Am. J. Hum. Genet. 47:62–72, 1990

Cloning and Chromosomal Localization of the Human Cytoskeletal α-Actinin Gene Reveals Linkage to the β-Spectrin Gene

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Summary

We report the cloning and characterization of a full-length cDNA encoding the human cytoskeletal isoform of α-actinin (αA), a ubiquitous actin-binding protein that shares structural homology with spectrin and dystrophin. The gene encodes 891 amino acids with 96%–98% sequence identity at the amino acid level to chicken nonskeletal muscle αA. Transient expression in COS cells produces a protein of ~104 kD that comigrates on SDS-PAGE with native αA. This αA gene is localized to chromosome 14q22-q24 by somatic cell hybrid and in situ hybridization analyses. Pulsed-field gel analysis of human genomic DNA revealed identically sized fragments when cDNA probes for αA and erythroid β-spectrin were used; the latter gene has been previously localized to chromosome 14, band q22. These observations indicate that the genes for cytoskeletal αA and β-spectrin are, in all likelihood, closely physically linked and that, in accordance with their similar structural features, they arose by partial duplication of an ancestral gene.

Introduction

Alpha-actinin (αA) was initially isolated from rabbit skeletal muscle as a factor that induces the gelation of F-actin and promotes the superprecipitation of actomyosin (Ebashi et al. 1965). Subsequently, a number of different isoforms have been isolated from both muscle and nonmuscle cells (Burridge and Feramisco 1981; Endo and Masaki 1982; Bennett et al. 1984; Duhaiman and Bamburg 1984; Landon et al. 1985) and from a wide variety of organisms (Pollard 1981; Condeelis and Vahey 1982; Mabuchi et al. 1985). The native molecule is thought to consist of a homodimer of two 97-kD subunits arranged in antiparallel fashion (Suzuki et al. 1976; Singh et al. 1977). In myofibrillar cells, αA constitutes a major component of Z-disks in striated muscle and of the functionally analogous dense bodies and dense plaques in smooth muscle. In nonmuscle cells, it is distributed along microfilament bundles and is thought to mediate their attachment to the membrane at adherens-type junctions (for review, see Buck and Horwitz 1987). Muscle αA and nonmuscle αA are also distinguished by their sensitivity to Ca^{2+}. Unlike the myofibrillar form, cytoskeletal αA is inhibited by micromolar concentrations of Ca^{2+} in its activity to bind actin or induce gelation (Burridge and Feramisco 1981; Bennett et al. 1984). The smooth-muscle form also does not appear to be sensitive to Ca^{2+} (Duhaiman and Bamburg 1984).

Identification of αA cDNA clones from *Dictyostelium discoideum* and from chicken skeletal muscle, smooth muscle, and fibroblast has been reported recently (Baron et al. 1987*a*, 1987*b*; Noegel et al. 1987; Arimura et al. 1988). Although some of these clones were partial sequences, comparison of the predicted amino acid sequence reveals a relatively high degree of similarity between *Dictyostelium* and chicken sequences and suggests the presence of three structural domains: (1) an N-terminal domain (amino acid residues 1–243), (2) a central motif of four repeats (amino acid residues 244–711), and (3) a C-terminal domain (amino acid residues 712–891). The N-terminal domain been shown to bind actin (Imamura et al. 1988) and has sequence

Received November 30, 1989; revision received February 14, 1990.

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0002-9297/90/4701-0009$02.00

similarity to putative actin-binding domains found in dystrophin (Hammonds 1987), β-spectrin (Byers et al. 1989), and actin-binding protein (filamin) (Gorlin et al. 1989). The middle domain exhibits more limited similarity to the repeats found in spectrin (Davison and Critchley 1988) and appears to be responsible for dimerization of the protein (Mimura and Asano 1987; Imamura et al. 1988). The C-terminal domain contains EF-hand-like Ca^{2+}-binding domains and presumably influences the actin-binding ability of the adjacent N-terminal domain in the native antiparallel dimer. In chicken, the skeletal muscle isoform of αA has only 80% sequence identity with nonmuscle αA and is most likely encoded by a separate gene from the smooth-muscle or cytoskeletal forms (Arimura et al. 1988). The genetic organization of the smooth-muscle and cytoskeletal isoforms, on the other hand, is less clear. Initial Southern blot analysis of chicken genomic DNA revealed a relatively complex pattern of hybridization with chicken fibroblast cDNA (Baron et al. 1987*a*), suggesting the existence of a gene family as a possibility, but later studies were more consistent with the view that the fibroblast form is also encoded by a single gene (Baron et al. 1987*b;* Arimura et al. 1988).

We have been interested in the genetic mechanisms that generate different tissue isoforms of αA, the molecular basis for the varying Ca^{2+} sensitivity of the different αA isoforms, and the genetic relationship between αA and related proteins. We report here the isolation and characterization of a full-length cDNA for αA from human endothelial cells.

Material and Methods

Materials

Oligonucleotides, including 5′-AAGATGACCCTGGGCATGATCTGGACC (HA-5) and 5′-ATCATCCTGCGGTTTGCCATCCAGGACATC (HA-6) were synthesized on an Applied Biosystems DNA synthesizer and purified by electrophoresis on 15% polyacrylamide gels. A monoclonal antibody raised against chicken gizzard αA was the gift of I. Virtanen (Narvanen et al. 1987). The human erythroid spectrin cDNA clone, β28 (Winkelmann et al. 1988), was provided by B. Forget.

Isolation of cDNA Clones

A human endothelial cDNA library cloned in lambda gt11 (Ginsburg et al. 1985) was screened with the synthetic oligonucleotide probes HA-5 and HA-6. Labeling of probes, hybridization conditions, and phage DNA preparation were all performed according standard methods (Maniatis et al. 1982). Filters were hybridized at 55°C and washed at 50°C.

DNA Sequencing

Phage inserts were subcloned into the *Eco*RI site of the Bluescript vector (Stratagene), and nested deletions were generated by progressive digestion with exonuclease III (Henikoff 1984). Sequencing of plasmid and M13 DNAs was performed using Sequenase (U.S. Biochemical). All sequence data shown were determined on both strands. Sequence data was analyzed using the Genetics Computer Group Sequence Analysis Software Package (Devereux et al. 1984).

Nucleic Acid Analysis

Southern blots were hybridized with uniformly ^{32}P-labeled probes (Feinberg and Vogelstein 1983). Agarose gels were also directly hybridized with end-labeled oligonucleotide probes (3×10^6 cpm/ml), after denaturation, neutralization, and drying of the gels (Youssoufian et al. 1986). Hybridization was performed for 4 h at 60°C. The strips were washed in 6 × SSC/0.05% Na pyrophosphate at 4° C for 15 min and at 22°C for 45 min, followed by a final wash for 2 min at 65°C–70°C. Pulsed-field gel electrophoresis was performed at a pulse of 45–60 s and 170–240 V for 16–24 h, according to a method described elsewhere (Cutting et al. 1988). Conditions for DNA transfer and hybridization were identical to those used for standard Southern analysis.

COS-1 Cell Expression

The isolated full-length αA cDNA clone was subcloned in the vector CDM8 (Seed 1987) and was expressed in COS-1 cells (Kwiatkowski et al. 1989). Cells were rinsed with PBS and were lysed with 1% Triton X-100 in the presence of 1 mM benzamidine, 1 mM leupeptin, and 2 mM phenylmethyl sulfonylfluoride (Kwiatkowski 1988). The polypeptide composition of cell lysates was resolved by SDS-PAGE (Laemmli 1970). Immunoblot analysis was performed using the anti-αA MAb, following transfer to Immobilon-P (Millipore, New Bedford, MA), according to a method described elsewhere (Kwiatkowski et al. 1989). For immunofluorescence, COS-1 cells were fixed in methanol at −20°C for 10 min, treated with anti-αA MAb in PBS/0.2% gelatin, rinsed, and stained with fluorescein-conjugated goat anti-mouse IgG (Cappel, Cochranville, PA).

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CCCGCCAGCCCAGCCCAGCCCAACCCTACTCCCTCCCCACGCCAGGGCAGCAGCCGTTGCTCAGAGAGAAGGTGGAGGAAGAAATCCAGACCCTAGCACGCGCGCCACCATCATGGACCAT  120
                                                                                                                    D H    2

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 Y D S Q Q T N D Y M Q P E E D W D R D L L L D P A W E K Q Q R K T F T A W C N S   42

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 K M R V H K I S N V N K A L D F I A S K G V K L V S I G A E E I V D G N V K M T   122

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 L G M I W T I I L R F A I Q D I S V E E T S A K E G L L L W C Q R K T A P Y K N   162

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 N L N T A F D V A E K Y L D I P K M L D A E D I V G T A R P D E K A I M T Y V S   242

AGCTTCTACCACGCCTTCTCTGGAGCCCAGAAGGCGGAGACAGCAGCCAATCGCATCTGCAAGGTGTTGGCCGTCAACCAGGAGAACGAGCAGCTTATGGAAGACTACGAGAAGCTGGCC  960
 S F Y H A F S G A Q K A E T A A N R I C K V L A V N Q E N E Q L M E D Y E K L A   282

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AAGCCGCCCAAGGTGCAGGAGAAGTGCCAGCTGGAGATCAACTTCAACACGCTGCAGACCAAGCTGCGGCTCAGCAACCGGCCTGCCTTCATGCCCTCTGAGGGCAGGATGGTCTCGGAC 1200
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TGGATGGAGGGGGCCATGGAGGACCTGCAGGACACCTTCATTGTGCACACCATTGAGGAGATCCAGGGACTGACCACAGCCCATGAGCAGTTCAAGGCCACCCTCCCTGATGCCGACAAG 1800
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GAGCGCCTGGCCATCCTGGGCATCCACAATGAGGTGTCCAAGATTGTCCAGACCTACCACGTCAATATGGCGGGCACCAACCCCTACACAACCATCACGCCTCAGGAGATCAATGGCAAA 1920
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 T A L Y G E S D L *                                                                                                    891

TTCTCCACTGAAAAATCCTCTTTCTTTGGGGTTTTTCTTTCTTTCTTTTTTGATTTTGCACTGGACGGTGACGTCAGCCTGTACAGGCTCCCAGGGGTGGCGTCAAATGCTATTGAAATT 3000

GCGCTGAATCGTATGCTTTTTCCTTTTGATAAATAAACAATGTAAAAATGTTTCAAAAACCTAATAAAATAAATAAATACGAAAAAAAAAAAAAAA                        3075
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Figure 1 Sequence of human cytoskeletal αA cDNA. The sequence of p5αA and the predicted translation product are shown (submitted to GenBank as M31300). Four polyadenylation signals (AATAAA), three of which are partially overlapping, and a 5′ 40-mer are underlined.

Chromosomal Localization

In situ hybridization to metaphase spreads of human chromosomes was performed according to a method described by Morton et al. (1984). By nick-translation the full-length αA cDNA was radiolabeled with all four ^{3}H-labeled deoxynucleotide triphosphates to a specific activity of 1.5×10^7 cpm/μg. Metaphase chromosomes were obtained from peripheral blood lymphocyte cultures established from karyotypically normal human males. For somatic cell hybrid analysis, DNA from human-rodent hybrid cells containing known complements of human chromosomes (Bruns et al. 1979;

Kwiatkowski and Bruns 1988) was analyzed by Southern blotting.

Results and Discussion

Sequence of Human Endothelial αA cDNA

Four hundred thousand clones of a human endothelial cDNA library were screened with two oligonucleotides, HA-5 and HA-6. The oligonucleotides were based on regions of identical sequence in the N-terminal domains of chicken αA and human dystrophin (Hammonds 1987), by using eukaryotic codon preference rules. Eight clones hybridizing with both probes were identified, and the one containing the largest (3.1-kb) insert (p5αA) was characterized in detail. Sequence analysis (fig. 1) showed 114 bp of 5′ untranslated sequence, followed by an initiator methionine codon that conforms to the Kozak (1984) rule (ATCATG), a long open-reading frame extending to the stop codon TAA at position 2788, and a 3′ untranslated sequence containing four polyadenylation signal sequences (AATAAA) and ending with a poly(A) tail. Because the initiator methionine is likely removed during posttranslational processing, this sequence predicts a mature protein of 891 amino acids and of molecular weight 102.9 kDa. Comparison of the human endothelial αA sequence with that of chicken smooth-muscle αA and fibroblast αA (Baron et al. 1987*b*; Arimura et al. 1988) shows 96% and 98% identity, respectively, of aligned amino acid residues (table 1). The identity is even higher (99%) in the N-terminal, actin-binding domain (amino acids residues 1–243) and is intermediate (97%) in the middle, tandem-repeat domain (amino acids residues 244–711). In the COOH-terminal domain (amino acids residues 712–891), there is a region of marked divergence between the chicken smooth-muscle isoform (Baron et al. 1987*a*, 1987*b*), on the one hand, and the chicken fibroblast isoform (Arimura et al. 1988) and human endothelial isoform, on the other hand. This region contains the first EF-hand sequence (Kretsinger 1980) present in the COOH-terminal domain. This observation suggests that alternative splicing occurs in this region, to generate two αA isoforms that may exhibit different Ca^{2+} sensitivity.

Expression of Human Endothelial αA

To further establish that the clone encoded human αA, the p5αA insert was subcloned into the expression vector CDM8 (Seed 1987), and the encoded protein was expressed in COS-1 cells. Coomassie blue–stained SDS-PAGE gels of transfected and control cell lysates suggested the presence of increased amounts of a protein of size ~104 kDa in the transfected cells (fig. 2*A*). Parallel immunoblot analysis using an anti-αA MAb confirmed that increased amounts of αA were expressed in the transfected cells. Examination of the transfected cells by immunofluorescence using the same MAb indicated that ~25% had taken up and expressed the applied DNA (fig. 2*B*). The positive cells were much brighter by immunofluorescence, but their morphology was similar to that of adjacent untransfected cells. Prominent membrane localization of αA was noted in some regions of the transfected cells. We estimate from the immunoblot and immunofluorescence data that a 20–50-fold increase in expression of αA occurred in transfected cells. In transient-expression experiments, it is difficult to draw firm conclusions regarding the effects that overexpression has on cell morphology.

Table 1

Conservation of Amino Acid–Residue Sequences between Human and Chicken αA

Amino Acid–Residue Sequence,[a] Region	Amino Acid Identity[b] (%)	
	Smooth Muscle[c]	fibroblast[d]
1–243, Actin-binding domain	240/243 (99)	207/208 (99.5)
244–711, Central repeats	454/467 (97)	454/467 (97)
712–891, EF-hand region	158/180 (88)	174/180 (97)
749–777, EF-hand 1	19/29 (66)	29/29 (100)
778–789, Spacer	5/12 (42)	11/12 (92)
790–818, EF-hand 2	28/29 (97)	28/29 (97)

[a] Numbering refers to the mature human cytoskeletal αA.

[b] Lack of identity is scored for both mismatches and insertions/deletions.

[c] Source: Baron et al. (1987*b*).

[d] Source: Arimura et al. (1988). Because the chicken fibroblast sequence is incomplete, the comparison begins at amino acid residue 36 of human cytoskeletal αA.

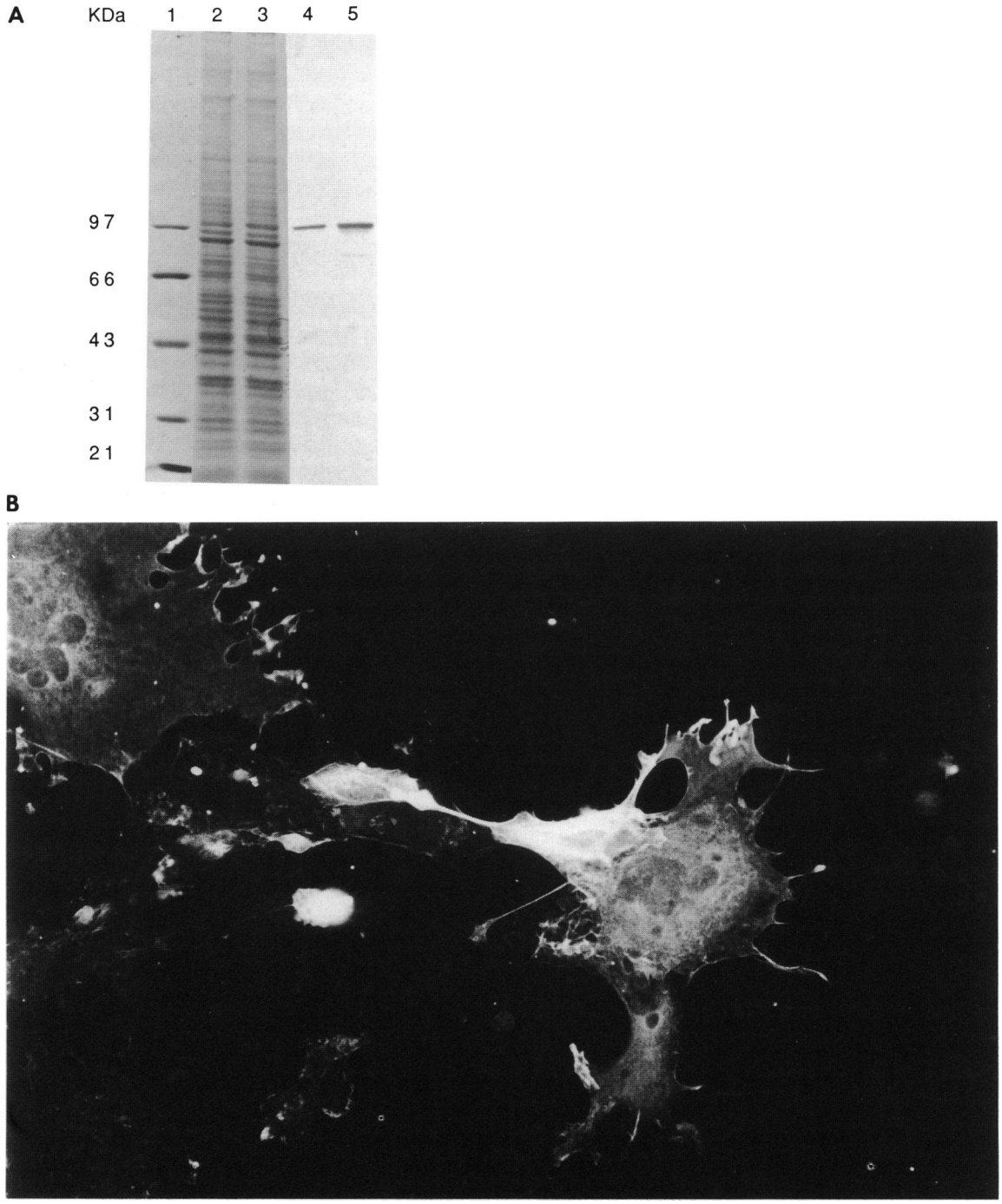

Figure 2 Expression of human cytoskeletal αA in COS-1 cells. *A*, Coomassie blue–stained 5%–15% polyacrylamide gel (lanes 2 and 3) and parallel immunoblot using anti-αA MAb (lanes 4 and 5) of control COS-1 cell lysates (lanes 2 and 4) and COS-1 cells transfected with CDM8/p5αA (lanes 3 and 5). Molecular-weight standards (lane 1) are indicated on the left of the figure. KDa = kilodaltons. *B*, Immunofluorescence photomicrograph of transfected cos cells, when the anti-αA MAb is used. In this are shown several cells, two of which have taken up and expressed the applied DNA. Membrane staining is particularly notable. Cells not expressing the transfected DNA are only faintly visible but have similar morphology.

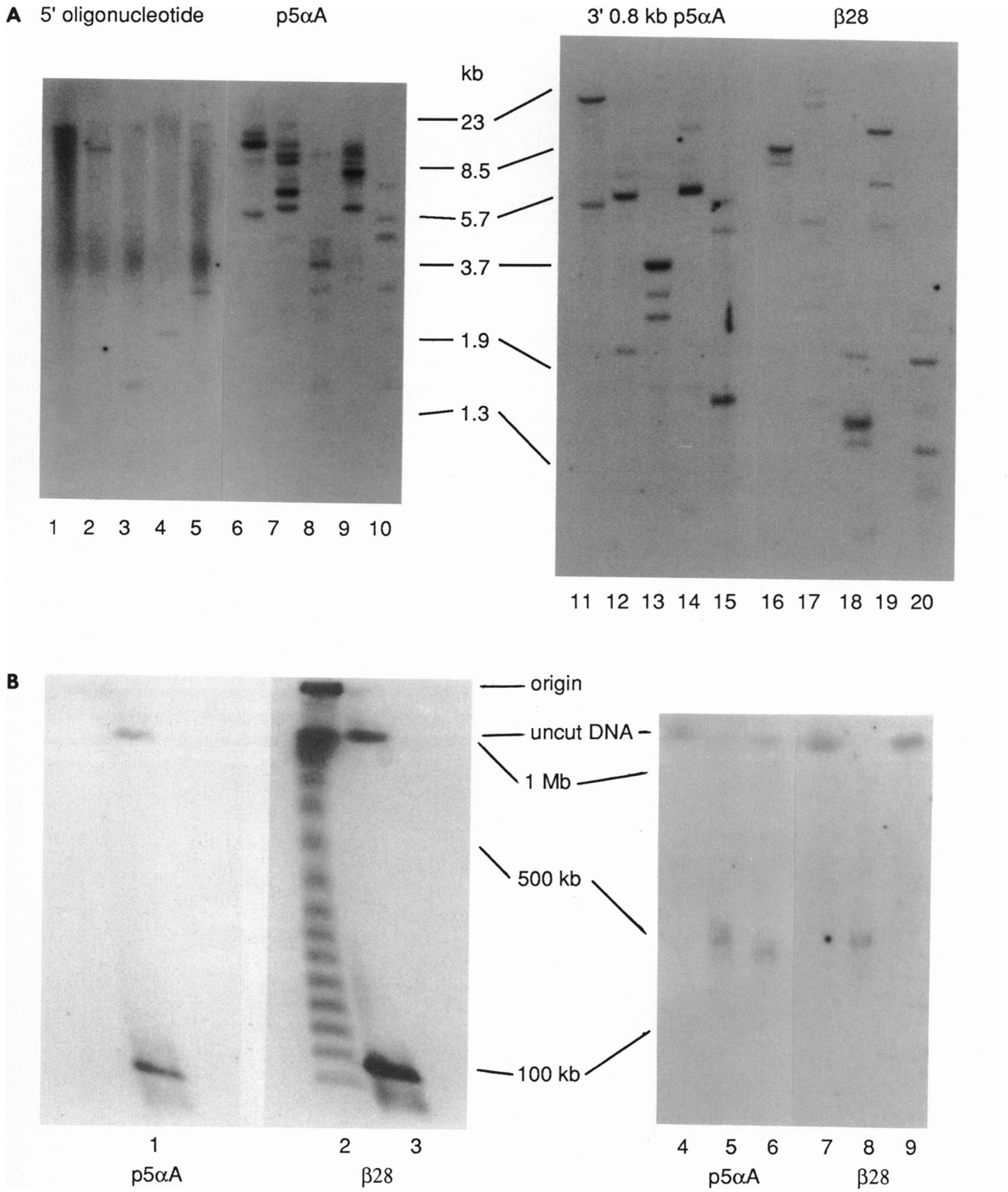

Figure 3 Southern blot analysis of human genomic DNA when αA and β-spectrin gene probes are used. *A,* Human genomic DNA digested with *Eco*RI (lanes 1, 6, 11, and 16), *Hin*dIII (lanes 2, 7, 12, and 17), *Pst*I (lanes, 3, 8, 13, and 18). *Bam*HI (lanes 4, 9, 14, and 19), or *Pvu*II (lanes 5, 10, 15, and 20). After electrophoresis, identical gel strips were used for either standard Southern blotting and hybridization with p5αA (lanes 6–10) or gel drying and in situ hybridization with an αA-specific oligonucleotide (lanes 1–5) encompassing bases 75–114 of p5αA (see fig. 1). Single hybridizing bands are seen in lanes 1–5. A pair of filters derived from a second gel were probed with the 3′ 0.8-kb fragment of p5αA (lanes 11–15) and with the β-spectrin β28 probe (lanes 16–20). The hybridizing bands seen in lanes 11–15 are a fraction of those seen in lanes 6–10, while those seen in lanes 16–20 are clearly distinct from those seen in lanes 6–10. Size markers are indicated. *B, left,* Sequential autoradiographs are of a Southern blot of pulsed-field gel-separated human genomic DNA digested with *Sfi*I and hybridized with the p5αA probe (lane 1) or β28 probe (lane 3), as well as with 0.1 ng of labeled lambda DNA (lane 2). The origin, the site of migration of uncut DNA, and a ladder of lambda multimers are indicated. Between the two rounds of hybridization, the filter was stripped in 50% formamide/1 × SSC for 1 h at 65°C and absence of the original signal was confirmed by autoradiography. The α-actinin hybridizing and β-spectrin hybridizing species comigrate. *B, right,* Parallel pulsed-field gel Southern blots of human DNA digested with *Mlu*I (lanes 4 and 7), *Sac*II (lanes 5 and 8), and *Sal*I (lanes 6 and 9). Lanes 4–6 were probed with p5αA; lanes 7–9 were probed with β28. The cognate bands for *Mlu*I (>1 Mbp) and *Sac*II (~500 kb) comigrate.

Genomic Organization of Human Cytoskeletal αA

Southern blot analysis of human genomic DNA by the p5αA insert showed a complex pattern of hybridization (fig. 3*A*, lanes 6–10), which did not change with increasingly stringent hybridization or washing conditions. Hybridization of an identical gel with a synthetic oligonucleotide derived from the 5′ untranslated region showed a single band with several different enzymes (fig. 3*A*, lanes 1–5). Similarly, hybridization with a 3′ 0.8-kb probe also showed a relatively simple pattern (fig. 3*A*, lanes 11–16). Southern blot analysis of pulsed-field gel-separated *Sfi*I-digested, high-molecular-weight human genomic DNA by using the p5αA insert as probe showed a single ~100-kb band (fig. 3*B*). These data indicate that the gene encoding human cytoskeletal αA most likely exists in a single copy per haploid genome.

To establish the chromosomal location of the αA gene, a somatic cell hybrid panel was examined by Southern blot analysis using the 3′ 0.8-kb fragment of p5αA. A representative autoradiograph is shown in figure 4, demonstrating concordant segregation among the hybrids of the two reactive bands. Complete analysis using a previously established somatic cell hybrid panel (Bruns et al. 1979) indicated exclusive segregation of the αA gene with human chromosome 14 (table 2). To determine the regional localization of the gene on chromosome 14, chromosomal in situ hybridization was performed, using the p5αA probe, to metaphase spreads of human chromosomes. A single peak of hybridization was observed to chromosome 14, in bands q22-q24 (fig. 5). Analysis of 105 metaphase preparations from a normal male indicated that 15% of spreads had silver-grain deposition at bands q22-q24 of at least one chromosome 14. The sixteen grains that were deposited over this region constituted 8% of the 204 total grains observed and 52% of the 31 grains located on or beside chromosome 14.

Previously, the gene for erythroid β-spectrin had also been mapped to chromosome 14 bands q22-q24 (Prchal et al. 1987; Winkelmann et al. 1988). Therefore, we sought to test the possibility of physical linkage between the genes for cytoskeletal αA and β-spectrin. After stripping of the p5αA probe (confirmed by autoradiography), the above pulse-field blot was rehybridized to a 2.7-kb cDNA clone from the C-terminal half of human erythroid β-spectrin cDNA, β28 (Winkelmann et al. 1988). The hybridization pattern seen with the β28 probe was identical to that seen with the αA probe (compare fig. 3*B left* and fig. 3 *right*). To confirm that this did not occur because of cross-hybridization of the probes, standard Southern blot analysis using the same β28 probe was performed (fig. 3*A*, lanes 16–20). This demonstrated that the αA and β28 probes recognized distinct genomic DNA fragments. A second pair of par-

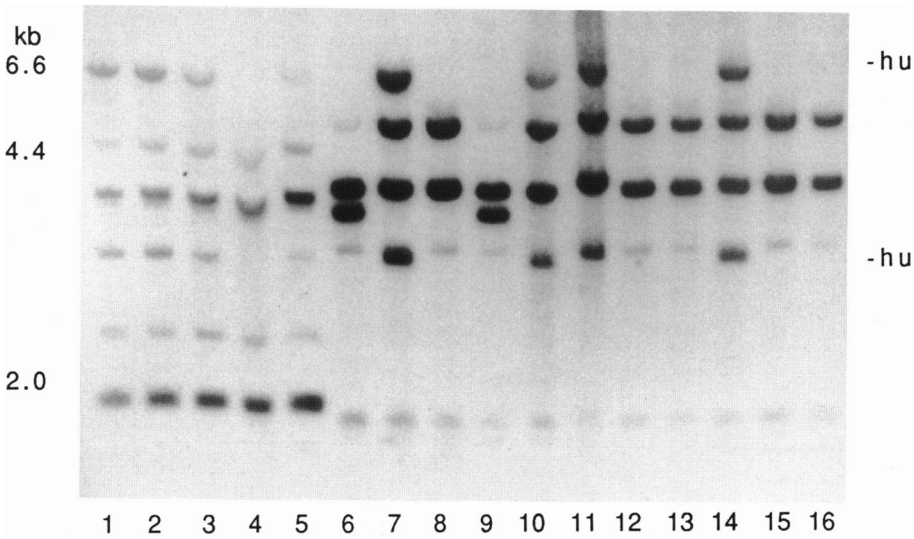

Figure 4 Southern blot analysis of somatic cell hybrid DNA when the 3′ 0.8-kb αA probe is used. A representative autoradiograph is shown. Ten micrograms of DNA digested with *Sac*I was loaded in each lane. Lane 1, Mouse parent DNA. Lanes 2–5, Human-mouse hybrid DNA. Lanes 6–16, Human-hamster hybrid DNA. The human bands at 6.5 and 3.0 kb (confirmed by separate Southern blot of human DNA [not shown]) segregate concordantly among the hybrids and are designated hu. An RFLP of a 5-kb hamster band is notable in lanes 6 and 9.

allel pulsed-field gel blots of human DNA were also hybridized with the αA and β28 probes (fig. 3*B*). These studies demonstrated that identical bands of size ~500 kb were recognized by the probes in *Sac*II digests, whereas distinct bands occurred in *Sal*I digests, and in *Mlu*I digests for both probes the bands had size >1 Mbp. Although definitive proof will require isolation of overlapping cosmid (or other) clones containing the two genes, these data suggest that the human αA and β-spectrin genes are syntenic and closely physically linked.

Erythroid spectrin is a major structural protein of the erythrocyte cytoskeleton, occurs as a heterodimer of α (size 250 kDa) and β (size 225 kDa) subunits, and assembles into tetramers and higher-order structures. The spectrin tetramer is an elongated molecule, the distal ends of which are thought to be responsible for its ability to bind to and cross-link F-actin into the two-dimensional array that occurs beneath the membrane of red blood cells (Cohen et al. 1980; Glenney et al. 1982). Erythroid spectrin is closely related to nonerythroid spectrins (fodrins), which have been found in many eukaryotic cells. Structurally, β-spectrin consists of multiple repeats of length 106 amino acids (Speicher and Marchesi 1984; Prchal et al. 1987; Winkelmann et al. 1988). Recently, determination of the primary sequence of *Drosophila* β-spectrin led to the recognition of an N-terminal sequence in the protein which is similar to the N-terminal actin-binding domains of αA and dystrophin (Byers et al. 1989). The 18 106-residue internal repeats of β-spectrin also share weak structural homology with the four central 113–121-residue repeats of αA (Winkelmann et al. 1988; Byers et al. 1989; Blanchard et al., in press). On the basis of these observations, αA, α- and β-spectrins, and dystrophin have been grouped into a protein superfamily. Members of this superfamily share (*a*) N-terminal actin-binding domains (αA, β-spectrins, and dystrophin); (*b*) central repeat segments of length 100–125 residues (αA, α- and β-spectrins, and dystrophin); and (*c*) COOH-terminal EF-hand Ca^{2+} binding domains (αA and α-spectrins) but are markedly different in total size (αA = 103 kDa; spectrin subunits = 225–270 kDa; dystrophin monomer = 480 kDa) and tissue distribution. The physical linkage of the genes for human cytoskeletal αA and β-spectrin suggests that a single ancestral gene may have generated the two genes by an intrachromosomal event. The most likely mechanism to account for this occurrence would be partial gene duplication, followed by amplification, with αA being the ancestral gene. This evolutionary sequence has been postulated by Dubreuil et al. (1989), on the basis of detailed sequence comparison between *Drosophila* α- and β-spectrins and αA. The physical linkage of these two genes in humans strengthens this hypothesis.

Our analysis of human endothelial αA also reveals remarkable identity with the chicken fibroblast sequence. It has been reasoned that, because actin is highly conserved in phylogeny, heterologous proteins that bind actin specifically also tend to show a high degree of conservation in their actin-binding domains (Blanchard

Table 2

Segregation Pattern of Human Cytoskeletal αA Gene in Human-Rodent Somatic Cell Hybrids

Chromosome[a]	Hybridization Pattern[b]				Discordant Fraction[c]
	+ / +	− / −	+ / −	− / +	
1	2	7	6	0	.40
2	3	5	4	1	.38
3	3	6	5	1	.40
4	4	5	3	0	.25
5	2	6	6	1	.47
6	5	6	3	0	.21
7	3	3	5	3	.57
8	4	5	4	2	.40
9	2	5	6	1	.50
10	4	4	4	2	.43
11	2	5	5	2	.50
12	3	5	4	1	.38
13	4	6	3	1	.29
14	8	7	0	0	.00
15	4	6	4	1	.33
16	3	5	5	2	.47
17	3	5	5	2	.47
18	6	6	2	1	.20
19 and 19q+[d]	8	3	0	4	.27
20	5	4	3	3	.40
21	6	5	2	1	.21
22	2	5	6	1	.50
X and Xq−[e]	1	4	7	3	.67
Y	0	7	8	0	.53

[a] Human chromosome complements of mouse-human or hamster-human hybrids.

[b] Column designations are as follows: + / + = hybridization signal and chromosome both present; − / − = hybridization and chromosome both absent; + / − = hybridization present but chromosome absent; and − / + = hybridization absent but chromosome present.

[c] Hybrids having a rearranged chromosome or those in which the chromosome was present in less than 15% of cells were excluded from the calculation of discordant fractions.

[d] For the 13 hybrids derived from fusions with white blood cells from two female carriers of different X;19 translocation chromosomes, this category represents the der(19) translocation chromosomes.

[e] Includes hybrids with the X or der(X) translocation chromosomes.

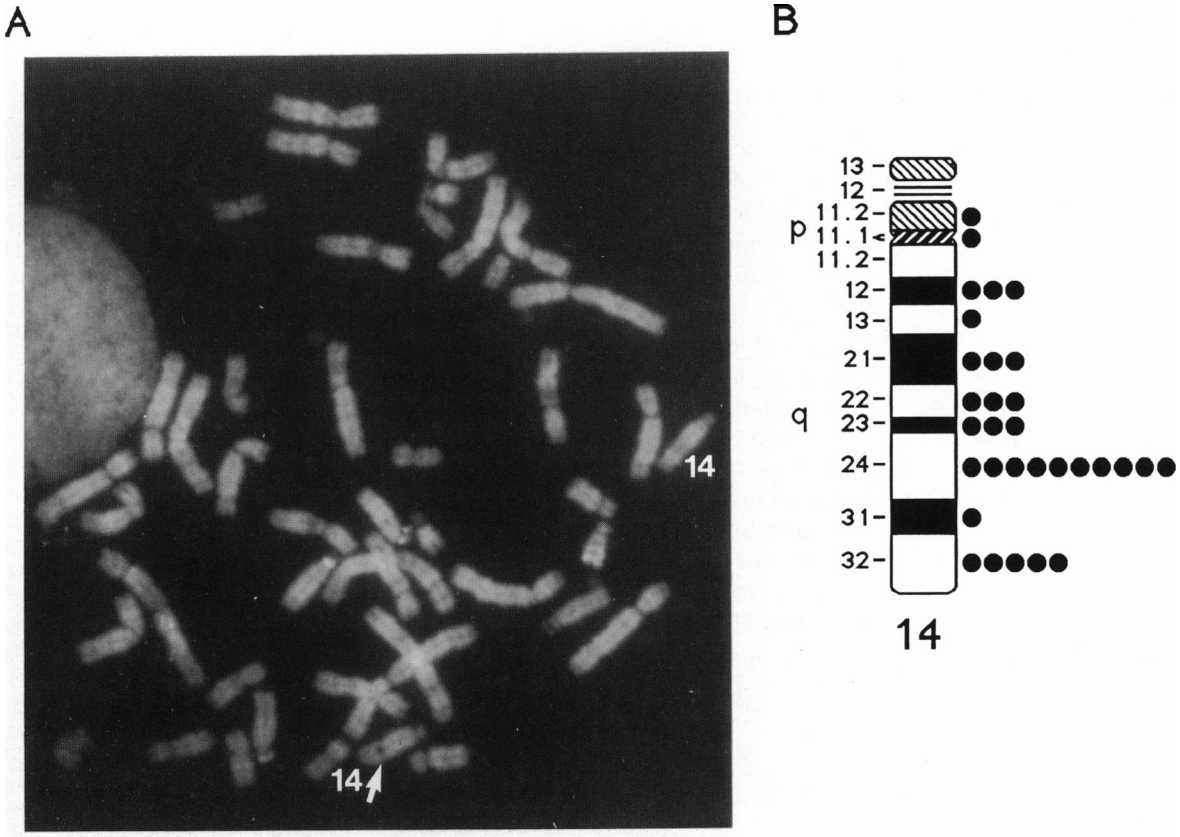

Figure 5 Chromosomal assignment of αA gene by in situ analysis. The photograph (*A*) shows αA hybridization to metaphase chromosomes from a normal male, and the ideogram of chromosome 14 (*B*) shows distribution of labeled sites. A representative metaphase spread is shown as visualized by incident UV and transmitted visible light. αA is assigned to 14q22-q24, and a silver grain is shown in 14q24. Each dot on the ideogram represents one labeled site observed in the corresponding band on chromosome 14.

et al. 1989). Comparison of the N-terminal globular head of αA with dystrophin (Davison and Critchley 1988) supports this notion. However, the extreme sequence conservation of cytoskeletal αA between chicken and human extends throughout the whole molecule. This observation suggests that other portions of αA must have important structural or functional properties. These include binding sites for vinculin (Wachsstock et al. 1987), lipids (Burn et al. 1985), Ca^{2+}, the fibronectin receptor (Otey et al. 1989), and possibly other currently unrecognized intracellular ligands.

Acknowledgments

We wish to acknowledge Thomas P. Stossel for helpful discussions; Cynthia C. Morton, in whose laboratory the in situ chromosomal hybridization was performed; David Critchley, Masaki Nishiyama, and Mehmet Ozturk for sharing unpublished sequence data; Garry Cutting, Anil Menon, and Lance Whaley for assistance with pulsed-field gel electrophoresis; and Gail A.P. Bruns for provision of somatic cell hybrid DNA samples and assistance with data interpretation. This work was funded by NIH Physician-Scientist Award HL02277 (to H.Y.), NIH grant HL01582 (to D.J.K.), a National Science Foundation Graduate Fellowship (to M.M.), and an American Cancer Society institutional grant (to D.J.K.).

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