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Genetics

Polymorphic gene for human carbonic anhydrase II: A molecular disease marker located on chromosome 8

(osteopetrosis and renal tubular acidosis/glutathione reductase/multigene enzyme family/evolution/cell hybrids)

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ABSTRACT **A panel of 28 mouse–human somatic cell hybrids of known karyotype was screened for the presence of the human carbonic anhydrase II (CA II) gene, which encodes one of the three well-characterized, genetically distinct carbonic anhydrase isozymes (carbonate dehydratase; carbonate hydro-lyase, EC 4.2.1.1). The human and mouse CA II genes can be clearly distinguished by Southern blot analysis of *Bam*HI-digested genomic DNA with a mouse CA II cDNA hybridization probe. The two major hybridizing fragments in mouse were 15 and 6.0 kilobase pairs, and in human they were 5.0 and 4.3 kilobase pairs. Analysis of the somatic cell hybrids by this technique identified those containing human CA II gene sequences. Segregation analysis of the molecular marker and chromosomes in cell hybrids indicated a clear correlation between the presence of chromosome 8 and the human CA II gene (*CA2*). This finding provides the second polymorphic marker for human chromosome 8 and, moreover, a molecular disease marker, because human CA II deficiency has recently been linked to an autosomal recessive syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification.**

Carbonic anhydrase (carbonate dehydratase; carbonate hydrolyase, EC 4.2.1.1) is a zinc metalloenzyme that catalyzes the reversible hydration of carbon dioxide (1–3). Because what appear to be evolutionarily homologous forms of this enzyme have been found in almost all organisms, including bacteria, carbonic anhydrase was probably present in the earliest organisms over two billion years ago (4). In reptiles, birds, and mammals (amniotes), three isozymes of carbonic anhydrase, designated CA I, CA II, and CA III, have now been characterized; and in mammals, it appears that the CA I and CA II genes are closely linked (for reviews see refs. 4 and 5). All of these isozymes are monomeric (M_r ≈29,000) enzymes that, although exhibiting a considerable degree of sequence homology, appear to have diverse physiological roles (4–6). It is also possible that a seemingly distinct, membrane-associated human carbonic anhydrase, tentatively designated CA IV (7), and a mammalian mitochondrial carbonic anhydrase (8, 9) may represent additional isozymes. So far, in human tissues and cells, CA I has been detected primarily in erythrocytes, CA II in a wide variety of tissues, CA III mainly in red skeletal muscle, and CA IV in lung (4, 5, 7). Several electrophoretic variants of human erythrocyte CA I and CA II have been described, some of which occur at polymorphic frequencies (10). An electrophoretically silent variant of human skeletal muscle CA III has recently been reported to occur at high frequencies in both Black and White populations (11). In addition, the virtually complete absence of human erythrocyte CA I has been reported in individuals homozygous for a CA I deficiency gene (12). No clinical defects have been found in individuals that are homozygous for any of these variant alleles. Recently, however, a deficiency of human CA II has been identified as the primary cause of an inherited syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification (13).

Although all three carbonic anhydrase isozyme genes exhibit polymorphisms, it has not been possible to map them to human chromosomes by cell hybridization methods because of the inability to detect their gene products in somatic mouse–human cell hybrids. Using a cloned mouse CA II cDNA probe and a panel of mouse–human somatic cell hybrids, we have found that the structural gene for human CA II (designated *CA2*) is located on chromosome 8. This chromosomal assignment of *CA2* in humans (*i*) provides a useful polymorphic marker for population and family studies, (*ii*) adds an important clinically related marker to the human gene map, and (*iii*) gives the chromosomal location for at least one and possibly two or more carbonic anhydrase isozyme genes.

MATERIALS AND METHODS

Parental and Hybrid Cells. Cells from 10 unrelated humans and 3 different mouse cell lines were employed for somatic cell hybridization. These parental cells were fused in monolayer or suspension cultures with inactivated Sendai virus or with polyethylene glycol, and cell hybrids were isolated on selection medium as described (14–16). Cell hybrid clones were isolated from 11 separate fusion experiments, and the hybrid sets were designated WIL (WI-38 × LTP) (14); REW (WI-38 × RAG) (17); JWR (JoVa × RAG) (18); XER (GM2859 × RAG) (19); JSR (JoSt × RAG) (20); NSL (GM2836 × LM/TK$^-$) (21); TSL (GM2808 × LM/TK$^-$) (22); ATR (A1Tr × RAG) (23); XTR (GM194 × RAG) (22); and EXR (GM3322 × RAG) (19). The individual cell hybrids examined are given in Table 1.

On the same cell passage, hybrid cells were homogenized for enzyme marker examination (15, 24) and harvested for chromosome analyses (20), and DNA was isolated for Southern filter hybridization testing (25). Parental cells were analyzed by using the same procedures.

Hybridization Probe. The mouse CA II cDNA clone was developed from mRNA from spleens of anemic BALB/c mice and identified by positive hybridization, *in vitro* translation, and nucleotide sequence analysis (26). The insert of the mouse CA II clone was removed from the *Pst* I site of plasmid pBR322 by partial digestion to avoid cleavage of both internal *Pst* I sites. The intact fragment was purified on a 5% acrylamide gel. It was then inserted into the *Sph* I site of pBR322 by G·C-tailing (27), introduced into competent bacteria by transformation, and grown up by standard procedures (28). One hundred micrograms of

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Abbreviations: CA, carbonic anhydrase; kb, kilobase pair(s).

this plasmid was cut with *Sph* I, and 25 μg of insert was purified on a sucrose gradient (29). The 1.5-kilobase-pair (kb) insert was labeled by nick translation with ^{32}P to 1–2 × 10^8 cpm/μg (28).

Blotting, Hybridization and Washing. Ten micrograms of DNA from each cell line was cut with the restriction endonuclease *Bam*HI at 2 units per μg of DNA for 3 hr at 37°C, using the buffer conditions suggested by the supplier. DNA fragments were separated by agarose electrophoresis in a 0.8% gel in a horizontal apparatus and were transferred onto nitrocellulose by the method of Southern (30). Before hybridization, filters were incubated for 3–5 hr at 42°C in 10 ml of solution containing 35% (vol/vol) formamide, 0.75 M NaCl, 75 mM sodium citrate, 50 mM sodium phosphate buffer (pH 6.5), sheared and denatured salmon sperm DNA at 200 μg/ml, and 5× Denhardt's solution (31). Filters were hybridized for 24–48 hr in the same solution to which 10% dextran sulfate and 1–2 × 10^6 cpm of labeled probe per ml had been added. Filters were washed for 5 min in 0.3 M NaCl/30 mM sodium citrate/0.1% sodium dodecyl sulfate at 50°C. The washing procedure was repeated five times, and the filters were then exposed to Kodak AR5 x-ray film with an intensifying screen (DuPont Cronex Lightning Plus) at −70°C for 1–4 days.

Human Gene Nomenclature. Following human gene nomenclature guidelines adopted by the Fifth Human Gene Mapping Workshop (32), it is proposed that the genes for CA I, CA II, CA III, and CA IV be designated *CA1*, *CA2*, *CA3*, and *CA4*, respectively.

RESULTS

Identification of Mouse and Human DNA Restriction Fragments. The banding patterns produced by hybridizing the electrophoretically separated mouse and human DNA restriction fragments with the labeled mouse CA II cDNA probe are shown in Fig. 1. The major fragments for the mouse are 15 and 6.0 kb, and those for the human fragments are 5.0 and 4.3 kb. In addition, for both species, several weaker bands are seen that are not present with more stringent hybridization and washing conditions. These weaker bands may be derived from the genes of the other isozymes (i.e., CA I, CA III, etc.), those portions (i.e., exons) of the human CA II gene that do not hybridize strongly with the mouse CA II probe, or pseudogenes that have sequence homologies with CA II (4, 5). Examination of Fig. 1 shows that there are two banding patterns for cell hybrids; one is identical to the mouse parental pattern (lanes 4–6), and the other demonstrates both mouse and human bands (lanes 7–9). The mouse pattern was scored negative, and the mixture of human and mouse bands was scored positive for retaining the human CA II gene. The lower intensities of the major human bands relative to the mouse bands are presumably due to the reduced cross-hybridization of the mouse CA II probe with the human CA II sequences, as well as the fact that not all cells in a given cell hybrid line retain the chromosome bearing *CA2*.

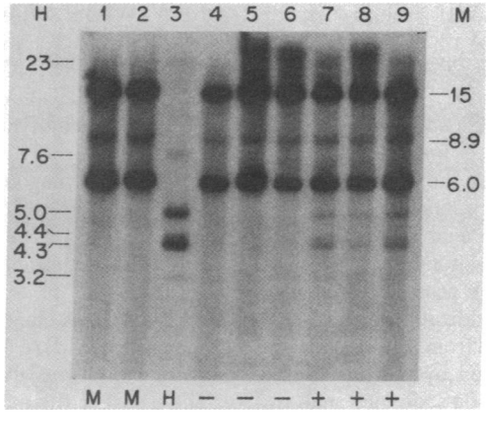

FIG. 1. Hybridization of the mouse CA II cDNA probe to mouse, human, and cell hybrid DNA. The probe hybridizes with mouse (lanes 1 and 2) and human (lane 3) unique fragments when cut with *Bam*HI. Cell hybrids without human bands are in lanes 4–6 and are scored negative. Positive hybrids are in lanes 7–9. Lanes: 1, mouse LM/Tk$^-$; 2, mouse RAG; 3, human WI-38; 4, REW-11; 5, TSL-2; 6, WIL-15; 7, REW-10; 8, WIL-7; and 9, WIL-2. Fragment sizes are given in kb on the left side for human (H) bands and the right side for mouse (M) bands.

Cosegregation of the CA II Gene (*CA2*) with a Human Chromosome in Cell Hybrids. A chromosome assignment panel (33) consisting of 28 cell hybrids characterized for human chromosomes (20) and enzyme markers (33) was employed. In this panel human enzyme markers confirmed the presence of human chromosomes with an exception bearing on the regional assignment of *CA2*. The panel is redundant so that more than one cell hybrid will rule out a particular chromosome for gene assignment. Parental cells consisted of 10 unrelated human lines and 3 different mouse cell lines, eliminating unknowns possibly attributed to a single parental cell line.

Each cell hybrid was examined on the same cell passage for human chromosomes, human enzyme markers, and hybridization of the human CA II gene with the mouse CA II cDNA probe. The results are presented in Table 1; they show that the presence of the CA II gene (*CA2*) in cell hybrids correlates with the presence of chromosome 8. This is determined by comparing the scores for *CA2* with the presence or absence of each human chromosome in all 28 cell hybrids. The presence of both *CA2* and a chromosome, or their shared absence, is a concordant score. The presence of *CA2* and not the chromosome, or the presence of a chromosome and not *CA2*, is a discordant score. Concordancy determines chromosome assignment and discordancy determines nonassignment. The percent discordance for each chromosome is substantial except for chromosome 8. The complete concordancy between chromosome 8 and *CA2* demonstrates its assignment to chromosome 8. One cell hybrid, JSR-26C, was positive for the chromosome 8 enzyme marker glutathione reductase (*GSR*) and *CA2*, but an intact chromosome 8 was not seen by karyotyping. These findings indicate that fragments of chromosome 8 are present but not detectable by microscopy. Because *GSR* has been located in region 8p211 on the short arm of chromosome 8 (35), and because JSR-26C retains both *GSR* and *CA2* but not a detectable chromosome 8, it appears that *CA2* is located near *GSR* on the short arm of human chromosome 8.

Identification of the other cross-hybridizing bands and their chromosomal assignments was not possible in the cell hybrids because of their lower intensities (Fig. 1).

DISCUSSION

The *CA2* gene, coding for CA II, was assigned to human chromosome 8 by using somatic cell hybrids and Southern filter hybridization techniques. Of the amino acid sequences available for human CA I, CA II (4, 36), and CA III (D. Hewett-Emmett, personal communication), the derived amino acid sequence of mouse CA II (ref. 26 and unpublished data) is 81% identical to human CA II but only 63% to human CA I and 56% to human CA III. This high degree of sequence homology between the mouse and human CA II isozymes makes it reasonably certain that the gene we are detecting by hybridization is human *CA2*. In comparison with other chromosomes, chromosome 8 has had few genes assigned to it; nevertheless, these genes are of con-

Table 1. Distribution of CA II and human chromosomes in human–mouse cell hybrids

Hybrid*	*CA2* score	Chromosomes†																						Translocations‡
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	X	
WIL-1	+	−	−	−	−	−	−	−	+	−	−	−	−	−	+	−	−	+	−	−	−	+	−	+	
WIL-2	+	−	−	−	−	−	−	−	+	−	−	−	+	−	−	+	−	+	−	−	−	+	−	+	
WIL-5	−	−	−	−	+	−	−	−	−	−	+	−	−	−	−	−	−	+	+	−	−	+	−	+	
WIL-6	+	−	−	−	+	+	+	+	+	−	+	+	−	−	+	−	−	+	−	+	+	+	−	+	
WIL-7	+	−	+	+	−	+	+	−	+	−	+	+	−	+	+	−	−	+	+	−	−	+	−	+	
WIL-8	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	−	+	
WIL-8X	+	−	−	+	+	+	−	+	+	−	+	+	+	−	+	−	−	+	+	+	+	+	−	+	
WIL-13	−	−	−	−	−	+	−	−	−	−	−	−	−	−	−	−	−	+	+	−	−	+	+	−	
WIL-14	+	−	−	+	−	−	−	−	+	−	+	−	−	−	+	−	−	+	−	−	−	−	−	+	
WIL-15	−	−	+	+	+	−	−	+	−	−	+	+	+	+	+	−	−	+	+	−	+	+	−	+	
REW-5	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	−	+	+	+	
REW-7	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
REW-10	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	−	+	
REW-11	−	−	−	−	+	−	−	−	−	−	−	+	+	+	−	−	+	−	−	−	+	+	−	+	
REW-15	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	+	+	+	+	+	+	+	
JWR-22H	−	−	−	−	+	−	+	−	−	−	+	+	−	−	+	−	−	+	+	−	+	+	−	−	2/1 (ref. 18)
JWR-26C	+	−	+	+	+	+	+	+	x§	+	+	+	+	−	+	+	+	+	+	−	+	+	−	+	1/2 (ref. 18)
XER-7	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	−	−	+	+	−	−	−	+	11/X (ref. 19)
XER-11	+	+	−	+	+	−	+	+	+	−	+	−	+	+	−	+	+	+	+	+	+	+	+	−	X/11 11/X (ref. 19)
JSR-17S	+	+	−	+	−	+	−	−	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	−	7/9 (ref. 20)
NSL-5	−	+	−	−	−	−	−	−	−	−	+	−	+	−	+	−	+	−	+	−	+	−	−	−	17/9 (ref. 21)
NSL-9	+	−	−	−	−	+	−	−	+	−	+	−	+	+	+	+	+	+	−	−	+	+	+	−	17/9 (ref. 21)
DUA-1A	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	X/15 (ref. 34)
TSL-2	−	−	+	−	−	+	+	−	−	−	+	−	+	−	−	−	−	−	+	−	+	+	−	+	17/3 (ref. 22)
ATR-13	+	+	+	+	+	+	+	+	+	−	+	−	+	+	+	+	+	+	+	+	−	−	−	−	5/X (ref. 23)
XTR-22	+	−	+	−	+	+	+	−	+	−	+	+	−	−	−	+	−	−	+	+	+	+	+	−	X/3 (ref. 22)
XTR-3BSAGB	−	−	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−	−	−	−	+	−	−	−	3/X 10q⁻¶
EXR-5 CSAz	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	−	+	+	+	+	+	+	−	X/11 (ref. 19)
% discordant‖		39	39	21	36	21	29	29	0	50	25	36	36	32	21	18	54	21	39	25	46	32	43	29	

* Cell hybrids described in *Materials and Methods*.

† Chromosomes have been determined as described (20). Enzyme markers were determined to confirm the chromosome analysis. The enzyme markers employed have been described (33).

‡ Cell hybrids with well-characterized reciprocal translocations from human parental cells have been included in this panel for possible regional assignments. References to these translocations are indicated.

§ In this cell hybrid the chromosome 8 marker glutathione reductase located at 8p211 on the short arm is present but an intact chromosome 8 was not observed, demonstrating a broken chromosome 8 in this cell hybrid.

¶ 10pter→q23 (unpublished data).

‖ Discordant means the presence of *CA2* and not a specific chromosome or the presence of a chromosome and not *CA2*. This value indicates the degree of discordant segregation between a chromosome and *CA2* in this panel and establishes a level of confidence for nonassignment. No discordance determines chromosome assignment.

siderable interest because of their association with molecular disease and cancer. In addition to *CA2* the other genes are (*i*) glutathione reductase (*GSR*), a polymorphic marker informative in family and cell genetic studies and potentially valuable for linkage analysis to *CA2* (35); (*ii*) a fibronectin cell surface marker (*FNS*) (37, 38) that is one of several genes involved in the expression of fibronectin; (*iii*) glutamate pyruvate transaminase (*GPT2*) (39); (*iv*) the oncogene *mos* that has been localized to the 8q22 region on the long arm of chromosome 8 (40, 41); and (*v*) the oncogene *myc* localized to the 8q24 long arm region and involved in lymphomas of various origins (40, 42–44). Because of the recently discovered association of a CA II deficiency with the syndrome of osteopetrosis with renal tubular acidosis and cerebral calcification (13), this assignment marks the location of a human molecular disease that may be useful in understanding phenotypes associated with aberrations involving chromosome 8.

The *CA2* gene promises to be useful in gene linkage studies to determine the linear order and organization of genes on human chromosome 8. Currently, four of the nine electrophoretic variants known for human erythrocyte CA II (i.e., CA II_2, CA II Australia, CA II Bombay, and CA II Baniwa) are known to occur at polylmorphic frequencies (see ref. 10 for review). Of these, the allele for CA II_2 is polymorphic (8–10%) in most Black populations and CA II Australia enjoys a widespread polymorphism (2.2%) in Australian Aborigines; the other two CA II polymorphisms exhibit more limited distributions. It should be noted that these CA II polymorphisms can be readily typed in hemolysates by examination of the electrophoretic patterns (45). If *CA2* is located on the short arm of chromosome 8 as suggested by a chromosomal rearrangement (Table 1), then with *GSR* located in the 8p211 region (35) there will be two polymorphic loci on the short arm of chromosome 8 for linkage and population studies. Because the glutathione reductase (*Gr-1*) and carbonic anhydrase II (*Car-2*) loci are not on the same chromosome in the mouse (46), it should be possible to test the antiquity of the *GSR-CA2* linkage. For example, *GSR* has been mapped to chromosome 7 in chimpanzee and gorilla and to chromosome 6 in orangutan (47).

The fact that the genes for CA I and CA II appear to be closely linked in the mouse (*Mus musculus*) (48), guinea pigs (*Cavia* spp.) (49), and the pigtail macaque (*Macaca nemestrina*) (50) suggests that these two genes are probably also closely linked in humans. In view of the probable presence of CA I and CA

II in birds and reptiles (5), it appears that at least the CA I and CA II genes have remained closely linked for more than 300 million years. Why duplicated genes that have diverged in tissue-specific expression and probably physiological function as well have remained closely linked for such a long period of time remains an intriguing question. Because of the possibility that the carbonic anhydrase multigene family could be clustered on the same chromosome, it will be of considerable interest to determine the chromosomal localization of the other carbonic anhydrase isozyme genes.

Note Added in Proof. We have recently screened a YBR mouse cosmid library (51) and isolated a genomic clone containing a complete CA II gene with *Bam*HI fragments that correspond to the 15- and 6-kb bands seen in Fig. 1. In addition, we have isolated several genomic clones from a human λ phage library constructed by T. Maniatis; one of these contains the 5′ region of a CA II gene and hybridizes to the 23- and 3.2-kb *Bam*HI fragments of human genomic DNA seen in Fig. 1. The identities of the CA isozyme genes in mouse and human clones were determined by DNA sequence analysis.

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1. Maren, T. H. (1967) *Physiol. Rev.* **47,** 595–781.
2. Lindskog, S., Henderson, L. E., Kannan, K. K., Liljas, A., Nyman, P. O. & Strandberg, B. (1971) in *The Enzymes*, ed. Boyer, P. D. (Academic, New York), Vol. 5, pp. 587–665.
3. Pocker, Y. & Sarkanen, S. L. (1978) *Adv. Enzymol.* **47,** 149–274.
4. Tashian, R. E. (1977) in *Isozymes: Current Topics in Biological and Medical Research*, eds. Rattazzi, M. C., Scandalios, J. G. & Whitt, G. S. (Liss, New York), Vol. 2, pp. 21–62.
5. Tashian, R. E., Hewett-Emmett, D. & Goodman, M. (1983) in *Isozymes: Current Topics in Biological and Medical Research*, eds. Rattazzi, M. C., Scandalios, J. G. & Whitt, G. S. (Liss, New York), Vol. 7, pp. 79–100.
6. Carter, M. J. (1972) *Biol. Rev.* **47,** 465–513.
7. Whitney, P. L. & Briggle, T. V. (1982) *J. Biol. Chem.* **257,** 12056–12059.
8. Dodgson, S. J., Forster, R. E., Storey, B. T. & Mela, L. (1980) *Proc. Natl. Acad. Sci. USA* **77,** 5562–5566.
9. Vincent, S. H. & Silverman, D. N. (1982) *J. Biol. Chem.* **257,** 6850–6855.
10. Tashian, R. E., Kendall, A. G. & Carter, N. D. (1980) *Hemoglobin* **4,** 635–651.
11. Hewett-Emmett, D., Welty, R. J. & Tashian, R. E. (1982) *Am. J. Human Genet.* **34,** 55A (abstr.).
12. Kendall, A. G. & Tashian, R. E. (1977) *Science* **29,** 471–472.
13. Sly, W. S., Hewett-Emmett, D., Whyte, M. P., Yu, Y.-S. L. & Tashian, R. E. (1983) *Proc. Natl. Acad. Sci. USA* **80,** 2752–2756.
14. Shows, T. B. (1972) *Proc. Natl. Acad. Sci. USA* **69,** 348–352.
15. Shows, T. B. & Brown, J. A. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 2125–2129.
16. Davidson, R. & Gerald, P. S. (1976) *Somat. Cell Genet.* **2,** 165–176.
17. Lalley, P. A., Brown, J. A., Eddy, R. L., Haley, L. L., Byers, M. G., Goggin, A. & Shows, T. B. (1977) *Biochem. Genet.* **15,** 367–382.
18. Shows, T. B. & Brown, J. A. (1975) *Cytogenet. Cell Genet.* **14,** 251–255.
19. Owerbach, D., Bell, G. I., Rutter, W. J., Brown, J. A. & Shows, T. B. (1981) *Diabetes* **30,** 267–270.
20. Shows, T. B., Brown, J. A., Haley, L. L., Byers, M. G., Eddy, R. L., Cooper, E. S. & Goggin, A. P. (1978) *Cytogenet. Cell Genet.* **21,** 99–104.
21. Shows, T. B., Sakaguchi, A. Y., Naylor, S. L., Goeddel, D. V. & Lawn, R. M. (1982) *Science* **218,** 373–374.
22. Naylor, S. L., Elliott, R. W., Brown, J. A. & Shows, T. B. (1982) *Am. J. Hum. Genet.* **34,** 235–244.
23. Lalley, P. A., Brown, J. A. & Shows, T. B. (1976) *Cytogenet. Cell Genet.* **16,** 188–191.
24. Shows, T. B. (1977) in *Isozymes: Current Topics in Biological and Medical Research*, eds. Rattazzi, M. C., Scandalios, J. G. & Whitt, G. S. (Liss, New York), Vol. 2, pp. 107–158.
25. Naylor, S. L., Sakaguchi, A. Y., Shows, T. B., Law, M. L., Goeddel, D. V. & Gray, P. W. (1983) *J. Exp. Med.* **57,** 1020–1027.
26. Curtis, P. J. (1983) *J. Biol. Chem.* **258,** 4459–4463.
27. Roychoudhury, R. & Wu, R. (1979) *Methods Enzymol.* **65,** 43–63.
28. Maniatis, T., Fritsch, E. F. & Sambrook, J. (1982) *Molecular Cloning* (Cold Spring Harbor Laboratory, Cold Spring Harbor, NY).
29. El-Gewely, M. R. & Helling, R. B. (1980) *Anal. Biochem.* **102,** 423–428.
30. Southern, E. M. (1975) *J. Mol. Biol.* **98,** 503–517.
31. Denhardt, D. T. (1966) *Biochem. Biophys. Res. Commun.* **23,** 641–646.
32. Shows, T. B., Alper, C. A., Bootsma, D., Dorf, M., Douglas, T., Huisman, T., Kit, S., Klinger, H. P., Kozak, C., Lalley, P. A., Lindsley, D., McAlpine, P. J., McDougall, J. K., Meera Khan, P., Meisler, M., Morton, N. E., Opitz, J. M., Partridge, C. W., Payne, R., Roderick, T. H., Rubinstein, P., Ruddle, F. H., Shaw, M., Spranger, J. W. & Weiss, K. (1979) *Cytogenet. Cell Genet.* **25,** 96–116.
33. Shows, T. B., Sakaguchi, A. Y. & Naylor, S. L. (1982) in *Advances in Human Genetics*, eds. Harris, H. & Hirschhorn, K. (Plenum, New York), Vol. 12, pp. 341–452.
34. Sakaguchi, A. Y. & Shows, T. B. (1982) *Somat. Cell Genet.* **8,** 83–94.
35. Meera Khan, P. & Robson, E. B. (1979) *Cytogenet. Cell Genet.* **25,** 39–46.
36. Tashian, R. E., Hewett-Emmett, D., Stroup, S. K., Goodman, M. & Yu, Y.-S. L. (1980) in *Biophysics and Physiology of Carbon Dioxide*, eds. Bauer, C., Gros, G. & Bartels, H. (Springer, Berlin), pp. 165–176.
37. Owerbach, D., Doyle, D. & Shows, T. B. (1978) *Proc. Natl. Acad. Sci. USA* **75,** 5640–5644.
38. Koch, G. A., Schoen, R. C., Klebe, R. J. & Shows, T. B. (1982) *Exp. Cell Res.* **141,** 293–302.
39. Robson, E. B. & Meera Khan, P. (1982) *Cytogenet. Cell Genet.* **32,** 144–152.
40. Neel, B. G., Jhanwar, S. C., Chaganti, R. S. K. & Hayward, W. S. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7842–7846.
41. Prakash, K., McBride, O. W., Swan, D. C., Devore, S. G., Tronick, S. R. & Aaronson, S. A. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 5210–5214.
42. Taub, R., Kirsch, I., Morton, C., Lenoir, G., Swan, D., Tronick, S., Aaronson, S. & Leder, P. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7837–7841.
43. Dalla-Favera, R., Bregni, M., Erickson, J., Patterson, D., Gallo, R. C. & Croce, C. M. (1982) *Proc. Natl. Acad. Sci. USA* **79,** 7824–7827.
44. Sakaguchi, A. Y., Lalley, P. A. & Naylor, S. L. (1983) *Somat. Cell Genet.* **9,** 391–405.
45. Tashian, R. E. & Carter, N. D. (1976) in *Advances in Human Genetics*, eds. Harris, H. & Hirschhorn, K. (Plenum, New York), Vol. 7, pp. 1–56.
46. Womack, J. E. (1982) *Genet. Maps* **2,** 286–293.
47. Pearson, P. L., Roderick, T. H., Davisson, M. T., Garver, D., Warburton, D., Lalley, P. A. & O'Brien, S. J. (1982) *Cytogenet. Cell Genet.* **25,** 82–95.
48. Eicher, E. M., Stern, R. H., Womack, J. E., Davisson, M. T., Roderick, T. H. & Reynolds, S. C. (1976) *Biochem. Genet.* **14,** 651–660.
49. Carter, N. D. (1972) *Comp. Biochem. Physiol.* **43B,** 743–747.
50. DeSimone, J., Linde, M. & Tashian, R. E. (1973) *Nature (London) New Biol.* **242,** 55–56.
51. Venta, P. J., Wiebauer, K. & Tashian, R. E. (1983) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **42,** 1971 (abstr.).