The EMBO Journal Vol.2 No.11 pp.1963 – 1968, 1983

Human-mouse hybrids with an embryonal carcinoma phenotype continue to transcribe HLA-A,B,C

F.J.Benham*, M.A.Quintero[1] and P.N.Goodfellow

Human Molecular Genetics Laboratory, Imperial Cancer Research Fund, Lincoln's Inn Fields, London, WC2A 3PX, UK

Communicated by L.V.Crawford
Received on 1 July 1983; revised on 8 August 1983

We previously constructed a hybrid cell line, MCP6, which contains an X/6 translocation chromosome as its sole human genetic component in a mouse embryonal carcinoma (EC) cell background. This chromosome, which carries the major histocompatibility complex (MHC) originated from a human B cell which expresses class I and class II MHC antigens. EC cells do not express class I or class II antigens on their cell surface. Northern blot analysis has now shown that in the MCP6 hybrid, human class I genes, i.e., HLA-A,B,C, continued to be transcribed, and cellular levels of the transcripts were similar to, or only slightly lower than, levels in hybrids with a non-EC phenotype. However, very low levels of mRNA species recognised by a mouse class I gene (H-2) probe were also detected in EC cells and EC hybrids. Comparison of the relative levels of H-2 and HLA class I gene transcripts in the EC hybrids and non-EC hybrids indicated that the introduced HLA-A,B,C genes were not appropriately regulated in the EC cell but were subject at least in part to *cis* control. In contrast to the class I genes, no class II gene (i.e. HLA-DRα) transcripts were detected in MCP6. Hybrid EC lines thus provide a system to investigate the different levels of control of MHC gene expression during development and may help to elucidate mechanisms whereby the embryonic genome programs expression of differentiated cell functions.

Key words: HLA expression/teratocarcinoma hybrids

Introduction

Mechanisms which control gene activity during early embryonic mammalian development are not well understood. Introduction of foreign genetic material into mouse embryonal carcinoma (EC) cell lines provides a means of investigating gene regulation in embryogenesis, as these EC lines share many properties with early embryonic cells, including the capacity to differentiate *in vivo* and *in vitro*, and have been widely used as models for early development (for review, see Martin, 1980). Exogenous genetic material has been introduced into mouse EC cells in the form of RNA and DNA tumour viruses (Peries *et al.*, 1977; Swartzendruber and Lehman, 1975; Teich *et al.*, 1977; Boccara and Kelly, 1978; Stewart *et al.*, 1982; Gautsch and Wilson, 1983), cloned gene sequences (Wagner and Mintz, 1982; Bucchini *et al.*, 1983) and by somatic cell hybridisation (Miller and Ruddle, 1976, 1977; McBurney, 1977; Andrews and Goodfellow, 1980; Rousset *et al.*, 1980; Gmur *et al.*, 1980; Benham *et al.*, 1983).

We have previously constructed a series of human/mouse somatic cell hybrid lines which contain a single human chromosome within a mouse EC cell background, in this case PCC4 AO (henceforth referred to as PCC4) (Goodfellow *et al.*, 1982; Benham *et al.*, 1983). These hybrid lines retain the phenotypic characteristics of the parental EC line, including the ability to differentiate *in vitro* (Benham *et al.*, 1983). One of these lines, MCP6, contains, as its sole human genetic material, an X/6 translocation chromosome, and this chromosome carries the complete major histocompatibility complex (MHC) (Goodfellow *et al.*, 1982). The MHC has been studied extensively in mouse and man, and detailed genetic and biochemical information about the genes and gene products of this system is available (for review, see Bodmer, 1981). Thus the MCP6 line provides a tool to investigate mechanisms which control expression of specific genes within the MHC during development. Class I genes of the MHC (H-2 K and D antigens in mouse and HLA-A,B,C antigens in man) are expressed at the cell surface of most nucleated cells in association with β_2-microglobulin. Cells which form exceptions to this general rule and do not express cell surface class I antigens include human trophoblast (Goodfellow *et al.*, 1976; Page-Faulk *et al.*, 1977) and mouse EC cells (Artzt and Jacob, 1974), and the weight of evidence indicates that these antigens are probably not expressed in mouse embryogenesis until post-implantation development (reviewed by Erikson, 1983). In contrast with class I antigens, class II antigens (Ia in mouse, DR in man) show a very restricted tissue distribution and are expressed mainly in cells of lymphoid origin.

Regulation of H-2K and D antigen expression has previously been investigated in intra-specific somatic cell hybrids made between non-expressing mouse EC cells and antigen expressing mouse thymocytes or splenocytes. In hybrids made by some investigators, expression of H-2K and D appeared to be subject to *cis* regulation, i.e., antigens of the thymocyte haplotype continued to be expressed whereas antigens of the EC haplotype remained unexpressed (Miller and Ruddle, 1977; Andrews and Goodfellow, 1980; Correani and Croce, 1980). However, other investigators reported conflicting results: inhibition of splenocyte H-2K and D antigen expression was observed in hybrids (Rousset *et al.*, 1980; Gmur *et al.*, 1980). The reasons for these different findings are not clear but may include the sensitivity of the assays used and chromosomal segregation. Introduction into an EC cell of the MHC from a different species, such as in the human/mouse MCP6 hybrid, has the advantage that the introduced material can be readily distinguished from the mouse EC cell counterpart at the DNA, the RNA and the gene product levels. In addition, stable retention in the hybrid of the human chromosome containing the MHC is achieved by HAT selection by virtue of its carrying the X-linked HPRT gene.

The human chromosome present in the MCP6 hybrid originally came from an adult B cell lymphoid line which expresses both class I (HLA-A,B,C) and class II (HLA-DR) antigens at the cell surface. We previously showed by radioimmunoassay that the MCP6 hybrid expresses neither mouse H-2 nor human HLA class I antigens at the cell surface (Goodfellow *et al.*, 1982). However, HLA-A,B,C heavy chains were found in the cell cytoplasm, suggesting that

[1]Present address: Laboratorio de Fisiopatologia, Departamento de Medicina Experimental, I.V.I.C., Apto. 1827, Caracas 1010A, Venezuela.
*To whom reprint requests should be sent.

Table I. Origins and phenotypes of somatic cell hybrid and parent cell lines

		Phenotype	Human chromosomes present	MHC transcription summary			Reference
				HLA class I	HLA class II	H-2 class I	
Hybrid							
GIR6	Fusion between G3.32.2 and the mouse L cell 1R	L cell	X/6[a], 15, plus others	+	−	+ + +	Goodfellow *et al.*, 1982
MCP6	Microcell transfer from GIR6 to PCC4	EC	X/6[a] only	+	−	±	Goodfellow *et al.*, 1982
MP1	Retinoic acid induced differentiated derivative of MCP6	? fibroblastic	X/6[a] only	+	−	±	Benham *et al.*, 1983
MP2	Retinoic acid induced differentiated derivative of MCP6	parietal endoderm	X/6[a] only	+	−	±	Benham *et al.*, 1983
PCG.G6	Fusion between G3.32.2 and PCC4	EC	X/6[a] and 15	+	−	±	Quintero, 1983
PC21A1	Fusion between 2102Ep and PCC4	EC	X, 11, 15, 17	−	−	±	Benham *et al.*, 1983
GBS1	Fusion between G3.32.2 and BAL17	B cell	X/6 plus others	nd	+	nd	Quintero, 1983
GBS1R	Revertant of GBS1	B cell	Several autosomes, no X/6 or 6	nd	−	nd	Quintero, 1983
Parent							
PCC4	Mouse teratocarcinoma	EC	−	−	−	±	Jakob *et al.*, 1973
G3.32.2	Human Burkitt's Lymphoma	B cell	aneuploid	+ + +	+ + +	−	Povey *et al.*, 1973
2102Ep	Human testicular teratocarcinoma	EC	aneuploid	+ +	−	−	Andrews *et al.*, 1982
BAL17R1	Mouse B cell lymphoma	B cell	−	−	−	nd	Kim *et al.*, 1978
RAG	Mouse adenocarcinoma	−	−	−	−	+ + +	Klebe *et al.*, 1970

[a]The X/6 (Xqter-Xq13:6p21-6qter) translocation is described in detail in Goodfellow *et al.*, 1982
nd = not done

neither transcription nor translation of the HLA-A,B,C genes was extinguished by being in the EC cell, and that absence of cell surface expression was due to a lack of sufficient β_2-microglobulin. Here, we present direct evidence from Northern blot analysis that the HLA-A,B,C genes are transcribed in the MCP6 hybrid, and comparison of the relative levels of HLA and H-2 class I gene transcripts in this and various other hybrids suggested that the EC cell cannot appropriately regulate the introduced class I genes, nor can the introduced genetic material regulate, i.e., increase expression of, the homologous H-2 genes of the EC cell. In contrast, the HLA-DRα (a class II gene) was not transcribed in MCP6. Thus, different genes of the MHC may be subject to different levels of regulation during development, and the EC hybrids such as MCP6, in which genes are introduced into an embryonic-like cell but are still contained within their differentiated chromosomal environment, provide a tool to investigate these different mechanisms.

Results

Table I lists the origins and phenotypes of the somatic cell hybrid lines used in these investigations. The MCP6 hybrid was made by the microcell fusion technique in which the human chromosome donor cell was a human/mouse hybrid line (GIR 6), made by fusing a mouse L cell with a human B cell. The human MHC is carried on the portion of the X/6 translocation chromosome which is the sole human genetic component in the MCP6 hybrid line, and karyotype and Southern blot analysis using cloned MHC genes as probes has previously shown that the human chromosome remains intact (Goodfellow *et al.*, 1982; Trowsdale *et al.*, 1983). This line retains the EC phenotypic characteristics of the mouse parent cell PCC4 (Benham *et al.*, 1983). Northern blot analysis using the HLA-A,B,C cDNA clone pHLA-A as probe revealed the presence of a 1.6-kb mRNA species in the MCP6 hybrid (Figure 1). This 1.6-kb transcript was also present in the following cells: the human B cell parent of MCP6 called G3.32.2, in other HLA-A,B,C expressing human lines such as fibroblasts, in the hybrid GIR6, the chromosome donor for MCP6 which has a non-EC phenotype and which contains several other human chromosomes, and in the hybrid PCG.G6 which is another hybrid with EC morphology made by direct fusion of PCC4 with the human line G3.32.2 and which contains the same X/6 translocation as MCP6. The blots show that the abundance of the 1.6-kb HLA-A,B,C transcript is much greater in the human cells than in the hybrid lines MCP6, PCG.G6 and GIR6, a finding consistent with observations that human gene products in rodent-human hybrids are generally found at lower levels than in human cultured cells (S.Povey, personal communication). Two constitutively differentiated cell lines, MP1 and MP2, derived from the MCP6 hybrid following culture with retinoic acid were also shown to have HLA-A,B,C transcripts, at levels appropriately equivalent to those in the MCP6 EC progenitor. No mRNA species recognised by the pHLA-A probe were detected in the mouse EC line PCC4 or in the hybrid line PC21A1, which has PCC4 as its mouse parent and retains a characteristic EC phenotype but which does not have the human MHC chromosomal material.

Class I antigens of the MHC in mouse, H-2K and D, have not been detected on the cell surface of mouse EC cells. We used an H-2 class I cDNA probe, pH-211, to determine whether any mouse class I transcripts were present in the PCC4 parent cells or the hybrids with an EC phenotype. On Northern blots (Figure 2) this probe hybridised to a unique mRNA species of 1.7-kb in all non-EC mouse cells examined, such as the adenocarcinoma-derived line RAG (Klebe *et al.*, 1970). A very weak cross-reaction of this probe with the slightly smaller, 1.6-kb human HLA-A,B,C mRNA was seen in human cells where the HLA-A,B,C mRNA is very abundant (e.g., G3.32.2). On short exposures (e.g., 1 − 2 days) no

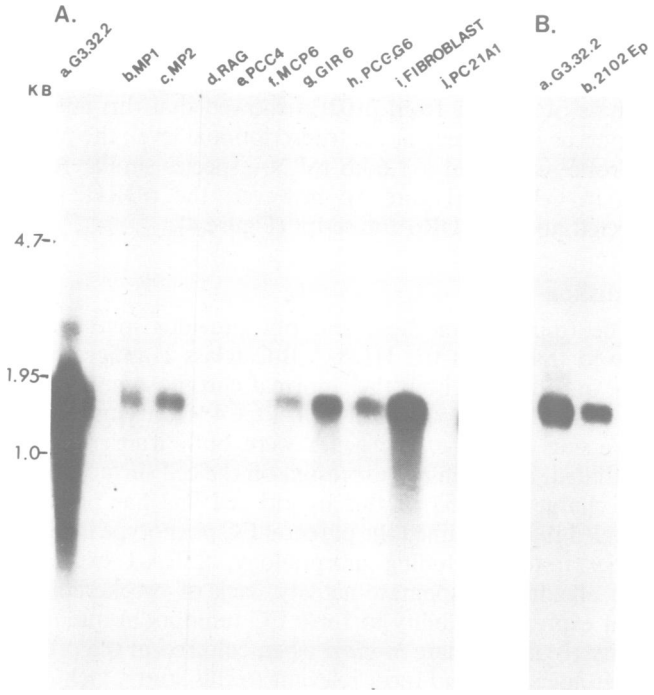

Fig. 1. Northern blot analysis showing poly(A)$^+$ mRNA species recognised by the HLA class I probe, pHLA-A. **(A)** Filter exposed for 7 days; **(B)** filter exposed for 1 day. **(A) (a)** G3.32.2 (human B cell); **(b)** MP1 (differentiated hybrid containing X/6); **(c)** MP2 (differentiated hybrid containing X/6); **(d)** RAG (mouse control); **(e)** PCC4 (mouse EC cell); **(f)** MCP6 (EC hybrid containing X/6); **(g)** GIR6 (non-EC hybrid containing X/6); **(h)** PCG.G6 (EC hybrid containing X/6); **(i)** human fibroblast; **(j)** PC21A1 (EC hybrid containing X/6). **(B) (a)** G3.32.2 (human B cell); **(b)** 2102Ep (human teratocarcinoma).

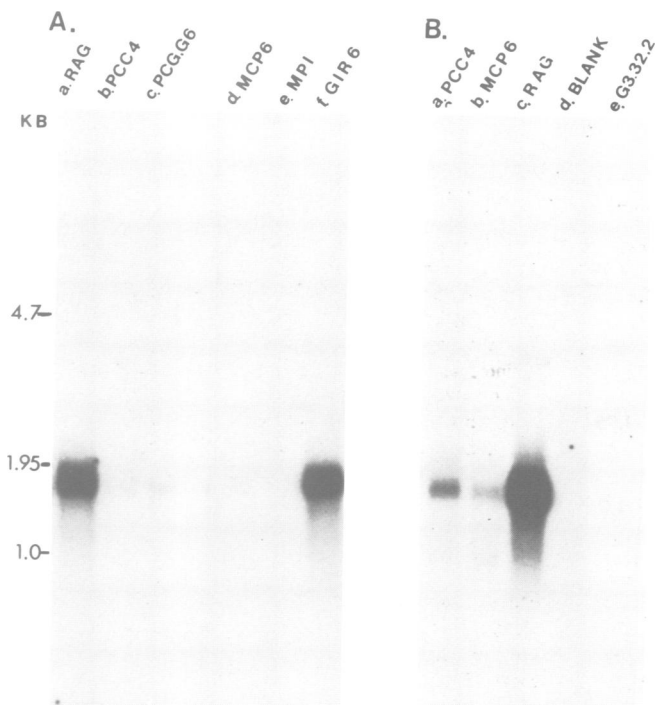

Fig. 2. Northern blot analysis showing poly(A)$^+$ mRNA species recognised by the H-2 class I probe, pH-211. **(A)** filter exposed for 7 days; **(B)** filter exposed for 14 days. **(A) (a)** RAG (mouse control); **(b)** PCC4 (mouse EC cell); **(c)** PCG.G6 (EC hybrid containing X/6); **(d)** MCP6 (EC hybrid containing X/6); **(e)** MP1 (differentiated hybrid containing X/6); **(f)** GIR6 (non-EC hybrid containing X/6). **(B) (a)** PCC4 (mouse EC); **(b)** MCP6 (EC hybrid containing X/6); **(c)** RAG (mouse control); **(d)** blank **(e)** G3.32.2 (human B cell).

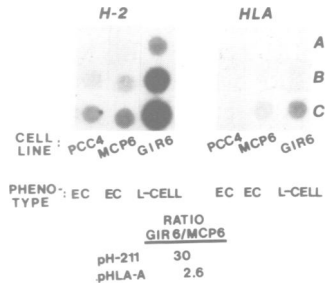

Fig. 3. Dot blot analysis of H-2 (pH-211 probe) and HLA (HLA-A probe) class I mRNA. **(A)** 0.1 μg poly(A)$^+$ mRNA; **(B)** 0.5 μg poly(A)$^+$ mRNA; **(C)** 1.0 μg poly(A)$^+$ mRNA. The ratio given from densitometer scans of the dot blots in the linear range is an average of four separate determinations.

bands were seen in tracks containing 5 μg of poly(A)$^+$ mRNA from PCC4. However, longer exposures (3 – 10 days) revealed a weak 1.7-kb H-2 mRNA species. Weak bands of similar intensities were seen in the EC hybrids MCP6, PCG.G6, PC21A1 and in MP2, the differentiated derivative of MCP6 which has a parietal endoderm phenotype. Another derivative, MP1, which has a fibroblast-like morphology showed no detectable mRNA recognised by the pH-211 probe. It is possible that the low level of H-2 class I mRNA seen in cells with an EC phenotype, both the PCC4 parent and hybrids, was due to the appearance of a small proportion of differentiated cells in the cultures as they became confluent, although no differentiated derivatives were seen by microscopic examination. We therefore isolated mRNA from both sparse, subconfluent cultures and from confluent cultures and compared the H-2 class I mRNA levels on Northern blots. However, no difference in levels was observed between the two types of cultures, suggesting that the EC cells do synthesise low levels of H-2 class I mRNA (data not shown). This interpretation is supported by the finding of similarly low levels of H-2 class I mRNA in F9 cells, an EC line which differentiates very poorly without the addition of inducers (Sherman and Miller, 1978).

In the hybrid with a non-EC phenotype, GIR6, (and other such hybrids, data not shown), H-2 class I mRNA was present at much higher levels than in the EC cells, approximating levels in the mouse parent cells.

The Northern blots indicated that the steady state levels of HLA class I mRNA in both the EC hybrid MCP6 and in the non-EC hybrid GIR6 were apparently much lower than the mRNA levels in the parental human cells. In contrast, H-2 class I mRNA levels apparently remained high (i.e., equivalent to parental cells) in hybrids with a non-EC phenotype, whereas all EC hybrids showed very low H-2 mRNA levels. This observation suggested that the lower level of HLA class I mRNA in the EC hybrid MCP6 compared with the parental human cell was at least partly due to the hybrid nature of the cell, rather than to the specific EC background. To quantitate this observation, dot blot hybridisations were used to measure and compare the ratios of HLA and H-2 class I mRNA in GIR6 and MCP6. Figure 3 shows the results of a typical experiment in which poly(A)$^+$ mRNA from PCC4, MCP6 and GIR6 was probed with the H-2 or HLA

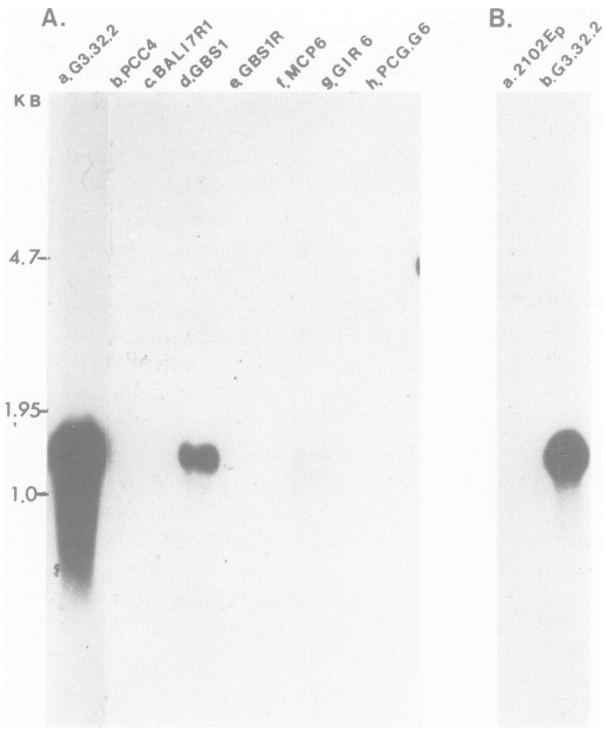

Fig. 4. Northern blot analysis showing poly(A)$^+$ mRNA species recognised by the HLA-DRα clone, pDRH2. **(A)** Filter exposed for 7 days; **(B)** filter exposed for 3 days. **(A) (a)** G3.32.2 (human B cell); **(b)** PCC4 (mouse EC cell); **(c)** BAL17R1 (mouse B lymphoma); **(d)** GBSI (B cell hybrid containing X/6); **(e)** GBSIR (B cell hybrid not containing X/6); **(f)** MCP6 (EC hybrid containing X/6); (g) GIR6 (non-EC hybrid containing X/6); **(h)** PCG.G6 (EC hybrid containing X/6). **(B) (a)** 2102Ep (human teratocarcinoma); **(b)** G3.32.2 (human B cell).

class I cDNA clones. Densitometer tracing of the blots showed that the levels of H-2 class I mRNAs were ~30-fold lower in MCP6 than in GIR6, whereas levels of HLA class I mRNA were only 2.6 times lower in MCP6 (and PCG.G6) than in GIR6. These results confirmed the Northern blot analysis, and in addition, showed that levels of H-2 mRNA were equivalent in PCC4 and the EC hybrids MCP6, PC21A1 and PCG.G6.

Northern blot analysis using the HLA-DRα cDNA clone, pDRH2, as probe revealed the presence of a 1.5-kb mRNA species in the human B cell line G3.32.2 (Figure 4). mRNA isolated from other human cell types which do not express DRα at the cell surface did not show any bands with this probe, as expected. The EC hybrids MCP6 and PCG.G6 also showed no detectable HLA-DRα transcripts, even after prolonged exposure. The MCP6 hybrid has previously been shown to contain the DNA sequence which codes for the DRα gene, and the chromosomal arrangement in MCP6 of the DRα gene is identical to that in the human parent cell as far as can be detected by Southern blot analysis. The non-EC hybrid GIR6 also failed to show any HLA-DRα mRNA species. In contrast, hybrids with a B cell-like phenotype constructed between G3.32.2 and the Ia antigen-positive mouse B cell lymphoma line BAL17R, called GBS1, did have the 1.5-kb HLA-DRα transcript. The transcript was not present in a segregant of GBS1, GBS1R, which had lost the human X/6 translocation chromosome and is HLA-DRα negative.

Detailed analysis by Andrews *et al.* (1983) has shown that the human testicular teratocarcinoma derived cell line, 2102Ep, expresses low levels of HLA-A,B,C antigens at the cell surface, but in common with most other non-lymphoid cells, does not express HLA-DRα antigens. Northern blot analysis of mRNA from 2102Ep showed that this antigenic phenotype is also seen at the transcriptional level: the pHLA-A probe recognised a 1.6-kb mRNA species similar to that seen in G3.32.2 (Figure 1); however, the pDRH2 probe detected no HLA-DRα transcript (Figure 4).

Discussion

The results from these and our previous investigations showed that when the HLA-A,B,C (class I antigen) genes were introduced within their normal chromosomal environment into the mouse EC cell PCC4 from an antigen-expressing cell type, the genes were both transcribed and translated, although not expressed on the cell surface. Extensive characterisation of this hybrid cell line has previously revealed that it retained the parental EC phenotype in all properties tested, including morphology, SSEA-1 expression, high alkaline phosphatase activity, lack of cytokeratin filament expression, ability to form EC tumours in nude mice, ability to differentiate *in vitro* when cultured in the presence of retinoic acid, and most relevant to this study, lack of cell surface H-2 expression (Benham *et al.*, 1983). Previous detection in the MCP6 cell cytoplasm of HLA-A,B,C heavy chains had suggested that the HLA-A,B,C genes were under *cis* regulation (Goodfellow *et al.*, 1982). Detection of HLA-A,B,C mRNA transcripts supports this hypothesis. However, the detection of very small amounts of mRNA species recognised by an H-2 class I gene probe in the mouse EC lines PCC4 and F9 and in the EC hybrids raised the possibility the that the EC cell was in fact appropriately regulating transcription of the HLA-A,B,C genes, especially since the HLA-A,B,C steady-state transcripts were less abundant in the EC hybrid than in the original human parent cell. However, further quantitative experiments indicated that the most likely interpretation of the data is still that the introduced HLA-A,B,C genes are refractory to EC cell regulation and are at least partly under some form of *cis* control, and that the reduced levels of mRNA in MCP6 compared with the parent cell are mainly due to the hybrid nature of the cell rather than to regulation by the EC background. This interpretation is supported by two main lines of evidence. Firstly, steady state levels of H-2 class I mRNA in the EC hybrids MCP6 and PCG.G6 were ~30-fold lower than in the non-EC hybrid, GIR6, whereas in contrast, levels of HLA class I mRNA in MCP6 and PCG.G6 were only ~2.5-fold lower than those in GIR6, which contains the same X/6 translocation chromosome. The reason for the 2.5-fold lower levels of HLA-A,B,C mRNA in the EC hybrids as compared with the non-EC hybrid are unclear, but may involve gene dosage, i.e., the loss of the homologue (chromosome 6) present in GIR6, differential stability of the mRNA in different cell backgrounds, or partial regulation of the genes by the EC cell control mechanisms. Secondly, in the hybrid PCG.G6, which is similar to MCP6 except that it also contains human chromosome 15, HLA-A,B,C antigens were detected at the cell surface in association with the chromosome 15-coded β_2-microglobulin (Benham *et al.*, 1983). In contrast, however, cell surface H-2 class I antigens were not detected, suggesting that levels of H-2 heavy chains were much lower than levels of HLA heavy chains and thus insufficient to be detected in association with β_2-microglobulin on the cell surface. Alter-

natively, it is possible that the mRNA species recognised by the H-2 class I gene probe pH-211 in the EC cells are transcribed from class I genes not normally expressed in adult cells.

The presence of active HLA-A,B,C genes in the EC hybrids did not show any evidence of dominant or *trans* influence on the expression of the homologous mouse class I genes, since levels of H-2 class I transcripts were similar in all the EC cells examined, including the PCC4 parent, hybrids which contained the human MHC, and hybrids which did not contain the human MHC.

A different, though not necessarily exclusive interpretation of the HLA-A,B,C expression data is that HLA-A,B,C antigens are expressed in human embryonic cells of the stage represented by the typical mouse EC cell. The presence of HLA-A,B,C on the human testicular teratocarcinoma-derived cell line 2102Ep is consistent with this notion, but until more is known about human embryology this question will remain unresolved.

In contrast with the HLA-A,B,C genes, the DRα (class II) gene present in the MHC and expressed in the human parental cell was not transcribed at detectable levels in the MCP6 or the PCG.G6 hybrids. However, the absence of expression was not specific to the EC cell background, since no HLA-DRα transcripts were detected in the GIR6 hybrid. Nevertheless, this gene was capable of continued expression in hybrids which were made by fusing the G3.32.2 human parent with a mouse B cell line which expresses the equivalent mouse class II genes. Thus, the HLA-DRα gene falls into the category of specialised function genes whose expression is extinguished when cells of two different differentiated phenotypes are fused to form hybrids (reviewed in Ringertz and Savage, 1976). In contrast, the class I genes did not show extinction at the transcriptional level when introduced into cells not expressing adult class I antigens, a finding which suggests that these genes, unlike their class II counterparts, may be subject to irreversible *cis* regulatory mechanisms. This EC hybrid cell and its related lines thus provide a tool to dissect the mechanisms whereby tissue-related gene expression is controlled. It may be possible to combine this hybrid system with other systems previously used to analyse HLA transcription, such as the induction with interferon (Burrone and Milstein, 1982).

Clues to mechanisms by which gene expression is controlled during embryogenesis have been obtained from experiments where exogenous DNA or RNA viruses have been introduced into embryonic or EC cells. Such cells are unable to express viral functions, although proviral or viral sequences do become integrated into the host genome (Swartzendruber and Lehman, 1975; Jaenisch *et al.*, 1981; Jaenisch, 1980; Teich *et al.*, 1977). In contrast, differentiated cells do express viral functions, indicating that pluripotent cells differ from their differentiated counter-parts in mechanisms which promote at least some types of gene expression (Swartzendruber and Lehman, 1975; Boccara and Kelly, 1978; Jaenisch, 1980; Jaenisch *et al.*, 1981). Differences in DNA methylation processes in the two types of cells are thought to be partly responsible (Jahner *et al.*, 1982; Stewart *et al.*, 1982; Gautsch and Wilson, 1983), but additional, though as yet undefined, processes may be involved (Gautsch and Wilson, 1983). EC hybrids such as the MCP6 cell line provide an additional system for investigation of such processes. Preliminary results from methylation studies in our laboratory suggest that the introduced HLA-A,B,C genes are hypermethylated in EC cells as compared with the cell of origin, the human B cell. This observation is consistent with the studies suggesting that methylation of DNA sequences is not the only mechanism for regulating suppression of gene expression (Gautsch and Wilson, 1983). The EC hybrids are capable of at least limited differentiation *in vitro* and *in vivo* (Benham *et al.*, 1983), thus it should be possible to determine the extent to which the EC cell can program and/or reprogram the genes contained on chromosomes derived from a differentiated cell. Attempts in our laboratory to obtain differentiated derivatives of the PCC4 or the PCC4 hybrids which express H-2 class I or class II antigens have so far been unsuccessful; however other EC lines are available which do differentiate into H-2 expressing cell types (McBurney *et al.*, 1982), and these are now being used in our experiments. Recently, a cloned mammalian gene sequence coding for β-globin has been introduced into and expressed in EC cells (Pellicer *et al.*, 1980; Wagner and Mintz, 1982). Comparison of expression patterns of such integrated gene sequences with those of genes introduced within their chromosomal environment may help to define mechanisms, including those which act by local chromosomal influence, by which embryonic cells program their own differentiation.

Materials and methods

Cell culture

Cells were grown in Dulbecco's modified Eagles medium, or RPMI 1640 (G3.32.2, GIR6, GBS1, GBS1R and PCG.G6), supplemented with 10% foetal bovine serum, penicillin and streptomycin. Where appropriate, retention of the human HPRT gene was ensured by supplementing the medium with 100 μM hypoxanthine, 10 μM methotrexate and 16 μM thymidine. Cells which grew attached were subcultured with trypsin: EDTA (0.25% and 0.1%, respectively). Cells which grew in suspension (G3.32.2, GBS1 and GBS1R) were subcultured by dilution.

Cells

The human cell lines used were the following: N2102Ep2A6 (referred to as 2102Ep), a clonal derivative of a human testicular teratocarcinoma (Andrews *et al.*, 1983); and G3.32.2, a B cell lymphoid line derived from a Burkitt's lymphoma and which is probably a subline of Jijoye (Povey *et al.*, 1973). G3.32.2 is the original donor of the human X/6 translocation chromosome. The mouse cells were: PCC4AO (referred to as PCC4), a ouabain resistant clone of PCC4Aza^R (Jakob *et al.*, 1973); and BAL17R1, a 6-thioguanine resistant clone of BAL17, an Ia-expressing B cell lymphoma (Kim *et al.*, 1978). The origins and phenotypes of the human-mouse somatic cell hybrids are described in Table I.

DNA clones

The clone pHLA-A [gift of J.Trowsdale (ICRF, London)] contains a portion of a human HLA class I gene cloned into the vector pAT. The insert is a cDNA sequence which encodes ~100 amino acids of the COOH terminus of an HLA-A,B,C molecule, probably HLA-A2 plus ~300 nucleotide pairs of 3'-non-coding region. The clone pDRH2 contains a nearly full length cDNA copy of the human HLA-DRα gene cloned into the vector pAT 153 (Lee *et al.*, 1982). For probing Northern and dot blots, the human inserts were isolated and labelled with [^{32}P]dCTP by nick-translation (Rigby *et al.*, 1977) to obtain a specific activity of at least 5 x 10^8 c.p.m./μg. The clone pH-211 (Steinmetz *et al.*, 1981) contains a cDNA copy of a portion of an H-2 class I gene. The plasmid contains an insert 1150 nucleotide pairs of which code for amino acids 107–352 of a class I heavy chain molecule plus 600 nucleotide pairs of the untranslated region. The plasmid DNA was kindly provided by A.Mellor. Nick-translation of the plasmid DNA yielded a specific activity 10^9 c.p.m./μg.

RNA isolation

Total cell RNA was obtained by homogenising freshly harvested cells in a 4 M guanidinium isothiocyanate and sedimenting out the RNA on a 5.7 M CsCl gradient (Chirgwin *et al.*, 1979). The polyadenylated mRNA fraction was separated by oligo(dT)-cellulose chromatography of the total RNA (Efstratiadis and Kafatos, 1976).

Northern blotting

5 μg of poly(A)$^+$ mRNA was denatured by treatment with 1 M glyoxylic acid and was separated by electrophoresis through a 1.1% agarose gel as described by Thomas (1980). The RNA was transferred by blotting to nitrocellulose filters, and the filters were probed with the appropriate ^{32}P-labelled DNA probe under hybridisation conditions essentially as described by Thomas (1980), but without the addition of dextran sulfate. The non-hybridised radioactivity was eliminated by washing the filters to 0.1 x SSC with 0.1% SDS at 50°C. Filters were exposed to Fuji X-ray film at −70°C with intensifying screens from 1 to 10 days. The sizes of the mRNA species recognised by the labelled probes were determined from a calibration curve made from the mobilities of fragments of rRNA of known mol. wt. from 2−5 A (oligonucleotide) treated mouse cell lysates (gift of R.Silverman, ICRF, London).

Dot blot hybridisations

Poly(A)$^+$ mRNA (0.25−2.5 μg) was spotted onto nitrocellulose filters previously soaked in 5 x SSC, using a Schleicher and Schuell Minifold apparatus. The filters were baked and hybridised to ^{32}P-labelled DNA as described for Northern blot analysis. The X-ray films were exposed for 2−6 days and scanned using a Joyce-Lobel, Chromoscan 3, Densitometer. The relative intensities of the signals from each spot were assessed by calculating the area under the signal peaks.

Acknowledgements

We thank Drs. S.Povey, D.Swallow and E.Solomon for help with characterisation of the hybrids, Drs. J.Trowsdale and J.Lee for providing the plasmids, and M.Wiles and G.Banting for technical assistance.

References

Andrews,P.W. (1983) in Damjanov,I., Knowles,B.B. and Solter,D. (eds.), *Human Teratomas*, Humana Press, NJ, pp. 285-311.
Andrews,P.W. and Goodfellow,P.N. (1980) *Somatic Cell Genet.*, **6**, 271-284.
Artzt,K. and Jacob,F. (1974) *Transplantation*, **17**, 632-634.
Benham,F.J., Wiles,M.V. and Goodfellow,P.N. (1983) *Mol. Cell Biol.*, in press.
Boccara,M. and Kelly,W. (1978) *Ann. Microbiol. (Paris)*, **129A**, 227-238.
Bodmer,W.F. (1981) in Russell,E.M. (ed.), *Mammalian Genetics and Cancer: The Jackson Laboratory 50th Anniversary Symposium*, Alan R.Liss, NY, pp. 213-239.
Bucchini,D., Lasserre,C., Kunst,F., Lovell-Badge,R., Pictet,R. and Jami,J. (1983) *EMBO J.*, **2**, 229-232.
Burrone,O.R. and Milstein,C. (1982) *EMBO J.*, **1**, 345-349.
Chirgwin,J.M., Przybyla,A.E., MacDonald,R.J. and Rutter,J. (1979) *Biochemistry (Wash.)*, **18**, 5294-5299.
Correani,A. and Croce,C.M. (1980) *J. Cell. Physiol.*, **105**, 73-99.
Efstratiadis,A. and Kafatos,F.C. (1976) in Last,J. (ed.), *Methods in Molecular Biology*, Vol. **8**, Dekker, NY, pp. 1-124.
Erikson,R. (1983) in Goodfellow,P.N. (ed.) *Receptors and Recognition: Series B*, Vol. **13**, Chapman and Hall, London, in press.
Gautsch,J.W. and Wilson,M.C. (1983) *Nature*, **301**, 32-37.
Gmur,R., Solter,D. and Knowles,B.B. (1980) *J. Exp. Med.*, **151**, 1349-1358.
Goodfellow,P.N., Barnstable,C.J., Bodmer,W.F., Snary,D. and Crumpton, M.J. (1976) *Transplantation*, **22**, 595-603.
Goodfellow,P.N., Banting,G., Trowsdale,J., Chambers,S. and Solomon,E. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 1190-1194.
Jaenisch,R. (1980) *Cell*, **19**, 181-188.
Jaenisch,R., Jahner,D., Nobis,P., Simon,I., Lohler,J., Harbers,K. and Grotkopp,D. (1981) *Cell*, **24**, 519-529.
Jahner,D., Stuhlmann,H., Stewart,C.L., Harbers,K., Lohler,J., Simon,I. and Jaenisch,R. (1982) *Nature*, **298**, 623-628.
Jakob,H., Boon,T., Gaillard,J., Nicolas,J.F. and Jacob,F. (1973) *Ann. Microbiol.*, **1246**, 269-282.
Kim,J., Wembaum,F.I., Matherson,B.J., Machaver,B.E. and Asofky,R. (1978) *J. Immunol.*, **121**, 339-344.
Klebe,J., Chen,T. and Ruddle,F.H. (1970) *J. Cell Biol.*, **45**, 74-82.
Lee,J., Trowsdale,J. and Bodmer,W.F. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 545-549.
McBurney,M.W. (1977) *Cell*, **12**, 653-662.
McBurney,M.W., Jones-Villeneuve,E.M.V., Edwards,M.K.S. and Anderson,P.J. (1982) *Nature*, **299**, 165-167.
Martin,G.R. (1980) *Science (Wash.)*, **209**, 768-776.
Miller,R.A. and Ruddle,F.H. (1976) *Cell*, **9**, 45-56.
Miller,R.A. and Ruddle,F.H. (1977) *Dev. Biol.*, **56**, 157-173.
Page-Faulk,W., Sanderson,A. and Temple,A. (1977) *Transplant. Proc.*, **9**, 1379-1384.
Pellicer,A., Wigler,M., Axel,R. and Silverstein,S. (1980) *Cell*, **14**, 133-141.
Peries,J., Alves-Cardoso,E., Canivet,M., Devons-Guillemin,M.C. and Lasneret,J. (1977) *J. Natl. Cancer Inst.*, **59**, 463-465.
Povey,S., Gardiner,S.E., Watson,B., Mowbray,S., Harris,H., Arthur,E., Steel,C.M., Blenkinsop,C. and Evans,H.J. (1973) *Ann. Hum. Genet.*, **36**, 247-266.
Quintero,M. (1983) D. Phil. Thesis, Oxford University.
Rigby,P.W.J., Dieckmann,M., Rhodes,C. and Berg,P. (1977) *J. Mol. Biol.*, **113**, 237-251.
Rousset,J.P., Jami,J., Dubois,P., Aviles,D. and Ritz,E. (1980) *Somatic Cell Genet.*, **6**, 419-433.
Sherman,M.I. and Miller,R.A. (1978) *Dev. Biol.*, **63**, 27-34.
Steinmetz,M., Frelinger,J.G., Fisher,D., Hunkapiller,T., Pereira,D., Weissman,S.H., Uehara,H., Nathenson,S. and Hood,L. (1981) *Cell*, **24**, 125-131.
Stewart,C.L., Stuhlman,H., Jahner,D. and Jaenisch,R. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4098-4102.
Swartzendruber,D.E. and Lehman,J.M. (1975) *J. Cell Physiol.*, **85**, 179-188.
Teich,N.M., Weiss,R., Martin,G.R. and Levy,D.R. (1977) *Cell*, **12**, 973-982.
Thomas,P.S. (1980) *Proc. Natl. Acad. Sci. USA*, **77**, 5201-5205.
Trowsdale,J., Lee,J., Carey,J., Grosveld,F., Bodmer,J. and Bodmer,W. (1983) *Proc. Natl. Acad. Sci. USA*, **80**, 1972-1976.
Wagner,E.F. and Mintz,B. (1982) *Mol. Cell Biol.*, **2**, 190-198.