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Genetics

Lysosomal arylsulfatase deficiencies in humans: Chromosome assignments for arylsulfatase A and B

(somatic cell hybrids/human chromosomes 5 and 22/metachromatic leukodystrophy/Maroteaux–Lamy syndrome/multiple sulfatase deficiency)

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ABSTRACT **Genetics of human lysosomal arylsulfatases A and B (aryl-sulfate sulfohydrolase, EC 3.1.6.1), associated with childhood disease, has been studied with human–rodent somatic cell hybrids. Deficiency of arylsulfatase A (ARS_A) in humans results in a progressive neurodegenerative disease, metachromatic leukodystrophy. Deficiency of arylsulfatase B (ARS_B) is associated with skeletal and growth malformations, termed the Maroteaux–Lamy syndrome. Simultaneous deficiency of both enzymes is associated with the multiple sulfatase deficiency disease, suggesting a common relationship for ARS_A and ARS_B. The genetic and structural relationships of human ARS_A and ARS_B have been determined by the use of human–Chinese hamster somatic cell hybrids. Independent enzyme segregation in cell hybrids demonstrated different chromosome assignments for the structural genes, ARS_A and ARS_B, coding for the two lysosomal enzymes. ARS_A activity showed concordant segregation with mitochondrial aconitase encoded by a gene assigned to chromosome 22. ARS_B segregated with β-hexosaminidase B encoded by a gene assigned to chromosome 5. These assignments were confirmed by chromosome analyses. The subunit structures of ARS_A and ARS_B were determined by their electrophoretic patterns in cell hybrids; a dimeric structure was demonstrated for ARS_A and a monomeric structure for ARS_B. Although the multiple sulfatase deficiency disorder suggests a shared relationship between ARS_A and ARS_B, independent segregation of these enzymes in cell hybrids did not support a common polypeptide subunit or structural gene assignment. The evidence demonstrates the assignment of ARS_A to chromosome 22 and ARS_B to chromosome 5. A third gene that affects ARS_A and ARS_B activity is suggested by the multiple sulfatase deficiency disorder.**

Two forms of arylsulfatase (aryl-sulfate sulfohydrolase, EC 3.1.6.1) are found in human lysosomes and are designated arylsulfatase A (ARS_A) and B (ARS_B). Deficiencies of these lysosomal arylsulfatases (1, 2) are inherited as autosomal recessive traits in humans and are associated with diverse pathological conditions, including early death. A deficiency of ARS_A, a cerebroside-3-sulfatase (3), leads to an accumulation of metachromatic sulfatides in the white matter of the central and peripheral nervous system, which is characteristic of metachromatic leukodystrophy (MLD). Progressive disintegration of myelin and neurodegeneration in the patient are followed by death. Classical metachromatic leukodystrophy may appear as a late infantile, a juvenile, or an adult form (see ref. 4). All three are characterized by a deficiency of ARS_A. A deficiency of ARS_B, an *N*-acetylgalactosamine-4-sulfate sulfatase (5, 6), is associated with increased excretion of dermatan sulfate and a broad array of clinical signs (7), including hepatosplenomegaly, corneal opacity, skeletal malformation, and growth retardation, which comprise the Maroteaux–Lamy syndrome (MLS). A rare disorder, sometimes considered to be a variant form of MLD, has been described which is characterized by multiple sulfatase deficiencies (4). The simultaneous deficiency of arysulfatases A, B, and C and the steroid sulfatases raises the possibility that all share a common property.

Interspecific somatic cell hybrids have been used to dissect genetic and structural components required for the final expression of enzymes and to characterize molecular defects in inherited deficiency diseases (8). We have used human–Chinese hamster hybrids to determine whether there exists a genetic or structural relationship between the lysosomal arylsulfatases and to map the structural genes associated with the enzyme deficiencies. We have demonstrated separate and independent chromosomal localization for their structural genes. ARS_A segregated with mitochondrial aconitase ($ACON_M$), whose structural gene has been assigned to chromosome 22 in humans (9, 10). ARS_B segregated with β-hexosaminidase B (HEX_B), assigned to chromosome 5 (11, 12). Assignments of ARS_A and ARS_B to chromosomes 22 and 5, respectively, were confirmed by chromosome analysis. Independent segregation of the two lysosomal activities in somatic cell hybrids is compatible with their separate expression in single sulfatase deficiencies, as seen in MLD and MLS, and suggests genetic independence of the two diseases. The cell hybrid evidence excluded a common subunit for ARS_A and ARS_B as a possible explanation for their joint deficiency in the multiple sulfatase deficiency disorder. Taken together, this disorder and the gene assignment data suggest that an additional independent gene functions in affecting the expression of sulfatase activity in humans.

MATERIALS AND METHODS

Parental Cells. Human fibroblast lines (13) for cell fusion were AlTr (X/5 translocation), DUV, JoVa, TS-495, and GM 1696 (14). Fresh human leukocytes used for fusion were ChDe and PeLa (13). Other human lines were WI-38 (ATCC no. CCL 75) and three lines from the Human Genetic Mutant Cell Repository (Camden, NJ): GM 78 and GM 197, from MLD patients; and GM 519, from a patient with MLS. All human fibroblast lines were maintained on Eagle's (diploid) basal medium (GIBCO) with 10% fetal calf serum and antibiotics. Chinese hamster lines (13) used were CHW-1102 [hypoxanthine phosphoribosyltransferase (HPRT) deficient] and A3 (thymidine kinase deficient) maintained on Dulbecco's modified Eagle's medium (GIBCO) with 10% fetal calf serum and antibiotics.

Human–Rodent Cell Hybrids. Human and Chinese hamster

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Abbreviations: ARS_A and ARS_B, arylsulfatases A and B, respectively; MLD, metachromatic leukodystrophy; MLS, Maroteaux–Lamy syndrome; $ACON_M$, mitochrondrial aconitase; HEX_B, β-hexosaminidase B; HPRT, hypoxanthine phosphoribosyltransferase; HAT, hypoxanthine/aminopterin/thymidine.

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cells were fused with inactivated Sendai virus (15). Hybrid cells were cloned and maintained on the HAT selection medium (Dulbecco's modified Eagle's medium with hypoxanthine, aminopterin, and thymidine) containing 10% fetal calf serum and antibiotics (15).

ARS_A activity was examined in 29 independent primary hybrids from separate fusions of cells from five individuals with CHW-1102 and eight primary hybrids from separate fusions of cells from three individuals with the A3 line. The cell hybrids (and human parental lines) made with CHW-1102 were designated ATC (AlTr), CDC (ChDe), JVC (JoVa), PLC (PeLa), and TCH (TS 495). Those made with A3 were designated JVA (JoVa), PLA (PeLa), and TAC (TS 495). Thirty-eight secondary clones (subclones), including four different human lines and both hamster lines, were used for the ARS_A studies.

A total of 50 hybrids was examined for ARS_B activity. These included 39 primary and 11 secondary clones from fusions of cells from eight individuals with CHW-1102. The hybrid sets (and human parental lines) were designated ARC (GM 197), ATC (AlTr), CDC (ChDe), DUC (DUV), JVC (JoVa), PLC (PeLa), TCH (TS 495), and XSC (GM 1696).

Electrophoresis and Enzyme Analysis. Confluent monolayers of parental and hybrid cells were harvested and extracts prepared by homogenization in 50 mM Tris·HCl (pH 7.0) (15). Homogenates equivalent to 10^8 cells per ml were rapidly frozen and thawed five times and centrifuged at 21,000 × *g* for 45 min at 0°C. The supernatant fluid was used for analysis. Electrophoresis for ARS_A and ARS_B was carried out on Cellogel sheets, 500 μm thick (Kalex Scientific Co., Manhasset, NY), by a modification of the method of Rattazzi *et al.* (16). The buffer contained 36 mM sodium barbital/36.6 mM sodium acetate/glacial acetic acid, pH 7.0, and 0.5 mM dithiothreitol. Cellogel electrophoresis was carried out at 25 mA, constant current, for 3 hr at room temperature. Both activities could be visualized on the same Cellogel sheet; however, conditions were varied to best resolve ARS_A or ARS_B. The long axis of the 10 × 17 cm Cellogel sheets was placed parallel to the electrode chambers for ARS_A and across (at right angles to) the chambers for ARS_B determinations. The enzyme assay mixture contained 20 mg of 4-methylumbelliferyl sulfate, potassium salt (Research Products International Corp., Elk Grove Village, IL), in 5 ml of 0.5 M sodium acetate buffer, pH 5.0 or 5.6 for ARS_A or ARS_B, respectively. Both activities were visualized with long-wave UV light after enhancement of the fluorescent product for 2–3 min with ammonia vapor. Further enhancement was possible with an overlay soaked in 1 M carbonate/bicarbonate, pH 9.6.

Methods for analysis of the enzyme markers encoded on each chromosome, including $ACON_M$ on 22 and HEX_B on 5, have been described (10, 12, 13, 17–20).

Chromosome Analysis. Giemsa/trypsin banding procedures were used to identify human chromosomes as described (21). Human chromosome classification followed that recommended by the Paris Conference (1971) (22).

RESULTS

Phenotypic Expression of ARS in Human Tissues and Cultured Cells. Multiple molecular forms of ARS expressed after electrophoresis on Cellogel are shown in Fig. 1. The pattern seen in an extract of human liver (channel 1) is shown to be duplicated in WI-38, a cultured human diploid fibroblast (channel 2). The deficiency of ARS_A in MLD is shown in cells cultured from a patient in channel 3; the ARS_B deficiency is observed in cells cultured from a patient exhibiting the MLS (channel 4). The identity of ARS_A and ARS_B with the anodal and cathodal bands, respectively, was verified by DEAE-cellulose chromatography (23).

ARS_A Expression in Parental Cells and Hybrids. Human

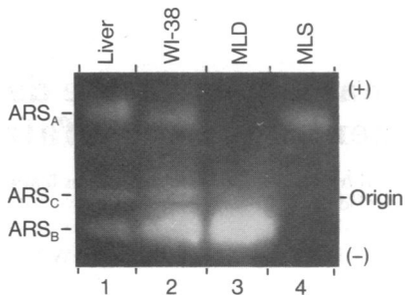

FIG. 1. Electrophoretic patterns for ARS activity in extracts of human cells. Channel 1, human liver; channel 2, human diploid fibroblast, WI-38, derived from embryonic lung; channel 3, skin fibroblast line, GM 78, derived from a patient with MLD; channel 4, skin fibroblast line, GM 519, from a patient exhibiting the MLS. ARS_A and ARS_B are lysosomal enzymes; ARS_C is the microsomal form.

ARS_A (Fig. 2, channel 2) can readily be distinguished from the more anodally migrating Chinese hamster band (channel 3). After cell fusion, two ARS_A phenotype patterns were observed in cell hybrids. One pattern is identical to that seen in the hamster parent (channels 6 and 7). The second contained the hamster band and also a band intermediate in mobility between the two parental bands (channels 4 and 5). Occasionally a very faint human ARS_A band could be seen cathodal to the intermediate band. The intermediate band most likely represents a heteropolymer formed between human and hamster subunits in hybrid cells (24). This is consistent with the dimeric structure shown for ARS_A by physical-chemical studies (25). When this intermediate band was observed, the hybrid was scored positive for the human gene, *ARS_A*. Involvement of a human gene in this phenotype was supported by its segregation among hybrid clones. No intermediate band was observed when the two parental lines were cocultivated or when extracts of the parental cells were merely mixed *in vitro* and incubated at 37°C.

Segregation of ARS_A Activity in Hybrids. Extracts were tested for the presence or absence of the hybrid ARS_A band and its distribution in relation to 30 other enzymes representing 21 of the 24 different human chromosomes. The results are shown in Table 1. ARS_A activity was expressed concordantly with $ACON_M$ activity in 97% of the hybrids examined. Concordant

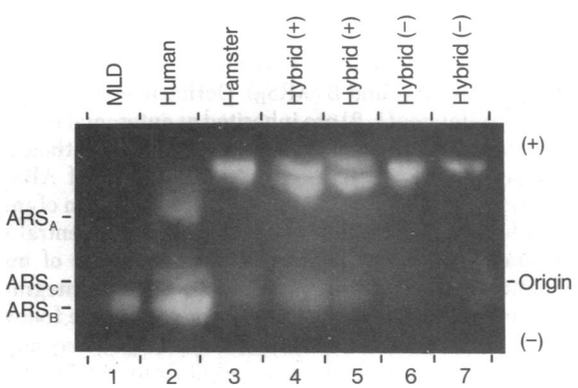

FIG. 2. Cellogel electrophoretic patterns for ARS_A activity in parental cells and hybrid clones. Channel 1, skin fibroblast line, GM 197, from a patient with MLD; channel 2, WI-38, human diploid fibroblast line; channel 3, CHW-1102, HPRT-deficient Chinese hamster line. Channels 4–7, human–Chinese hamster hybrid clones: channels 4 and 5, hybrids expressing the human gene for ARS_A; channels 6 and 7, hybrids lacking the human gene for ARS_A. The intermediate heterodimer migrating between the hamster and human parental enzymes was scored positive for ARS_A (channels 4 and 5). The human band was sometimes weakly visible in cell hybrids and was difficult to demonstrate photographically. Relative band intensities of parental enzymes and heteropolymers has been discussed for expression of multimeric enzymes in cell hybrids (13).

segregation was not observed for any of the other markers tested. These results demonstrate that ARS_A segregates discordantly with all enzyme markers except the chromosome 22 marker, $ACON_M$. The two exceptions in the ARS_A–$ACON_M$ cosegregation pattern may be explained by chromosome breakage or sensitivity of enzyme assays used.

Assignment of Gene Locus for ARS_A. Reliable enzyme markers were not available for human chromosomes 3 and 13 in human–Chinese hamster hybrids. In order to test these for the assignment of *ARS_A*, several primary and secondary hybrid clones were subjected to chromosome analysis where all human chromosomes could be identified. The distribution of human chromosomes in these hybrids is shown in Table 2. Human ARS_A segregated independently of chromosome 3 and 13 (30 and 70% discordancy, respectively). ARS_A segregated without exception with both $ACON_M$ activity and chromosome 22 in these hybrids. All other chromosomes were excluded through discordant segregation.

Table 1. Distribution of ARS activity and enzyme markers in somatic cell hybrids

Chromosome	Enzyme marker	ARS_A		ARS_B	
		Concordant	Discordant	Concordant	Discordant
1	ENO-1	25(4)*	11(2)	14	7
	PGM_1	25(4)	12(6)	17	9
	PEPC	25(5)	12(5)	19	12
2	ACP_1	15(4)	5(0)	8	2
	IDH_S	28(1)	9(26)	22	9
	MDH_S	17(9)	8(5)	9	1
4	PEPS	7(13)	4(1)	11	10
5	HEX_B	26(29)	11(4)	31	0
6	ME_S	13(2)	6(5)	14	11
7	GUS	26(24)	11(14)	25	6
8	GSR	6(4)	6(4)	8	13
9	AK_1	24(3)	11(24)	19	11
10	GOT	24(14)	12(22)	22	9
11	ACP_2	14(5)	13(9)	8	2
	ESA_4	21(7)	14(6)	22	8
	LDH_A	23(26)	13(8)	21	10
12	LDH_B	20(4)	16(24)	19	12
14	NP	14(4)	20(19)	17	11
15	HEX_A	25(15)	12(7)	20	11
	MPI	25(18)	12(6)	18	12
	PK_{M2}	24(18)	13(8)	19	12
16	APRT	9(6)	5(5)	5	10
17	GALK	4(4)	2(2)	5	3
18	PEPA	11(17)	9(8)	19	6
19	GPI	19(24)	17(7)	18	10
	MAN_B			9	5
20	ADA	20(17)	14(2)	19	10
21	SOD_S	16(18)	14(9)	21	9
22	$ACON_M$	36(37)	1(1)	16	7
X	G6PD	22(28)	15(8)	20	10
	PGK	15	10	7	5

Symbols for marker enzymes, their chromosome assignments, and electrophoretic procedures have been described (10, 12, 13, 17–20). The concordant segregation columns show the numbers of hybrid clones in which ARS activity and the respective enzyme marker(s) were either present or absent together. Indicated in the discordant columns are the numbers of clones in which only ARS or the enzyme marker was expressed in a given homogenate. Totals differ within and between syntenic (linkage) groups since activity for each enzyme marker was not always determined for every hybrid. When an enzyme marker was tested, its activity and that for ARS was always determined on the same homogenate (cell passage). Note that ARS_A is coexpressed with $ACON_M$, a chromosome 22 marker, and that ARS_B is jointly expressed with HEX_B, a chromosome 5 marker.

* Additional subclones (in parentheses) of primary hybrids were tested for ARS_A, adding to the total cell hybrids examined.

Phenotypic Expression of ARS_B in Cultured Cells. Fig. 3 demonstrates the expression of ARS_B activity in cell hybrids after electrophoresis. Hamster ARS_B activity (channels 3–5) migrated more anodally than human ARS_B (channel 1). Hybrids displayed two phenotypes for ARS_B. Some showed both parental bands (channel 4) and, since the hamster band was often weak (channel 2) or not seen at all, some expressed only the human band (not shown). Hybrids negative for human ARS_B showed only the hamster band (channels 3 and 5). No heteropolymers of ARS_B were observed, confirming the monomeric nature of this enzyme (25). Any hybrid showing the human band was scored positive for *ARS_B*. The expression of ARS_B in cell hybrids was independent of ARS_A (Fig. 3, channels 3 and 4).

Segregation and Assignment of ARS_B. ARS_B activity was examined in 31 primary hybrid clones for cosegregation with 31 enzyme markers encoded on specific human chromosomes. The results are shown in Table 1. ARS_B segregated concordantly, without exception, with HEX_B; the *HEX_B* gene was previously assigned to chromosome 5 (11, 12). Joint segregation of ARS_B with enzymes representing the other human chromosomes was not observed (Table 1). After chromosome analysis, ARS_B was found to segregate concordantly with chromosome 5 and HEX_B in all hybrids examined (Table 2). Discordant segregation was observed between ARS_B and chromosomes 3, 13, and all other chromosomes (Table 2).

Hybrids made from human cells (AlTr) containing an X;5 translocation chromosome [46,X,t(Xq;5q)(q22;q35)] were selected on hypoxanthine/aminopterin/thymidine (HAT)-supplemented medium and examined for ARS_B activity (12). In this cell line, the X chromosome region encoding *HPRT* is translocated to the terminal region of the long arm of chromosome 5. To survive in HAT medium, the *HPRT* gene on the X;5 translocation must be retained (12, 21). All surviving hybrids expressed ARS_B and HEX_B in addition to the X chromosome markers, HPRT and glucose-6-*P* dehydrogenase (Table 3). After counterselection on 8-azaguanine-supplemented medium, growth is possible only if *HPRT* and the X;5 translocation are not retained, through chromosome loss (21). Surviving clones, after counterselection against *HPRT* encoded on the X;5 translocation, were negative for ARS_B and HEX_B and the X-linked markers, HPRT and glucose-6-*P* dehydrogenase. The cosegregation of ARS_B with HEX_B, HPRT, and glucose-6-*P* dehydrogenase on the X;5 translocation further supports the assignment of ARS_B to chromosome 5.

DISCUSSION

Of the several arylsulfatases known to exist in mammalian cells, the lysosomal forms, ARS_A and ARS_B, have been studied extensively and are of interest since both enzymes are associated with fatal inherited disorders (4). Although the two lysosomal arylsulfatases may both be conveniently assayed with a common substrate, 4-methylumbelliferyl sulfate, they act independently of one another, with each displaying distinct specificity toward natural substrates (3, 5, 6). ARS_A is a cerebroside-3-sulfatase, ARS_B exhibits a specificity for *N*-acetylgalactosamine-4-sulfate. Each has a distinct pH optimum and electrophoretic mobility and each is subject to different inhibitors (26). There is also no evidence for immunological crossreactivity between the two forms (27, 28). ARS_A is a dimer with an apparent molecular weight around 107,000 at neutral pH, about twice that of ARS_B, a monomer. ARS_A can be dissociated into two subunits of molecular weight about 50,000–60,000 each, and neither subunit has the activity of ARS_B (25). Our cell hybrid data support this subunit structure for ARS_A and ARS_B. Like other lysosomal enzymes, both arylsulfatases are glycoproteins; ARS_A is acidic and ARS_B is basic. Treatment of ARS_A with neurami-

Table 2. Segregation of human chromosomes and selected enzymes in human–Chinese hamster hybrids

Hybrid clones	Enzymes				No. cells examined	Human chromosomes																				
	ARS_A	$ACON_M$	HEX_B	ARS_B		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X
CDC-2	+	+	+	+	16	9		7		12	9			6	2			1	10		4	12	9	5	5	8	11	13
CDC-4	+	+	+	+	21					7	15	7		3	2	9			5		9		13		8	13	14	9
CDC-5	−	−	−	−	18								9		8				2		9			10		18		13
CDC-13	+	+	−	−	11											2	2						1	1			9	10
CDC-16	+	+	+	+	20			5	7	13		13		11	18				12	4	20	20		2			9	19
CDC-19	+	+	−	−	16			4	1		1										2	8	1		3	12	14	8
JVC-1	+	+	+	+	21			11	11	14		5				10		8		13			5	14	11		13	17
JVC-1B	+	+	+	+	21			13		10						5				7			10		13		10	18
JVC-1J	+	+	+	+	14			9	5	6		11				8				8				6	4		9	12
ATC-13M*	+	+	+	+	11					2													2	10			8	3

Each enzyme was scored for the presence (+) or absence (−) of the human or hybrid phenotype in a clone. Human chromosomes were identified by Giemsa banding and morphology according to the Paris Conference, 1971 (22). The number of cells examined for each hybrid is indicated. The number of cells that contained each chromosome is listed under each human chromosome. Both the enzyme analyses and chromosome identification were performed on replicate flasks from the same cell passage.

* X;5 translocation was never observed in these cells although human HPRT was observed, indicating chromosome breakage.

nidase retards its mobility toward the anode but does not convert it to ARS_B (25, 29). The different physical-chemical characteristics of ARS_A and ARS_B and their distinct physiologic specificities suggest these enzymes are coded by different structural genes.

The diverse pathological conditions resulting from the absence of ARS_A or ARS_B in deficiency diseases strengthen the argument for genetic independence for the two enzymes. Deficiency of ARS_A activity results in the accumulation of metachromatic granules and neurodegeneration, which characterize the sulfatide lipidosis termed MLD (4). Deficiency of ARS_B is associated with accumulation of dermatan sulfate and growth and skeletal abnormalities comprising the MLS, a type of mucopolysaccharidosis (4). Deficiency of one sulfatase activity in the presence of the other argues strongly for independence between the two autosomally inherited disorders. Deficiency of ARS_A and ARS_B in MLD and MLS, respectively, probably results from defects in the structural genes coding for these two enzymes. Antibodies directed against each ARS have shown crossreacting protein (CRM) in tissues of patients in amounts equivalent to that in normal subjects (27, 28). Some residual enzyme activity with reduced specific activity has been observed. Electrophoretic properties of the defective molecules were identical to enzyme extracted from normal fibroblasts (28). The evidence suggests that the structural genes coding for ARS_A and ARS_B are abnormal in MLD and MLS, respectively.

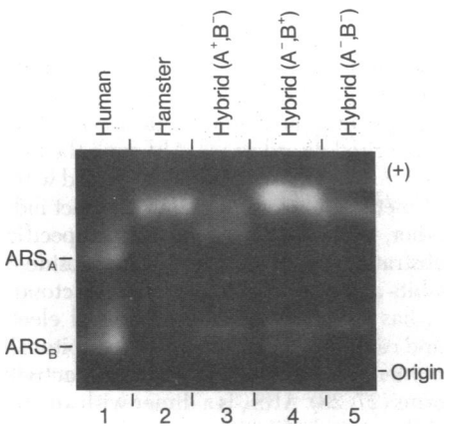

FIG. 3. Electrophoretic expression of ARS_B activity in parental cells and hybrid clones. Channel 1, human fibroblast line WI-38; channel 2, CHW-1102, HPRT-deficient Chinese hamster line. Channels 3–5, human–Chinese hamster hybrid clones: channel 3, arylsulfatase A^+B^- hybrid; channel 4, arylsulfatase A^-B^+ hybrid; and channel 5, arylsulfatase A^-B^- hybrid (ARS activity was indicated for human phenotypes only).

The somatic cell hybrid method allows for the dissection of multiple genes responsible for the expression of a complex isozyme system. We have shown that ARS_A and ARS_B are coded by structural genes on different human chromosomes. ARS_A activity segregated concordantly with $ACON_M$ in 97% of all human–Chinese hamster somatic cell hybrids tested; chromosome analysis confirmed the syntenic relationship between ARS_A and $ACON_M$ and showed the concordance between these two enzymes and human chromosome 22. This is in agreement with the assignment evidence reported by Bruns *et al.* (30). Enzyme segregation data, based on enzyme markers for only 20 human chromosomes, previously suggested synteny between *ARS_A* and *GUS*, assigned to chromosome 7 (31). Enzyme markers were lacking for chromosomes 3, 13, and 22; the Y chromosome was ruled out since positive hybrids were isolated from fusions involving both male and female parent lines. Expansion of our data by generating new hybrids and secondary clones, the availability of $ACON_M$ as a marker for chromosome 22, and direct chromosome analysis enabled us to exclude chromosome 7 as well as 3 and 13. Concordant segregation between ARS_B, HEX_B, and chromosome 5 was found without exception in all somatic cell hybrids examined. This assignment supports the evidence reported by Hellkuhl and Grzeschik (32) with human–mouse hybrids. Sex chromosome linkage was ruled out on the same basis as for ARS_A. Assignment of ARS_B to chromosome 5 was verified with a set of hybrids containing a

Table 3. Segregation of ARS_B, HEX_B, and selection markers in X;5 translocation hybrids

Selection medium	Hybrid sets	No. of clones	Enzyme markers			
			G6PD	HPRT	HEX_B	ARS_B
HAT*	ATC	8 primary	+	+	+	+
		3 secondary	+	+	+	+
8-Azaguanine†	ATC-8	2	−	−	−	−
	ATC-11	3	−	−	−	−

Each enzyme was scored for the presence (+) or absence (−) of the human phenotype in each hybrid clone selected on hypoxanthine/aminopterin/thymidine medium or counterselected on 8-azaguanine-supplemented medium (21). For each hybrid, all enzymes were determined on the same homogenate. Glucose-6-phosphate dehydrogenase (G6PD) and HPRT are coded by X-linked genes translocated to chromosome 5.

* Dulbecco's modified Eagle's medium supplemented with hypoxanthine, aminopterin, and thymidine. HPRT must be present for the cells to survive.

† Dulbecco's modified Eagle's medium supplemented with 8-azaguanine. HPRT must be absent (through chromosome loss) for the cells to survive.

human X;5 translocation by using the selection/counterselection principle with different selection media. These somatic cell genetic data show that independent structural genes code for ARS_A and ARS_B on chromosomes 22 and 5, respectively.

The rare multiple sulfatase deficiency disorder, sometimes referred to as a variant form of MLD, is characterized by a combination of clinical symptoms for both MLD and MLS with deficient activity of ARS_A, ARS_B, the microsomal ARS_C, and the steroid sulfatases (33). This indicates that a common property is shared among these arylsulfatases. There are examples of structural interdependence for other lysosomal enzymes. At least two of the HEX isozymes share a common subunit. HEX_A is composed of two different subunits, α and β; HEX_B is composed of β subunits only. The gene coding for the α subunit has been assigned to chromosome 15, and the β subunit is encoded on chromosome 5. Thus, expression of HEX_A is dependent upon HEX_B by virtue of the shared subunit (12). There is also a shared polypeptide for the lysosomal acid phosphatases-2 and -3 (34). However, ARS_A and ARS_B do not share a common subunit since cell hybrids can be isolated that contain human chromosome 22 in the absence of chromosome 5 and express only ARS_A activity. Hybrids that expressed the opposite phenotype were also observed. On this basis, a shared subunit was ruled out.

Recently, Fluharty *et al.* (35) have shown that deficient activities of arylsulfatases A, B, and C in cultured fibroblasts from patients with multiple sulfatase deficiency disorder could be elevated, simultaneously, to near normal levels by altering growth medium. This report suggested the defect in this disorder to be under reversible environmental control, perhaps by posttranslational modification of the enzymes. Earlier, Austin (36) reported that an inactive polypeptide could be determined in multiple sulfatase deficiency disorder with antibody raised to ARS_A from normal tissues, which suggests a structural gene defect, a specific inhibitor, or a lack of a posttranslation modification.

We have presented evidence suggesting a common posttranslation modification for other lysosomal enzymes (37, 38). The abnormal electrophoretic mobilities of several lysosomal enzymes in fibroblasts from individuals with mucolipidoses II and III were attributed to abnormal sialylation. Such an abnormal processing could be the property shared by the deficient enzymes in multiple sulfatase deficiency disorder.

The somatic cell hybrid strategy has been used effectively to dissect the genetic characteristics of enzyme deficiencies associated with human disease (8). We have used this approach to better understand the genetics of ARS_A and ARS_B associated with MLD and MLS in humans and have mapped the structural genes, ARS_A and ARS_B, to chromosomes 22 and 5, respectively. Reports on the properties of the deficient enzymes suggest that the MLD mutation alters the ARS_A structural gene, while the MLS mutation alters the ARS_B structural gene. Properties of the multiple sulfatase deficiency disorder mutation suggest a third gene that affects activity of ARS_A and ARS_B, possibly by control or posttranslation modification mechanisms. These data indicate that several genes are required for the final expression of arylsulfatase enzymes.

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