HMG (high-mobility-group)-14/17-like proteins in calf thyroid

Thyrotropin-dependent phosphorylation and comparison with calf thymus proteins

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Two-dimensional polyacrylamide-gel electrophoresis of acid extracts of thyroid and thymus tissue, and of thyroid nuclei, revealed the presence of three HClO_4 -soluble nuclear proteins, PS.1, PS.2 and PS.3, whose electrophoretic mobilities closely resembled those of HMG (high-mobility-group) proteins 14 and 17. PS.1 co-migrated with HMG 14 on CM-Sephadex column chromatography. Like HMG 14, PS.2 and PS.3 were phosphorylated in calf thyroid slices; ^{32}P -labelling of PS.3 was stimulated by thyrotropin. Thyrotropin also induced a rapid increase in the labelling of A5, an HMG-14/17-like protein found in whole calf thyroid and thymus tissue, but not in thyroid nuclei.

The high-mobility-group (HMG) proteins HMG 14 and HMG 17 are closely related non-histone chromatin proteins that are preferentially associated with actively transcribed chromatin (Weisbrod, 1982). They undergo a variety of post-translational modifications (Allfrey, 1982) that may serve as a means by which their functions are modulated.

Electrophoretic analysis of tissue, cell and nuclear extracts containing HMG proteins often reveals the presence of minor proteins that are clearly resolved from the major forms of HMG 14 and 17. Some of these could be modified or variant forms of HMG 14 and 17 (Levy-Wilson, 1981; Lund *et al.*, 1981; Nicolas & Goodwin, 1982; Bhorjee *et al.*, 1983; D'Anna *et al.*, 1983; Walton & Gill, 1983); others may be similar proteins or proteolytic-degradation products (Sterner *et al.*, 1979; Levinger *et al.*, 1981; Saffer & Glazer, 1982; Barsoum *et al.*, 1982; D'Anna *et al.*, 1983; Walton & Gill, 1983). In view of the importance of HMG 14 and 17, and the possible significance both of closely related proteins and HMG modifications in the regulation of active chromatin function, more detailed investigations are clearly warranted.

In the present paper we describe the presence of several HMG-14/17-like proteins in calf thyroid and

thymus, and give a brief account of their ^{32}P -labelling in calf thyroid slices in the presence and absence of thyrotropin.

Materials and methods

Extraction of salt/acid-soluble proteins from whole calf thyroid tissue and thyroid nuclei

Nuclei were prepared from 25 g of minced fresh calf thyroid tissue (Cooper *et al.*, 1982); salt/acid-soluble proteins were then extracted with 0.5 M-NaCl/0.4 M-HCl (Cooper *et al.*, 1981).

Salt/acid-soluble proteins were obtained directly from calf thyroid tissue (10 g) frozen in liquid N_2 immediately after slaughter of the calves, or tissue (5 g) carried on ice from the slaughterhouse and minced within 30 min of slaughter. Tissues were extracted twice with 0.625 M-NaCl/0.5 M-HCl (1:4, w/v). Homogenates were centrifuged for 20 min at 2500 g or 10 min at 9000 g; supernatants from the first and second extractions were pooled. During extractions, tissues and extracts were kept at or below 4°C at all times.

Extraction of HClO_4 -soluble proteins from whole calf thyroid tissue

Calf thyroids were frozen in liquid N_2 immediately after slaughter of the calves. HClO_4 -soluble proteins were obtained from 5 g of frozen tissue by extracting twice directly into 7.5 ml of 0.75 M- HClO_4 . Homogenates were centrifuged for

Abbreviations used: HMG protein, high-mobility-group protein; Hepes, 4-(2-hydroxyethyl)-1-piperazine-ethanesulphonic acid.

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10 min at 9000g; proteins were then precipitated from pooled supernatants (13.5 ml) by addition of 0.45 ml of conc. HCl and 81 ml of acetone, followed by centrifugation for 20 min at 9000g. Precipitates were washed once with acidified acetone (acetone/conc. HCl, 120:1, v/v) and twice with acetone, and dried in air. During extractions, tissues and extracts were kept at or below 4°C at all times.

Preparation of HMG proteins from calf thymus tissue

HMG proteins were isolated from calf thymus (1300g) by a modification of the methods of Goodwin *et al.* (1977) and Sanders (1977). Batches of minced calf thymus (78–355g) were extracted twice with 0.45 M-HClO₄ (2:3, w/v). Homogenates were centrifuged for 20 min at 2500g and the supernatants retained. Combined supernatants were filtered through cheesecloth; 3 vol. of acetone were added to the filtrates, which were then made 0.1 M in HCl by addition of conc. HCl. Precipitates were removed by centrifugation for 20 min at 8000g. HMG proteins were then precipitated by addition of a further 3 vol. of acetone, followed by centrifugation for 20 min at 8000g. Precipitates were washed once with acidified acetone (acetone/conc. HCl, 120:1, v/v) and twice with acetone, and dried in air at 4°C.

To obtain an HMG fraction enriched in HMG 14 and 17, total HMG proteins (1.1 g) were redissolved in 0.1 M-HCl at a concentration of 50 mg/ml, and 12.5 vol. of ethanol/conc. HCl (99:1, v/v) were added (Goodwin *et al.*, 1977). The 'HMG-14/17-enriched' precipitate was collected by centrifugation for 1 h at 4000g, washed with acetone and dried. The protein obtained was prepared for ion-exchange column chromatography on CM-Sephadex C25 and chromatographed as described by Goodwin *et al.* (1977). Proteins in the major peaks were, in most cases, precipitated from pooled fractions by acidifying to 0.1 M-HCl and adding 6 vol. of acetone (Goodwin *et al.*, 1977; Sanders, 1977); precipitates were collected by centrifuging for 20 min at 8000g. Alternatively, pooled fractions were dialysed against distilled water and freeze-dried.

³²P-labelling of thyroid tissue slices

Fresh calf thyroid slices (0.4–0.6g) were incubated at 37°C for 30 min in 5 ml of modified Krebs–Ringer bicarbonate buffer containing 40 μM-sodium phosphate/10 mM-sodium bicarbonate/2 mg of D-glucose/ml and 20 mM-Hepes, pH 7.4. Slices were then transferred to 2 ml of the same buffer containing 0.5 mM-methylisobutylxanthine and 0.25–0.35 mCi of [³²P]P_i, and incubated for 1 h at 37°C. Thyrotropin (50 munits/ml) was added to half

the incubation flasks and incubation was continued for 10 min or 2 h.

After incubation, tissues were rinsed and immediately frozen in liquid N₂. Salt/acid-soluble proteins were extracted as follows. Tissues were homogenized in 2 ml of 0.5 M-NaCl/0.4 M-HCl; 2 or 3 ml of 0.5 M-NaCl/0.4 M-HCl were then added, and the suspensions mixed and placed on ice for 30–60 min. Salt/acid-soluble and -insoluble material were separated by centrifugation for 30 min at 1750g or 10 min at 10000g.

Protein and phosphoprotein analysis

The supernatants obtained after salt/acid extraction of ³²P-labelled tissue slices, and portions of the salt/acid extracts of whole thyroid tissue and nuclei, were dialysed against 0.9 M-acetic acid at 4°C and freeze-dried.

Protein extracts were redissolved in 0.9 M-acetic acid/6 M-urea/10% (v/v) glycerol/5% (v/v) β-mercaptoethanol and portions were subjected to two-dimensional polyacrylamide-gel electrophoresis in a system employing 0.9 M-acetic acid/6 M-urea in the first dimension and 0.1% SDS in the second (Cooper *et al.*, 1982). After electrophoresis, SDS was removed from the gels by extensive washing with methanol/acetic acid/water (5:1:5, by vol.); gels were then stained overnight with 0.04% Coomassie Brilliant Blue R250 in methanol/acetic acid/water (5:1:5, by vol.) and destained in methanol/acetic acid/water (2:1:8, by vol.).

Autoradiography was carried out as described by Cooper *et al.* (1981); ³²P-labelled protein spots were cut out of the gels and counted by Čerenkov radiation. Radioactivity in each phosphoprotein was expressed as c.p.m. per batch of tissue slices, after correction for background (22–24 c.p.m.) and ³²P decay.

Results

Fig. 1(a) shows the pattern of protein staining obtained with salt/acid extracts of calf thyroid nuclei. Five spots are clearly visible in the HMG-14/17 region. The most prominent are HMG 14, HMG 17 and a protein with electrophoretic mobility similar to that of HMG 17, designated 'PS.3'. Two other proteins, with mobilities similar to that of HMG 14, are designated 'PS.1' and 'PS.2'. All of these proteins were observed, in similar proportions, in salt/acid extracts of minced calf thyroid tissue (Fig. 1b), in salt/acid and HClO₄ extracts of calf thyroid tissue that had been frozen in liquid N₂ immediately after slaughter of the calves (results not shown), and in an HMG-14/17-enriched fraction prepared from HClO₄ extracts of calf thymus (Fig. 1c). PS.1, PS.2 and PS.3, like HMG 14 and 17, displayed metachromatic staining with Coomassie

Fig. 1. Protein-separation patterns obtained by two-dimensional polyacrylamide-gel electrophoresis of (a) a salt/acid extract of calf thyroid nuclei (sample representing 170 mg of whole tissue), (b) a portion of a direct salt/acid extract of calf thyroid (representing 85 mg of tissue), and (c) 50 µg of an HMG-14/17-enriched fraction prepared from HClO_4 extracts of calf thymus tissue

The positions of the histones and the HMG and HMG-like proteins are indicated. In order to clearly detect the minor spots in the HMG-14/17 region of the gels, electrophoresis was carried out on relatively large amounts of material, thereby grossly overloading the histone regions. Histone H1 was resolved into two major spots, designated H1_I and H1_s. Histones H2A and H2B were not resolved, and their position is designated 'H2'. The identities of the HMG proteins were confirmed by co-electrophoresis with purified HMG proteins kindly provided by Dr. Leroy Kuehl (Department of Biochemistry, University of Utah, Salt Lake City, UT, U.S.A.) and Dr. Gordon Walton (Department of Medicine, University of California at San Diego, CA, U.S.A.).

Blue, having a distinctly pink colour, whereas almost every other protein stained blue.

Salt/acid extracts of whole thyroid slices contained numerous proteins with electrophoretic mobilities similar to those of HMG 14 and HMG 17 in the SDS dimension (Fig. 1*b*). We have designated one of these 'A5', since it appears, on the basis of its

acid-solubility, electrophoretic mobility in two dimensions and phosphorylation in thyrotropin-treated and control thyroid slices (see below), to be identical with the protein 'A5' whose thyrotropin-dependent phosphorylation we have described previously (Spaulding & Schubart, 1978). We found this protein to be prominent in HClO_4 extracts of

Fig. 2. Autoradiographs of two-dimensional gels containing salt/acid extracts of calf thyroid tissue slices (0.5–0.6 g) that had been incubated with $^{32}\text{P}_i$ (0.3 mCi) in the absence (a) or presence (b) of thyrotropin (50 munits/ml for 2 h). Samples analysed represented approx. 180 mg of tissue slices. The positions of the ^{32}P -labelled histones, and HMG and HMG-like proteins, are indicated (for comparison with stained gels, see Fig. 1*b*); phosphoprotein AS.1 has been described previously (Cooper *et al.*, 1982; Cooper & Spaulding, 1983).

whole thyroid (results not shown) and thymus tissue (Fig. 1c).

The HMG-14/17-enriched fraction prepared from calf thymus (as shown in Fig. 1c) was subjected to ion-exchange column chromatography on CM-Sephadex C-25. The flow-through and the HMG 1, HMG 2, HMG 14 and HMG 17 peaks were analysed by electrophoresis in two dimensions. PS.1 co-eluted with HMG 14; PS.2, PS.3 and A5 were not detected in any of the fractions analysed.

The phosphorylation of PS.1, PS.2, PS.3 and A5 was investigated by exposing thyroid tissue slices to ³²P_i in the presence or absence of thyrotropin (50munits/ml for 2h) and analysing salt/acid extracts of the whole slices. Autoradiographs are shown in Figs. 2(a) and 2(b). Thyrotropin increased the ³²P-labelling of HMG 14, A5 and the histones H1 and H3 (but had no effect on the H2 histones, histone H4, or non-histone protein AS.1), as previously reported (Spaulding & Schubart, 1978; Cooper *et al.*, 1981, 1982, 1983; Cooper & Spaulding, 1983). Little or no ³²P was incorporated into

HMG 17 or PS.1; labelling of PS.3 was readily observed only in thyrotropin-treated samples. Labelling of PS.2 was clearly evident in both thyrotropin-treated and control samples, but did not appear to be affected by thyrotropin. Quantitative data confirming these observations are provided in Table 1. In controls, PS.2 and HMG 14 contained similar amounts of ³²P, even though much less PS.2 was present (as indicated by protein staining, Fig. 1b); thus the specific activity of PS.2 appeared to be much higher than that of HMG 14.

Table 2 shows the results of an experiment in which thyroid slices were exposed to thyrotropin (50munits/ml) for 10min. ³²P-labelling of the histones and HMG 14 was affected only slightly, or not at all, within this time. Our identification of A5 was supported by the rapidity and extent of its phosphorylation in response to thyrotropin, which we have described previously (Spaulding & Schubart, 1978); labelling of this protein was increased 4–5-fold by thyrotropin in 10min (Table 2).

We did not measure [³²P]ATP concentrations in the present study; however, the observed increases in ³²P-labelling of proteins in response to thyrotropin could not have been simply the result of changes in

Table 1. Effects of thyrotropin (50munits/ml for 2h) on the incorporation of ³²P into various salt/acid-soluble proteins

Batches of calf thyroid slices (0.5–0.6g) were incubated with 0.27mCi of ³²P_i for 3h, without thyrotropin or with thyrotropin (50munits/ml) for the final 2h. Salt/acid-soluble proteins were extracted, and analysed by two-dimensional polyacrylamide-gel electrophoresis, as described in the Materials and methods section. For identification of phosphoproteins, see Figs. 1(b) and 2. Values are means ± s.e.m. for ³²P (c.p.m. per batch of tissue slices) in each phosphoprotein (determinations in quadruplicate). Statistical significance: ^a*P* < 0.005; ^b*P* < 0.001; ^c*P* < 0.0005, thyrotropin versus control, by two-tailed *t*-test. To compensate for differences in the amount of ³²P taken up by different batches of tissue slices, we calculated the ratios of ³²P in each protein to ³²P in H2, which confirmed thyrotropin stimulation of the labelling of H1, H3, A5, HMG 14 and PS.3 and failed to reveal other hormone-dependent changes (results not shown).

Protein	³² P radioactivity (c.p.m.)	
	Control	Thyrotropin
AS.1	2779 ± 995	2710 ± 334
H1	4596 ± 862	22957 ± 4083 ^a
H2	4744 ± 1092	5769 ± 1094
H3	536 ± 109	1896 ± 200 ^b
H4	709 ± 248	827 ± 215
A5	495 ± 75	1615 ± 145 ^c
HMG 14	925 ± 103	2250 ± 239 ^a
PS.2	679 ± 107	851 ± 89
HMG 17	245 ± 35	303 ± 22
PS.3	456 ± 63	1325 ± 101 ^c

Table 2. Effects of thyrotropin (50munits/ml for 10min) on the incorporation of ³²P into various salt/acid-soluble proteins

Batches of calf thyroid slices (0.4–0.6g) were incubated with 0.34mCi of ³²P_i for 70min without thyrotropin or with thyrotropin (50munits/ml) for the final 10min. Salt/acid-soluble proteins were extracted, and analysed by two-dimensional polyacrylamide-gel electrophoresis, as described in the Materials and methods section. For identification of phosphoproteins, see Figs. 1(b) and 2. Values are means ± s.e.m. for ³²P (c.p.m. per batch of tissue) in each phosphoprotein (all determinations in quadruplicate). Statistical significance: ^a*P* < 0.05; ^b*P* < 0.005, thyrotropin versus control, by two-tailed *t*-test. To compensate for differences in the amount of ³²P taken up by different batches of slices, we calculated the ratios of ³²P in each protein to ³²P in H2. This analysis confirmed the thyrotropin-dependent increase in ³²P-labelling of A5, but not of H3; no other hormone-dependent changes were revealed (not shown).

Protein	Radioactivity (c.p.m.)	
	Control	Thyrotropin
AS.1	765 ± 103	1047 ± 193
H1	895 ± 47	1305 ± 219
H2	2397 ± 204	3180 ± 610
H3	402 ± 7	634 ± 82 ^a
H4	231 ± 22	304 ± 48
A5	119 ± 19	618 ± 105 ^b
HMG 14	456 ± 53	548 ± 95

labelled-precursor-pool size, because the labelling of half of the proteins analysed was not significantly affected by thyrotropin, and the labelling of each stimulated protein was increased to a different extent, from 150 to 400% (Table 1).

Discussion

The present study shows that calf thyroid and thymus tissue contain three nuclear proteins, PS.1, PS.2 and PS.3, with properties (salt/acid-solubility, HClO_4 -solubility, electrophoretic mobility in two dimensions) similar to those of HMG 14 and HMG 17. Our data confirm previous observations (Cooper *et al.*, 1982, 1983; Cooper & Spaulding, 1983) of thyrotropin-stimulated ^{32}P -labelling of HMG 14, and very little ^{32}P incorporation into HMG 17, in calf thyroid slices; however, they show that the pattern of ^{32}P -labelling of nuclear proteins resembling HMG 14 and HMG 17 is more complex than previously recognized. Labelling of PS.2, a protein with very similar electrophoretic mobility to HMG 14, is unaffected by thyrotropin; on the other hand, thyrotropin clearly stimulates the phosphorylation of PS.3, a protein with similar mobility to HMG 17.

There are good reasons for believing that PS.1, PS.2 and PS.3 are modified or variant forms of HMG 14 and 17, or very similar proteins, rather than experimental artefacts resulting from proteolytic degradation.

(i) They are all consistently found, in similar relative proportions, in direct HClO_4 and salt/acid extracts of rapidly frozen calf thyroid tissue, as well as in unfrozen thyroid and thymus tissue and thyroid nuclei.

(ii) They all display metachromatic staining, having a distinctly pink colour, whereas almost every other acid-soluble protein except HMG 14 and HMG 17 stains blue. Thus it would seem most likely that if they are proteolytic degradation products, they are derived from HMG 14 or HMG 17 [but see (iii)–(vi) below].

(iii) PS.1 and PS.2 are unlikely to be proteolytic degradation products of HMG 17, because they have lower mobilities than HMG 17 in the SDS dimension of our electrophoresis gels.

(iv) PS.3 is unlikely to be a proteolytic degradation product of HMG 17, because it incorporates more ^{32}P than does HMG 17 even though it is less heavily stained, and its ^{32}P -labelling is stimulated by thyrotropin whereas that of HMG 17 is not.

(v) PS.1 is unlikely to be a proteolytic degradation product of HMG 14, because it does not appear to be ^{32}P -labelled in thyroid slices. Proteolytic degradation of thyroid HMG 14 could, of course, remove segments containing ^{32}P -labelled sites; however, removal of both of the sites that we have found to be phosphorylated, serine-6 and serine-24

(G. M. Walton, G. N. Gill, E. Cooper & S. W. Spaulding, unpublished work), would require removal of at least 20% of the *N*-terminal region. This would be expected to result in a much larger difference in electrophoretic mobility than that observed between PS.1 and HMG 14.

(vi) PS.2 is unlikely to be a proteolytic degradation product of HMG 14 (or of HMG 17) because its specific radioactivity in control thyroid slices was found to be much higher than that of HMG 14 (or of HMG 17); furthermore, its labelling was not stimulated by thyrotropin, as was that of HMG 14.

In numerous investigations of HMG 14 and 17 phosphorylation *in vivo* and *in vitro*, ^{32}P -labelled HMG proteins have been analysed by electrophoresis in one dimension, in a single gel system (Inoue *et al.*, 1980; Arfmann & Baydoun, 1981; Arfmann *et al.*, 1981; Bhorjee, 1981; Levy-Wilson, 1981; Saffer & Glazer, 1982; Harrison & Jungmann, 1982). In view of the ease with which HMG 14, HMG 17 and similar proteins may be misidentified, the results of such studies may have to be reassessed. A recent report by Bhorjee *et al.* (1983) illustrates this point, and our own data re-emphasize it. In our study, one-dimensional SDS/polyacrylamide gels would have failed to resolve PS.2 from HMG 14; similarly, one-dimensional acetic acid/urea gels would have failed to resolve unlabelled PS.1 from ^{32}P -labelled HMG 14, and ^{32}P -labelled PS.3 from unlabelled HMG 17.

Although there have been accounts of the presence of HMG-14/17-like proteins, or (putative) modified HMG proteins, in a variety of tissue, cell and nuclear extracts, we know of very few descriptions of proteins derived from calf thyroid or thymus tissue that might correspond to PS.1, PS.2 or PS.3. Albright *et al.* (1980) reported the presence, in purified preparations of calf thymus HMG 14, of contaminants that migrated ahead of HMG 14 in one-dimensional SDS/polyacrylamide gels. The most prominent of these, which was not described in detail, had an electrophoretic mobility similar to that of PS.1, and like PS.1 was not separated from HMG 14 by CM-Sephadex column chromatography. Nicolas & Goodwin (1982) reported recently that purified calf thymus HMG 14 and 17 are both resolved into two bands when analysed by polyacrylamide-gel electrophoresis at neutral pH. Sterner *et al.* (1979) described the presence, in 0.35 M-NaCl extracts of calf thymus chromatin, of a protein ('HMG-X') that co-migrated with HMG 17 on SDS/polyacrylamide gradient gels, but this protein appeared to be a proteolytic degradation product of HMG 1 or HMG 2. We have argued above that PS.1, PS.2 and PS.3 are not proteolytic degradation products; further, since PS.1, PS.2 and PS.3 all have slightly different mobilities to that of HMG 17 in the SDS dimension of our two-

dimensional gels, it is unlikely that any of them corresponds to 'HMG-X'.

The protein we have designated 'A5' is intriguing, for two reasons. First, of the nine or ten most abundant HClO_4 -soluble proteins observed in thyroid and thymus tissue, it was the only one not found in thyroid nuclei. Although it could, of course, be nuclear in origin, it may turn out to be a non-nuclear protein with rather unusual physical properties. Second, ^{32}P -labelling of A5 was increased very rapidly in response to thyrotropin, far more so than any of the other acid-soluble proteins studied. It seems likely that increased phosphorylation of A5 is involved in the very early stages of thyroïdal response to thyrotropin.

In summary, we have shown in the present study that acid extracts of calf thyroid and thymus tissue contain three proteins, PS.1, PS.2 and PS.3, that have similar electrophoretic mobilities to HMG 14 and HMG 17, and appear to be nuclear in origin. Two of these proteins, PS.2 and PS.3, are phosphorylated in calf thyroid slices, and the ^{32}P -labelling of one, PS.3, is stimulated by thyrotropin. We have also shown that thyrotropin rapidly stimulates the phosphorylation of an HClO_4 -soluble protein (A5) found in extracts of whole calf thyroid and thymus tissue but not in nuclei.

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References

- Albright, S. C., Wiseman, J. M., Lange, R. A. & Garrard, W. T. (1980) *J. Biol. Chem.* **255**, 3673–3684
- Allfrey, V. G. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., ed.), pp. 123–148, Academic Press, London
- Arfmann, H.-A. & Baydoun, H. (1981) *Z. Naturforsch.* **36**, 319–322
- Arfmann, H.-A., Haase, E. & Schroter, H. (1981) *Biochem. Biophys. Res. Commun.* **101**, 137–143
- Barsoum, J., Levinger, L. & Varshavsky, A. (1982) *J. Biol. Chem.* **257**, 5274–5282
- Bhorjee, J. S. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 6944–6948
- Bhorjee, J. S., Mellon, I. & Kifle, L. (1983) *Biochem. Biophys. Res. Commun.* **111**, 1001–1007
- Cooper, E. & Spaulding, S. W. (1983) *Endocrinology* **112**, 1816–1822
- Cooper, E., Palmer, R. J. & Spaulding, S. W. (1981) *Nucleic Acids Res.* **9**, 3389–3401
- Cooper, E., Palmer, R. J. & Spaulding, S. W. (1982) *Endocrinology* **110**, 1459–1461
- Cooper, E., Abe, Y., Palmer, R. J. & Spaulding, S. W. (1983) *Biochim. Biophys. Acta* **740**, 179–184
- D'Anna, J. A., Becker, R. R., Tobey, R. A. & Gurley, L. R. (1983) *Biochim. Biophys. Acta* **739**, 197–206
- Goodwin, G. H., Rabbani, A., Nicolas, R. H. & Johns, E. W. (1977) *FEBS Lett.* **80**, 413–416
- Harrison, J. J. & Jungmann, R. A. (1982) *Biochem. Biophys. Res. Commun.* **108**, 1204–1209
- Inoue, A., Tei, Y., Hasuma, T., Yukioka, M. & Morisawa, S. (1980) *FEBS Lett.* **117**, 68–72
- Levinger, L., Barsoum, J. & Varshavsky, A. (1981) *J. Mol. Biol.* **146**, 287–304
- Levy-Wilson, B. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 2189–2193
- Lund, T., Holtlund, J., Kristensen, T., Ostvold, A. C., Sletten, K. & Laland, S. G. (1981) *FEBS Lett.* **133**, 84–88
- Nicolas, R. H. & Goodwin, G. H. (1982) in *The HMG Chromosomal Proteins* (Johns, E. W., ed.), pp. 41–68, Academic Press, London
- Saffer, J. D. & Glazer, R. I. (1982) *J. Biol. Chem.* **257**, 4655–4660
- Sanders, C. (1977) *Biochem. Biophys. Res. Commun.* **78**, 1034–1042
- Spaulding, S. W. & Schubart, U. K. (1978) *Endocrinology* **103**, 2334–2341
- Sterner, R., Vidali, G. & Allfrey, V. G. (1979) *Biochem. Biophys. Res. Commun.* **89**, 129–133
- Walton, G. M. & Gill, G. N. (1983) *J. Biol. Chem.* **258**, 4440–4446
- Weisbrod, S. (1982) *Nature (London)* **297**, 289–295