The Biology of Isolated Chromatin

Chromosomes, biologically active in the test tube, provide a powerful tool for the study of gene action.

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Chromosomes, as they are present in the nucleus in their extended form between mitotic cell divisions, are of interest because it is in this condition that they carry out both DNA replication and RNA synthesis. Such chromosomes, known collectively as chromatin, have been studied by cytology, electron microscopy, and radioautography, and much has become known about them, particularly in such instances as the giant salivary gland chromosomes of Diptera and the lampbrush chromosomes of amphibian oocytes. During recent years, it has become possible to isolate chromatin from the interphase nucleus and to study it directly by the methods of biophysics and biochemistry.

Such isolated chromatin is composed of DNA complexed with proteins and some RNA. In such chromatin, only a portion of the genetic material is available for transcription by RNA polymerase, and the genes thus accessible are the same ones that are accessible and transcribed in life. In this article we summarize some aspects of our knowledge of chromatin and point out the directions in which the further study of isolated chromatin may be expected to yield insight relevant to the control of genetic activity and of developmental processes.

Preparation of Chromatin

Detailed consideration of methodology for the preparation of chromatin from plant and animal tissues is given in Bonner *et al.* (*1*) [see also Zubay and Doty (*2*), Frenster *et al.* (*3*), and Dingman and Sporn (*4*)]. These methods are all based upon disruption of the tissue by homogenization, removal of tissue fragments and membranes by filtration through Miracloth, selective sedimentation of nuclei or chromatin by low-speed centrifugation, washing of the crude chromosomal pellet by repeated suspension and sedimentation, and final purification by sedimentation through 1.7*M* sucrose.

The clear gelatinous pellet, resuspended in and dialyzed against 0.01*M* tris(hydroxymethyl)aminomethane (*p*H 8), exhibits little or no turbidity (optical density at 320 nanometers), less than 0.05 that at 260 nanometers, ratios of protein to DNA in the range 1.3 : 1 to 2 : 1, and ratios of RNA to DNA of 0.2 or less. Purified chromatins migrate in zone electrophoresis as single homogeneous materials. Their melting profiles indicate stabilization of the DNA by the proteins complexed to it.

Generation of RNA

Isolated chromatin generally contains RNA polymerase which is bound to, and is an integral component of, the genetic material. The presence of RNA polymerase activity can be detected by virtue of the fact that isolated chromatin catalyzes the synthesis of RNA from the four nucleoside triphosphates. The enzyme in those instances in which it has been studied possesses the general properties of bacterial RNA polymerase, is dependent for its activity upon the presence of a reduced thiol group, and is destroyed by heat (for example, 60°C). The enzyme can be dissociated from DNA by salt of high concentration (for example, 4*M* CsCl) and separated by sedimentation of the DNA from the solution. A portion of the RNA polymerase, approximately half in the case of pea-plant chromatin, remains in the supernatant and may be selectively extracted from it. Chromosomal RNA polymerase has in this way been purified from pea-plant chromatin (*5*). A soluble polymerase has been purified from corn seedlings (*6*) and from various mammalian tissues (*7*).

When lysed nuclei of plant or animal cells are treated with high salt concentrations, for example 0.4*M* KCl, an aggregate containing the bulk of the nuclear DNA forms and may be wound out from the solution. Among the proteins still bound to this DNA is a portion of the chromosomal RNA polymerase originally present. This type of preparation, the so-called aggregate enzyme of Weiss (*8*), therefore possesses the power to catalyze the synthesis of RNA from the four riboside triphosphates. Study of the aggregate system, while appropriate for the qualitative purpose of demonstrating the presence of RNA polymerase, is less appropriate for other purposes, since some chromosomal proteins may be lost in the salt treatment and the aggregate is highly contaminated with adherent ribosomes and other nonchromosomal constituents (*9*).

Chromatin as Template for Exogenous Polymerase

The RNA polymerase activity of all chromatins which we have studied is relatively low, although whether this represents the true state of affairs inside the cell or the loss of polymerase activity during purification of chromatin is uncertain. In any case, chromatin can in general serve as template for the synthesis of RNA by added RNA polymerase (*10*). By this technique the amount of RNA transcribed from a given chromatin template can be greatly increased above that possible with endogenous polymerase alone. It will be shown that RNA transcribed from isolated chromatin by exogenous poly-

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Table 1. Chemical compositions of varied chromatins.

Source of chromatin	Content, relative to DNA, of				Template activity (% of DNA)
	DNA	Histone	Nonhistone protein	RNA	
Pea embryonic axis	1.00	1.03	0.29	0.26	12
Pea vegetative bud	1.00	1.30	.10	.11	6
Pea growing cotyledon	1.00	0.76	.36	.13	32
Rat liver	1.00	1.00	0.67	.043	20
Rat ascites tumor	1.00	1.16	1.00	.13	10
Human HeLa cells	1.00	1.02	0.71	.09	10
Cow thymus	1.00	1.14	.33	.007	15
Sea urchin blastula	1.00	1.04	0.48	.039	10
Sea urchin pluteus	1.00	0.86	1.04	.078	20

merase is identical with the RNA transcribed from the same chromatin in life by endogenous chromosomal polymerase.

Escherichia coli RNA polymerase (*11*) and micrococcal polymerase (*12*) appear to be equally effective in transcription of the chromosomal template. The kinetics of polymerase-template interaction are shown in Fig. 1. When reaction mixtures containing a fixed amount of *E. coli* polymerase are incubated with increasing amounts of chromatin or of DNA prepared from such chromatin, the rate of RNA synthesis increases until saturation is reached. At this maximum, all polymerase is bound essentially irreversibly by the template (*13*). If twice as much polymerase is used per reaction mixture, then twice as much template is required for saturation of the enzyme. The amount of template required to saturate a given amount of enzyme is the same for chromatin and for deproteinized chromosomal DNA. We take this to signify that the number and availability of polymerase binding sites are identical in chromatin and in purified DNA. In reaction mixtures containing excess polymerase, net syntheses can be achieved with the amount of RNA synthesized exceeding, by 50 or more times, the amount of template DNA used.

Comparative Template Activities

Template activity for RNA synthesis catalyzed by RNA polymerase is less for DNA in the form of liver chromatin than for deproteinized liver DNA (Fig. 1). Such restriction of the template activity of DNA for RNA synthesis is characteristic of all isolated chromatins that have thus far been studied. Liver chromatin possesses a template activity approximately 0.2 that of liver DNA. The template activities of other chromatins assembled in Table 1 range from 6 to 30 percent of that of the corresponding DNA. The template activity for RNA synthesis of chromatins isolated from cells which in vivo exhibit low rates of RNA synthesis are correspondingly low (for example, chromatin of duck erythrocytes, dormant buds, and early developmental stages of sea urchins). Template activities of chromatins isolated from cells which are highly active in RNA synthesis are in general higher.

That a given amount of DNA as chromatin supports a lower rate of RNA synthesis with a given amount of added polymerase than does a corresponding amount of deproteinized DNA could in principle be due either to the availability of only a portion of the template of DNA of chromatin for transcription or to a lowered rate of transcription of all of the chromosomal DNA. That the former is the correct interpretation is shown by several independent lines of evidence. Thus, the RNA transcribed from chromatin by RNA polymerase in vitro exhibits a base composition different from that of the RNA transcribed from DNA (*14*). That the enzymatic reaction between template, polymerase, and substrate monomer is similar regardless of whether DNA or chromatin serves as template is shown by the identity of the nucleoside triphosphate affinities in the two cases.

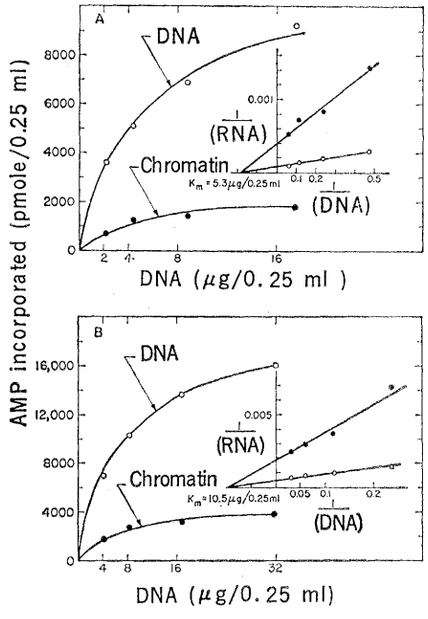

Fig. 1. Template activities of rat-liver chromatin and of the deproteinized DNA of same. The reaction mixtures (0.25 ml) were of the composition specified by Marushige and Bonner and contained *E. coli* RNA polymerase F_4, 30 μg (A), or 60 μg (B) [after Marushige and Bonner (*14*)].

Hybridization studies also show that a restricted portion of chromosomal DNA is transcribed by RNA polymerase. This was first reported by Paul and Gilmour (*15*), and their findings have been extended by Bekhor *et al.* (*16*). An example taken from the data of Paul and Gilmour is shown in Fig. 2; RNA transcribed in vitro from whole nuclear DNA hybridizes with approximately 15 times more denatured nuclear DNA than does RNA transcribed from thymus chromatin in vitro. Clearly, only a portion of the DNA of chromatin is available for transcription by RNA polymerase. That the portions available for transcription are different in chromatins from different tissues of the same organism has also been shown by appropriate competition experiments (*17*).

Non-Artifactuality of Isolated Chromatin

That the state of genetic repression characteristic of chromatin in life is maintained in isolated chromatin was first indicated by the work of Bonner *et al.* (*10*) for a single genetic complex of the pea plant, that coding for pea-seed globulin. This protein is made only in developing pea cotyledons; it is not made in other organs of the pea plant at other times in the life cycle. Chromatin isolated from pea buds or from developing pea cotyledons was therefore incubated in the presence of the four riboside triphosphates and RNA polymerase to form a system that would generate messenger RNA. This system was in turn coupled to a system of *E. coli* ribosomes that synthesized protein. The protein synthesized with pea-bud chromatin as template for messenger RNA synthesis contains no pea-seed globulin detectable by immuno-

chemical assay. The protein synthesized with messenger RNA generated from developing pea-cotyledon chromatin as template contains pea-seed globulin in the same proportion of total protein as in life (Table 2).

That the state of repression of isolated chromatin is similar to that in the living cell has been further studied by Paul and Gilmour (*17*) by the method of DNA-RNA hybridization. For this work, RNA is generated from isolated chromatin, exogenous RNA polymerase being used, and hybridized with whole nuclear DNA. Competition experiments between this kind of RNA and whole unlabeled RNA prepared from living tissue show that RNA transcribed from chromatin in the test tube contains no sequences not also found in life in the same tissue (Fig. 3). The reverse experiment, competition of unlabeled RNA transcribed in vitro against labeled RNA formed in vivo, shows in addition that RNA made in vivo contains no sequences not found in the population of RNA molecules synthesized in vitro. The populations of RNA molecules transcribed from chromatin of different cell types in vitro do, however, contain different sequences as expected. McCarthy and Church (*18*) have also found that the RNA transcribed from liver chromatin in vitro is generally similar to that made in life.

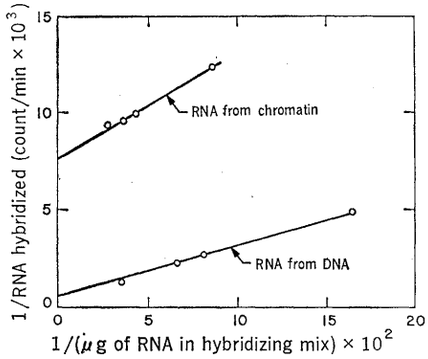

Fig. 2. Hybridization of RNA transcribed in vitro from either calf-thymus DNA or calf-thymus chromatin to calf-thymus nuclear DNA. The reciprocal of the amount of RNA hybridized is plotted as a function of the reciprocal of the concentration of RNA in the hybridization reaction mixture. The intercept on the vertical axis indicates the amount of RNA hybridized at infinite RNA concentration. The data show that RNA transcribed from chromatin hybridizes with approximately 0.07 times as much nuclear DNA as does RNA transcribed from DNA [after Paul and Gilmour (*15*)].

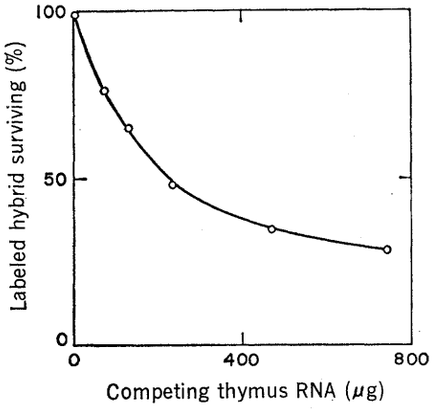

Fig. 3. RNA transcribed from calf-thymus chromatin in vitro is subjected to hybridization competition by whole calf-thymus nuclear RNA synthesized in vivo. The RNA synthesized in vivo is a successful competitor of that synthesized in vitro. Extrapolation of the curve to infinite concentration of competitor RNA synthesized in vivo indicates that all labeled RNA synthesized in vitro would be competed against [after Paul and Gilmour (*15*)].

Basis for the Restriction of Chromosomal Template Activity

We now consider the material basis for restriction of template activity of the DNA of chromatin. To do this it is necessary first to consider the composition of chromatin in more detail. Chromatin is composed of DNA, of the characteristic basic chromosomal proteins—the histones, of nonhistone proteins, and of a small amount of RNA. The chromosomal compositions summarized in Table 1 indicate that the mass ratios of histone to DNA of the several chromatins tabulated varies over the range 0.8 to 1.35. The amino acid composition of histones is such that a mass ratio of histone to DNA of 1.35 is required for establishment of the stoichiometric complex of histone and DNA (one basic group of protein to one phosphate group of DNA). The several chromatins of Table 1 vary therefore from fully to less than fully complexed with histone. The mass ratios of nonhistone protein to DNA vary over the range 0.1 to 1.04. The nonhistone protein content of pea chromatins appears to vary inversely with histone content. The ratios of RNA to DNA in chromatins vary from less than 0.01 in the case of thymus chromatin, to approximately 0.3 in the case of pea-embryo chromatin.

That histones are the agents responsible for restriction of chromosomal template activity is shown by the collective weight of several kinds of experiments. Thus Huang and Bonner (*5*) dissolved chromatin in 4*M* CsCl, sedimented the DNA, recombined the chromosomal nonhistone protein with DNA, and showed that the template activity of the resulting product had been greatly increased by removal of histone. In the method of Marushige and Bonner (*14*), chromatin is sedimented from 0.2*N* HCl at 0°C. In the acid solution, histone is soluble, nonhistone protein and DNA are not. Nonhistone protein and DNA sediment as

Table 2. Synthesis of pea-seed globulin by messenger-RNA dependent *E. coli* ribosomal system in response to messenger RNA generated by transcription by *E. coli* RNA polymerase of apical-bud chromatin and cotyledon chromatin [after Bonner, Huang, and Gilden (*10*)]. The reaction mixture containing all materials required for both RNA and protein synthesis was incubated at 37°C for 30 minutes. All particulate material was then centrifuged off, and the content of pea-seed globulin in soluble protein was determined by immunochemical assay. Since DNA of phage T2 contains no information about pea-seed globulin, the protein generated in response to this DNA serves as a control on the specificity of the immunochemical assay. The assay detects 0.13 percent globulin when none is present. This is the background of the assay.

C^{14}-leucine incorporation		Globulin/ total protein (%)
Total soluble protein (count/min)	Globulin (count/min)	
Apical-bud chromatin		
15,650	16	0.10
41,200	54	.13
Cotyledon chromatin		
8,650	623	7.2
6,500	462	6.9
DNA of phage T2		
59,400	77	0.13

Table 3. Effect of removal of histone by acid treatment on the template activity of rat-liver chromatin for RNA synthesis [after Marushige and Bonner (*14*)]. Template activity equals rate of RNA synthesis by chromatin divided by the rate of RNA synthesis by DNA in the standard RNA synthesis reaction mixture containing *E. coli* RNA polymerase. Chromatin was suspended in 0.2*N* HCl at 0°C and the complex of insoluble DNA and nonhistone protein was separated from soluble histone by centrifugation.

Template	Chemical composition		Template activity for RNA synthesis
	Histone/ DNA	Nonhistone protein/ DNA	
Rat-liver DNA	0.00	0.02	1.00
Rat-liver chromatin	.94	.64	0.28
Chromatin treated with 0.2*N* HCl	.03	.55	.86

Table 4. Amino- and carboxy-terminal amino acids of pea-bud and calf-thymus histone fractions [after Fambrough and Bonner (*26*) and Fambrough (*31*)]. Amino acids: (Arg) arginine, (Lys) lysine, (Ser) serine, (Ala) alanine, (Gly) glycine, (Pro) proline.

Histone fraction	Synonym	Description	Amino-terminal	Carboxy-terminal
Pea Iab		Lys-rich	Blocked	Lys
Calf thymus Iab	f(1)	Lys-rich	Blocked	Lys
Pea $IIa_{1,2}$		Slightly Lys-rich	Pro	Ser Ala
Pea IIb		Slightly Lys-rich	Blocked	Ala
Calf thymus IIb_1	f2a2	Slightly Lys-rich	Blocked	Lys
Calf thymus IIb_2	f2b	Slightly Lys-rich	Pro	Lys
Pea III		Arg-rich	Ala	Ala
Calf thymus III	f(3)	Arg-rich	Ala	Ala
Pea IV		Arg-rich	Blocked	Gly
Calf thymus IV	f2a1	Arg-rich	Blocked	Gly

a complex, whereas the histone remains in solution. The redissolved chromatin, now without histones, possesses essentially the template activity of totally deproteinized DNA (Table 3). Alternatively, the chromatin may be dissolved in and sedimented from a series of salt concentrations. Histones are dissociated from DNA at lower salt concentrations than is a portion of the nonhistone proteins. A salt concentration may be selected at which the bulk of the histone is removed while the bulk of the nonhistone protein remains complexed to DNA (Fig. 4). It is clear, for example, that with calf-thymus chromatin 0.5*M* sodium perchlorate removes 90 percent of the histone, but only 40 percent of the nonhistone protein. The template activity of the resulting chromatin is essentially equal to that of totally deproteinized DNA. Finally, the chromatin of pea embryo (*19*) or of liver (*20*) can be physically separated into two fractions, one with high template activity for RNA synthesis, the other with low template activity. In both cases, that portion of the chromatin possessing low template activity consists of DNA and histone in nearly stoichiometric ratio, whereas the portion with high template activity consists of DNA to which is bound the bulk of the nonhistone protein of the chromatin.

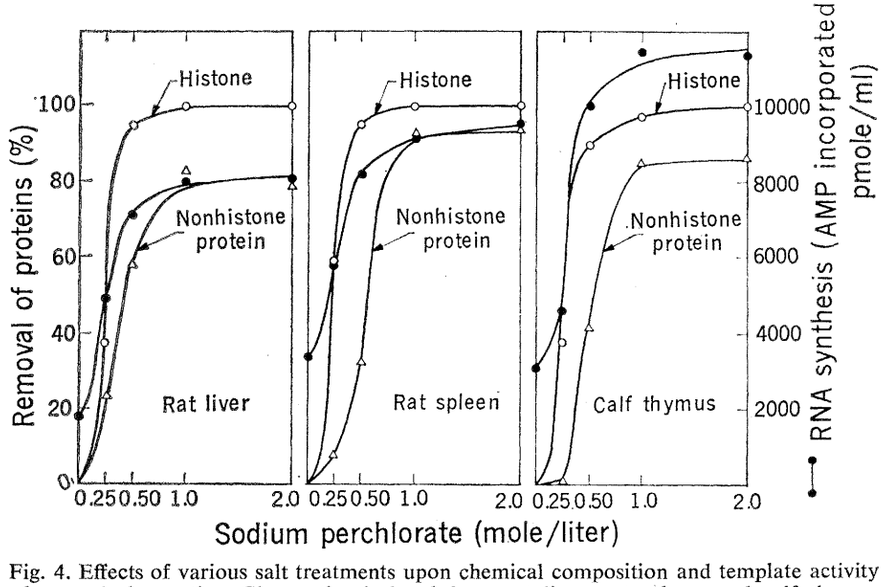

Fig. 4. Effects of various salt treatments upon chemical composition and template activity of several chromatins. Chromatins isolated from rat liver, rat spleen, and calf thymus were treated with $NaClO_4$ at concentrations ranging from 0.25 to 2.0 mole/liter. The ratios of histone to DNA for these chromatins, subjected to the same treatment, but without salt, were 1.10, 0.87, and 1.14; the ratios of nonhistone protein to DNA were 0.72, 0.24, and 0.33, respectively. For the determination of template activity, 10 μg of DNA in the form of chromatin was incubated with 100 μg of RNA polymerase F_3 in the standard reaction mixture for RNA synthesis. (○) Fraction of histone removed; (●) template activity of remaining nucleoprotein; (△) fraction of nonhistone protein removed [after Marushige and Bonner (*14*)].

Histone Chemistry

Histones have been studied extensively during the past 100 years, but only relatively recently have methods become available for the quantitative separation of the individual histone components from one another and for the characterization of the pure products thus made available. Two principal methods for the fractionation of histones have been developed, that of Johns and Butler (*21*) [see also Phillips and Johns (*22*), Hnilica and Bess (*23*)] and that developed in the laboratory of Luck (*24, 25*). We have found the latter method especially suitable for the study of histones of both animals and plants (*26*), particularly when combined with disc electrophoresis for analysis of purity of individual fractions.

Most studies of histone chemistry are made with preparations of histones extracted from chromatin with acid. It is imperative to use purified chromatin as a starting material for histone preparation. The acid extraction of whole tissues or even of nuclei yields preparations which include other acid-soluble proteins such as the basic proteins of ribosomes, which are often present in much larger amounts than the histones themselves and which obscure the actual number of species of histone proteins present in chromatin. In the method of Rasmussen *et al.* (*24*) histones, extracted from chromatin with 0.2*N* H_2SO_4 and precipitated with ethanol, are separated from one another on a weak cation-exchange resin (Amberlite CG-50) with a gradient of guanidinium chloride buffered at neutral *p*H. The details of the gradient and the resulting elution patterns of pea and of thymus histones are illustrated in Fig. 5.

A small amount of protein is eluted from the column after one void volume of eluant. This consists of weakly basic protein, which appears to be associated with histones in the chromosomal structure, and of which more will be said later. Ribosomal proteins, if they are present, also appear in this "runoff" peak. The initial peak proteins are followed by incompletely resolved histones Ia and Ib which are rich in lysine; then by histones IIa and IIb, which are slightly rich in lysine; and finally by incompletely resolved histones III and IV, which are rich in arginine.

The individual fractions are not pure proteins, as shown by their patterns in disc electrophoresis (Fig. 6). By re-

chromatography or preparative disk electrophoresis, or both, pure histone fractions have been prepared for all the histone components except pea histone IIa, which appears to consist of two species of molecules. The histone fractions from both calf thymus and pea bud have been thus prepared, and each fraction appears to be homogeneous as judged by the additional criteria of carboxy-terminal and amino-terminal analyses (Table 4).

It has in the past appeared that the histones constituted an enormously heterogeneous group of many different proteins. It comes as some surprise, therefore, that the true number of histones is probably quite small. The heterogeneity found in the past appears now to be attributable to (i) contamination by ribosomal proteins, (ii) the formation of aggregates of histones with one another and with ribosomal proteins, and (iii) proteolysis of histones during preparation.

The elution profile of the histones of calf-thymus chromatin on the Amberlite CG-50 column is remarkably similar to that of pea-bud histones. The comparable histone fractions of pea and calf thymus generally resemble one another in behavior in ion-exchange chromatography, in amino acid composition, in amino-terminal and carboxy-terminal amino acids, in electrophoretic mobility, and (as discussed below) in the strength of their native binding to DNA. Histones of rat-liver chromatin are also closely similar to those of pea and calf. Less extensive chemical studies permit comparison of the histones of a wide range of plants and animals. It would appear that the histones of higher plants, higher animals, and of some invertebrates which have been examined, are generally similar in properties. This has led to the hypothesis that the histones appeared early in evolution, and, because of the importance of their primary structures to the performance of their biological tasks, their freedom to be altered by nonlethal mutational changes has been severely restricted (*26*).

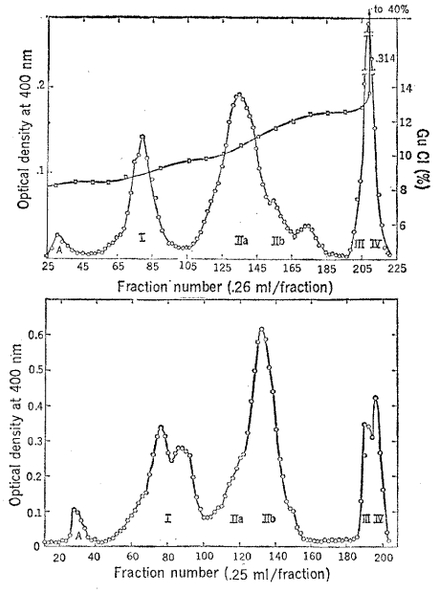

Fig. 5. Fractionation of pea-bud and calf-thymus histones by column (0.6 by 55 cm) chromatography on Amberlite CG-50. Protein concentration in the effluent fractions was determined by absorption (400 nm) of the turbid solutions resulting when the 0.26-ml fractions were mixed with 1.1*M* trichloroacetic acid in a total volume of 1.56 ml. (Top) Fractionation of pea-bud histones; (○) protein concentration; (□) concentration of guanidinium chloride (GuCl). (Bottom) Fractionation of calf-thymus histones [after Fambrough and Bonner (*26*)].

The histones of HeLa metaphase chromosomes have been studied by Sadgopal (*27*), who has compared them with the histones of interphase chromosomes from the same cell line. The ratios of histone to DNA for metaphase and interphase chromosomes are identical, and the same spectra of histone components are found in both. Metaphase chromosomes but not interphase chromosomes contain, in an amount equal to that of histone, a mixture of nonhistone proteins that are soluble in acid (HCl but not H_2SO_4). This material, as judged by its electrophoretic and chromatographic properties, is most probably acid-soluble ribosomal protein. It may arise from ribosomes which are associated with metaphase chromosomes and whose presence was first detected (*28*) on the basis of their ribosomal RNA content.

Selective Removal of Individual Histones

Although all histones are linked to DNA by ionic bonds, the several different classes of histones are nonetheless liberated from DNA at different ionic strengths of solution. This is indicated, for example, by studies of the binding of histone to DNA by equilibrium dialysis in solutions of varying ionic strengths (*29*). A greater salt concentration is required to dissociate histones III and IV from DNA than is required to dissociate histone I. For detailed study of this matter, native nucleohistone is dissolved in solutions of varied ionic strength, and the DNA is sedimented. The histones which remain in the supernatant, as well as those which remain associated with the DNA in the pellet, have been investigated by disc electrophoresis and column chromatography. These investigations (*30*) show that histone I is completely dissociated from the chromosomal DNA by 0.6*M* NaCl. Histones of class II are liberated over the concentration range 0.8 to 1.8*M* NaCl, whereas histones III and IV are liberated from DNA over the concentration range 0.9 to 2.0 mole/liter. In their dissociation characteristics, histones of pea-bud chromatin resemble those of calf-thymus chromatin (*31*).

Nucleohistones which result from dissociation of part of the histone from DNA at salt concentrations less than 2 mole/liter exhibit a template activity for RNA synthesis greater than that of the original chromatin. The relation

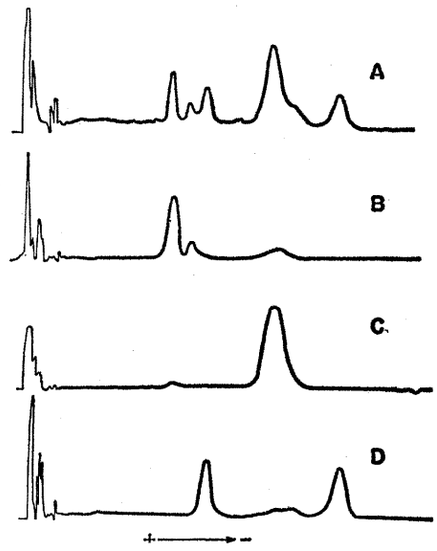

Fig. 6. Fractionation of pea-bud histones by disc electrophoresis in polyacrylamide gel according to Bonner *et al.* (*1*). Electrophoresis in polyacrylamide gel (15 percent) containing 6*M* urea. The figures are microdensitometric tracings on the Canalco Model E microdensitometer. The gels were stained with amido black and destained electrophoretically. The gels were then scanned. Tracing A shows the electrophoretic fractionation of whole pea-bud histone; tracings B, C, and D show, respectively, the electrophoretic fractionation of histones I, IIa, and III and IV all prepared by column chromatography on Amberlite CG-50. Peaks at far left indicate origin of gel, not stained material. The forward shoulder on the histone IIa peak in tracing A is histone IIb [after Fambrough and Bonner (*26*)].

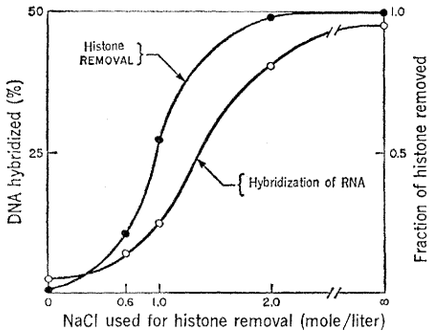

Fig. 7. Removal of histone from pea-bud nucleohistone as a function of NaCl concentration together with the fraction of nuclear DNA hybridized at saturation by RNA transcribed by *E. coli* RNA polymerase from nucleohistone treated with each of these same NaCl concentrations [after Bekhor *et al.* (*16*)].

of template activity to degree of histone removal has been indicated in Fig. 4.

The technique of RNA-DNA hybridization may also be used to discover which classes of histones are responsible for restriction of transcription (*16*). The data of Fig. 7 (*16*) show that RNA transcribed from native pea-bud chromatin hybridizes (at saturation) with approximately 2.5 percent of denatured pea DNA, whereas RNA transcribed from completely deproteinized pea DNA hybridizes with about 50 percent of denatured pea DNA. Removal of histone I provides a template, the transcription of which yields RNA which hybridizes with about 7 percent of denatured pea DNA. Removal of further classes of histones by treatment with still higher salt concentrations provides templates for synthesis of RNA which hybridizes with still more nuclear DNA. It is clear, then, that, although histone I is responsible for the suppression of transcription of a portion of the chromosomal DNA, histones of other classes are also involved. Georgiev *et al.* (*32*) from their similar study of the hybridization of RNA made by transcription of ascites tumor chromatin from which part of the histones has been removed arrived at the conclusion that only histone I is responsible for suppression of transcription. Their findings, which are contrary to our own, are apparently ascribable to the fact that their RNA made in vitro, even if transcribed from DNA, hybridized with only a very low proportion of DNA.

A fraction of chromatin that is a less active template has been separated from one that is more active. Frenster, Allfrey, and Mirsky (*3*) sonically disrupted whole chromatin and then separated the more aggregated, less active template fraction from the soluble, more active portion by centrifugation. These workers have studied in detail the chemical and metabolic differences between these two fractions. The more active fraction contains, for example, about one *N*-acetyl group per histone molecule; the less active fraction contains about one-half as much (*33*). These acetyl groups turn over independently of turnover of the rest of the histone molecule. Similarly, histones contain serine and threonine phosphomonoesters which can turn over independently (*34*). The more active fraction contains somewhat more ester phosphate than the less active fraction does (*35*). Such phosphate ester groups are concentrated in the histones I and III and IV, which contain less than one phosphate group per histone molecule (*36*). The significance of these minor groups in histones cannot at present be assessed although it is possible that, like the minor bases in transfer RNA, they may have important biological meaning.

Distribution and Functions of Individual Histones

The distribution of histone I along the chromosomal DNA can be investigated by determining the length of the deproteinized DNA regions formed by selective removal of histone I. This has been done by Olivera (*37*) using the melting profile method. If the bare patches are sufficiently long they should melt like deproteinized DNA rather than like DNA of nucleohistone in which the DNA is stabilized against melting. Olivera found, however, that nucleohistone after removal of histone I contains no DNA which melts as free DNA. The stretches bared by removal of histone I must therefore be shorter than the 200 base pairs estimated as required to show the collective collapse characteristic of the melting of high-molecular-weight DNA. A stretch of DNA 200 base pairs long would accommodate about eight histone I molecules. It may be concluded that rarely, if ever, do eight histone I molecules lie side by side along the chromosomal axis.

The DNA of nucleohistone is shortened and fattened compared to pure DNA of the same molecular weight. This is most probably due to the assumption by DNA in nucleohistone of a supercoiled configuration, which not only shortens the molecule by about 35 percent, but also decreases a portion of its hypochromicity (*38*) and of its flow birefringence and flow dichroism (*39*). Removal of histone I from nucleohistone results in no loss of any of these features. Histone I is therefore not responsible for the structural peculiarities of DNA in nucleohistone. Removal of even a small portion of histone II results in dramatic shifts of the properties of chromosomal DNA toward those of deproteinized DNA. Histones II and possibly III and IV are therefore concerned with the structural features of chromosomal DNA as well as with regulation of template activity.

Specificity of Interaction between Histone and DNA

Since the binding of histone to DNA is in a large measure through ionic bonds between the basic groups of the histone and the acidic phosphate groups of the DNA, it is difficult to see how a histone molecule might recognize base sequence and hence bind selectively to DNA of particular information content. The fact that there are only a few species of histones and that these bind to a major portion of the DNA itself suggests little specificity. The experimental facts indicate, indeed, that there is little selectivity in the binding of histone to DNA. Hurwitz *et al.* (*40*) have found in experiments in which histones of the several classes were added to DNA before use of the latter as template for RNA synthesis that RNA formed in the presence of DNA-histone I complex is somewhat impoverished in adenine and uracil, compared to RNA transcribed from protein-free DNA of the same base composition. Selective binding of histone I to adenine-thymine regions is therefore implied. Conversely, RNA formed from DNA in the presence of histones III and IV is somewhat impoverished in guanine and cytosine. These effects have been confirmed by Widholm (*41*), who has studied the template properties of crab poly-dAT (polydeoxyadenylic acid-deoxythymidylic acid) in the presence of histones of the several classes. The selectivity thus indicated is, however, of a low order. Leng and Felsenfeld (*42*) have investi-

gated possible selectivity in protein-DNA interactions in model systems of DNA and polylysine or polyarginine. Under conditions in which ionic interaction is minimized, concentrations of NaCl of 0.5 mole/liter or greater, polylysine bound preferentially to adenine-thymine pairs, and polyarginine bound to guanine-cytosine base pairs. These more specific interactions, which must depend upon hydrophobic properties of the histones, can be of biological significance in some as yet unknown way. It is important that they apparently operate only in ionic strengths high enough to be detrimental to biological structure. In general, histone and DNA interactions would appear to depend upon factors other than information content of the DNA. Thus, pea histone binds as well to calf-thymus DNA as to pea DNA, and vice versa (*43*). It is upon this basis, namely that histones do not, on the surface of it, appear to be able to decipher base sequence, that the significance of histones as repressors of genetic information has occasionally been denied.

Chromosomal RNA

The RNA of chromatin is present in part as a complex with the chromosomal protein. Recognition of the presence of such RNA has resulted from attempts to find ways of separating native histones from chromatin by other than the usual acid-extraction procedure. One alternative method is to dissolve chromatin or native nucleohistone in a solution containing in final concentration 2.09 molar CsCl, 0.01 molar tris(hydroxymethyl)aminomethane (*p*H 8.0). The chromatin is then centrifuged to density gradient equilibrium. At the ionic strength of 2.09 molar CsCl, histone dissociates from DNA. At the density of 2.09 molar CsCl (approximately 1.28) DNA sediments, whereas chromosomal proteins assume a position of neutral buoyancy at approximately the center of the CsCl gradient formed (Fig. 8). The banded chromosomal protein contains associated RNA (*44*). Even more simply, chromatin may be dissolved in 4*M* CsCl and centrifuged until the DNA is sedimented (18 hours). The chromosomal proteins float, forming a skin which also contains the chromosomal RNA. Chromosomal RNA associated with protein, in an amount 0.01 to 0.05 that of DNA, has been found in all chromatins which have thus far been appropriately investigated. These include the chromatins from pea bud, pea cotyledon, chick embryo (*45*), rat ascites tumor (*46*), rat liver (*47*), and calf thymus (*48*).

Chromosomal RNA is bound in the structure of chromatin in such a way as to be resistant to ribonuclease (*49*). It is made susceptible to ribonuclease by either destruction of chromosomal DNA by deoxyribonuclease, or by the heating of chromatin to 60°C. These facts suggest, although they do not prove, that chromosomal RNA is bound to DNA, presumably by base pairing.

Chromosomal RNA is characterized by short chain length (chain length 40 to 60 nucleotides by end-group analysis, sedimentation coefficient 3.2*S* by band centrifugation) and by its composition, which includes a relatively high content (5 to 25 moles per 100 moles) of dihydrouridylic acid. Chromosomal RNA is bound covalently to chromosomal protein (*45*). This complex is in turn bound to histone through bonds which are broken by guanidinium chloride; these are presumably hydrogen bonds (*19*).

The population of base sequences in chromosomal RNA is heterogeneous, and this RNA hybridizes with a substantial fraction of nuclear DNA. Chromosomal RNA of pea chromatin freed of DNA by centrifugation in CsCl, freed of protein by treatment with pronase, and freed of peptides by chromatography on a diethylaminoethyl-Sephadex column with a gradient of NaCl in 7*M* urea has been hybridized with whole genomal pea DNA (*50*). At saturation it hybridizes with slightly over 5 percent of nuclear DNA. We conclude, both from this measure, and from the slow rate of hybridization of chromosomal RNA to DNA that chromosomal RNA is exceedingly heterogeneous in base sequences and consists of many species of RNA, each represented but a small number of times. Neither transfer nor ribosomal RNA competes with chromosomal RNA in hybridization. The populations of chromosomal RNA sequences which occur in different organs of the same organism are only in part identical (Fig. 9). As might be expected, chromosomal RNA of one organism does not hybridize with the DNA of an unrelated creature, although the degree of hybridization possible between chromosomal RNA and nuclear DNA of closely related creatures remains to be studied. Chromosomal RNA is, finally, confined to the nucleus and must therefore execute a nuclear rather than any cytoplasmic function (*50*).

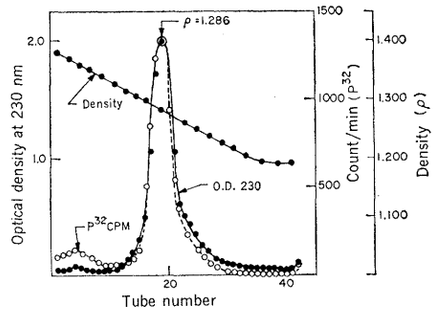

Fig. 8. Density-gradient centrifugation (CsCl) of pea-bud nucleohistone labeled with P^{32}. The nucleohistone has been previously sedimented from 0.4*M* $NaClO_4$ to remove histone I. The pellet was redissolved in 2.09*M* CsCl and centrifuged at 39,000 rev/min to equilibrium. The DNA of the nucleohistone is sedimented to the bottom of the tube and is not recovered. Histone and associated RNA bands with the peak density as indicated [after Huang and Bonner (*44*)].

What are we to make of a chromosomal complex consisting of RNA and chromosomal protein? One possibility is that the RNA contributes to base sequence recognition by the complex as a whole; that the complex of RNA and protein binds to that portion of the chromatin in which the RNA is complementary to an appropriate DNA sequence, and in which the histone is then ionically bound. The properties of chromosomal RNA—short chain length, sequence heterogeneity, principally nuclear occurrence—are in

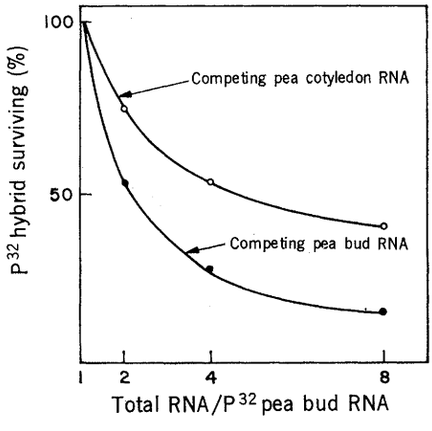

Fig. 9. Hybridization competition between pea-bud chromosomal RNA labeled with P^{32} and unlabeled pea-bud or pea-cotyledon chromosomal RNA. The concentration of labeled pea-bud chromosomal RNA is 37.5 μg/ml throughout. Pea-cotyledon chromosomal RNA contains sequences which pea-bud chromosomal RNA does not.

accord with the speculation that chromosomal RNA may function as a sequence detector for chromosomal protein.

Our approach to the further study of this problem has been to dissociate histone from chromatin with salt, to next reassociate histone and DNA under varied experimental conditions, and to then determine by hybridization competition whether RNA transcribed from the reconstituted chromatin is similar to that transcribed from native chromatin. Our results (*16*) show that: (i) when chromatin is reconstituted by dialyzing away the salt which caused dissociation, histones are deposited randomly on DNA, that is, the RNA transcribed from it does not compete for hybridization to DNA with RNA transcribed from native chromatin; (ii) the condition of reconstitution which yields chromatin similar to native chromatin by the hybridization (of transcribed RNA) competition criterion is removal of salt in the presence of a concentration of urea which permits hybridization of RNA to DNA; (iii) such specific reconstitution of dissociated chromatin does not occur if the chromosomal RNA is removed.

Relation to Bacterial Control Systems

In the two bacterial control systems of which we have detailed knowledge, it appears that proteins produced under the control of the regulator gene possess the ability to bind specifically (and by implication to repress) the appropriate operator gene (*51*). Transcriptional control of this kind, if it is general, would require the presence, in the bacterial cell, of a great number of kinds of repressor proteins each specific to its relevant operator. In nucleated cells, as detailed above, a major portion of transcriptional control is effected by a small number of kinds of repressor proteins which do not of themselves appear, however, to possess gene specificity and which must therefore acquire such specificity through auxiliary mechanisms.

Significant questions of our time are: In how far are the transcriptional control systems of higher organisms truly different from those of microorganisms? Is it possible that as we gain more detailed knowledge of the nonhistone chromosomal proteins we shall find gene-specific repressors analogous to those of bacteria? The argument presented earlier in this review indicated that such specific repressor proteins, if they are present in higher organisms, are responsible for but a small proportion of the total repression. Their existence could therefore have been thus far overlooked. By the same token we may ask whether further studies on the control of bacterial transcription may reveal a class or classes of proteins which bind to DNA as do histones and which by such binding cause the DNA to which they are bound to be no longer transcribable. It is clear that bacteria do not possess proteins which chemically resemble histones (*52*). Whether they possess proteins which fulfill the biological role of histones is unclear. In any case, it would still appear profitable to try to discover ways in which bacterial and nuclear control of transcription are similar, as well as ways in which they are different.

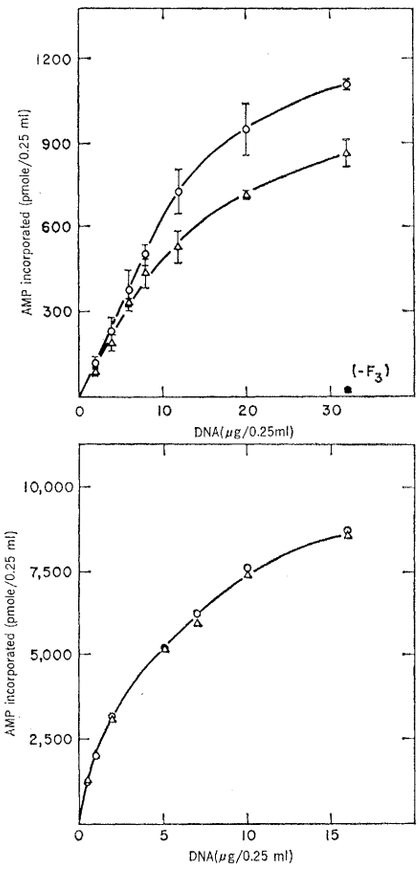

Fig. 10. Template activity of rat-liver chromatin isolated 4 hours after treatment of adrenalectomized rats with either hydrocortisone (○) or control saline (△). The incubation mixtures (0.25 ml) contained various concentrations of DNA either as chromatin (top) or as deproteinized DNA of the same preparations (bottom), as well as the standard reaction mixture for RNA synthesis and equal amounts of *E. coli* RNA polymerase [after Dahmus and Bonner (*55*)].

Pure and Applied Chromosomology

Our discussion thus far has concerned the properties of chromatin, pure chromosomology. We will now consider how this information can serve in the generation of further understanding of biological processes, applied chromosomology. Our first example is that of embryonic development.

Differential activation and inactivation of genes appears to play a key role in development. The quantitative changes in availability of genes for transcription can be followed during development by examining the template activity for RNA synthesis of chromatin isolated from cells of different developmental stages. During the development of frog embryos, an increase in the ability of chromatin to support RNA synthesis has been found from gastrulas to a later larval stage (*53*). In sea urchin embryos, the template activity of chromatin isolated from plutei is twice that of chromatin isolated from blastulas (*54*). This difference in template activity between two chromatins is accompanied by differences in their chemical compositions. The more active chromatin contains slightly less histone, and substantially more nonhistone protein than does less active chromatin from blastulas. The same individual histones are found in both chromatins. The difference in template activity between these two chromatins is associated with chromosomal proteins. This follows from the fact that removal of proteins causes an increase in template activity, and yields DNA's of equal template activity from blastula and pluteus chromatin.

Our second example of applied chromosomology concerns derepression. Particular small molecules can induce in appropriate target organs the derepression of genes previously repressed, and the consequent *de novo* synthesis of particular enzymes. Of small molecules with this activity in higher creatures the hormones are the most studied and best known. For example, administration of cortisone to adrenalectomized rats causes within 15 minutes an increased rate of DNA-dependent RNA synthesis in the liver. The increased rate of RNA synthesis is followed by the appearance of a series of enzymes of gluconeogenesis and transamination. The template activity of the isolated liver chromatin for RNA synthesis (in the presence of added RNA polymerase) is increased by such cortisone treatment (*55*) (Fig. 10). The template activity of

the chromatin of adrenalectomized rats for RNA synthesis is about one-seventh that of DNA; the template activity of chromatin isolated from liver after the administration of cortisone is about one-fifth that of the corresponding DNA, an increase of 30 percent. The increase in template activity is due in some way to alteration in the posture of the chromosomal proteins or RNA since removal of all protein and RNA from the chromatin of rats treated and not treated with cortisone results in DNA of identical template activity. Exactly similar results have been obtained for the template activity of chromatin isolated from the uteri of ovariectomized rats before and after the administration of estrogen (*56*). Even more dramatic is the increase in template activity of the chromatin of dormant potato buds after their treatment with ethylene chlorohydrin, an agent which mimics the natural hormone gibberellic acid in the breaking of bud dormancy. The chromatin of dormant potato buds (of freshly harvested potato tubers) is almost devoid of template activity for RNA synthesis in the presence of added RNA polymerase. Template activity is increased approximately 20-fold (*57*) in buds treated with ethylene chlorohydrin.

There is no doubt that the addition of hormone to appropriate target organ derepresses genetic material previously repressed. In all of the instances which have been so far studied, addition of hormone directly to isolated chromatin exerts no effect upon the template activity of such chromatin [see, for example, Dahmus and Bonner (*55*)]. Addition of, for example, cortisone to isolated liver nuclei of adrenalectomized rats does, however, cause increased rate of RNA synthesis, just as it does in the intact animal (*58*). It is clear, therefore, that the effect of the inducer must be mediated by some substance or substances present in the nucleus but not isolated with purified chromatin. The nature of the nuclear factor responsible for the mediation of such hormonal effects has been studied by Maurer and Chalkley (*59*), Jensen and Jacobson (*60*), Jensen (*61*), and Toft and Gorski (*62*). The nuclei of endometrial cells, for example, contain a soluble protein which can specifically bind β-estradiol, an estrogenically active substance. Other such materials (for example, diethylstilbesterol) complete with β-estradiol for the binding site. Estrogenically inactive substances such as α-estradiol do not. The material has been partially purified by Jensen and by Gorski, who used its properties of estradiol binding as a guide. Maurer and Chalkley have shown that the substance that binds estradiol, once it has bound the hormone, must bind to some component of endometrial chromatin since it is isolated together with it. Clearly, the detailed study of the molecular basis of the interaction between hormones and binding protein, and of their interaction with chromatin provides a key to the understanding of the molecular basis of derepression.

Summary

The isolated chromatin of higher organisms possesses several properties characteristic of the same chromatin in life. These include the presence of histone bound to DNA, the state of repression of the genetic material, and the ability to serve as template for the readout of the derepressed portion of the genome by RNA polymerase. The important respect in which isolated chromatin differs from the material in vivo, fragmentation of DNA into pieces shorter (5×10^6 to 20×10^6 molecular weight) than the original, does not appear to importantly alter such transcription. The study of isolated chromatin has already revealed the material basis of the restriction of template activity; it is the formation of a complex between histone and DNA. Chromatin isolated by the methods now available, together with the basis provided by our present knowledge of chromatin biochemistry and biophysics, should make possible and indeed assure rapid increase in our knowledge of chromosomal structure and of all aspects of the control of gene activity and hence of developmental processes.

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The Matthew Effect in Science

The reward and communication systems of science are considered.

Robert K. Merton

This paper develops a conception of ways in which certain psychosocial processes affect the allocation of rewards to scientists for their contributions—an allocation which in turn affects the flow of ideas and findings through the communication networks of science. The conception is based upon an analysis of the composite of experience reported in Harriet Zuckerman's interviews with Nobel laureates in the United States (*1*) and upon data drawn from the diaries, letters, notebooks, scientific papers, and biographies of other scientists.

The Reward System and "Occupants of the Forty-First Chair"

We might best begin with some general observations on the reward system in science, basing these on earlier theoretical formulations and empirical investigations. Some time ago (*2*) it was noted that graded rewards in the realm of science are distributed principally in the coin of recognition accorded research by fellow-scientists. This recognition is stratified for varying grades of scientific accomplishment, as judged by the scientist's peers. Both the self-image and the public image of scientists are largely shaped by the communally validating testimony of significant others that they have variously lived up to the exacting institutional requirements of their roles.

A number of workers, in empirical studies, have investigated various aspects of the reward system of science as thus conceived. Glaser (*3*) has found, for example, that some degree of recognition is required to stabilize the careers of scientists. In a case study Crane (*4*) used the quantity of publication (apart from quality) as a measure of scientific productivity and found that highly productive scientists at a major university gained recognition more often than equally productive scientists at a lesser university. Hagstrom (*5*) has developed and partly tested the hypothesis that material rewards in science function primarily to reinforce the operation of a reward system in which the primary reward of recognition for scientific contributions is exchanged for access to scientific information. Storer (*6*) has analyzed the ambivalence of the scientist's response to recognition "as a case in which the norm of disinterestedness operates to make scientists deny the value to them of influence and authority in science." Zuckerman (*7*) and the Coles (*8*) have found that scientists who receive recognition for research done early in their careers are more productive later on than those who do not. And the Coles have also found that, at least in the case of contemporary American physics, the reward system operates largely in accord with institutional values of the science, inasmuch as quality of research is more often and more substantially rewarded than mere quantity.

In science as in other institutional realms, a special problem in the workings of the reward system turns up when individuals or organizations take on the job of gauging and suitably rewarding lofty performance on behalf of a large community. Thus, that ultimate accolade in 20th-century science, the Nobel prize, is often assumed to mark off its recipients from all the other scientists of the time. Yet this assumption is at odds with the well-known fact that a good number of scientists who have not received the prize and will not receive it have contributed as much to the advancement of science as some of the recipients, or more. This can be described as the phenomenon of "the 41st chair." The derivation of this tag is clear enough. The French Academy, it will be remembered, decided early that only a cohort of 40 could qualify as members and so emerge as immortals. This limitation of numbers made inevitable, of course, the exclusion through the centuries of many talented individuals who have won their own immortality. The familiar list of occupants of this 41st chair includes Descartes, Pascal, Molière, Bayle, Rousseau, Saint-Simon, Diderot, Stendahl, Flaubert, Zola, and Proust (*9*).

What holds for the French Academy holds in varying degree for every other institution designed to identify and reward talent. In all of them there are occupants of the 41st chair, men outside the Academy having at least the same order of talent as those inside it. In part, this circumstance results from errors of judgment that lead to inclusion of the less talented at the expense of the more talented. History serves

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