Somatic cell genetics of adenosine deaminase expression and severe combined immunodeficiency disease in humans*

(human chromosomes 2, 6, and 20/somatic cell hybrids/adenosine deaminase-complexing protein/adenosine deaminase deficiency)

GEORGE KOCH AND THOMAS B. SHOWS

Biochemical Genetics Section, Roswell Park Memorial Institute, New York State Department of Health, Buffalo, New York 14263

Communicated by Victor A. McKusick, April 21, 1980

ABSTRACT The somatic cell hybrid method has been used to study the number and different types of human genes involved in the expression of adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) in normal cells and cells from a patient with ADA-deficient severe combined immunodeficiency disease (SCID). Genetic and biochemical characterization of ADA in SCID and the ADA tissue-specific isozymes in normal human cells indicates that additional genes, besides the ADA structural gene on chromosome 20, are involved in ADA expression. Human chromosome 6 encodes a gene, *ADCP-1*, whose presence is necessary for the expression of an ADA-complexing protein in human-mouse somatic cell hybrids [Koch, G. & Shows, T. B. (1978) *Proc. Natl. Acad. Sci. USA* 75, 3876-3880]. We report the identification of a second gene, *ADCP-2*, on human chromosome 2, that is also involved in the expression of the ADA-complexing protein. The data indicate that these two *ADCP* genes must be present in the same cell for that cell to express the complexing protein. Human-mouse somatic cell hybrids, in which the human parental cells were fibroblasts from an individual with ADA-deficient SCID, also required human chromosomes 2 and 6 to express the ADA-complexing protein, indicating that neither *ADCP-1* nor *ADCP-2* is involved in the ADA deficiency in SCID. The SCID-mouse hybrid cells expressed no human ADA even when human chromosome 20 had been retained. The deficiency of human ADA in these hybrids maps to human chromosome 20, and therefore is not due to the repression or inhibition of ADA or its product by unlinked genes or gene products. We propose that the expression of the polymeric ADA tissue isozymes in human cells requires at least three genes: ADA on chromosome 20, *ADCP-1* on chromosome 6, and *ADCP-2* on chromosome 2. A genetic scheme is presented and the different genes involved in ADA expression and their possible functions are discussed.

Adenosine deaminase (ADA; adenosine aminohydrolase, EC 3.5.4.4) catalyzes the hydrolytic deamination of adenosine to inosine during purine nucleotide salvage and consists of differentially expressed isozymes in human tissues. The genetic processes that result in (i) the generation of the tissue-specific ADA isozymes, whose expression reflects the signals of cellular differentiation (1); and (ii) the deficiency of ADA, which is associated with an autosomal recessive form of severe combined immunodeficiency disease (SCID) (2, 3) remain largely unresolved. The genetic dissection of these two aspects of human ADA expression, using somatic cell hybrids, is investigated here.

Human ADA consists of six different isozymes. The catalytic moiety of each of these isozymes is the product of the ADA locus on chromosome 20 (4), weighs 36,000 daltons (5), and is present as a monomer in erythrocytes and certain other tissues; this isozyme has been designated erythrocyte ADA, or ADA-1 (1). The remaining five ADA tissue isozymes have molecular weights of $\approx 220,000$ and are multimers of ADA-1 and an ADA-complexing protein (6-8). The ADA tissue isozymes are

not present in erythroid cells; their presence requires the expression of human genes in addition to ADA (6). The tissue specificity of these isozymes further suggests that more than one type of gene may be required for the appearance of these isozymes in human cells. We have described *ADCP* (adenosine deaminase complexing protein), on human chromosome 6, whose product is necessary for the expression of the ADA fibroblast isozyme, ADA-d, in somatic cell hybrids (6). We showed that the *ADCP* gene product was involved in the conversion of ADA-1 to ADA-d, in agreement with the biochemical evidence for the existence of an ADA-complexing protein in human kidney (8) and lung (9). A second *ADCP* gene (10) is described here, which is encoded on human chromosome 2; its presence is also required for ADA-d expression in human-mouse somatic cell hybrids.

Identifying the type of genetic defect in SCID would provide information on the metabolic defect and whether different genes from those above are involved in ADA expression. Several genetic mechanisms have been proposed to explain the defect that results in ADA-deficient SCID, including control, inhibitor, and structural gene mutations (11-13). It is possible to differentiate among these mechanisms and to determine the linkage relationships of genes that may be involved in ADA-deficient SCID by using cell hybrids formed between SCID fibroblasts and mouse cells. We have isolated such hybrids; the data indicate that the lack of ADA in ADA-deficient SCID is due either to the altered expression of, or a mutation in, the ADA locus on chromosome 20. In either case, no human genes unlinked to ADA are involved in the ADA-deficient phenotype of primary SCID (14).

This genetic description of ADA realization in interspecific somatic cell hybrids thus defines the involvement of at least three unlinked genes in the expression of human ADA tissue isozymes: ADA on chromosome 20, *ADCP-1* on chromosome 6, and *ADCP-2* on chromosome 2. A mutant ADA gene on chromosome 20 is suggested for SCID, and further evidence suggests that additional, as yet unassigned, genes appear to be involved in the processing of the complexing protein.

MATERIALS AND METHODS

Human, Rodent, and Hybrid Cells. Human-mouse somatic cell hybrids were isolated from the fusion of human fibroblasts or leukocytes with A₉ (hypoxanthine phosphoribosyltransferase-deficient), LM/TK⁻, or RAG (hypoxanthine phosphoribosyltransferase-deficient) mouse cells. Human fibroblast parental cells were SH 421 (15), WI-38 (American Type Culture Collection no. CCL 75), AnLy (16), DUV (17), JoSt (18), GM 194 (carrying an X/3 translocation) (19), GM 469 (ADA-deficient line established from a SCID patient) (20), and GM 1006

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: ADA, adenosine deaminase; SCID, severe combined immunodeficiency disease; ADCP, ADA-complexing protein; ITP, inosine triphosphatase.

* Preliminary reports of this work have been presented (10, 14).

(a mucopolipidosis type II line) (21, 22). Human leukocytes were from JoVa (23) and AITr (24). The fusions were performed by using either inactivated Sendai virus (25) or polyethylene glycol (26). Interspecific cell hybrid clones were isolated and maintained as described (25). The cell hybrids used in this study were from the independent hybrid sets: DUA (DUV-A₉), DUM (DUV-RAG), ICL (GM 1006-LM/TK⁻), ICR (GM 1006-RAG), ALA (AnLy-A₉), ALR (AnLy-RAG), JWR (JoVa-RAG), ATR (AITr-RAG), JSR (JoSt-RAG), RAS (SH 421-RAG), XTR (GM 194-RAG), and SIR (GM 469-RAG), derived from nine different human parental cell types.

Gel Electrophoresis. ADA isozymes were separated in 12% or 13% Electrostarth gels (lots 307 and 392, respectively; Otto Hiller, Madison, WI) at 170 V for 18 hr (6). The human-mouse somatic cell hybrids expressing ADA-d contained the ADA-complexing protein. In hybrids lacking all forms of human ADA, the complexing protein was assayed by mixing a human erythrocyte lysate, containing ADA-1, with the hybrid cell extract and incubating the mixture at 37°C for 1 hr before electrophoresis. The presence, after starch gel electrophoresis, of ADA-d in the mixed extracts indicated that the ADA-complexing protein was present in the human-mouse cell hybrids (6).

RESULTS

ADA-Complexing Protein Expression in Cell Hybrids Requires Human Chromosomes 2 and 6. We have described the expression of the ADA isozymes in cultured human cell lines and their hybrids with mouse cells (6) (Fig. 1). Two isozymes, ADA-1 and ADA-d, can occur in human fibroblast lines (1, 6, 27). These two isozymes are present in various proportions, which depend on the tissue origin of the fibroblasts, culture conditions, and transformed state of the human cells (1, 27, 28). In human-mouse somatic cell hybrid clones also, human ADA activity is expressed as ADA-1 and/or ADA-d (6) (Fig. 1, channels 4-6). The structural gene for ADA-1 has been assigned to human chromosome 20 (4); when human chromosome 20 is not present in a hybrid clone, that clone expresses neither ADA-1 nor ADA-d (6), indicating that ADA-1 is required for the formation of ADA-d in hybrid cells, as it is in human cells (7, 29). Extracts of ADA-deficient SCID tissues can convert exogenous ADA-1 to the appropriate tissue isozymes *in vitro* (7), a phenomenon that has been attributed to the presence of tissue-specific ADA-complexing proteins (8, 9) that spontaneously complex with ADA-1 to form the ADA tissue isozymes.

In a previous paper (6), we demonstrated that ADA-complexing protein activity (recognized by the formation of ADA-d from ADA-1 *in vivo*, or from exogenous ADA-1 *in vitro*) was expressed in some human-mouse somatic cell hybrids. We observed that complexing protein activity was present if human chromosome 6 had been retained in the hybrid clones. Karyotypic analysis of 39 additional hybrid clones has confirmed the existence of an ADCP gene on human chromosome 6 and has shown that human chromosome 2 also contains a gene that is involved in the expression of the ADA-complexing protein (Table 1). The data in Table 1 show that ADA-complexing protein activity is present in a human-mouse somatic cell hybrid clone only when human chromosomes 2 and 6 are present in the hybrid cells; if either of these two chromosomes has been lost in the clone, ADA-complexing protein activity is not expressed. Our previous results demonstrate that the presence of human chromosome 2 alone is not sufficient for ADA-complexing protein activity in human-mouse somatic cell hybrids (6). When all other human chromosomes and enzyme markers for chromosomes were examined, no other pairs of human chromosomes concordantly segregated with the ADA-com-

FIG. 1. Starch gel electrophoresis of human and mouse ADA isozymes in somatic cell hybrids (6). The ADA phenotype of the parental mouse cells is shown in channel 1; the electrophoretic identities of the ADA-d in human cells (channel 2), in human-mouse hybrids (channel 4), and that formed *in vitro* by mixing (channel 8) are shown. The three phenotypes of human-mouse somatic cell hybrids are shown in channels 4-10: ADA-d⁺, channel 4; ADA-1⁺, ADA-d⁻, channels 5 and 6; ADA-1⁻, ADA-d⁻, channels 7 and 9. Channels 7 and 8 show that SIR-5 can convert exogenous ADA-1 (supplied as the erythrocyte lysate in channel 3) to ADA-d, and therefore contains the ADA-complexing protein; channels 9 and 10 show that SIR-8 does not express the ADA-complexing protein. The complexing protein activity in human ADA-1⁻ hybrids was assayed with exogenous ADA-1.

plexing protein activity in our hybrids (data not shown). Herbschleb-Voogt *et al.* (30) have also presented evidence for the involvement of a gene on chromosome 2 in ADA complexing protein expression.

To define the genetic and molecular events that result in the expression of the ADA-complexing protein in hybrids con-

Table 1. Segregation of ADA-complexing protein with human chromosomes 2 and 6, singly (A and B) and in pairs (C)

A.	Chromosome 2	ADA-complexing protein		B.	Chromosome 6	ADA-complexing protein	
		+	-			+	-
	+	19	5		+	19	8
	-	0	15		-	0	12
C.	Chromosomes		ADA-complexing protein		ADA-complexing protein		
	2	6	+	-	+	-	
	+	+	19	0			
	-	+	0	8			
	+	-	0	5			
	-	-	0	7			

Trypsin/Giemsa banding was used to identify human metaphase chromosomes (23). Replicate flasks of the hybrid clones were used for chromosome preparations and determinations of ADA-complexing protein activity. Numbers of clones with the indicated characteristics are tabulated.

FIG. 2. (Upper) Starch gel electrophoresis of ITP (31) in SCID-RAG hybrid clones. The mouse phenotype is shown in channel 1, the human phenotype in channel 2. The hybrids shown in channels 3-5 expressed a human-mouse ITP heteropolymer, and had therefore retained human chromosome 20 [as confirmed by chromosome analysis (Fig. 3)]. (Lower) Starch gel electrophoresis of ADA in SCID-RAG hybrid clones. The mouse parental cell phenotype is shown in channel 1, the normal human phenotype in channel 2. The SCID-RAG hybrids (channels 3-5) and the human SCID parental cell lines (ref. 20) exhibited no human ADA activity (ref. 6).

taining human chromosomes 2 and 6, we examined the individual metaphases from each of the 19 ADCP⁺ hybrid clones and an additional three clones that appeared, through enzyme marker and chromosome analysis, to have retained both human chromosome 2 and chromosome 6, yet did not express the ADA-complexing protein. In each ADCP⁺ clone, at least 15%

of the individual metaphases examined contained both chromosome 2 and chromosome 6; the three ADCP⁻ 2⁺ 6⁺ clones never retained both human chromosomes in the same cell. Furthermore, extracts of chromosome 2⁺ 6⁻ hybrids and chromosome 2⁻ 6⁺ hybrids did not complement *in vitro* to form an ADA-complexing protein. These results indicate that the gene products of the two distinct ADCP loci on human chromosomes 2 and 6 are required in the same cell for that cell to express an ADA-complexing protein. Presently, it is premature to describe specifically the polypeptide products of these two genes. We have therefore designated them ADCP-1 (on chromosome 6) and ADCP-2 (on chromosome 2) solely to describe their phenotype in human-mouse somatic cell hybrids (see Discussion).

Molecular Defect in ADA-Deficient SCID is on Human Chromosome 20. It was of interest to investigate the genetic variant that results in an ADA deficiency in human cells because of the variety of genetic mechanisms that have been proposed to explain this phenotype (11-13) and to determine if any genetic relationship exists between the ADA-complexing protein and SCID. We used the somatic cell hybrid strategy to investigate the ADA deficiency in cell hybrid clones between an ADA-deficient SCID fibroblast line (GM 469) and mouse RAG cells. The ADA phenotype of the 16 SCID-RAG hybrid clones is represented by three clones in Fig. 2 Lower: none of the SCID-RAG hybrids expressed human ADA, although all expressed mouse ADA. To determine if human chromosome 20, which contains the structural gene for ADA-1, had been retained in these hybrids, we analyzed the human ADA⁻ hybrids for the syntenic marker (32) inosine triphosphatase (ITP). Nine of the hybrid clones contained a human-mouse ITP heteropolymer, demonstrating that human chromosome 20 had been retained in these human ADA⁻ hybrids (Fig. 2 Upper). These human ITP⁺ ADA⁻ clones also contained a structurally intact human chromosome 20 (Fig. 3); the hybrid from which the karyotype in Fig. 3 was derived contained at least one copy of chromosome 20 in 10 of the 16 metaphases examined. Similar results were obtained for the karyotypic analysis of other SCID-RAG hybrid clones. Thus, an intact human chromosome 20, presumably containing a human ADA gene, had been retained in the human ITP⁺ ADA⁻ hybrids.

In human-mouse somatic cell hybrids, the ITP-ADA linkage group segregates independently of the remaining 23 human linkage groups (4, 32). If the absence of human ADA in the ITP⁺ ADA⁻ SCID-RAG hybrids is caused by another gene, unlinked to ADA on chromosome 20, whose product has a regulatory or inhibitory influence on human ADA expression,

FIG. 3. Trypsin/Giemsa-banded human chromosomes in ADA⁻ SCID-RAG hybrid clone SIR-5; the mouse chromosomes have been omitted. A structurally intact human chromosome 20 has been retained in this hybrid clone (see Fig. 2 for the ADA and ITP phenotypes of this clone).

the ITP⁺ ADA⁻ phenotype could be explained by the consistent loss or retention, respectively, of a syntenic group containing that gene. We therefore analyzed the nine ITP⁺ ADA⁻ clones for human enzymes representing syntenic groups that have been assigned to each human chromosome. Human karyotypes were also prepared for three of these clones. The data from both the enzyme and chromosome analyses show that the absence of human ADA in the ITP⁺ ADA⁻ hybrids could not be correlated with the presence or absence of any human linkage group, except those assigned to chromosome 20 and X (Table 2). Because primary ADA-deficient SCID is inherited as an autosomal recessive phenotype, neither the human X nor the Y chromosome is involved in the human ADA deficiency. Therefore, the data indicate that the molecular defect resulting in the human ADA deficiency in these hybrids is encoded on human chromosome 20 alone, which chromosome contains the ADA structural gene.

DISCUSSION

We have used the somatic cell hybrid method to define genes designated *ADCP-1* and *ADCP-2* on human chromosomes 6 and 2, respectively, that are required for the presence of the ADA-complexing protein in human-mouse somatic cell hybrids. The individual products of these two genes do not interact *in vitro* to form a functional ADA-complexing protein; rather, the presence of *ADCP-1* and *ADCP-2* in the same cell is required for that cell to express the complexing protein.

The ADA-complexing protein assay was based on the established ability of the purified human protein to combine with ADA-1 to form the tissue isozyme ADA-d (8, 9). The ADA-d in our hybrids that express both *ADCP-1* and *ADCP-2* has an

Table 2. Segregation analysis of human linkage groups in ADA⁻ ITP⁺ SCID-RAG hybrid clones

Chromosome	Marker enzymes	Enzyme marker phenotype	
		+	-
1	ENO1, PEPC	6	2
2	IDH, MDH	5	4
3	GLB1	5	4
4	PEPS	3	2
5	HEXB	6	3
6	ME1, SOD2	7	2
7	GUSB	7	2
8	GSR	1	3
9	AK1, ACO1	3	2
10	GOT1	7	2
11	LDHA	7	2
12	LDHB, PEPB	7	2
13	ESD	4	5
14	NP	7	2
15	HEXA, MPI	6	3
16	APRT	8	1
17	GALK	3	5
18	PEPA	7	2
19	GPI	3	2
20	ITP	9	0
21	SOD1	6	3
22	ACO2	3	4
X	G6PD	9	0

The human enzyme markers encoded on each human chromosome were all analyzed on the same homogenate to identify each linkage group (33, 34). The + and - columns indicate, respectively, the number of ITP⁺ ADA⁻ clones that had retained or lost the marker enzymes representative of each human linkage group. The chromosome assignments, gene symbols, and electrophoretic and visualization procedures have been described (33-35).

electrophoretic mobility that is identical to that found in human cells and tissues. We have examined the ADA phenotypes of mouse and human brain, liver, spleen, lung, colon, skeletal muscle, and heart. In the mouse, these diverse tissues all have ADA phenotypes identical to the phenotype of mouse erythrocyte ADA, whereas all of the corresponding human tissues contain unique ADA tissue isozyme patterns (1, 6, 7). Extracts of the mouse tissues do not alter the mobility of either mouse or human erythrocyte ADA *in vitro*, whereas extracts of the human tissues convert exogenous human, but not mouse, erythrocyte ADA to the appropriate tissue isozymes. These results demonstrate that ADA-complexing proteins and electrophoretically discernible tissue isozymes are not present in mouse tissues. The presence of complexing protein activity in human-mouse somatic cell hybrids, therefore, must be due to the expression of a human gene(s) encoding an ADA-complexing protein. Thus, at least one of the two genes we have described is an *ADCP* structural gene.

The remaining *ADCP* gene probably serves one of three functions in human cells. The second *ADCP* gene might encode a second polypeptide subunit of the ADA-complexing protein, because the protein purified from human kidney is a glycoprotein dimer with subunits of similar, if not identical, molecular weights (8, 36, 37). Alternatively, the second *ADCP* gene may function in the processing of the *ADCP* structural gene product. Biochemical evidence from the mucopolidoses suggests that the correct formation of ADA-d requires several genetically distinct processing steps of the ADA-complexing protein (38); one *ADCP* gene could then be involved in a step of this processing that is essential for ADA-complexing protein activity. Finally, the second *ADCP* gene could positively regulate the expression of the *ADCP* structural gene. That a genetic modulation of ADA-complexing protein activity occurs is demonstrated by the various levels of complexing protein activity in human tissues (1, 7, 9, 27) and by the decrease in cellular levels of ADA-d after simian virus 40 transformation (27) or lymphocyte stimulation (39). A scheme of ADA tissue realization is shown in Fig. 4.

Interspecific somatic cell hybrids between human fibroblasts derived from a SCID patient and mouse cells were constructed to characterize genetically the ADA deficiency in this disease. Each of the hybrid clones that retained human chromosomes 2 and 6 expressed the complexing protein as tested by the ability to convert exogenous ADA-1 to ADA-d. This observation demonstrates that the complexing protein is not implicated in

FIG. 4. Genetic description of ADA tissue isozyme realization. Two genes, *ADCP-1* on chromosome 6 and *ADCP-2* on chromosome 2, encode products that are essential for the formation of the dimeric ADA-complexing protein (M_r 190,000). At least one of the *ADCP* genes is a structural gene for a subunit(s) of the complexing protein. The remaining *ADCP* gene is a structural, processing, or control gene whose product interacts with the *ADCP* structural gene product (see text). Tails on complexing protein subunits (circles) represent carbohydrate. The ADA-complexing protein dimer (8, 36) and ADA-1 (M_r 36,000) spontaneously assemble to form a tetrameric ADA tissue isozyme (M_r 220,000). In SCID, ADA or a gene linked to it is defective, resulting in a deficiency of all forms of ADA.

ADA-deficient SCID, confirming the observations of Hirschhorn (7). Indeed, karyotypic and enzyme analysis of these hybrids demonstrated that only human chromosome 20, containing the ADA structural gene, can be implicated in the ADA deficiency in this SCID (Table 2). Similar preliminary results obtained by using an obligate heterozygote for the ADA deficiency have been reported (40).

Three mechanisms have been proposed for the lack of ADA activity that can occur in SCID: (i) the presence of an ADA inhibitor, resulting in reduced amounts of ADA activity (12); (ii) a defective modulation of the ADA structural gene, resulting in a decreased amount of ADA enzyme (11); and (iii) a mutation in the ADA structural gene itself, which yields a catalytically altered ADA (13). We have not observed an inhibition of mouse ADA activity in any of our SCID-RAG hybrids or a reappearance of human ADA in the SCID-RAG hybrids that had retained human chromosome 20. Our somatic cell hybrid data, therefore, indicate that the lack of ADA in ADA-deficient SCID is not caused by genes encoding an inhibitor of ADA activity or repressing the expression of ADA. Indeed, the residual ADA activity in SCID tissues and cultured fibroblasts exhibits an altered heat stability and electrophoretic mobility (13). These observations are consistent with the interpretation that the altered ADA is encoded by an ADA gene carrying a mutation in the coding sequence for ADA-1, and are consistent with our results demonstrating that human ADA activity is not detectable on starch gels in SCID-RAG hybrids that have retained human chromosome 20 (Table 2). Because the catalytic moiety of ADA, ADA-1, is a monomer (1, 5, 40, 41), it is not surprising (if ADA deficiency is due to a structural gene mutation) that the mouse genome does not complement the ADA deficiency of SCID in somatic cell hybrids to form an enzymatically active human-rodent heteropolymeric ADA.

In summary, at least three human genes are required for the expression of the ADA tissue isozymes: ADA on chromosome 20, ADCP-1 on chromosome 6, and ADCP-2 on chromosome 2. The ADA deficiency in SCID segregates with chromosome 20 alone in interspecific somatic cell hybrids, suggesting that a structural gene mutation at the ADA locus is the primary cause of ADA-deficient SCID.

We recognize the expert assistance of M. G. Byers, R. L. Eddy, L. L. Haley, and C. Young. The contributions and advice of Dr. J. A. Brown to the chromosome analysis of the cell hybrids are gratefully acknowledged. This work was supported by National Institutes of Health Grants HD 05196 and GM 20454.

- Edwards, Y. H., Hopkinson, D. A. & Harris, H. (1971) *Ann. Hum. Genet.* **35**, 207-219.
- Giblett, E. R., Anderson, J. E., Cohen, F., Pollara, B. & Meuwissen, H. J. (1972) *Lancet* **ii**, 1067-1069.
- Parkman, R., Gelfand, E. W., Rosen, F. S., Sanderson, A. & Hirschhorn, R. (1975) *N. Engl. J. Med.* **292**, 714-719.
- Tischfield, J. A., Creagan, R. P., Nichols, E. & Ruddle, F. H. (1974) *Hum. Hered.* **24**, 1-11.
- Schrader, W. P., Stacy, A. R. & Pollara, B. (1976) *J. Biol. Chem.* **251**, 4026-4032.
- Koch, G. & Shows, T. B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 3876-3880.
- Hirschhorn, R. (1975) in *Isozymes*, ed. Markert, C. L. (Academic, New York), Vol. 2, pp. 583-599.
- Daddona, P. E. & Kelley, W. N. (1978) *J. Biol. Chem.* **253**, 4617-4623.
- Nishihara, H., Ishikawa, S., Shinkai, K. & Akedo, H. (1973) *Biochim. Biophys. Acta* **302**, 429-447.
- Koch, G. A. & Shows, T. B. (1979) *Cytogenet. Cell Genet.* **25**, 174 (abstr.).
- Siciliano, M. J., Bordelon, M. R. & Kohler, P. O. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 936-940.
- Trotta, P. P., Smithwick, E. M. & Balis, M. E. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 104-108.
- Hirschhorn, R., Beratis, N. & Rosen, F. S. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 213-217.
- Koch, G. A. & Shows, T. B. (1979) *Cytogenet. Cell Genet.* **25**, 173 (abstr.).
- Rattazzi, M. C., Brown, J. A., Davidson, R. G. & Shows, T. B. (1976) *Am. J. Hum. Genet.* **28**, 143-154.
- Cohen, M. M., Lin, C. C., Sybert, V. & Orecchio, E. J. (1972) *Am. J. Hum. Genet.* **24**, 583-597.
- Solomon, E., Bobrow, M., Goodfellow, P. N., Bodmer, W. F., Swallow, D. M., Povey, S. & Noel, B. (1976) *Somatic Cell Genet.* **2**, 125-140.
- 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.
- de la Chapelle, A., Schröder, J., Klinger, H. P., Aronson, M. M., Miller, R. C., Greene, A. E. & Coriell, L. L. (1975) *Cytogenet. Cell Genet.* **15**, 404-405.
- Chen, S.-H., Scott, C. R. & Swedberg, K. R. (1975) *Am. J. Hum. Genet.* **27**, 46-52.
- Champion, M. J. & Shows, T. B. (1977) *Proc. Natl. Acad. Sci. USA* **74**, 2968-2972.
- Champion, M. J. & Shows, T. B. (1977) *Nature (London)* **270**, 64-66.
- Shows, T. B. & Brown, J. A. (1975) *Cytogenet. Cell Genet.* **14**, 251-255.
- Lalley, P. A., Brown, J. A. & Shows, T. B. (1976) *Cytogenet. Cell Genet.* **16**, 188-191.
- Shows, T. B. (1972) *Proc. Natl. Acad. Sci. USA* **69**, 348-352.
- Davidson, R. L. & Gerald, P. S. (1976) *Somatic Cell Genet.* **2**, 165-176.
- Shows, T. B. (1975) in *Isozymes*, ed. Markert, C. L. (Academic, New York), Vol. 3, pp. 619-636.
- van Cong, N., Weil, D., Gross, M. S., Jami, J. & Frezal, J. (1979) *Cytogenet. Cell Genet.* **25**, 214 (abstr.).
- Hirschhorn, R. (1975) *J. Clin. Invest.* **55**, 661-667.
- Herbschleb-Voogt, E., Grzeschik, K.-H., de Wit, J., Pearson, P. L. & Meera Khan, P. (1979) *Cytogenet. Cell Genet.* **25**, 163 (abstr.).
- Hopkinson, D. A., Povey, S., Solomon, E., Bobrow, M. & Gormley, I. P. (1976) *Cytogenet. Cell Genet.* **16**, 159-160.
- Meera Khan, P., Pearson, P. L., Wijnen, L. L. L., Doppert, B. A., Westerveld, A. & Bootsma, D. (1976) in *Baltimore Conference (1975): Third International Workshop on Human Gene Mapping*, Birth Defects: Original Article Series (Natl. Found., New York), Vol. 12, No. 7, pp. 420-421.
- 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.
- Naylor, S. L., Klebe, R. J. & Shows, T. B. (1978) *Proc. Natl. Acad. Sci. USA* **75**, 6159-6162.
- Shows, T. B. & McAlpine, P. (1978) *Cytogenet. Cell Genet.* **22**, 132-145.
- Schrader, W. P. & Stacy, A. R. (1977) *J. Biol. Chem.* **252**, 6409-6415.
- Swallow, D. M., Evans, L. & Hopkinson, D. A. (1977) *Nature (London)* **269**, 261-262.
- Champion, M. J. & Shows, T. B. (1977) *Am. J. Hum. Genet.* **29**, 149-163.
- Hirschhorn, R. & Levytska, V. (1974) *Cell. Immunol.* **12**, 387-395.
- Herbschleb-Voogt, E., Vossen, J. M., Pearson, P. L. & Meera Khan, P. (1979) *Cytogenet. Cell Genet.* **25**, 764 (abstr.).
- Osborne, W. R. A. & Spencer, N. (1973) *Biochem. J.* **133**, 117-123.
- Spencer, N., Hopkinson, D. A. & Harris, H. (1968) *Ann. Hum. Genet.* **32**, 9-14.