FACTORS INFLUENCING THE ABILITY OF ISOLATED CELL NUCLEI TO FORM GELS IN DILUTE ALKALI*

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INTRODUCTION

In this short paper we intend to present a summary of various procedures which can be used in obtaining isolated cell nuclei that are capable of forming gels in dilute alkali, or in strong sodium chloride solution at pH 7 or above. We believe that the common effect of all these procedures is to prevent the action of an intramitochondrial enzyme (see preceding paper (14)) which is capable of destroying the gel-forming capacity of the nuclei (as outlined in the preceding paper) by detaching the desoxyribonucleic acid (DNA) from some protein of the cell nucleus to which it is normally rather firmly attached. It has been shown previously that nuclei which can form gels contain firmly bound DNA whereas those which cannot form gels contain only loosely bound DNA which can be extracted, together with histone, in approximately molar sodium chloride solutions at neutral pH (1).

EXPERIMENTAL

In Table I is given a summary of various conditions for isolating cell nuclei, together with data showing whether the nuclei can or cannot form gels. We know definitely of three procedures for obtaining nuclei which will form gels, all of which are illustrated by material in the table. A fourth procedure of somewhat doubtful significance will also be discussed. The first and probably the soundest method is to use a technique such as that outlined in the preceding article wherein mitochondrial integrity is maintained throughout the isolation procedure, since the "degelling" enzyme is an intramitochondrial enzyme which can act only after the mitochondria are ruptured.

A second method for obtaining nuclei that can form gels is to use a pH of 4.0 or lower during the isolation procedure. This results in an inactivation (but not complete destruction) of the degelling enzyme, so that even though the mitochondria are disrupted, as they are by the Waring blendor when the latter is used in the isolation of nuclei, the enzyme cannot act.

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A third way to isolate "gellable" nuclei is to use an excess of organic solvent in the isolation procedure, such as 80 per cent glycerol or ethylene glycol (2); or even a completely anhydrous procedure such as that involved in the technique of Behrens (2). Since large excesses of organic solvents are known

TABLE I

Gel Forming Capabilities of Various Types of Isolated Nuclei

Type of nuclei*	Type of homogenization*	Conditions of isolation*	Gel formation
Rat liver, lamb kidney, dog kidney, lamb pancreas	Waring blendor, colloid mill, or new homogenizer	pH 6.0, dilute citric acid in water	−
Rat liver	Waring blendor or colloid mill	pH 6.0, 0.25 M sucrose	−
Rat liver	Waring blendor	pH 6.0, 0.44 M sucrose	‡
Rat liver, Walker carcinoma 256	Waring blendor, colloid mill, or new homogenizer	pH 4.0 or less, dilute citric acid in water	+
Rat liver	New homogenizer	pH 6.0, dilute citric acid in 0.44 M sucrose	+
Rat liver, Walker carcinoma 256	New homogenizer	0.005 M $CaCl_2$ in 0.44 M sucrose for homogenate; dilute citric acid in 0.44 M sucrose for washings	+
Rat liver	Waring blendor, colloid mill, or new homogenizer	70 per cent glycerol or 70 per cent ethylene glycol	+
Rat liver	Waring blendor, colloid mill, or new homogenizer	0.002 M $CaCl_2$ in 0.9 per cent NaCl§	+
Rat liver or Walker carcinoma 256	Ball mill	Behrens' procedure	+
Chicken erythrocyte	Simple stirring	pH 6.8, saponin in 0.9 NaCl + phosphate buffer	+

* See reference 2 for description of methods.

‡ Method usually fails owing to breakage of nuclei. Once nuclei were obtained which gelled partially.

§ The use of 0.9 per cent NaCl causes condensation of chromatin, and when condensed the latter is resistant to the action of the mitochondrial enzyme.

to diminish enzyme activity as a rule, and since anhydrous conditions must also block enzyme activity, it is not surprising that "gellable" nuclei can be obtained when such conditions prevail, even though the mitochondria may be damaged or completely disrupted.

As can be seen from an inspection of Table I, "gellable" nuclei can also be isolated when 0.85 to 0.9 per cent NaCl is used as homogenizing medium near a neutral pH. The reason for the action of the sodium chloride is not

entirely clear. It might be connected with the pronounced condensation of chromatin which invariably occurs when physiological saline is used. If this is the case, the action of the sodium chloride should be explainable by solubility (and possibly steric) effects. It is not yet certain, however, whether the use of 0.9 per cent saline as homogenizing medium will in all cases lead to the isolation of nuclei that can form gels. It should be noted that mitochondria are not present in chicken erythrocytes, and this fact might explain the ease with which nuclei that can form gels can be obtained from these cells in isotonic saline.

The ability of certain types of isolated cell nuclei to form gels in dilute alkali or approximately molar NaCl can be destroyed by incubation of the nuclei with suspensions of broken mitochondria, as indicated in the previous paper, but in addition, heating, especially in dilute alkali or acid, and the action of x-irradiation can also prevent gel formation. This is shown by the following two experiments:—

A. Destruction by Heat of the Capacity of Isolated Nuclei to Form Gels in Alkali.—Three samples of nuclei capable of forming gels (previously isolated at pH 4.0 in distilled water) were extracted at pH 7 with 0.9 per cent NaCl twice to remove globulin. (The pH was brought to 7 by the addition of dilute NaOH.) One of the extracted samples was then suspended in 0.1 N HCl, another in distilled water, and the third in 0.1 N NaOH. All three samples were then placed in a bath of boiling water and heat-treated for 7 minutes, after which the tubes were quickly cooled and the suspended material from the tubes at neutral and acid pH was centrifuged down and washed once with a small quantity of distilled water. The washed residues were then suspended in small equal quantities of distilled water and a drop of dilute NH_4OH was added to each to induce gel formation. The samples which had been heated at acid pH failed to form gels, yielding only viscous solutions, while those heated at neutral pH in water formed weak gels. The samples heated in alkali, which had been gels before the heat treatment, were no longer in the form of gels after the heat treatment. Gel formation normally occurs over such a wide range of concentrations that exact adjustment of concentrations of the nuclear suspensions is unnecessary.

B. Destruction by X-Irradiation of the Capacity of Isolated Nuclei to Form Gels in Alkali[1].—Equal aliquots of a suspension of nuclei (previously isolated at pH 4.0 from distilled water) were subjected to irradiation by filtered hard x-rays of varying doses. The aliquots were each adjusted to pH 9.0 with dilute NaOH prior to irradiation, in order to induce gel formation. The gels were exposed to the x-irradiation in open beakers.

The effects of the irradiation were measured approximately by ascertaining what dilution with water was required just to break the gels. A dose of 300 r resulted in a measurable weakening of the gel, and 900 r produced a very marked effect. When dose of x-irradiation was plotted against dilution, an approximately linear curve was obtained.

DISCUSSION

In this paper we have discussed factors which are responsible for the loss in the ability of certain preparations of cell nuclei to form gels. In a previous publication (1) we have shown that when cell nuclei isolated at pH 6.0 in

[1] The authors are greatly indebted to Mr. K. I. Altman of the Atomic Energy section of this University for collaborating in this experiment.

very dilute citric acid, which do not form gels, are extracted with approximately molar saline, there is little or no DNA left in the residue. However, when one extracts in the same way nuclei isolated at pH 4.0 that will form gels, only a small proportion of the DNA passes into solution. We have subsequently found that if gels are formed in alkali or strong saline from other types of nuclei capable of forming gels, the gel can be compressed by centrifugation, carrying with it a large proportion of the DNA originally present.

On the basis of some of these results, we have already proposed that DNA normally is bound to some constituent, presumably a protein, of cell nuclei, probably by linkages other than those of a simple ionic nature, and that loss in the ability of the nuclei to form gels is an indication that DNA has been detached from this protein. This simple deduction apparently has not received wide acceptance, however, and the theory of purely ionic binding of the DNA has persisted. We now wish to show that our deduction concerning the mode of binding of DNA to nuclear protein is in agreement with the results of certain recent work dealing with the isolation of DNA with the aid of detergents (3) and with modern concepts regarding the probable role of DNA.

In the past, DNA has often been isolated by extraction from tissue, nuclei, or chromosomes as a "nucleohistone," followed by some procedure for the removal of the protein component (4–6). However, two facts in connection with such isolation techniques seldom are mentioned, namely that considerable time is generally required for the extraction of the nucleohistone, and that the yields of DNA generally are much less than they should be in theory (7), mainly because of inability to extract all of the DNA.

When detergents are used, some time is still required for the extraction of DNA, but eventually almost 100 per cent of the latter becomes soluble. Sodium dodecyl sulfate will moreover extract DNA almost quantitatively even from nuclei isolated at pH 4.0 which will form gels in alkali (3). It is our contention that the action of the detergent is gradually to break non-ionic linkages binding the DNA to protein of the nucleus, possibly through an exchange type of reaction in which the detergent takes the place of the DNA. It is difficult to understand why the extraction of DNA should generally be extremely slow in the absence of detergent and somewhat slow even in the presence of the latter, and also why the use of the detergent should generally so markedly increase the amounts of DNA that can be extracted if nothing more is involved than the breaking of ionic bonds between DNA and histone.

We interpret the necessity for prolonged extraction in the absence of detergent as indicating a slow autolytic reaction, which may be brought about by the mitochondrial enzyme referred to above, and which gradually causes detachment of the DNA from protein. In the case of Mirsky's extraction of nucleohistone (4), a high speed mixer is used in the initial stages, and it is

possible that some breakdown of the large molecular aggregation consisting of DNA and protein can be caused by the action of the mixer. It is perfectly conceivable that with large enough molecules, even covalent bands might thus be broken. In any case, the yield of nucleohistone does not seem to be quantitative.

In regard to modern concepts of the biological role of DNA, there is considerable evidence that the latter acts as gene material or as an important component of gene material (8, 9). Evidence against this point of view recently has been offered (10, 11), but it is concerned only with the apparent lack of DNA in certain egg cells, and in our opinion is not conclusive. If DNA does indeed function as gene material, it follows at once from the known facts of genetics that it must be firmly bound at definite locations on the chromosome. If it were present as "nucleohistone," held in place only by electrostatic bonds, it is very difficult to understand how it could maintain definite loci. Indeed we consider the banded distribution of DNA in chromosomes (especially the giant chromosomes of the salivary glands of *Drosophila*) as a fairly direct indication that DNA is not a simple nucleohistone as it exists within the living cell, but instead is firmly attached to the chromosomal structure at definite loci.

The work described in this paper and in the preceding one has made it possible for us to understand why certain isolated nuclei show gel formation upon the addition of strong alkali, whereas others do not. The principal factor of importance in determining whether the nuclei will or will not form gels is the mitochondrial enzyme already discussed. It now seems clear that the easily extractable nucleohistone, such as that described by Luck *et al.* (12, 13) is in a sense an artifact produced by the isolation procedure, since some factor (generally the mitochondrial enzyme) must liberate the DNA from the protein to which it is normally attached in order for it to become easily extractable together with the histone in strong salt solutions. Work is in progress in our laboratory which it is hoped will show definitely to which component of the nuclear protein the DNA is bound.

SUMMARY

1. Known methods for isolating cell nuclei are divided into two classes, depending on whether or not the nuclei are capable of forming gels in dilute alkali or strong saline solutions. Methods which produce nuclei that can form gels apparently prevent the action of an intramitochondrial enzyme capable of destroying the gel-forming capacity of the nuclei. Methods in the other class are believed to permit this enzyme to act on the nuclei during the isolation procedure, causing detachment of DNA from some nuclear constituent (probably protein).

2. It is shown that heating in alkaline solution and x-irradiation can destroy

nuclear gels. Heating in acid or neutral solutions can destroy the capacity of isolated nuclei to form gels.

3. Chemical and biological evidence is summarized in favor of the hypothesis that DNA is normally bound firmly to some nuclear component by non-ionic linkages.

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