

## Elevated levels of a specific class of nuclear phosphoproteins in cells transformed with *v-ras* and *v-mos* oncogenes and by co-transfection with *c-myc* and polyoma middle T genes

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**Transformation of a rat thyroid epithelial cell line (FRTL5-C12) with Kirsten and Harvey murine sarcoma viruses (carrying the *ras* oncogenes) results in elevated levels of three perchloric acid-soluble nuclear phosphoproteins. These three proteins are also induced to high levels in the PC-C13 thyroid epithelial cell line when transformed by the myeloproliferative sarcoma virus (carrying the *v-mos* oncogene) and when transformed by transfection with the *c-myc* proto-oncogene followed by infection with the polyoma leukaemia virus (PyMuLV) carry the polyoma middle T antigen gene. Neither *c-myc* or PyMuLV alone induced high levels of the three nuclear proteins. Untransformed thyroid fibroblasts have high levels of two of the three proteins and can be transformed by PyMuLV alone resulting in the appearance of the third protein. Transformation with Harvey sarcoma virus also results in the induction of the third protein. The three phosphoproteins have been purified by h.p.l.c. and shown to be related to the HeLa protein HMGI already described. The results of these studies indicate that elevated levels of these HMGI-like proteins are associated with neoplastic transformation and/or with an undifferentiated phenotype.**

**Key words:** HMG proteins/oncogenes

### Introduction

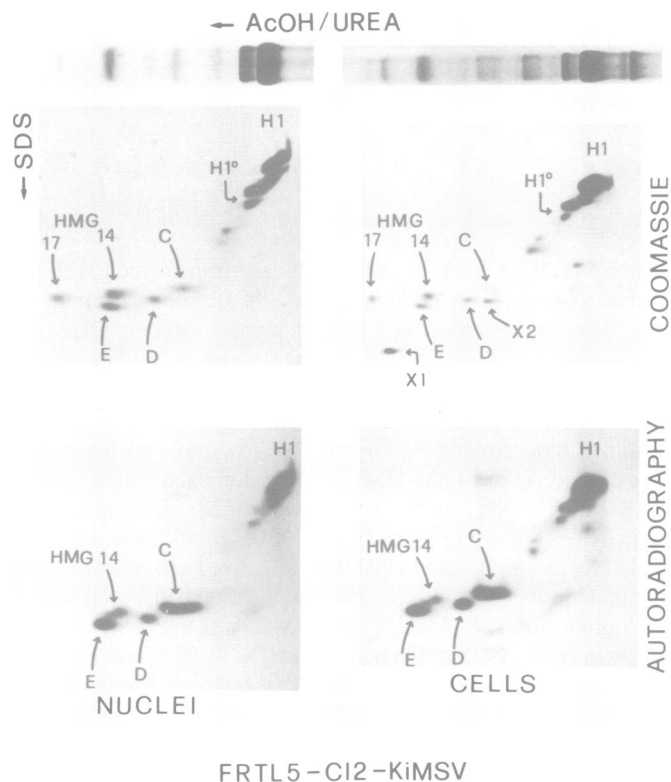
Neoplastic transformation of eukaryotic cells is a multi-step process involving changes in structure and expression of proto-oncogenes (Farber and Cameron, 1980), altered production and response to growth factors (Sporn and Roberts, 1985) and, more generally, extensive changes in the population of mRNA molecules in the cell [e.g. it has been estimated that ~3% of the mRNA molecules in SV40-transformed mouse cells are present at much higher levels in the transformed cells as compared with their untransformed counterparts (Scott *et al.*, 1983)]. It is not unreasonable to expect that some of the genes expressed at elevated levels in transformed cells are involved in the biochemical processes of DNA replication and cell division occurring in the cell nucleus. We have previously documented the induction of elevated levels of a nuclear protein of the high mobili-

ty group (HMG) when rat fibroblasts are transformed with the avian sarcoma virus (Goodwin *et al.*, 1985a). Similarly epithelial cells, when transformed with Kirsten murine sarcoma virus, exhibit elevated levels of a similar protein and two other related proteins (Giaccotti *et al.*, 1985).

All terminally differentiated mammalian and avian cells so far examined have two well-characterized low mol. wt basic proteins termed HMG14 and HMG17 (Goodwin and Johns, 1983). Proliferating cells such as untransformed fibroblasts and thymocytes (but not epithelial cells) have, in addition, elevated levels of a protein termed HMGI which have been isolated from human and rat cells and has been shown to have a similar amino acid composition to HMG14 and HMG17 (Lund *et al.*, 1983; Goodwin *et al.*, 1985a). On transformation of rat fibroblasts with the avian sarcoma virus, such cells express high levels of a second additional protein termed HMGI'. In the case of rat epithelial cells, untransformed proliferating cells do not exhibit these additional HMG-like proteins, but on transformation three proteins C, D and E appear at elevated levels (Giaccotti *et al.*, 1985), proteins C and D having similar electrophoretic properties to the fibroblast HMGI' and I, respectively. Similar proteins have been described in human and mouse transformed cell lines (Lund *et al.*, 1983, 1985). The previously described studies (Goodwin *et al.*, 1985a; Giaccotti *et al.*, 1985) suggested the possibility that the additional HMG proteins may arise as a consequence of neoplastic transformation and may be necessary components of this transition. To examine more widely the correlation between the elevated levels of these proteins and the cell phenotype, we have analysed these proteins in a number of rat thyroid epithelial and fibroblast cell lines at different stages of differentiation and malignancy. We have also purified the three HMG-like proteins from transformed epithelial cells and show that all three proteins are very similar in composition to the previously described HMGI protein from human, mouse and rat cells and a homologous protein, HMG $\alpha$  from monkey cells (Strauss and Varshavsky, 1984).

### Results

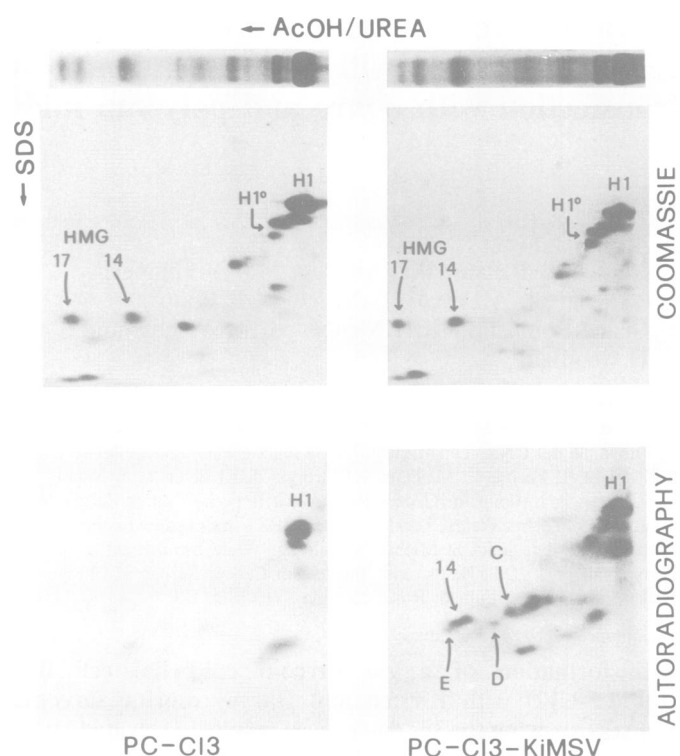
The HMG proteins from two rat thyroid epithelial cell lines and one rat thyroid fibroblast cell line were analysed before and after infection with various murine viruses containing oncogenes. As a control, one cell line was infected with a virus which does not have an oncogene. Table I summarizes the cells that have been analysed in this study. The properties of these cells have been documented elsewhere (Fusco *et al.*, 1981, 1982, 1985a,b; Ambesi-Impiombato *et al.*, 1980). The two epithelial parental cell lines FRTL5-C12 and PC-C13 express thyroid differentiation markers, e.g. synthesis of thyroglobulin, uptake of iodide and dependence on thyroid-stimulating hormone (TSH) for growth. Infection of these cells with a wide variety of retroviruses results in a loss of one or more of the differentiation markers but does not invariably give rise to the transformed phenotype. FRTL5-C12 and PC-C13 differ in that the former are derived



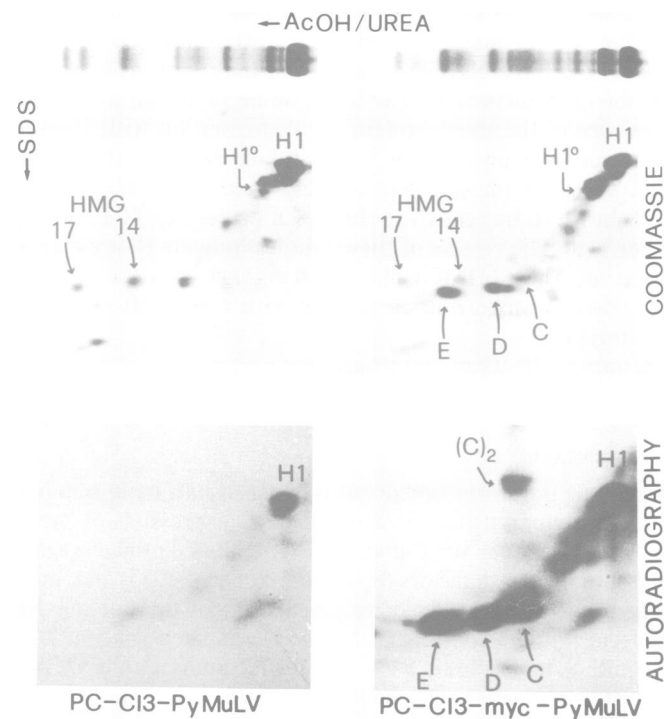
**Fig. 1.** Two-dimensional gel electrophoresis of PCA-extracted proteins from FRTL5-C12-KiMSV nuclei (two panels on left) and whole cells (two panels on right). The top pair of two-dimensional gels were stained with Coomassie Blue and the bottom panels are autoradiographs of the top two gels. The first dimension electrophoresis in acetic acid-urea is shown above the two-dimensional gels.

from young rat thyroid and the latter from adult rat thyroid. FRT-fibro is a thyroid fibroblast cell line which, after infection with all the retroviruses so far tested, always results in the production of highly malignant cells. The three cell lines were infected with: (i) Kirsten murine sarcoma virus (KiMSV) carrying the *v-Ki-ras* oncogene; (ii) Harvey murine sarcoma virus (HaMSV) carrying the *v-Ha-ras* oncogene; (iii) myeloproliferative sarcoma virus (MPSV) carrying the *v-mos* oncogene (Pragnell *et al.*, 1981); (iv) polyoma leukaemia virus (PyMuLV) carrying the polyoma middle T gene (Donoghue *et al.*, 1984); (v) Moloney murine leukaemia virus (Mo-MuLV) (no oncogene); (vi) transfection with plasmid pMCGMI carrying the human *c-myc* proto-oncogene (Spandidos, 1985); and (vii) transfection with pMCGMI followed by infection with PyMuLV.

The HMG proteins were quantitatively and selectively extracted (together with histone H1) from the nuclei or whole cells with 5% perchloric acid (PCA) and analysed by two-dimensional gel electrophoresis using equal loadings of histone H1. Figure 1 shows such an analysis of the proteins from KiMSV-transformed FRTL5-C12 cells and nuclei and their level of phosphorylation after incubating the cells with  $^{32}\text{PO}_4$ . As described previously, in the low mol. wt HMG region of the gel, three prominent proteins, C, D and E, in addition to HMG14 and 17, are observed. The three proteins C, D and E are not observed (or were present at very low levels) in the uninfected FRTL5-C12 cells (Giaccotti *et al.*, 1985). HaMSV-transformed cells behaved in the same way (data not shown). Two proteins marked X1 and X2 are observed in whole cell extracts and not nuclear extracts, and are



**Fig. 2.** Two-dimensional gel electrophoresis of PCA-extracted proteins from PC-C13 (two panels on left) and PC-C13-KiMSV cells (two panels on right). The top pair of two-dimensional gels were stained with Coomassie Blue and the bottom two panels are autoradiographs of the top two gels.



**Fig. 3.** Two-dimensional gel electrophoresis of PCA-extracted proteins from PC-C13-PyMuLV cell (two panels on left) and PC-C13<sup>myc</sup>-PyMuLV cells (two panels on right). The top pair of two-dimensional gels were stained with Coomassie Blue, the bottom two are autoradiographs of the top two.

therefore probably cytoplasmic proteins, and are not discussed further in this paper. The autoradiographs of Figure 1 show that the proteins C, D, E and HMG14 are phosphorylated. Careful examination of the autoradiographs suggests that protein C may be composed of more than one phosphorylated species and that there may be an additional protein migrating between HMG14 and protein E.

FRTL5-C12 cells were also infected with Mo-MuLV (which has no oncogene) but proteins C, D and E were not observed (data not shown) demonstrating that the induction of these proteins is not simply the consequence of viral infection but requires the presence of an oncogene in the virus. Proof that infection had occurred was confirmed by reverse transcriptase assays.

PC-C13 cells can be infected with KiMSV but, in contrast to the FRTL5-C12 cells, this fails to transform the cells. Proof that the virus has infected the cells comes from dot-blot RNA hybridization (data not shown) and from the fact that the cells have a changed morphology and are no longer dependent on TSH for growth. Such infected but untransformed cells do not express high levels of the proteins C, D and E (Figure 2). Although not detected by Coomassie staining, the small amount of C, D and E proteins present can be detected after labelling with  $^{32}\text{PO}_4$ . However, the level of labelling is very much lower than that seen with FRTL5-C12-KiMSV cells. These results again demonstrate that viral infection alone does not invariably result in elevated levels of the C, D and E proteins and that further changes that occur during oncogenesis are required for manifestation of the elevated levels of these proteins. There is some indication from the comparison of the two autoradiographs of Figure 2 that viral infection leads to elevated phosphorylation of the proteins. Interestingly, PC-C13 cells infected with the related virus Ha-MSV which also contains a *ras* oncogene, *v-Ha-ras*, are also not transformed but they do express high levels of protein C, but not D and E (data not shown), again suggesting that the expression of all three proteins C, D and E is a marker of neoplastic transformation.

PC-C13 cells are not transformed when transfected with the plasmid containing the human *c-myc* gene and they do not express high levels of the C, D and E proteins (data not shown). However, when these *c-myc* transfectants are subsequently infected with PyMuLV virus (carrying the polyoma middle T gene), the cells become transformed, showing a highly malignant phenotype as demonstrated by invasive tumour formation 7–10 days after mice are injected with such cells. This leads to animal death. Such cells now exhibit high levels of proteins C, D and E, especially protein E (Figure 3). PC-C13 cells can be transformed with PyMuLV alone but they differ from the *myc*-PyMuLV transformants in a number of respects. The PyMuLV transformants are less tumourigenic, taking 4 weeks to produce small tumours in nude mice which are not invasive, and the animals survive. PyMuLV transformants retain their differentiation markers but they lose growth factor dependence (Table I), whilst *myc*-PyMuLV transformants show a complete suppression of thyroid differentiation function. Proteins C, D and E are not detectable in PyMuLV-transformed PC-C13 cells (Figure 3). PC-C13 cells could also be transformed to a highly neoplastic phenotype with MPSV alone (carrying the *v-mos* gene). All three proteins C, D and E were expressed at a high level (data not shown) and again protein E was particularly prominent.

Also to be noted in the two-dimensional gels of Figure 3 is that protein C (mol. wt  $\sim 15\ 000$ ) forms a dimer ( $\text{C}_2$ ) ( $\sim 30\ 000$  mol. wt) when the first dimension gel is stained and

Table I. Properties of the investigated cell lines

Cells	Oncogene	Diff. markers <sup>a</sup>	Growth factor dep. <sup>b</sup>	Neo-plastic pheno <sup>c</sup>	Elevated protein levels		
					C	D	E
FRTL5-C12		+	+	–	–	–	–
+ KiMSV	<i>v-Ki-ras</i>	–	–	+	+	+	+
+ HaMSV	<i>v-Ha-ras</i>	–	–	+	+	+	+
+ Mo-MuLV	None	+	+	–	–	–	–
PC-C13		+	+	–	–	–	–
+ KiMSV	<i>v-Ki-ras</i>	(+) <sup>d</sup>	–	–	–	–	–
+ MPSV	<i>v-mos</i>	–	–	+	+	+	++
+ <i>myc</i>	<i>c-myc</i>	+	+	–	–	–	–
+ PyMuLV	middle T	+	–	(+) <sup>e</sup>	–	–	–
<i>myc</i> PyMuLV	<i>c-myc</i> middle T	–	–	+	+	+	++
+ HaMSV	<i>v-Ha-ras</i>	–	–	–	+	–	–
FRT-Fibro				–	–	+	+
+ HaMSV	<i>v-Ha-ras</i>			+	+	+	+
+ PyMuLV	middle T			+	+	+	+

<sup>a</sup>Differentiation markers are TGB synthesis and secretion (as evaluated by radioimmunoassay and dot-blot RNA hybridization) and iodide uptake.

<sup>b</sup>Cells were assayed for the dependence of growth on the following growth factors: insulin, hydrocortisol, transferrin, thyrotropin, somatostatin and glycyl-histidyl-lysine.

<sup>c</sup>Neoplastic phenotype was assayed by cloning efficiency in soft agar and s.c. injection of  $2 \times 10^6$  cells into athymic mice.

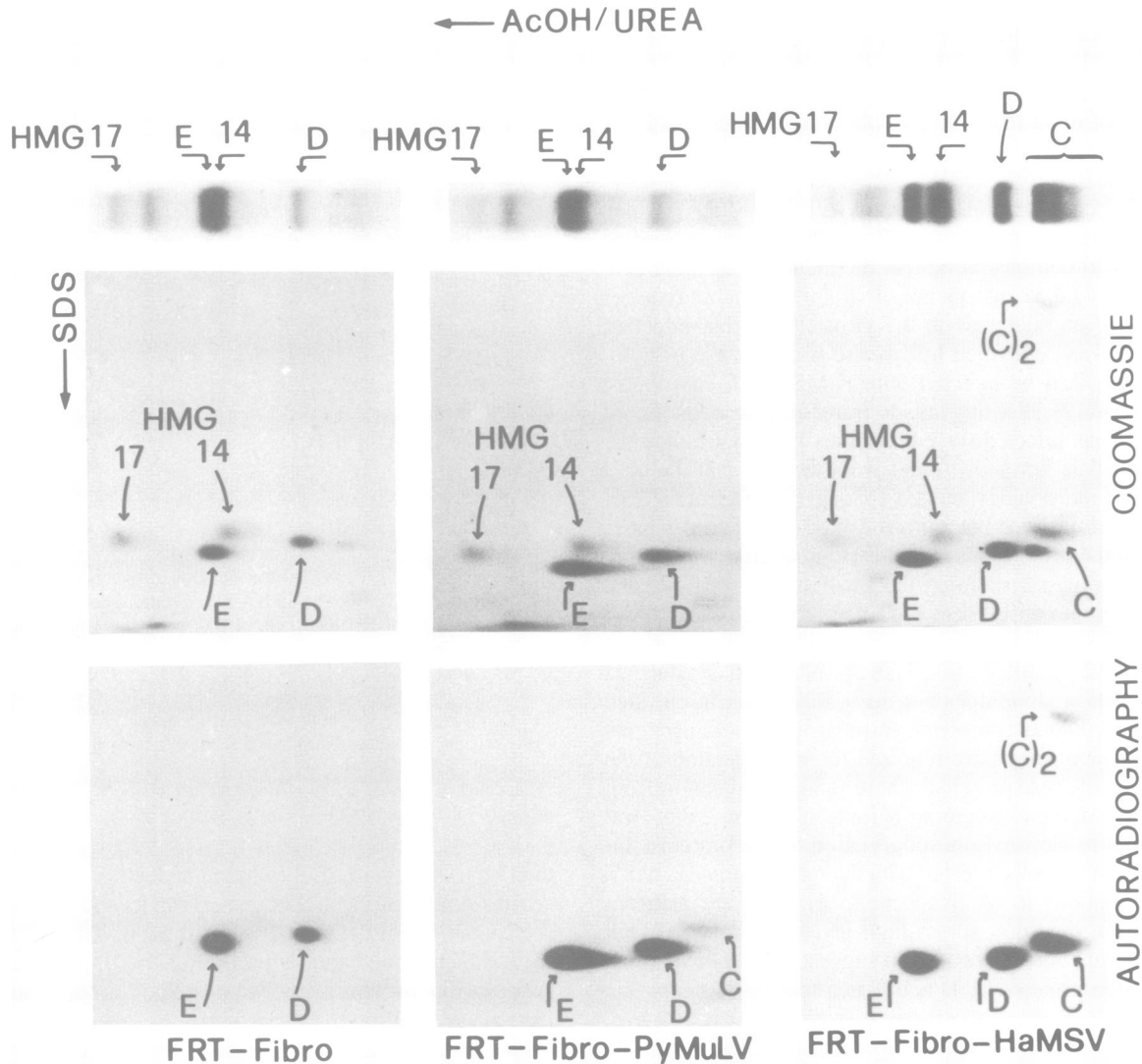
<sup>d</sup>This cell line expressed TGB synthesis at one-tenth the level of that expressed by PC-C13.

<sup>e</sup>This cell line showed a low colony forming efficiency in soft agar and produced small tumours (1–2 cm) in athymic mice.

then equilibrated in SDS (without reducing agent) prior to the second dimension electrophoresis. Trimers and tetramers are also seen at the top of the gel (data not shown). These forms are not seen if mercaptoethanol is used after the first dimension. This suggests that protein C has two cysteine residues capable of oxidation (see below).

Similar experiments were carried out with the Fisher rat thyroid fibroblast cell line FRT-Fibro (Figure 4). As in the case of the rat-1 embryo fibroblast cell line (Goodwin *et al.*, 1985a), the untransformed fibroblasts already have high levels of protein D (or HMGI, see below) and the protein E, and these are phosphorylated. Transformation of rat-1 cells with avian sarcoma virus (ASV) results in high levels of protein HMGI' (Goodwin *et al.*, 1985a). Similarly, transformation of FRT-Fibro with HaMSV results in the high levels of protein C (HMGI', see below). Thus, it would appear that the fully transformed phenotype of both epithelial and fibroblast cells is associated with elevated expression of all three proteins. FRT-Fibro cells can be transformed with PyMuLV alone. In this case, the three HMG proteins C, D and E are induced but protein C is not elevated to such a high level as that seen with HaMSV-transformed cells, FRT-Fibro-HaMSV (Figure 4). It is of interest to note that when injected into nude mice, PyMuLV-transformed fibroblasts have a much longer latency period before the appearance of tumours as compared with HaMSV-transformed cells (A. Fusco *et al.*, unpublished data).

To characterize the epithelial proteins more fully and to relate them to the previously described HMGI proteins (Lund *et al.*, 1983; Goodwin *et al.*, 1985a), h.p.l.c. was carried out on PCA-extracted proteins that were obtained from tumours induced in athymic mice inoculated with PC-C13 *myc*-PyMuLV-transformed cells (Figure 5). As we have described previously, the low mol.

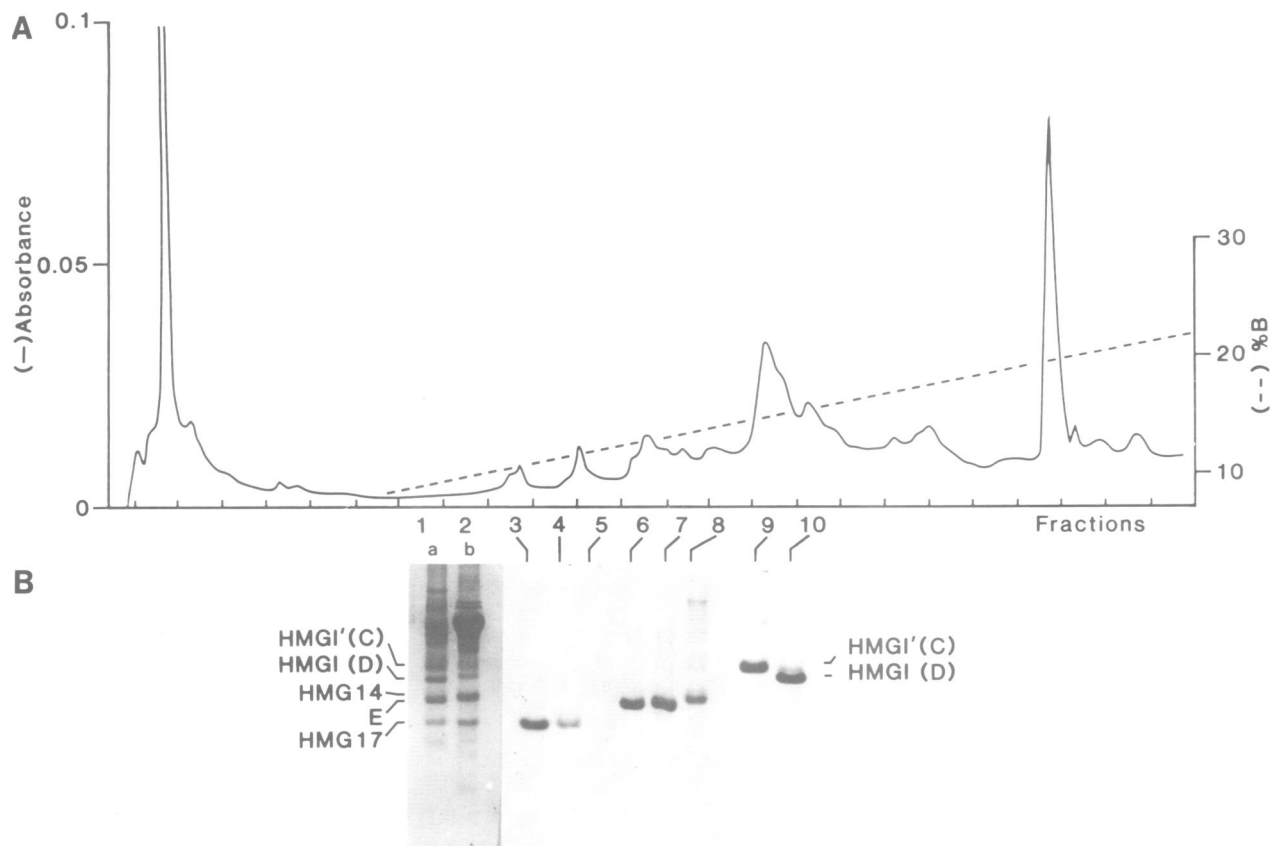


**Fig. 4.** Two-dimensional gel electrophoresis of PCA-extracted proteins from FRT-Fibro (two panels on left), FRT-Fibro-PyMuLV (two panels in centre), and FRT-Fibro-HaMSV cells (two panels on right). The top three two-dimensional gels are Coomassie Blue stained and the bottom three are autoradiographs of the top three.

wt HMG proteins elute between ~10 and 15% acetonitrile. Histone H1, HMG1 and 2 elute much later above 30% acetonitrile and their elution is not shown in Figure 5A. To identify the eluted proteins, the fractions from that part of the elution profile of interest at the start of the gradient were analysed on acid-urea gels (Figure 5B) and on two-dimensional gels (not shown). HMG17 elutes in fractions 3 (and 4), protein E elutes in fractions 6 and 7, HMG14 in fraction 8, protein C in fraction 9 and protein D in fraction 10. Judging from the elution profile, protein E in fractions 6 and 7 is composed of several species. By comparing electrophoretic mobilities of the *myc*-PyMuLV transformed epithelial cell proteins with the ASV-transformed fibroblasts described previously (Figure 5B), it is apparent that protein D corresponds to the previously isolated HMGI and protein C to HMGI'. The identification of the HMG14 (fraction 8) and 17 (fraction 3) proteins was confirmed by N-terminal sequencing. The first 26 amino acid residues of fraction 3 were identical to that of calf HMG17 (Walker *et al.*, 1977) and the first 24 residues of fraction 8 were the same as that of rat thymus HMG14 (data not shown). HMGI (D), HMGI' (C) and protein E in fractions 6 and 7 all have blocked N termini.

The amino acid compositions of the HMG-like proteins C, D and E in fractions 6, 7, 9 and 10 are given in Table II, together with those of rat thymus HMG14, 17 and HMGI. The amino acid compositions are all typical of the low mol. wt HMG proteins in having high levels of basic and acidic amino acid and having no aromatic and few hydrophobic residues. The three proteins are very similar to one another and to the previously purified rat thymus HMGI, except the threonine and lysine figures are somewhat lower and the proline figures somewhat higher. The three proteins C, D and E differ from HMG14 and 17 in having lower aspartic acid and lysine values and having higher serine, arginine and possibly proline values.

From the amino acid compositions, the three proteins C, D and E contain one or two cysteine residues assuming the proteins are the same size as HMG14, about 100 residues in length (Table II). The multiple oxidation products of protein C seen in Figure 3 suggest that this protein has two cysteines. When the purified proteins are oxidized by shaking aqueous solutions for several hours, protein C forms a dimer (as revealed by polyacrylamide gel electrophoresis) but proteins D and E are unaffected (data not shown). It would appear therefore that the



**Fig. 5.** Reverse phase h.p.l.c. fractionation of PCA-extracted proteins from tumours of PC-C13 *myc*-PyMuLV cells. (A) Elution profile with 1-ml fractions collected. (B) Acid-urea gel electrophoresis of h.p.l.c. fractions 1–10. (a) PCA-extracted proteins from PC-C13 *myc*-PyMuLV tumours. (b) PCA-extracted proteins from rat fibroblasts transformed by avian sarcoma virus (VIT cells).

cysteine groups of protein C more readily form intermolecular disulphide bridges than those of proteins D and E.

### Discussion

Three proteins C, D and E have been isolated from rat epithelial cells transformed with *c-myc* and polyoma middle T genes. These proteins form a group of closely related proteins, having very similar amino acid compositions and having blocked N termini. Since one of the proteins (protein D) is identical to a previously described protein HMGI, we have collectively termed the three proteins as the HMGI-like proteins. All three HMGI-like proteins are phosphorylated and in this respect they resemble HMG14. The HMGI-like proteins are a common feature of proliferating and transformed cells. Laland and co-workers (Lund *et al.*, 1983, 1985) have described three proteins in HeLa cells and Ehrlich ascites, termed HMGI, HMGY and HMG, the latter being specific for metaphase cells and is a highly phosphorylated form of HMGI. The HMGI in their paper and ours are probably the same. HMG has a similar electrophoretic mobility of HMGI' (protein C), but, since we observe HMGI' in unsynchronized cells at high levels, the relationship between the two is not clear. HMGY seems to be similar to the protein E described in this paper. This HMGY is not to be confused with the HMGY described in chicken oviduct (Goodwin *et al.*, 1981). The protein HMG $\alpha$  from monkey CVI cells described by Strauss and Varshavsky (1984) would appear to be a member of the HMGI-like proteins, as is the phosphorylated protein from CHO cells described by D'Anna *et al.* (1983).

From the results of this paper and the preceding reports, there

appears to be a strong correlation between the neoplastic transformation of cells and the appearance of high levels of all three HMGI-like proteins. It is interesting to note that two of the proteins (D and E) can be present in cells without them being transformed (as is the case with rat thyroid fibroblasts); the third only appears on transformation. It is tempting to speculate that the induction of high levels of all three HMGI-like proteins is one of the steps required for neoplastic transformation. However, we must consider the possibility that the presence of these proteins may be markers for some other biological transition. It is possible that the proteins are markers of undifferentiated cells, since we have observed HMGI-like proteins in rat embryos (G.H. Goodwin, unpublished data). Transformation may result in 'dedifferentiation' of the cells with the concomitant induction of HMGI expression. It is certainly true that many of the transformed cells examined in this study lose their differentiation markers (Table I). The fact that PyMuLV-transformed PC-C13 cells have low levels of HMG-I proteins and retain their differentiated phenotype whilst having a transformed phenotype (albeit less malignant than in the co-transfected *myc*-PuMuLV transformants which have high levels of HMG-I proteins) would support the view that the HMGI proteins are markers of the undifferentiated phenotype rather than the neoplastic phenotype *per se*. It is however, also conceivable that the high levels of the three HMGI-like proteins are markers of a highly malignant phenotype which leads to invasive tumour growth in animals, as seen with *myc*-PyMuLV-transformed cells. That high levels of the three HMGI-I like proteins may be characteristic of undifferentiated rather than transformed cells is also supported

**Table II.** Amino acid compositions (% mol) of h.p.l.c. fractions 6, 7, 9 and 10 and of previously purified rat thymus HMGI, HMG14 and HMG17 (Goodwin *et al.*, 1985a)

	PC-C13-myc-PyMuLV h.p.l.c fractions				Rat thymus HMG proteins		
	6 (E)	7 (E)	9 (C,HMGI')	10 (D,HMGI)	HMGI	HMG14	HMG17
Cys <sup>a</sup>	1.5 (2.1)	0.8	1.6 (2.0)	0.8 (1.3)	N.D.	N.D.	N.D.
Asx	4.6	4.7	5.2	4.5	4.9	7.8	13.7
Thr	5.4	5.3	5.0	5.5	7.1	4.6	1.8
Ser	9.3	10.2	8.9	9.7	11.9	8.2	2.8
Glx	18.2	18.9	20.6	18.5	17.8	16.4	9.0
Pro	10.6	8.8	11.3	10.8	9.0	7.3	10.0
Gly	11.0	11.6	10.1	11.4	11.1	8.1	9.3
Ala	6.8	7.2	9.3	7.5	5.2	13.2	20.2
Val	2.9	2.8	2.8	3.7	4.7	3.2	2.6
Met	0.3	—	0.3	0.2	—	0.1	—
Ile	1.6	1.5	1.1	1.4	1.0	1.1	1.1
Leu	2.4	3.2	1.1	2.4	1.6	2.3	—
Tyr	0.6	0.6	0.5	0.4	—	—	—
Phe	0.7	0.6	0.6	0.5	—	—	—
His	0.4	0.8	0.6	0.5	0.6	0.2	—
Lys	14.7	13.9	12.9	14.1	16.5	20.9	25.8
Arg	8.6	9.4	8.2	7.9	9.2	7.8	4.1

<sup>a</sup>Cysteine was determined by oxidation to cysteic acid or by carboxymethylation (figures in brackets).

by studies on avian erythroid cells transformed with the avian erythroblastosis virus (Beug *et al.*, 1982). In fact, these neoplastic cells are halted in the differentiation pathway at a late stage (CFU-E) just prior to terminal differentiation and we have not observed HMGI-like proteins in these cells (G.H. Goodwin, unpublished observations). Transformed avian fibroblasts have HMGI-like proteins as judged by two-dimensional electrophoretic gels but the proteins have not been characterized.

One other consistent feature that has been observed with all the transformed cells used in this study is that they can grow in the absence of exogenous growth factors, including thyrotropin, suggesting that the cells now produce their own growth factors or that mechanisms that govern growth have been uncoupled from growth factor-dependent biochemical reactions. The presence of the HMGI proteins does not correlate with this loss of growth factor control since PC-C13 cells infected with KiMSV or PyMuLV lose growth factor dependence but do not have high levels of the HMGI proteins.

The function of the HMGI-like proteins is not known. One of the HMGI-like proteins, HMG $\alpha$  (or HMGI), binds preferentially to DNA containing stretches of A and T and it has been suggested that this protein is associated with satellite chromatin (Strauss and Varshavsky, 1984; Solomon *et al.*, 1986). In our studies, preliminary quantitative analyses indicate that concomitant with the appearance of high levels of the HMGI proteins, there is a decrease in HMG14 and HMG17. This is visually evident from the gels of Figures 1–4. It is possible, therefore, that the HMGI proteins could be replacing the structurally similar HMG14 and 17 proteins on the nucleosomes in undifferentiated and transformed cells. Given that the HMG14 and 17 proteins may be specifically associated with transcriptionally active genes (Weisbrod, 1982), this replacement could result in the changes in gene expression seen with transformed cells. It should be pointed out that extraction of HMGI proteins from ASV-transformed fibroblast nuclei does not alter the structure of active genes as revealed by DNase I digestion studies (Goodwin *et al.*, 1985b). Another possibility is that, although high levels of the HMGI proteins are not apparently required for cell growth

*per se* (since normal epithelial cells growing in culture do not have high levels of HMGI proteins), replacement of HMG14 and 17 by the HMGI proteins may alter the growth properties of the cells in some way. Since the HMGI proteins are phosphoproteins, like HMG14 they could be substrates for cyclic nucleotide-dependent kinases (Walton *et al.*, 1984). Thus cyclic nucleotides may exert their mitogenic effects via the phosphorylation of the HMG14 protein in normal cells and via the HMGI proteins in transformed and undifferentiated cells. It is of interest to note that the HMGI-like proteins resemble the *c-myc* proto-oncogene nuclear protein, a phosphoprotein which is expressed at high levels in undifferentiated cells and declines when cells terminally differentiate (Reitsma *et al.*, 1983; Gonda and Metcalf, 1984; Lackman and Skoultschi, 1984; Dyson *et al.*, 1985; Gross and Pitot, 1985).

## Materials and methods

The characterization and the establishment of the epithelial rat thyroid FRTL5-C12 and PC-C13 cell lines and the fibroblastic FRT-Fibro cell line have been described previously (Ambesi-Impombati *et al.*, 1980; Fusco *et al.*, 1985a,b). The epithelial cells were cultivated in Coons modified Ham's F12 medium supplemented with 5% calf serum and six growth factors: the thyrotropic hormone (TSH), insulin, transferrin, somatostatin, hydrocortisone and the tripeptide glycyl-histidyl-lysine. FRT-Fibro were cultivated in the same medium supplemented with 5% fetal calf serum without the addition of the growth factors. Both normal and infected cells were grown to confluence and left for >24 h before harvesting. In experiments using the <sup>32</sup>P label, the culture medium was replaced by one lacking phosphate 6 h before harvesting. Four hours before harvesting, the medium was replaced with one including 40  $\mu$ Ci of <sup>32</sup>PO<sub>4</sub> per ml.

HMG proteins with histone H1 were extracted from whole cells or from isolated nuclei with 5% perchloric acid (Giancotti *et al.*, 1985) and precipitated with acetone:HCl. H.p.l.c. was carried out as described previously using a BioRad Hipore butyl reverse phase column (4.6  $\times$  250 mm) (Goodwin *et al.*, 1985a). H1/HMG (3 mg) was loaded in 1 ml of buffer A [0.1% (v/v) trifluoroacetic acid], the column washed with 3 ml of 8% buffer B and then HMG14, HMG17 and the HMGI proteins eluted with a 30-ml gradient of 8–30% buffer B. Buffer B is 95% (v/v) acetonitrile in water, 0.1% (v/v) trifluoroacetic acid. Proteins were analysed using acid-urea and SDS single- and two-dimensional electrophoresis as described previously (Giancotti *et al.*, 1985). In order to compare perchloric acid extracts from different tissues on two-dimensional gels equal masses of protein (determined by preliminary electrophoresis and staining of gels) were loaded onto the gels and stained with Coomassie Blue.

N-terminal amino acid sequences were analysed using the applied Biosystems 470A gas phase sequencer and the PTH-amino acid residues determined by the Applied Biosystems 120A amino acid analyser.

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