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Human-Mouse Cell Hybrids: A Suggestion of Structural Mutation for Dipeptidase-2 Deficiency in Mouse Cells

Abstract. *The dipeptidase-2 enzyme is inactive in certain cultured cell lines from the mouse. In somatic cell hybrids between such deficient cells and diploid human fibroblasts, the mouse deficiency was complemented when the homologous human peptidase-A was retained. The results suggested that the murine peptidase deficiency was the result of a structural mutation, rather than a regulatory one.*

The complementary effect of adding a homologous genome, or part of a genome, to a cell has contributed to studies on the characterization of genes, gene function, linkage relationships, and enzyme structure in lower organisms (*1*). The capability of isolating somatic cell hybrids from combinations of human, mouse, and hamster cell lines has extended these studies to mammals. Proliferating somatic cell hybrids can be isolated after fusion of two cell lines in which one or both possess an enzyme deficiency that is complemented in the hybrid cell (*2*). Complementation between structurally inactive enzyme mutants at the same locus may be explained by the formation of an active hybrid enzyme between inactive subunits (*1, 3*). We report the formation of an active human-mouse hybrid enzyme from inactive mouse dipeptidase-2 and homologous active human peptidase-A subunits in hybrid cells and in in vitro enzyme hybridization experiments. Peptidase expression in mouse-mouse hybrids from dipeptidase-2 positive and negative cells is evidence against a regulatory mutation. The results demonstrate the importance of somatic cell hybrids for characterizing genetic mutants in mammals, and for understanding mutant expression in a "heterozygous" condition.

Dipeptidase-2 (Dip-2) from the mouse is separated from other peptidases by gel electrophoresis (Fig. 1). The enzyme is capable of hydrolyzing several dipeptides (*4*) and is expressed in a majority of mouse tissues and tissue culture cells. In mouse LM/TK$^-$ tissue culture cells (*5*) and in a subline, LTP (*6*), Dip-2 was not expressed (Fig. 1). The mutation apparently originated in LM/TK$^-$ cells because C3H mouse tissues and LM cells (*7*), the mouse strain and cell line of origin, had Dip-2 activity. Human peptidase-A (Pep-A) has an electrophoretic mobility that is different from Dip-2 (Fig. 1) and demonstrates substrate and structural characteristics which are suitable for in vivo and in vitro hybridization studies to characterize the enzyme deficiency.

Human WI-38 lung fibroblasts (*8*), which are karyotypically normal and Pep-A positive, were fused to mouse LTP heteroploid cells, which are Dip-2 negative (Dip-2$^-$), with inactivated Sendai virus. Clones and subclones of human-mouse somatic cell hybrids were selected as previously described under conditions which eliminated parental cells (*6*). All hybrid clonal populations posessed human and mouse chromosomes and enzymes (*6*). Human components were reduced in number with extended growth of the hybrid cells (*2*). Control hybrid clones were isolated from fusions between RAG (*9*),

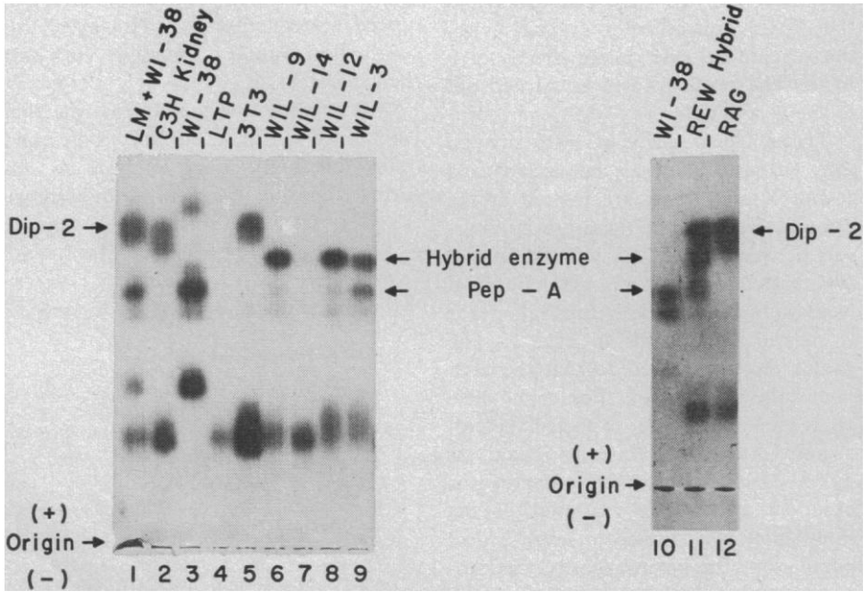

Fig. 1. Peptidase zymograms of cell homogenates with L-valylleucine as substrate. Human Pep-A$^+$ parent, channels 3 and 10; mouse Dip-2$^+$ homogenates, channels 5 and 12; mouse Dip-2$^-$ parent, channel 4; human-mouse hybrids of Dip-2$^-$ mouse cells and human WI-38 cells, channels 6 to 9; hybrid between Dip-2$^+$ RAG cells and WI-38 cells, channel 11. The interspecific hybrid enzyme is expressed in channels 6, 8, 9, and 11, and absent in channel 7. A mixture of LM (Dip-2$^+$) and WI-38 (Pep-A$^+$) cell homogenates is in channel 1. Mouse kidney homogenate is in channel 2. The human peptidase activity anodal to Pep-A in channel 3 is Pep-C; peptidase activity cathodal to Pep-A is Pep-S (*14*). Starch gel electrophoresis was performed in a tris(hydroxymethyl)aminomethane, borate, ethylenediaminetetraacetic acid buffer, *p*H 8.6, employing 12 percent electrostarch (*16*); the staining procedures and substrates have been described (*4, 14*). Cells were harvested at amounts of 0.8 $\times 10^8$ to 1.0 $\times 10^8$ cells per milliliter and homogenized (*6*).

a Dip-2 positive (Dip-2$^+$) mouse cell line, and the WI-38 human cell line by the same procedures. Thirty clones (designated REW), which possessed human chromosomes and enzymes, were isolated. Those which retained the human *Pep-A* gene expressed the Pep-A phenotype, the mouse Dip-2 phenotype, and an intermediate or hybrid zone of activity (Fig. 1, channels 10 to 12). A three-zoned isozyme pattern is the expected heterozygote phenotype for variants of a dimeric enzyme (*10*) as, for example, the heterozygote phenotype of Pep-A variants in man (*11*).

Thirteen independent hybrid clones of LTP and WI-38 (designated WIL), were tested for peptidase activity. Ten of these clones expressed the human Pep-A phenotype and a second peptidase which migrated to an intermediate or hybrid enzyme position between Pep-A and the Dip-2 zone (Fig. 1, channels 6, 8, and 9). The position of intermediate activity was similar to that of the REW hybrid enzyme. Dipeptidase-2 was never expressed in WIL hybrids nor was it expressed in 50 subclones; hybrids negative for Pep-A did not express the hybrid enzyme (Fig. 1, channel 7). In subclones from peptidase positive hybrids, Pep-A and the hybrid peptidase were lost together when human chromosomes were reduced. The presence of a new band in WIL hybrids strongly suggests that a structurally altered inactive polypeptide was synthesized in hybrid cells, which could dimerize with a homologous polypeptide. The parental Dip-2 and Pep-A phenotypes were usually expressed as a double-banded pattern in our system (Figs. 1 and 2). The REW hybrid enzyme was also expressed as a doublet, but the WIL hybrid enzyme was present as a single band. The doublet is likely the result of conformational differences of the peptidases (*12*). The Dip-2 structural change apparently alters properties of the hybrid enzyme.

Confirming evidence for the presence of the hybrid enzyme in WIL hybrids was obtained from in vitro enzyme hybridization experiments (*13*) (Fig. 2). Mixed homogenates of human and mouse cells were treated with urea to dissociate the dimeric peptidases which were then renatured. A hybrid enzyme was formed in control mixtures of human and mouse LM (Dip-2$^+$) cell homogenates similar to that in REW hybrids. When homogenates of WI-38 and LTP (Dip-2$^-$) cells were mixed and treated, a single hybrid enzyme was observed that was identical in mobility and expression to that found in WIL hybrids. Therefore, (i) an inactive protein is synthesized in LTP cells, as in WIL hybrids, and (ii) the formation of the hybrid enzyme is not related to the actions of other structural and regulatory genes which function in the hybrid cells. Separately treated homogenates of WI-38, LTP, and LM cells did not generate a hybrid enzyme (Fig. 2).

The substrate specificities of Dip-2 and Pep-A were tested for several peptides. The results confirm the similar specificities reported for the mouse and human peptidases (*4, 14*). Peptidase-A and Dip-2 expressed a preference for L-valylleucine, which distinguishes them from the other peptidases. Little or no activity was expressed by Pep-A and Dip-2 for lysylleucine while human Pep-C and mouse Dip-1 were active. When leucylglycylglycine was employed, Pep-B and Trip-1 activity was observed but not Dip-2 and Pep-A activity. The WIL, REW, and in vitro hybrid enzymes expressed the same substrate requirements as Pep-A and Dip-2 (Figs. 1 and 2). The WIL hybrid enzyme appeared to stain the gel more intensely than the REW hybrid enzyme composed of normal subunits (Fig. 1). This finding suggests that the WIL enzyme composed of mutant and human polypeptides may be more active. However, since the intensity of the hybrid component reflects the number of human *Pep-A* genes in each hybrid population, and this varies between clones, it was not possible to accurately compare staining intensities between WIL and REW hybrid enzymes.

As a test of whether Dip-2$^-$ is a regulatory mutant, mouse-mouse cell hybrids of LM/TK$^-$ (Dip-2$^-$) and RAG (Dip-2$^+$) cells were isolated (*9*).

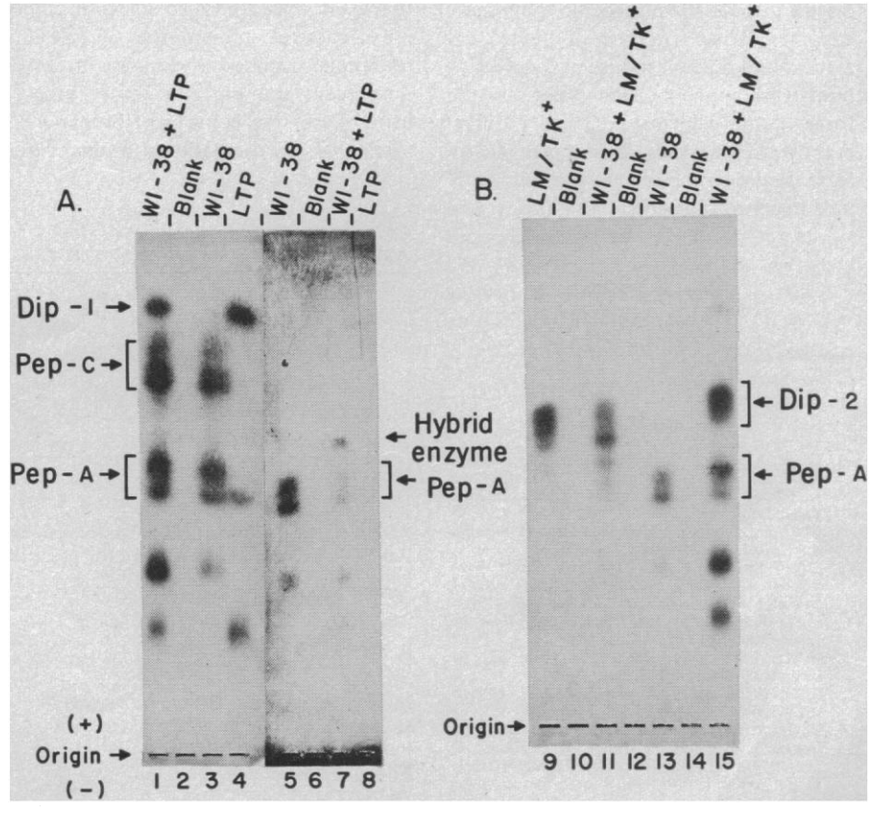

Fig. 2. Peptidase zymograms after in vitro hybridization (*13*) of human Pep-A and mouse Dip-2. (A) Peptidase hybridization was carried out in mixtures of homogenates of Dip-2$^-$ mouse and Pep-A$^+$ human cells. The substrate was leucyltyrosine (Leu-Tyr). Untreated samples are in channels 1, 3, and 4; treated samples are in channels 5, 7, and 8. The hybrid enzyme was observed only in the treated mixture in channel 7. The murine peptidase Trip-1, which has some affinity for Leu-Tyr, has a similar migration to human Pep-A in channel 4. (B) Hybridization of peptidase subunits was carried out on homogenates of Dip-2$^+$ mouse and Pep-A$^+$ human cells. Valylleucine was employed as substrate. Treated samples are in channels 9, 11, and 13. The hybrid enzyme was generated in a treated mixture in channel 11, but not in an untreated mixture in channel 15. Electrophoresis and staining were performed as described in legend to Fig. 1.

If Dip-2$^-$ is a regulatory mutant preventing synthesis of a structural protein, no enzyme activity would be expected. However, Dip-2 was expressed in hybrids, presumably by the RAG genome. These results and the formation of a hybrid enzyme in human-mouse cell hybrids and in in vitro mixtures indicate that the Dip-2 deficiency is not a regulatory mutant.

The fact that in vivo and in vitro hybridization occurred between mouse inactive peptidase Dip-2 and human active Pep-A indicated (i) a structural mutation for the *Dip-2* locus, (ii) structural homologies for the human and mouse enzymes, (iii) that the murine peptidase mutation did not eliminate subunit synthesis, and (iv) that the mutation did not eliminate the ability of inactive Dip-2 subunits to dimerize with the human gene product and form an active enzyme. The hybrid enzyme possessed the substrate affinities of human Pep-A and mouse Dip-2, and the staining intensities indicated that the hybrid enzyme was not less active than a hybrid enzyme composed of normal subunits.

Interspecific hybrid enzymes for several enzymes have been described in human-mouse cell hybrids (*6, 15*), and no restrictions have been described in the formation of heteropolymers. Thus, somatic cell hybrids may prove important in the study of human biochemical diseases that affect specific polymeric enzymes. The formation of hybrid enzymes with normal mouse and defective human components, and especially the formation of electrophoretically different hybrid enzymes, could provide information on genetic heterogeneity, subunit structure, structural alterations, and substrate specificities of mutant human enzymes.

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Virions from Progressive Multifocal Leukoencephalopathy: Rapid Serological Identification by Electron Microscopy

Abstract. *Virions were extracted directly from the brain of a patient with progressive multifocal leukoencephalopathy (PML). They were treated with antiserum to SV40, with rabbit antiserum to previous PML isolates, or with serum from another patient with the same disease and observed directly by electron microscopy. This procedure could be used for the rapid identification of the antigenic nature of virions in cases of PML.*

Progressive multifocal leukoencephalopathy (PML) is a subacute human demyelinating disease in which large numbers of papovavirus-like particles have been repeatedly found by electron microscopy (*1*). In the past year virus has been isolated from the brains of three patients with this disease (*2, 3*). Padgett *et al.* (*2*) inoculated a homogenate from brain tissue of a patient with PML into monolayers of human fetal brain cells and isolated a virus; this agent appeared distinct from mouse polyoma virus, simian virus 40 (SV40), or human papilloma virus by immunofluorescent staining. Weiner *et al.* (*3*) using dispersion cultures of human cells (derived from the patients' brains) fused to monkey cells [primary African green monkey kidney (PAGMK)] recovered viruses from two patients with PML. A close serological relation between the virus isolated by Weiner *et al.* and SV40 virus was demonstrated.

The isolation of new agents in cell culture systems always raises the possibility of contamination, and the isolation of an agent in simian cells with the antigenic phenotype of a simian virus raises the possibility that recombination of the agent with latent agents has occurred (*4*). Therefore, a method of serologic identification of virions extracted directly from diseased tissues would provide a valuable adjunct to cell culture methods.

Table 1. Serologic interaction of virions from brain and from related viruses; +, definite aggregation; −, no agglutination; space, not done.

Serums	Virions extracted from brain (patient A)	SV40	Previous virus isolates in PAGMK		Polyoma virus
			Patient A	Patient B	
Monkey antiserum to SV40	+	+	+	+	−
Horse antiserum to SV40	+	+			
Rabbit antiserum to virus isolated from patient A	+		+	+	−
Rabbit antiserum to virus isolated from patient B	+		+	+	−
Serum from patient A	−	−	−	−	
Serum from patient B	+	+	+	+	−
Rabbit antiserum to polyoma	−	−			+
Control serums					
Monkey serum before immunization to SV40	−	−	−	−	
Horse serum before immunization to SV40	−	−		−	
Rabbit antiserum to PAGMK cells	−		−	−	
Normal human serum		−		−	