Review Letter

CHROMATIN STRUCTURE AND SPECIFICITY REVEALED BY IMMUNOLOGICAL TECHNIQUES

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1. Introduction

Chromatin is a nucleoprotein complex in which DNA, RNA, histone and non-histone proteins are organized in a manner which allows for regulation of the expression of the genetic message encoded in DNA (for review see [1]). Structural studies on chromatin have to take into account the dynamic properties of this complex. The exact organization of chromosomal components may undergo temporal variations during the life cycle of a cell. The changes which occur in the gross structure of chromatin are most obvious when the diffuse appearance of interphase chromatin is compared to the well defined superstructure of chromosomes.

Unfortunately, most chromosomal proteins do not have an assayable function which allows one to follow their fate during various developmental stages of a tissue or during the life cycle of a cell.

Approaches to the study of the structure of chromatin range from a characterization of the individual (and often denatured) components, to the investigation of 'native, unfractionated' nucleoprotein. The first yields important information but seldom reveals how the individual components interact with each other. The study of 'native' chromatin, even though attacked by a great variety of methods, suffers from the inability to distinguish the contribution of the individual chromosomal components to the various parameters measured.

Immunochemical techniques may bridge the two approaches and provide information which is very difficult to obtain by other methods. Specific antibodies can be used to identify a chromosomal component complexed in chromatin, being synthesized on the ribosome [2] or free in solution. Serological reactions conceivably can be used to follow conformational changes or structural rearrangements occuring in chromatin or chromosomes. These techniques are mild; requiring neutral pH, low temperatures and moderate ionic strength; therefore, they do not lead to drastic structural changes. They can be used to visualize the organization of a chromosomal component at the level of resolution afforded by both the light microscope (immunofluorescence) and the electron microscope.

It is the purpose of this review to summarize the information obtained about the structure and specificity of both chromatin and chromosomes by immunological techniques.

2. Antisera

A variety of antisera can potentially be useful in probing the structure of chromatin. Antisera against double-stranded DNA occur spontaneously. Antibodies against single-stranded DNA, RNA, dinucleotides, trinucleotides and against several bases and base analogs can be experimentally induced. The immunology of nucleic acids has been recently reviewed by Stollar [3]. In addition, antisera against the following chromosomal components have been elicited:

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chromatin [4–8], reconstituted nucleoprotein [3,9], histone-depleted chromatin [10–12], nonhistone proteins, [8,13–15], the major histone fractions (H1, H2A, H2B, H3, H4, H5) [2,7,15–25], H1 histone subfractions [26–28], and histone fragments [28,29]. The techniques for eliciting and assaying these antibodies, as well as examples of their use, have been described by Chytil [30] and Stollar [3,31].

Unfortunately, the specificity of some of the antisera used as probes for chromatin and chromosomes has not been well defined. Limited investigation of the specificity of antisera may be misleading since two different antigens, which may show immunological specificity when tested with highly diluted antisera, may show considerable cross reaction when tested at lower sera dilution. Determination of the immunological distance [27,68] between two related proteins or between a protein and its derivative (obtained by chemical modification or by treatment with various denaturing reagents) can provide a reasonable quantitave measure of the degree of cross reaction between two antigens. The question of cross reactivity is of particular relevance to immunofluorescence studies when relatively low dilutions of antisera are used.

3. Specificity studies

Immunological specificity refers to a situation where it is possible to distinguish between antigens by the use of an antisera. The specificity of antibodies is sufficiently sharp to distinguish between two related proteins in which a single amino acid replacement occured in an immunogen region [32,33]. It is difficult to elucidate the molecular basis of immunologic specificity in complex antigens such as chromatin and chromosomes; however, the immunologic specificity is a sensitive analytical tool to detect differences between such antigens, which by other criteria may seem identical.

3.1. *Histones*

While immunological tests for determination of protein specificity are not as rigorous as determination of primary sequence they are sufficiently convenient so that the specificity of a large number of proteins can be rapidly screened. Antisera elicited against calf thymus histones, H2A, H2B, and H4, failed to distinguish between these histones and those obtained from chicken erythrocyte, frog liver, human spleen, and lobster hepatopancreas reflecting the conservation in the primay structure of these proteins during evolution [17]. On the other hand, antisera elicited against unfractionated H1 derived from calf thymus [26] or several rat tissues [34] revealed both species and organ specificity in this histone class which is in agreement with other studies [1]. Specific antibodies elicited against chromatographically purified H1 subfractions derived from rat thymus revealed that some of the subfractions have sequence differences as high as 20% [27]. Detailed analysis of purified H1 subfractions from rat liver, rat thymus, and calf thymus revealed that the organ specificity is of a much lower order than the species specificity [27]. The range of difference between the various H1 subfractions contained in one tissue is of the same order as that between H1 sub-fractions derived from various species suggesting that each H1 subfraction evolved independently. H1 subfractions derived from tumors retain the immunological specificity of subfractions derived from normal tissue [28]. The species and tissue specificity is also observed in the C-terminal fragments derived from H1 by cleavage at the single tyrosine residue with *N*-bromo-succinimide [28].

Serological reactions can detect conformational changes in histones. Thus, Mihalakis et al. [23], using antisera to salt extracted H1, detected irreversible conformational changes in acid- or urea-treated H1. *N*-Bromosuccinimide cleaves H1 into 2 large segments; trypsin digestion produces many smaller peptides. The serological reaction of intact H1 could not be reconstituted with any combination of fragments, suggesting the presence of conformational determinants in this histone [29]. Maleylation [35], phosphorylation [36], nitration [29], and dinitrophenylation [29] of H1 also result in changes which can be detected immunologically. However since the immunologic distance [68] between the histone and its derivative have not been determined it is difficult to estimate the magnitude of the change.

3.2. *Non-histone proteins*

With the exception of protein D1 from *Drosophila*

[15], antibodies specific to a particular nonhistone component have not been described. In this review nonhistone chromosomal proteins are defined as proteins (excluding histones) that are purified from isolated chromatin or chromosomes [1]. Stumph et al. [13] have elicited antibodies against a particular molecular weight non-histone protein sub-fraction by immunization with protein–SDS complexes eluted from polyacrylamide gels. Thus, immunological studies on the specificity of nonhistone proteins, using anti-nonhistone sera, have not been performed yet. However, since native DNA is not immunogenic [3] and chromatin-bound histones are very poor immunogens [3,7,10,12,18], studies using antisera elicited against 'native' chromatin or dehistonized chromatin often measure determinants contained in the chromatin-bound nonhistone proteins.

3.3. *Chromatin and dehistonized chromatin*

Antisera elicited by either chromatin or dehistonized chromatin exhibits a remarkable specificity for the immunogen. The degree of specificity has not been quantitated; however, the differences reported seem to be larger than those detected by other techniques.

By immunizing with a partially purified nucleoprotein preparation, immunological differences between normal and leukemic white blood cells [4] and between normal and malignant canine tissue [5] were detected by the immunodiffusion technique and by passive hemagglutination. Absolute specificity was claimed after cross-adsorption of the antisera with heterologous tissue. By immunofluorescence with cells grown in tissue culture, antisera against chromatin from 3T6 mouse fibroblasts and from W7-38 human fibroblasts revealed distinct species specificity in nuclear staining [37]. However, in each case, cytoplasmic fluorescence was observed raising the specter of cytoplasmic contamination in partially purified chromatins.

The problem of cytoplasmic contamination is almost completely avoided if chromatin is isolated from purified nuclei. Essentially all the histones and about 30% of nonhistone proteins can be extracted by dissociation in 2.0 M NaCl, 5 M urea pH 6, and prolonged centrifugation. DNA and tightly bound nonhistones are recovered as a pellet. Using such a pellet (NP–DNA) obtained from chick oviduct, Chytil and Spelsberg [10] obtained a tissue specific antiserum which could distinguish between NP–DNA complexes and chromatins derived from other chick tissues. The antigenic sites were pinpointed to the nonhistone protein fraction. Histones and DNA were not antigenic even in native chromatin. Addition of histones to NP–DNA masked over 80% of antigenic determinants. The NP–DNA complexes could be completely dissociated with concomitant loss of almost all immunological reactivity. Upon reassociation, immunological reactivity was restored. Nonhistone proteins from chick oviduct (the immunogen) can be complexed with DNA derived from other sources to form hybrid chromatins. In each case, regardless of the origin of DNA, hybrid chromatins containing chick oviduct nonhistone proteins were serologically active, while hybrid chromatin containing non-histone proteins from another tissue were unreactive [38]. The hybrid chromatin resembles the chromatin serving as the source of nonhistone proteins both immunologically and as measured by template activity. This suggests that acidic proteins recombine with DNA in a manner which reconstitutes the antigenic sites of untreated chromatin.

The immunological tissue and species specificity of NP–DNA were confirmed by Wakabayashi and Hnilica [39]. However, the latter group reports that immunological activity could be restored only in complexes containing homologous DNA. DNA isolated from other species, as well as other polyanions, did not reconstitute a tissue specific complex as measured by complement fixation [12]. The difference in the reconstitution results, could be due to minor differences in obtaining the NP–DNA fraction. While the first group obtained the pellet with 2.0 M NaCl, 5.0 M urea, 1.0 mM $MgCl_2$, 10 mM phosphate pH 6.0; the second group used 2.5 M NaCl, 5.0 M urea, 50 mM sodium phosphate. Other differences in extracting the chromatin, separating the tightly bound nonhistones from DNA, and the exact protocol for reconstitution, could account for the discrepancies on this point.

Using the approaches described above, antisera elicited against chromatin or NP–DNA complexes was used to follow changes in chromatin during ontogeny of rat liver [40]; to detect differences between chromatins derived from neoplastic and normal tissues [11,12,41]; to detect differences

between fast growing and slow growing tumors [12,41]; and to detect difference between chromatins derived from human WI-38 fibroblast and their SV_{40} transformed counterparts [6].

The specificity observed with antisera elicited by NP–DNA complexes is higher than that detected in nonhistone protein fractions by other techniques. The specificity may be due either to tissue specific composition of these proteins, to tissue specific structural relationship of some proteins with DNA, or both [10]. It is clear that at least part of the antigenic determinants are conformational determinants which detect specific structural features of chromatin. Isolated nonhistone proteins or DNAase digest of NP–DNA complexes show a markedly diminished, or a complete loss of, immunoreactivity [10,12,38,39]. All the experiments described above used the complement fixation technique [31,42] to study the specificity of the antisera. This technique is extremely sensitive, maximizing the differences between various antigens. The availability of antisera elicited in chickens which allows measuring immunological reaction at high ionic strength [43] where chromosomal components are soluble, introduces an additional immunological technique for the study of chromosomal components.

Antisera elicited against chromatin antibodies react with a variety of chromosomal components. These antibodies could be useful for detecting changes between chromatins derived from various sources or occuring in chromatin during development, neoplasia, hormonal stimulation, or other physiological changes. For more detailed structural analyses, antisera of a defined, narrow specificity may prove more useful.

4. Structure of chromatin

The application of immunochemical techniques to the study of the structure of chromatin and chromosomes is complicated by a conflicting situation. Absolute specificity requires pure antigens, yet chromosomal components are closely and specifically organized so that their purification requires steps which often alter their native structure. Thus, the degree of exposure of antigenic determinants is of paramount importance and the immunological reaction obtained cannot always be correlated with results obtainable by other techniques. For example, it could be expected that the amount of DNA exposed to digestion by nucleases [69] or available to titration by polylysine [70] or certain dyes [71] will be substantially higher than that available to interact with antibody. Nevertheless, immunological techniques can be very useful as the information obtainable ranges from insight into the overall organization of the nucleus to pinpointing and visualization of the location of a particular histone in nucleosomes.

4.1. *DNA*

Antisera specific for double-stranded DNA and for single-stranded DNA were used to quantitate the exposure of determinants specific for these structures in chromatin. Using quantitative microcomplement fixation, Stollar [3,9] found that only 2–5% of the DNA in chromatin was as exposed as naked DNA. The unavailability of determinants specific for double-stranded DNA reflected specific organization of the chromosomal components since dissociation followed by reassociation of chromatin resulted in irreversible changes in which up to 20% of DNA became reactive. In artificial DNA: histone complexes (at ratios similar to those reported in chromatin) 50% of the DNA was reactive.

A similar search for single-stranded DNA in chromatin indicated that less than 0.01% of DNA is single-stranded [44]. However, using anti-thymine antibody some single-stranded DNA in rat liver nuclei was detected by immuno-electron microscopy [45]. The indirect immunofluorescence technique with antisera specific to double-stranded DNA and single-stranded DNA has been used to search for the existence of such structures during the cell cycle [47]. Native DNA determinants were available adjacent to nuclear membrane in G1, while single-stranded DNA determinants were available in S phase. DNA–histone determinants were visible throughout the cell cycle. Other immunofluorescence studies on the exposure of a variety of chromosomal determinants during the cell cycle were recently summarized by Stollar [3].

Immunofluorescence studies with whole nuclei or with cells do not yield quantitative data and the results may be further complicated since the ability of antibodies to penetrate the cell and nuclear membrane has to be taken in account. Nevertheless,

such studies may provide information on gross structural changes occurring within the nucleus.

4.2. *Organization of histones in chromatin*

The availability of antisera specific for each of the major histone fractions H1, H2A, H2B, H3, H4 and H5 as well as against H1 subfractions makes it possible to probe the organization of these proteins in chromatin.

4.2.1. Immunofluorescence

The gross organization of the histones in the interphase nucleus was studied by immunofluorescence. Anti-H1 stained nuclei of rat liver [16,22,34,48] as well as nuclei of human carcinoma, Syrian hamster, rat embryonal tissue, and human lymphocyte [22,48]. They did not stain spermatozoa or nuclei from mature chick red blood cell [48]. It should be noted that in most cases the antibodies were prepared against histones purified from calf thymus. While only cross-reacting determinants are measured, this procedure also minimizes the possibility that the fluorescence is due to some tissue-specific chromosomal protein. The method of fixing the cells affects the results obtained, probably because of extraction of histone or other chromosomal components during fixation and because of conformational changes. Thus, anti-H4 stained acetone-fixed but not methanol-fixed cells [22]. The fluorescence pattern obtained with anti-H1 is different from that obtained with anti-H4 in that H4 determinants were preferentially exposed at the peripheral part of the nucleus [22]. It is perhaps significant, that when determinants of native DNA react with antibody they are also located adjacent to nuclear membrane [47]. It is possible that this portion of chromatin has a less condensed structure than the bulk of the nuclear chromatin. Huang [49] used the immunofluorescence technique to follow the appearance of H5 determinants during the development of the chick red blood cell. The number of H5-positive cells increased during development and there seemed to be a correlation between the fluorescence pattern of a nucleus and its stage of development suggesting differences in the gross organization of chromatin at the various developmental stages. The degree of compactness of chromatin seems to affect the exposure of histone determinants in nuclei since in some cases nuclei stained with anti-H3, H2A and H2B only after swelling at pH 9.0 in the presence of dithiothreitol and trypsin [48].

4.2.2. Immunoadsorption studies

Anti-histone sera react weakly with chromatin in the microcomplement fixation test suggesting that some histone determinants are masked in native chromatin [7,18]. However, by using chromatin as an immunoadsorbant it is possible to measure the availability of antigenic determinants in chromatin-bound histones in a sensitive and semi-quantitative way [7,18]. In the immunoadsorption technique a constant amount of specific antibody is added to various amounts of chromatin, the chromatin and antibodies bound to it are separated from non-bound antibodies by centrifugation and the remaining antibodies are tested against the homologous immunogen. The method allows the use of a high concentration of chromatin (which in a direct test often is anticomplementary) and yields semiquantitative data. Sequential adsorption with antisera specific to the various histones indicated that addition of antibodies to chromatin does not cause marked alterations in the structure of chromatin and that the determinants of the various histones are spaced in a way which does not cause significant steric hindrance, probably in some ordered fashion [7]. Current models on nucleosome organization suggest several possibilities for ordered histone organization in chromatin [50,51]. The availability of histone determinants in chromatin is probably dependent on steric factors such as the extent to which the histone is buried inside the macromolecular nucleoprotein complex, and conformational changes occurring in the molecule upon binding to DNA or upon complexing with other chromosomal components. A value defined as 'equivalent antigenicity' allows semi-quantitative comparison of the ability of determinants in chromatin-bound histones to that of non-bound histones to interact with homologous antibody [18]. Using this value, it could be shown that in chromatin, histone H1 and H2B are more exposed than histones H3 and H4; histone H2A being the less exposed as measured by this technique. Sonication changed the availability of determinants in histone H3 and H4 [18]. Antisera specific to the five H1 histone subfractions present in rat thymus [27] were used to study the organization of each of the sub-fractions in chromatin [52]. The results indicated

that in chromatin the subfractions are arranged in a similar manner. The determinants which are exposed are determinants which are shared among the H1 subfractions [52]. While the complement fixation technique is very sensitive, it yields only semiquantitative data and requires stringent conditions (pH, ionic strenght) thereby minimizing the experimental parameters which can be varied. These problems are minimized when antibodies which have been purified by affinity chromatography and labeled with ^{125}I are used [53]. The amount of ^{125}I antibody bound to chromatin is directly proportional to the amount of antibody added. The amount of antibody bound by a given amount of chromatin-bound histone can be measured. The ability of rat thymus chromatin to bind ^{125}I anti-H1 antibody was higher than that of rat brain chromatin suggesting differences between the two types of chromatin [53].

The immunoadsorption techniques may be most useful for comparative studies. In such studies differences between chromatins derived from various sources [54], at different stages of the cell cycle, of development, of neoplasia, etc., can be checked. However since the binding of a specific antibody to its chromatin-bound determinant depends on numerous parameters, it will be difficult to define the molecular basis of a detected difference.

5. Chromosome structure

The metaphase chromatin is condensed into well defined superstructures – chromosomes. The organization of chromosomal components has been studied by the indirect immunofluorescence technique.

5.1. *DNA*

Antibodies specific to each A, T, C, G, and 5-methyl-C [55,59], when reacting with chromosomes denatured by treatment with formamide [55], or exposed to ultraviolet [57,58] or photoxidized in presence of methylene blue [56] give specific banding patterns. The antibodies react only with single stranded regions. Generally, the banding with anti-A or anti-T is similar to that obtained with guanine or giemsha and is the reverse of that obtained with anti-C. Anti-5-methylcytosine stained most intensely the centromere regions of human chromosome 1, 9, 15, 16 and Y [57,58]. Results using the immunoperoxidase technique [57] were identical to those obtained by immunofluorescence [58] but the former has several technical advantages. It has been suggested that the banding patterns represent the organization of large amounts of repetitive DNA into distinct bands [56]. Antibody to bromodeoxyuridine was used to detect sister chromatid exchange and to follow DNA replication [60].

5.2. *Histones and non-histones*

Both metaphase and polytene chromosomes give positive immunofluorescence with antisera elicited by chromosomal proteins [20,62]. The distribution of nonhistone proteins of various molecular weight in polytene squashes from *Drosophila* salivary glands have been visualized [14] using antisera elicited by SDS–protein complexes [13]. The fluorescent patterns observed, while quite complex, are sufficiently distinct to allow correlation between traditional bands recognizable by phase microscopy and availability of determinants reacting with an antiserum to a nonhistone protein subfraction. It is possible that in some cases the fluorescent pattern may be dependent on the developmental stage of the chromosome. More detailed information on the location of a particular nonhistone protein in the same polytene chromosome was recently obtained with antisera specific to protein D1 [15]. This protein, rich in both basic and acidic amino acids, is localized mainly in a few, intensely fluorescent, regions near the chromocentromere. Additional faintly fluorescent bands were scattered throughout the genome raising the possibility that the organization of this protein differs from one region of the chromosome to another. In contrast to anti-D1, antisera elicited with H1 and H2B obtained from *Drosophila* stain all the bands resolvable by phase contrast microscopy [15].

Antisera elicited against purified calf thymus histone fractions have been used to visualize the location of histones in polytene chromosomes from *Chironomus thummi* [63]. Use of cross reacting sera insures that all the fluorescent bands indeed contain histones however, species specific antigenic determinants will not be visualized. Antisera to H1 and H2A (which may be relatively species specific) gave a uniform weak (albeit positive) staining of all 4 chromosomes. Antisera towards the other 3 histone

types stained brightly most of the bands visualized by phase contrast or by orcein staining. The resolution of individual bands by fluorescence microscopy is almost of the same order as that observed by phase microscopy. The results indicate that the number of antigenic sites of chromosome-bound histones is proportional to the amount of DNA in a band and that each band contains each histone.

The distribution of the various histone fractions in metaphase chromosomes, in which protein extraction and rearrangement has been minimized by cross linking with glutaraldehyde, has also been visualized [64]. Immunofluorescence studies revealed that each of the five histone fractions is located along the entire length of each chromosome present in a tissue [64]. This result suggests that the chromatin fiber is built of units containing all histone types in agreement with current concepts of nucleosome structure [46]. Chromosomes from which histones have been extracted by controlled acid treatment [21,64] display banding or spotty appearance when treated with antihistone sera. The fluorescent pattern depended on the extraction procedure used and on the type of antisera applied and differed from one chromosome to another. The extraction of histones from chromosomes may be dependent on the packing density of the chromatin fiber within the chromosome. It is therefore possible that the use of antisera, specific for each histone in conjunction with differential extraction may serve as a tool for chromosome identification by the histone pattern.

6. Nucleosome structure and composition

Chromatin of eukaryotic organisms is organized into repeating subunits each containing a 180–205 base-pair segment of DNA associated with a core of 8 histones. The repeating units called nucleosomes have been visualized in the electron microscope as spherical particles about 70–120 Å in diameter depending on the exact state of hydration [46,65]. Since it has been shown that specific antihistone antibodies specifically bind to chromatin and chromosomes it is possible that the detailed organization and composition of histones in nucleosomes can be visualized by immuno-electron microscopy. Using anti-chromatin antibodies as a model for interaction of antibodies with chromatin, Bustin, Goldblatt and Sperling [66] found that the beaded network appearance of chromatin is preserved when antisera are added to chromatin spread on grids precoated with bovine serum albumin. Upon specific binding of antibodies to chromatin (verified by the use of a ferritin labeled anti-antibody) the diameter of the nucleosome increases up to 300 Å. This size is compatible with a model where one layer of gamma globulin 110 Å in diameter encircles a chromatin sphere 100 Å in diameter. Thus a significant enlargement of the diameter of a nucleosome upon interaction with antibodies reveals the presence of an antigenic site in this nucleosome. Using this technique, it could be shown that over 95% of nucleosomes react with anti-chromatin sera which contain antibodies to nonhistone proteins but not to DNA or free histones. Since the multiplicity of nonhistone proteins is so large that a single nucleosome cannot contain all the types of nonhistone proteins, the immunological results indicate that the nucleosomes differ from each other in their content on nonhistone proteins.

Visualization of histone H2B content in nucleosomes by immuno-electron microscopy suggests that at least 90% of nucleosomes contain this histone.

Nucleosomes isolated from chromatin by controlled nuclease digestion sediment on isokinetic sucrose gradients with an $s_{20,w}$ of 11 [46]. To assess the heterogeneity and composition of nucleosomes Simpson and Bustin [67] reacted isolated, purified nucleosomes with antibodies against histone H2B purified by affinity chromatography. Incubation of 7 S IgG antibodies with 11 S nucleosomes resulted in a particle of an increased mass. A direct correlation exists between the amount of antibody added and amount of nucleosome sedimenting as heavy material. Over 98% of nucleosomes can be made to sediment faster than an 11 S particle suggesting that each nucleosome contains this histone. Quantitative analyses indicate that, at the lowest IgG concentration sufficient to move all nucleosomes from the original 11 S position, from 1–3 IgG molecules bound per nucleosome. Thus, when nucleosomes are reacted with an unsaturating amount of antibodies they are dispersed and sediment as a relatively broad peak. The histone content in each fraction across the broadened peak was determined by electrophoresis in polyacrylamide gels. The quantitative ratios among the

four histones H2A, H2B, H3 and H4 was constant suggesting that each nucleosome has an identical histone content.

It seems that a systematic study with a variety of antisera using either the immuno-electron microscopic or the immunosedimentation approach will provide useful information on the heterogeneity and composition of the nucleosome. While the size of the probe (the IgG molecules) enforces obvious limits of resolution it seems that a combination of the two approaches may prove a powerful tool in understanding the fine structure of the nucleosome.

7. Summary

The relation between the structure and function of chromatin is complex and not fully understood; therefore, a variety of experimental techniques have to be employed to elucidate the structural basis of the regulation of the genetic message encoded in DNA. The present review attempts to point out and summarize the use of antibodies as probes to study the structure and specificity of chromatin, chromosomes and their components.

The applicability of immunological techniques requires two major steps. The first step requires elucidation of a large repertoire of antibodies specific for the various forms and components of chromatin and chromosomes. The usefulness of antibodies as probes for chromatin structure is directly dependent on the number of specific, well characterized antisera. The available immunochemical techniques allow elucidation and purification of antibodies against almost any conceivable antigen. Indeed, as indicated in this review, a large number of antisera is already available. However the list of potentially useful antisera is almost unlimited. The second step involves adaptation of various serological and immunochemical techniques to the study of chromatin and chromosomes. Unfortunately most of the techniques used are not quantitative. However, the information obtainable on both the gross and fine arrangement of individual components in chromatin and chromosomes is not easily available by other techniques.

As the techniques for preparing 'clean', well-defined nuclei, soluble chromatin, well-defined nucleosomes or polynucleosomes, and unfixed chromosomes improve the information obtainable by immunological techniques will be more exact and defined. Table 1 lists some uses of serological techniques in the study of chromatin, chromosomes and their components.

Table 1
Uses of serological techniques in the study of chromatin and chromosomes

(1)	Specificity of chromosomal proteins studied by: (a) immunodiffusion (b) complement fixation (c) immunofluorescence
(2)	Specificity of chromatin studied by: (a) immunodiffusion (b) direct complement fixation (c) complement fixation after immunoadsorption (d) radio-immunoadsorption (e) immunofluorescence (f) precipitin
(3)	Organization of nucleic acids and proteins in chromatin, studied by: (a) complement fixation (b) immunofluorescence
(4)	Organization of chromosomal components in the cell studied by: (a) immunofluorescence
(5)	Localization of proteins and nucleosides in chromosomes: (a) immunofluorescence (b) peroxidase labeled antibodies
(6)	Organization of histones in nucleosome studied by: (a) immuno-electron microscopy (b) immunosedimentation
(7)	Organization of components in eu- and heterochromatin: (a) immunofluorescence (b) immuno-electron microscopy

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