Biochem. J. (1985) **229**, 233–240
Printed in Great Britain

Heterogeneity of high-mobility-group protein 2

Enrichment of a rapidly migrating form in testis

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(*Received 28 January 1985; accepted 15 March 1985*)

A determination of the absolute amounts of high-mobility-group proteins 1 and 2 (HMG1 and HMG2) in rat tissues demonstrated that amounts of HMG2 were low in non-proliferating tissues, somewhat higher in proliferating and lymphoid tissues, but were extremely elevated in the testis. This increase was due to a germ-cell-specific form of HMG2 with increased mobility relative to somatic HMG2 on acid/urea/polyacrylamide-gel electrophoresis. To determine if the findings in the rat were a general feature of spermatogenesis, testis (germinal), spleen (lymphoid), and liver (non-proliferating) tissues from various vertebrate species were examined for their relative amounts of HMG1 and HMG2, and for HMG2 heterogeneity. Bull, chimpanzee, cynomologus monkey, dog, gopher, guinea pig, hamster, mouse, opossum, rabbit, rat, rhesus monkey, squirrel and toad (*Xenopus*) tissues were analysed. Nearly all species showed relatively high contents of HMG2 in testis tissue, whereas HMG1 contents were similar in all species and tissues. Ten of thirteen species showed a rapidly migrating HMG2 subtype in testis tissue, separable by acid/urea/polyacrylamide-gel electrophoresis. *Xenopus*, which lacks HMG2 in somatic tissues, showed an HMG2-like protein in testis tissue. Although the rapidly migrating HMG2 subtype in species other than rat was not testis-specific, it was always enriched in the testis. This study indicates that increased amounts of HMG2 and the enrichment of a rapidly migrating HMG2 subtype are general features of spermatogenic cells.

High-mobility-group proteins 1 and 2 (HMG1 and HMG2) are closely related and are believed to have important functions in chromatin (Goodwin & Mathew, 1982; McCarty *et al.*, 1982), although their exact role is not yet known. HMG1 and HMG2 appear to be bound to internucleosomal regions of chromatin (Peters *et al.*, 1979), although the binding is not very strong, and chromatin-bound HMG1 and HMG2 may be in equilibrium with a cytoplasmic pool (Wu *et al.*, 1981). The fate and modifications of HMG1 and HMG2 in cells undergoing various processes have been examined to reveal possible correlations with cellular events, and several observations have been made.

Abbreviations used: HMG1 and HMG2, high-mobility-group proteins 1 and 2.

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First, some investigators have reported that HMG1 and HMG2 are enriched in nuclease-sensitive regions of chromatin (Jackson *et al.*, 1979; Levy-Wilson *et al.*, 1979; Vidali *et al.*, 1977), whereas others find no preferential association (Goodwin & Johns, 1978; Hyde *et al.*, 1979). Variation in results may be due to the known loss and redistribution of HMG1 and HMG2 during preparation of nuclei before nuclease-digestion studies (Bucci *et al.*, 1984; Gordon *et al.*, 1981; Isackson *et al.*, 1980; Kuehl *et al.*, 1979).

Secondly, HMG1 and HMG2 are able to unwind coiled DNA (Bonne *et al.*, 1982; Isackson *et al.*, 1979; Javaherian *et al.*, 1979; Shepelev *et al.*, 1982), indicating a possible association with DNA replication, repair, or genetic recombination. Increased amounts of HMG2 relative to HMG1 in proliferating tissues, as opposed to non-proliferating tissues (Bucci *et al.*, 1984; Pipkin *et al.*, 1981; Seyedin & Kistler, 1979), suggest a role in

proliferation. However, cultured cell lines show no correlation between relative HMG2 contents and proliferation rates (Seyedin *et al.*, 1981*b*).

Thirdly, additional changes in HMG amounts have been associated with spermatogenesis. Increases in ratios of HMG1 and HMG2 to histone in late spermatids of the rooster testes led to a proposed function of HMG1 and HMG2 in nucleohistone disassembly and replacement by protamine (Chiva & Mezquita, 1983). In rat spermatogenesis, the absolute amounts of HMG2, but not HMG1, have been shown to increase dramatically in pachytene spermatocytes (Bucci *et al.*, 1984), a non-proliferating cell type undergoing genetic recombination and very active RNA synthesis (Bellve, 1979). Furthermore, this increase is due to a testis-specific form of HMG2 with increased mobility in acid/urea/polyacrylamide gels (Bucci *et al.*, 1984).

If variations in amounts and heterogeneity of HMG2 indeed have a functional role, the same observations seen in the rat should be found in other mammalian species. We have analysed HMG2 amounts and heterogeneity in testis and somatic tissues of other species to determine whether the observations for rat tissues and cells are applicable to other species, and constitute a general finding in spermatogenesis.

Experimental

Source of animals

Adult male Sprague–Dawley rats (*Rattus norvegicus*) were purchased from Harlan (Indianapolis, IN, U.S.A.), Charles River (Wilmington, MS, U.S.A.), or Timco (Houston, TX, U.S.A.). Chinese hamsters (*Cricetus cricetus*), guinea pigs (*Cavia porcellus*), rabbits (*Oryctolagus cuniculus*), dogs (*Canis familiaris*), rhesus monkeys (*Macaca mulatta*), cynomologus monkeys (*Macaca cynomologus*) and chimpanzees (*Pan troglodytes*) were provided by the Division of Veterinary Medicine and Surgery, The University of Texas System Cancer Center at Houston and Bastrop, TX, U.S.A. Bull tissues were obtained from a local abattoir. Mice (*Mus musculus*, C3H/Kam) were an inbred, specific-pathogen-free, strain maintained by the Department of Experimental Radiotherapy, The University of Texas System Cancer Center at Houston. Adult male *Xenopus laevis* toads were graciously given by Dr. David Wright, Department of Genetics, The University of Texas System Cancer Center at Houston. Adult male gophers (*Geomyidae* sp.), grey fox squirrels (*Sciurus niger*) and opossum (*Didelphis virginialis marsupialis*) were trapped in their natural habitat. Verification of active spermatogenesis was made by histological examination of testes, or in some instances by presence of sonication-resistant testicular sperm heads.

Protein extraction and gel electrophoresis

HMG proteins were extracted from fresh or frozen whole tissues by 0.2M-H_2SO_4, followed by precipitation of non-HMG proteins by 3% (w/v) trichloroacetic acid. The remaining soluble proteins were precipitated by 25% trichloroacetic acid, resulting in 3%-trichloroacetic acid-soluble, acid-extractable proteins (HMG extract) (Bucci *et al.*, 1984; Seyedin & Kistler, 1979). HMG extracts prepared in this manner have previously been shown, by using tracer amounts of radioactively labelled HMG2, to recover HMG proteins reproducibly and quantitatively from tissues (Bucci *et al.*, 1984). DNA was measured by the diphenylamine assay (Burton, 1956). Acid/urea/polyacrylamide slab gels (0.16cm × 17cm × 30cm) were run in 8M-urea by the procedure of Panyim & Chalkley (1969). Stacking gels consisted of 7.5% (w/v) acrylamide, 0.2% bisacrylamide, 2.5M-urea, 0.25% *NNN'N'*-tetramethylethylenediamine and 0.3% ammonium persulphate. Gels were pre-electrophoresed for at least 12h, and samples were electrophoresed at a constant current of 3mA/cm^2. HMG2 forms were separated by allowing HMG2 to migrate at least 18cm from the origin, corresponding to at least 48h of electrophoresis. Gels were stained with Amido Black as previously described (Bucci *et al.*, 1984).

Results

Tissue contents of HMG1 and HMG2 in the rat

Amounts of HMG proteins per unit of DNA in various rat tissues were analysed for two purposes. First, we wished to extend the range of tissues studied to determine whether the high amounts of HMG2 in the testis represented a unique situation. Second, we wanted to test whether HMG2/HMG1 and HMG2/histone-H1 ratios were valid indicators of the absolute content of HMG2 per unit of DNA. Amounts of HMG1 and HMG2 as well as HMG2/HMG1 and HMG2/H1 ratios determined from various tissues in the rat are shown in Table 1. Whereas HMG1 amounts in other tissues were not significantly different (by Student's *t* test) from the testis value of 13.5 μg of HMG1/mg of DNA, HMG2 contents varied widely. Three groups of HMG2 contents were observed: low, intermediate and high. All non-proliferating fully differentiated tissues had low contents of HMG2 (1–5 μg/mg of DNA). The HMG2/HMG1 ratios in all of these tissues never exceeded 0.50, and the HMG2/H1 ratio never exceeded 0.04. All proliferating tissues or those that possessed a high proportion of lymphoid tissue (capable of rapid proliferation

Table 1. *Absolute and relative amounts of HMG1 and HMG2 from rat tissues*

HMG1, HMG2 and DNA were quantified in whole rat tissues, and results are expressed as μg of protein/mg of DNA. Errors represent S.E.M. for *n* preparations, and values without errors are from one determination. For HMG2/H1 ratios, H1 includes all H1 variants and $H1^0$. XRT testes are testes sterilized by γ-irradiation (Trostle-Weige *et al.*, 1982), and analysed for relative ratios of HMG1 and HMG2 56 days after irradiation. At this time, testes contained only somatic cells, all non-proliferating. Abbreviation: ND, not determined.

Tissue	HMG1	HMG2	HMG2/HMG1	HMG2/H1	*n*
Non-proliferating					
Brain	13.4±6.2	0.8±0.6	0.20	0.02	2
Epididymis	18	5	0.48	0.04	1
Heart	11.3±0.7	3	0.22	0.02	2
Kidney	16.6±8.4	2.6	0.36	0.02	2
Liver	15.7±1.6	3.2±0.7	0.18	0.01	7
Lung	13.7±4.7	4.9±1.5	0.42	0.03	3
Pancreas	11.4±4.3	2.6±1.4	0.22	0.02	3
Seminal vesicle	9	3	0.38	0.02	1
XRT testes	ND	ND	0.07	0.01	1
Lymphoid and proliferating					
Bone marrow	6.8±0.1	13.4±0.4	2.52	0.06	2
Caecum	7.7±0.5	5.2±0.5	0.68	0.04	2
Leucocytes	7.1	10.2	1.43	0.06	1
Small intestine	10.8±2.6	8.5±1.8	0.78	0.04	2
Spleen	11.4±0.7	8.1±0.8	0.85	0.04	6
Thymus	13.6±3.6	8.0±1.8	0.69	0.04	5
Germinal					
Testis	13.5±1.1	31.8±2.0	2.33	0.21	16

given the proper stimulus) had intermediate contents of HMG2 (5–14 μg/mg of DNA). HMG2/HMG1 ratios in all of these tissues were 0.6–2.5; the HMG2/H1 ratios were all between 0.04 and 0.06. The testis was the only rat tissue with a high content of HMG2 (32 μg/mg of DNA). Testis HMG2/HMG1 (2.3) and HMG2/H1 (0.21) ratios were both significantly elevated relative to all but one of the somatic tissues studied. The bone marrow contains a slightly higher HMG2/HMG1 ratio, but the HMG2/H1 ratio is much lower than in the testis. Thus testis is unique among rat tissues in that it contains a large amount of HMG2. Also, HMG2/HMG1 and HMG2/H1 ratios do indeed reflect the absolute contents of HMG2 per unit of DNA.

Tissue contents of HMG1 and HMG2 in other species

The large amount of HMG2 in rat testis tissue is associated with a testis-specific HMG2 subtype with increased mobility relative to somatic HMG2 on acid/urea/polyacrylamide gels (Bucci *et al.*, 1984). On the basis of those results, tissues from other species representing non-proliferating (liver), lymphoid (spleen) and germinal (testis) tissue were examined for HMG1 and HMG2 protein contents, and for presence of HMG2 subtypes by acid/urea/polyacrylamide-gel electrophoresis. Testis tissue from all species contained more HMG2 relative to HMG1 than did somatic tissues, with the exception of the bull and rabbit (Table 2). In addition, HMG2/H1 ratios were higher in the testes of all species than in the spleen and, with the exception of the guinea pig and rabbit, higher in the testes than in the liver. HMG1/H1 ratios (results not shown) for each tissue from all the different species were averaged. HMG1/H1 ratios from testis (0.074), spleen (0.050) and liver (0.070) indicated that HMG1 contents were similar for the tissues tested. HMG2/H1 ratios from different species were similarly averaged for each tissue and showed higher values in testis (0.096) than in spleen (0.024) or liver (0.023). These results indicate that the increase in HMG2 in testis tissue is a general finding associated with spermatogenesis in all species.

HMG2 heterogeneity

On acid/urea/polyacrylamide gels, rat testis HMG2 could be resolved into at least two bands (forms). The fast forms were unique to the testis (Fig. 1), and were present only in germ cells (Bucci *et al.*, 1984). Nine of thirteen other species examined showed two HMG2 forms resolved by acid/urea/polyacrylamide-gel electrophoresis (Fig. 2). Exceptions, which showed only one band of HMG2, were the opossum, the cynomologus monkey (results not shown) and the rhesus monkey

Table 2. *Relative amounts of HMG2 in testis, spleen and liver of various species*

HMG and H1 contents and HMG2 heterogeneity in various species were analysed from acid/urea/polyacrylamide gels. Errors represent the S.E.M. from the numbers of separate determinations for each tissue (indicated by the number in parentheses). Abbreviation: ND, not determined.

Species	No. of animals used	HMG2/HMG1			HMG2/H1			HMG2 subtypes
		Testis	Spleen	Liver	Testis	Spleen	Liver	
Bull	1	0.59 ± 0.02 (4)	0.73 ± 0.01 (2)	ND	0.04 ± 0.001 (4)	0.03 ± 0.001 (2)	ND	+
Chimpanzee	1	2.12 ± 0.16 (2)	0.45 ± 0.05 (2)	0.18 (1)	0.08 ± 0.001 (2)	0.01 ± 0.001 (2)	0.01 ± 0.000 (2)	+
Cynomologus monkey	1	1.09 ± 0.03 (2)	0.51 (1)	0.14 (1)	0.07 ± 0.000 (2)	0.03 (1)	0.01 (1)	−
Dog	1	0.82 ± 0.05 (6)	0.51 ± 0.05 (6)	0.78 ± 0.11 (5)	0.14 ± 0.007 (5)	0.04 ± 0.004 (3)	0.06 ± 0.008 (2)	+
Gopher	2	1.47 ± 0.04 (3)	0.32 (1)	0.81 (1)	0.08 ± 0.001 (3)	0.01 (1)	0.04 (1)	+
Guinea pig	1	3.02 ± 0.26 (2)	0.62 ± 0.17 (2)	1.62 ± 0.01 (2)	0.11 ± 0.009 (2)	0.02 ± 0.002 (2)	0.13 ± 0.001 (2)	+
Hamster	1	2.51 ± 0.15 (3)	ND	0.08 (1)	0.15 ± 0.011 (2)	ND	0.004 (1)	+
Mouse	4	1.89 ± 0.04 (6)	0.51 ± 0.04 (3)	0.14 ± 0.04 (2)	0.11 ± 0.002 (4)	0.05 ± 0.012 (2)	0.02 ± 0.002 (2)	+
Opossum	1	1.15 (1)	0.54 ± 0.01 (2)	1.07 ± 0.18 (3)	0.18 (1)	0.04 ± 0.001 (2)	0.04 ± 0.006 (3)	−
Rabbit	1	1.17 ± 0.04 (3)	0.50 ± 0.02 (3)	1.21 ± 0.09 (3)	0.06 ± 0.002 (2)	0.02 ± 0.002 (2)	0.07 ± 0.005 (2)	+
Rat	16	2.34 ± 0.05 (55)	0.85 ± 0.03 (17)	0.18 ± 0.04 (9)	0.17 ± 0.007 (52)	0.04 ± 0.003 (10)	0.01 ± 0.001 (8)	+
Rhesus monkey	3	1.16 ± 0.03 (8)	0.42 ± 0.05 (4)	0.74 ± 0.15 (5)	0.10 ± 0.003 (7)	0.03 ± 0.004 (4)	0.04 ± 0.015 (5)	−
Squirrel	1	0.78 ± 0.03 (3)	0.51 ± 0.04 (3)	0.22 ± 0.02 (3)	0.09 ± 0.003 (3)	0.02 ± 0.002 (3)	0.01 ± 0.000 (2)	+
Xenopus toad*	1	0.56 (1)	0	0	0.19 (1)	0 (2)	0 (2)	(−)

* *Xenopus* somatic tissues did not contain HMG2, whereas testis tissue did, but HMG2 forms were not seen.

Fig. 1. *Detection of rat HMG2 forms from rat tissue HMG extracts by acid/urea/polyacrylamide-gel electrophoresis*
Lanes are marked to indicate tissues from which proteins were extracted: RL, rat liver; RS, rat spleen; RT, rat testis. The slower HMG2 form (HMG2S) is denoted by ●, and the faster HMG2 form (HMG2F) by ◀. Note the lack of HMG2F in spleen and liver samples. The protein that migrates slightly faster than HMG1 in lane 1 is not an HMG, but a liver-specific cytoplasmic protein. H1at consists of two H1 variants, H1a and H1t, which co-migrate in this gel system, and H1bcde consists of the H1 variants H1b, H1c, H1d and H1e. Although the H1 proteins were indeed overloaded in some of the slots in this and subsequent Figures, the resolution of the H1 bands is lost, owing to the photographic techniques necessary to reproduce the rather faint HMG2 bands. Direction of migration is from top (+) to bottom (−).

(Fig. 2*f*). The amphibian *Xenopus* was unique in that somatic tissues did not contain HMG2, but the testis showed an HMG2-like protein, as characterized by its acid-solubility and electrophoretic mobility (Fig. 3). Although, in general, fast HMG2 forms were not testis-specific, in nine of ten species the fast band was highest in amount in the testis, usually comprising the predominant fraction of total HMG2 in testis tissue (Fig. 2, Table 3). When testis and spleen samples from rat were mixed and electrophoresed, HMG2 migrated clearly as two bands (results not shown). Identical results were obtained from mouse testis and spleen, indicating that the slower subtype of HMG2 is common to all tissues, and the faster form is either unique to or enriched in the testis. HMG1 from all tissues of all species tested co-migrated, and no subtypes were apparent on acid/urea/polyacrylamide gels. Conversely, electrophoretic mobilities of HMG2 differed among species, indicating that HMG2 had a greater heterogeneity than HMG1.

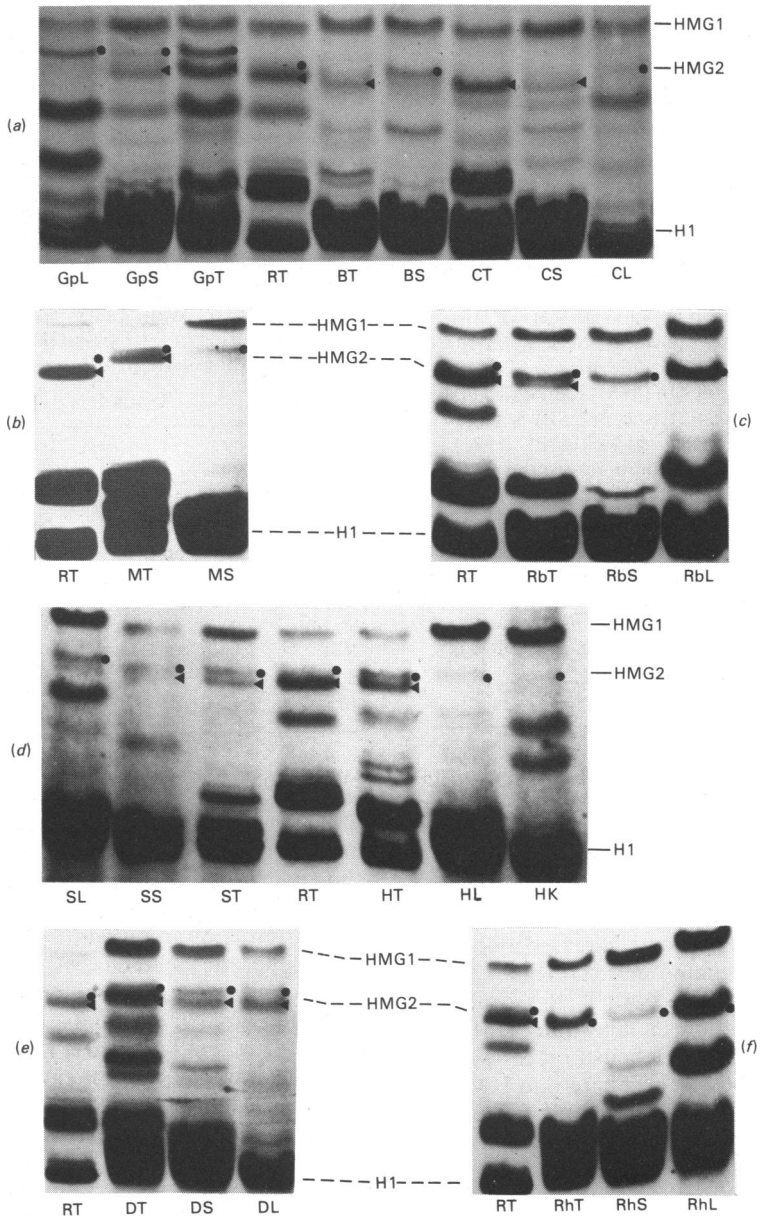

Fig. 2. *Detection of HMG2 forms by acid/urea/polyacrylamide-gel electrophoresis of HMG extracts from testis, spleen and liver tissues of various species*

Slower HMG2 forms are denoted by ●, and faster HMG2 forms by ◀. Note the presence of increased proportions of faster HMG2 forms in testis tissues, as compared with somatic tissues, for each species. Lanes are marked to indicate tissues from which proteins were extracted (*a*) GpL, guinea-pig liver; GpS, guinea-pig spleen; GpT, guinea-pig testis; RT, rat testis; BT, bull testis; BS, bull spleen; CT, chimpanzee testis; CS, chimpanzee spleen; CL, chimpanzee liver. (*b*) Mouse tissue HMG extracts. Mouse liver possessed such a low quantity of HMG2 that photographic reproduction was not presented. RT, rat testis; MT, mouse testis; MS, mouse spleen. (*c*) Rabbit tissue HMG extracts. RT, rat testis; RbT, rabbit testis; RbS, rabbit spleen; RbL, rabbit liver. (*d*) Squirrel and Chinese-hamster tissue HMG extracts. Slower migration of squirrel liver HMG species is due to an overload of H1. SL, squirrel liver; SS, squirrel spleen; ST, squirrel testis; RT, rat testis; HT, hamster testis; HL, hamster liver; HK, hamster kidney. (*e*) Dog tissue HMG extracts. RT, rat testis; DT, dog testis; DS, dog spleen; DL, dog liver. (*f*) Rhesus-monkey tissue HMG extracts. RT, rat testis; RhT, rhesus testis; RhS, rhesus spleen; RhL, rhesus liver. On this and several other gels (not shown), rhesus tissues did not exhibit HMG2 forms. Direction of migration is from top (+) to bottom (−) for each gel.

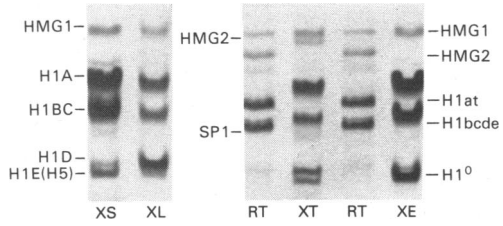

Fig. 3. *Detection of HMG extracts from Xenopus laevis tissues by acid/urea/polyacrylamide-gel electrophoresis*
Labels identifying known *Xenopus* H1 variants (Risley & Eckhardt, 1981) and HMG1 (Kleinschmidt *et al.*, 1983) are shown at the left. Labels identifying *Xenopus* testis-specific proteins are in the centre; HMG2 denotes the HMG2-like protein found only in *Xenopus* testis tissue, and SP1 is a late-spermatidal protein (Risley & Eckhardt, 1981). Labels identifying rat testis proteins are at the right. Lanes are marked to indicate tissues from which proteins were extracted. XS, *Xenopus* spleen; XL, *Xenopus* liver; RT, rat testis, showing that rat and *Xenopus* HMG1 co-migrate; XT, *Xenopus* testis, showing the presence of an HMG2-like protein migrating slightly faster than HMG1; RT, rat testis; XE, *Xenopus* erythrocyte. Direction of migration is from top (+) to bottom (−).

Table 3. *Percentage of fast band of HMG2 in total HMG2 in tissues of various species*
Values represent the proportion of total HMG2 that is contained in the fast form [(HMG2F/HMG2) × 100%]. Errors are S.E.M., with numbers of determinations in parentheses. Contents of HMG2 in mouse liver were too low for determination of percentage of HMG2F. In all animals, except the guinea pig, the proportion of HMG2F is highest in the testis, suggesting that an enrichment of the fast form is a general feature of spermatogenesis. Rat was the only species that showed HMG2F to be testis-specific. Also, fewer species contained fast HMG2 forms in liver tissue than in spleen or testis tissues. Abbreviation: ND, not determined.

Species	Testis	Spleen	Liver
Bull	73±7 (2)	29 (1)	ND
Chimpanzee	96 (1)	90 (1)	0 (1)
Dog	86±1 (5)	54±2 (5)	53±11 (4)
Gopher	100 (1)	21 (1)	72 (1)
Guinea pig	66 (1)	87 (1)	0 (1)
Hamster	65±1 (3)	ND	0 (2)
Mouse	61±1 (2)	13 (1)	ND
Rabbit	40±1 (2)	13 (1)	0 (1)
Rat	83±1 (7)	0 (5)	0 (3)
Squirrel	55±1 (3)	42±1 (3)	40±5 (2)

Preliminary data from non-equilibrium isoelectric focusing of rat testis and spleen HMG2 shows that at least four forms are present, thus indicating charge heterogeneity, the nature of which is still unknown.

Since reports exist that show phosphorylation of some HMG proteins can lead to increased mobility on acid/urea/polyacrylamide gels (D'Anna *et al.*, 1983), rat testes were injected with [^{32}P]phosphate to assay for incorporation of label into HMG2. HMG protein extracts from labelled rat testes did not show incorporation of phosphate into HMG1 or HMG2, whereas H1 did show incorporation (results not shown). Thus postsynthetic phosphorylation is not likely to be responsible for the increased mobility of rapidly migrating HMG2.

H1 variants

Although not the main subject of this study, we have observed changes in H1 variant patterns in the testis of most species studied. Rat testis contains high amounts of both H1a, a variant found only in low amounts in somatic tissues, and H1t, a testis-specific variant (Seyedin *et al.*, 1981*a*; Bucci *et al.*, 1982). These two proteins co-migrate on acid/urea/polyacrylamide gels (Fig. 1). The testis of all species studied, except *Xenopus*, showed higher amounts of H1 bands migrating in the region of rat H1a and H1t than did spleen and liver tissues (Fig. 2).

Discussion

The increase in HMG2 in testis tissue found here for a variety of species is supported by results shown in other studies. Lanneau & Loir (1982) investigated mammalian spermatid-specific basic nuclear proteins and used the same extraction and electrophoretic methods as the present study. Ram, bull and breeding hedgehog testes all exhibited greater amounts of HMG2 than of HMG1 (Fig. 2 in Lanneau & Loir, 1982). However, non-breeding hedgehogs exhibited less HMG2 than HMG1, consistent with the findings in rats that the increase in testis HMG2 is due to germinal cells (Bucci *et al.*, 1984). In addition, Seyedin *et al.* (1981*a*) showed a greater amount of HMG2 than of HMG1, analysed on both acid/urea/- and sodium dodecyl sulphate/-polyacrylamide gels, from rat, hamster, rabbit and mouse testes.

Additional evidence that the fast bands of HMG2 seen in the present study are truly HMG2 comes from (1) the co-migration of testis and somatic HMG2 on sodium dodecyl sulphate/polyacrylamide gels, (2) the finding that amounts of HMG2 from rat testis cells determined by sodium dodecyl sulphate/- and acid/urea/-polyacrylamide-gel electrophoresis were equivalent, and (3) that the amino acid composition of rat testis HMG2 (83% fast form) was almost identical with that of calf thymus HMG2 (Bucci *et al.*, 1984; Seyedin & Kistler, 1979).

The only non-mammal included in this study, *Xenopus laevis*, represents the most extreme case of association of increased HMG2 with spermatogenesis. *Xenopus* somatic tissues, as well as *Xenopus* oocytes (Kleinschmidt *et al.*, 1983), contained only HMG1 (see Fig. 3), but testis tissue contained an HMG2-like protein, which probably represents a testis-specific HMG, consistent with the increase in HMG2 content and the enrichment of a fast-migrating HMG2 form seen in mammalian testes. *Xenopus* testis HMG2-like protein is not a contaminating spermatidal basic nuclear protein, since it was not seen in late-spermatid nuclei (Risley & Eckhardt, 1981).

Primary sequence variants of the histones (Hohmann, 1978; von Holt *et al.*, 1979; Sperling & Wachtel, 1981) have been characterized in greater detail than have those of the HMG proteins. Chicken HMG2, but not HMG1, has sequence variants (Sterner *et al.*, 1978; Walker, 1982). The trout HMG1- and HMG2-like protein HMGT, which migrates as two bands on acid/urea/polyacrylamide gels, also has sequence variants (Brown *et al.*, 1980). Data on sequences of mammalian HMG1 and HMG2 are limited to calf thymus, and thus no conclusion about the existence of mammalian HMG2 variants can be made. HMG1 and HMG2 from mammals and chickens can be separated into discrete forms by isoelectric focusing (Mathew *et al.*, 1979; Nicolas & Goodwin, 1982; Tyrell *et al.*, 1982) and h.p.l.c. (Mazrimas *et al.*, 1984), but the nature of these subtypes is still unknown. Tryptic-peptide maps of rat testis HMG2 were indistinguishable from those of rat spleen HMG2 (Bucci *et al.*, 1984), indicating that the fast HMG2 form might not be a sequence variant. Similarly, the two forms of calf thymus HMG2 separated by h.p.l.c. had very similar amino acid compositions, indicating that the two forms may not be sequence variants, but may be modified species (Mazrimas *et al.*, 1984).

The possibility of a change in the mobility of rat testis HMG2 as a result of postsynthetic phosphorylation was found to be unlikely, owing to lack of incorporation of [^{32}P]phosphate (Bucci, 1983). Oxidation or degradation of HMG1 or HMG2 was also considered. Rat spleen HMG2 (containing only the slow form) was oxidized, and produced bands that migrated faster than the testis form of HMG2 (Bucci, 1983). Rat testis nuclei extracted with 0.35M-NaCl and stored for 24h also showed bands on acid/urea/polyacrylamide gels that migrated faster than the testis form of HMG2 (L. R. Bucci, unpublished work). These bands corresponded to the HMG3 band and other known degradation products of HMG proteins, which are not seen when tissues or nuclei are extracted directly with acid (Goodwin *et al.*, 1978; Isackson & Reeck, 1982). Studies of radioactive lysine incorporation into isolated rat testis cells showed that spermatogonia synthesized only the slower form (L. R. Bucci, unpublished work). This supports the hypothesis that the fast HMG2 form is not a parental form, but may be caused by postsynthetic processing. Several known postsynthetic modifications (acetylation, glycosylation or ADP-ribosylation) would decrease the positive charge of HMG2, resulting in decreased mobility on acid/urea/polyacrylamide gels. The fast band would have to represent the unmodified parental form, which is unlikely. It is also possible that the fast form of HMG2 may be due to some form of postsynthetic modification not yet considered, such as controlled proteolytic cleavage (Allis *et al.*, 1980), although the *N*-terminal amino acid of rat testis HMG2 is glycine, the same as that of calf thymus HMG2 (Bucci *et al.*, 1984). Regardless of the cause of HMG2 heterogeneity, the increase in testes of many species of a tissue-specific or -enriched subtype of HMG2 with increased mobility on acid/urea/polyacrylamide gels relative to somatic-tissue HMG2 suggests a unique role for HMG2 in the testis.

The observations in this paper and several others (Bucci *et al.*, 1984; Seyedin & Kistler, 1979) imply that HMG1 and HMG2 could have different functions. HMG1 amounts remain remarkably constant among tissues and cells of various states of differentiation, and the charge homogeneity of HMG1 between species is likewise remarkably conserved. On the other hand, HMG2 exhibits both quantitative and qualitative changes during cell proliferation, and its amounts increase greatly in male germ cells. HMG2 exhibits testis-specific or -enriched forms, whereas HMG1 does not, and HMG2 shows interspecific charge heterogeneity, unlike HMG1.

The appearance of fast HMG2 forms in testis might represent another manifestation of the major changes of gene expression that occur during the primary spermatocyte stage. Known examples of these changes during spermatogenesis are seen in surface antigens (Bellve, 1979), histone variants (Bucci *et al.*, 1982; Meistrich *et al.*, 1981; Seyedin *et al.*, 1981*a*; Trostle-Weige *et al.*, 1982) and isoenzyme expression (Bellve, 1979).

The presence of increased amounts of HMG2 forms in testis also suggests a definite role for HMG2 in testis-specific nuclear functions, such as switching patterns of gene expression, genetic recombination, meiotic reductive divisions, or replacement of nucleosomes by late-spermatidal basic nuclear proteins during nuclear condensation of spermatids. Although a significant amount of HMG2 is lost during preparation of nuclei (Bucci *et al.*, 1984), the losses of the fast and slow forms

are equivalent, indicating at least a partial nuclear location of these proteins. Further research to elucidate roles for HMG2 should not ignore germ cells as a unique, useful, system for study.

We thank Patricia Trostle-Weige for technical help, Dr. Lewis V. Rodriguez for advice and the use of isoelectric-focusing apparatus, and Ann McCarver and Sheri Chase for typing this manuscript. This investigation was supported in part by Grants HD-16843, CA-17364 and CA-06294 from the National Institute of Child Health and Human Development, National Cancer Institute, U.S. Department of Health and Human Services. This manuscript is partial fulfilment for the Ph.D. requirements of L. R. B., a recipient of the Rosalie B. Hite Memorial Fellowship.

References

Allis, C. D., Bowen, J. K., Abraham, G. N., Glover, C. V. C. & Gorovsky, M. A. (1980) *Cell* **20**, 55–64

Bellve, A. R. (1979) *Oxford Reviews of Reproductive Biology* (Finn, C. A., ed.), vol. 1, pp. 159–261, Clarendon Press, Oxford

Bonne, C., Sautiere, P., Duguet, M. & de Recondo, A. M. (1982) *J. Biol. Chem.* **257**, 2722–2725

Brown, E., Goodwin, G. H., Mayes, E. L. V., Hastings, J. R. B. & Johns, E. W. (1980) *Biochem. J.* **191**, 661–664

Bucci, L. R. (1983) Ph.D. Dissertation, University of Texas Graduate School of Biomedical Sciences at Houston

Bucci, L. R., Brock, W. A. & Meistrich, M. L. (1982) *Exp. Cell Res.* **140**, 111–118

Bucci, L. R., Brock, W. A., Goldknopf, I. L. & Meistrich, M. L. (1984) *J. Biol. Chem.* **259**, 8840–8846

Burton, K. (1956) *Biochem. J.* **62**, 315–323

Chiva, M. & Mezquita, C. (1983) *FEBS Lett.* **162**, 324–328

D'Anna, J. A., Becker, R. R., Tobey, R. A. & Gurley, L. R. (1983) *Biochim. Biophys. Acta* **739**, 197–206

Goodwin, G. H. & Johns, E. W. (1978) *Biochim. Biophys. Acta* **519**, 279–284

Goodwin, G. H. & Mathew, C. G. P. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., ed.), pp. 193–221, Academic Press, New York

Goodwin, G. H., Walker, J. M. & Johns, E. W. (1978) *Biochim. Biophys. Acta* **519**, 233–242

Gordon, J. S., Bruno, J. & Lucas, J. L. (1981) *J. Cell Biol.* **88**, 373–379

Hohmann, P. (1978) *Subcell. Biochem.* **5**, 87–127

Hyde, J. E., Igo-Kemenes, T. & Zachau, H. G. (1979) *Nucleic Acids Res.* **7**, 31–48

Isackson, P. J. & Reeck, G. R. (1982) *Biochim. Biophys. Acta* **697**, 378–380

Isackson, P. J., Fishback, J. L., Bidney, D. L. & Reeck, G. R. (1979). *J. Biol. Chem.* **254**, 5569–5572

Isackson, P. J., Bidney, D. L., Reeck, G. R., Neihart, N. K. & Bustin, M. (1980) *Biochemistry* **19**, 4466–4471

Jackson, J. B., Pollock, J. M., Jr. & Rill, R. L. (1979) *Biochemistry* **18**, 3739–3748

Javaherian, K., Sadeghi, M. & Liu, L. F. (1979) *Nucleic Acids Res.* **6**, 3569–3580

Kleinschmidt, J. A., Scheer, U., Dabauville, M., Bustin, M. & Franke, W. W. (1983) *J. Cell Biol.* **97**, 838–848

Kuehl, L., Lyness, T., Watson, D. C. & Dixon, G. H. (1979) *Biochem. Biophys. Res. Commun.* **90**, 391–397

Lanneau, M. & Loir, M. (1982) *J. Reprod. Fertil.* **65**, 163–170

Levy-Wilson, B., Connor, W. & Dixon, G. H. (1979) *J. Biol. Chem.* **254**, 609–620

Mathew, C. G., Goodwin, G. H., Gooderham, K., Walker, J. M. & Johns, E. W. (1979) *Biochem. Biophys. Res. Commun.* **87**, 1243–1251

Mazrimas, J. A., Laskaris, M., Corzett, M. & Balhorn, R. (1984) *J. Liq. Chromatogr.* **7**, 907–916

McCarty, K. S., Sr., Kellner, D. N., Wilke, K. & McCarty, K. S., Jr. (1982) in *Genetic Expression in the Cell Cycle* (Padilla, G. M. & McCarty, K. S., Sr., eds.), pp. 55–102, Academic Press, New York

Meistrich, M. L., Trostle, P. K. & Brock, W. A. (1981) in *Bioregulators of Reproduction* (Jagiello, G. & Vogel, H. J., eds.), pp. 151–166, Academic Press, New York

Nicolas, R. H. & Goodwin, G. H. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., ed.), pp. 41–68, Academic Press, New York

Panyim, S. & Chalkley, R. (1969) *Arch. Biochem. Biophys.* **130**, 337–346

Peters, E. H., Levy-Wilson, B. & Dixon, G. H. (1979) *J. Biol. Chem.* **254**, 3358–3361

Pipkin, J. L., Hinson, W. G., Hudson, J. L., Anson, J. & Pack, L. D. (1981) *Biochim. Biophys. Acta* **655**, 421–431

Risley, M. S. & Eckhardt, R. A. (1981) *Dev. Biol.* **84**, 79–87

Seyedin, S. M. & Kistler, W. S. (1979) *J. Biol. Chem.* **254**, 11264–11271

Seyedin, S. M., Cole, R. D. & Kistler, W. S. (1981*a*) *Exp. Cell Res.* **136**, 399–405

Seyedin, S. M., Pehrson, J. R. & Cole, R. D. (1981*b*) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 5988–5992

Shepelev, V. A., Kosaganov, Y. N., Lazurkin, Y. S., Lindigkeit, R. & Grade, K. (1982) *Stud. Biophys.* **87**, 163–164

Sperling, R. & Wachtel, E. J. (1981) *Adv. Protein Chem.* **34**, 1–60

Sterner, R., Boffa, L. C. & Vidali, G. (1978) *J. Biol. Chem.* **253**, 3830–3836

Trostle-Weige, P. K., Meistrich, M. L., Brock, W. A., Nishioka, K. & Bremer, J. W. (1982) *J. Biol. Chem.* **257**, 5560–5567

Tyrell, D., Isackson, P. J. & Reeck, G. R. (1982) *Anal. Biochem.* **119**, 433–439

Vidali, G., Boffa, L. C. & Allfrey, V. G. (1977) *Cell* **12**, 409–415

von Holt, C., Strickland, W. N., Brandt, W. F. & Strickland, M. W. (1979) *FEBS Lett.* **100**, 201–218

Walker, J. M. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., ed.), pp. 69–87, Academic Press, New York

Wu, L., Rechsteiner, M. & Kuehl, L. (1981) *J. Cell Biol.* **91**, 488–496