# Strategies for the Reconstitution of Chromatin

Alexandra Lusser & James T. Kadonaga*

Section of Molecular Biology, University of California, San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0347 USA

*Corresponding author: jkadonaga@ucsd.edu

**In eukaryotes, chromatin is the natural form of DNA in the nucleus. For hundreds of millions of years, DNA-binding factors have evolved with chromatin. It is therefore more desirable to study the molecular mechanisms of DNA-directed processes with chromatin than with naked DNA templates. To this end, it is necessary to reconstitute DNA and histones into chromatin. Fortunately, there are a variety of methods by which a nonspecialist can prepare chromatin of high quality. Here we describe strategies and techniques for the reconstitution of chromatin *in vitro.***

In the eukaryotic nucleus, DNA is packaged into a nucleoprotein complex termed chromatin[1-3]. The basic repeating unit of chromatin is the nucleosome, which typically consists of 180 to 200 bp DNA, a core histone octamer (which contains two copies each of the core histones H2A, H2B, H3, and H4), and a molecule of the linker histone H1 (**Fig. 1**). The nucleosome core comprises about 147 bp of DNA wrapped approximately 1.7 times around the core histone octamer in a left-handed superhelix[4]. In chromatin, nucleosome cores are joined together by linker DNA. The linker histone H1 interacts with both the nucleosome core as well as the linker DNA, and promotes the higher-order folding and compaction of chromatin. In metazoans, there is approximately one molecule of histone H1 per nucleosome[5].

In recent years, it has become clearly evident that the structure and composition of chromatin is critically important for the proper functioning of processes that involve DNA. For instance, ATP-dependent chromatin remodeling factors have been found to participate in gene regulation, DNA repair, DNA replication, and homologous DNA recombination (for reviews, see:[6-12]). At the biochemical level, these chromatin remodeling factors exhibit activities that include the assembly and disassembly of nucleosomes as well as the mobilization ('sliding') of nucleosomes. In addition, the core histones are subjected to a broad range of posttranslational modifications [such as acetylation, methylation, ubiquitylation, poly(ADP)ribosylation, and phosphorylation] that affect the biophysical properties of chromatin as well as serve as signals for the specific interaction of regulatory factors with the chromatin template (for reviews, see:[13-16]). Thus,

chromatin is an integral component in the function of DNA-directed processes, and it is important to use chromatin templates in the biochemical analysis of these phenomena.

In this review, we will describe methods for the reconstitution and analysis of chromatin *in vitro*. We seek, in particular, to provide assistance to nonspecialists who are interested in incorporating chromatin into their research programs.

**Overview of Methods for Chromatin Reconstitution**

The minimal nucleosome reconstitution process involves the DNA template, core histones, and a 'chaperone' that acts both to allow the proper deposition of positively-charged histones onto the negatively-charged DNA and to prevent undesired interactions of the histones with other molecules. This 'chaperone' function can be mediated by histone-binding proteins [such as NAP-1 or nucleoplasmin (for reviews, see:[17-23])], by negatively-charged polymers (such as polyglutamate[24,25] or RNA[26]), or by relatively high concentrations of a salt such as NaCl (see, for example:[27-31]). In the absence of a chaperone, core histones and DNA will form an insoluble, non-nucleosomal aggregate at low ionic strengths (such as in a buffer containing 0.1 M NaCl).

There are two main approaches to the reconstitution of chromatin *in vitro* – the ATP-independent random deposition of histones onto DNA, and the ATP-dependent assembly of periodic nucleosome arrays. The ATP-independent reconstitution process involves core histones, DNA, and a histone 'chaperone' (such as NaCl or NAP-1), and typically results in the random deposition of histones onto DNA. The assembly of periodic nucleosome arrays (similar to those seen in bulk native chromatin) is an ATP-dependent reaction[32], and requires ATP-utilizing chromatin assembly factors such as ACF (ATP-utilizing chromatin assembly and remodeling factor)[33-37] or RSF (remodeling and spacing factor)[38-40], or related activities that are present in crude chromatin assembly extracts (see, for instance:[32,41,42]).

**Mononucleosomes or Extended Nucleosome Arrays?**

Before proceeding to the actual preparation of chromatin, it will be necessary to determine what 'type' of chromatin is best suited for your studies. The first question, which will be discussed in this section, is whether or not to use reconstituted mononucleosomes.

The classical core particle comprises ~147 bp of DNA and a core histone octamer[43-45]. Native core particles can be generated by extensive digestion of chromatin with micrococcal nuclease[43,44]. It is relatively simple to reconstitute mononucleosomes *in vitro* with purified core histones and DNA fragments that range from about 150 bp to 250 bp in length. [Note that reconstituted species with greater than 147 bp DNA are not identical to classical core particles, and are often termed 'mononucleosomes'.] If desired, di- or tri-nucleosomes can also be reconstituted with longer DNA fragments. Typically, nucleosomes are reconstituted *in vitro* onto short DNA fragments by using a salt (NaCl) dialysis method.

Core particles/mononucleosomes are useful for a variety of studies. Mononucleosomes can be used to characterize the binding of proteins, such as sequence-specific DNA-binding factors or non-histone chromosomal proteins, to nucleosome cores (see, for example:[46-49]). Mononucleosomes can also be used to study the activities of ATP-dependent chromatin remodeling factors, such as their ability to facilitate the binding of sequence-specific DNA-binding factors (see, for example:[50,51]) or to catalyze the movement of the histone octamer relative to the DNA ('nucleosome sliding';[52,53]). Mononucleosomes can serve as substrates for the analysis of histone-modifying enzymes[13-16]. Furthermore, biophysical studies of chromatin often employ mononucleosomes. For instance, the high-resolution x-ray crystal structure of the nucleosome core was solved with reconstituted core particles[54].

Some of the benefits of the use of reconstituted core particles or mononucleosomes are as follows. First, mononucleosomes can be prepared with well-defined, purified components – core histones and DNA. Second, the binding of factors to nucleosomes can often be detected by gel shift analysis of nucleosomes in a nondenaturing gel as well as by DNase I footprinting (see, for example:[46-49]). Third, nucleosomes can be positioned at specific locations by using inherently

curved or bent DNA templates that bind to the histone octamer with higher affinity than DNA with less curvature (see, for example:[55-58]). Fourth, by native gel electrophoresis, it is possible to distinguish between mononucleosome species that differ by the positions of the histone octamers on the DNA[59,60]. In such experiments, it is possible to monitor the enzyme-catalyzed movement ('sliding') of nucleosomes from one position to another location on the DNA fragment[52,53].

On the other hand, there are also some limitations or potential problems with the use of mononucleosomes/core particles. First, mononucleosomes are an incomplete model for chromatin, which mainly consists of extended periodic arrays of nucleosomes. For example, factors may interact with the free DNA ends in mononucleosomes that would not be present in a nucleosome array. In addition, the interactions of the core histone tails (*i.e.*, the relatively unstructured N- and C-terminal ends of the core histones that extend beyond the central globular core) may be different in mononucleosomes than in extended nucleosome arrays. For instance, the histone H2A C-terminal tail interacts differently with nucleosomes in the presence versus the absence of linker DNA[61]. Second, it should be noted that reconstituted 'mononucleosomes' can be prepared in which less than 147 bp of DNA is associated with the octamer. In an early demonstration of this point, a 145 bp DNA fragment was reconstituted into a 'mononucleosome' in which only 128 bp of DNA was found to be associated with the core histone octamer[62]. Thus, for most applications, it is important to determine that 147 bp of the DNA fragment is indeed associated with the histone octamer.

Thus, mononucleosomes are probably most useful when precise positioning of a histone octamer is needed with a relatively simple experimental system.

**Periodic or Randomly-distributed Nucleosomal Arrays?**

If mononucleosomes are not appropriate for the planned studies, there are different methods to consider for the preparation of extended arrays of nucleosomes. Depending upon the method of assembly, the reconstituted chromatin will consist of randomly-distributed (**Fig. 2a**) or periodically-spaced nucleosome arrays (**Fig. 2b–d**).

Randomly Distributed Nucleosome Arrays. The ATP-independent reconstitution of nucleosomes, such as by the salt dialysis technique, generates chromatin that consists of randomly-distributed nucleosomes (**Fig. 2a**). One advantage of this approach is that it can be used to produce pure chromatin (consisting of only purified core histones and DNA) that is devoid of histone chaperones or other large molecules that could interfere with subsequent applications. For example, we have used salt dialysis-reconstituted chromatin for studies of homologous recombination and chromatin remodeling by the Rad54 ATPase[63] as well as for the analysis of chromatin remodeling by ACF[33]. It is important to note, however, that chromatin with randomly-distributed nucleosomes may contain stretches of naked DNA. Therefore, in the characterization of such chromatin, useful conclusions can be drawn if the reconstituted chromatin possesses properties that are distinct from those of naked DNA. In such instances, the unique activities seen with chromatin could not be attributed to the presence of stretches of naked DNA in the chromatin templates.

Periodic arrays of nucleosomes. ATP-dependent chromatin assembly can be used to prepare periodic nucleosome arrays with any DNA sequence of indefinite length. This reaction can be performed either with crude cell extracts (see, for example:[32,41,42]) or with purified ATP-utilizing motor proteins, such as ACF and RSF (see, for example:[37,40]). A key feature of chromatin assembled by ATP-dependent methods is that it consists of periodic nucleosome arrays that resemble those of bulk native chromatin. When natural promoter sequences have been assembled into chromatin in the presence of their cognate sequence-specific regulatory proteins, the resulting structures of the chromatin were observed to resemble those of the native genes *in vivo* (see, for example:[64-66]). In addition, chromatin assembled with the ATP-dependent factors has been found to be an excellent substrate for biochemical studies of transcriptional regulation (see, for example:[38,67-76]), chromatin remodeling (see, for example:[64,77-79]), and homologous strand pairing[63]. It should be noted, however, that the ATP-utilizing assembly factors could potentially

affect the properties of the chromatin in subsequent applications. If chromatin that is completely devoid of assembly factors is desired, then it would be necessary either to purify the chromatin from the assembly factors or to reconstitute nucleosomes by the salt dialysis method.

ATP-dependent chromatin assembly yields periodic but not specifically-positioned nucleosomes (**Fig. 2c**). It is possible, however, to establish local regions of nucleosome positioning by the use of sequence-specific DNA-binding proteins (see, for example:[35,79]; **Fig. 2d**). The nucleosomes are located adjacent to the DNA-binding protein, and the nucleosome positioning is propagated as far as about two to three nucleosomes on each side of the DNA-binding protein.

It is also possible to reconstitute stretches of periodic nucleosome arrays onto tandemly-repeated positioning sequences by using the ATP-independent salt dialysis methodology (**Fig. 2b**). These DNA templates contain multiple (typically, about 12 to 18), tandem copies of a nucleosome positioning sequence, such as a segment of the sea urchin 5S rRNA gene[56] or a synthetic, high affinity sequence[58]. When the templates are reconstituted into chromatin by salt dialysis methodology, a positioned nucleosome is formed on each repeated sequence, thereby creating a series of evenly-spaced nucleosomes. Such tandemly-repeated nucleosomes have been used to study a variety of phenomena, which include the change in the DNA linking number per core particle[30], transcriptional elongation in chromatin[80], the effect of histone acetylation upon chromatin folding[81], and chromatin remodeling by the SWI/SNF complex[82].

## Chromatin Reconstitution

There are many different potential approaches to the reconstitution of chromatin. In this section, we will discuss a variety of specific issues that are relevant to chromatin reconstitution.

Native Chromatin in the Experimental Organism. It is important to consider the natural state of chromatin in the experimental organism. In general, native chromatin consists mostly of regularly-spaced arrays of nucleosomes. The unit amount of DNA in a single nucleosome in a periodic

array is termed the repeat length. In metazoans, chromatin typically has a repeat length of about 180 to 200 bp and approximately 1 molecule of histone H1 per nucleosome. However, in the yeast *Saccharomyces cerevisiae*, bulk chromatin has a repeat length of about 165 bp and does not appear to contain canonical histone H1 molecules. Thus, before initiating the reconstitution of chromatin *in vitro*, it would be useful to study the properties of the chromatin in your experimental organism. For instance, it would be appropriate to include histone H1 in the study of chromatin in humans but not in yeast.

Histones. The core histone octamer consists of a central $(H3\text{-}H4)_2$ tetramer that is flanked by two H2A-H2B dimers. Interactions among the histones are affected by attractive hydrophobic forces as well as by repulsive ionic forces. At high salt, such as 2 M NaCl, the repulsive forces are reduced, and the core histones assemble into octamers. In contrast, in buffers that are typically used for biochemical reactions (which might contain about 50 mM to 100 mM NaCl), the histones exist mostly as H2A-H2B dimers and $(H3\text{-}H4)_2$ tetramers[83,84].

There are many different methods for the purification of histones, and most of these procedures probably yield core histones that are of sufficient purity and integrity for the reconstitution of high quality chromatin. von Holt and colleagues[85] have provided a comprehensive description of histone purification. Many methods include a step in which the histones are subjected to acidic conditions, such as 0.25 M HCl. Although such treatment sounds harsh, the histones are probably not adversely affected by these conditions.

For our reconstitution experiments, native core histones lacking histone H1 are purified by using a relatively mild procedure[41], which is a slight modification of the method of Simon and Felsenfeld[86]. We have also developed a nondenaturing method for the purification of histone H1[87]. In addition, recombinant, bacterially-synthesized core histones can be used for the reconstitution of chromatin[35,38,54,76]. Recombinant histones lack posttranslational modifications (except for removal of the N-terminal Met residue), are devoid of any contaminating eukaryotic

factors (such as chromatin remodeling factors that might copurify with native histones), and can be synthesized and purified in various mutant forms.

Histones are atypical proteins. They often stick to plastic and glass, and hence, the use of siliconized plasticware and glassware is recommended. When subjected to 18% polyacrylamide-SDS gel electrophoresis, the order of migration of the core histones from slowest to fastest (*i.e.*, top to bottom of the gel) is H3, H2B, H2A, and H4. Histone H3 is the most proteolytically sensitive core histone. The quality of a preparation of core histones can often be judged by examination of the histone H3. As H3 becomes degraded, the full-length H3 (top band) disappears, and the proteolyzed H3 appears as a new band between histones H2A and H4. It is also recommended to test for the presence of contaminating histone H1.

DNA Template. It is important to use highly purified DNA that does not contain contaminating RNA, which is difficult to detect by the commonly used method of ethidium bromide staining. Histones can interact with RNA as well as DNA, and hence, the presence of contaminating RNA can lead to variable and inconsistent results. We use DNA that has been subjected to two successive CsCl gradient purification steps. DNA that is purified with commercially available kits should also be suitable for chromatin assembly.

Histone:DNA Ratio. Perhaps the most critical parameter for successful chromatin reconstitution is the histone:DNA mass ratio. If this ratio is too low, then chromatin reconstitution will be incomplete. On the other hand, if the histone:DNA ratio is too high, then the excess histones will cause the formation of undefined protein-DNA aggregates. Therefore, in initial experiments (in the absence of histone H1), it is useful to begin with an estimated histone:DNA mass ratio of 1.0:1.0 (which corresponds to one nucleosome per 160 bp), and then to perform a series of titration reactions in which the histone:DNA ratio is varied by ~10% increments (*e.g.*, 0.8:1.0, 0.9:1.0, 1.0:1.0, 1.1:1.0, 1.2:1.0; see, for example:[37]).

ATP-independent Reconstitution of Nucleosomes. The wrapping of DNA around the core histone octamer in the nucleosome results in a change of the linking number of approximately –1.0[30,88]. Thus, the reconstitution of one nucleosome onto a closed circular plasmid DNA generates one positive supercoil, which will relieve superhelical tension in a negatively supercoiled template. This effect has been observed to result in more rapid and efficient nucleosome reconstitution onto negatively supercoiled DNA relative to relaxed DNA[89]. Hence, in practical terms, the ATP-independent reconstitution of nucleosomes occurs more rapidly and efficiently with negatively supercoiled DNA than with relaxed circular DNA or linear DNA.

There are a variety of methods for the ATP-independent preparation of mononucleosomes or chromatin. We prefer the salt dialysis method because it involves only purified histones and DNA, and the resulting chromatin can be separated from free histones and naked DNA (or suboptimally reconstituted chromatin) by sucrose gradient sedimentation. In the salt gradient procedure, the core histones are combined with DNA in 2 M NaCl, and then, the NaCl concentration is slowly decreased by dialysis or dilution of the mixture. The resulting chromatin is usually purified by sucrose gradient sedimentation. Chromatin that is prepared by this method can be stored for at least a month at 4˚C, but should not be frozen. The methodology for salt gradient dialysis as well as other techniques for the ATP-independent reconstitution of nucleosomes are described elsewhere (see, for example[25,30,31,90]).

One additional advantage of the salt gradient dialysis method is that the nucleosomes reconstituted by this technique have been extensively characterized. In contrast, it is not clearly evident whether some of the other methods for ATP-independent nucleosome reconstitution result in the generation of canonical nucleosomes. For instance, the ATP-independent transfer of histones to negatively supercoiled DNA by the NAP-1 chaperone was found to lead to the formation of irregular nucleoprotein structures that are larger than canonical nucleosomes, as assessed by atomic force microscopy[91].

Lastly, if only a trace amount of radioloabelled DNA is being reconstituted into chromatin, then it might be preferable to use the histone transfer method, in which histones are transferred

from unlabelled, native bulk chromatin to the radiollabelled probe DNA (see, for example:[90]). The major shortcoming of this method is that the radiolabelled chromatin constitutes only a small fraction of the total chromatin.

ATP-dependent assembly systems. Periodic nucleosome arrays can be assembled in an ATP-dependent reaction with either crude extracts (see, for instance:[32,41,42]) or purified factors (for methods, see:[37,40]). The crude extracts have been used successfully by many laboratories, but the complexity of the extracts can be problematic for many applications. In addition, the preparation of these extracts requires relatively large quantities of *Drosophila* embryos or *Xenopus* oocytes, which may not be readily accessible to some researchers. Fortunately, purified recombinant chromatin assembly systems for ATP-dependent chromatin assembly are now available[37,40]. These assembly systems employ either ACF or RSF as the ATP-utilizing assembly factor (**Box 1**). For most applications, it is probably best to use the purified factors for chromatin assembly.

An interesting feature of the ATP-dependent assembly systems is the effect of ionic strength and histone H1 upon the nucleosome repeat length[37,92,93]. In the absence of histone H1, the nucleosome repeat length of the chromatin increases as the ionic strength is increased. Thus, the repeat length of the chromatin can be varied by alteration of the ionic conditions of the reaction. In the presence of histone H1 (which resembles a multivalent cation, as it is rich in lysine residues), the repeat length increases with increasing H1 concentration until a 1:1 ratio of H1:core histone octamers is achieved [37].

In our laboratory, we have extensively used ACF in conjunction with NAP-1 for the assembly of chromatin. We have found that chromatin assembly occurs efficiently with a broad range of DNA sequences as well as with supercoiled, relaxed circular, or linear DNA templates. To minimize the nonspecific ATP-independent transfer of histones from NAP-1 to the DNA, we use DNA that has been previously relaxed with purified topoisomerase I. In addition, topoisomerase I is included in the reaction to relieve the positive superhelical tension that is generated as nucleosomes are being formed (see above). Chromatin that is assembled with the purified ACF

system can be stored at 4˚C for several days with no apparent decrease in quality, but should not be frozen. The detailed methodology is described elsewhere[37].

Lastly, before embarking on the assembly of chromatin with the purified factors, it should be noted that these experiments are significantly more difficult to perform than ATP-dependent chromatin assembly with crude extracts or ATP-independent nucleosome reconstitution. For instance, each of the reaction components must be highly purified and carefully titrated. Therefore, the establishment of the purified ATP-dependent assembly systems requires a substantial commitment of time and resources.

**Analysis of Chromatin Reconstituted *In vitro***

It is important to analyze the reaction products carefully and thoroughly to determine whether or not chromatin has been reconstituted properly. We have described the step-by-step methodology for many of these assays elsewhere (see, for example:[37,41]). In this section, we will provide a brief description of some of these assays.

DNA Supercoiling Assay. The formation of one nucleosome causes a change in the linking number of approximately -1.0[30,88]. Thus, the reconstitution of *N* nucleosomes onto a closed circular DNA template in the presence of topoisomerase I will yield a supercoiled DNA species with *N* negative supercoils after removal of the histones. In this manner, the DNA supercoiling assay can provide a quantitative assessment of the efficiency of nucleosome assembly (**Fig. 3a**). The extent of DNA supercoiling is most commonly measured by standard one-dimensional agarose gel electrophoresis. If the distribution of topoisomers needs to be determined with higher precision, then the topoisomers can be more clearly resolved by two-dimensional agarose gel electrophoresis[94]. The DNA supercoiling assay is useful for the analysis of chromatin that is reconstituted by either ATP-independent or ATP-dependent processes. However, it is best used in conjunction with the micrococcal nuclease digestion assay (see below), because the generation of

negative supercoils upon the addition of histones to DNA (in the presence of topoisomerase I) can also be caused by the formation of non-nucleosomal histone-DNA species (see, for example:[95]).

Micrococcal Nuclease Digestion Assay. The micrococcal nuclease digestion assay reveals the periodicity of the nucleosomes as well as the nucleosome repeat length[43] (**Fig. 3b**). In this assay, the chromatin is partially digested with micrococcal nuclease, which cleaves both strands of DNA in the linker region between the nucleosomal cores. The resulting mono- and oligo-nucleosomal fragments are deproteinized, and the DNA fragments are then resolved by agarose gel electrophoresis. A ladder corresponding to DNA derived from mono- and oligo-nucleosomes is observed if the nucleosomes are efficiently assembled and regularly spaced. This assay is of critical importance to the analysis of chromatin that is assembled by the ATP-dependent processes that yield periodic nucleosome arrays. The number of distinct oligonucleosomal DNA bands that can be visualized is a good indication of the 'quality' (in terms of nucleosome periodicity and efficiency of assembly) of the chromatin. We typically view chromatin that yields six or more distinct bands (*i.e.*, DNA species derived from mononucleosomes to at least hexanucleosomes) as being of high quality. The micrococcal nuclease assay should also be used for the analysis of irregularly-distributed nucleosomes that are prepared by ATP-independent histone deposition methods. Although only mononucleosome (~147 bp DNA) and perhaps dinucleosome bands are usually observed with such chromatin, it is important to see that nucleosomes are reconstituted and that subnucleosomal particles, which yield DNA fragments less than 147 bp in length, are not formed.

Polyacrylamide-SDS Gel Electrophoresis. It is important to determine whether the chromatin contains roughly equimolar amounts of each of the core histones. Typically, 18% polyacrylamide-SDS gels are used. We recommend staining the proteins with Coomassie Brilliant Blue R-250. Some specific silver staining methods (*e.g.*, those with silver nitrate in $NaOH/NH_3$) are effective for the detection of core histones, whereas other silver staining methods are not recommended.

Gel Mobility Shift Analysis. This method is useful for characterization of reconstituted mononucleosomes. Mononucleosomes migrate more slowly than their corresponding free DNA species in nondenaturing gels. Thus, the gel shift assay reveals the relative proportions of mononucleosomes and free DNA. As noted above, the gel shift assay can be used to determine the specific positions of nucleosomes on the DNA fragments[52,53,59,60].

Electron Microscopy or Atomic Force Microscopy. It is often useful to visualize the chromatin samples as single molecules. Electron microscopy (as well as atomic force microscopy) reveals features of the chromatin that are not apparent in bulk biochemical assays. For instance, histone H1-containing chromatin typically has a zig-zag appearance at low ionic strength[96]. Also, the folding of the chromatin into higher order structures could be visualized by electron or atomic force microscopy.

## Conclusions

The purpose of this review is to provide practical information that serves as a guide for the nonspecialist who is interested in reconstituting chromatin *in vitro*. Fortunately, at the present time, there are reliable methods for the reconstitution of chromatin with well-defined, purified components. Some of these experiments are moderately challenging to perform. Once the appropriate skills are acquired, however, the results are highly reproducible.

In this review, we focussed almost entirely upon the reconstitution of the simplest form of chromatin that consists of DNA and the core histone octamers. It is also possible to reconstitute periodic arrays of chromatin with histone H1 (see, for example:[37]). In the future, it will be important to extend the methodology for the reconstitution and analysis of specific forms of chromatin. For instance, it would be useful to study chromatin that contains specific covalent modifications (such as acetylation, methylation, phosphorylation, etc.; see, for example[97]) of the core histones. In addition, chromatin can be reconstituted that contains core histone variants. Also,

it will be important to incorporate abundant nonhistone chromosomal proteins, such as the high mobility group (HMG) proteins and heterochromatin protein-1 (HP-1). In this manner, it will be possible to investigate the broad spectrum of mechanisms by which chromatin participates in fundamental processes in the nucleus.

## References


1. van Holde, K.E. *Chromatin*, (Springer Verlag, New York, 1989).
2. Wolffe, A. *Chromatin: Structure and Function*, (Academic Press, San Diego, 1998).
3. Kornberg, R.D. & Lorch, Y. Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome. *Cell* **98**, 285-294 (1999).
4. Davey, C.A., Sargent, D.F., Luger, K., Maeder, A.W. & Richmond, T.J. Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 a resolution. *J. Mol. Biol.* **319**, 1097-1113 (2002).
5. Bates, D.L. & Thomas, J.O. Histones H1 and H5: one or two molecules per nucleosome? *Nucleic Acids Res.* **9**, 5883-5894 (1981).
6. Vignali, M., Hassan, A.H., Neely, K.E. & Workman, J.L. ATP-dependent chromatin-remodeling complexes. *Mol. Cell. Biol.* **20**, 1899-1910 (2000).
7. Flaus, A. & Owen-Hughes, T. Mechanisms for ATP-dependent chromatin remodelling. *Curr. Opin. Genet. Dev.* **11**, 148-154. (2001).
8. Fyodorov, D.V. & Kadonaga, J.T. The many faces of chromatin remodeling: SWItching beyond transcription. *Cell* **106**, 523-525. (2001).
9. Narlikar, G.J., Fan, H.Y. & Kingston, R.E. Cooperation between complexes that regulate chromatin structure and transcription. *Cell* **108**, 475-487. (2002).
10. Becker, P.B. & Horz, W. ATP-dependent nucleosome remodeling. *Annu. Rev. Biochem.* **71**, 247-273 (2002).
11. Lusser, A. & Kadonaga, J.T. Chromatin remodeling by ATP-dependent molecular machines. *Bioessays* **25**, 1192-1200 (2003).
12. Peterson, C.L. & Cote, J. Cellular machineries for chromosomal DNA repair. *Genes Dev.* **18**, 602-616 (2004).
13. Strahl, B.D. & Allis, C.D. The language of covalent histone modifications. *Nature* **403**, 41-45 (2000).

14. Zhang, Y. & Reinberg, D. Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev.* **15**, 2343-2360. (2001).

15. Berger, S.L. Histone modifications in transcriptional regulation. *Curr. Opin. Genet. Dev.* **12**, 142-148 (2002).

16. Bannister, A.J., Schneider, R. & Kouzarides, T. Histone methylation: dynamic or static? *Cell* **109**, 801-806 (2002).

17. Ito, T., Tyler, J.K. & Kadonaga, J.T. Chromatin assembly factors: a dual function in nucleosome formation and mobilization? *Genes Cells* **2**, 593-600 (1997).

18. Adams, C.R. & Kamakaka, R.T. Chromatin assembly: biochemical identities and genetic redundancy. *Curr. Opin. Genet. Dev.* **9**, 185-190 (1999).

19. Verreault, A. De novo nucleosome assembly: new pieces in an old puzzle. *Genes Dev.* **14**, 1430-1438 (2000).

20. Mello, J.A. & Almouzni, G. The ins and outs of nucleosome assembly. *Curr. Opin. Genet. Dev.* **11**, 136-141 (2001).

21. Tyler, J.K. Chromatin assembly. Cooperation between histone chaperones and ATP-dependent nucleosome remodeling machines. *Eur. J. Biochem.* **269**, 2268-2274 (2002).

22. Ito, T. Nucleosome assembly and remodeling. *Curr. Top. Microbiol. Immunol.* **274**, 1-22 (2003).

23. Haushalter, K.A. & Kadonaga, J.T. Chromatin assembly by DNA-translocating motors. *Nat. Rev. Mol. Cell. Biol.* **4**, 613-620 (2003).

24. Stein, A., Whitlock, J.P., Jr. & Bina, M. Acidic polypeptides can assemble both histones and chromatin *in vitro* at physiological ionic strength. *Proc. Natl. Acad. Sci. USA.* **76**, 5000-5004 (1979).

25. Stein, A. Reconstitution of chromatin from purified components. *Methods Enzymol.* **170**, 585-603 (1989).

26. Nelson, T., Wiegand, R. & Brutlag, D. Ribonucleic acid and other polyanions facilitate chromatin assembly *in vitro*. *Biochemistry* **20**, 2594-2601 (1981).

27. Axel, R., Melchior, W., Jr., Sollner-Webb, B. & Felsenfeld, G. Specific sites of interaction between histones and DNA in chromatin. *Proc. Natl. Acad. Sci. USA.* **71**, 4101-4105 (1974).

28. Oudet, P., Gross-Bellard, M. & Chambon, P. Electron microscopic and biochemical evidence that chromatin structure is a repeating unit. *Cell* **4**, 281-300 (1975).

29. Thomas, J.O. & Butler, P.J. The nucleosome core protein. *Cold Spring Harb. Symp. Quant. Biol.* **42 Pt 1**, 119-125 (1978).

30. Simpson, R.T., Thoma, F. & Brubaker, J.M. Chromatin reconstituted from tandemly repeated cloned DNA fragments and core histones: a model system for study of higher order structure. *Cell* **42**, 799-808 (1985).

31. Luger, K., Rechsteiner, T.J., Flaus, A.J., Waye, M.M. & Richmond, T.J. Characterization of nucleosome core particles containing histone proteins made in bacteria. *J. Mol. Biol.* **272**, 301-311 (1997).

32. Glikin, G.C., Ruberti, I. & Worcel, A. Chromatin assembly in *Xenopus* oocytes: *in vitro* studies. *Cell* **37**, 33-41 (1984).

33. Ito, T., Bulger, M., Pazin, M.J., Kobayashi, R. & Kadonaga, J.T. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**, 145-155 (1997).

34. Ito, T., Levenstein, M.E., Fyodorov, D.V., Kutach, A.K., Kobayashi, R. & Kadonaga, J.T. ACF consists of two subunits, Acf1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. *Genes Dev.* **13**, 1529-1539 (1999).

35. Levenstein, M.E. & Kadonaga, J.T. Biochemical analysis of chromatin containing recombinant *Drosophila* core histones. *J. Biol. Chem.* **277**, 8749-8754 (2002).

36. Fyodorov, D.V. & Kadonaga, J.T. Dynamics of ATP-dependent chromatin assembly by ACF. *Nature* **418**, 897-900 (2002).

37. Fyodorov, D.V. & Kadonaga, J.T. Chromatin assembly *in vitro* with purified recombinant ACF and NAP-1. *Methods Enzymol.* **371**, 499-515 (2003).

38. Loyola, A., LeRoy, G., Wang, Y.H. & Reinberg, D. Reconstitution of recombinant chromatin establishes a requirement for histone-tail modifications during chromatin assembly and transcription. *Genes Dev.* **15**, 2837-2851 (2001).

39. Loyola, A., Huang, J.Y., LeRoy, G., Hu, S., Wang, Y.H., Donnelly, R.J., Lane, W.S., Lee, S.C. & Reinberg, D. Functional analysis of the subunits of the chromatin assembly factor RSF. *Mol. Cell. Biol.* **23**, 6759-6768 (2003).

40. Loyola, A. & Reinberg, D. Histone deposition and chromatin assembly by RSF. *Methods* **31**, 96-103 (2003).

41. Bulger, M. & Kadonaga, J.T. Biochemical reconstitution of chromatin with physiological nucleosome spacing. *Methods in Molecular Genetics* **5**, 241-262 (1994).

42. Becker, P.B., Tsukiyama, T. & Wu, C. Chromatin assembly extracts from *Drosophila* embryos. *Methods Cell Biol.* **44**, 207-223 (1994).

43. Noll, M. & Kornberg, R.D. Action of micrococcal nuclease on chromatin and the location of histone H1. *J. Mol. Biol.* **109**, 393-404 (1977).

44. Finch, J.T., Lutter, L.C., Rhodes, D., Brown, R.S., Rushton, B., Levitt, M. & Klug, A. Structure of nucleosome core particles of chromatin. *Nature* **269**, 29-36 (1977).

45. Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D. & Klug, A. Structure of the nucleosome core particle at 7 A resolution. *Nature* **311**, 532-537 (1984).

46. Perlmann, T. & Wrange, O. Specific glucocorticoid receptor binding to DNA reconstituted in a nucleosome. *EMBO J.* **7**, 3073-3079 (1988).

47. Cirillo, L.A. & Zaret, K.S. An early developmental transcription factor complex that is more stable on nucleosome core particles than on free DNA. *Mol. Cell* **4**, 961-969 (1999).

48. Mardian, J.K., Paton, A.E., Bunick, G.J. & Olins, D.E. Nucleosome cores have two specific binding sites for nonhistone chromosomal proteins HMG 14 and HMG 17. *Science* **209**, 1534-1536 (1980).

49. Sandeen, G., Wood, W.I. & Felsenfeld, G. The interaction of high mobility proteins HMG14 and 17 with nucleosomes. *Nucleic Acids Res.* **8**, 3757-3778 (1980).

50. Cote, J., Quinn, J., Workman, J.L. & Peterson, C.L. Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53-60 (1994).

51. Kwon, H., Imbalzano, A.N., Khavari, P.A., Kingston, R.E. & Green, M.R. Nucleosome disruption and enhancement of activator binding by a human SW1/SNF complex. *Nature* **370**, 477-481 (1994).

52. Hamiche, A., Sandaltzopoulos, R., Gdula, D.A. & Wu, C. ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* **97**, 833-842 (1999).

53. Langst, G., Bonte, E.J., Corona, D.F. & Becker, P.B. Nucleosome movement by CHRAC and ISWI without disruption or trans- displacement of the histone octamer. *Cell* **97**, 843-852 (1999).

54. Luger, K., Mader, A.W., Richmond, R.K., Sargent, D.F. & Richmond, T.J. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* **389**, 251-260 (1997).

55. Chao, M.V., Gralla, J. & Martinson, H.G. DNA sequence directs placement of histone cores on restriction fragments during nucleosome formation. *Biochemistry* **18**, 1068-1074 (1979).

56. Simpson, R.T. & Stafford, D.W. Structural features of a phased nucleosome core particle. *Proc. Natl. Acad. Sci. USA.* **80**, 51-55 (1983).

57. Shrader, T.E. & Crothers, D.M. Artificial nucleosome positioning sequences. *Proc. Natl. Acad. Sci. USA.* **86**, 7418-7422 (1989).

58. Lowary, P.T. & Widom, J. New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J. Mol. Biol.* **276**, 19-42 (1998).

59. Linxweiler, W. & Horz, W. Reconstitution of mononucleosomes: characterization of distinct particles that differ in the position of the histone core. *Nucleic Acids Res.* **12**, 9395-9413 (1984).

60. Meersseman, G., Pennings, S. & Bradbury, E.M. Mobile nucleosomes--a general behavior. *EMBO J.* **11**, 2951-2959 (1992).

61. Usachenko, S.I., Bavykin, S.G., Gavin, I.M. & Bradbury, E.M. Rearrangement of the histone H2A C-terminal domain in the nucleosome. *Proc. Natl. Acad. Sci. USA.* **91**, 6845-6849 (1994).

62. Ramsay, N., Felsenfeld, G., Rushton, B.M. & McGhee, J.D. A 145-base pair DNA sequence that positions itself precisely and asymmetrically on the nucleosome core. *EMBO J.* **3**, 2605-2611 (1984).

63. Alexiadis, V. & Kadonaga, J.T. Strand pairing by Rad54 and Rad51 is enhanced by chromatin. *Genes Dev.* **16**, 2767-2771 (2002).

64. Tsukiyama, T., Becker, P.B. & Wu, C. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**, 525-532 (1994).

65. Pazin, M.J., Sheridan, P.L., Cannon, K., Cao, Z., Keck, J.G., Kadonaga, J.T. & Jones, K.A. NF-kappa B-mediated chromatin reconfiguration and transcriptional activation of the HIV-1 enhancer *in vitro. Genes Dev.* **10**, 37-49 (1996).

66. Armstrong, J.A. & Emerson, B.M. NF-E2 disrupts chromatin structure at human beta-globin locus control region hypersensitive site 2 *in vitro. Mol. Cell. Biol.* **16**, 5634-5644 (1996).

67. Pazin, M.J., Kamakaka, R.T. & Kadonaga, J.T. ATP-dependent nucleosome reconfiguration and transcriptional activation from preassembled chromatin templates. *Science* **266**, 2007-2011 (1994).

68. Sheridan, P.L., Sheline, C.T., Cannon, K., Voz, M.L., Pazin, M.J., Kadonaga, J.T. & Jones, K.A. Activation of the HIV-1 enhancer by the LEF-1 HMG protein on nucleosome-assembled DNA *in vitro. Genes Dev.* **9**, 2090-2104 (1995).

69. Mizuguchi, G., Tsukiyama, T., Wisniewski, J. & Wu, C. Role of nucleosome remodeling factor NURF in transcriptional activation of chromatin. *Mol. Cell* **1**, 141-150 (1997).

70. Mayall, T.P., Sheridan, P.L., Montminy, M.R. & Jones, K.A. Distinct roles for P-CREB and LEF-1 in TCR alpha enhancer assembly and activation on chromatin templates *in vitro*. *Genes Dev.* **11**, 887-899 (1997).

71. Orphanides, G., LeRoy, G., Chang, C.H., Luse, D.S. & Reinberg, D. FACT, a factor that facilitates transcript elongation through nucleosomes. *Cell* **92**, 105-116 (1998).

72. Armstrong, J.A., Bieker, J.J. & Emerson, B.M. A SWI/SNF-related chromatin remodeling complex, E-RC1, is required for tissue-specific transcriptional regulation by EKLF *in vitro*. *Cell* **95**, 93-104 (1998).

73. Kraus, W.L. & Kadonaga, J.T. p300 and estrogen receptor cooperatively activate transcription via differential enhancement of initiation and reinitiation. *Genes Dev.* **12**, 331-342 (1998).

74. Naar, A.M., Beaurang, P.A., Zhou, S., Abraham, S., Solomon, W. & Tjian, R. Composite co-activator ARC mediates chromatin-directed transcriptional activation. *Nature* **398**, 828-832 (1999).

75. Jiang, W., Nordeen, S.K. & Kadonaga, J.T. Transcriptional analysis of chromatin assembled with purified ACF and dNAP1 reveals that acetyl-CoA is required for preinitiation complex assembly. *J. Biol. Chem.* **275**, 39819-39822 (2000).

76. An, W., Palhan, V.B., Karymov, M.A., Leuba, S.H. & Roeder, R.G. Selective requirements for histone H3 and H4 N termini in p300-dependent transcriptional activation from chromatin. *Mol. Cell* **9**, 811-821 (2002).

77. Tsukiyama, T. & Wu, C. Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* **83**, 1011-1020 (1995).

78. Varga-Weisz, P.D., Wilm, M., Bonte, E., Dumas, K., Mann, M. & Becker, P.B. Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature* **388**, 598-602 (1997).

79. Pazin, M.J., Bhargava, P., Geiduschek, E.P. & Kadonaga, J.T. Nucleosome mobility and the maintenance of nucleosome positioning. *Science* **276**, 809-812 (1997).

80. O'Neill, T.E., Smith, J.G. & Bradbury, E.M. Histone octamer dissociation is not required for transcript elongation through arrays of nucleosome cores by phage T7 RNA polymerase *in vitro*. *Proc. Natl. Acad. Sci. USA.* **90**, 6203-6207 (1993).

81. Garcia-Ramirez, M., Rocchini, C. & Ausio, J. Modulation of chromatin folding by histone acetylation. *J. Biol. Chem.* **270**, 17923-17928 (1995).

82. Logie, C. & Peterson, C.L. Catalytic activity of the yeast SWI/SNF complex on reconstituted nucleosome arrays. *EMBO J.* **16**, 6772-6782 (1997).

83. Kornberg, R.D. & Thomas, J.O. Chromatin structure; oligomers of the histones. *Science* **184**, 865-868 (1974).

84. Ruiz-Carrillo, A. & Jorcano, J.L. An octamer of core histones in solution: central role of the H3-H4 tetramer in the self-assembly. *Biochemistry* **18**, 760-768 (1979).

85. von Holt, C. *et al.* Isolation and characterization of histones. *Methods Enzymol.* **170**, 431-523 (1989).

86. Simon, R.H. & Felsenfeld, G. A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. *Nucleic Acids Res.* **6**, 689-696 (1979).

87. Croston, G.E., Lira, L.M. & Kadonaga, J.T. A general method for purification of H1 histones that are active for repression of basal RNA polymerase II transcription. *Protein Expr. Purif.* **2**, 162-169 (1991).

88. Germond, J.E., Hirt, B., Oudet, P., Gross-Bellark, M. & Chambon, P. Folding of the DNA double helix in chromatin-like structures from simian virus 40. *Proc. Natl. Acad. Sci. USA.* **72**, 1843-1847 (1975).

89. Pfaffle, P. & Jackson, V. Studies on rates of nucleosome formation with DNA under stress. *J. Biol. Chem.* **265**, 16821-16829 (1990).

90. Rhodes, D. & Laskey, R.A. Assembly of nucleosomes and chromatin *in vitro*. *Methods Enzymol.* **170**, 575-585 (1989).

91. Nakagawa, T., Bulger, M., Muramatsu, M. & Ito, T. Multistep chromatin assembly on supercoiled plasmid DNA by nucleosome assembly protein-1 and ATP-utilizing chromatin assembly and remodeling factor. *J. Biol. Chem.* **276**, 27384-27391 (2001).

92. Blank, T.A. & Becker, P.B. Electrostatic mechanism of nucleosome spacing. *J. Mol. Biol.* **252**, 305-313 (1995).

93. Bulger, M., Ito, T., Kamakaka, R.T. & Kadonaga, J.T. Assembly of regularly spaced nucleosome arrays by *Drosophila* chromatin assembly factor 1 and a 56-kDa histone-binding protein. *Proc. Natl. Acad. Sci. USA.* **92**, 11726-11730 (1995).

94. Peck, L.J. & Wang, J.C. Energetics of B-to-Z transition in DNA. *Proc. Natl. Acad. Sci. USA.* **80**, 6206-6210 (1983).

95. Camerini-Otero, R.D. & Felsenfeld, G. Supercoiling energy and nucleosome formation: the role of the arginine-rich histone kernel. *Nucleic Acids Res.* **4**, 1159-1181 (1977).

96. Thoma, F., Koller, T. & Klug, A. Involvement of histone H1 in the organization of the nucleosome and of the salt-dependent superstructures of chromatin. *J. Cell Biol.* **83**, 403-427 (1979).

97. An, W., Kim, J. & Roeder, R.G. Ordered Cooperative Functions of PRMT1, p300, and CARM1 in Transcriptional Activation by p53. *Cell* **117**, 735-748 (2004).

98. LeRoy, G., Orphanides, G., Lane, W.S. & Reinberg, D. Requirement of RSF and FACT for transcription of chromatin templates *in vitro. Science* **282**, 1900-1904 (1998).

99. Corona, D.F. *et al.* Two histone fold proteins, CHRAC-14 and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex (CHRAC). *EMBO J.* **19**, 3049-3059 (2000).

Correspondence should be addressed to J.T.K. (e-mail: jkadonaga@ucsd.edu).

**Acknowledgments.** We thank Timur Yusufzai, Tammy Juven-Gershon, Jer-Yuan Hsu, Chin Yan Lim, Barbara Rattner, and Tom Boulay for critical reading of the manuscript. Our research on chromatin assembly and function was supported by grants from the NIH (GM 46995 and GM58272) and the Volkswagen Stiftung (I/77 995) to J.T.K. A.L. is the recipient of a fellowship from the Austrian Programme for Advanced Research and Technology (APART) of the Austrian Academy of Sciences.

## Figure Legends

**Figure 1.** Schematic diagram of chromatin. The core histone octamers are shown in yellow, and histone H1 is shown in blue. The fundamental repeating unit of chromatin is the nucleosome, which typically consists of 180-200 bp DNA, a core histone octamer, and a molecule of the linker histone H1. An isolated nucleosome core (histone octamer and 147 bp DNA) is termed a core particle, whereas a slightly larger species containing about 166 bp DNA and one molecule of histone H1 is termed a chromatosome.

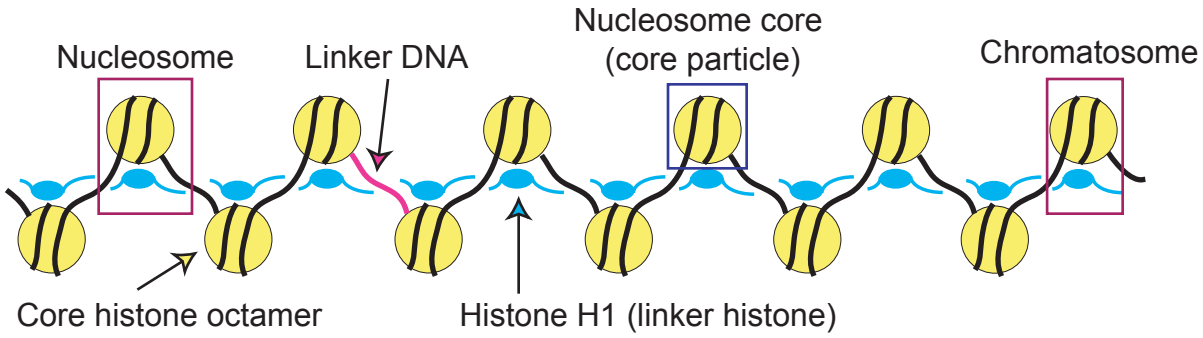
**Figure 2.** Different types of chromatin that can be reconstituted *in vitro* with purified components.

**Figure 3.** Assays for chromatin reconstitution.
**a.** DNA supercoiling analysis. Chromatin is relaxed with topoisomerase I. The samples are deproteinized, and the resulting DNA species are subjected to either one-dimensional (left) or two-dimensional (right) agarose gel electrophoresis. Two-dimensional agarose gel electrophoresis is typically carried out in the absence of chloroquine during the first dimension and in the presence of chloroquine during the second dimension. The lower part of the figure depicts representative DNA patterns. The numbers in the panel for two-dimensional gel electrophoresis specify topoisomers containing 0, 6 and 14 negative supercoils. N denotes the position of nicked DNA.
**b.** Micrococcal nuclease digestion assay. Chromatin is partially digested with micrococcal nuclease. Then, the sample is deproteinized, and the resulting DNA fragments are subjected to agarose gel electrophoresis. DNA fragments corresponding to mononucleosomes (denoted 1-mer), dinucleosomes (2-mer), *etc.* are symbolized by black bars.

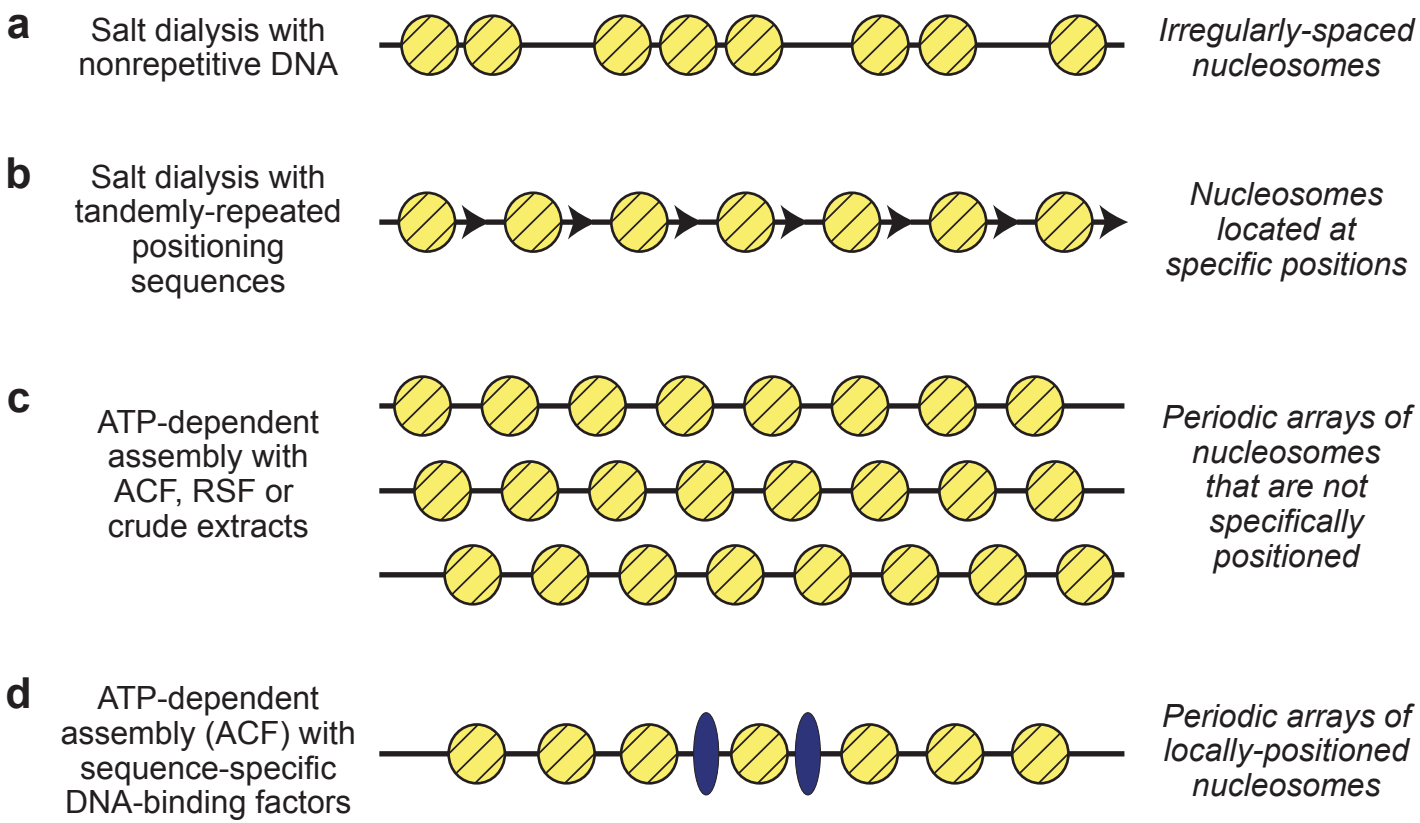
**Box 1**. ACF and RSF: Two ATP-utilizing Chromatin Assembly Factors

The assembly of periodic nucleosome arrays requires an ATP-utilizing factor such as ACF or RSF. ACF has been studied mainly in *Drosophila* and consists of two subunits – Acf1 and the ISWI ATPase[33,34]. RSF was identified in humans and comprises two subunits – p325 (Rsf-1), which is related but not homologous to Acf1, and hSNF2H, which is homologous to the *Drosophila* ISWI ATPase[39,98]. Recombinant ACF and RSF can be produced by cosynthesis of their respective subunits in Sf9 cells by using a baculovirus expression system. Subsequent affinity purification of the complexes via a Flag-tagged subunit (Acf1 or Rsf-1) yields purified, highly active protein[37,40].

ACF processively catalyzes the transfer of core histones from the NAP-1 chaperone to DNA to yield a periodic array of nucleosomes[36]. ACF-mediated chromatin assembly can be performed with purified recombinant ACF, purified recombinant NAP-1, purified native or recombinant core histones, DNA (relaxed circular, supercoiled, or linear), and ATP. *Drosophila* ACF is able to assemble *Drosophila*, *Xenopus*, or human histones into chromatin. In addition, ACF can catalyze the assembly of histone H1-containing chromatin[37]. Like ACF, RSF uses the energy of ATP hydrolysis to catalyze the assembly of nucleosomes[38]. Unlike ACF, RSF does not require a core histone chaperone. Efficient RSF-mediated nucleosome assembly requires the acetylation of histones H2A and H2B, such as that catalyzed by the p300 acetyltransferase[38,40]. In contrast, ACF efficiently assembles unacetylated histones into chromatin[35,76]. It should also be noted that ACF is closely related to another factor termed CHRAC (chromatin accessibility complex). CHRAC was purified on the basis of its ability to increase the access of restriction enzymes to DNA in chromatin[78]. ACF and CHRAC are identical except for the presence of two additional subunits, CHRAC-14 and CHRAC-16, in the CHRAC complex[99].



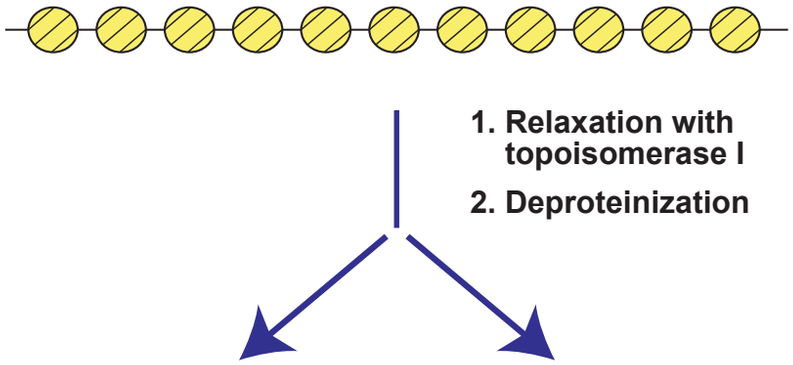

Lusser and Kadonaga (Fig. 1)




Lusser and Kadonaga (Fig. 2)

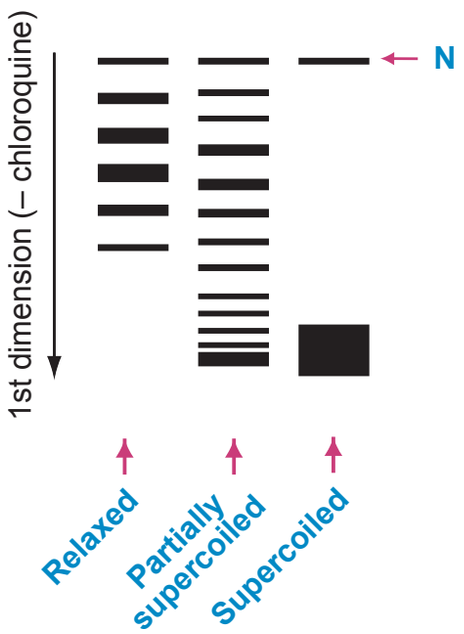
a

## DNA supercoiling analysis

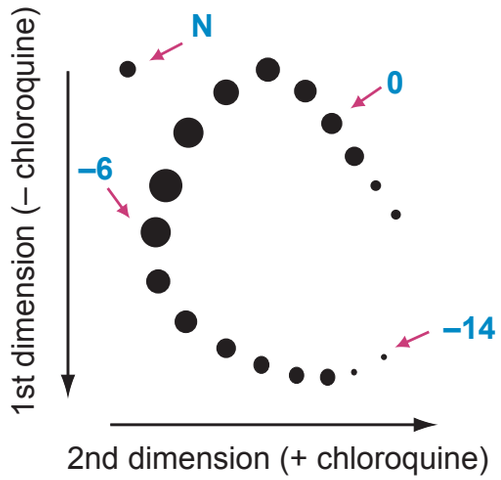


1. Relaxation with topoisomerase I
2. Deproteinization

**1-D agarose gel electrophoresis**



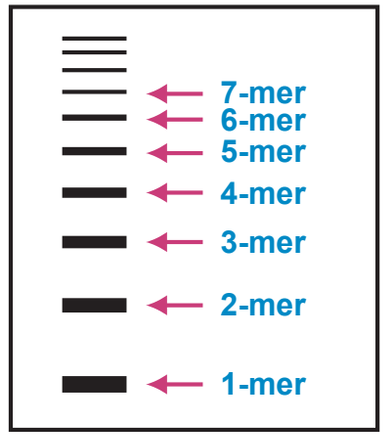

**2-D agarose gel electrophoresis**




b

## Micrococcal nuclease digestion assay

1. Partial digestion with micrococcal nuclease
2. Deproteinization
3. Agarose gel electrophoresis




Lusser and Kadonaga (Fig. 3)