles are only 30 min each (Hara et al., 1980). Second, chromatin assembly keeps pace with this rapid rate of replication, as demonstrated by normal chromosome condensation and separation at each division. Third, egg cytoplasm can induce resting nuclei to undergo these rapid division cycles after micro-injection (Graham et al., 1966; Gurdon et al., 1975).

A crucial feature of the egg's ability to divide rapidly is illustrated in Table 1. During the growth of the oocyte in the ovary (oogenesis), a large stockpile of materials is prefabricated to fulfill two main roles. First there is a store of materials to support transcription and translation, such as polymerases, ribosomes, mRNA and tRNA, to sustain the embryo while its genome is replicating maximally. Second there is a store of materials involved directly in chromosomal replication, such as DNA polymerases, deoxynucleoside triphosphates and histones (for references see Table 1). The histone pool was originally predicted by Adamson & Woodland (1974) on the basis of a meticulous study of histone synthesis rates. Thus they found that the rate of histone synthesis was too low to keep pace with DNA synthesis in early development, but that histones were synthesized during oogenesis in the absence of DNA synthesis. They suggested that the histones synthesized during oogenesis were stored for subsequent use during development. On the basis of this prediction that histones are stored in Xenopus eggs, we chose Xenopus egg homogenates as starting material to develop a cell-free system for assembling nucleosomes at physiological ionic strength.

The small circular DNA molecule from simian virus 40 (SV40) was chosen for assays of nucleosome assembly for three reasons. First, it provides a homogenous population of well-characterized molecules. Second, it occurs in infected cells as a minichromosome, being complexed to host histones in the form of nucleosomes. Third, it is a covalently closed circle, so its superhelical density can be used as a measure of nucleosome number as illustrated in Fig. 2. Thus, when double-stranded DNA is wrapped around the surface of a protein cylinder, it becomes constrained. DNA topoisomerase I (nicking–closing enzyme) relaxes this constraint by nicking one DNA strand, allowing the strands to rotate. It then ligates the nick. Therefore when the assembled DNA is depolymerized it has acquired a number of superhelical turns which is proportional to the number of nucleosomes assembled (Fig. 2).

We used this correlation to test if egg homogenates assemble nucleosomes. Relaxed DNA circles were prepared by treating

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Table 1. Accumulation of materials in oocytes of Xenopus laevis

<table>
<thead>
<tr>
<th>Material</th>
<th>Amount per egg</th>
<th>Ratio to amount in larval cells</th>
<th>Principal source of data</th>
</tr>
</thead>
<tbody>
<tr>
<td>Histones</td>
<td>190 ng</td>
<td>3000</td>
<td>Adamson &amp; Woodland (1974); Woodland &amp; Adamson (1977)</td>
</tr>
<tr>
<td>DNA polymerases</td>
<td>—</td>
<td>100 000</td>
<td>Benbow et al. (1975)</td>
</tr>
<tr>
<td>RNA polymerases</td>
<td>8 000 ng</td>
<td>60 000–100 000</td>
<td>Roeder (1974)</td>
</tr>
<tr>
<td>Ribosomess</td>
<td>80 ng</td>
<td>20 000</td>
<td>Brown &amp; Litina (1964);</td>
</tr>
<tr>
<td>tRNA</td>
<td>40 ng</td>
<td>10 000</td>
<td>Brown &amp; Litina (1966)</td>
</tr>
</tbody>
</table>

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Fig. 1. Schematic model for the structure of the nucleosome in which two turns of DNA are wound around the surface of a histone octamer (Finch et al., 1977; Thoma et al., 1979)

Fig. 2. Monitoring nucleosome assembly by DNA supercoiling

When a circular form of chromatin (a) such as the SV40 minichromosome, which has approx. 20 nucleosomes) is depolymerized, the DNA released is supercoiled (b). This can be relaxed (c) by DNA topoisomerase I (nicking–closing enzyme). Thus when relaxed DNA (c) is assembled into nucleosomes (d) and then treated again with DNA topoisomerase I (a) followed by denaturation (b), the number of nucleosomes assembled can be estimated by measuring the number of superhelical turns inserted into the DNA (see Fig. 3, for example).
SV40 DNA with DNA topoisomerase I in vitro. The circles were incubated in a 145,000g max. supernatant of eggs for various times and the DNA was re-extracted, deproteinized and electrophoresed in an acrylamide gel, to resolve topoisomers with different numbers of superhelical turns (Fig. 3). Superhelical turns were inserted over the subsequent 1h to reach the same superhelical density as DNA from naturally occurring SV40 nucleohistone (Laskey et al., 1977). In addition, after incubation in the egg homogenate the DNA had the buoyant density and sedimentation properties of nucleoprotein, not free DNA (Laskey et al., 1977, 1978b). Examination in the electron microscope (Fig. 4) revealed that the nucleoprotein formed had the discontinuously beaded appearance of a chain of nucleosomes (Laskey et al., 1978b), and digestion with micrococcal nuclease (Laskey et al., 1977) revealed a series of protected DNA fragments of periodicity identical with that obtained by digestion of native chromatin (Hewish & Burgoyne, 1973; Noll, 1974).

A final confirmation that the structures formed were nucleosomes came from the observation that when excess DNA is added to a limited amount of the homogenate the amount of DNA assembled can be greatly increased by adding exogenous histones to the reaction.

Therefore the product obtained when DNA is incubated in Xenopus egg homogenates can be identified as a chain of nucleosomes by the criteria of (1) sedimentation, (2) buoyant density, (3) beaded appearance in the electron microscope, (4) local protection from micrococcal nuclease at a periodicity of 200 base-pairs, (5) superhelical winding of DNA and (6) histone-dependence at high DNA concentrations.

These observations also provided direct confirmation of Adamson & Woodland's (1974) prediction of a stored histone pool and they allow a minimum estimate of its size. Thus the supernatant from each homogenized unicellular egg is able to assemble a mass of DNA equivalent to at least 6000 diploid nuclei into nucleosomes (Laskey et al., 1977), and when homogenates are diluted before centrifugation this value is doubled. Assembly occurs independently of protein synthesis and DNA synthesis. In conditions where less than 0.5% of the template replicates, essentially all the DNA (>90%) is assembled into nucleosomes. Assembly is non-co-operative. When excess DNA is added, or at intermediate incubation times, all molecules become partly assembled, suggesting that there is no predisposition for a nucleosome to form next to pre-existing nucleosomes (Laskey et al., 1977).

Fractionation of the nucleosome assembly system from eggs of Xenopus laevis

Although egg homogenates were able to assemble nucleosomes from exogenous histones and DNA, the homogenate was found to be essential. Directly mixing histones and DNA resulted in precipitation, suggesting that eggs provided more than histones and that an additional assembly factor(s) was involved.

When homogenates were fractionated by size, some of the histones co-fractionated with the assembly activity, suggesting...
An egg supernatant was centrifuged through a 10–40% sucrose gradient and each fraction was analysed in three ways (fractions nearest the top of the gradient are on the right in each panel): (a) electrophoretic separation of the polypeptides in each gradient fraction; (b) assay of each fraction for insertion of superhelical turns into relaxed $^3$H-labelled SV40 DNA; (c) as (a), but each fraction was heated for 10 min at 80°C and centrifuged to remove precipitated proteins. One thermostable polypeptide co-purifies with assembly activity (arrow). (Reproduced with permission from Laskey et al., 1978a.)

Fig. 5. Nucleosome assembly activity from Xenopus eggs co-purifies with a thermostable polypeptide of 29000 daltons (arrow in c).

An egg supernatant was centrifuged through a 10–40% sucrose gradient and each fraction was analysed in three ways (fractions nearest the top of the gradient are on the right in each panel): (a) electrophoretic separation of the polypeptides in each gradient fraction; (b) assay of each fraction for insertion of superhelical turns into relaxed $^3$H-labelled SV40 DNA; (c) as (a), but each fraction was heated for 10 min at 80°C and centrifuged to remove precipitated proteins. One thermostable polypeptide co-purifies with assembly activity (arrow). (Reproduced with permission from Laskey et al., 1978a.)

that they were bound in a relatively large complex. Furthermore this complex adsorbed to positively charged, and not negatively charged, resins, unlike free histones, which are themselves positively charged. This suggested that the egg's histones were complexed to a large negatively charged molecule which might itself be an assembly factor (Laskey et al., 1978b). An attempt to identify its nature by testing its sensitivity to degradative enzymes produced disturbing results. Assembly activity resisted ribonuclease A, micrococcal nuclease, trypsin and chymotrypsin, though after treatment with trypsin or chymotrypsin it required addition of histones. Furthermore it resisted heating for 10 min at 80°C. In the forlorn hope that it was a proteinase-resistant thermostable protein, we incubated homogenates with the general proteinase, proteinase K, at pH 10. This abolished activity, whereas pH 10 alone or proteinase K that had been inactivated by treatment with the inhibitor phenylmethanesulphonyl fluoride failed to abolish activity.

If indeed a thermostable protein was responsible for assembly, then heating for 10 min at 80°C should provide a valuable purification step, since most cellular proteins are precipitated by this treatment. Fig. 5 illustrates the effect of fractionating a homogenate by sucrose-gradient centrifugation followed by heating each fraction for 10 min at 80°C. A prominent thermostable polypeptide of 29000 apparent molecular weight is observed co-sedimenting with assembly activity. Furthermore the same polypeptide was co-eluted from DEAE-cellulose with assembly activity. When the separations were combined sequentially, to fractionate by size, charge and thermostability, the protein was homogeneously pure (Laskey et al., 1978a). Assembly activity remained associated with this polypeptide after chromatography on Bio-Gel A-0.5 m, phenyl-Sepharose, phosphocellulose and hydroxyapatite.

The purified protein assembles nucleosome cores from histones and DNA, as shown by the criteria listed in Table 2. For the reason explained in the next section, we have proposed that this protein should be called nucleoplasmin (Laskey & Earnshaw, 1980).

Nucleoplasmin

The properties of nucleoplasmin are summarized in Table 3. It consists of at least two types of subunit of 29000–30000 daltons (Laskey et al., 1978a). Gel filtration and sedimentation indicate that the native protein has a molecular weight greater than 100000, and cross-linking with dimethyl suberimidate indicates that nucleoplasmin exists as a pentamer in solution (Earnshaw et al., 1980), an unusual multimer for a soluble protein. It is acidic, with pI approx. 5, but both types of subunit streak on isoelectric focusing (Fig. 6). Krohne & Franke (1980b) suggest that this is at least partly due to variable extents of phosphorylation. Nucleoplasmin is rich in charged amino acids. About 42% of its amino acid residues are potentially charged and about 30% of the protein is Glx and Aax (Laskey et al., 1979). It is soluble in 1% trichloroacetic acid and resistant to denaturation by boiling.

If the ability of nucleoplasmin to assemble nucleosome cores in vitro reflects a physiological role, then it should be localized within the nucleus. Unfertilized eggs of Xenopus are arrested in the mitotic metaphase and therefore do not have the nucleus, but their developmental precursors, oocytes, contain large nuclei which can be isolated by hand. Therefore we separated nuclei and cytoplasm from oocytes by hand and fractionated their proteins to determine the intracellular location of nucleoplasmin (Mills et al., 1980). Fig. 7 shows that nucleoplasmin is localized within the nucleus. Two-dimensional electrophoresis reveals that it is exclusively localized within the nucleus. Its concentration is remarkable. Thus it is the most abundant protein of the Xenopus oocyte nucleus, occurring at 3–5 mg/ml and representing about 10% of the total nuclear protein. Its intranuclear location was confirmed by iodinating a sample of the purified protein and micro-injecting it into oocyte cytoplasm. Within
Fig. 6. Two-dimensional electrophoresis of purified nucleoplasmin performed as described by Mills et al. (1980)

Table 2. Properties of the nucleoprotein assembled by nucleoplasmin from histones and DNA

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Properties observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sedimentation</td>
<td>Same as nucleohistone</td>
<td>Laskey et al. (1978a, 1979)</td>
</tr>
<tr>
<td>Electron microscopy</td>
<td>Discontinuously beaded and compacted, resembles chromatin</td>
<td>Laskey et al. (1978a, 1979)</td>
</tr>
<tr>
<td>Superhelicity</td>
<td>Same superhelix density as native chromatin</td>
<td>Laskey et al. (1978a, 1979); Earnshaw et al. (1980)</td>
</tr>
<tr>
<td>Resistance to micrococcal nuclease</td>
<td>146 base-pairs of DNA protected</td>
<td>Earnshaw et al. (1980)</td>
</tr>
<tr>
<td>Resistance to deoxyribonuclease I</td>
<td>Characteristic pattern of protected fragments at 10 base-pair intervals,</td>
<td>Earnshaw et al. (1980)</td>
</tr>
<tr>
<td>Incorporation of radioactive histones and DNA</td>
<td>Both incorporated into nucleoprotein product</td>
<td>Laskey et al. (1978a); Earnshaw et al. (1980)</td>
</tr>
<tr>
<td>Incorporation of radioactive nucleoplasmin</td>
<td>Does not enter nucleoprotein product; remains free in solution</td>
<td>Earnshaw et al. (1980)</td>
</tr>
</tbody>
</table>

Table 3. Properties and distribution of nucleoplasmin

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Properties observed</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apparent mol.wt. of subunits</td>
<td>29000</td>
<td>Laskey et al. (1978a)</td>
</tr>
<tr>
<td>Number of subunits</td>
<td>5</td>
<td>Earnshaw et al. (1980)</td>
</tr>
<tr>
<td>Isoelectric point</td>
<td>4.5–5.5 (many isoelectric variants)</td>
<td>Mills et al. (1980)</td>
</tr>
<tr>
<td>Thermostable</td>
<td>Acid-soluble</td>
<td></td>
</tr>
<tr>
<td>GLy + Ala (%)</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>Lys + Arg (%)</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Modifications</td>
<td>Phosphorylated</td>
<td></td>
</tr>
<tr>
<td>Intracellular location</td>
<td>Nucleoplasm</td>
<td></td>
</tr>
<tr>
<td>Concentrations in Xenopus oocyte nucleus</td>
<td>5–8 mg/ml</td>
<td></td>
</tr>
<tr>
<td>Percentage of oocyte nuclear protein in Xenopus</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>Known distribution to date</td>
<td>Nucleoplasm of amphibia, birds and mammals</td>
<td>Krohne &amp; Franke (1980a,b)</td>
</tr>
<tr>
<td>Cells which lack nucleoplasmin</td>
<td>Erythrocytes and mature spermatozoa</td>
<td>Krohne &amp; Franke (1980b)</td>
</tr>
</tbody>
</table>

24 h it migrated into the nucleus and was no longer detectable in the cytoplasm (Mills et al., 1980). The ability to migrate into the nucleus resides in the mature protein, unlike many other trans-membrane transport systems in which additional signal peptides are present in transient precursors. However, nucleoplasmin leaks rapidly from isolated nuclei, emphasizing the problem of the forces which initiate and maintain its intranuclear location.

The question of whether nucleoplasmin occurs in other types of nucleus and in other species has been addressed by Krohne & Franke (1980a,b), using an antiserum raised against nucleoplasmin from Xenopus oocytes. It cross-reacts with a component from a wide range of vertebrate nuclei, and in all the cases examined so far these components have been polypeptides of roughly similar size and charge to nucleoplasmin, suggesting that nucleoplasmin may be an abundant component of most types of vertebrate somatic nucleus. Studies on invertebrates have not yet been reported. Nucleoplasmin appears to be absent from mature spermatozoa and from the nucleated erythrocytes of amphibians and birds (Krohne & Franke, 1980a,b), suggesting that it is present only in nuclei undergoing nucleic acid synthesis.

The antibody studies by Krohne & Franke (1980a,b) and the micro-injection studies by Mills et al. (1980) indicate that nucleoplasmin is not associated with condensed chromatin but is distributed throughout the nucleoplasm. Thus it is not found associated with metaphase chromosomes, nucleoli or the lambrush chromosomes of oocytes. Similarly it does not bind to DNA or to the nucleoprotein which it assembles in vitro (Earnshaw et al., 1980). Because of its high concentration in the nucleoplasm, we have proposed that the protein should be called nucleoplasmin (Laskey & Earnshaw, 1980; Earnshaw et al., 1980).

Although nucleoplasmin does not bind detectably to DNA or to nucleosomes, it does bind histones in vitro (Laskey et al., 1978a, 1979; Earnshaw et al., 1980). The complex formed sediments as a discrete peak through sucrose gradients and binds to anion-exchange resins at neutral pH. Thus nucleoplasmin mimics a property of unfractionated Xenopus histones and generates in causing histones to bind to positively charged resins and not to negatively charged resins, in contrast with the behaviour of free histones (Earnshaw et al., 1980). When the complex between histones and nucleoplasmin is isolated by sucrose-gradient centrifugation and incubated with DNA, histones transfer from the complex to the DNA with the formation of nucleosome cores (Laskey et al., 1979). It is not clear yet if histones are stably bound to nucleoplasmin in vivo (Krohne & Franke, 1980a,b; Earnshaw et al., 1980),
Several observations suggest that the interaction between histones and nucleoplasmin may not involve stereospecific bonding. First, different histones compete with each other for binding to nucleoplasmin. For example, histones H2A and H2B compete against H3 and H4 for binding (Earnshaw et al., 1980). Second, there is an obvious symmetry mismatch between pentameric nucleoplasmin and naturally occurring histone oligomers (dimers, tetramers and octamers). Third, the role of nucleoplasmin in nucleosome core assembly can be mimicked by polyglutamic acid (Stein et al., 1979). This may suggest that clustering of negative charges is important for assembly by nucleoplasmin, since other acidic proteins of similar isoelectric point do not substitute for nucleoplasmin in the assembly reaction (Earnshaw et al., 1980).

The stoichiometry of the histone-nucleoplasmin complex is not clear, so it is not known if nucleoplasmin assembles histone octamers or lower oligomers. However, polyglutamic acid has been shown by cross-linking to assemble histone octamers in the absence of DNA (Stein et al., 1979); perhaps suggesting by analogy that nucleoplasmin may do the same. Unfortunately this question has proved extraordinarily difficult to answer by cross-linking nucleoplasmin to histones, because the lysine residues in nucleoplasmin also cross-link to each other and to histones, and histone dimers co-electrophorese with nucleoplasmin monomeric subunits on sodium dodecyl sulphate/polyacrylamide gels (J. O. Thomas, W. C. Earnshaw & R. A. Laskey, unpublished work). Regardless of whether nucleoplasmin is able to assemble histone octamers in the absence of DNA, it is clear that octamer formation is not essential for its action. Thus, if only either histones H2A and H2B or H3 and H4 are provided, these will be transferred to DNA in the complete absence of the other histone pair, which can then be added later to complete the nucleosome core (Earnshaw et al., 1980). Thus the most attractive explanation of the mechanism of nucleosome core assembly by nucleoplasmin is first that it promotes hydrophobic interactions between individual histones by neutralizing their strong positive charges and competing against their mutual repulsion, and second that it acts as a ‘molecular chaperone’ competing against the strong electrostatic attraction between histones and DNA and thus allowing only a specific subset of interactions to occur. If nucleoplasmin acts as an electrostatic shield in this way, it might have other roles, perhaps mediating in other interactions between the many highly charged components of the nucleus.

Alternative methods of assembling nucleosome cores at physiological ionic strength

Since the isolation of nucleoplasmin, other methods have been developed for assembling nucleosome cores at physiological ionic strength. One of these, using polyglutamic acid as a substitute for nucleoplasmin (Stein et al., 1979), has been discussed above. In addition, Germond et al. (1979) have found that DNA topoisomerase I (nicking-closing enzyme) can act as an assembly factor in a reaction which differs from that mediated by nucleoplasmin: 20 times more topoisomerase is required to assemble nucleosome cores than the amount required to relax DNA, representing about one topoisomerase molecule per nucleosome core assembled. Assembly requires preincubation of DNA with topoisomerase before addition of histones. Since DNA topoisomerase I binds to DNA, this observation suggests that nucleosome assembly by this pathway involves interaction of histones with a complex between DNA and topoisomerase (Fig. 8). Both DNA topoisomerase I and nucleoplasmin occur in the nucleus at high enough concentrations to assemble nucleosomes. Since one of these factors interacts with histones and the other interacts with DNA, the possibility that they perform complementary roles in the assembly process within the nucleus is attractive (Fig. 8c).

The two other methods for assembling nucleosome cores at physiological ionic strength do not require assembly factors, but require careful control over the mixing conditions. Ruiz-Carrillo et al. (1979) were able to assemble nucleosome cores at physiological ionic strength by slowly pumping preformed histone dimers (H2A + H2B) and tetramers (two each of H3 and H4) into an excess of DNA. Stein et al. (1979) were able to assemble nucleosome cores by directly mixing histones and DNA, but only by prolonging the incubation for 16 h to redissolve the precipitate which formed on direct mixing. Although both methods are rather limited in the precise conditions which allow nucleosome cores to assemble, they do clearly establish that the topological information required for assembly rests in histones and DNA themselves. This is consistent with the view expressed above, that assembly factors may act by competing against electrostatic side reactions which would lead to precipitation rather than to correct assembly.

The cellular mechanism of nucleosome assembly

In the absence of suitable mutants, it is difficult to prove that either nucleoplasmin or DNA topoisomerase I is essential for
nucleosome assembly in vitro. However, their intranuclear concentrations are consistent with such a role. Furthermore, in the Xenopus egg the ratio of nucleoplasmin to stored histones found by Mills et al. (1980) is the same as the ratio that was previously reported to be optimal for assembly in vitro (Laskey et al., 1978a). In addition, the Xenopus egg has a 20000-fold excess of histones over its chromosomal DNA and, of the various assembly methods in vitro, only nucleoplasmin allows more than a 1:1 ratio of histones to DNA. Finally Earnshaw et al. (1980) have shown that the rate of nucleosome core assembly by nucleoplasmin in vitro is sufficient to account for the rapid rate of chromatin replication during the early development of Xenopus.

One structural feature of nucleosome chains has proved to be remarkably difficult to reconstruct in vitro. This is the regular periodic spacing of nucleosomes at 200-base-pair intervals. So far none of the assembly systems in vitro which use purified materials has been able to reconstitute this feature. However, crude homogenates of Xenopus eggs or Drosophila embryos are able to reconstitute the native spacing at 200-base-pair intervals (Laskey et al., 1977; Nelson et al., 1979). The mechanism which spaces nucleosomes regularly is unknown, but it is clear that it is independent of DNA replication (Laskey et al., 1977) and therefore cannot arise from the discontinuous synthesis of DNA as Okazaki fragments. Similarly, since it can arise de novo on naked DNA, it does not require copying of a pre-existing spacing from parental chromatin (Laskey et al., 1977), nor does it require a periodic signal from the DNA structure, since it can occur readily on prokaryotic DNA, which is not naturally assembled into nucleosomes. One attractive possibility is that it involves the transient modifications which occur on newly synthesized histones (Louie & Dixon, 1972; Ruiz-Carrillo et al., 1975; Jackson et al., 1976).

Future prospects

By using the assembly systems in vitro described above, it has become possible to reassemble purified DNA into nucleosome cores or regularly spaced chains of nucleosomes at physiologic ionic strength. However, it has not yet been possible to assemble regularly spaced chains of nucleosomes by using purified histones. A fully purified system for positioning nucleosomes precisely on DNA is extremely desirable as a means of analysing the relationship between nucleosome position and DNA sequence, since several laboratories have found that this relationship is not random but is likely to play an important role in gene function (Ponder & Crawford, 1977; Musich et al., 1977; Wittig & Wittig, 1979; Varshavsky et al., 1979; Wu et al., 1979).

Secondly, the influence of specific non-histone nuclear proteins on chromatin function is a rapidly growing area of interest. For example, Weisbrod & Weintraub (1979), Weisbrod et al. (1980) and Levy & Dixon (1978) have found that transcriptionally active nucleosomes are characterized by the presence of HMG 14 and HMG 17, two small proteins of the 'high mobility group' (Goodwin et al., 1978). Questions of how their presence influences transcriptional activity and of how certain regions of the genome are rigorously selected for assembly of nucleosomes containing these proteins can be approached via nucleosome assembly.

Thirdly, recent attention has been focused on the organization of interphase and mitotic chromatin into loop-shaped domains (Benyajati & Worcel, 1976; Cook et al., 1976; Paulson & Laemmli, 1977; Pardoll et al., 1980). The possibility that such domains represent functional units for expression and perhaps replication has been suggested repeatedly.

Recently it has become relatively easy to isolate and amplify isolated segments of the eukaryotic genome by growth in bacteria. The opportunity to assemble genes isolated in this way back into the ordered nucleoprotein structures of chromatin provides a direct approach to understanding the function of the structures of the eukaryotic nucleus.

I am deeply grateful to the Biochemical Society and to Unilever for the encouragement which this award provides. I also wish to thank warmly Sydney Brenner, Aaron Klug, Max Perutz, Guido Pontecorvo and especially Lionel Crawford, John Gurdon and Ann Laskey for their encouragement, and my numerous past and present colleagues for their essential contributions to this award.

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