A gene on human chromosome 6 functions in assembly of tissue-specific adenosine deaminase isozymes
(somatic cell hybrids/gene mapping/immune deficiency disease)

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ABSTRACT In human tissues, adenosine deaminase (ADA) (adenosine aminohydrolase; EC 3.5.4.4) activity can be separated by gel electrophoresis into several isozymes. A structural gene (ADA) on chromosome 20 codes for the "erythrocyte" iso-enzyme, ADA-1, which is also expressed in some nonerythroid tissues. Nonerythroid cells also differentially express five ADA "tissue isozymes" of a greater molecular weight than ADA-1. Each ADA tissue isozyme has a characteristic electrophoretic mobility and tissue distribution. It has been suggested that these ADA tissue isozymes are composed of ADA-1 and other components. We report that the expression of one of these tissue isozymes, ADA-d, is dependent upon ADA on chromosome 20 and another gene on chromosome 6 which functions in the assembly of the ADA tissue isozymes. In human–mouse hybrids segregating human chromosomes, chromosome 6*+20+ hybrids express both ADA-1 and ADA-d; chromosome 6*+20+ hybrids express only ADA-1; while 6*+20+ hybrids have no human ADA activity. ADA-d formation also occurs in vitro by self-assembly when an extract of human erythrocytes or chromosome 6*+20+ hybrids is mixed with a homogenate of chromosome 6*+20+ hybrids. The gene on chromosome 6, designated ADCP, codes for an adenosine deaminase complexing protein. The product of ADCP presumably combines with ADA-1 to form the ADA tissue isozymes. The data are consistent with the hypothesis that the distribution of enzymatic activity between ADA-1 and the tissue isozymes depends on the expression of the gene for ADA complexing protein, while the differences in the electrophoretic mobilities of the ADA isozymes, except ADA-1, are generated, as suggested by others, by the degree of glycosylation of the complexing protein.

Differentiated cell types in higher organisms are the result of specific programs of gene expression. Differentiation occurs primarily as the result of a patterned activation of certain genes and a repression of others. Tissue-specific molecular forms of an enzyme activity (isozymes) illustrate this control of gene expression in development. Isozymes specific for differentiated tissues are generated basically by two mechanisms. Multiple forms of an enzyme activity are coded by different structural genes, or they are derived from a single gene product that undergoes one or more post-translational modifications. The first mechanism includes isozymes whose subunits can combine to form both homopolymers and heteropolymers (e.g., lactate dehydrogenase, ref. 1) and those isozymes of the same enzymatic activity that do not share subunits (e.g., phosphoglucomutase, ref. 2). The second mechanism includes tissue-specific isozymes that are formed, for example, by the addition of carbohydrate moieties to an enzyme (e.g., acid phosphatase, ref. 3) or enzymatically inactive proteins to a catalytically active enzyme (β-glucuronidase, ref. 4). This report describes the genetics and a possible mechanism of the process that controls the formation of tissue-specific isozymes of human adenosine deaminase (ADA) (adenosine aminohydrolase; EC 3.5.4.4).

Human ADA can be separated by starch gel electrophoresis into several isozymes (5), of which there are two classes: (i) erythrocyte ADA (ADA-1), the form present in erythrocytes and most other tissues, and (ii) a variety of tissue-specific isozymes (ADA-a, -b, -c, -d, and -e, in order of decreasing electrophoretic mobility) that occur in varying amounts of nonerythroid tissues. The tissue isozymes are thought to be derived from the association or assembly of ADA-1 and other, noncatalytic components (6). A deficiency of ADA-1 has been implicated as the cause of certain autosomal recessive forms of severe combined immunodeficiency disease (SCID), an inherited defect of both T- and B-lymphocyte functions (7, 8). Although tissue extracts from patients with SCID exhibit no ADA activity on starch gels, the characteristic tissue-specific isozymes self-assemble when SCID tissue extracts are mixed with erythrocyte lysates from unaffected individuals (6, 9). This observation demonstrates the dependence of the tissue-specific isozymes on the presence of ADA-1.

The segregation of human chromosomes and adenosine deaminase isozymes (10) in human–mouse cell hybrids suggested that the identification and mapping of genes involved in the expression of ADA tissue-specific isozymes expressed in cultured cells was possible by a somatic cell hybrid approach. In this report we identify a gene, ADCP (adenosine deaminase complexing protein), which is necessary for the conversion of adenosine deaminase activity from ADA-1 to ADA-d in human–mouse somatic cell hybrids. ADCP is on human chromosome 6 and is therefore not linked to the structural gene (ADA) for ADA-1, on chromosome 20 (11).

MATERIALS AND METHODS

Human, Rodent, and Hybrid Cells. The human parental cells used in cell hybrids were the fibroblasts SH 421 (12), WI-38 (ATCC no. CCL 75), AnLy (13), DUV (14), GM-1006 (15), and JoSt (16); and Jo Va (17) and AITr (18) leukocytes. Rodent parental cells were CHW-1102 (HPRT−) Chinese hamster cells and A9 (HPRT−), RAG (HPRT−), and LM/TK− mouse cells (see ref. 19). Human–mouse and human–hamster somatic cell hybrids were fused by using inactivated Sendai virus; hybrid cells were selected and maintained in HAT medium consisting of Dulbecco's modified Eagle's medium (GIBCO), HAT (hypoxanthine/aminopterin/thymidine), 10% fetal calf serum, and antibiotics (20). The cell hybrids used in this study were taken 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), ATC (AITr × CHW-1102), ATR (AITr × RAG), JSR

Abbreviations: ADA, adenosine deaminase; ADCP, gene coding for ADA complexing protein; SCID, severe combined immunodeficiency disease.
(JoSt × RAG), and RAS (SH 421 × RAG) (19) derived from seven individuals. Cell homogenates for electrophoresis were prepared in 50 mM Tris-HCl (pH 7.5) at 0.8–1.0 × 10⁶ cells/ml (20).

STARCH GEL ELECTROPHORESIS. Adenosine deaminase isozymes were separated in 12% starch gels (Electrostarch) at 200 V for 18 hr. The gel buffer was 5 mM sodium barbital (pH 8.0) and the bridge buffer was 50 mM sodium barbital (pH 8.0). The staining mixture for visualization of ADA isozymes in starch gels was (per ml) 1 mg of adenosine, 0.2 mg each of MTT (a tetrazolium dye) and phenazine methosulfate, 0.005 unit of xanthine oxidase, and 0.1 unit of nucleoside phosphorylase in a 25-ml 1% agar overlay containing 0.25 M Tris-HCl (pH 8.0) (21). Additional enzyme markers necessary for identifying each human chromosome except Y were analyzed on each independent hybrid clone (see Results).

CHROMOSOME ANALYSIS. Trypsin–Giemsa banding was used to identify human metaphase chromosomes in hybrid cells (22).

RESULTS

Human ADA can be separated by starch gel electrophoresis into at least six isozymes in tissues (5). Of these, only two—ADA-1 and ADA-d—are expressed in cultured cells, but their expression is not without exception. For example, ADA-1 is present in some human fibroblasts (AnLy, Fig. 1, channel 8) but not in others (Wi-38, channel 6); it is also present in a proportion of human–mouse somatic cell hybrid clones (Fig. 1, channels 4 and 9, and Fig. 2, channels 5 and 6). The electrophoretic pattern of erythrocyte ADA in Fig. 1 was separated, as reported (21), into multiple bands in erythrocytes, fibroblasts, and cell hybrids. After storage, the pattern shifted in the anodal direction. The erythrocyte ADA-1 (Fig. 1, channel 2) has shifted from the initially tested major cathodal band to a less intense anodal band. ADA electrophoretic variants exist in the human population (21); however, all tissues, fibroblasts, and human parental cells for cell hybrid studies were of the ADA-1 phenotype when tested in fresh homogenates. Only the major form of ADA-1 is expressed in Fig. 2. ADA-d is expressed in human fibroblast lines (Fig. 1, channels 6 and 8), kidney (channel 7), and in some ADA-1+ human–mouse hybrids (channel 9), but not in erythrocytes (channel 2). When erythrocyte lysates or homogenates of ADA-1+ and ADA-d− (channel 4) human–mouse hybrids are mixed with extracts of cultured fibroblasts (GM-469) derived from a patient with SCID (ADA-i−, ADA-d−; channel 1), the ADA-1 is converted to a tissue isozyme, ADA-d (channels 3 and 5, respectively). The ADA-d thus formed is identical in electrophoretic mobility with that in extracts of WI-38, AnLy fibroblasts, and human kidney. Mouse ADA (channel 10) does not appear to interact with ADA-1 or ADA-d.

Because the hybrids studied preferentially segregate human chromosomes and enzyme markers, including ADA-d (10), it was possible to assign the gene involved in the formation of ADA-d to a specific human chromosome. The expression of ADA-d was investigated in 102 independent human–mouse somatic cell hybrid clones derived from fibroblasts or leukocytes from seven different humans and three different mouse parental cell lines. We had observed that human chromosome 20, coding for ADA-1, is required, but is not sufficient, for ADA-d expression in human–mouse hybrids; so initially only those hybrids that were ADA-1+ were examined. The presence or absence of ADA-d in ADA-1+ hybrids segregated concordantly with the chromosome 6 markers malic enzyme (ME₆) (23) and superoxide dismutase (SOD₆) (24) in 60 of 62 hybrids (Table 1A), demonstrating the involvement of a gene(s) syntenic to ME₆ and SOD₆ in the expression of ADA-d in ADA-1+ human–mouse hybrids. In the hybrids chosen for this study, SOD₆ and ME₆ always segregated concordantly.

If ADA-d and the other tissue isozymes are derived from the assembly of ADA-1 and other subunits or complexing proteins (6, 9, 24), then the results shown in Table 1 indicate that complexing protein genes are not on chromosome 20. Therefore, human–mouse hybrids that have lost chromosome 20 may have retained the component(s) that generate ADA-d in vitro. We assayed extracts from ADA-1− human–mouse hybrids for the ability to generate ADA-d from exogenously supplied ADA-1 (Fig. 2). Homogenates of ADA-1−, ADA-d− cell hybrids (Fig. 2, channel 1) were incubated with an equal volume of either human hemolysates or homogenates of ADA-1+, ADA-d− human–hamster hybrid clones (channel 2). Some ADA-1−, ADA-d− hybrids (channel 1) can form ADA-d from ADA-1 in vitro (channels 3 and 4), while other hybrid clones apparently

![Starch Gel Electrophoresis of ADA Isozymes](image)

**Fig. 1.** Starch gel electrophoresis of ADA isozymes, showing the generation of ADA-d when SCID fibroblast homogenates were used in mixing experiments. Equal volumes (20 μl) of SCID fibroblast extract (channel 1) and human erythrocyte lysate (channel 2) or human–mouse hybrids (channel 4) were mixed (channels 3 and 5) for 2 hr at room temperature. Also shown are human–mouse hybrid and human tissue extracts that contain ADA-d (channels 6–9). Mouse cultured cells (channel 10) have no detectable ADA activity in the human region of the gel. Human SCID fibroblasts were GM-469 obtained from The Human Genetic Mutant Cell Repository, Camden, NJ.

<table>
<thead>
<tr>
<th>Table 1. Segregation of ADA-d and chromosome 6 markers</th>
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<tbody>
<tr>
<td><strong>(A)</strong> ADA-1+ hybrids</td>
<td><strong>(B)</strong> ADA-1− hybrids*</td>
</tr>
<tr>
<td>ME₆</td>
<td>SOD₆</td>
</tr>
<tr>
<td>+</td>
<td>−</td>
</tr>
<tr>
<td>−</td>
<td>+ 38 0</td>
</tr>
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</table>

ADA-d: 2 22 | ADA-d: − 0 23

All enzyme analyses were done on the same sample.

* An aliquot (20 μl) of a homogenate from each ADA-1− clone was mixed with 20 μl of an ADA-1+, ADA-d− human–hamster hybrid cell extract or a human erythrocyte lysate. The mixtures were incubated for 2–3 hr at room temperature before electrophoresis. For each hybrid clone, ME₆ and SOD₆ were analyzed on the same cell homogenate, but before it was mixed with the ADA-1+ extracts.
lack this ability (channel 7). Erythrocyte ADA-1 in these mixtures (Fig. 2) does comigrate with ADA-1 of cultured cells. When 40 independent human–mouse hybrids (ADA-1−, ADA-d−) were examined in this way, the ability to generate ADA-d from ADA-1 in vitro also segregated with the chromosome 6 markers ME5 and SODM (Table 1B). When considered with results obtained in other laboratories, these data indicate a gene coding for an adenosine deaminase complexing protein which is syntenic to ME5 and SODM and can generate ADA-d from exogenous ADA-1. We have designated the gene coding for this component as ADACP. The possible synteny of ADACP with a gene grouping other than that which has been assigned to human chromosome 6 was excluded by the independent segregation of the ADA complexing protein with enzyme markers assigned to all human chromosomes except Y (Table 2). The Y chromosome could be excluded from consideration for the location of ADACP since, in some hybrids that expressed ADA-d, the human fibroblast parental cells were female.

The assignment of ADACP to human chromosome 6 was confirmed in 20 mouse–human hybrids that had been examined karyotypically (Table 3) (24). Ten of these hybrids were ADA-1−, ADA-d−; a homogamete of each of these clones was mixed with extracts of ADA-1+, ADA-d− human–hamster hybrids or with human hemolysates to determine the presence or absence of the complexing protein. ADACP segregated concordantly with chromosome 6 with no exceptions. In addition, ADA-d and chromosome 6 segregated concordantly in all ten ADA-1+ hybrid clones examined for chromosomes.

\[
\begin{array}{|c|c|c|c|}
\hline
\text{Chromosome} & \text{Marker enzymes} & \text{Concordant} & \text{Discordant} \\
\hline
1 & Eno1, AK2, PGM1, PEPC, & 65 & 16 \\
 & FH & & \\
2 & ACP2, IDH, MDH & 55 & 20 \\
3 & \beta GAL & 35 & 12 \\
4 & PEPS & 16 & 12 \\
5 & HEXb & 57 & 35 \\
6 & ME5, SODM & 100 & 2 \\
7 & GUS & 61 & 31 \\
8 & GSR & 21 & 23 \\
9 & AK1, ACONs & 46 & 42 \\
10 & GOTs & 65 & 27 \\
11 & ACP2, ESAa, LDHA & 66 & 12 \\
12 & LDHb, PEPB & 65 & 22 \\
13 & ESD & 61 & 31 \\
14 & NP & 64 & 28 \\
15 & MPI & 66 & 25 \\
16 & APRT & 53 & 19 \\
17 & TK & 7 & 9 \\
18 & PEPA & 64 & 28 \\
19 & GPI & 54 & 38 \\
20 & ADA & 65 & 27 \\
21 & SOD1 & 61 & 30 \\
22 & ACONM & 17 & 9 \\
X & G6PD, HPRT, PGK & 57 & 24 \\
\hline
\end{array}
\]

All enzymes were analyzed on the same homogenate of each hybrid clone. The concordant column indicates the number of clones in which the marker enzymes and the ADA-d phenotype were present or absent together. The discordant column gives the number of clones in which only ADA-d or an enzyme, but not both, was present. The chromosome assignments, gene symbols, and electrophoretic procedures for each marker enzyme have been described (19).

* Forty of these 102 clones were ADA-1−; their ability to convert ADA-1 to ADA-d was tested in vitro as described in the legend to Table 1.

† The presence of human TK was inferred since human–mouse hybrids in which the mouse parent was TK− grew in HAT medium.

**DISCUSSION**

We have identified a gene, ADACP (adenosine deaminase complexing protein), which functions in the assembly of a tissue-specific isozyme, ADA-d. It was demonstrated that this isozyme can be spontaneously assembled by using somatic cell hybrid extracts possessing the appropriate chromosomes. ADA-d formation occurs in vivo and in vitro only when ADA complexing protein and ADA-1 are present. ADA complexing protein itself has no detectable ADA activity. ADACP is on

\[
\begin{array}{|c|c|c|c|}
\hline
\text{No.} & \text{Enzyme} & \text{Chromosome} \\
\hline
6 & + & + & + & + & + & + \\
4 & + & - & - & - & - & + \\
5 & - & - & + & + & + & + \\
5 & - & - & - & - & - & - \\
\hline
\end{array}
\]

All homogenates and chromosome preparations were made from hybrid cells at the same passage from replicate flasks.

* After mixing with an extract of an ADA-1+, ADA-d− human–hamster hybrid or with a human erythrocyte lysate.
human chromosome 6; since the structural gene for ADA-1 is on chromosome 20, it follows that both chromosomes 6 and 20 are required for ADA-d expression in human–mouse somatic cell hybrids. The segregation of human chromosomes and enzyme markers in 102 human–mouse hybrid clones indicated that no human chromosomes other than 6 and 20 are involved in ADA expression.

Information on the biochemical function of ADA complexing protein in the biogenesis of the ADA tissue isozymes comes from two lines of study. First, a "conversion factor" (M, 73,000), purified from human lung and present in other organs that contain the tissue isozymes of ADA, combines with ADA-1 (Mr 33,000) in vitro to form an ADA of a molecular weight similar to that of the tissue isozymes (M, 230,000) (25). A complexing protein with similar characteristics also has been isolated from human kidney (26). These complexing proteins are antigenically unrelated to ADA-1 (25, 26), indicating that the tissue isozymes are not multimers consisting only of ADA-1. Second, ADA isozymes specific for each tissue can be generated by mixing hemolysates, containing only ADA-1, with various tissue extracts from those patients with SCID who are grossly deficient in all ADA activities (6, 9). These assembly components in SCID tissues, the complexing proteins from human tissues, and homogenates of chromosome 6, 20 human–mouse hybrids all affect exogenous ADA-1 in the same way, i.e., by converting it to an ADA tissue isozyme. The evidence therefore indicates that ADCP, on human chromosome 6, is the gene that is responsible for the formation of ADA-d in human tissues, and that its product, an ADA complexing protein, is the same protein present in normal and SCID tissues which combines with ADA-1 to form the tissue isozymes.

The molecular identity of each ADA tissue isozyme is apparently determined by the identity of the proteins complexed with the ADA-1 enzyme (6, 9). Since each of the five ADA tissue isozymes has a characteristic electrophoretic mobility, the complexing proteins must be different in each isozyme. There are two possible genetic explanations for the origin of the different ADA complexing proteins. The tissue-specific complexing proteins could be the products of separate genes or the variably modified product of a single gene. Evidence for the latter possibility comes from the observation that neuraminidase treatment of tissue extracts decreases the mobility of ADA-a, -b, and -c to that of ADA-d (27). This suggests that ADA-d is the initial, or least modified, form of the complex of ADA-1 and ADA complexing protein (27). Neuraminidase has no effect on the electrophoretic mobility of ADA-1. Thus, the complexing proteins of all of the ADA tissue isozymes may have the same primary structure, while the differences in the electrophoretic mobilities of the tissue isozymes reflect the different degrees of glycosylation of the complexing protein. Since the ADA complexing protein is necessary for assembly of ADA-d, it is probable that processed ADA complexing protein also functions in the assembly of ADA-a, -b, and -c. Processing enzymes, perhaps of the same kind that modify ADA complexing protein, are also involved in the post-translational modification of other enzymes, since ADA-d is one of several enzymes with an altered electrophoretic mobility in mucolipidosis II (15), a rare and fatal genetic disease. The primary defect in mucolipidosis II is thought to be in a post-translational step common to the biogenesis of the affected enzymes since some of the electrophoretic abnormalities, including that of ADA-d, can be corrected in vitro by neuraminidase treatment (15).

The genetic profile of the ADA tissue isozymes can be summarized by the following hypothesis: ADA complexing protein is encoded at the ADCP locus on human chromosome 6. The protein is glycosylated by the action of an unassigned gene product(s) in a fashion characteristic of each tissue. After processing, the protein complexes with ADA-1, encoded at the ADA locus on chromosome 20; the complex of ADA-1 with ADA complexing protein is an ADA tissue isozyme. A mechanism similar to that proposed above for the generation of ADA tissue isozymes also operates in the distribution of β-glucuronidase activity between lysosomes and microsomes in mouse liver (28). In most strains of mice, a complexing glycoprotein, designated egasyn, combines with lysosomal β-glucuronidase to form microsomal β-glucuronidase (4). The attachment of β-glucuronidase enzymatic activity to the membrane requires egasyn (28). Also, GUS and Eg, the structural gene for egasyn, are unlinked (28), as are ADCP and ADA. ADCP and ADA are under independent genetic control in human–rodent hybrids and in human cells. This is demonstrated by the continued synthesis of ADA complexing protein in the absence of ADA-1 in SCID fibroblasts and chromosome 6+, 20 human–mouse hybrids, while human erythrocytes and chromosome 6−, 20 human–rodent hybrids contain ADA-1 but not ADA complexing protein. Since ADA-1 is required for the expression of ADA activity (6, 29), the ubiquitous presence of this activity in human cells (1) defines ADA as a constitutively expressed gene. The expression of ADCP, on the other hand, reflects the requirement of a particular cell type for the appropriate distribution of ADA activity between ADA-1 and the ADA tissue isozymes, and probably occurs in response to the signals that accompany cellular differentiation. These signals presumably also effect the synthesis of enzymes that modify ADA complexing protein in a tissue-specific fashion (27). The significance of ADCP, then, assigned here to human chromosome 6, is its ability to function in the elaboration of a differentiated phenotype: the expression of the ADA tissue isozymes.

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