The Characterization of a Non-Histone Protein Isolated from Histone F1 Preparations

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A non-histone protein was obtained by extraction of nuclei derived from rat liver or thymus or ascites-hepatoma cells with 5% (w/v) HClO_4 . Separation from histone F1 was achieved by chromatography on DEAE-cellulose. The purified component P1 was characterized and the formation of complexes with histone F1 and polylysine was studied.

Although histones in general display conservatism of structure over a wide range of species and tissues, histone F1 shows both species and tissue specificity (Kincade & Cole, 1966). Histone F1 is phosphorylated (Ord & Stocken, 1966), the degree of phosphorylation being related to the rate of DNA synthesis (Ord & Stocken, 1968, 1969). The histone fraction normally described as histone F1 has been shown to contain non-histone protein components (Johns, 1964), and Buckingham & Stocken (1970) reported an increased phosphorylation of histone F1 during liver regeneration without a concomitant increase in phosphorylation of the associated non-histone protein. Since it is known that several non-histone nuclear proteins are phosphorylated (Kleinsmith *et al.*, 1966) and are important in conferring specificity on chromatin (Paul & Gilmour, 1968), it was considered that the non-histone component of histone F1 might be closely involved in the function of the histone and should therefore be more precisely characterized.

Methods

Preparation of nuclei. Nuclei were prepared from sheep thymus by the method of Allfrey *et al.* (1964) and from rat liver and ascites-hepatoma cells by the method of Chauveau *et al.* (1956) or that of Dabeva & Tsanev (1966), the layer of 1.8M-sucrose being omitted.

Preparation of histone F1. Nuclei were washed with 10mM-Tris-HCl buffer, pH 7.4, containing 5mM- MgCl_2 , and with 1mM-HCl, and histone F1 was extracted with 5% (w/v) HClO_4 . The extract was made 20% (w/v) with respect to trichloroacetic acid and centrifuged at 3000g for 10 min. The precipitated histone was dissolved in water and reprecipitated in trichloroacetic acid (20%, w/v). It was then redissolved in water and dialysed to give 'crude histone F1' (Johns, 1964).

Amino acid analyses. The method of Spackman *et al.* (1958) was used.

Protein determination. This was carried out by the method of Lowry *et al.* (1951); a reference solution

for the assay was obtained from an amino acid analysis of the appropriate purified protein.

Phosphate determination. Total phosphate was determined by the method of Bartlett (1959), with 60% (w/v) HClO_4 as ashing agent.

Polyacrylamide-gel electrophoresis. This was carried out with the 6.25M-urea–0.9M-acetic acid system of Panyim & Chalkley (1969). Polyacrylamide-gel electrophoresis in the presence of sodium dodecyl sulphate was performed by the method of Weber & Osborn (1969).

Formation of complexes. The formation of histone and non-histone protein complexes was followed turbidimetrically by measuring the extinction at 450nm 15 min after the addition of various amounts of histone F1 or polylysine to a solution of component P1 (see below) (100 μg in 2ml of 25mM-Tris-HCl buffer, pH 7.4) (T. Langan & L. Smith, personal communication).

Histone kinase. Histone kinase was used in a system similar to that described by Pawse *et al.* (1971). A 100 μg portion of substrate protein, 2.5 μmol of MgCl_2 , 10 μmol of Tris-HCl buffer, pH 7.4, 0.21 μmol of [γ - ^{32}P]ATP (specific radioactivity 28000 d.p.m./nmol) and 0.1 unit of the enzyme were contained in a final volume of 0.2ml. (One unit is the activity of enzyme required to transfer 1nmol of phosphate from ATP to histone F1 in 10 min at 37°C.)

Results

Separation of non-histone. The non-histone contaminants were separated from histone F1 on a DEAE-cellulose column (Whatman DE52). 'Crude histone F1' was applied to the column in 50mM-Tris-HCl buffer, pH 7.4. The non-histone protein was retained on the column, and after a washing with Tris buffer was eluted by 0.5M-NaCl in 50mM-Tris-HCl buffer, pH 7.4 (Buckingham & Stocken, 1970). In all three tissues the yield of non-histone protein was 5–8% by weight of the histone F1.

Electrophoresis on polyacrylamide gels containing acetic acid–urea showed two major bands, whose

mobilities relative to that of histone F1 were 0.86 and 0.33. The latter band was not observed when the applied protein solution contained β -mercaptoethanol, showing it to be an oxidized form of the former. In addition to the major bands, a number of minor bands with relative mobilities less than 0.3 were also observed.

To purify the major band, designated component P1, the solution, containing 10mg of protein, was applied to a column of CM-Sephadex (CM-50) ($20\text{cm} \times 3.0\text{cm}^2$) washed with 6M-urea–0.3M-NaCl–50mM-Tris–HCl buffer, pH7.4, and eluted in 6M-urea – 1M-NaCl – 50mM-Tris – HCl buffer, pH7.4. When dialysed against water, the eluate containing component P1 sometimes became cloudy. It was clarified by making the solution 5% (w/v) with respect to HClO_4 and centrifuging at 10000g for 20 min. The clear solution runs as a single band in sodium dodecyl sulphate gels but as a doublet in the system described by Panyim & Chalkley (1969). The protein may be the same as the non-histone band found in acid extracts of 'nucleoprotein' by Lake *et al.* (1972).

Electrophoresis in the presence of sodium dodecyl sulphate suggests a molecular weight of about 20000. The amino acid composition of component P1 is shown in Table 1. Cysteine is present, which accounts for the major slow-running band observed only in the absence of β -mercaptoethanol. Although the protein contains an unusually high proportion of basic and acidic residues (over 50%), the electrophoretic

results suggest that most of the acidic residues must be present as amides.

The phosphate contents of histone F1 and the non-histone components are shown in Table 1. It is evident that the purified histone F1 contains considerably less phosphate than does the original extract, and that observations on the phosphate content of unpurified histone F1 are the sum of two different populations of phosphate.

Component P1 is a substrate for histone kinase, although it is less effective than the histone F1 itself. The ratio of ^{32}P radioactivity accepted by histone F1 to that accepted by component P1 is 4.8 ± 0.9 (mean $\pm$ s.d. of three experiments).

Acidic nuclear proteins form insoluble complexes with histones (T. Langan & L. Smith, personal communication; Wang & Johns, 1968), and component P1 also forms complexes (Fig. 1) with histones or poly-L-lysine. However, the inhibition of template activity of DNA for *Escherichia coli* RNA polymerase *in vitro* by histone F1 is not relieved by component P1, possibly because the assay mixture contains β -mercaptoethanol, which prevents the formation of complexes (Fig. 1).

Discussion

The status of non-histone nuclear proteins has occasionally been questioned, as nucleohistone readily adsorbs cytoplasmic proteins during preparation

Table 1. *Amino acid analyses and phosphate contents of components of 'histone F1'*

Amino acid content is expressed in mol/100mol; phosphate content is expressed in nmol/mg of protein.

	Ascites histone F1	Ascites non-histone components	Thymus histone F1	Thymus non-histone components	Thymus component P1
Asx	4.0	10.8	3.5	11.6	11.7
Thr	4.1	2.9	6.1	3.1	2.6
Ser	12.1	7.3	7.6	5.4	5.9
Glx	5.5	15.0	4.8	15.8	17.8
Pro	9.0	5.0	8.3	4.7	4.1
Gly	7.4	6.2	5.8	5.2	4.7
Ala	21.0	9.8	20.7	9.2	8.3
CyS	0	0.8	0	0.9	0.3
Val	5.0	2.0	5.2	4.0	2.3
Met	0.45	7.5	1.25	4.6	1.8
Ile	1.5	2.3	1.25	2.1	1.9
Leu	4.2	3.5	4.6	2.6	2.2
Tyr	0.6	1.1	0.6	3.8	{ 2.9 3.9
Phe	0.9	2.6	0.8		
His	0.35	1.2	0.3	1.3	1.1
Lys	32.5	16.7	27.0	20.1	23.9
Arg	3.5	4.1	2.4	4.7	4.0
Phosphate	13.5	140	2.4	81	14.7

Fig. 1. Formation of complexes between component P1 and histone F1 in the absence and in the presence of β -mercaptoethanol and between component P1 and poly-L-lysine

The formation of complexes between component P1 (100 μ g in 2ml) and histone F1 alone (●), histone F1 in the presence of β -mercaptoethanol (○) and poly-L-lysine (▲) was followed as described in the text.

(Johns & Forrester, 1969). Since we have isolated the nuclei before extracting the histone, and since the amount of component P1 in HClO_4 extracts from nuclear preparations from different tissues was remarkably constant, cytoplasmic contamination seems unlikely. The yield of non-histone protein, however, was lower than that observed from whole-tissue extracts (Johns, 1964), which may well contain other cytoplasmic proteins. The molecular weight of component P1, together with its different amino acid composition, preclude its being a degradation product of histone F1.

Histones undergo a variety of chemical modifications, of which phosphorylation (Ord & Stocken,

1966) and thiolation (Ord & Stocken, 1966) are related to replication. It is evident that component P1 could also be subject to modification by phosphorylation and the redox state of the nucleus, which in turn could affect the formation of histone complexes and so provide a further means for the control of nuclear function.

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