in control experiments they show minimal variation in length of neurite outgrowth. Fourteen other ascites fluids containing different antibodies of comparable concentrations did not influence outgrowth of retinal axons when tested in this system.

Time lapse recordings of retinal axons growing over either a monolayer of retinal cells[7], or a collagen or laminine-coated surface revealed that immediately on addition of T61/3/12 antibody most of the growth cones stopped their searching movements, and remained firmly attached to the substrate. As inhibition of growth was observed on several substrates, it cannot easily be explained by cross-linking of the growth cones to the substrate by T61/3/12. Further work with Fab fragments will help to exclude cross-linking phenomena. Filopodia were still visible and at least some of them were actively moving in the medium. None of these axons could be seen to grow again during the observation period of 5 h after addition of the antibody. A minority seemed to be unaffected and continued to move. We do not know whether these axons represent neurites from a different ganglion cell class. Inhibition of neurite outgrowth was reversible, because axons started to regrow about 1 h after thorough washing of the explants with culture medium.

Dorsal root ganglia, explanted from 9-day-old chick embryos and cultured for 3 days in the presence of T61/3/12 antibody (T61/3/12-ascites, 1:20 in Dulbecco's modified Eagle's medium + 10% heat-inactivated fetal calf serum + 20ng ml^{-1} β-nerve growth factor) on polylysine-coated microtitre plates or on laminine-coated coverslips were not inhibited in their axonal outgrowth by the antibody. The axons reached the same length as in cultures without antibody or with the addition of a control ascites fluid (antibody P12, directed against chromosomal proteins of *Drosophila melanogaster* (ref. 5); given by P. Symmons). This result was confirmed by cinematographic studies. The growth cones of dorsal root ganglion axons that had grown *in vitro* for 1–3 days were unaffected by the addition of T61/3/12. Also, the indirect immunofluorescence test showed that they do not bind the antibody.

The effect of T61/3/12 antibody does not seem to be due to general cytotoxicity. In monolayer cultures of 9-day-old retinae, where ~40% of the cells express the antigen, no dead cells could be observed after brief or overnight incubation with the antibody, as judged by trypan blue exclusion. Furthermore, the very fast inhibition of growth cone movement suggests a specific mode of action.

A preliminary investigation of the species distribution of the antigen revealed that retinal cells from 2-day-old postnatal mice as well as frozen sections from adult mouse retina show no antibody binding. Retinal cells from 8-day-old quail embryos are stained with the same intensity as chick cells.

The immunoglobulin class of the antibody has been shown by immunoprecipitation and immunofluoresecence with class-specific antibodies to be IgG3, but the chemical nature of the antigen has not been determined. It seems that the molecule is relatively insensitive to mild digestion by a 0.1% solution of trypsin or papain. However, on incubation with 0.1% pronase, antibody binding is reduced to ~50% (R. Babiel, personal communication), suggesting that the antigen might be a protein or glycoprotein. Fixation of cells with 96% ethanol does not abolish antibody binding capacity and thus the antigen does not seem to be a ganglioside.

Previous studies have identified several molecules involved in cell aggregation in the chick retina. The T61/3/12 antigen, although not yet identified, can be distinguished from the cell adhesion molecule (CAM)[9] by its stability to trypsin and its absence in mouse retina[10] and chick dorsal root ganglia[11]. The antigen differs from cognin[12] in being present on freshly trypsinized retinal cells and by its expression on central nervous system tissue other than the retina. The strong binding of T61/3/12 antibody to plexiform layers shows some resemblance to the properties of RET5 antibody described by Lemmon and Gottlieb[13]. In contrast to T61/3/12, however, this antibody does not stain cell perikarya.

The apparently specific inhibitory action of the T61/3/12 antibody on outgrowth *in vitro* of chick retinal ganglion cell axons suggests that the antibody is directed against a molecule that might either have an important role in the interaction between growth cone and substrate or may serve as a receptor for growth factors that stimulate neurite outgrowth. Characterization of the chemical nature of the antigen (work in progress) may help to determine its mode of action.

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Genetic dissection of monoamine neurotransmitter synthesis in *Drosophila*

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The biogenic monoamine neurotransmitters octopamine, dopamine and serotonin have been detected in nervous tissue from many insects[1]. We report here that intact *Drosophila melanogaster* brains, when incubated with the radioactive amino acids tyrosine and tryptophan, synthesized and accumulated labelled monoamines. In two mutant strains monoamine synthesis was abnormal. The *per°* mutation abolishes the normal circadian rhythm[2–4]. Brains from *per°* flies, when incubated in tritiated tyrosine, accumulated one-third as much labelled octopamine as did brains from wild-type flies, but had normal dopamine and serotonin synthesis. In contrast, dopa decarboxylase (*Ddc*) mutations[5,6] decreased dopamine and serotonin synthesis but did not affect octopamine synthesis. These results suggest that there are two different aromatic amino acid decarboxylases in *Drosophila* brains, one that decarboxylates L-dopa and 5-hydroxytryptophan and another that decarboxylates tyrosine. Direct measurement of L-dopa, 5-hydroxytryptophan and tyrosine decarboxylase activities in the different strains confirmed this suggestion.

The study of monoamine neurotransmitters in flies is complicated by the fact that dopamine and possibly other monoamines are important intermediates in cuticle tanning[7–9], and consequently have a high rate of synthesis and metabolism in the epidermis[10]. To study monoamines in their role as neurotransmitters we developed a technique in which brains were dissected intact from either adult or larval *Drosophila*, and incubated in a small hanging drop with radioactive precursors to measure monoamine synthesis and accumulation. The brains were incubated for 30–60 min in oxygenated saline containing tritiated tyrosine or tryptophan and the radioactive products were separated by high-voltage electrophoresis. The major metabolites

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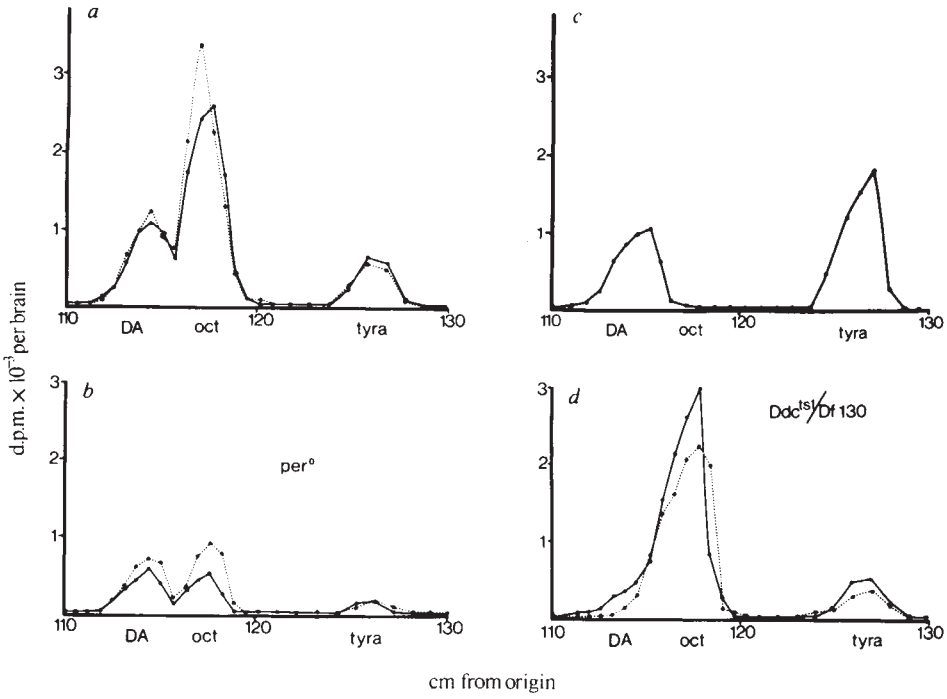

Fig. 1 High-voltage electrophoresis profile of *Drosophila* brains incubated in ^{3}H-tyrosine. Dotted and solid lines represent duplicate experiments. Eight intact adult brains with cuticle removed were incubated for 30 min at 25 °C in a 10-μl hanging drop of *Drosophila* saline[18] containing 10 μCi ^{3}H-tyrosine. In *c*, the incubation medium also contained 10 mM nonradioactive tyramine, which blocks the conversion of the ^{3}H-tyramine to octopamine. The tritiated metabolites (DA, dopamine; oct, octopamine; tyra, tyramine) were then analysed by high-voltage paper electrophoresis[19]. The chemical identity of each peak was confirmed by chromatographing the paper after electrophoresis in a second dimension in two different solvent systems: methyl ethyl ketone/propionic acid/water (25:8:7) or sec-butanol/pyridine/acetic acid/water (30:0.2:2:5). During electrophoresis L-dopa runs so close to tyrosine that any accumulation of L-dopa would not have been detected. The apparent increased height of the dopamine peak in wild-type (*a*) compared with that of *per°* (*b*) is due to the tail of the octopamine region. Electrophoresis of ^{3}H-octopamine alone yields a profile similar in shape to the octopamine peaks in *d* with an elevation of the baseline at the dopamine peak by about 15% of the height of the octopamine peak. Flies with a deficiency for *Ddc* (maintained as a heterozygous stock with the balancer chromosome *CyO:Df(2L)130/CyO*) were mated with flies that had a temperature-sensitive allele of *Ddc* (*Ddc^{ts1}/CyO*). We raised the F_1 progeny flies at a permissive temperature (20 °C) until 3 days after eclosion to obtain adults with fully sclerotized cuticles. We then selected the *Ddc^{ts1}/Df(2L)130* flies from among the progeny and shifted them to a non-permissive temperature (29 °C) for at least 3 days before biochemical experiments.

synthesized from tyrosine were dopamine, octopamine and tyramine (Fig. 1*a*). When incubated in tritiated tryptophan, fly brains synthesized and accumulated labelled serotonin (not illustrated).

We examined several behaviourally interesting mutants of *Drosophila*, including those affecting learning, for their ability to synthesize and accumulate labelled monoamine transmitters when incubated with tritiated precursors. One mutant that had a clearly abnormal monoamine profile was *per°*, previously isolated in a screen to select for mutants lacking normal circadian rhythms[2]. Two other alleles of the same gene locus also alter these rhythms, producing abnormally long (28 h) or short (19 h) circadian cycles[2–4].

Table 1 Tyrosine, L-dopa and 5-hydroxytryptophan decarboxylase activities in different mutants as a percentage of the wild-type activity

	Tyrosine decarboxylase	Dopa decarboxylase	5-HTP decarboxylase
$\frac{+}{+}, \frac{+}{+}$ (wild-type)	100±4	100±19	100±6
$\frac{per^o}{per^o}, \frac{+}{+}$	35±8	83±19	110±6
$\frac{+}{+}, \frac{Ddc^{ts1}}{Df(2)130}$	72±7	1.0±0.1	12±11
$\frac{per^o}{per^o}, \frac{Ddc^{ts1}}{Df(2)130}$	33±4	1.1±1.4	

Decarboxylase activity was measured by incubating head homogenates with ^{3}H-tyrosine, ^{3}H-L-dopa or ^{3}H-5-hydroxytryptophan and then separating precursor from products by high-voltage electrophoresis. Incubation mixtures (20 μl total) contained 0.2 M sodium phosphate *p*H 7.2, 0.1 mM pyridoxal phosphate, 2 mM tyramine, 2 mM dopamine, 2 mM serotonin, 5 μCi tritiated precursor and either 10 μl (for tyrosine and 5-hydroxytryptophan decarboxylase determinations) or 5 μl (for L-dopa decarboxylase determinations) tissue homogenate (1 head per 10 μl). Incubations were for 30 min at 25 °C. In these conditions the decarboxylation reactions were linear with time and amount of homogenate.

When *per°* brains were incubated in ^{3}H-tyrosine, they accumulated less than one-third as much ^{3}H-octopamine and ^{3}H-tyramine as wild-type brains, whereas the amount of dopamine accumulated was about the same (Fig. 1*a*, *b*). (The absolute height of the dopamine peak is greater in wild type than in *per°*, but this is because of the overlap between the trailing edge of the octopamine region and the dopamine peak.) There are several possible explanations for this result: the *per°* mutation could either decrease the number or vigour of octopaminergic neurones or it could affect any step in octopamine synthesis (tyrosine uptake, tyrosine decarboxylation, tyramine hydroxylation, octopamine storage or octopamine release).

To determine if the *per°* mutation affects any of the enzymes involved in octopamine synthesis we first established (Fig. 1*c*) that *Drosophila* synthesizes octopamine by the pathway shown in Fig. 2. We then measured separately the activities of the two enzymes required for octopamine synthesis. *per°* brains had normal levels of tyramine β-hydroxylase activity (not illustrated) but decreased levels of tyrosine decarboxylase activity (Table 1). Reduced levels of tyrosine decarboxylase would account for the observed decrease in accumulation of both octopamine and tyramine. We measured tyrosine decarboxylase activity in three other *per°* stocks that had different genetic backgrounds and all three strains had less than 50% of the wild-type activity. Other mutant alleles of the *per* locus also showed reduced tyrosine decarboxylase activity: *per^{long}* had 50% of the wild-type activity and *per^{short}* 60%.

The finding that dopamine synthesis was normal in *per°* brains suggests that the amino acid decarboxylase used in the synthesis of dopamine must be different from the tyrosine decarboxylase used in the synthesis of octopamine. Wright and his colleagues have determined the genetic locus that codes for dopa decarboxylase and have isolated several mutant alleles and deficien-

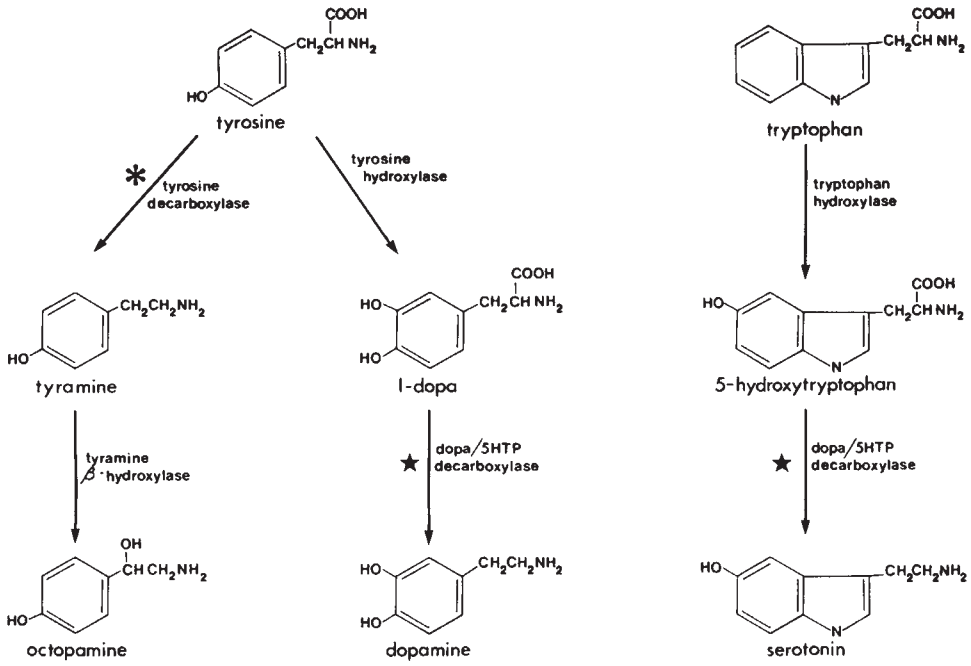

Fig. 2 Pathways for synthesis of octopamine, dopamine and serotonin[7-9,20]. The asterisk indicates a reaction partially blocked by the *per°* mutation, and the stars indicate reactions blocked by *Ddc* mutations.

cies for this locus (*Ddc*)[5,6]. Dopa decarboxylase is present in both cuticle and brain and this activity is reduced by mutations in the *Ddc* locus[11,12], but the effect of *Ddc* mutations on neurotransmitter synthesis has not been explored. Dopamine is an essential intermediate in cuticle sclerotization, and so, in order to be able to study the neurochemistry and behaviour of strains with severe reductions in dopa decarboxylase levels, we used temperature-sensitive alleles of *Ddc* in combination with a deficiency for the region containing the *Ddc* locus (*Df(2L)130*). These flies were raised at a permissive temperature (20 °C) until their cuticles were fully sclerotized and then shifted to a non-permissive temperature (29 °C) 3 days before biochemical testing. When the brains of $Ddc^{ts1}/Df(2L)130$ flies were incubated in ^{3}H-tyrosine they showed no detectable dopamine synthesis but normal amounts of octopamine and tyramine (Fig. 1*d*). When incubated with radioactive tryptophan, they showed no synthesis of serotonin whereas both wild-type and *per°* brains showed serotonin synthesis.

The results with *Ddc* mutants again suggest that wild-type flies have two different amino acid decarboxylases, one that decarboxylates tyrosine and another that decarboxylates both L-dopa and 5-hydroxytryptophan. We tested this directly by measuring each decarboxylase activity in whole head homogenates in the different mutants. Table 1 shows that *per°* head homogenates had reduced tyrosine decarboxylase activity but normal L-dopa and 5-hydroxytryptophan decarboxylase activities. In contrast, $Ddc^{ts1}/Df(2L)130$ homogenates showed almost undetectable levels of L-dopa- and 5-hydroxytryptophan-decarboxylase activities but almost normal levels of tyrosine decarboxylase activity. The finding that both L-dopa and 5-hydroxytryptophan decarboxylase activities were abolished by mutations in the structural gene for dopa decarboxylase implies that both substrates are decarboxylated by the same gene product.

Purified *Drosophila* dopa decarboxylase will decarboxylate tyrosine, but it has a much lower affinity for tyrosine than for L-dopa[13,14]. Therefore it is possible that the small amount of tyrosine decarboxylase activity remaining in *per°* flies is due to the activity of the dopa decarboxylase enzyme on an anomalous substrate, tyrosine. To assess this possibility we tested flies mutant for both *per°* and *Ddc*, that is, *per°/per°*; $Ddc^{ts1}Df(2L)130$ (temperature shifted as described in Fig. 1). These flies showed the same 35% residual tyrosine decarboxylase activities as did *per°/per°* flies not mutant at the *Ddc* locus (Table 1). Therefore, the residual tyrosine decarboxylase

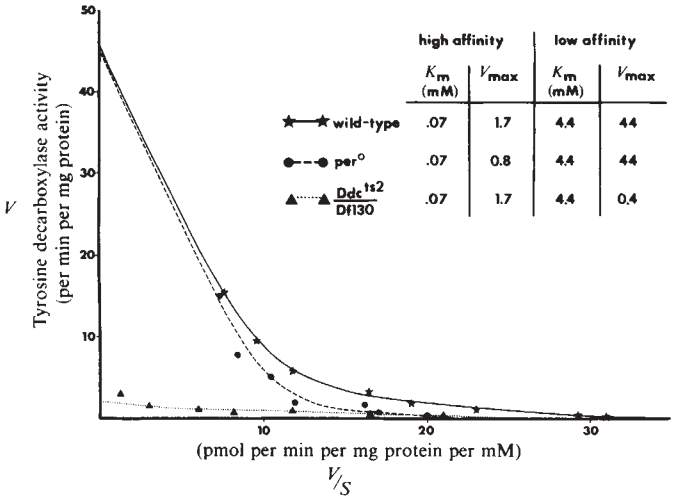

Fig. 3 Eadie–Hofstee plot[21,22] of tyrosine decarboxylase kinetics: wild-type tyrosine decarboxylase activity has both high affinity ($K_m = 0.07$ mM) and low affinity ($K_m = 4.4$ mM) components. Tyrosine decarboxylase activity was measured by the organic extraction method of McCaman *et al.*[23]. Incubation conditions were identical to those described in Table 1 legend except that instead of tritiated precursors, incubations contained ^{14}C-tyrosine (specific activity = 100 Ci mol^{-1}) at concentrations from 0.01 to 3 mM. Protein was determined by the method of Lowry[24]. The kinetic parameters were determined by assuming that the K_ms for the two components were the same for all three mutants and then calculating the V_{max} values by using a linear least-squares curve fitting program[25]. The lines were calculated from the parameters listed.

activity in *per°* flies is not due to dopa decarboxylase activity, and the *per°* mutation must not completely eliminate tyrosine decarboxylase activity.

The reason we did not see any tyrosine decarboxylation by the enzyme dopa decarboxylase in the experiment shown in Table 1 is probably that the assays were done at 8×10^{-6} M tyrosine, a concentration well below the K_m of dopa decarboxylase for tyrosine. At higher concentrations of tyrosine, however, the action of both decarboxylases was evident, in that tyrosine decarboxylation showed biphasic kinetics (Fig. 3). Furthermore, the two mutations reduced different components of this activity. In extracts from per° flies the high affinity component was reduced, whereas in extracts from $Ddc^{ts1}/Df(2L)130$ flies the low affinity component was missing.

If per^o is a weak allele of the structural gene for tyrosine decarboxylase, one would expect tyrosine decarboxylase activity to be reduced more severely by a deficiency of the *per* locus, and to be elevated by a duplication of the gene. Neither effect was observed. We measured tyrosine decarboxylase activities in flies heterozygous for per^o and three different deficiencies that delete the *per* locus: $Df(1)w^{rJ1}/per^o$, $Df(1)w^{rJ2}/per^o$ and $Df(1)64c4/per^o$ (ref. 15). All of the deficiencies had the same level of tyrosine decarboxylase activity as did per^o/per^o flies, but not lower levels. These results suggest that per^o is a null mutation, that is, it has the same effect as a deficiency of the gene. (per^o likewise acts as a null mutation in its behavioural phenotype[4].) We found that a duplication of the *per* region (attached to the Y chromosome (w^+Y)) in combination with a per^o mutation on the X chromosome elevated tyrosine decarboxylase activity to wild-type levels, but the duplication in combination with the wild-type allele did not further elevate the enzyme levels above the wild-type levels. Thus, tyrosine decarboxylase activity was not directly correlated with the number of per^+ gene copies and it therefore seems unlikely that the *per* locus codes for the enzyme tyrosine decarboxylase. Apparently, *per* mutations reduce enzyme levels indirectly, perhaps by affecting octopaminergic neurones.

The use of *Drosophila* mutants has enabled us to distinguish two separate aromatic amino acid decarboxylases. One, which has a high affinity for tyrosine, is used in the synthesis of octopamine and is reduced indirectly by mutations at the *per* locus. The other, encoded by the *Ddc* gene, can decarboxylate all three transmitter precursors but is necessary for the synthesis of only two neurotransmitters: dopamine and serotonin. Octopamine is a major neurotransmitter in many invertebrates[1], but most vertebrates probably do not have specific octopaminergic neurones[16]. We expect that the tyrosine-specific decarboxylase described here will be present in the nervous systems of only those organisms that use octopamine as a neurotransmitter.

Many pharmacological experiments indicate that monoamines act as modulators and may modify or determine behavioural states[17]. In *Drosophila* we can alter monoamines genetically rather than pharmacologically to explore their role in behaviour. The per^o mutation seems to affect octopaminergic function, and per^o flies have abnormal biological rhythms but normal learning and memory (not shown). In contrast, the *Ddc* mutation abolishes dopamine and serotonin synthesis but does not affect octopamine synthesis. *Ddc* mutant flies have normal circadian rhythms (unpublished observations) but fail to learn in either negatively or positively reinforced olfactory discrimination tests (B.L.T., M.S.L. and W. G. Quinn, unpublished observations).

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Assignment of the gene for human melanoma-associated antigen p97 to chromosome 3

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p97 is a 97,000 molecular weight cell-surface glycoprotein, which is present in human melanomas but in only trace amounts in normal adult tissues[1–4]. Amino acid sequence and iron binding studies have shown that p97 is structurally and functionally related to transferrin[5]. Reports that the genes for the transferrin receptor (TR)[6–8] and possibly transferrin[9–13] are located on chromosome 3 prompted us to investigate the chromosomal localization of the p97 gene. Our strategy was to characterize interspecies somatic cell hybrids derived from human fibroblasts or lymphocytes for expression of p97 and presence of human chromosomes. Although fibroblasts and lymphocytes express only small amounts of p97[3,4], we were able to type the hybrids for p97 by using monoclonal antibodies in highly sensitive and specific immunoassays. Of 14 hybrids, 6 contained chromosome 3 and expressed p97, and 8 were negative for both. We conclude that the p97 gene, like the transferrin and TR genes, is located on chromosome 3.

The first hybrids tested (designated PEP 2B, 6A, 12B, 13B) were obtained by fusing human fibroblasts with HGPRT mouse melanoma cells[14]. The hybrids, which had been stored in liquid nitrogen, were expanded in culture and tested for p97 by a binding assay with ^{125}I-labelled monoclonal antibody, unlabelled antibody being used as competitor to distinguish specific binding to p97 from nonspecific binding to the cells[3]. The hybrids were also tested for p97 by a double determinant immunoassay (DDIA), which involves monoclonal antibodies to two distinct epitopes of p97[3]. The two assays gave similar results but, since the DDIA was more sensitive, it was used for most of the study. Representative data are shown in Table 1. Two hybrids were found to be positive for p97 (300 molecules per cell), and two were negative (fewer than 10 molecules per cell). The human chromosome contents of the four cell lines[14] are shown in Table 2. Only chromosome 3 was present in both p97-positive hybrids and absent from both p97-negative hybrids. The simplest interpretation of the data is thus that the p97 gene resides on that chromosome. The amount of p97 on the positive hybrids, although some three orders of magnitude lower than on melanoma cells, is comparable with levels in fibroblasts[3]. The hybrids were also tested for TR by a binding assay with ^{125}I-labelled monoclonal antibody B3/25 (refs 15, 16). The two hybrids that contained chromosome 3 and

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