Some Chemical Properties of Isolated Pea Nucleoli*, ‡

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ABSTRACT

Isolated nuclei and nucleoli of ungerminated pea embryos have been analyzed chemically for their content of DNA, RNA, zinc, iron, phosphorus, and protein sulfhydryl groups. The values obtained cannot be considered to represent the whole of the living nucleolar body as an undetermined amount of material is extracted from nucleoli in the course of their isolation. Only negligible amounts of DNA have been found in the isolated nucleoli; most of the DNA released on disruption of nuclei appears in a fraction showing very few structures under the light microscope. RNA is more concentrated in the nucleolus than in the nucleus or cytoplasm, but since nucleolar protein is 6 per cent of nuclear and less than 1 per cent of cytoplasmic protein, the total amount of nucleolar RNA is comparatively small. None of the other components listed occurs in high concentration in either nucleus or nucleolus.

INTRODUCTION

Reports on the chemical composition of nucleoli are controversial (1, 2). The main issues concern the concentration of RNA (ribonucleic acid), and the presence of DNA (deoxyribonucleic acid). Cytologists with few exceptions have long supposed the nucleolus to be a seat of high RNA concentration and to contain virtually no DNA. However, nucleoli isolated from starfish oocytes (1) and from rat liver (2) have been found by direct chemical analysis to contain relatively little RNA. Furthermore, it has been claimed that nucleoli isolated from rat liver contain large amounts of DNA (2).

We report here a partial chemical analysis of isolated pea nucleoli. The components include nucleic acids, phosphorus, iron, zinc, and protein sulfhydryl. Of these, only RNA appears to be markedly concentrated in the nucleolus.

Methods

Nuclei and nucleoli of "Arthur" peas were isolated as previously described (3). Subcellular fractions were suspended in 2.0 M sucrose from which appropriate volumes were withdrawn for analysis. Total and protein nitrogen were determined for each of the fractions. Protein was precipitated by acidifying the samples with 50 per cent (*w/v*) TCA (trichloracetic acid) to a final concentration of 5 per cent. The precipitates thus formed were digested according to the procedure of Campbell and Hanna (4) and the nitrogen contents determined by nesslerization (19). For sulfhydryl measurements the proteins were precipitated with 5 per cent sulfosalicylic acid, suspended in 1 per cent "Tween 80," and titrated amperometrically with $AgNO_3$ (5) in the presence of 1 per cent sodium lauryl sulfate. In order to avoid precipitation of the lauryl sulfate during titration, ethyl alcohol was added to a final concentration of 10 per cent. The specificity of the titration was demonstrated by the zero thiol values obtained after addition of 0.001 M *N*-ethyl-maleimide to the protein suspensions.

Nucleic acids were precipitated from the sucrose medium by adding 50 per cent TCA and 95 per cent ethanol to final concentrations of 5 per cent and 25 per cent, respectively. The ethanol assures a quantitative precipitation. Samples thus treated were centrifuged so as to yield a clear supernatant solution. The residues were washed twice with 5 per cent TCA, then twice with 95 per cent ethanol. They were then extracted for 1 hour with a 2:1 mixture of alcohol: ethyl ether at 50°C., washed twice with anhydrous ether, and freed of ether under vacuum. Nucleic acids were extracted from the dried powders either by the method of Ogur and Rosen (8) or by means of hot 10 per cent NaCl (14) containing 0.05 M Tris buffer, pH 7. Phosphorus was determined colorimetrically, using amidol to reduce

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the phosphomolybdate complex (9). Deoxyribose was measured by the method of Burton (10).

Zinc was measured by ashing samples at 500°C. starting with a cool muffler furnace and raising the temperature gradually (6). An HCl-solution of the ash was evaporated to dryness on a steam bath. The residue was taken up in 5 ml. of 0.12 N HCl, 0.6 ml. of 20 per cent sodium acetate, 0.25 ml. of 0.1 per cent gelatin, and sufficient water to give a final volume of 10 ml. The flocculent precipitate which formed within a few minutes after mixing the reagents was removed by centrifugation and the dissolved zinc determined polarographically. Iron was estimated by the method of McCance and Shipp (7) in a separate ashing.

RESULTS

The concentrations of nucleic acids in the embryo fractions analyzed are listed in Table I.

The striking feature of Table I is the pronounced concentration of RNA and the virtual absence of DNA in isolated nucleoli. It would be difficult to decide whether the small amount of DNA found in the nucleolar fraction was due to contamination or whether it was an actual component of the nucleolus. Foreign particles were present in the nucleolar fraction (3), and these might contain DNA even though most of the DNA released upon disruption of the nuclei in the 2 M sucrose medium could not be sedimented after 2 hours of centrifugation at 140,000 *g*. However, even if the DNA found in the nucleolar fraction were native to the nucleolus, the result would not be at all in harmony with that of Litt *et al.* who found DNA to be a major component of rat liver nucleoli (2).

TABLE I

Distribution of Nucleic Acids in Fractions of the Pea Embryo

Fraction	Concentration per mg. total nitrogen			Concentration per mg. protein nitrogen		
	RNA-P*	DNA-P*	Residual-P‡	RNA-P	DNA-P	Residual-P
Embryo (less cotyledons)	19.5	2.5	12.0	27	3.4	17.0
Nuclei	20	47.0	4.0	20	47.0	4.0
Nucleoli	59	2.0	4.0	59	2.0	4.0

Phosphorus values are expressed in micrograms.

* RNA and DNA were determined as described under Methods.

‡ "Residual-P" represents acid-insoluble phosphorus less that accounted for as nucleic acid.

On the basis of protein content the nucleolus contains a high RNA concentration, relative to other cellular fractions. It is clear, however, that the ratio of RNA to protein in the isolated nucleoli is much lower than the 15 to 30 per cent found in nucleoprotein particles prepared from pancreatic microsomes (20). Using 6.25 and 11.0, respectively, as conversion factors from N to protein and P to nucleic acid, it can be calculated from Table I that on a weight basis RNA is about 10 per cent of the nucleolar protein. A similar value for the RNA/dry weight ratio was obtained when a weighed amount of the fat-free dry powder was assayed for RNA content. Thus, in line with the observations of Vincent (1) on starfish oocyte nucleoli, it appears that quantitatively, the major constituent of the fat-extracted isolated pea nucleolus is protein. On the other hand, unlike oocyte nucleoli, there is no large amount of phosphorus in the protein fraction. Hot 10 per cent NaCl or cold 1 N perchloric acid each removed about 93 per cent of the phosphorus, all of the latter being accountable as nucleic acid on the basis of ultraviolet absorption measurements of the extracts which had sharply defined spectra. The remaining phosphorus, much of which is unextractable even in hot 0.5 N perchloric acid, is presumed to be present as phosphoprotein, but, as Table I shows, such phosphoprotein is more concentrated in the cytoplasm than in nucleus or nucleolus.

Although the ratio of RNA/protein in the isolated nucleolus is higher than that in the nucleus or in the cytoplasm as a whole, it may be shown from the total amounts of protein in each of the cellular fractions that the proportion of nucleolar RNA in the cell is small. In preparations where account was kept of nitrogen distribution about 4 per cent of the protein nitrogen of the nucleus was found in the nucleolar fraction. From a rough count of nucleoli in this and the two other fractions, it would appear that the recovery was approximately 70 per cent. It may, therefore, be assumed that nucleoli account for about 6 per cent of the nuclear proteins, a value which is consistent with the nucleolar volume approximated from electron micrographs of *in situ* sections (18). Thus, of all the RNA in the nucleus, only about 20 per cent is found in the isolated nucleolus. It can be shown from the dilution of the DNA/protein ratio in the nucleus by the DNA-free cytoplasm (11), that the isolated nucleus accounts for only 14 per cent of the cellular protein. The isolated nucleus, therefore, contains only 8 per cent, and the isolated nucleolus

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TABLE II

Some Chemical Components of Pea Embryo Fractions

Fraction	Protein-SH		Zinc	Iron
	Untreated	Treated*		
	μmoles	*μmoles*	*μg.*	*μg.*
Embryo (less cotyledons)	0.27	0.36	1.85	3.88
Nuclei	0.24	0.22	1.44	1.95
Nucleoli	0.19	0.22	1.66	2.16

All values expressed as per mg. protein-nitrogen.

* "Treated" protein —SH refers to protein suspensions treated with sodium thioglycollate at pH 7.0 to reduce any easily accessible —SS— groups or —SH groups which might have been oxidized in the course of preparation of the various fractions.

only 1.6 per cent of the total RNA of the embryonic cell.

The nature of the proteins present in the isolated nucleoli has not been resolved. Although several histochemical studies (15, 16) have shown sulfhydryl groups of proteins to be concentrated in chromosomes and nucleoli, we have found by direct titration that protein —SH is more or less evenly distributed among the subcellular fractions examined (Table II). A similar lack of localization was found in the case of bound zinc and iron. The distribution of histones was not determined because about 70 per cent of the proteins in the pea embryo (less cotyledons) and over 50 per cent of those in the nuclei were soluble in cold 0.2 N HCl, a medium commonly used for histone extraction. These values were determined by extracting the tissues for 1 hour in 0.2 N HCl at 0°C., centrifuging the suspension for 20 minutes at 140,000 *g*, and precipitating the protein of the clear supernatant solution with 5 per cent TCA. Thus, even if a major proportion of pea embryo protein were histone, a much more selective method than acid extraction would be necessary to identify chromosome-associated histones.

DISCUSSION

Two considerations apply to any conclusions drawn from these data. First, the nucleoli are derived from physiologically dormant cells, and second, intranucleolar material has been lost in the course of isolation. Even if no qualitative changes occur in the composition of the nucleolus as a consequence of germination, it is most likely that quantitative ones do. RNA particularly varies markedly with metabolic activity, and so does nucleolar size and stainability. It is, therefore, possible that the metabolically responsive moieties of the nucleolus are present in minimal concentration in the dormant embryo and that upon initiation of germination the concentration of components such as RNA rises in the nucleolus. Thus, growing tissues, which are histochemically the ones usually associated with large RNA-rich nucleoli, may not be as lacking in RNA as Vincent supposes (1). However, the second consideration listed—the loss of nucleolar material during isolation—applies more strongly to the differences in RNA content between isolated nucleoli and those examined *in situ* histochemically. It is commonly assumed that the materials lost by the two preparative procedures are similar quantitatively and qualitatively, but this is clearly not so. Osmic acid fixation of intact nuclei retains materials which are lost when the nuclei are ruptured (3). The difference in composition obtained by the two methods cannot, therefore, be resolved in favour of conventional chemical analysis on the grounds of its quantitative accuracy. It must be allowed that components present in the living nucleolus may be absent from the isolated one, but not from one in histological section. Should the material lost from the nucleoli after nuclear rupture contain RNA, then the concentration measured in the isolated preparation would be much below that present *in situ*. It seems probable that the absence of a membrane around the nucleolus is largely responsible for the loss of material in the course of isolation. It would be difficult to decide whether such material properly belongs to the nucleoplasm rather than to the nucleolus, but there can be little doubt the material in question is intimately associated with the activities of the nucleolus in the living cell.

The most striking observations from the chemical data are the very low concentration of DNA and the high concentration of RNA in the isolated nucleoli. To what extent the observed RNA concentration has been affected by isolation techniques and what changes occur with the breaking of dormancy are not known. It is apparent, nevertheless, that the findings are consistent with the many observations of rapid RNA turnover in nucleoli of intact cells (17).

The results here reported on the sedimentation properties of DNA released by disruption of nuclei

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have a bearing on claims that plant mitochondria and chloroplasts contain DNA (12, 13). These claims are based on the presence of DNA in isolated preparations of these subcellular particles, nuclear contamination being denied either because there are no stainable nuclear fragments in the preparations (13) or because it is believed that nuclei are not readily fragmented and hence nuclear DNA should be restricted to an easily sedimentable fraction (12). Neither of these arguments, however, appears to be valid in the case of pea and wheat embryos. In the case of peas, most of the nuclear DNA is in a fraction which shows few, if any, structures under the light microscope; in the case of wheat germ nuclei disrupted by low speed blending in the absence of calcium, the DNA released is sedimented by a variety of centrifugal forces covering a broad range of particle sizes. With respect to fragility, we have observed in both wheat and pea embryos that nuclei of freshly broken cells are readily disrupted, but that they become increasingly resistant to disruption upon standing in suspension. Most nuclear fragmentation thus occurs at the time of cell rupture so that the released nuclear DNA would be dispersed through the homogenate before any physical separation was made.

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