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Genetics

The α-spectrin gene is on chromosome 1 in mouse and man

(human chromosome mapping/elliptocytosis/*in situ* hybridization)

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ABSTRACT **By using α-spectrin cDNA clones of murine and human origin and somatic cell hybrids segregating either mouse or human chromosomes, the gene for α-spectrin has been mapped to chromosome 1 in both species. This assignment of the mouse α-spectrin gene to mouse chromosome 1 by DNA hybridization strengthens the previous identification of the α-spectrin locus in mouse with the *sph* locus, which previously was mapped by linkage analysis to mouse chromosome 1, distal to the *Pep-3* locus. By *in situ* hybridization to human metaphase chromosomes, the human α-spectrin gene has been localized to 1q22–1q25; interestingly, the locus for a non-*Rh*-linked form of elliptocytosis has been provisionally mapped to band 1q2 by family linkage studies.**

Native spectrin, composed of two large subunits, α-spectrin (M_r 240,000) and β-spectrin (M_r 220,000), is the major protein component of the erythrocyte membrane skeleton (for reviews, see refs. 1 and 2). This membrane skeleton, also containing actin, ankyrin, and several other proteins, is responsible for maintenance of erythrocyte shape and flexibility, and defects in specific components of the erythrocyte membrane skeleton are the cause of various hemolytic anemias in mouse (3–5) and man (1, 2, 6–8). In the mouse, hemolytic anemias are inherited as autosomal recessives, and it has been shown recently that one mutation is due to a deficiency in ankyrin, one is due to a β-spectrin defect, and several mutations at the *sph* locus (3, 9) are due to defective α-spectrin molecules (3).

Abnormalities in the erythrocyte membrane skeleton also have been shown for hemolytic anemias in man (for reviews, see refs. 1 and 2). In type I hereditary spherocytosis, the molecular defect is in the NH_2-terminal end of the spectrin β chain (2, 10, 11); for type II hereditary spherocytosis, which may be a heterogeneous group of diseases, the molecular defect(s) has not yet been pinpointed (2). There is a rare, recessive form of spherocytosis (6) that is similar to the mouse disease and is probably due to a deficiency of α-spectrin (3, 6). For some forms of hereditary ellipsocytosis, the defect may be in spectrin, specifically in α-spectrin (12), or in a protein called band 4.1 (13). In hereditary pyropoikylocytosis, the defect has been localized to domain I of α-spectrin (14). Clearly much has been learned about the normal and abnormal erythrocyte membrane skeleton by biochemical analysis and linkage studies using the various hemolytic anemia diseases of both mouse and man; but for a finer analysis of molecular defects in the various diseases and for a detailed understanding of the interactions between the various proteins involved in the erythrocyte membrane skeleton, cloned DNA probes for the genes encoding these proteins will provide an invaluable tool.

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As a first step in the molecular analysis of the α-spectrin locus in mouse and man, we have used cDNA clones for murine and human α-spectrin (15, 16) in conjunction with panels of somatic cell hybrids segregating mouse or human chromosomes to determine the chromosomal location of murine and human α-spectrin genes.

MATERIALS AND METHODS

Cell Lines. Isolation, propagation, and characterization of parental cells and somatic cell hybrids of the Chinese hamster–mouse panel (17–20) and the mouse–human panel (21–23) have been described.

Molecular Probes. The mouse α-spectrin cDNA probe was a 750-base-pair (bp) *Pst* I insert excised from pMαSp1, a pUC9 plasmid containing the coding sequence for the repeated segments α16, α17, and α18 of mouse α-spectrin (15, 16).

The human α-spectrin cDNA probe, pHαSp6, consisted of a 720-bp *Pst* I fragment derived from a human α-spectrin cDNA clone, which was obtained by using the mouse α-spectrin cDNA (15, 16); sequencing showed that this DNA fragment is human α-spectrin cDNA (16).

For hybridization with nitrocellulose filters, the purified α-spectrin cDNA inserts were labeled by nick-translation (24) to a specific activity of $\approx 1 \times 10^9$ cpm/μg with [α-^{32}P]dNTPs and $\approx 1 \times 10^8$ cpm were used per filter hybridization.

***In Situ* Hybridization.** Total pHαSp6 plasmid DNA was nick-translated with [^{3}H]dATP (51.9 Ci·mmol^{-1}; 1 Ci = 37 GBq), [^{3}H]dGTP (39.9 Ci·mmol^{-1}), [^{3}H]dTTP (100.1 Ci·mmol^{-1}), and [^{3}H]dCTP (62.0 Ci·mmol^{-1}) and was hybridized to human metaphase chromosome preparations from peripheral blood cells of a normal male. The techniques used for *in situ* hybridization were essentially as described by Harper and Saunders (25). The probe had a specific activity of 3.0×10^7 cpm·μg^{-1}. Forty microliters of probe solution at 280 ng·ml^{-1} in hybridization solution [50% formamide containing 2× NaCl/Cit (1× NaCl/Cit= 0.15 M NaCl/0.015 M sodium citrate, pH 7), 10% dextran sulfate, and 100 μg of sonicated salmon sperm DNA per ml] were placed on each slide at 37°C. Autoradiography was performed by using NTB3 Kodak emulsion for 10 days before development. The slides were stained for 10 min in 6:1 mixture of borate buffer (50 mM Na_2SO_4/2.5 mM $Na_2B_4O_7$, pH 9.2), and Wright's Giemsa stain solution (2.4 g of Wright stain and 1.4 grains of Giemsa stain in 1 liter of methanol).

DNA Extraction and Southern Blot Analysis. Mouse, Chinese hamster, human, and hybrid DNAs were isolated as described (26) and digested with a 5-fold excess of restriction endonuclease *Hin*dIII. Digested DNAs (10 μg per lane) were separated electrophoretically in 0.8% agarose gels and blotted onto nitrocellulose filters (27). Filters were hybridized under stringent conditions [50% formamide containing 0.9 M

Abbreviation: bp, base pair(s).

NaCl, 50 mM Hepes, 5 mM EDTA, 0.2 mg of sonicated salmon sperm DNA per ml, and 1× Denhardt's solution (0.02% polyvinylpyrrolidone/0.02% Ficoll/0.02% bovine serum albumin)] at 42°C for 16 hr with ^{32}P-labeled mouse or human α-spectrin cDNA probe. After hybridization, filters were washed in 0.1× NaCl/Cit containing 0.5% sodium dodecyl sulfate at 68°C for 3 hr. Hybridized filters were exposed to Kodak XAR-1 film at −70°C in the presence of intensifying screens for 9 hr to 3 days.

RESULTS

Murine α-Spectrin. In *Hin*dIII digests of mouse cellular DNA, the mouse α-spectrin cDNA insert from pMαSp1 detected bands of 5.0 and 2.5 kbp; in *Hin*dIII digests of Chinese hamster DNA, bands of 10.0 and 2.6 kbp were detected. Thus, this probe was used to test for the presence or absence of the mouse α-spectrin gene in *Hin*dIII digests of cellular DNA from a panel of well-characterized Chinese hamster–mouse hybrids (17–20) by Southern blot (27) analysis. Results of these hybridizations are shown in Table 1 and demonstrate cosegregation of mouse chromosome 1 with the mouse α-spectrin gene. Bodine *et al.* (3) have concluded from analyses of mutant erythrocyte membranes that the mouse *sph* locus is the structural locus for the mouse α-spectrin gene, which they have localized by linkage studies distal to the *Pep-3* locus on mouse chromosome 1. Our localization of the mouse α-spectrin gene to chromosome 1 by cDNA hybridization is fully consistent with the conclusions of Bodine *et al.* (3). In fact, the one Chinese hamster–mouse hybrid that does not contain an intact mouse chromosome 1 (see the footnote in Table 1) carries a noncentromeric fragment of chromosome 1 and two mouse chromosome 1 markers, *Bxv-1* and *Pep-3* (20), and is positive for mouse α-spectrin.

Table 1. Correlation of presence of α-spectrin gene and specific mouse chromosomes in 16 Chinese hamster–mouse hybrids

Mouse chromosomes	Number of hybrid clones with α-spectrin/chromosome retention +/+	−/−	+/−	−/+	% discordant
1	7	8	1*	0	6*
2	4	5	4	3	44
3	5	5	3	3	38
4	4	7	4	1	31
5	1	7	7	1	50
6	5	6	3	2	31
7	5	3	3	5	50
8	4	8	4	0	25
9	3	7	5	1	38
10	3	8	5	0	31
11	0	8	8	0	50
12	4	3	4	5	56
13	3	5	5	3	50
14	2	7	6	1	44
15	7	2	1	6	44
16	5	6	3	2	31
17	7	5	1	3	25
18	6	6	2	2	25
19	5	5	3	3	38
X	6	6	2	2	25

DNA (10 μg) from 16 Chinese hamster–mouse hybrids (17–19) was digested with a 5-fold excess of restriction endonuclease *Hin*dIII, subjected to electrophoresis, and blotted. Filters were hybridized with nick-translated ^{32}P-labeled mouse α-spectrin cDNA probe as described.

*Present as a translocation (chromosome fragment lacking centromere) but containing the mouse *Pep-3* isozyme and *Bxv-1* markers for mouse chromosome 1 (20).

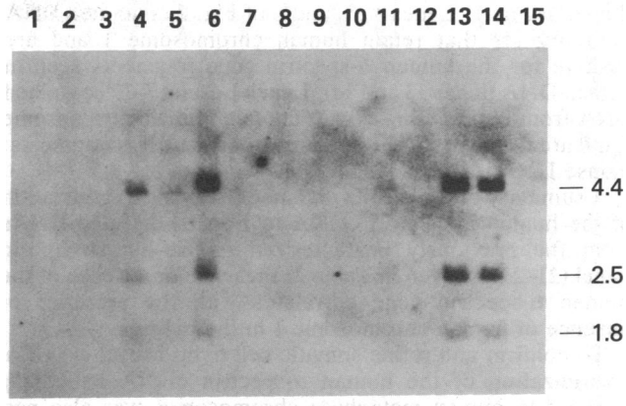


FIG. 1. Hybridization of human α-spectrin probe to DNA from mouse–human hybrids. *Hin*dIII-digested DNA was from 53-87-1 c1 21 (lane 1), Nu9 (lane 2), 77B10 c1 30 (lane 3), 77B10 c1 31 (lane 4), 77B10 c1 33 (lane 5), 77B10 c1 5 (lane 6), DSK 1B2A5 c1 20 (lane 7), DSK 1B2A5 c1 2 suba (lane 8), GM × LM c1 6b (lane 9), GM × LM c1 5 (lane 10), GM × LM c1 3a (lane 11), PAF × BALB IV c1 5 (lane 12), human peripheral blood lymphocytes (lane 13), human peripheral blood lymphocytes from another donor (lane 14), and F9 mouse teratocarcinoma cell line (lane 15).

Human α-Spectrin. In *Hin*dIII digests of human DNA, three prominent bands of 4.4, 2.5, and 1.8 kbp were detected by the human α-spectrin cDNA insert from pHαSp6, while the mouse α-spectrin gene was not detected under the hybridization conditions used. An example of a Southern blot using this probe on various mouse–human hybrid DNAs is

Table 2. Correlation of presence of α-spectrin gene and specific human chromosomes in 20 mouse–human hybrids

Human chromosomes	Number of hybrid clones with α-spectrin/chromosome retention +/+	−/−	+/−	−/+	% discordant
1	5	15	0	0	0
2	0	14	5	1	30
3	3	10	2	5	35
4	0	13	5	2	35
5	4	11	1	4	30
6	1	12	4	3	35
7	0	9	5	6	55
8	4	9	1	6	35
9	4	10	1	5	30
10	5	13	0	2	10
11	1	12	4	3	35
12	1	10	4	5	45
13	4	13	1	2	15
14	5	5	0	10	50
15	0	13	5	2	35
16	2	12	3	3	30
17	0	8	5	7	60
18	4	12	1	3	20
19	0	13	5	2	35
20	4	10	1	5	30
21	0	11	5	4	45
22	2	7	3	8	55
X	4	10	1	5	30

DNA from a panel of hybrid cells that has been characterized for the presence of specific human chromosomes by isozyme analysis and in some cases by karyotypic analysis and DNA·DNA hybridization (using DNA probes for genes that have been mapped to specific chromosomes) was analyzed for the presence of the human α-spectrin gene.

shown in Fig. 1. Lanes 4, 5, and 6 of Fig. 1 contained DNA from hybrids that retain human chromosome 1 and are positive for the human α-spectrin gene fragments seen in human DNA (lanes 13 and 14). Lanes 1–3 and 7–12 contained DNA from hybrids that do not contain human chromosome 1 and are negative for the human α-spectrin DNA fragments; mouse DNA is in lane 15.

A summary of the results obtained following hybridization of the human α-spectrin cDNA to *Hin*dIII-digested DNAs from the previously characterized mouse–human hybrid panel (21–23) is given in Table 2. Presence or absence of the human α-spectrin gene correlates with the presence or absence of human chromosome 1 in the hybrids.

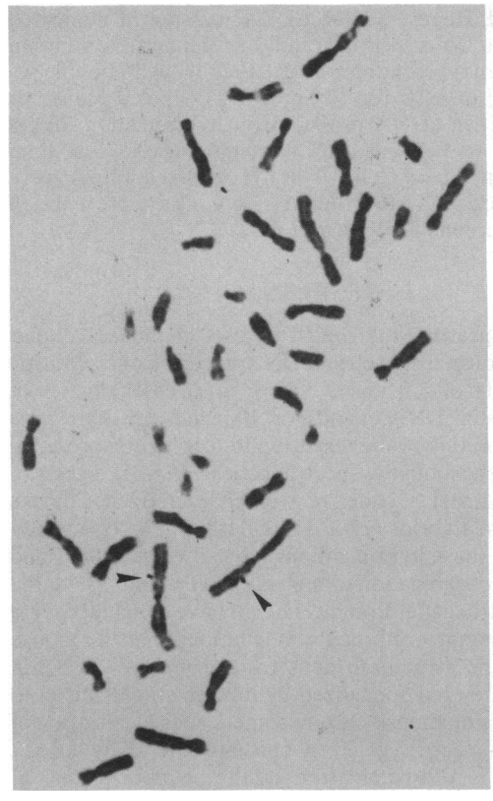
To confirm and refine somatic cell hybrid results, *in situ* hybridization of the human α-spectrin cDNA containing plasmid to human metaphase chromosomes was also performed; results of this analysis are shown in Fig. 2. After autoradiography, the metaphase spreads were analyzed for grain localization. An example of a spread is shown in Fig. 2 *Upper*. Over 22% of all grains were located on the long arm of chromosome 1. Over 97% of the 1q grains were between 1q22 and 1q25, with most grains at 1q24. A histogram depicting the silver grain distribution along the human chromosomes is shown in Fig. 2 *Lower*. The long arm of chromosome 1 represents approximately 4.59% of the haploid genome, and our observation that more than 22% of the human α-spectrin probe hybridization was localized to the proximal half of this region is highly significant ($P < 0.01$). Thus, cytological hybridization localizes the human α-spectrin gene to the q2 region of chromosome 1.

DISCUSSION

The localization of the erythrocyte α-spectrin gene to chromosome 1 in mouse and man extends the homology of part of mouse chromosome 1 with part of human chromosome 1; the structural gene(s) for renin is on chromosome 1 in man and mouse (28, 29) and the gene for homologous forms of peptidase (mouse *Pep-3* and human *PEPC* is on chromosome 1 in both species (30–32). The order of the three murine genes on chromosome 1, with respect to the centromere, is: structural gene for renin, *Pep-3*, and gene for α-spectrin; the order of the three genes on human chromosome 1 is not yet precisely known.

In preliminary studies using DNA from normal donors and two donors with hereditary spherocytosis, we have not observed α-spectrin restriction fragment length polymorphisms using several restriction enzymes; nor have we detected more than one locus for α-spectrin in the human or mouse genome (see also ref. 16). Further study will be required to determine if the α-spectrin-like molecules found in nonerythroid tissues (33) are products of the same α-spectrin locus detected by this human α-spectrin cDNA clone and whether this locus carries one or more genes for α-spectrin.

The localization of the human α-spectrin gene to 1q22–1q25 has certain implications with respect to the underlying cause of some hereditary anemias. The gene for one form of hereditary ellipsocytosis, *Rh*-linked hereditary ellipsocytosis, has been mapped by linkage to the *Rh* locus to the short arm of chromosome 1 (34), and recently another ellipsocytosis locus (non-*Rh*-linked) has been provisionally assigned to 1q2 (35) by linkage to the Duffy (*Fy*) blood group. Thus, this non-*Rh*-linked form of hereditary ellipsocytosis, whose gene maps in the same region of chromosome 1 as does human α-spectrin gene, could conceivably be due to an α-spectrin defect. On the other hand, genes of some forms of human hereditary spherocytosis have been assigned provisionally to chromosomes other than 1. In linkage studies of families in which hereditary spherocytosis was segregating



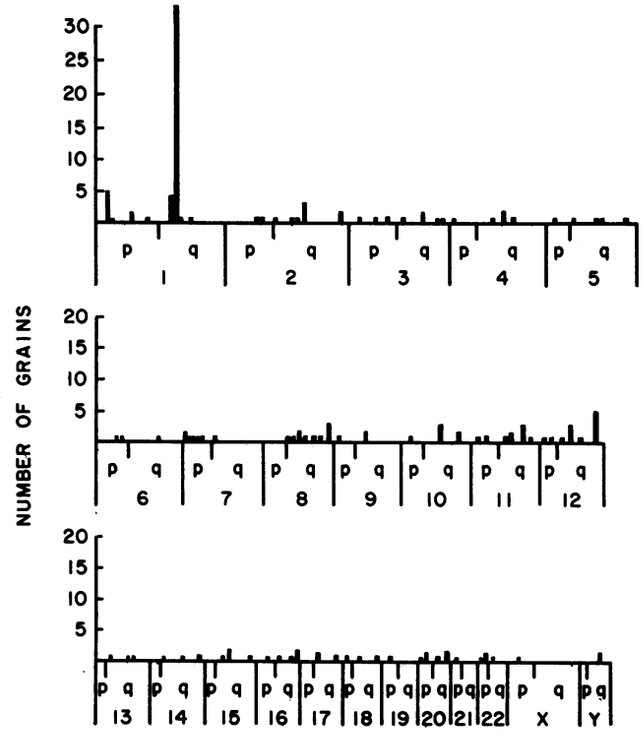


FIG. 2. Localization of α-spectrin gene in the human genome by *in situ* hybridization analysis. (*Upper*) Photograph of a G-banded lymphocyte metaphase spread hybridized with the human α-spectrin probe. Arrowheads indicate grains found in the proximal portion of chromosome 1q. (*Lower*) Diagram showing the grain distribution of 80 metaphases. The abscissa represents the chromosomes in their relative size proportion; the ordinate shows the number of silver grains. The distribution of 159 grains on 80 spreads was scored; 35 were found over 1q22–>1q25.

with translocated chromosomes (t8:12, t3:8) (36, 37), the gene for one form of hereditary spherocytosis has been mapped to 8p11 (36, 37). The gene for another type of hereditary spherocytosis, the Denver type, has been provisionally mapped to 14q by linkage studies (38). Thus, assuming these chromosome assignments are correct, neither of the two types of hereditary spherocytosis whose genes have been mapped in the human could be due primarily to a defect in the α-spectrin gene, whereas the recessively inherited murine spherocytosis is clearly due to a defect in α-spectrin (3). It should now be possible, in the murine form of hereditary spherocytosis (sph) and in the α-spectrin-deficient human anemias, to define by fine mapping of α-spectrin genomic clones, the genetic defect that causes these diseases.

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