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Comparison of the high-mobility-group chromosomal proteins in rainbow-trout (*Salmo gairdnerii*) liver and testis

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Chromatography and characterization of the proteins extracted by 5% (w/v) $HClO_4$ from rainbow-trout (*Salmo gairdnerii*) liver and testis show that the two tissues present a characteristically different spectrum of high-mobility-group (HMG) proteins. A variant subfraction of HMG C is found in liver, but is not detectable in testis, where even the main fraction of HMG C is present in only very low quantity. A protein, F, which appears to be related to protein H6 has similarly been isolated only from liver and not from testis. Quantification of the HMG proteins in total 5%-$HClO_4$ extracts of trout liver and testis nuclei shows that, in relation to DNA, levels of HMG T1 and T2, and D are more than 2-fold, and C, 20-fold higher in liver than in testis. However, these differences do not result merely from the sequential withdrawal of HMG proteins at the same time that histones are replaced by protamines in the developing spermatid, since in testis, at some stages of maturation, levels of H6 are almost 2-fold higher than in liver. The implications of these findings for the function of HMG proteins are discussed.

The HMG proteins are a group of well-characterized non-histone proteins extractable from chromatin with 0.35 M-NaCl or 5% $HClO_4$, possessing an unusually high proportion of charged residues, which are asymmetrically distributed within the molecule. The function of the HMG proteins is still unknown: a structural role was originally proposed for them (Goodwin *et al.*, 1973) and an association with transcriptionally active chromatin has been suggested (Levy *et al.*, 1977; Weisbrod & Weintraub, 1979). However, they are present not only in mature erythrocytes (Rabbani *et al.*, 1978) and condensed chromosomes (Smith *et al.*, 1978; Kurth & Bustin, 1981; Lund *et al.*, 1981), but also in satellite chromatin (Mathew *et al.*, 1981; Levinger *et al.*, 1981; Reudelhuber *et al.*, 1982). They have so far been found in all vertebrate tissues examined except sperm (Kennedy & Davies, 1980; the present paper), although there are considerable qualitative and quantitative variations in the complement of HMG-like proteins from different species [for a review, see Mayes & Johns (1982)]. There have been several reports that the levels of the larger HMG proteins 1 and 2 or their subfractions vary not only between different species but in different tissues (Mathew *et al.*, 1979; Seyedin & Kistler, 1979; Gordon *et al.*, 1980). It has been proposed that a specific distribution of the larger HMG proteins could play a part in cellular differentiation by establishing a conformation of chromatin characteristic for a particular tissue (Gordon *et al.*, 1981). We have previously shown that, in the rainbow trout, *Salmo gairdnerii*, there are two proteins, HMG T1 and HMG T2, which are similar to mammalian/avian HMG 1 and HMG 2 (Brown *et al.*, 1980), and three proteins, D, C and H6, similar to mammalian/avian HMG 14 and HMG 17 (Walker *et al.*, 1980) and avian HMG Y (Goodwin *et al.*, 1981). Further detailed investigation of trout HMG proteins has shown that there are at least two more members of the C/D/H6 family, which are differently distributed in the two tissues. It was moreover noted that the levels in liver of not only the higher-molecular weight proteins, T1 and T2, but also the smaller proteins, D, C and H6, differed consistently from those in testis. HMG proteins from each tissue were therefore quantified in relation to DNA to establish whether or not the distribution of the HMG proteins in trout also was tissue-specific. The comparison of liver and testis was particularly interesting, as in liver the rate of cell replacement is

Abbreviations used: HMG protein, high-mobility-group protein; SDS; sodium dodecyl sulphate; PMSF, phenylmethanesulphonyl fluoride.

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low and the rate of transcription high, whereas in testis the rate of replication is high and, as protamines replace the histones (Dixon & Smith, 1968), transcriptional activity decreases.

Experimental

Extraction of protein from unfractionated testis, liver and ova

Trout tissues were obtained from mid-October to the beginning of December. One batch of testis was obtained in January and processed separately. Tissues were frozen in solid CO_2 within 2 min of the death of the fish and stored at −80°C. To obviate any possibility of the degradation or differential extraction of protein, they were thawed to 4°C in, and then blended at high speed for 3 × 2 min in, 5% $HClO_4$. The supernatants were combined and the addition of 2 vol. of acetone precipitated a large amount of unidentified material from liver and ova that was shown by polyacrylamide-gel electrophoresis to contain no histone nor HMG protein. The extract was made 0.3 M with respect to HCl and the addition of 1.5 vol. of acetone (relative to the original extract) precipitated histone H1, which was removed by centrifugation, and a further 2.5 vol., the HMG proteins.

Extraction of protein from sperm

Fully mature sperm were obtained by manual expression of the milt in January. The sperm tails were removed by twice-repeated gentle hand homogenization in 0.25 M-sucrose/3 mM-$MgCl_2$/1 mM-PMSF and centrifugation at 4000 ***g*** for 10 min at 4°C. Sperm heads were extracted with 5% $HClO_4$ as above, and protein precipitated by the addition of HCl (to 0.3 M) and 6 vol. of acetone.

Column chromatography

Column chromatography was carried out as described in the legends to Figs. 1, 2 and 3 below. The possibility that protein recovery was affected by the slightly different conditions used to optimize separation of T1 from T2 in testis, and C from D in liver, was checked by chromatographing testis proteins under the conditions used for liver proteins and vice versa. No difference in the amount of protein recovered was found.

Quantification of protein in relation to DNA

(*a*) *Preparation of nuclei and extraction of protein.* Nuclei from testis and liver were prepared simultaneously by exactly the same procedures. Each tissue was thawed at 4°C, blended at low speed for 45 s in a buffer of 0.25 M-sucrose/3 mM-$MgCl_2$/1 mM-PMSF and centrifuged at 4000 ***g*** for 5 min. Each nuclear pellet was purified by centrifugation through 1.8 M-sucrose/3 mM-$MgCl_2$/1 mM-PMSF for 1 h at 30000 ***g***. The purity of the nuclei was checked by electron microscopy. Proteins were extracted from the nuclei with 5% $HClO_4$ as described above for sperm. Precipitated protein was redissolved in a measured volume of distilled water and stored at −20°C.

(*b*) *Determination of DNA.* The DNA content of measured portions taken from the suspension of nuclei was measured by A_{260} (the absorbance of a 1 mg/ml solution of DNA in 1 M-NaOH was taken as 26.5), by the indole method of Ceriotti (1952) as adapted by Hubbard *et al.* (1970), and by the diphenylamine method of Dische (1930).

In the case of testis, a difference of only 2% was found between the estimates obtained by the three methods, which were averaged. On account of the presence of considerable quantities of RNA in liver, the estimates obtained by the indole and diphenylamine methods only were averaged. These differed by 5%.

(*c*) *Quantification of protein.* Proteins were electrophoresed on SDS/15%-polyacrylamide gels as described by Laemmli (1970), or on acid/urea gels as described by Panyim & Chalkley (1969). The gels were stained with Procion Navy and protein quantified as described by Smith *et al.* (1980). The protein content of stock solutions of standards was accurately determined by total amino acid analysis on a Rank–Hilger Chromospek analyser.

Results

Isolation and characterization of HMG proteins from unfractionated tissue

Typical chromatographies are shown of 5%-$HClO_4$-extracted proteins from trout testis (Fig. 1*a*) and liver (Fig. 2*a*). Proteins were recovered and analysed from all the peaks, and those from fractions relevant to the present paper are shown after electrophoresis in an SDS/polyacrylamide gel in Figs. 1(*b*) and 2(*b*) respectively, in correspondingly lettered lanes. Such chromatographies were carried out more than ten times each for both liver and testis, and were highly reproducible. In the case of C, D and H6, protein recovery corresponded to peak size, and it can be seen that quantities of these proteins vary in relation to each other in each tissue, and that levels of C in relation to D and H6 are notably diminished in testis. Pure samples of both C and D were prepared by rechromatography of fractions in which they were eluted together or with other liver proteins (Fig. 2*b*), on a column of phosphocellulose. A typical elution profile is shown in Fig. 3(*a*), and fractions containing C, D and two unknown proteins migrating slightly faster than C are shown after electrophoresis in an SDS/polyacrylamide gel in Fig. 3(*b*) in correspondingly lettered lanes. The amino acid analyses of fractions a, b, d and e from

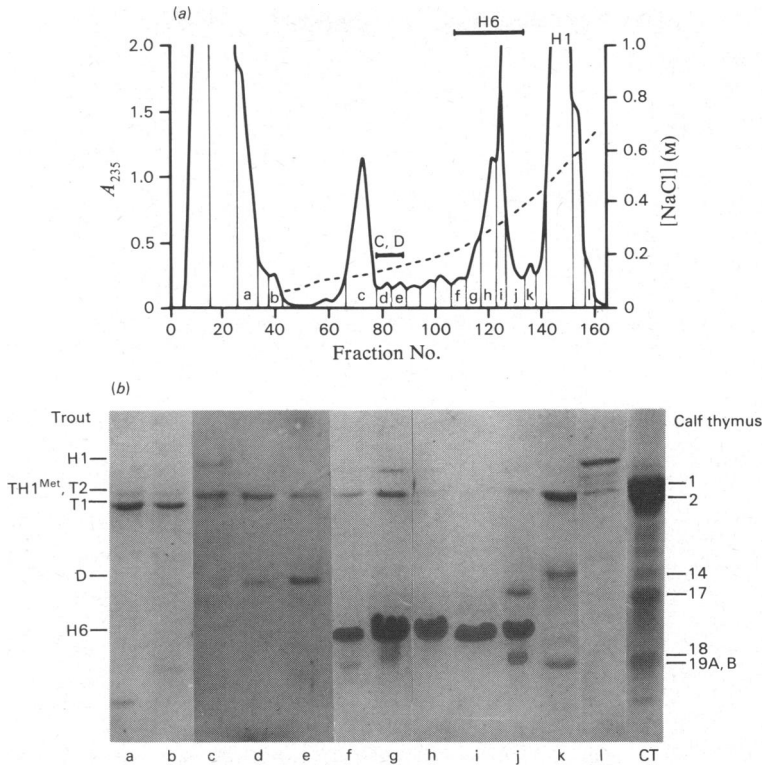

Fig. 1. *Chromatography and SDS/polyacrylamide-gel electrophoresis of proteins from a 5%-$HClO_4$ extract of trout testis*
(*a*) Proteins were chromatographed on a column (5 cm × 30 cm) of CM-cellulose C-52 equilibrated with 7.5 mM-sodium borate buffer, pH 9.0. Protein was dissolved in buffer containing 9 M-urea and 0.05% (v/v) mercaptoethanol. Buffer (250 ml) was pumped through the column, followed by a 700 ml linear gradient of buffer containing 0–0.3 M-NaCl and then by a 1.2-litre linear gradient from 0.3 to 1.2 M-NaCl. ——, A_{235}; ----, [NaCl]. (*b*) Proteins recovered from the peaks in (*a*) are shown, after electrophoresis, in correspondingly lettered lanes. a and b, HMG-T1; c–g, HMG-T2; d and e, HMG-D; f–i, H6; CT, HMG proteins from a 5%-$HClO_4$ extract of calf thymus (minus excess H1).

Fig. 3(*b*) are given in Table 1. On account of its amino acid analysis, migration in an acid/urea gel and lack of characteristic metachromatic staining by Coomassie Blue, the protein in fraction f was considered to be completely unrelated to C and D (results not shown). Protein C was eluted in fraction b. A smaller peak (a) contained a protein of similar amino acid analysis, but differences in the content of glutamic acid, alanine, threonine, serine and methionine showed that it was a variant and not a phosphorylated or otherwise modified form of C. Two forms of D were already known to exist, as the *N*-terminal sequence of one has a residue of glutamine inserted between residues 4 and 5 (Walker *et al.*, 1980), and it was possible that fractions d and e corresponded to these two variants, since there was a small difference in their content of glutamic acid/glutamine. Other slight dissimilarities in analysis were consistent with the possibility of further differences of one molecule for any one type of amino acid but, as such, were so small (1.1% for a protein of about 90 residues) that they could have resulted merely from experimental variation. When C- and D-containing fractions from testis were chromatographed on a phosphocellulose column under identical conditions, no variant fraction (a) and only a very small fraction (b) of protein C was present, whereas D was again eluted as two immediately consecutive peaks (results not shown).

As both C and D had been shown to be microheterogeneous, it was possible that the successive peaks containing H6 which were reproducibly present in all fractionations carried out of trout testis (Fig. 1*a*, f–i) and liver (Fig. 2*a*, i and j) also represented variant rather than modified forms, but amino acid analysis of these fractions proved inconclusive, since, as in the case of D, the variations found never exceeded a possible difference of one residue for any one amino acid. However, a protein, designated protein F, which stains metachromatically with

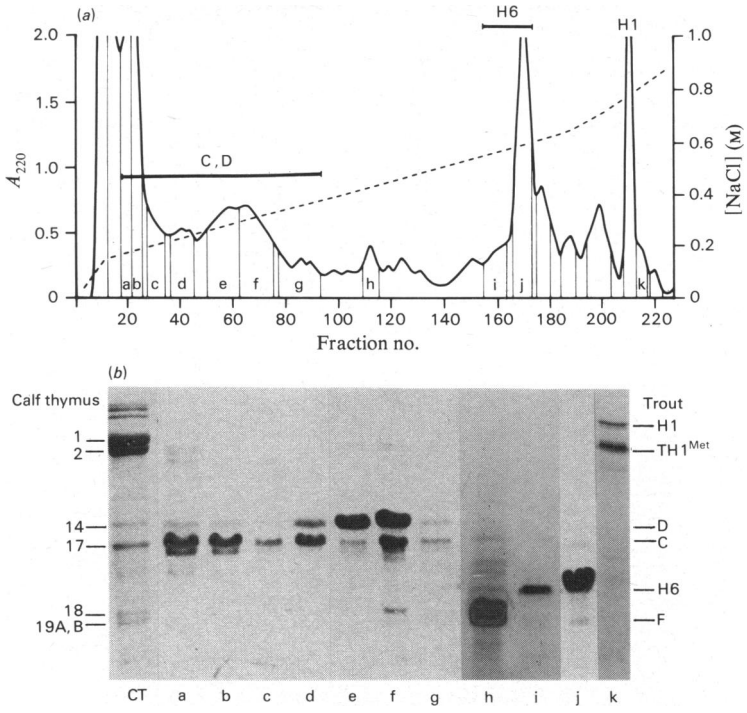

Fig. 2. *Chromatography and SDS/polyacrylamide-gel electrophoresis of proteins from a 5%-$HClO_4$ extract of trout liver* (*a*) Proteins were chromatographed on a column (5 cm × 30 cm) of CM-Sephadex C25, equilibrated with 0.05 M-NaCl/7.5 mM-sodium borate buffer, pH 8.9. Buffer (250 ml) was pumped through the column, followed by a 1.6-litre linear gradient from 0.05 to 0.6 M-NaCl and then by a 1-litre linear gradient from 0.6 to 1.2 M-NaCl in 7.5 mM-sodium borate buffer. ——, A_{220}; ----, [NaCl]. (*b*) Proteins recovered from the peaks in (*a*) are shown, after electrophoresis, in correspondingly lettered lanes. a–g, HMG proteins C and D; i and j, H6; h, protein F; traces only of T1 and T2 are seen in a and f; the protein running in the same position in lane k is a subfraction of H1, THI^{Met} (Brown & Goodwin, 1983); CT, HMG proteins from a 5%-$HClO_4$ extract of calf thymus (minus excess H1).

Coomassie Blue and has *N*-terminal proline has been consistently isolated from liver (Figs. 2*a* and 2*b*, fraction h), but never from testis. It runs faster than H6 on an SDS/-, but in the same position on an acid/urea/-polyacrylamide gel, and also its amino acid analysis strongly resembles that of H6 (Table 1). As it contains a residue of histidine, it cannot be a degradation product of H6, but is probably closely related to the latter.

Quantification of the levels of HMG proteins extracted with 5% $HClO_4$ from trout liver and testis nuclei in relation to DNA

Since chromatography and subsequent characterization of proteins had revealed the presence in trout liver of appreciable quantities of a methionine-containing subfraction of H1, THI^{Met}, (Figs. 2*a* and 2*b*, fraction k), which runs in the same area of an SDS/polyacrylamide gel (Fig. 1*b*, lane l) as HMG proteins T1 (lanes a and b) and T2 (lanes c–g), T1 and T2 were quantified together in an acid/urea/gel system in which they migrate more slowly than H1, whereas THI^{Met} continues to run ahead of it (Brown & Goodwin, 1983). Since it was important to quantify C and D separately and they have almost identical mobilities in an acid/urea system, they were electrophoresed in an SDS/polyacrylamide gel despite the presence in 5%-$HClO_4$ extracts of testis (Fig. 1*b*) and nuclei (results not shown) of two proteins which run in the position (lane j) of C and (lane k) of D: however, electrophoresis of these unknown proteins in an acid/urea/gel system showed that they were unrelated to C or D (results not shown). The contribution of protein in lane j to total protein running in this position is negligible, but the protein in lane k has been estimated from the proportion seen to be present and the total weight of protein recovered in this fraction, to constitute up to 10% of the protein running in the position of D.

Fig. 4(*a*) shows the profile of proteins from 5%-$HClO_4$ extracts of trout testis and liver nuclei after electrophoresis on an SDS/- and Fig. 4(*b*) on an acid/urea/-polyacrylamide gel (the latter has been truncated to show only the relevant portion). The

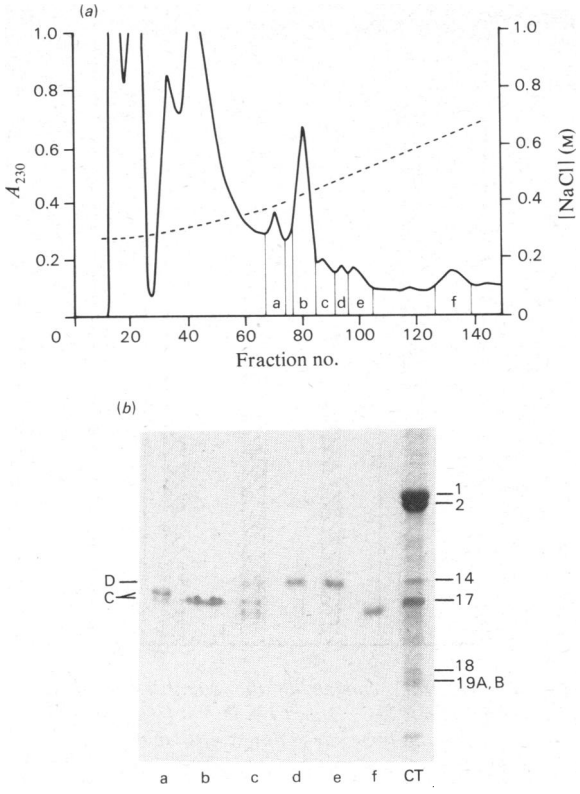

Fig. 3. *Separation by chromatography and SDS/polyacrylamide-gel electrophoresis of trout liver HMG proteins C and D*

(*a*) Fractions a–d of Fig. 2(*a*) were recovered and loaded on a column (2.5 cm × 20 cm) of phosphocellulose equilibrated with 0.05 M-NaCl/10 mM-sodium acetate, pH 5.0. A 40 ml portion of 10 mM-sodium acetate/0.05 M-NaCl, then 40 ml of 10 mM-sodium acetate/0.3 M-NaCl buffer were pumped through the column, followed by a 700 ml linear gradient from 0.3 to 0.8 M-NaCl in sodium acetate buffer. Protein-containing fractions were pooled as indicated, dialysed against 50% (v/v) ethanol/10 mM-HCl, and protein was precipitated by acidification to 0.1 M-HCl and the addition of 6 vol. of acetone. The peaks eluted first from the column resulted from the use of 9 M-urea and 0.05% (v/v) mercaptoethanol in the loading buffer and from non-proteinaceous material absorbing strongly at 260 nm contained in fraction a of Fig. 2(*a*). ——, A_{230}; ----, [NaCl]. (*b*) Proteins recovered from the peaks in (*a*) are shown, after electrophoresis, in correspondingly lettered lanes. a, Subfraction of C; b, main fraction of C; c, mixture of C, D and an unidentified protein; d and e, protein D; f, a protein unrelated to C or D; CT, HMG proteins from a 5%-$HClO_4$ extract of calf thymus (minus excess H1).

Table 1. *Amino acid analyses of trout proteins belonging to the HMG 14/17 family*

Columns a, b, d and e correspond to the fractions shown in Figs. 3(*a*) and 3(*b*) and are single analyses. F is an average for three samples.

Amino acid	Content (mol/100 mol)							
	a	b	d	e	F	H6*	C†	D†
Asx	8.5	6.6	9.3	9.2	6.6	6.7	6.8	9.8
Thr	4.4	2.5	3.7	3.7	2.0	1.6	3.1	3.6
Ser	6.0	3.9	5.5	5.8	5.1	5.6	5.5	5.0
Glx	19.1	23.1	20.0	19.3	8.5	6.1	21.9	18.8
Pro	12.2	11.8	6.5	7.4	6.3	12.3	11.1	7.8
Gly	5.3	3.5	5.0	5.5	8.0	7.4	4.3	6.0
Ala	13.2	17.1	17.2	16.6	22.4	25.4	15.7	15.7
Val	4.4	4.2	2.9	2.8	5.1	3.4	4.0	3.2
Cys	1.3	1.0	0.0	0.5	0.0	0.0	1.0	0.0
Met	0.6	0.1	0.0	0.6	0.0	0.0	0.2	0.3
Ile	0.6	0.9	0.6	0.8	0.0	0.0	0.8	0.5
Leu	1.5	1.8	1.7	2.0	2.6	1.2	1.8	1.6
Tyr	0.3	0.2	0.0	0.0	0.0	0.0	0.2	0.4
Phe	0.6	0,5	0,5	0.5	0.8	0.0	0.3	0.6
Lys	17.3	17.7	22.0	20.4	29.1	23.1	18.4	20.8
His	1.0	0.6	1.5	1.7	1.2	0.0	0.5	1.3
Arg	3.6	4.4	3.5	3.1	4.7	7.2	4.2	4.4

* From Wigle & Dixon (1971).

† Analyses of unfractionated proteins C and D purified from trout by CM-Sephadex and gel-filtration chromatography (Brown, 1982). Each analysis is the average of at least 12 samples.

bands of protein running faster than HMG T1/T2 in liver (Fig. 4*b*, peak 3) have been included in their quantification as, unless special care is taken to reduce these proteins with mercaptoacetic acid, multiple oxidized forms are often seen (Brown *et al.*, 1980). The protein running slightly more slowly than C (Fig. 4*a*, peak 1) has been included in the quantification of C, as it probably corresponds to the variant form of C identified in Fig. 3(*b*), lane a. The protein running slightly faster (peak 2) has not been included, as it probably incorporates the unknown proteins seen in Fig. 3(*b*), lanes c and f. The amount of each protein in the two tissues is shown in relation to DNA in Fig. 5.

The variation of the quantities of C, D and H6 in relation to each other in each tissue already observed during the chromatography of unfractionated tissue was confirmed (and, conversely, showed that no degradation or differential extraction of protein had occurred during the preparation of nuclei). Additionally, it was shown that, in relation to DNA, D is present in appreciably, and C in strikingly larger, amounts in liver, whereas H6, at least during some stages of development, is present in greater quantities in testis. Although great care was taken to try and quantify D as accurately as possible, the deduction or not of 10% from the total (indicated in Fig. 5 by a dotted line) in fact makes no difference at all to these conclusions. For HMG T1/T2, it was impossible to confirm the results shown in Fig. 5 by comparing peak size or weights of protein recovered after the

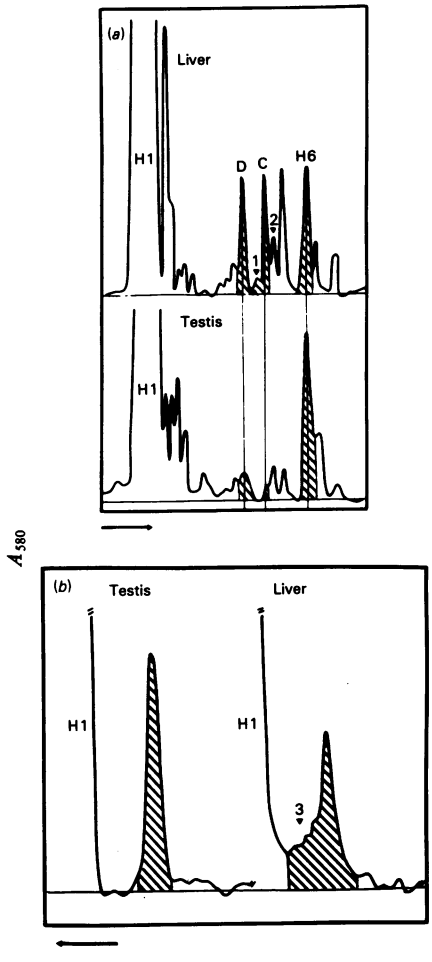

Fig. 4. *Scans of total proteins from a 5%-$HClO_4$ extract of trout liver nuclei and testis nuclei after SDS/-(a) and acid/urea (b)/-polyacrylamide-gel electrophoresis*
(*b*) has been truncated to show only histone H1, and HMG proteins T1 and T2. The direction of migration is indicated by an arrow. Gels were scanned at 580nm with a Gilford 250 spectrophotometer equipped with a linear-transport system. Each scan was recorded on a chart, and peak areas were cut out as indicated and weighed. The amount of protein present was assessed by reference to a standard curve made by electrophoresing at least four concentrations of purified protein standards on the same gel so that electrophoresis, staining and destaining of sample and marker proteins took place under identical conditions.

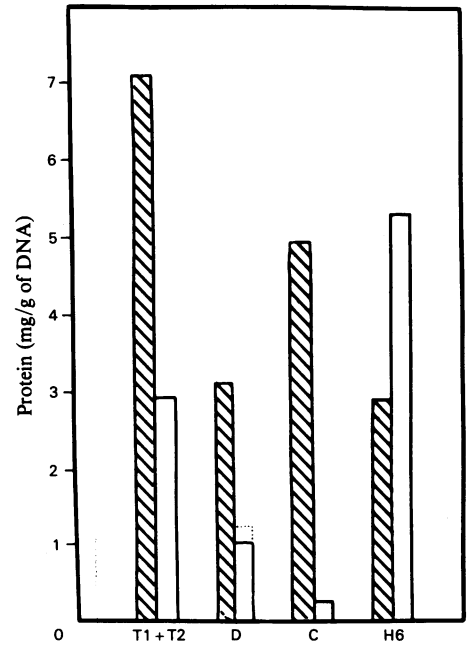

Fig. 5. *Histogram comparing the quantities of HMG proteins T1 and T2, C, D and H6 in 5%-$HClO_4$ extracts of trout liver nuclei and testis nuclei*
The amount of each protein quantified as shown in Fig. 4 was related to the quantity of DNA present in the nuclei from which the proteins were extracted. Liver proteins are indicated by hatched, and testis proteins by plain, areas.

chromatography of extracts of unfractionated tissue, since in liver, probably as a result of protein aggregation on the column, only traces of HMG T1 and T2 were ever seen (Fig. 2*b*, lanes a and f).

These comparisons were made between liver and testis collected between mid-October and the beginning of December, a period when the amounts of proteins equivalent in the winter flounder to HMG T and H6 in trout are maximal (Kennedy & Davies, 1980). Separate extracts of January trout testis and expressed sperm were made, and results are shown in Fig. 6. As in the winter flounder, known HMG proteins are almost completely absent in sperm, although three new proteins are present in January testis (Fig. 6, lane b) and probably also in sperm (lane d), which run slightly faster than, and are possibly variant forms of, C and D. In January testis, the level of H6 is no longer higher than in liver, as it has dropped from 5.2mg/g of DNA (Fig. 5) to 0.1mg/g of DNA. When proteins from a 5%-$HClO_4$ extract of ova (lane e) collected at the same time as the testis in lane f were electrophoresed on an SDS/polyacrylamide gel to distinguish between C and D, C was seen to be present in greater quantities than D (results not shown), so that the particularly low level of C in testis is characteristic of the developing male gamete only.

Discussion

Qualitative comparisons based on the isolation and characterization of proteins from 5%-$HClO_4$

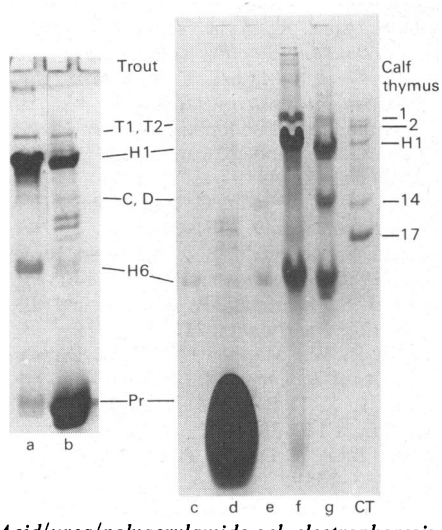

Fig. 6. *Acid/urea/polyacrylamide-gel electrophoresis of 5%-$HClO_4$ extracts of trout testis, liver, ova or sperm*
Proteins from a 5%-$HClO_4$ extract of testis (a, b and f), sperm (d), ova (e) and liver (g) are shown; c, standard marker of H6 (the extracts shown in lanes e, f and g are from tissues collected on the same occasion); CT, HMG proteins from a 5%-$HClO_4$ extract of calf thymus (minus excess H1); Pr, protamine.

extracts of unfractionated tissue showed (i) that in trout testis and liver, levels of HMG proteins C, D and H6 varied in relation to each other; (ii) that the C/D/H6 family of proteins is microheterogeneous; and (iii) that proteins belonging to this family are differently distributed in the two tissues, as neither the quantitatively minor variant of C nor the H6-resembling protein F were ever detected in testis. Quantitative studies confirmed the variation in levels of C, D and H6 in relation to each other, and furthermore showed that, in each tissue, HMG protein levels also varied in relation to DNA.

Calculations based on the data of Fig. 5 and the assumption that 200 base-pairs of DNA constitute a nucleosomal repeat length, indicate that, in trout liver, one molecule of either T1 or T2 is present for about every 30 nucleosomes, and one for every 75 in testis, a much lower estimate than 1 in 15 (Goodwin & Mathew, 1982) or 1 in 6 (Smith *et al.*, 1980) for calf thymus HMG 1 and 2. In the case of the lower-molecular-weight HMG proteins there is no such striking numerical species difference: in trout liver, there is about 1 molecule of C, D or H6 for every 13, 26 or 18 nucleosomes respectively, a total of 1 in 6, and in testis, there is about 1 molecule of D in every 60, and 1 of H6 in every 10 nucleosomes, a total of 1 in 9; in calf thymus, 1 molecule of HMG 14 or 17 has been estimated to be present for every 10 nucleosomes (Goodwin & Mathew, 1982). However, there is a striking qualitative difference between the two species, since in trout this family comprises three major members, C, D and H6, whereas no protein of similar molecular weight and amino acid analysis to H6 is present in comparable amount in calf thymus or liver (Brown, 1982). [In chickens, HMG-Y, a protein with a molecular weight similar to that of H6, also constitutes a third, though less abundant, member of the HMG 14/17 family (Goodwin *et al.*, 1981).] There are thus phylogenetic differences between the HMG proteins of different species, a conclusion also drawn by Christensen & Dixon (1981). However, unlike the latter authors, we additionally find that there are variations in the distribution of the HMG proteins in different tissues.

Seyedin & Kistler (1979) were the first to report that levels of HMG 2 parallelled the proliferative activity of a tissue. Mathew *et al.* (1979) found that the levels of HMG subfractions 2a and 2b differed in chicken thymus and erythrocytes, whereas Gordon *et al.* (1980) found that the relative amounts of HMG proteins 1, 2 and E differed in chicken liver, brain, thymus and erythrocytes. Our own findings lead to a similar conclusion for HMG-T1 and T2 in trout, where levels of T1/T2 are about 2-fold higher in liver than testis; they also extend the observed quantitative variations to the proteins of lower molecular weight, C, D and H6, the levels of which vary to a far greater extent. In relation to DNA, there is a 20-fold difference in the amount of C present in the two tissues.

Several authors have found examples of microheterogeneity in trout HMG proteins (B. Levy-Wilson, unpublished work cited in Levy-Wilson *et al.*, 1980; Brown *et al.*, 1980; Bhullar *et al.*, 1981) and similar microheterogeneity continues to be found in other species as well. Just as there are two variants of D, there are known to be two forms of chicken erythrocyte 14 (Isackson *et al.*, 1980) and probably of HMG-Y also (Goodwin *et al.*, 1981). The question immediately arises whether there are similar variants of calf thymus 14 and 17. Two-dimensional electrophoresis, using isoelectric focusing in the first dimension and SDS/polyacrylamide-gel electrophoresis in the second showed that there may be many subfractions of HMG 14 (Nicholas & Goodwin, 1982).

Since striking variations are found between the complement of HMG proteins in liver and testis, a tissue in which an extensive rearrangement of chromatin is taking place to achieve compaction within the sperm head, a correlation immediately suggests itself between changes in HMG proteins and chromatin structure. If on the other hand the observed changes in the levels of C, D and H6 are related to the transcriptional activity of the testis, it must be postulated that different members of the

family are associated with the activation of different sets of genes, a premise for which there exists no supportive evidence at the moment relating to the genomes of mammals and birds.

The rate of RNA synthesis in rainbow trout decreases during spermatogenesis, and is virtually absent in late spermatids and spermatozoa; RNA polymerase II decreases over 200-fold, and polymerase I over 400-fold, in activity (Gillam *et al.*, 1979). About 20000 polyadenylated mRNA sequences of average length 6×10^5 Da are present in trout liver and only about 6000 in October testis (Levy & Dixon, 1977). The difference observed between the presence of 1 molecule of C/D/H6 for every 6 nucleosomes in liver, and 1 in every 9 nucleosomes in October/November/early December testis is therefore unexpectedly small, but not so small that an involvement for these proteins in transcription is thereby completely excluded if it is supposed, for example, that 2 molecules of H6 are needed to replace 1 of C, or that the quantities of mRNA found in the cytoplasm are not proportional to the complexity of mRNA within the nucleus.

The finding that HMG proteins are differently distributed both qualitatively and quantitatively in trout liver and testis is thus not completely incompatible with a role for them in transcription, but is more easily accommodated within the broader framework of the suggestion by Gordon *et al.* (1981) that the variable distribution of the HMG proteins may be associated with the process of differentiation; no evidence exists at the moment, however, to indicate whether it might initiate, implement or merely result from such a process.

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