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Provisional Mapping of the Gene for a Cell Surface Marker, GA-1, in the Red-necked Wallaby *Macropus rufogriseus*

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Abstract

A series of *M. rufogriseus*–mouse somatic cell hybrids was constructed and analysed cytologically, enzymatically and immunologically. A monoclonal antibody, GA-1, was prepared against an *M. rufogriseus* cell surface antigen on an *M. rufogriseus*–mouse somatic cell hybrid. A gene determining the expression of this antigen was provisionally assigned to the long arm of the *M. rufogriseus* chromosome 3. The monoclonal antibody also reacted with an *M. rufus* (red kangaroo)–mouse somatic cell hybrid containing only the *M. rufus* chromosome 5, the G-banded chromosome identical to *M. rufogriseus* 3q. The results also suggest synteny of the genes for the marsupial enzymes hypoxanthine phosphoribosyltransferase and phosphoglycerate kinase-A.

Introduction

As a result of the rapid growth of information on mammalian gene maps, it has become apparent that groups of genes which are syntenic in one species may also be syntenic in other distantly related species (Human Gene Mapping 7 1984). Comparative gene mapping has provided a 'new' approach to phylogenetic studies (Roderick *et al.* 1984).

Most mammalian gene mapping to date has been carried out in eutherian mammals, in particular man and mouse; only a handful of gene-mapping studies have been reported for the other major extant group of mammals, the marsupials (Cooper *et al.* 1975; Graves *et al.* 1979; Donald and Adams 1981; Donald and Hope 1981). These two mammalian groups have evolved separately since they last shared a common ancestor, some 130 million years ago (Air *et al.* 1971) and it is therefore of particular interest to compare the chromosomal gene arrangements in eutherian and marsupial mammals.

Most of the genes assigned to mammalian chromosomes are structural genes for enzymic genetic markers. Somatic cell hybrids have also been used in the assignment to specific chromosomes of genes determining the expression of cell surface antigens (Buck *et al.* 1976; Kao *et al.* 1976). By using, for example, human–mouse cell hybrids as immunogens in the appropriate syngeneic mouse strain, the number of antigenic recognition sites is restricted to determinants encoded by genes on human chromosomes in the hybrids. Single antigenic specificities can then be detected on the hybrid cells using monoclonal antibodies (Köhler and Milstein 1976). Genes determining the expression of human X-linked cell surface antigens include *Xg* (Mann *et al.* 1962), *SA-X* (Buck and Bodmer 1976) and *MIC-5* (Hope *et al.* 1982) and on the basis of Ohno's theory of conservation of the mammalian X chromosome (Ohno 1967), homologous loci would be expected to exist in other mammals, including marsupials.

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Apart from the tentative assignment of an X-linked gene determining a cell surface antigen in *Macropus rufus* (Sykes and Hope 1978) no genes determining cell surface antigens have been assigned to a chromosome in any marsupial species.

In this study, a number of marsupial–mouse somatic cell hybrids were made. These were used as immunogens in the production of monoclonal antibodies to marsupial cell surface antigens. Along with cytological and immunological studies of these hybrids the data derived were analysed to facilitate the mapping of genes determining enzymic and antigenic markers to *M. rufogriseus* chromosomes.

Materials and Methods

Cells

PG19 is a hypoxanthine phosphoribosyltransferase-deficient ($HPRT^-$) cell line drived from a C57BL/6J mouse melanoma (Jonasson *et al.* 1977). A primary fibroblast line was established from ear tissue of an *M. rufogriseus* (red-necked wallaby) female. PGMR2-4 is an *M. rufus* (red kangaroo) × PG19 somatic cell hybrid (Sykes and Hope 1978). The 1RMR hybrids (*M. rufus* × 1R) are described in Donald and Hope (1981). P3/NS1/1Ag4/1 (referred to as NS1), is an $HPRT^-$, 8-azaguanine-resistant, non-secreting myeloma cell line derived from a BALB/c mouse (Köhler and Milstein 1976). Marsupial lymphocytes were separated from whole blood using Ficoll–Hypaque density gradients.

Cell Culture

Cell culture conditions for PG19, NS1, marsupial × mouse hybrid cells and marsupial × mouse fusions have previously been described (Sykes and Hope 1985). Marsupial × mouse hybrid cells were selected and grown in standard medium supplemented with hypoxanthine, aminopterin and thymidine (HAT medium) (Littlefield 1964). Revertant subclones were maintained in a concentration of 5 μg/ml 6-thioguanine (6TG).

Hybrid Cell Nomenclature

M. rufogriseus (lymphocytes) × PG19 hybrid cells have been designated REP hybrids. The primary hybrids, only one of which was isolated from each Petri dish after fusion, are distinguished by a number, e.g. REP1, REP2. The remaining cells from each Petri dish, consisting of a mixture of cells from other hybrid colonies and possibly also from the primary colony, were harvested together and referred to as bulk (B) hybrids, e.g. REPB1, REPB2, etc. HAT-selected subclones of REP hybrids are designated as subclasses of the primary or bulk hybrids, e.g. REP3-1, REP3-2, etc. REP3-7-1 is a subclone of REP3-7 which is itself a subclone of REP3. REP3 R1 is a subclone of the primary hybrid REP3 which has been back-selected for growth in the presence of 5 μg/ml 6TG. 6TG-resistant clones are referred to as 'revertants'.

Monoclonal Antibody, GA-1

GA-1 is a monoclonal antibody which reacts with a cell surface antigen on fibroblasts of the Macropodidae and Tarsipedidae. The production of GA-1 and the immunofluorescence assay are described in detail in Sykes and Hope (1985). Cells are scored as ± (faint non-specific binding), + (weak fluorescence), + + (bright fluorescence) and + + + (very bright reaction, halo of fluorescence). Dead cells were identified by their homogeneous fluorescence. By scoring 100 cells the percentage of total cells having a given fluorescence score was estimated.

Chromosomes

Standard methods were used for chromosome preparations.

Chromosomes were C-banded using the method of Sumner (1972) and G-banded according to the methods of Seabright (1971) and Yunis *et al.* (1978). For each chromosome preparation, 50 C- and/or 50 G-banded metaphase spreads were analysed for chromosomal content.

Electrophoresis

Frozen phosphate-buffered saline-washed cell pellets were rapidly thawed and one drop of lysis solution (0·1% v/v β-mercaptoethanol; 0·1% v/v Triton X-100 and 1·19 mM NADP) added to the pellet and the cells rapidly frozen and rethawed twice. Hybrids were examined for hypoxanthine phosphoribosyltransferase (HPRT, EC 2.4.2.8) activity using the method of Watson *et al.* (1972). Phosphoglycerate kinase-A (PGK-A, EC 2.7.2.3) isozymes were separated using a 0·1 M Tris-citrate buffer, pH 8·6, on cellulose

acetate ('Cellogel-250'). After pre-running the gel for 10 min at 200 V, samples were loaded onto the gel and run for 3 h at 200 V at room temperature. The gel was subsequently stained and counterstained (Meera Khan 1971). Mouse and *M. rufogriseus* glucose-6-phosphate dehydrogenase (G6PD, EC 1.1.1.49) have very similar electrophoretic mobilities and considerable difficulty was encountered in separating the two isozymes. The best separation was obtained on Cellogel-250 using a Tris–glycine buffer, pH 9·1, at 340 V for 35 min at 4°C (Migeon *et al.* 1979). The staining mixture was adapted from the method of Johnston *et al.* (1975). Lactate dehydrogenase (LDH, EC 11.1.1.7) isozymes were separated on Cellogel-250 using either a 0·02 M phosphate buffer, pH 7·0, or a 0·04 M sodium barbitone buffer, pH 9·9. The gel was run at 200 V for 2 h at room temperature, and stained according to (Meera Khan 1971).

Results

Marsupial–Mouse Hybrids

The various REP hybrids isolated are listed in Table 1.

Table 1. Chromosomal and enzymatic constitution, and reaction with the monoclonal antibody GA-1, of the *M. rufogriseus*–mouse cell hybrids

Hybrid	Approx. No. of hybrid cells (%) containing *M. rufogriseus* chromosomes[A]					*M. rufogriseus* enzymes expressed by hybrids		GA-1 reaction[B]
	t(3q,Xq)	Xq	3	6	7	HPRT	PGK-A	
REP1[C]	—	—	—	—	—	+	+	±
REP2[C]	—	—	—	—	—	+	+	±
REP3	27	—	18	18	9	+	+	+ + → + + + (20)
REP4[C]	—	—	—	—	—	+	+	+ → + + (30)
REP3-1	—	—	80	—	—	+	+	+ + + (90)
REP3-2	75	—	—	—	12	+	+	+ + + (90)
REP3-2-1	100	—	—	—	—	+	+	+ + + (95)
REP3-2-2	—	—	—	—	—	+	+	+ + + (100)
REP3-3	—	—	—	—	—	+	+	±
REP3-4	t(3q,?)[E]55	—	—	45	—	+	+	+ + → + + + (90)
REP3-5	—	—	—	—	—	+	+	±
REP3-6	—	—	—	—	—	+	+	+ + + (25)
REP3-7	—	—	i3q50	36	—	+	+	+ + + (100)
REP3-7-1	—	—	89	—	—	+	+	+ (90)
REP3-7-2[C]	—	—	75	—	—	+	+	+ + + (100)
REP3-7-3[C]	—	—	—	—	—	+	+	±
REP3-7-4	—	—	3q66	—	—	+	+	+ + + (100)
REP3-7-5[C]	—	—	—	—	—	+	+	+ + + (100)
REP3R1→ REP3R8	—	—	—	—	—	−	−	±
REPB1	—	—	—	—	—	+	+	±
REPB2	—	—	—	13	—	+	+	±
REPB3[D]	—	—	—	—	—	+	+	+ + → + + + (60)
REPB3-1	—	—	90	—	—	+	+	+ + + (100)
REPB3-2[C]	—	—	66	—	—	+	+	+ + + (70)
REPB3-3	—	4	50	—	—	+	+	+ + + (100)
REPB3-4	—	91	70	—	—	+	+	+ + + (95)
REPB3-5	—	—	3q50	95	—	+	+	+ + + (95)
REPB4	—	—	—	67	—	+	+	±
REPB5	—	—	—	36	—	+	+	±

[A] Chromosomes identified by C- and G-banding; — indicates that the particular *M. rufogriseus* chromosome was not found in the cells tested.
[B] Fluorescent score. Values in parenthesis are percentage of positive reacting cells.
[C] These cell lines were C-banded only.
[D] REPB3 was neither C- nor G-banded.
[E] The origin of the short arm of this chromosome was not established.

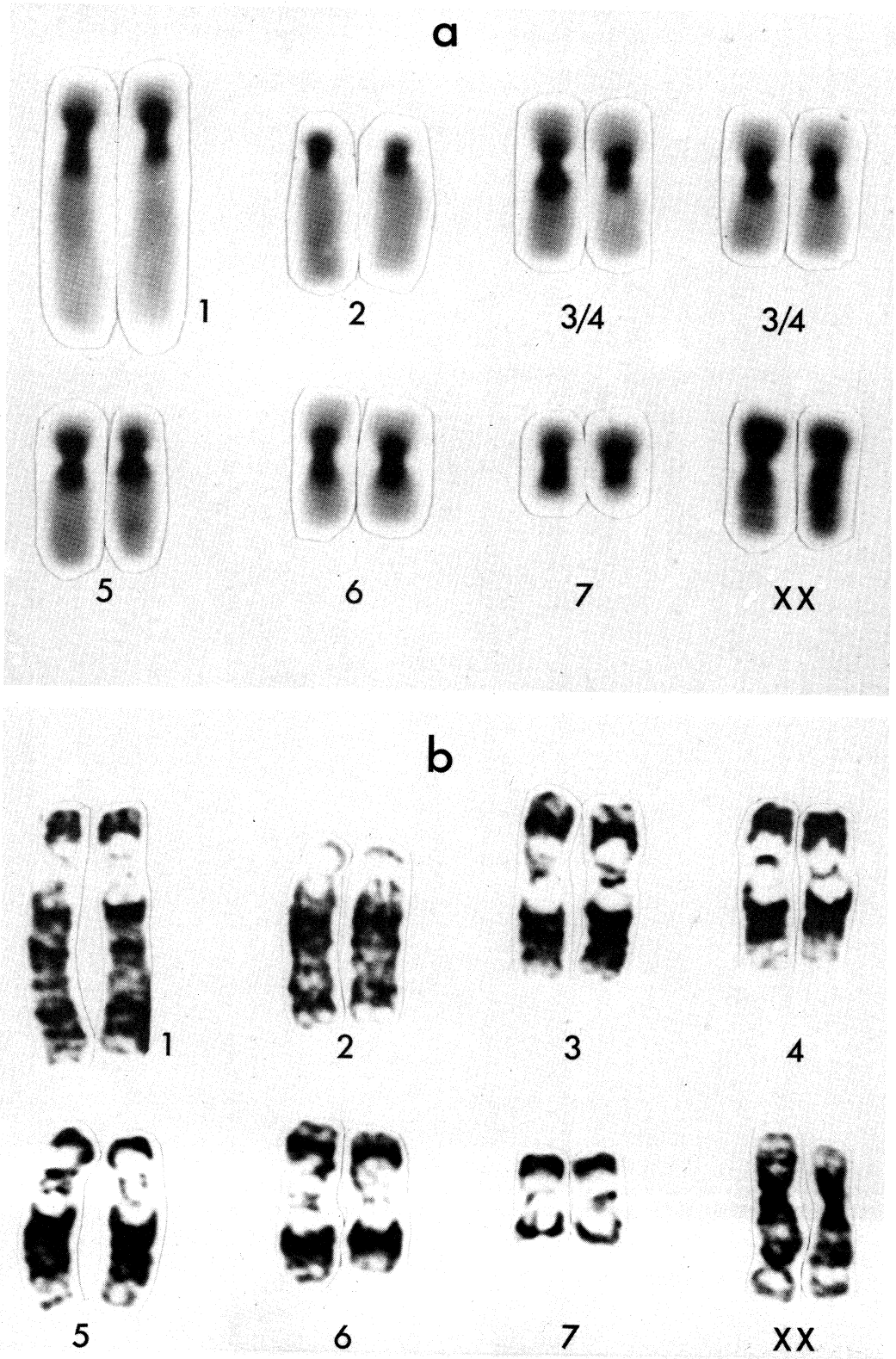

Fig. 1. *M. rufogriseus* karyotype. (*a*) C-banded. (*b*) G-banded.

Chromosomes

Chromosome preparations from *M. rufogriseus* lymphocyte and fibroblast cultures showed all 16 chromosomes to contain large areas of centromeric C-banding (Fig. 1*a*) compared with the much smaller centromeric C-band regions of PG19 chromosomes. Approximately two-thirds of the short arm of the *M. rufogriseus* X chromosome was heterochromatic, the distal one-third being euchromatic. The X chromosome also exhibited three interstitial C-bands on its long arm. These could be used to identify the presence of this chromosome, or at least its long arm, in cell hybrid chromosome preparations which had been C-banded. The *M. rufogriseus* autosomes had large pale G-bands at the centromeric region whereas the X chromosomes had dark centromeric G-bands. Each pair of *M. rufogriseus* chromosomes could be identified by their G-banding pattern (Fig. 1*b*). The C- and G-banding patterns were much clearer on *M. rufogriseus* chromosomes in the hybrid cells compared with chromosomes from fibroblast and lymphocyte preparations. Any one chromosome preparation of the hybrid cell lines exhibited a range of chromosome condensation between metaphase spreads enabling a progressive comparison of the same G-banded chromosome in different degrees of condensation. As a result of such comparison, confident identification of normal *M. rufogriseus* chromosomes in the hybrids was achieved. As has been previously reported for marsupial–mouse cell hybrids, preferential elimination of marsupial chromosomes was observed in all these hybrids (Hope and Graves 1978; Donald and Hope 1981). The *M. rufogriseus* chromosomes identified by C- and G-banding as being present in the REP hybrids are summarized in Table 1.

All the primary hybrids, REP1→REP4, and the five hybrids, REPB1→REPB5, had some cells containing chromosomes with very large blocks of C-banded material characteristic of *M. rufogriseus* chromosomes. A proportion of REP3 and REP3-2 cells contained a chromosome similar to the *M. rufogriseus* X chromosome but with a longer than normal euchromatic short arm. This was identified as a Robertsonian translocation chromosome consisting of the long arm of the *M. rufogriseus* X chromosome and the long arm of the *M. rufogriseus* number three autosome and is henceforth referred to as t(3q,Xq). The possible reciprocal translocation product was not identified in any of the hybrids. Some REP3 cells contained only chromosomes which were indistinguishable from PG19 chromosomes, while others contained the *M. rufogriseus* t(3q,Xq), 3, 6 or 7, identified by G-banding. *M. rufogriseus* chromosomes 1, 2, 4, and 5 were not identified in any of the hybrids. This chromosomal variation presumably resulted from continuing chromosome loss from the hybrid cells in the early stages of REP3 chromosome evolution. REP3 was chosen for more detailed study because it was the only primary hybrid containing a number of *M. rufogriseus* autosomes as well as at least the long arm of the *M. rufogriseus* X chromosome and it was the only primary hybrid to bind the monoclonal antibody GA-1 strongly. REP3 was subcloned with the aim of deriving a series of clones with different karyotypes. A photograph of a G-banded REP3-1 metaphase spread containing the *M. rufogriseus* 3 against a PG19 chromosome background is presented in Fig. 2. The resulting REP subclones (Table 1) were chromosomally more homogeneous than REP3 but still exhibited karyotypic heterogeneity. Two of the more heterogeneous subclones REP3-2 and REP3-7 were further subcloned in order to partition further this chromosome variation.

The HAT-selected REP hybrids referred to in Table 1 contained a small acrocentric chromosome with a G-banding pattern distinct from any of the PG19 or normal

M. rufogriseus chromosomes. The large dark G-band region near the centromere indicated that this chromosome was the short arm of the *M. rufogriseus* X chromosome. *M. rufogriseus* Xp was present in a proportion of all HAT-selected hybrid cell lines that were G-banded; however, the identification of Xp in the cell lines was sometimes inconclusive and Xp has not been included in Table 1 for this reason. All the REP3 revertant cell lines had C- and G-banded karyotypes similar to PG19 cells. No normal *M. rufogriseus* chromosomes could be identified in them.

The *M. rufogriseus* 3q was the only chromosome arm found in common in REP3-1, REP3-2 (and subclones), REP3-4, REP3-7 (and subclones) and the five REPB3 subclones.

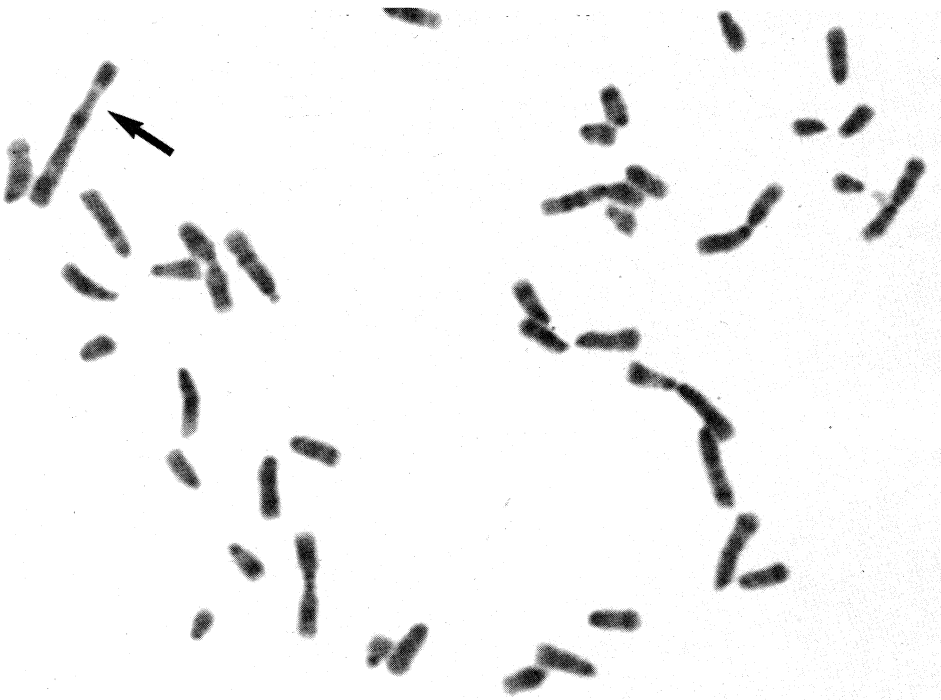

Fig. 2. G-banded cell of REP3-1. The *M. rufogriseus* chromosome 3 is arrowed.

Enzymes

The expression in the hybrids of HPRT and PGK-A is shown in Table 1. All the REP hybrid cell lines expressed marsupial HPRT. Neither *M. rufogriseus* nor mouse HPRT was expressed in revertant cell lines (Fig. 3*a*). All the HAT-selected REP hybrids and subclones expressed both *M. rufogriseus* and PG19 PGK-A (Fig. 3*b*). The 6TG-selected REP3 revertants expressed only the mouse form of PGK-A. The very faint slower band found in the *M. rufogriseus* controls is presumed to be the autosomally controlled *M. rufogriseus* PGK-B (Vandeberg *et al.* 1978).

The *M. rufogriseus* form of G6PD was not found in any of the cell lines tested. However, only a small electrophoretic separation of the mouse and *M. rufogriseus* G6PD enzymes was achieved, making interpretation of the gels difficult.

None of the hybrid clones, subclones or their revertants expressed the *M. rufogriseus* LDH-A gene.

Specificity of Monoclonal Antibody, GA-1

GA-1 bound (+ + +) to 100% of *M. rufogriseus* (♀) fibroblasts, approximately 30% of *M. rufogriseus* (♂) peripheral blood lymphocytes and approximately 20% of REP3 cells. It did not show any reaction with PG19.

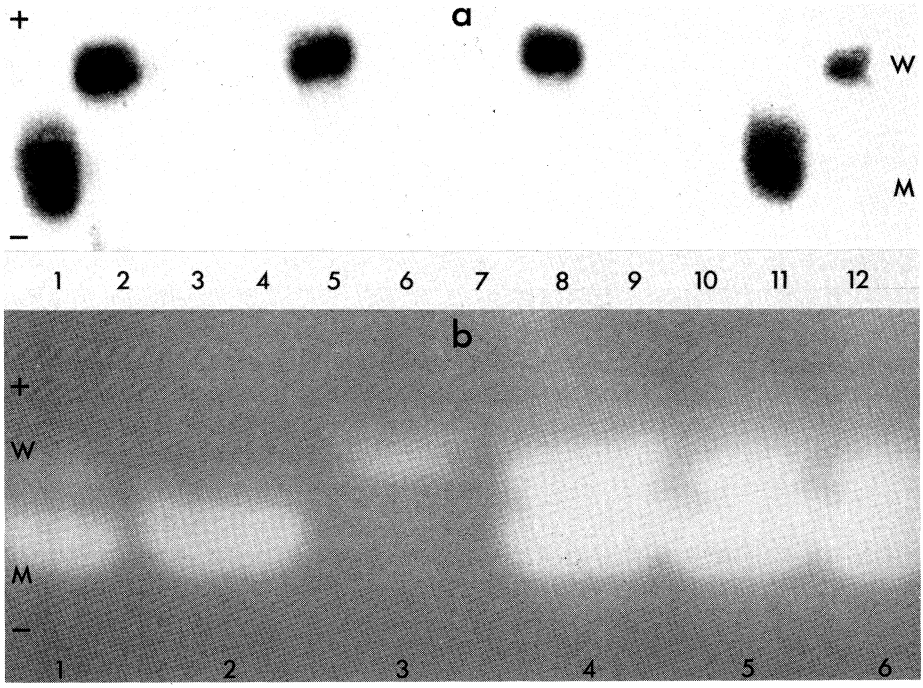

Fig. 3. (*a*) Autoradiograph of a starch gel showing hypoxanthine phosphoribosyl transferase (HPRT) activity. Channels 1 and 11 show the slow form of HPRT from mouse 3T3 cells (Matsuya and Green 1969). All the HAT-selected hybrids (channels 2, 5, 8 and 12) express the faster form of HPRT from red-necked wallaby (W) cells whereas the 6TG-selected revertants (channels 3, 4, 6, 7, 9 and 10) do not express either the mouse or marsupial forms of HPRT. (*b*) Electrophoresis of phosphoglycerate kinase (PGK) on cellogel. PG19 mouse PGK-A is shown in channel 2. *M. rufogriseus* PGK-A with the much weaker and slower PGK-B in the mouse PGK-A region is shown in channel 3. The HAT-selected hybrids express both the mouse and marsupial forms of PGK-A (channels 1, 4, 5 and 6). *M*, mouse; *W*, red-necked wallaby.

Table 2. Reaction of GA-1 with *M. rufus* × mouse somatic cell hybrids

Cells	*M. rufus* chromosomes present in hybrid cells		Reaction with GA-1	Cells reacting (%)
	X	5		
M. rufus fibroblasts	+	+	+ + +	100
1R	–	–	±	0
1RMR1	+	+	+ + → + + +	100
1RMR1R5A5	–	+	+ + → + + +	100
1RMR2-1	–	–	±	0
PGMR2-4	+	–	±	0

The results of reaction of GA-1 with the REP hybrids are given in Table 1. REP3 was the only primary cell hybrid in which a percentage of cells reacted strongly with GA-1. Of the bulk REP hybrids only REPB3 and its subclones reacted with GA-1. No reaction with GA-1 was observed for any of the REP3 revertants.

Donald and Hope (1981) have made and analysed a series of *M. rufus* (red kangaroo) × 1R hybrids, called the 1RMR hybrids. A number of these as well as PGMR2-4 (Sykes and Hope 1978) were tested for reaction with GA-1. Table 2 summarizes the chromosomal content of these hybrids along with their reaction with GA-1. It can be seen that reaction with GA-1 appears to be dependent on the presence in the *M. rufus*–mouse hybrids of the *M. rufus* chromosome 5.

Discussion

All 36 hybrid cell lines screened for the expression of *M. rufogriseus* HPRT and PGK-A showed concordance of expression of these two enzymes. The above results suggest synteny of the *Hpt* and *Pgk-A* loci. However, some caution must be exercised in drawing such a conclusion because (1) only four primary REP hybrids were studied; (2) many of the hybrids were subclones of REP3; and (3) the 'bulk' hybrids were not isolated independently of the primary hybrid colonies. If *Hpt* and *Pgk-A* are syntenic, then the hybrid cells expressing both enzymes must contain at least a fragment of the relevant *M. rufogriseus* chromosome. As the genes for HPRT and PGK-A have been found to be X-linked in all eutherian and marsupial species in which they have been mapped, presumably they are also X-linked in *M. rufogriseus*.

Family studies in marsupials give no indication of the possible X-linkage of HPRT. It is known from family studies that PGK-A is X-linked in a number of kangaroo species closely related to *M. rufogriseus* (Cooper *et al.* 1971; Vandeberg *et al.* 1973, 1977). PGK-A has been found to be monomorphic in all *M. rufogriseus* family studies carried out so far (P. Johnston, personal communication) and hence it has not been possible to demonstrate possible X-linkage of PGK-A in this species. Although X-linkage of *M. rufogriseus Hpt* and *Pgk-A* could not be confirmed in the present study, the data above suggests that these genes are at least syntenic. The *M. rufogriseus* Xp may be responsible for HPRT and PGK-A in the hybrids as Xp was the only *M. rufogriseus* chromosome arm identified in a proportion of all the HAT-selected hybrids.

Provisional Assignment of the Gene for the Antigen GA-1

None of the *M. rufogriseus* chromosomes showed absolute concordance with GA-1 expression. Non-concordance with GA-1 expression was particularly marked for the *M. rufogriseus* 6, 7 and X. However, *M. rufogriseus* 3q showed strong concordance with GA-1 expression, and this chromosome arm was therefore considered to be the most likely candidate for the location of the gene determining GA-1 expression.

Of the 23 REP hybrids analysed 21 were concordant for *M. rufogriseus* 3q (identified by G-banding) and binding of GA-1. This result suggests that the gene coding for GA-1 (or a gene determining the expression of this antigen) is present on *M. rufogriseus* 3q. It is possible that a fragment of 3q (containing the GA-1 gene) had been translocated onto a mouse chromosome in the two discordant hybrids, REP3-6 and REP3-2-2. There were no hybrids containing a clearly identifiable 3q arm which did not react with GA-1. The presence of a C-banded chromosome resembling the *M. rufogriseus* chromosome 3 in two GA-1$^+$ hybrids which were not G-banded, REP3-7-2 and

REPB3-2, supports the assignment of GA-1 to 3q. REP3-7-5, another GA-1$^+$ hybrid which was C- but not G-banded, did not contain the chromosome present in REP3-7-2 and REPB3-2. If only the *M. rufogriseus* 3q arm was present in REP3-7-5 it may have remained unidentified by C-banding. REP3-7-3, the one GA-1$^-$ hybrid which was not G-banded, did not contain the normal *M. rufogriseus* chromosome 3 identified by C-banding. This observation is also in accordance with the assignment of the gene for GA-1 expression to 3q.

In all hybrids except REP3-2-1, the percentage of cells that reacted with GA-1 was higher than the percentage of cells which contained the *M. rufogriseus* 3q. Hybrid cell lines which showed the largest differences were those containing 3q alone, rather than the normal *M. rufogriseus* chromosome 3 or t(3q,Xq). It was harder to identify the 3q arm by itself in the hybrids than the larger *M. rufogriseus* chromosome 3 or t(3q,Xq). It is possible, therefore, that some of the hybrid cells, e.g. in REP3-7-4, contained the unidentified 3q arm, and this may account for some of the differences observed.

The G11 staining technique has been used to identify human chromosomal fragments in human × rodent cell hybrids (Friend *et al.* 1976) and this method would have been of value in identifying *M. rufogriseus* chromosome fragments that may have been present in the hybrids. G11 staining was carried out on a number of REP hybrids known to contain *M. rufogriseus* chromosomes, but no differential colour staining between the mouse and marsupial chromosomes was observed. Human–mouse cell hybrids stained at the same time showed clear differentiation of human and rodent chromosomes.

Amongst the REP hybrids, there were exceptions to the concordance of GA-1 expression with *M. rufogriseus* 3q and therefore a firm assignment could not be based on these hybrids alone. Concordance of GA-1 with HPRT and PGK-A was not found, suggesting that the genes for HPRT and PGK-A are on a different chromosome to the gene for GA-1.

Cross-reaction of GA-1 with M. rufus*–Mouse Hybrids*

As GA-1 was shown to bind to *M. rufus* fibroblasts, assays for the presence of GA-1 were carried out on the 1RMR cell hybrids. For the following reason it is of special interest that GA-1 reacted with only those 1RMR hybrids which contained the *M. rufus* chromosome number 5 (Table 2). Rofe (1978) clearly demonstrated that the G-banding pattern of the *M. rufus* number 5 is identical to that of the long arm of the *M. rufogriseus* number 3 chromosome and that the *M. rufogriseus* chromosome 3 is a Robertsonian translocation product of the *M. rufus* chromosomes 5 and 8. This implies that GA-1 was reacting with a cell surface antigen present on 1RMR hybrids which is determined by a gene on *M. rufus* chromosome 5, the G-banded chromosome identical to the *M. rufogriseus* 3q. These results provide strong support in favour of the assignment of the GA-1 gene to the *M. rufogriseus* 3q.

Donald and Adams (1981) have assigned the gene for LDH-A using a series of *M. rufus*–mouse cell hybrids. *Ldh-A* and the gene for GA-1 are syntenic in *M. rufus*; however, LDH-A was not present in hybrids containing *M. rufogriseus* 3q. This was surprising in view of the close phylogenetic relationship of *M. rufogriseus* and *M. rufus* and the apparent G-band identity of the two relevant chromosomes.

The problems of small number of independent clones and extensive chromosome fragmentation observed in this study have been observed previously in rodent–marsupial somatic cell hybrids (Hope and Graves 1978). Although the presence of a normal *M. rufus* X chromosome has been observed in a number of *M. rufus* × mouse cell

hybrids (Sykes and Hope 1978; Donald and Hope 1981), attempts to make *M. robustus robustus* × eutherian cell hybrids containing the normal *M. robustus robustus* X chromosome (Graves *et al.* 1979), and attempts to obtain normal marsupial autosomes in somatic cell hybrids (B. Wainwright, personal communication) have also been unsuccessful. The extent of fragmentation of the marsupial chromosomes in marsupial–mouse hybrids may also depend on the origin of the mouse parent. Human–mouse hybrids made with PG19 are known to contain human chromosomes exhibiting extensive rearrangement. However, PG19 yields higher numbers of hybrids with marsupials compared with other rodent cell lines (Hope, unpublished data). Hope and Graves (1978) obtained *Pseudocheirus peregrinus* (ring-tail possum) lymphocyte × PG19 cell hybrids containing as many as 10 *P. peregrinus* chromosomes in contrast to studies where only a few or no marsupial chromosomes have been found in hybrids with macropodid species.

As a result of their studies with *P. peregrinus* × PG19 cell hybrids, Hope and Graves (1978) suggested that there may be a tendency for the hybrids to retain marsupial heterochromatin. The choice of *M. rufogriseus* as the marsupial parent of the hybrids described here was based on the belief that the large regions of conspicuous C-banding material in the chromosomes of this species would considerably aid in their identification. However, the C-banding regions of G-band-identified *M. rufogriseus* chromosomes were sometimes abnormal or even absent. This was particularly the case for *M. rufogriseus* chromosomes 3 and Xp. On the basis of chromosome size and G-banding, it appears that the C-banded material of these chromosomes was, in fact, physically present but remained unexpressed cytologically. On the other hand, the C-banding patterns of the *M. rufogriseus* chromosomes 6 and 7 remained unaltered in all cell lines in which they were present. Therefore, this phenomenon of variable C-band expression in the hybrids was not common to all *M. rufogriseus* chromosomes. No change in C-banding pattern was observed for any of the PG19 chromosomes. The reason for the C-banding variation of some *M. rufogriseus* chromosomes is not clear.

GA-1 is the only autosomal gene to be provisionally assigned to a chromosome in *M. rufogriseus* and only the second autosomal gene mapped in any marsupial.

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