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Human Acid Phosphatase in Somatic Cell Hybrids

Abstract. *The human enzyme, lysosomal acid phosphatase ACP_2, is expressed in man-rodent somatic cell hybrids as a dimeric molecule. The human-rodent heteropolymer, as well as the human and rodent homopolymer, is associated with lysosomes in these cells. The genes specifying lysosomal acid phosphatase ACP_2 and LDH A are syntenic.*

Somatic cell hybrids formed between aneuploid rodent cells and human fibroblasts or white blood cells selectively lose human chromosomes (*1, 2*). Human genes can be assigned to particular chromosomes by correlating the presence or absence of the gene product in a series of hybrid clones with the presence or absence of specific chromosomes (*3*). These hybrids provide a unique opportunity to study the organization and regulation of genes coding for enzymes and proteins associated with particular subcellular structures. We describe the expression of human lysosomal acid phosphatase ACP_2 in man-mouse and man-hamster somatic cell hybrids and the syntenic relationship of the gene specifying this enzyme with the gene specifying LDH A.

Rodent parental cells used for the preparation of hybrids included RAG, a mouse cell deficient in hypoxanthine–guanine phosphoribosyl transferase; 3T3 4E, a mouse cell deficient in thymidine kinase; and E 36, a hamster cell deficient in hypoxanthine–guanine phosphoribosyl transferase (*4*). Human parental cells included white blood cells from two normal males, white blood cells from two unrelated females carrying different translocations involving chromosomes X and 19, or diploid fibroblasts from two normal males. Parental cells were treated with Sendai virus inactivated by β-propiolactone (*2, 5*), and hybrid colonies were selected in Dulbecco's modification of Eagle's medium supplemented with 5 or 10 percent fetal calf serum, $1 \times 10^{-4}M$ hypoxanthine, $4 \times 10^{-7}M$ aminopterin, and $1.6 \times 10^{-5}M$ thymidine (*6*) (HAT medium). For electrophoretic analysis, cells from primary hybrid clones grown in HAT medium were harvested by trypsinization 9 to 12 weeks after hybridization.

Cell extracts were analyzed for acid phosphatase (E.C. 3.1.3.2.) by means of the starch gel systems of Lundin and Allison (*7*) and Swallow and Harris (*8*). Enzyme activity was detected with an α-naphthyl phosphate–fast garnet GBC salt or a β-glycerol phosphate–lead nitrate stain (*7*). The hybrid clones were analyzed by starch gel, agarose, acrylamide, or Cellogel electrophoresis for the presence of human and rodent forms of a number of enzymes which included: adenosine deaminase (E.C. 3.5.4.2), adenylate kinase 2 and 3 (E.C. 2.7.4.3), aspartate aminotransferase (E.C. 2.6.1.1), α-galactosidase (E.C. 3.2.1.22), β-glucuronidase (E.C. 3.2.1.31), glucose-6-phosphate dehydrogenase (E.C. 1.1.1.49), glucose phosphate isomerase (E.C. 5.3.1.9), hexosaminidase A and B, indophenol oxidase dimer and tetramer, isocitrate dehydrogenase (E.C. 1.1.1.42), lactate dehydrogenase A and B (E.C. 1.1.1.27), malate oxidoreductase (E.C. 1.1.1.37), malate oxidoreductase (decarboxylating) (E.C. 1.1.1.40), purine nucleoside phosphorylase (E.C. 2.4.2.1), peptidases A, B, and C, phosphoglucomutase 1 (E.C. 2.7.5.1), phosphogluconate dehydrogenase (E.C. 1.1.1.43), phosphoglycerate kinase (E.C. 2.7.2.3), and phosphopyruvate hydratase (E.C. 4.2.1.11) (*9*).

Starch gels of extracts of certain hybrid clones showed two new bands of acid phosphatase activity in addition to the rodent cathodal band (Fig. 1). The electrophoretic mobility of the anodal band is comparable to the major component of extracts of human diploid fibroblasts (see below). The second new component occupies a position intermediate to those of the rodent and human isozymes and presumably is a heteropolymer composed of rodent and human subunits. The formation of a heteropolymer is consistent with the

Table 1. Expression of human lysosomal acid phosphatase ACP_2 and human LDH A in man-mouse and man-hamster hybrid clones. The column designations are +/+, ACP_2 present and LDH A present; +/−, ACP_2 present and LDH A absent; −/+, ACP_2 absent and LDH A present; and −/−, ACP_2 and LDH A absent.

Source	Number of clones			
	+/+	+/−	−/+	−/−
RAG/WBC (X/19 translocation W)	3	0	0	5
RAG/WBC (X/19 translocation B)	7	0	0	7
RAG/WBC (D, normal male)	2	0	0	0
E 36/WBC (X/19 translocation W)	13	2	0	8
E 36/WBC (G, normal male)	2	0	0	1
3T3 4E/fibroblasts (normal male A)	3	0	0	1
3T3 4E/fibroblasts (normal male B)	4	0	0	1
Total	34	2	0	23

proposed dimeric structure of this enzyme (*8*). The heteropolymer was not observed when extracts of the two parental cells were mixed before electrophoresis, or when cell homogenates from parental cells were incubated together at 37°C for 2 hours. The electrophoretic mobilities of the putative human homopolymer, the heteropolymer, and the rodent homopolymer were not altered by incubation of cell extracts with neuraminidase (Sigma, type VI) for 3 hours at 37°C (*10*).

Shows and Lalley (*11*) observed the segregation of a human gene that extinguishes the expression of the cathodal mouse acid phosphatase isozyme in certain hybrid clones derived from fusions of LTP or LM/TK$^-$ mouse cells with human diploid fibroblasts. The expression of this gene is independent of expression of human acid phosphatase ACP_2. In none of our man-rodent hybrid clones was the expression of the cathodal rodent isozyme extinguished. This may reflect the nature of the rodent cell lines used in our hybrids.

The characteristics of the acid phosphatase isozymes in the parental cells and in the hybrids that expressed human acid phosphatase were investigated to determine whether these isozymes have the characteristics of lysosomal acid phosphatase. The single isozyme in each parental cell type and the three components in the hybrids (the rodent homopolymer, the heteropolymer, and the putative human homopolymer) have an acid *p*H optimum, are active on the substrate β-glycerol phosphate, show a lysosomal distribution after differential centrifugation of cell homogenates, and exhibit structure-linked latency (*12*). The electrophoretic mobility of the putative human homopolymer was compared to the mobility of lysosomal acid phosphatase in human diploid fibroblasts, in partially purified human placental lysosomes, and in white blood cells (Fig. 1). The mobility of the human homopolymer in hybrid cell extracts is comparable to that of the C isozyme of human lysosomal acid phosphatase, irrespective of whether the rodent parental cells are RAG, 3T3, 4E, or E 36 or whether the human parental cells are diploid fibroblasts or white blood cells (*12*). If the terminology of Swallow *et al.* (*13*) is used, the human lysosomal acid phosphatase isozyme expressed in our hybrids represents the product of the ACP_2 locus.

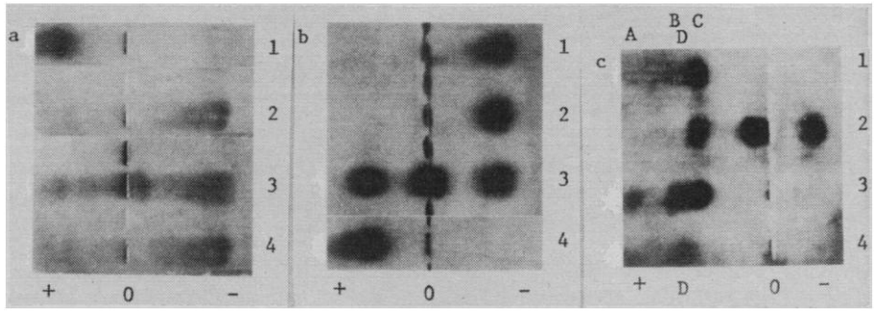

Fig. 1. Lysosomal acid phosphatase in man-rodent hybrid cells. Clones which expressed human acid phosphatase are designated positive hybrids. Negative hybrid clones did not express human acid phosphatase. (a) Man-mouse hybrids; 1, human fibroblasts; 2, negative clone from a hybridization of RAG (a mouse cell deficient in hypoxanthine–guanine phosphoribosyl transferase) with human white blood cells; 3, positive clone from the same hybridization; 4, RAG cells. (b) Man-hamster hybrids; 1, E 36 cells; 2, negative clone from a hybridization of E 36 with human white blood cells; 3, positive clone from the same hybridization; 4, human WI 38 cells. (c) Electrophoretic mobility of the human homopolymer of acid phosphatase in a man-hamster hybrid clone compared to the mobilities of the acid phosphatase isozymes in human cells; 1, human diploid fibroblasts; 2, positive man-hamster hybrid clone; 3, human placenta; 4, human white blood cells. The isozymes of human lysosomal acid phosphatase are designated A, B, C, and D (*8*). The D isozyme, which is associated with lymphocytes (*8*), has at least one subunit in common with the C isozyme (*22*) and may represent a dimer of modified C type subunits. The starch gel system of Lundin and Allison (*7*) was used in (a) and (b) and that of Swallow and Harris (*8*) in (c).

The expression of lysosomal acid phosphatase ACP_2 was correlated with the expression of 25 other human enzymes in the hybrid clones. The concordance in the presence or absence of LDH A and lysosomal acid phosphatase ACP_2 (Table 1) indicates a syntenic relationship of the genes specifying these two enzymes. The two discordant clones that expressed lysosomal acid phosphatase but not LDH A presumably arose by chromosome breakage. Lysosomal acid phosphatase ACP_2 segregated independently of the other 24 human enzymes that represent at least 12 additional syntenic groups. Since the LDH A locus has been assigned to chromosome 11 by Boone *et al.* (*14*), the locus specifying lysosomal acid phosphatase ACP_2 may now be assigned to chromosome 11. The gene specifying ACP_1, the nonlysosomal form of acid phosphatase present in red cells, is not linked to the ACP_2 locus but rather has been assigned to the short arm of chromosome 2 by family studies (*15*).

In our hybrid clones, human lysosomal acid phosphatase is expressed both as the human homopolymer and as a human-rodent heteropolymer. The association of the heteropolymer with subcellular particles, which behave as lysosomes by several criteria, suggests that lysosomes of man-rodent somatic cell hybrids can be comprised of hybrid components. The expression, in hybrid cells, of a human-hamster heteropolymer of the lysosomal enzyme α-galactosidase has been described by Grzeschik *et al.* (*16*). Human mitochondrial enzymes specified by the nuclear genome are present in mitochrondia of man-mouse hybrid cells which contain mouse but not human mitochondrial DNA (*17*). The human surface histocompatibility antigens and the H antigen are also expressed in man-mouse hybrid cells (*18*). Hence a number of complex cellular structures are formed from human and rodent proteins in somatic cell hybrids.

The human lysosomal enzymes hexosaminidase A, hexosaminidase B, and β-glucuronidase are also expressed in man-rodent hybrids (*12, 19–21*). The genes specifying these enzymes do not exhibit clustering in the human genome. A locus specifying hexosaminidase B has recently been assigned to chromosome 5 (*19*) and a locus specifying hexosaminidase A has been assigned to chromosome 7 (*20*). The locus specifying lysosomal β-glucuronidase is not syntenic with the loci specifying acid phosphatase ACP_2, hexosaminidase A, or hexosaminidase B (*12*). These observations have significance both for an understanding of gene organization in eukaryotic organisms and for the mechanism of assembly of lysosomes.

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Somatostatin: Hypothalamic Inhibitor of the Endocrine Pancreas

Abstract. *Somatostatin, a hypothalamic peptide that inhibits the secretion of pituitary growth hormone, inhibits basal insulin secretion in fasted cats and rats. In fasted baboons both basal and arginine-stimulated secretion of insulin and glucagon are inhibited. Somatostatin appears to act directly on the endocrine pancreas. The action is dose-related, rapid in onset, and readily reversed.*

Somatotropin release inhibiting factor (somatostatin, SRIF) has been shown to inhibit both basal (*1*) and stimulated growth hormone release (*1–4*) in a variety of in vivo and in vitro systems, with the use of rats, dogs, and humans. In addition, the increased thyrotropin (TSH) secretion caused by thyrotropin releasing hormone (TRH) is inhibited by SRIF, while the prolactin response to TRH was unaffected (*5–7*). The SRIF does not affect luteinizing hormone releasing hormone (LH-RH) stimulation of LH or follicle stimulating hormone (FSH) (*2, 4, 7*), nor does it alter the adrenocorticotropic hormone (ACTH)–corticosteroid response to hypoglycemia (*4*) in these studies. Basal secretion of pituitary hormones other than growth hormone has been unaffected.

While extending these studies in conscious, overnight-fasted, chair-restrained male baboons with permanently installed venous catheters, we noted that plasma glucose consistently fell during the intravenous administration of synthetic SRIF (the linear peptide form) and promptly returned to control levels upon termination of the infusion.

In order to determine whether this fall in glucose was attributable to a decrease in glucose production or an increase in glucose utilization, we performed an isotope dilution study (*8*) with uniformly labeled [^{14}C]glucose. In three studies the average steady-state glucose production rate was calculated to be 3.5 mg/kg · min (range 2.3 to 4.7). During a 30-minute SRIF infusion the specific activity of plasma glucose increased by an average of 22 percent (range, 18 to 26 percent), indicating a decrease in hepatic glucose production. If we assume that glucose utilization remains constant at the rate observed during the control period and calculate the magnitude by which the glucose concentration would fall in 30 minutes if production were completely inhibited, the value obtained is close to that observed experimentally. This implies that the observed hypoglycemia was largely accounted for by the inhibition of hepatic glucose production and that any change in glucose utilization must be small.

At this stage of fasting, factors which could rapidly reduce hepatic glucose production include (i) an increase in insulin secretion (*9*), (ii) an increase in parasympathetic activation of hepatic glycogen synthetase (*10*), (iii) a decrease in pancreatic glucagon secretion, or (iv) a decrease in adrenergic stimulation of hepatic phosphorylase (*11*). Alternatively, SRIF may act directly on the liver.

Preliminary attempts to look for direct effects of SRIF on hepatic glucose production in vitro were inconclusive. We therefore measured the plasma concentration of three of the above potential hormonal mediators during hypoglycemia caused by SRIF.

Concentrations of norepinephrine in the plasma (*12*) did not fall during SRIF-induced hypoglycemia in two animals. In contrast, plasma immunoreactive glucagon (*13, 14*) and insulin (*15*) changed during SRIF administration. Figure 1 shows the results obtained in four animals that had been given an intravenous loading dose of SRIF (25.0 μg/kg) which was followed by a 30-minute infusion of 0.83 μg/kg·min, a maximally effective hypoglycemic dose. Within 10 minutes venous plasma concentrations of both insulin and glucagon fell to less than 15 percent of pre-infusion values, while glucose had only fallen to 90 percent of pre-infusion values. Subsequently, insulin and glucagon could no longer be detected in three of the four animals studied, whereas glucose fell to 70 percent of the pre-infusion value at the end of the 30-minute SRIF infusion. When the somatostatin infusion was stopped, glucagon rebounded promptly to concentrations above those prior to infusion. This may be related to the lower glucose concentration acting as a stimulus to glucagon release once SRIF has been stopped. Glucose returned to normal within 30 minutes, while insulin recovered much more slowly—again perhaps reflecting the negative stimulus of hypoglycemia. With SRIF infusions of longer duration (up to 2 hours), insulin and glucagon remained very low until the end of the infusion. When added in vitro to standard hormone solutions, SRIF had no effect on the immunoassay systems.

The fall in basal insulin secretion could be either secondary to the fall in glucose concentration or could be due to a direct effect of somatostatin on the β cells of the pancreatic islets. The latter alternative is supported by the following observations: (i) At 3 and 6 minutes after SRIF was administered to baboons, insulin had fallen by 39 and 66 percent, respectively, whereas glucose remained unchanged. (ii) Soma-