

THE LOCUS FOR HUMAN ADENINE
PHOSPHORIBOSYLTRANSFERASE ON CHROMOSOME NO. 16¹

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ABSTRACT

Evidence for assigning the locus determining the structure of adenine phosphoribosyltransferase (APRT) to human chromosome No. 16 is presented. Hybrids of APRT-deficient mouse cells and of human fibroblasts having normal APRT were isolated by fusing the parental cells with Sendai virus, blocking *de novo* purine nucleotide synthesis with azaserine and selecting for hybrids that could use exogenous adenine. The hybrid clones that were studied had only APRT activity that was indistinguishable from human APRT with regard to electrophoretic migration and reaction with antibodies against the partially purified human enzyme. No. 16 was the only human chromosome consistently present in all of the clones, and in one clone, it was the only human chromosome detected. Selection against hybrid cells with 2,6-diaminopurine (DAP) yielded DAP-resistant survivors that lacked chromosome No. 16. One hybrid that originally had an intact No. 16 yielded adenine-utilizing subclones that lacked No. 16 but had a new submetacentric chromosome. The distribution of centromere-associated heterochromatin and the fluorescence pattern indicated that this chromosome consisted of a mouse telocentric chromosome and the long arm of No. 16. Cells having the submetacentric chromosome had human APRT. Both the enzyme and the chromosome were absent in DAP-resistant derivatives. These results suggest that the structure of APRT is defined by a locus on the long arm of human chromosome No. 16.

HUMAN genes can be mapped to their respective chromosomes in somatic cell hybrids by detecting concordant segregation patterns between specific phenotypic expressions and specific human chromosomes in interspecies hybrid cells. This principle is now well established, and one or more genes have already been assigned to most of the human chromosomes using this approach (RIDDLE 1973).

We report here evidence for assigning the locus determining the structure of adenine phosphoribosyltransferase (APRT; E.C. 2.4.2.7) (ENZYME NOMENCLATURE: RECOMMENDATIONS OF 1964, 1965), which converts adenine to adenosine-5'-monophosphate (AMP), to human chromosome No. 16. This

¹ Abbreviations: AG, 8-azaguanine; DAP, 2,6-diaminopurine; [AS], azaserine; APRT, adenine phosphoribosyltransferase; CS, calf serum; FCS, fetal calf serum; A[AS], adenine-azaserine selective medium; MNNG, N-methyl-N'-nitro-N-nitrosoguanidine; HGPRT, hypoxanthine-guanine phosphoribosyltransferase.

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enzyme is dispensable for growth under ordinary conditions, since cells can synthesize the nucleotide via a *de novo* pathway. If *de novo* synthesis is blocked cells having APRT can utilize exogenously supplied adenine and grow but APRT-deficient cells are arrested. Therefore, when APRT-deficient mouse cells are fused with normal human cells and the *de novo* synthesis of AMP is blocked in adenine-containing medium, mouse parental cells are eliminated and hybrid cells containing human APRT form distinct colonies against the background of persistent human cells. KUSANO, LONG and GREEN (1971) used alanosine to block *de novo* AMP synthesis in applying this principle and tentatively assigned genes for APRT to a human acrocentric chromosome. However, two ongoing research problems in this laboratory prompted us to independently determine the chromosomal location of APRT genes: (i) We have obtained variant diploid human fibroblasts that are resistant to 2,6-diaminopurine (DAP). At least some of the variants have deficient APRT activity and it was necessary to determine the location of APRT genes in order to genetically analyze the DAP-resistant variants. (ii) We were attempting to detect derepression of the human inactive X chromosome by selecting for expression of the HGPRT gene on the inactive X in hybrids of female fibroblasts with HGPRT-deficient mouse cells. The principle of these experiments is described elsewhere (KAHAN and DEMARS 1973). As an alternative, we thought that initial selection for APRT in hybrid cells would permit a condition of gratuity, in which events necessary for X-chromosome reactivation could occur without cell proliferation being immediately dependent on expression of previously inactive X-chromosomal genes. Such hybrids could subsequently be checked for expression of selectable, or even unselectable alleles of genes that were on the inactive X in the human cell parent. In the course of these hybridization studies we were able to assign the structural gene for APRT to human autosome No. 16. The assignment is probably reliable since it agrees with that made independently by TISCHFIELD and RUDDLE (1974) using different strains of cells and a different selection system. In addition, we have observed translocated chromosomes in the hybrid cells that lead us to assign the APRT gene to the long arm of No. 16.

MATERIALS AND METHODS

Details of cell propagation methods are presented in DEMARS and HELD (1972).

Cell lines: The human fibroblasts were derived from a skin biopsy of a female having normal APRT who was heterozygous for the A and B alleles of the X-chromosomal glucose-6-phosphate dehydrogenase (G6PD) locus. For purposes of X-chromosome derepression studies an HGPRT-deficient subclone was produced by a mutagenic exposure of strain No. 129 cells to 2.5 $\mu\text{g}/\text{ml}$ MNNG for 24 hours. Clone 129.22 was isolated in 3×10^{-5} M 8-azaguanine (AG) in F10 medium lacking hypoxanthine (HAM 1963) and containing 15% calf serum (CSF10 medium). Clone 129.22 is resistant to AG due to a deficiency in HGPRT enzyme activity, having 5% of the normal specific activity, and is unable to grow in HAT selective medium (SZYBALSKI, SZYBALSKA and RAGNI 1962), modified as previously described (DEMARS and HELD 1972). Diploid cells of the clone have a normal fluorescence karyotype.

The mouse line D⁷ was derived by AG selection (8×10^{-6} M) from 1D LM(TK-) cells (DUBBS and KIT 1964) and lacks detectable HGPRT activity. APRT-deficient cells of the 1D line were found to spontaneously exist as about 20% of the population. D⁷ is an APRT-deficient

subclone of 1D cells, and is therefore an APRT⁻, HGPRT⁻, TK⁻, triply deficient cell line. The modal chromosome number for D⁷ is 52, including 7 large metacentric chromosomes and one large marker chromosome having the appearance of a dicentric chromosome due to a prominent secondary constriction. The remaining chromosomes are acrocentrics. D¹¹ is an APRT⁺ subclone of 1D.

Formation and selection of hybrids: Hybrid cells were isolated and maintained in selective medium consisting of CS-F10 medium containing 10⁻⁶ M azaserine ([AS]) to block the *de novo* purine pathway, and 3 × 10⁻⁵ M adenine (A[AS] medium). Normal human fibroblasts and 1D mouse lines not deficient in APRT grow well in A[AS], whereas growth of APRT-deficient mouse D⁷ cells is completely inhibited.

Cells from 129.22 were fused with D⁷ cells following a modification of the monolayer technique of KLEBE, CHEN and RUDDLE (1970). On day 0, 10⁶ cells of each parental type were mixed in suspension and plated in a 60 mm diameter Falcon plastic petri dish (P60) in a total of 5 ml F10 medium supplemented with 15% fetal calf serum (FCSF10). The following day, the medium was aspirated and the dish was chilled at 4° for 10 minutes. 2000 HAU's of β-propiolactone inactivated Sendai virus in one ml of chilled 10% FCS-F10 was added, and the dish was incubated at 4° for 20 minutes. The cells were then returned to 37° and 4 ml warm 10% FCS-F10 was added. After 2 hours, the dish was rinsed twice and renewed with 5 ml volumes of FCSF10 medium. On day 2, the cells were suspended by trypsinization and distributed into 100 P60's containing FCSF10. On day 6, selection of hybrid cells was begun. The seven hybrid colonies that appeared were isolated 3 weeks later by encircling the colonies with 7 mm I.D. stainless steel cylinders and removing the trypsinized cells with a Pasteur pipette.

In experiments in which loss of APRT activity in hybrid cells was correlated with loss of a particular chromosome, hybrid cells resistant to the adenine analog 2,6-diaminopurine (DAP) were isolated in CSF10 medium containing 10⁻⁴ M DAP. The incidence of DAP-resistant colony formers was between 10⁻⁴ and 10⁻² in two independent hybrids.

Chromosome analysis: Slides of metaphase chromosomes were prepared by the air-dry method of ROHFELS and SIMINOVITCH (1958). For quinacrine banding patterns (CASPERSON *et al.* 1970), slides were stained for 30 minutes in an aqueous 0.5% Atebrin solution, rinsed three times with distilled water, and mounted in a 0.1 M KH₂PO₄ buffer, pH 5.5, 20% (w/v) sucrose solution. Photographs were obtained with a Zeiss fluorescence microscope fitted with a Planachromat 100x oil immersion objective with iris diaphragm (.8/1.25 N.A.) and dark field ultra-condensor, 1.2/1.4 N.A. Light was transmitted by an HBO 200W/4* mercury bulb through exciter filter BG12 and barrier filter 53. Kodak High Contrast Copy Film and Kodabromide F3-F5 paper were used for prints.

Constitutive heterochromatin patterns were obtained by treating 1-8-week-old slides with 90-95% hot formamide for 15-30 minutes, then staining with a 1% Giemsa solution for 30-60 minutes (DEV *et al.* 1972).

Enzyme assays: Cell monolayers containing about 1 to 3 × 10⁶ cells per P60 were rinsed twice with cold 0.155 M KCl and were collected in .2 ml of 0.155 M KCl per P60 by scraping with a rubber policeman. The cells were disrupted by 3 cycles of freezing and thawing and were then centrifuged at 4° for 20 minutes at 20,000 × g. 6-19 μg of cell protein was present in 50 μl of reaction mixture containing 5 × 10⁻² M Tris HCl, pH 7.4, 5 × 10⁻³ M MgSO₄, 10⁻³ M PRPP, 2.0 × 10⁻⁴ M [8-¹⁴C] adenine (0.235 mc/mmole, Schwarz-Mann), and 50 μg bovine serum albumin. Reactions proceeded for 30 minutes at 37°. Detection of converted purines was as described in RAPPAPORT and DEMARS (1973).

APRT electrophoresis: Two considerations motivated the choice of electrophoretic system: (1) for optimal separation of human and mouse forms of the enzyme, a buffer system near the isoelectric point of one of the species was sought; (2) for comparison purposes, a slab gel allowing side-by-side multiple runs is preferable. Therefore, electrophoresis was conducted in acrylamide slabs at pH 6.0 using an ORTEC electrophoresis system (ORTEC, Inc., Oak Ridge, Tenn.) (But see TISCHFIELD, BERNHARD and RUDDLE [1973] for what appears to be a better method.)

Gels were cast as suggested by ORTEC (ORTEC Inc., 1973). An acrylamide gradient separating gel of 8% (51 mm), 6% (9 mm) and 3½% (5 mm) acrylamide, and a well-forming gel of 8% (20 mm) acrylamide were used.

The buffer system within the gel was potassium acetate (pH 6.0). The tank buffer was potassium cacodylate (pH 6.0). The following buffer solutions were prepared: (1) Separating gel buffer: 0.48 M acetate, adjusted to pH 6.0 with 1N KOH, and containing 0.5 ml TEMED per 100 ml. The final separating gel contained 0.12 M acetate. (2) Well and cap buffer: 0.12 M acetate, adjusted to pH 6.0 with 1N KOH, and containing 0.5 ml TEMED per 100 ml. The final gel contained 0.3 M acetate. (3) Tank buffer: 0.0325 M cacodylate, adjusted to pH 6.0 with 1N KOH. (4) Extraction (EX) buffer: 0.3 M acetate adjusted to pH 6.0 with 1N KOH. (5) EX buffer containing 80% sucrose and a drop of .1% bromphenol blue.

The cell samples were prepared as described above for APRT enzyme assays, using EX buffer instead of 0.155 M KCl. 65 μ l containing 15 to 40 μ l of cell extract and at least 20% sucrose was placed in each well formed by an 8-place well former.

Electrophoresis was conducted at 400 v and 0.5 mF capacitance at 4°. The pulse settings during the run were as follows: t_{0-10} , 75 Hz (25 mA); t_{10-20} , 125 Hz (55 mA); t_{20-n} minutes, 225 Hz (82 mA).

The migration position of the enzyme was determined by a modification of the method of DER KALOUSTIAN *et al.* (1969), in which the reaction product is selectively adsorbed on an anion exchange paper and visualized by autoradiography. After electrophoresis, a piece of Whatman DE-81 (DEAE-cellulose) paper was tightly apposed to the gel saturated with reaction mixture containing 50 mM Tris buffer (pH 7.4), 5 mM MgCl₂, 1.0 mM PRPP, and 2.5 μ c/ml (8-14 C) adenine (Schwarz-Mann; specific activity = 46.9 mc/mmmole). The gel and paper were wrapped in Saran Wrap and incubated at 37° for 2 hours. The paper was then placed in a perforated container and rinsed with three changes of 2 liters 0.1 M Tris-HCl, pH 9.5. The paper was dried and taped to x-ray film (Kodak X0 MAT). The autoradiograph was developed after 3-7 days.

Immunological methods-preparation of cell extracts: Dishes containing monolayers of cultured fibroblasts were drained. The cells were scraped into cold saline and washed 3 times with saline using centrifugation at 1000 \times g for 15 min at 4°. The pellet was taken up in an equal volume of double distilled water and the cells were lysed by three cycles of rapid freezing and thawing using an acetone-dry ice bath. The lysates were centrifuged at 30,000 \times g for 20 min and the supernatant fluids were kept on ice prior to assay.

Immunoprecipitation: Immunoprecipitation reactions* were performed in 6 \times 50 mm KIMAX tubes. The 100 μ l reaction mixture contained various amounts of rabbit anti-APRT-serum supplemented with non-specific rabbit serum to a total of 6.25 μ l, 25 μ l of cell lysate, 10⁻⁴ M PRPP and 3% polyethylene glycol 6000 (HARRINGTON, FENTON and PERT 1971) in hemagglutination buffer (DIFCO). All reaction mixtures were incubated for 4 hours at room temperature (20 \pm 2°) followed by centrifugation at 2000 \times g for 60 min at 4°. Unprecipitated APRT activities were determined by incubating 20 μ l aliquots of the supernatant fluids for 2 hours at 37°. Activity was determined essentially as described by RAPPAPORT and DEMARS (1973) but 0.01 M Tris-HCl, pH 8.4, was used in the reaction mixture and bovine serum albumin was omitted. Cell lysates were preassayed and used at concentrations that converted 50-80% of the substrate in determinations of unprecipitated APRT activity after incubation with serum from unimmunized rabbits.

RESULTS

APRT activity: The human parental cells grew well in A[AS] selective medium. By twelve days after dispersal and dilution of the original cell hybridization mixture the mouse parental cells were eliminated and discrete colonies of morphologically distinctive hybrid cells were forming against a background of

* A manuscript by HELD *et al.* describing the details of the purification of APRT, the preparation of immune sera, and these immunoprecipitation reactions is in preparation.

TABLE 1

APRT activities of parental cells and of their adenine-utilizing hybrids

Each value is the average of duplicate determinations. (100% conversion would correspond to approximately 3300 cpm.)

Cells	Type	Percent conversion	μg . protein	p moles/ μg /hr
D ⁷	APRT ⁻ parental mouse cells	0	19.0	0
D ¹¹	APRT ⁺ mouse cells	9.0	18.5	98
129.22	APRT ⁺ parental human cells	7.5	10.5	146
722.2	hybrid (D ⁷ × 129.22)	2.0	5.5	72
722.3	hybrid (D ⁷ × 129.22)	5.0	17.0	60
722.4	hybrid (D ⁷ × 129.22)	7.2	15.5	92
722.5	hybrid (D ⁷ × 129.22)	3.7	12.5	60
722.6	hybrid (D ⁷ × 129.22)	7.3	19.0	77

human parental fibroblasts. Isolated hybrid colonies grew well in A[AS] selective medium, in which APRT function is required for conversion of exogenous adenine to adenine nucleotides. *In vitro* measurements of enzyme activity confirmed the biochemical basis of these growth patterns (Table 1). Unlike the parental mouse cells, each of five independent hybrid clones had substantial enzyme activity, with a specific activity approximately one-half that of the normal human parent.

Species identification of the APRT in hybrid cells: Two lines of evidence indicate the APRT in the hybrid cells is human in origin. First, the electrophoretic mobility of the human enzyme toward the anode is greater than that of the mouse enzyme (Fig. 1). The two forms are clearly resolved electrophoretically in mixed extracts. The enzyme of three different hybrid clones migrated in parallel with that from human cells.

Secondly, the pattern of immunoprecipitation of APRT in hybrid extracts with antisera prepared against purified human APRT clearly parallels that of extracts from normal, diploid, human fibroblasts (Fig. 2). The identity of these curves, in conjunction with the electrophoretic identity of hybrid and human enzymes, leads us to conclude that the APRT in hybrid cells is a human gene product.

Human chromosomes present in hybrid cells: Photographs of 10–38 fluorescent metaphases from each of five independent hybrid clones were analyzed for the presence of specific human chromosomes within 3½–4 months of hybrid initiation. In this cross, each of the 23 human chromosomes has a fluorescent pattern distinct from that of the mouse chromosomes. The results are tabulated in Table 2. The following points are evident: (1) Four of five hybrid clones have two mouse chromosome complements and various members of the human complement. (2) One hybrid clone has one complement of mouse chromosomes and only one human chromosome. (3) Just one human chromosome, No. 16, is common to all five hybrid clones. It is the only human chromosome present in

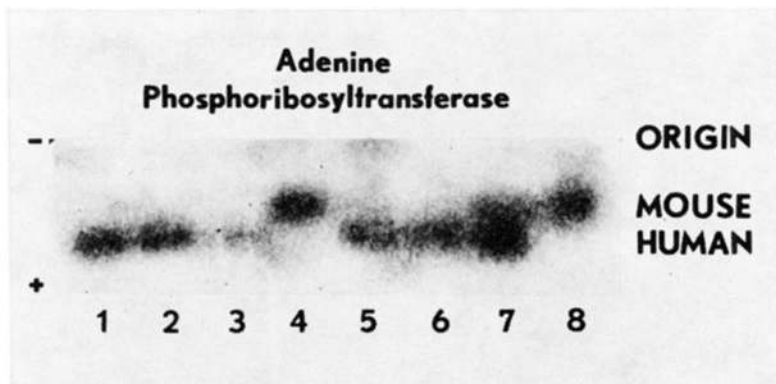


FIGURE 1.—Autoradiograph (7 day exposure) of APRT gel.

1. 722.4, hybrid
2. 129, human control
3. 722.3, hybrid
4. D¹¹, mouse control
5. 722.2, hybrid
6. 129, human control
7. D¹¹ + 129, mouse + human control
8. D¹¹, mouse control

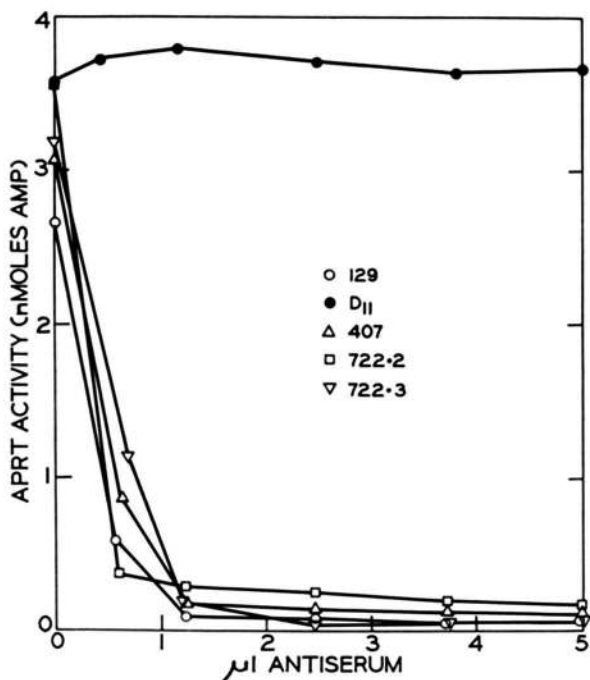


FIGURE 2.—Immunoprecipitation of APRT activity of mouse (D¹¹), human (129, 407), and hybrid (722.2, 722.3) cell extracts by anti-human APRT serum.

TABLE 2

Human chromosomes present in adenine-utilizing hybrid cells

Human chromosome	Percent of fluorescent metaphases examined having one or more members of a given human chromosome pair*				
	722.2	722.3	Hybrid clone 722.4	722.5	722.6
1				.27	.80
2					.70
3			.09	.47	.10
4				.60	.70
5		.45	.09		.40
6				.60	1.00
7			.18	.14	.80
8			.09		.40
9					.10
10			.09	.40	1.00
11			.09		.90
12			.18	.07	.50
13			.09		
14		.64	.18	.67	.10
15					.10
16	1.00	.91	.92	.93	.80
17			.09	.40	.90
18				.27	.70
19		.27			.60
20			.59	.07	.80
21		.91	.09		.90
22				.60	.80
X			.09	.33	.80
Total no. cells examined	38	11	12	15	10
Modal no. total chromosomes present/cell (range)	51 (45-52)	124 (95-146)	96 (54-143)	118 (72-135)	117 (96-122)
Modal no. human chromosomes present/cell (range)	1 (1-2)	4 (1-8)	1 (1-8)	8 (3-12)	15 (12-22)

* Blank indicates none present.

one hybrid clone, and it is present in every metaphase of that clone (Fig. 3). It is also the only one consistently present in nearly all cells of the remaining four hybrid clones. Almost all hybrid metaphases contained one human No. 16 chromosome; rarely 2-4 copies of the chromosome were observed in a single metaphase. The simplest explanation of these results is that the gene defining the structure of human APRT is on chromosome No. 16.

Correlation of loss of APRT with loss of chromosome No. 16: Cells that have APRT are sensitive to the adenine analog, DAP. Loss of APRT results in DAP-resistant cells. To correlate loss of human APRT with loss of a specific human chromosome, two hybrid clones (722.2 and 722.3) were subcloned both in DAP and A[AS] selective media. The chromosomes of three DAP subclones and three control A[AS] subclones of each of the two hybrid clones were compared. An

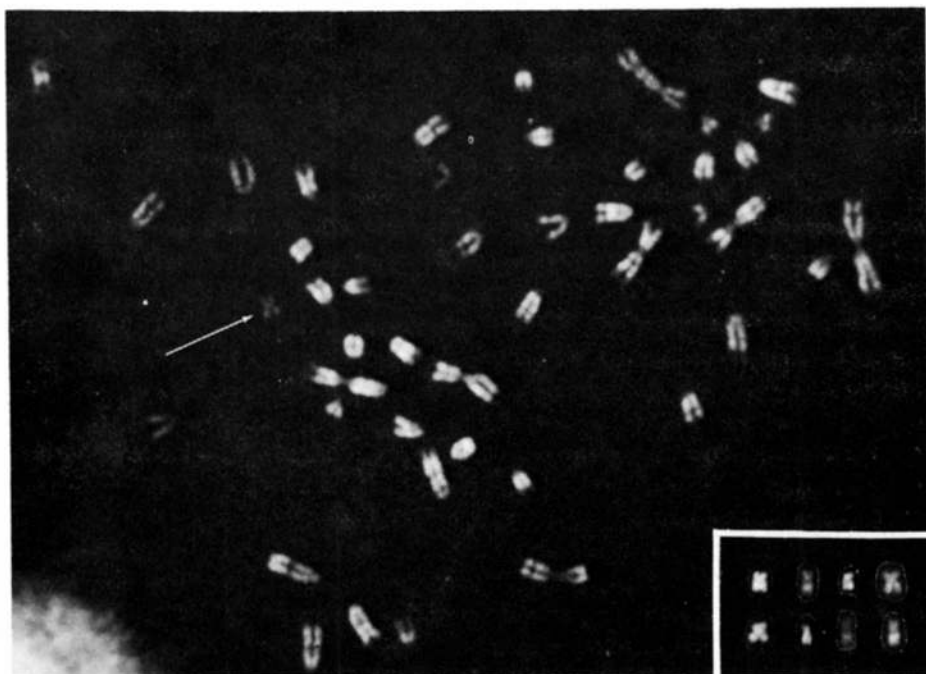


FIGURE 3.—Photomicrograph of a fluorescent metaphase of hybrid 722.2. Only one small, metacentric human chromosome (No. 16) is present (arrow). The other chromosomes (including the 1D marker "dicentric") are mouse cell chromosomes. The inset shows a sample of No. 16 chromosomes taken from a number of different hybrid 722.2 metaphases.

intact chromosome No. 16 was consistently present in each A[AS]-selected subclone of hybrid 722.3 and was the only human chromosome consistently absent in the DAP-resistant subclones. The other human chromosomes present in hybrid 722.3 (Table 2) occurred in both types of subclones.

No. 16 was also absent from all cells of the three DAP subclones of hybrid 722.2, in which it had previously been the only human chromosome present. However, an interact No. 16 was also missing from the three A[AS] control 722.2 subclones. Instead, a new submetacentric chromosome was present in over 90% of the cells of all three subclones. The fluorescent pattern of the short arm of this marker chromosome was consistent with that of the long arm of chromosome No. 16 (Fig. 4). Furthermore, this chromosome, the ability to grow in A[AS], and APRT activity were all absent in DAP-resistant survivors (selected at a cell density of 1 cell/20 mm²) of each subclone originally isolated in A[AS] (Fig. 4). Among 98 metaphases of the three DAP-selected populations of the 722.2 A[AS] subclones, 14 metaphases contained submetacentric chromosomes considered as possible candidates for being the marker submetacentric in question. However, all but two of these had fluorescent patterns clearly different from that of the putative mouse-human translocation chromosome. Even these two had an arm ratio that fell outside the range of that of the marker submetacentric in A[AS]-maintained subclones.

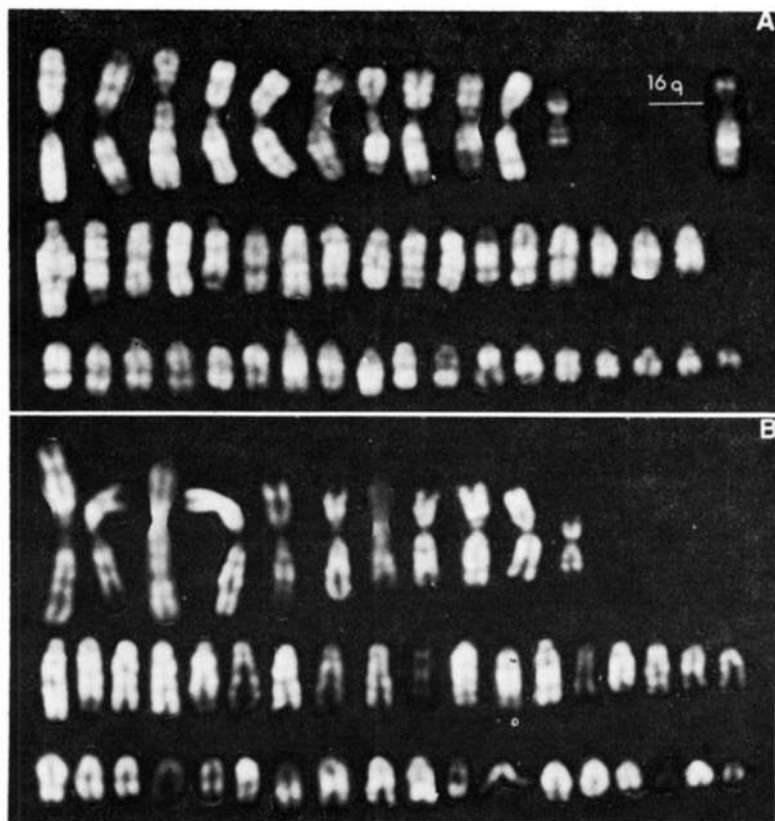


FIGURE 4.—A. Ideogram of an A[AS]-selected subclone of hybrid 722.2. An intact human No. 16 chromosome is missing, and a new submetacentric marker chromosome is present. The quinacrine banding pattern of the short arm matches that of the long arm of the human No. 16 chromosome. B. Ideogram of a DAP-selected derivative of the same subclone. The submetacentric marker chromosome is conspicuously missing.

From an analysis of photographs of fluorescent metaphases of the cells of hybrid 722.2 at three stages, (1) "pre-marker", in which all cells contained a clearly distinct human No. 16, (2) "post-marker" in three A[AS] subclones, in which nearly all cells contained a distinctive submetacentric chromosome considered to be a mouse-human translocation product, and (3) DAP-resistant derivatives of the three A[AS] subclones, we feel the following statements are possible.

The modal chromosome numbers of the three populations are (1) 51, (2) 47–48, and (3) 46–48. Cells from populations (2) and (3) had 2 to 6 more metacentric chromosomes than the average metaphase from population (1). Evidently the formation of a variety of metacentrics occurred relatively frequently in this hybrid clone. Selection with DAP did not result in a distinct reduction in the total number of chromosomes but, rather, in the specific disappearance of that

submetacentric possessing the fluorescence pattern corresponding to the long arm of chromosome No. 16.

Although hybrid cells contain 2-3 small mouse telocentrics of a size similar to that of human 16q, these telocentrics lack the relatively sharp medial fluorescence band of 16q, and of the short arm of the new marker submetacentric chromosome.

The fluorescence pattern of the long arm of the marker submetacentric is not compatible with that of one of the arms of the large metacentrics in the "pre-marker" clone; it is, therefore, unlikely this chromosome resulted from a terminal deletion of one arm of a pre-existing mouse metacentric chromosome.

One or two of the longer mouse telocentric chromosomes have a fluorescence pattern that is not discernibly different from that of the long arm of the marker submetacentric.

Our interpretation of these results is that during the five months following the first cytological analyses of the 722.2 hybrid clone, a translocation of human No. 16 to a mouse chromosome occurred. Possibly having a selective advantage, cells with this translocation chromosome became the majority cell type. If centro-

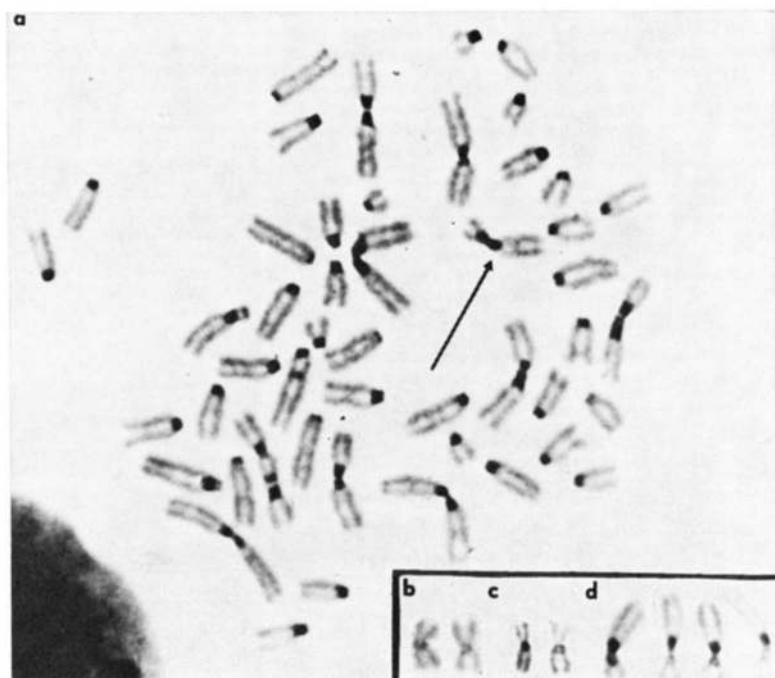


FIGURE 5.—(a) Centric heterochromatin patterns on the chromosomes of an A[AS]-selected subclone of hybrid 722.2. The submetacentric marker chromosome (arrow) has prominent heterochromatin on both sides of the centromere region, consistent with its derivation from the fusion of human 16q with a mouse chromosome.

- (b) Intact human No. 16's from an earlier culture of hybrid 722.2.
- (c) Human No. 16's from a culture of human fibroblasts strains 129.
- (d) Additional submetacentrics, presumed to be 16q-translocation chromosomes, from A[AS]-selected subclones of hybrid 722.2.

meric fusion of mouse and human chromosomes occurred, this observation would localize the gene for human APRT on the long arm of the chromosome No. 16.

To test this hypothesis, the pattern of centromeric heterochromatin present on the translocation chromosome and on the normal No. 16 were studied. Two possibilities were considered.

1) If a transfer of the long arm of human No. 16 to a mouse telocentric occurred, the translocation chromosome would have Giemsa-dense heterochromatin on both sides of the centromere: all mouse telocentrics and the long arm of human No. 16 have distinctive amounts of such centric heterochromatin.

2) If a mouse chromosome was joined to the short arm of the intact human No. 16, the centromeric stain should resemble that of the intact human No. 16, i.e., there would be Giemsa-denseness only on the short arm of the translocation chromosome.

Figure 5 shows that pattern (1) was observed. Conspicuous Giemsa stain was seen on both sides of the centromere, frequently with the stain of the short arm being less intense than that of the long arm. It has been previously observed that in hybrid cells, the centromeric heterochromatin of human chromosomes stains less intensely than that of the mouse chromosomes (CHEN and RUDDLE 1971).

Demonstration of human APRT in translocation hybrids: The interpretation that the locus specifying APRT is on the long arm of chromosome No. 16 and that the short arm of the marker submetacentric is human No. 16q requires that, in fact, human APRT is still present in the cells having the marker but lacking an intact human No. 16. This was demonstrated by showing that their APRT was inactivated by anti-human APRT at the same rate as the enzyme of human cells (Fig. 6).

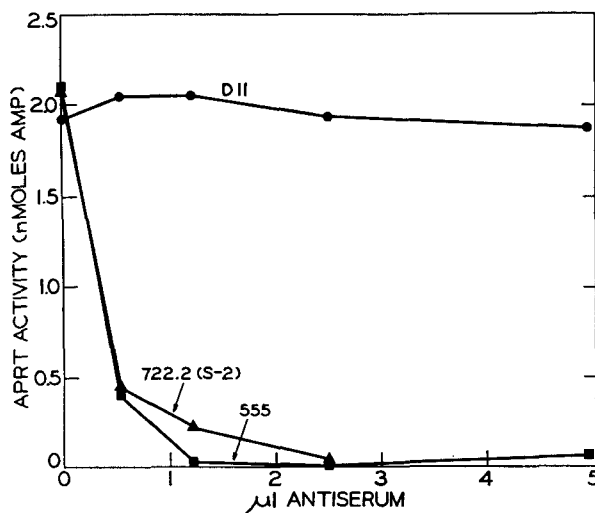


FIGURE 6.—Immunoprecipitation of APRT activity of mouse (D¹¹), human (555), and hybrid subclone [722.2 (S-2)] by anti-human APRT serum. The A[AS]-selected hybrid subclone lacks an intact human No. 16 chromosome, but contains a putative 16q-translocation product.

DISCUSSION

Our main conclusion is that human chromosome No. 16 bears a gene or genes specifying the structure of APRT. The isolation and propagation of the hybrid clones and subclones in A[AS]-selective medium and in DAP-selective medium required the presence and the absence of APRT, respectively: A[AS]-selected clones were not simply resistant to azaserine. We are fairly confident that the APRT expressed in the adenine-utilizing hybrids was of the human variety. Only one electrophoretic species and one degree of sensitivity to immunoprecipitation by anti-human APRT were observed in the APRT of the hybrids and they corresponded to those present in human cells. If mouse APRT protein was produced it was enzymatically inactive and did not copolymerize with human APRT in a way that altered the electrophoretic migration or immunologic specificity of the latter in our test systems.

In cases, such as the present one, when a conclusion depends on the species identity of a gene product in hybrid cells, it is sometimes desirable to use more than one criterion of species specificity. We used the most commonly applied criterion, electrophoretic mobility, and a criterion less frequently applied to enzymes in this context: immunologic specificity. The latter technique has also been successfully applied to human-mouse and human-hamster cell hybrids selected for on the basis of HGPRT expression (HELD *et al.*, manuscript in preparation).

No. 16 was the only human chromosome consistently present in primary hybrid clones requiring APRT activity for survival and all cells lacked this chromosome when APRT activity in hybrids was selected against with DAP. These results suggest the assignment of the structural gene for human APRT to chromosome No. 16. A more complicated interpretation, for which there is no firm evidence, is that No. 16 has *trans*-acting, regulatory genes necessary for the expression of human APRT and that genes determining APRT structure are on portions of other human chromosomes that were not recognized because they had been fragmented and translocated to mouse chromosomes. This hypothesis implies that a second intact human chromosome, in addition to No. 16, should consistently occur in some independent clones of A[AS]-selected hybrid cells. Such clones have not been described.

Several reports now indicate that human chromosomes in mouse-human hybrid cells undergo breakage and, in some cases, translocation to mouse chromosomes with relatively high frequency (MILLER *et al.* 1971; BOONE, CHEN and RUDDLE 1972; MIGEON and MILLER 1968). Because this phenomenon may obscure patterns of gene and chromosome segregation, it is advisable to characterize cell hybrids at an early stage of their evolution, and in several independent hybrid clones. On the other hand, such breaks and translocations allow a more specific regional localization of genes on a specific chromosome (BOONE, CHEN and RUDDLE 1972). Finally, chromosome breakage agents, such as adenovirus 12, affecting a chromosome maintained in hybrid cells may produce breakage patterns consistent with localization of genes to one chromosome band region,

such as a gene for thymidine kinase to 17q22 (McDOUGALL, KUCHERLAPATI and RUDDLE 1973). In one hybrid maintained longer than four months A[AS]-selected subclones contained not a normal 16, but a chromosome consistent with its being a mouse-human 16q translocation. The loss of this chromosome, like that of the intact 16, could be correlated with loss of human APRT. This observation would indicate that the human APRT gene is located on 16q. If 16p was retained in the hybrid cells it must have been translocated to another chromosome: no small acrocentric chromosome corresponding to 16p was detected. If one assumes that 16p was present and that the APRT gene is located on 16p, our results require that the 16p-bearing chromosome always segregate with the 16q translocation chromosome. We regard this explanation as very unlikely since it requires an unverifiable assumption and an improbable coincidence.

Heritable fragile sites on 16q (MAGENIS, HECHT and LOVRIEN 1970) might be used in somatic hybrids to more precisely locate the APRT locus, and to determine its relation to additional loci on chromosome 16, such as that for α -haptoglobin (ROBSON *et al.* 1969). Moreover, in this specific case, structural information for a liver cell-specific product, haptoglobin, can be maintained in hybrid cells by selection for the linked APRT genes. One can then study conditions permitting or preventing expression of haptoglobin synthesis without uncertainties about the presence of the structural genes. This uncertainty has plagued previous interpretations of experiments presumably demonstrating the extinction or reappearance of cell-specific products in hybrid cells.

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