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Structure, Chromosome Location, and Expression of the Human γ-Actin Gene: Differential Evolution, Location, and Expression of the Cytoskeletal β- and γ-Actin Genes

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The accumulation of the cytoskeletal β- and γ-actin mRNAs was determined in a variety of mouse tissues and organs. The β-isoform is always expressed in excess of the γ-isoform. However, the molar ratio of β- to γ-actin mRNA varies from 1.7 in kidney and testis to 12 in sarcomeric muscle to 114 in liver. We conclude that, whereas the cytoskeletal β- and γ-actins are truly coexpressed, their mRNA levels are subject to differential regulation between different cell types. The human γ-actin gene has been cloned and sequenced, and its chromosome location has been determined. The gene is located on human chromosome 17, unlike β-actin which is on chromosome 7. Thus, if these genes are also unlinked in the mouse, the coexpression of the β- and γ-actin genes in rodent tissues cannot be determined by gene linkage. Comparison of the human β- and γ-actin genes reveals that noncoding sequences in the 5′-flanking region and in intron III have been conserved since the duplication that gave rise to these two genes. In contrast, there are sequences in intron III and the 3′-untranslated region which are not present in the β-actin gene but are conserved between the human γ-actin and the *Xenopus borealis* type 1 actin genes. Such conserved noncoding sequences may contribute to the coexpression of β- and γ-actin or to the unique regulation and function of the γ-actin gene. Finally, we demonstrate that the human γ-actin gene is expressed after introduction into mouse L cells and C2 myoblasts and that, upon fusion of C2 cells to form myotubes, the human γ-actin gene is appropriately regulated.

Actin is one of the most highly conserved proteins in evolution (41). This conservation is likely due to its central role as the basic building block of cellular microfilaments which involves interactions between actin monomers and also specific interactions with a wide variety of actin-binding proteins (41). Mammalian nonmuscle cells synthesize two actins, denoted β and γ, which differ by only four conservative amino acid substitutions in the amino terminus of the 375-residue polypeptides (17, 53, 54). The two actins are presumed to be expressed in all mammalian nonmuscle cells in similar relative levels, although the weight of published data is not great. The most significant variation in the relative steady-state levels of β- and γ-actin has been detected in muscle rather than nonmuscle tissues. Vandekerckhove and Weber (57) found that mammalian nonmuscle cells in culture accumulate β- and γ-actin in the ratio 2:1 and in rat liver in the ratio 2.5:1. In contrast, rat aorta accumulated β-/γ-actin in the ratio 6:1, and in an avian brush border the ratio was 0.5:1 (57). In parallel, Otey et al. (39) have found that β-/γ-actin accumulates in ratios of 1.8:1 to 3.4:1 in rat brain, diaphragm, kidney, liver, lung, spleen, and thymus. Two tissues showing wider variation were rat testis (1:1) and heart (6:1). Thus, the data suggest that the relative expression of these two actin isoforms is subject to modulation in different cell types and the most extreme differences are found in muscle cells.

The coexpression but differential modulation of β- and γ-actin accumulations suggests that variation in the relative levels of these two proteins may be of physiological significance. However, there are several unanswered questions which may complicate interpretation of the data. First, it is unclear that the β- and γ-actin proteins measured in the above studies are the products of only two genes. The possible existence of other nonmuscle actins being included with either β- or γ-actin determinations cannot be excluded, particularly considering the demonstration of a third nonmuscle actin in chickens (5, 9) and the complex array observed in amphibians (52). Second, there are at least 16 γ-actin loci in the human genome (42), and it is unclear how many of these are functional genes. In the case of human β-actin, we have clearly demonstrated that only one functional gene exists amid a plethora of nonfunctional pseudogenes (37). Third, the mammalian β- and γ-actin genes are presumed to be the product of a gene duplication event during early amphibian evolution, but confirmation awaits characterization of a functional mammalian γ-actin gene (58). Finally, whether the differential accumulation of β- and γ-actin in different cell types is controlled at the level of transcript accumulation or at that of protein production and turnover is unknown.

To approach these problems, we have examined the accumulation of β- and γ-actin mRNAs in a variety of different mouse tissues, using actin isotype-specific cDNA probes. We demonstrate that there is strict coexpression of these two transcripts and that their relative accumulation varies over 2 orders of magnitude between different cell types. Second, we have cloned and sequenced the human γ-actin gene. By comparison with the human β-actin gene, we have identified regions unique to the γ-actin gene and conserved in evolution. Such regions are good candidates for domains which may confer unique functional properties on the γ-actin gene. We have similarly identified regions con-

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served in evolution and held in common between β- and γ-actin genes. These regions are likely to contribute functional properties common to the β- and γ-actin genes. Further, sequence analysis confirms the predicted evolutionary origin of these two genes by gene duplication. Third, we have determined the chromosome location of the γ-actin gene and demonstrate that the β- and γ-actin genes are located on separate chromosomes in humans.

MATERIALS AND METHODS

RNA and DNA isolation. Total RNA was prepared from mouse heart, skeletal muscle, and stomach by the phenol-chloroform method (40) and from nonmuscle organs by a modification of the guanidine-HCl method (10). Cytoplasmic RNA was isolated from C2 and Ltk$^-$ cells by LiCl-urea precipitation after cell lysis with 1% (vol/vol) Nonidet P-40 plus 0.5% (wt/vol) sodium deoxycholate (32). RNA was electrophoresed on 1% agarose gels containing 2.2 M formaldehyde and transferred to nitrocellulose (28). DNA was isolated from HeLa cells, digested with various restriction enzymes, size fractionated on 0.7% agarose gels, and transferred to nitrocellulose as previously described (14, 48).

Hybridizations. DNA fragments were radiolabeled by nick translation to a specific activity of 10^8 dpm/μg (44). The β- and γ-actin-specific cDNA probes, pHFβA-3'UT-HF and pHFγA-3'UT-*Hin*f, respectively (15), were nick translated in parallel to identical specific activities and hybridized to nitrocellulose filters in 6× SSC (1× SSC is 150 mM NaCl plus 15 mM sodium citrate, pH 7.0)–50 mM NaH_2PO_4 (pH 6.8)–5 × Denhardt solution (11)–10% (wt/vol) dextran sulfate at 60°C. These filters were washed in 2× SSC–0.1% sodium dodecyl sulfate (SDS) for 30 min at room temperature followed by three 30-min washes in 0.5× SSC–0.1% SDS at 50°C. The panels were autoradiographed in parallel, and relative expression of β- and γ-actin mRNA was quantitated by densitometry. The filters were stained with 0.04% (wt/vol) methylene blue to visualize the 28S and 18S rRNAs and to ensure that equal amounts of RNA were transferred to the filters (28).

All other hybridizations were performed in 4× SSC–50 mM NaH_2PO_4 (pH 6.8), 5× Denhardt solution (11)–10% (wt/vol) dextran sulfate at 65°C. These filters were washed for 30 min in 2× SSC–0.1% SDS at room temperature, followed by three 30-min washes in 0.5× SSC–0.1% SDS at 65°C.

Cloning of functional γ-actin gene. One milligram of HeLa DNA was restricted overnight with 1,600 U of *Bam*HI in 150 mM NaCl–10 mM Tris hydrochloride (pH 7.5)–10 mM $MgCl_2$–10 mM dithiothreitol at 37°C. The digestion products were size fractionated by agarose gel electrophoresis, and DNA fragments migrating within the size range 7.5 to 9.5 kilobases were isolated by electroelution (28). The genomic DNA was ligated to the λ EMBL 3A (16) arms that had been generated by *Bam*HI digestion. The ligation products were mixed with packaging extracts prepared from *Escherichia coli* BHB2688 and BHB2690 (Amersham Corp., Arlington Heights, Ill.). The packaging reaction mixture was diluted into 0.5 ml of 10 mM Tris hydrochloride (pH 7.4)–10 mM $MgSO_4$–0.01% gelatin and stored over chloroform at 4°C. A total of 10^5 PFU were used to infect *E. coli* LE392, and the resulting plaques were screened by in situ hybridization (4) with the γ-actin-specific probe pHFγA-3'UT-HX (15). Positive phage were plaque purified, phage DNA was prepared, and the human *Bam*HI inserts were cloned into the *Bam*HI site of pBR322 (28).

DNA sequencing. A majority of the DNA fragments prepared for sequence analysis were generated by Bal-31 digestion of plasmid pgHγNMA-1 carrying the human γ-actin gene. DNA fragments were cloned into M13 vectors mp10 and mp11 (31) and sequenced by the dideoxy chain terminator method (45). Sequence data were managed with the GEL program (IntelliGenetics, Inc., Mountain View, Calif.). Comparison of DNA sequence data was managed with the IFIND program (IntelliGenetics, Inc.) based on the Wilbur and Lipman algorithm (60).

Nuclease S1 mapping. A 618-base-pair (bp) *Bam*HI/*Pvu*II-generated fragment, which contains 474 bp of 5'-flanking sequence, exon 1, and 76 bp of intron I, was prepared from plasmid pgHγNMA-1. This fragment was ligated into the replicative form of M13 mp10 between the *Bam*HI and *Hin*cII sites. A 1-ng amount of M13 sequencing primer was annealed to 1 μg of single-stranded DNA in 10 μl of 20 mM Tris hydrochloride (pH 8.5)–10 mM $MgCl_2$ at 45°C for 3 h. The primer was extended on the M13 template in 50 μl of 10 mM Tris hydrochloride (pH 8.5)–10 mM dithiothreitol–20 μM dATP–20 μM dGTP–125 pmol of [α-^{32}P]dCTP–125 pmol of [α-^{32}P]dTTP (>400 Ci/mmol; Amersham Corp.) with *E. coli* DNA polymerase I (Klenow fragment) at 37°C for 30 min. The reaction products were extracted with phenol-chloroform and purified from unincorporated nucleotides by chromatography on a Sephadex G-50 column. The radiolabeled DNA products were restricted with *Eco*RI, and a single-stranded probe complementary to the M13 insert was isolated from a 5% acrylamide–urea gel (29). After denaturation at 85°C for 5 min, 10 μg of either total HeLa cell RNA or yeast tRNA was hybridized to 1/20 of the isolated probe in 30 μl of 80% formamide–0.5 M NaCl–40 mM piperazine-*N*,*N*'-bis(2-ethanesulfonic acid) (PIPES; pH 6.4) at either 52 or 57°C for 18 h. S1 nuclease digestion was performed by dilution of the hybridization mix into 200 μl of 0.28 M NaCl–50 mM sodium acetate (pH 4.6)–4.5 mM $ZnSO_4$–20 μg of denatured calf thymus DNA per ml containing between 250 and 500 U of nuclease S1 (Sigma Chemical Co., St. Louis, Mo.) for 10 min at 37°C. Carrier tRNA was added, and undigested nucleic acids were precipitated with isopropanol and analyzed on an 8% acrylamide-urea gel.

Cell hybrids and chromosome analysis. Somatic cell hybrids were constructed by fusing human fibroblasts or leukocytes from 13 unrelated individuals with four different mouse cell lines possessing selectable markers (22). The hybrid cells were maintained on a selective medium containing hypoxanthine-aminopterin-thymidine (46). Chromosome and enzyme marker-characterized hybrids were used for gene mapping.

Chromosomes, chromosome-specific enzyme markers, and hybridization of isolated DNA to a γ-actin-specific probe were all determined on the same cell passage. Trypsin-Giemsa was used to identify human chromosomes and specific regions retained in the human-mouse cell hybrids (47). Homogenates of parental and hybrid cell lines were prepared for electrophoresis as described before (46). Enzyme markers specific for each chromosome were tested as reported previously (22, 36).

Cell culture. Mouse L fibroblast cells deficient in thymidine kinase (Ltk$^-$) were obtained from Stanley Cohen. C2 cells are a myogenic cell line, originally isolated by Yaffe and Saxel (62) by selective serial passaging of myoblasts from mouse thigh muscle. A well-differentiating subclone, C2C12 (6), was a gift of Helen Blau. C2 cells were grown in Dulbecco modified Eagle medium (DMEM) supplemented with 20% fetal calf serum and 0.5% chicken embryo extract.

To induce C2 differentiation, cells were grown to approximately 90% confluency and then cultured for 48 h in DMEM containing 2% horse serum. Ltk$^-$ cells were cultured in DMEM plus 10% fetal calf serum.

Cell transfections. The plasmids to be used for cell transfections were prepared from cleared bacterial lysates by banding on two successive ethidium bromide-cesium chloride gradients. Both C2 and Ltk$^-$ cells were transfected at approximately 50% confluency in 100-mm-diameter dishes. DNA was transfected into cells by the calcium phosphate precipitation method (19). The calcium phosphate-DNA precipitate was allowed to form for 30 min at room temperature and added directly to the cells in 100-mm-diameter dishes covered with 10 ml of the appropriate growth medium. The precipitate was left over the cells for 4 h at 37°C. The cells were washed once with DMEM, subjected to an osmotic shock by incubation in 3 ml of 15% glycerol in HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid)-buffered saline for 2 min (30), and washed again in DMEM. The cells were incubated overnight at 37°C in 10 ml of the appropriate growth medium. The following day the cells were trypsinized from the dish and replated after a 1:2 dilution. The cells were selected in 400 μg (active concentration) of G418 (Geneticin; GIBCO Laboratories, Grand Island, N.Y.) per ml in the appropriate growth medium (49). The medium was changed every 2 to 3 days for a period of 3 to 4 weeks. More than 100 G418-resistant C2 and Ltk$^-$ colonies were obtained, representing a transfection efficiency of approximately 1 cell in 10^4. The resistant colonies were pooled and grown as a mass culture. Single-cell clones were obtained by dilution.

RESULTS

Nonmuscle actin mRNA levels vary between mouse tissues. The 3'-untranslated regions (UTRs) of the β- and γ-cytoskeletal actin genes are isotype specific and conserved in evolution (42, 61). We used cDNA probes derived from the 3'-UTRs of our human β- and γ-actin cDNA clones (15) to determine the steady-state amounts of these nonmuscle actin gene transcripts in a variety of mouse tissues. Total RNA was isolated from five nonmuscle tissues (brain, liver, kidney, lung, and testis), one smooth muscle (stomach), and two striated muscles (skeletal muscle and heart). A 2-μg portion of total RNA from each tissue was electrophoresed through agarose-formaldehyde gels, blot transferred to nitrocellulose, and hybridized to either the β-actin-specific (β-3'UT-HF) or γ-actin-specific (γ-3'UT-FNU) DNA probe (15). Autoradiographic exposures of these filters are shown in Fig. 1.

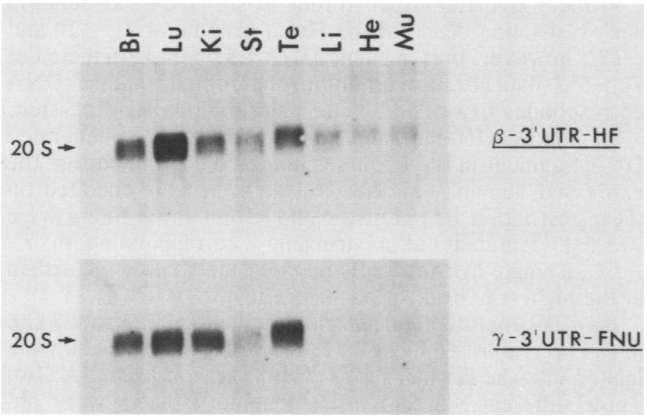

FIG. 1. Steady-state levels of the β- and γ-cytoskeletal actin mRNAs in mouse organs. A 2-μg portion of total RNA from eight different mouse organs was size fractionated by electrophoresis on a 1% agarose–2.2 M formaldehyde gel. The RNAs were immobilized on nitrocellulose filters and hybridized with either β-3'UT-HF or γ-3'UT-FNU (15) under identical conditions. The 3'-UTR probes are isotype specific (42). Br, Brain; Lu, lung; K, kidney; St, stomach; Te, testis; Li, liver; He, heart; Mu, muscle. The autoradiogram showing hybridization to γ-3'UT-FNU was exposed for a threefold-greater period of time than that for β-3'UT-HF.

TABLE 1. Actin transcripts in mouse tissues

Tissue	Transcript level (arbitrary units per μg of total RNA)[a]		
	β	γ	β/γ
Lung	350 (161)	70 (32)	5.0
Testis	141	83	1.7
Brain	112 (176)	51 (80)	2.2
Kidney	99 (85)	58 (50)	1.7
Stomach	49	17	2.9
Liver	46 (114)	0.4 (1)	114
Muscle	36	3	12
Heart	26	2	13

[a] Numbers in parentheses were derived by renormalizing the relative transcript levels in arbitrary units per microgram of DNA (rather than per microgram of RNA) by using the RNA/DNA ratios of 0.46, 0.86, 1.57, and 2.32 for rat lung, kidney, brain, and liver, respectively (20a), and assuming that similar values hold for these organs in the mouse.

Inspection of the autoradiograms clearly reveals the presence of β-actin mRNA in all tissues, both muscle and nonmuscle (Fig. 1). In contrast, γ-actin mRNA is just detectable in heart and skeletal muscle and undetected in liver. It was necessary to increase exposure times a further 5- to 10-fold to observe clear autoradiographic signals from the γ-actin mRNA in striated muscles and liver (not shown). Thus, we conclude that, whereas β- and γ-actin mRNAs are present in all eight tissues, their relative expression shows considerable variation among these tissues. As expected, the smooth and striated muscles express quite low levels of β- and γ-actin mRNA because they also express muscle actins as their major actin isoforms (55–57), but this does not account for the variation seen in the nonmuscle tissues. To quantitate this variation, the autoradiograms were subject to densitometry (Table 1).

We measured the levels of β- and γ-actin mRNA per microgram of RNA in all tissues. Table 1 shows that, between the nonmuscle tissues, β-actin mRNA levels per microgram of RNA vary eightfold between liver and lung. If we calculate the β-actin mRNA per microgram of DNA, this variation is substantially reduced, with the most extreme difference being twofold between brain and kidney (Table 1). In contrast, the level of γ-actin mRNA per microgram of RNA shows a 200-fold difference between liver and testis. This is due to the extraordinary low expression of γ-actin mRNA in liver (Table 1). If we eliminate liver from consideration, the levels of γ-actin mRNA per microgram of RNA are more similar to each other in nonmuscle tissues than when calculated per microgram of DNA. Thus, we conclude that, whereas β-actin mRNA tends to accumulate at similar levels per microgram of DNA and that of γ-actin mRNA does so per microgram of RNA, there is substantial variation between some mouse nonmuscle tissues.

Calculation of the ratio of β-/γ-actin mRNA levels in mouse tissues demonstrates that their relative expression

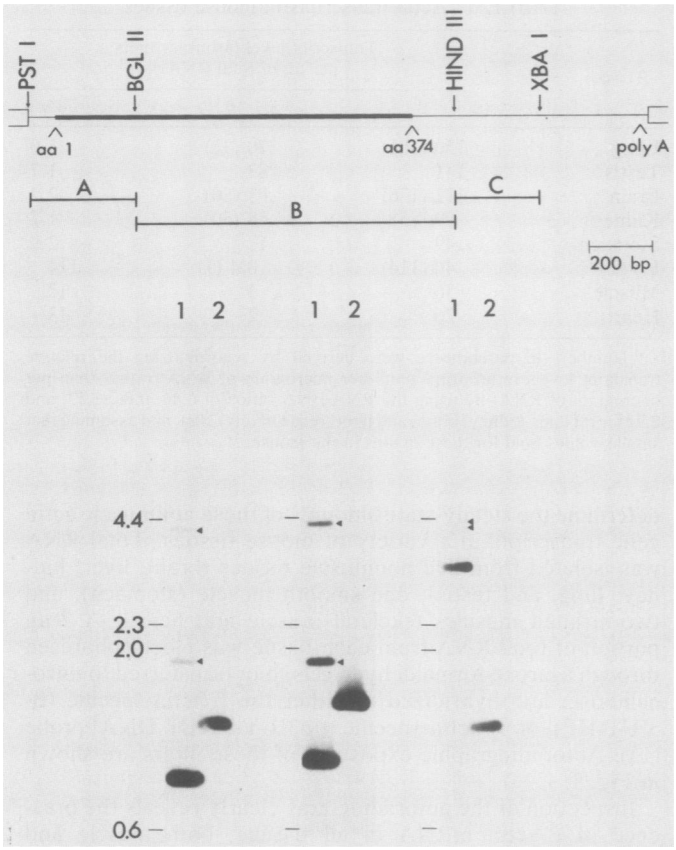

FIG. 2. Evidence that transcription unit of λgHγNMA-1 is interrupted. The γ-cytoskeletal actin cDNA, pHFγA-1 (lane 1), and λgHγNMA-1 (lane 2) DNAs were restricted with *Bgl*II and *Hin*dIII. The digestion products were electrophoresed on an agarose gel and blot transferred to nitrocellulose in triplicate. Each filter was hybridized in 4× SSC at 65°C with a different probe derived from pHFγA-1: a 340-bp *Pst*I/*Bgl*II fragment (A), a 1,010-bp *Bgl*II/*Hin*dIII fragment (B), and a 270-bp *Hin*dIII/*Xba*I fragment (C). The filters were washed in 0.5× SSC at 65°C and autoradiographed. The positions of λ*Hin*dIII marker fragments are shown to the left of each panel in kilobase pairs. Partial digestion products of pHFγA-1 are indicated by closed arrowheads.

can be modulated over 60-fold (Table 1). Mouse liver accumulates β/γ in the ratio of 114, striated muscle accumulates β/γ in the ratio of 12 to 13, and the other tissues do so in the range of 1.7 to 5.0. This indicates that, while the β- and γ-actin mRNAs appear to be truly coexpressed, they are subject to differential regulation between different cell types.

To evaluate the basis of this coordinate expression but differential modulation of the β- and γ-actin genes, it is necessary to isolate and characterize the corresponding genes. Although we have previously characterized in detail the human β-actin gene (35, 37), no data exist for either an avian or mammalian γ-actin gene. We therefore undertook the isolation of the human γ-actin gene corresponding to our human γ-actin cDNA.

Isolation of the human γ-cytoskeletal actin gene. We have previously demonstrated that isotype-specific probes from the 3′-UTR of the human γ-actin mRNA hybridize to many DNA fragments in restriction endonuclease digests of the human and mouse genomes (15, 42). Many of these DNA fragments from the human genome have been isolated and characterized (H. Erba, unpublished data). Our data indicate that the majority of these γ-related sequences represent processed pseudogenes which have been generated during mammalian evolution and are dispersed in the human genome. Furthermore, a γ-cytoskeletal actin pseudogene has recently been isolated from both the mouse (25, 50) and human (26) genomes. Both of these sequences display the characteristics of processed pseudogenes: they are flanked by direct repeats, possess a 3′ poly(A) tract, and are not interrupted by introns.

Since the processed pseudogenes are most likely not expressed, their sequences are not maintained by selective pressure and have been diverging away from that of the parent, expressed γ-cytoskeletal actin gene during mammalian evolution. A probe derived from the 3′-UTR of a γ-actin cDNA should therefore be most homologous to the expressed gene. Ponte et al. (42) attempted to identify the functional γ-actin gene by hybridizing at moderate stringency a 3′-UTR probe to an *Eco*RI digest of the human genome and washing the hybrids under increasingly stringent conditions. However, under their most stringent conditions all of the γ-related sequences could still be detected, and they were unable to identify which of the hybridizing genomic fragments produced the γ-cytoskeletal actin mRNA.

The kinetics of nucleic acid hybridization and duplex melting differ. Beltz et al. (2) have reported that performing filter hybridizations under high-stringency conditions may allow the discrimination of members of a multigene family, even though high-stringency washes of filter-bound DNA hybrids may not. We therefore attempted to identify the functional human γ-cytoskeletal actin gene by hybridizing an isotype-specific probe to genomic DNA under high-stringency conditions. HeLa DNA was digested with *Eco*RI, *Bam*HI, and *Sac*I, and the digestion products were size fractionated on 0.8% agarose gels. The genomic DNAs were blot transferred to nitrocellulose filters. Three such panels were hybridized with the γ-actin-specific probe γ-3′UT-HX (15) in 50% formamide–5× SSC at 57, 60, or 62°C. These hybridization conditions correspond to 10, 7, and 5°C, respectively, below the calculated (2) melting temperature (T_m) of this 200-bp probe. All three filters were washed at T_m −5°C.

Probe γ-HX hybridizes to four to six DNA fragments in each restriction digest of the HeLa genome at T_m −10 and −7°C; however, only a single DNA fragment is detected at T_m −5°C (data not shown). This probe from the γ-actin cDNA corresponded to a >23-kilobase-pair (kbp) *Eco*RI-generated, a 9.2-kbp *Bam*HI-generated, and a 4.4-kbp *Sac*I-generated DNA fragment in the human genome. We assumed that the expressed human γ-cytoskeletal actin gene is encoded on these restriction fragments. Although all three filters were washed identically at a stringency corresponding to T_m −5°C, a single hybridizing band could only be distinguished at the highest hybridization stringency.

*Bam*HI-generated HeLa DNA fragments of 7.5 to 9.5 kbp in size were purified by agarose gel electrophoresis and were ligated into the lambda replacement vector EMBL 3A (16). Three different γ-actin-related genomic isolates were obtained after screening the recombinant plaques in situ with γ-HX. Two of the three genomic clones contained a 9.2-kbp *Bam*HI fragment, but otherwise had different restriction maps (data not shown). The choice for initial analysis of DNA from the highly aneuploid HeLa cells may not have been optimal since in subsequent experiments (e.g., see Fig. 11) we found that DNA from normal individuals contained the γ-actin gene on restriction fragments that differed in size from the HeLa fragments.

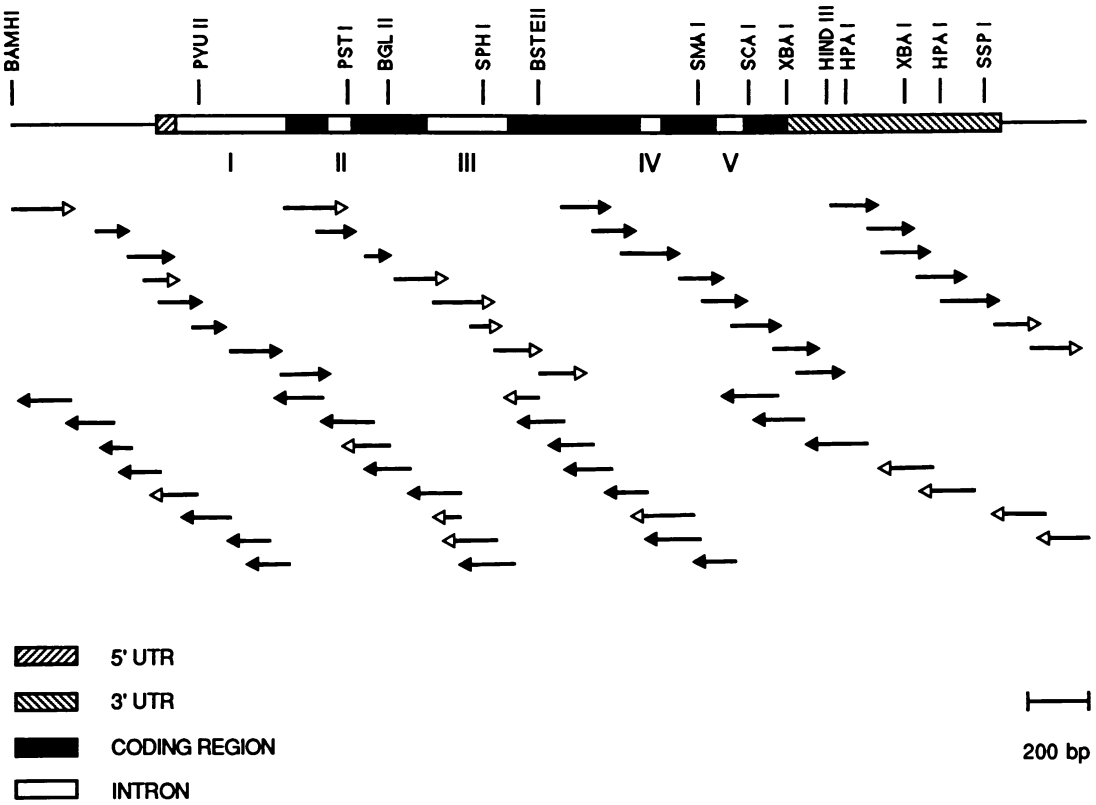

FIG. 3. Restriction map and sequencing strategy of pgHγNMA-1. Restriction fragments (open arrowheads) and Bal-31-generated DNA fragments (closed arrowheads) of pgHγNMA-1 were inserted into M13 cloning vectors. The DNA sequence was determined by the dideoxy chain terminator method. The introns are located in the 5'-UTR and in the coding region at codons 41/42, 121/122, 267, and 327/328. All restriction enzymes shown cut in this region of the γ-cytoskeletal actin gene only once, except for *Xba*I and *Hpa*I, which both recognize two sites in the 3'-UTR, and *Sma*I, which also cuts several times in the 5'-flanking region and intron I (not shown).

Intervening sequences have been shown to interrupt the coding region of every functional vertebrate β-cytoskeletal actin gene characterized (24, 35, 37, 38). Also, a *Xenopus borealis* type 1 nonmuscle actin gene has been recently characterized, the 3'-UTR of which is homologous to that of the human γ-cytoskeletal actin mRNA (G. S. Cross, H. P. Erba, and H. Woodland, unpublished data). The intron locations of this amphibian nonmuscle actin gene are identical to those of the β-actin genes. We assumed, therefore, that the expressed human γ-cytoskeletal actin gene is also interrupted by intron sequences in the 5'-UTR and in the coding region at codons 41/42, 121/122, 267, and 327/328.

The following assay was performed for each genomic clone to test for interruptions in the actin-coding region. DNA from each genomic clone was digested to completion with the restriction endonucleases *Bgl*II and *Hin*dIII. These restriction sites occur once each in the human γ-cytoskeletal actin cDNA, pHFγA-1 (23), at codon 84 and in the 3'-UTR, respectively (Fig. 2). The digest of the phage DNA (lane 2) was electrophoresed on an agarose gel with a *Bgl*II/*Hin*dIII digest of pHFγA-1 (lane 1) and blot transferred to nitrocellulose filters in triplicate. Three probes were prepared from the human γ-actin cDNA: a 340-bp *Pst*I/*Bgl*II fragment (A), a 1,010-bp *Bgl*II/*Hin*dIII fragment (B), and a 270-bp *Hin*dIII/*Xba*I fragment (C). Each filter was hybridized with one of these probes, washed, and autoradiographed. The results obtained with one of the genomic clones, λgHγNMA-1, are illustrated in Fig. 2.

Each probe hybridizes to a unique *Bgl*II/*Hin*dIII fragment in pHFγA-1, demonstrating the purity of each probe. The hybridization to partial digestion products (closed arrowheads in lane 1) allows the filters to be accurately aligned. Probes A and B do not hybridize to the same *Bgl*II/*Hin*dIII fragment of λgHγNMA-1, indicating that the *Bgl*II site of this actin gene is also at codon 84. Similarly, the nonoverlapping hybridization of probes B and C shows that the position of the *Hin*dIII site has been conserved in this gene. Furthermore, probe B detects a *Bgl*II/*Hin*dIII fragment in this genomic clone which is 450 bp larger than that of pHFγA-1. This result suggests that the coding region of this human γ-cytoskeletal actin gene is interrupted by 450 bp of intron sequence. However, the results of this assay are equally consistent with the insertion of a moderately repetitive element into the coding region of a processed pseudogene that has maintained the *Bgl*II and *Hin*dIII sites. Nonetheless, since λgHγNMA-1 was the only genomic clone to satisfy the criteria of this assay, it was chosen for further analysis.

Sequence of the human γ-cytoskeletal actin gene. The 9.2-kbp *Bam*HI fragment from the genomic clone λgHγNMA-1 was inserted into the *Bam*HI site of pBR322. A restriction map of this subclone, pgHγNMA-1, is presented in Fig. 3 accompanied by the strategy used to sequence this gene. The dideoxy chain terminator method (45) determined 92% of the nucleotide sequence from both strands. The complete sequence of the human γ-cytoskeletal actin gene, including the entire 474 bp of 5'-flanking sequence included on this 9.2-kbp *Bam*HI fragment, is presented in Fig. 4. The 3' end of the cDNA, pHFγA-1, corresponds to position 2832

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      GGAT CCGCTTGGAG CCCGAGGGGA AAGATTGCAA GGGACGTCCC AGGGAGCAAA GCCCCGCAGC GTGGCCGGGG   -401

ACTCGGGGAC CCACCCCAGC TCCGGCGCGG CCCCGCCCCG GGTCAGGCAG TTTCGGGGCG CAGCAGGGCC CTCCCCACGG   -321

CGCGGCAGTC CCGGGGAGGG CCAGGCCCGT CAGCCCTTCG GCCCTCCCGG CGACCCTCGG AGGGCGCCCC AACTCAGACC   -241

CGCCCGCCGG CCCGCGCAGG GTGCCGAGCG CGCGGGGGTG GCGCGGGCGG GGCCGTTGGG AGGCGGTGCG GGCTGGGGGC   -161

GGGGCGGGCC GGGGCCCCAC GTGTCCCTGC CCGGCGGGCC AATGGGTGCC CGGCTTTCGG AAAGATCGCC ATATATGGAC    -81

ATGTTCTGGG GCCGCGCGCG CCGCCGGGCG CCGCGGGCGC GCCGCTTCCG CTTAAATAAC GGCGGGGGAG GCCGCGGTCG     -1

GTCTCAGTCG CCGCTGCCAG CTCTCGCACT CTGTTCTTCC GCCCCTCCGC CGTCGCGTTT CTCTGCCG / GTGAGCGCCC     78

CGCCCCGGGG CCTGAGCTGG ACGTCGCAGG CCTGCGCCCC CCGACCCCGG CTGGCCCCGC TTCCAGCTGC CGAGGCCTCG    158

TCGCGCCTTC CCCGGGAACA AAAGGCGGGG TGGGCCGGGT CAGGCGGACG GGGCTGGGGG GGCGCCGGGG GTCCAGGGCG    238

GGGCCCGGCC GCAGTGCAGA CTTCCGAGCC GTGGACGTTA CGTAAAAGGC CCGGCCTTCG GTTCCAGGCG GTTGCGATCT    318

TGGAGGCCAC GGCCGCTGGG GTTGGGGCAG GGCCGAGGCT GCCCCGCGGG AGGGCATTAG GTGGCTGTGG GGAGGGGACC    398

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                                                      1
GTC ATT GAC AAT GGC TCC GGC ATG TGC AAA GCT GGT TTT GCT GGG GAC GAC GCT CCC CGA GCC GTG    537
Val Ile Asp Asn Gly Ser Gly MET Cys Lys Ala Gly Phe Ala Gly Asp Asp Ala Pro Arg Ala Val
     10                                  20                                  30
TTT CCT TCC ATC GTC GGG CGC CCC AGA CAC CAG /GTGAGTGGA TGGCGCCGCG GGGCTCCTGG GTTCTGCGTT    609
Phe Pro Ser Ile Val Gly Arg Pro Arg His Gln
                                    40
GCGGGGTGGG TTCGGTGTCG CCGGCGAGGC TGACGGATCG TCCCCTGCAG/ GGC GTC ATG GTG GGC ATG GGC CAG    683
                                                        Gly Val MET Val Gly MET Gly Gln

AAG GAC TCC TAC GTG GGC GAC GAG GCC CAG AGC AAG CGT GGC ATC CTG ACC CTG AAG TAC CCC ATT    749
Lys Asp Ser Tyr Val Gly Asp Glu Ala Gln Ser Lys Arg Gly Ile Leu Thr Leu Lys Tyr Pro Ile
 50                                  60                                  70
GAG CAT GGC ATC GTC ACC AAC TGG GAC GAC ATG GAG AAG ATC TGG CAC CAC ACC TTC TAC AAC GAG    815
Glu His Gly Ile Val Thr Asn Trp Asp Asp MET Glu Lys Ile Trp His His Thr Phe Tyr Asn Glu
                                80                                  90
CTG CGC GTG GCC CCG GAG GAG CAC CCA GTG CTG CTG ACC GAG GCC CCC CTG AAC CCC AAG GCC AAC    881
Leu Arg Val Ala Pro Glu Glu His Pro Val Leu Leu Thr Glu Ala Pro Leu Asn Pro Lys Ala Asn
                        100                                 110
AGA GAG AAG ATG ACT CAG /GTGAGGC TCGGCCGACG CCCGTGCTCC TCCCGTCCTT TCCCCAGTCA TTTTCTCGCC    956
Arg Glu Lys MET Thr Gln
                120
CGGCTTGATT TCTGACATTT AAGTGTTTCT TTCGCTGTTC CAGGCTCTGT TCCTCTCCCG GCATTTCCTC CCTGAAGCCT   1036

CCAGGTTTCT CATTTGGTTT CTGCCTGCGT TCTTTTCTTT TCTCCACACA TCACACTGGC ATGCAGCATG TTGTGGCGTG   1116

TGAGCATGGG GTGGCCGTGG GTCTCTGTCC CTGACTAAGC CGCCCCTTGT CCCTTCTCAG / ATT ATG TTT GAG ACC   1191
                                                                    Ile MET Phe Glu Thr

TTC AAC ACC CCG GCC ATG TAC GTG GCC ATC CAG GCC GTG CTG TCC CTC TAC GCC TCT GGG CGC ACC   1257
Phe Asn Thr Pro Ala MET Tyr Val Ala Ile Gln Ala Val Leu Ser Leu Tyr Ala Ser Gly Arg Thr
          130                                 140
ACT GGC ATT GTC ATG GAC TCT GGA GAC GGG GTC ACC CAC ACG GTG CCC ATC TAC GAG GGC TAC GCC   1323
Thr Gly Ile Val MET Asp Ser Gly Asp Gly Val Thr His Thr Val Pro Ile Tyr Glu Gly Tyr Ala
    150                                 160                                 170
CTC CCC CAC GCC ATC CTG CGT CTG GAC CTG GCT GGC CGG GAC CTG ACC GAC TAC CTC ATG AAG ATC   1389
Leu Pro His Ala Ile Leu Arg Leu Asp Leu Ala Gly Arg Asp Leu Thr Asp Tyr Leu MET Lys Ile
                                180                                 190
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CTC ACT GAG CGA GGC TAC AGC TTC ACC ACC ACG GCC GAG CGG GAA ATC GTG CGC GAC ATC AAG GAG   1455
Leu Thr Glu Arg Gly Tyr Ser Phe Thr Thr Thr Ala Glu Arg Glu Ile Val Arg Asp Ile Lys Glu
                          200                                 210
AAG CTG TGC TAC GTC GCC CTG GAC TTC GAG CAG GAG ATG GCC ACC GCC GCA TCC TCC TCT TCT CTG   1521
Lys Leu Cys Tyr Val Ala Leu Asp Phe Glu Gln Glu MET Ala Thr Ala Ala Ser Ser Ser Ser Leu
                    220                             230                234a
GAG AAG AGC TAC GAG CTG CCC GAT GGC CAG GTC ATC ACC ATT GGC AAT GAG CGG TTC CGG TGT CCG   1587
Glu Lys Ser Tyr Glu Leu Pro Asp Gly Gln Val Ile Thr Ile Gly Asn Glu Arg Phe Arg Cys Pro
                240                             250
GAG GCG CTG TTC CAG CCT TCC TTC CTG G / GTA GGTGTTGTGA GCTAAAGGTT TCTACTCTTT CATCCTCGGT   1658
Glu Ala Leu Phe Gln Pro Ser Phe Leu G
       260
GACACAGCAT CACTAAGGGA GGGCTCTGTC CCCTAG/ GT ATG GAA TCT TGC GGC ATC CAC GAG ACC ACC TTC   1729
                                          ly MET Glu Ser Cys Gly Ile His Glu Thr Thr Phe
                                                  270
AAC TCC ATC ATG AAG TGT GAC GTG GAC ATC CGC AAA GAC CTG TAC GCC AAC ACG GTG CTG TCG GGC   1795
Asn Ser Ile MET Lys Cys Asp Val Asp Ile Arg Lys Asp Leu Tyr Ala Asn Thr Val Leu Ser Gly
    280                                 290                                     300
GGC ACC ACC ATG TAC CCG GGC ATT GCC GAC AGG ATG CAG AAG GAG ATC ACC GCC CTG GCG CCC AGC   1861
Gly Thr Thr MET Tyr Pro Gly Ile Ala Asp Arg MET Gln Lys Glu Ile Thr Ala Leu Ala Pro Ser
                                  310                                     320
ACC ATG AAG ATC AAG / GTGAGTCGAG GGGTTGGTGG CCCTCTGCCT GGCTCGGGAG AGCTGACTGG GGGGCGCTCT   1936
Thr MET Lys Ile Lys

GCGAGCTGAA GCCGTGCCTG GCTGTCTTTG CAG /  ATC ATC GCA CCC CCA GAG CGC AAG TAC TCA GTG TGG   2005
                                        Ile Ile Ala Pro Pro Glu Arg Lys Tyr Ser Val Trp
                                                330
ATC GGC GGC TCC ATC CTG GCC TCA CTG TCC ACC TTC CAG CAG ATG TGG ATT AGC AAG CAG GAG TAC   2071
Ile Gly Gly Ser Ile Leu Ala Ser Leu Ser Thr Phe Gln Gln MET Trp Ile Ser Lys Gln Glu Tyr
340                                 350                                     360
GAC GAG TCG GGC CCC TCC ATC GTC CAC CGC AAA TGC TTC TAG ACGGACTCA GCAGATGCGT AGCATTTGCT   2142
Asp Glu Ser Gly Pro Ser Ile Val His Arg Lys Cys Phe  .
                              370
GCATGGGTTA ATTGAGAATA GAAATTTGCC CCTGGCAAAT GCACACACCT CATGCTAGCC TCACGAAACT GGAATAAGCC   2222

TTCGAAAAGA AATTGTCCTT GAAGCTTGTA TCTGATATCA GCACTGGATT GTAGAACTTG TTGCTGATTT TGACCTTGTA   2302

TTCAAGTTAA CTGTTCCCCT TGGTATTTGT TTAATACCCT GTACATATCT TTGAGTTCAA CCTTTAGTAC GTGTGGCTTG   2382

GTCACTTCGT GGCTGAGGTA AGAACGTGCT TGTGGAAGAC AAGTCTGTGG CTTGGTGAGT CTGTGTGGCC AGCAGCCTCT   2462

GATCTGTGCA GGGTATTAAC ATGTCAGGGC TGAGTGTTCT GGGATTTCTC TAGAGGCTGG CAAGAACCAG TTGTTTTTGT   2542

CTTGCGGGTC TGTCAGGGTT GGAAAGTCCA AGCCGTAGGA CCCAGTTTCC TTTCTTAGCT GATGTCTTTG GCCAGAACAC   2622

CGTGGGCTGT TAACTTGCCT TGAGTTGGAA GCGGTTTGCA TTTACGCCTG TAAATGTATT CATTCTTAAT TTATGTAAGG   2702

TTTTTTTTGT ACGCAATTCT CGATTCTTTA AAGAGATGAC AACAAATTTT GGTTTTCTAC TGTTATGTGA GAATATTAGG   2782

CCCCAGCAAC ACGTCATTGT GTAAGGAAAA ATAAAAGTGC TGCCGTAACC AATGAATGGC TCCTGTTTGG GGAAGTAGCA   2862

AGTGGGCTGG GAAAGACAGA CCAACCTGGA AGTATTGGGT AGTCTTGGGG GTGGGGGCGG TGGCTGCTGC TCAGCTTGGC   2942

TTCGTGGGCT GGTGAGAAAA CGGCTTAACA ATAAACTGAG CAGTTCGATT TCTTCCAAAT CGAAAGTGCA AGAACAAGGC   3022

AGCTAGTGGG TTTGGGTGTT GGAAATAACT GAAGCAACAT CATAAGCAGG CTGGGAGGGA AGTCCTGAGA CGGCTTTTTC   3102

CGTTATT                                                                                   3109
```

FIG. 4. Nucleotide sequence of the human γ-cytoskeletal actin gene. The nucleotides are numbered relative to the G residue of the mRNA cap site. Nucleotides 5′ of the cap site are designated by negative numbers. The 74-nucleotide 5′-UTR (bases 1 to 68, bases 442 to 447) is underlined. Intron sequences are enclosed by the / symbol. The amino acid sequence is numbered according to the convention of Lu and Elzinga (27). The 3′ end of the cDNA, pHFγA-1, is at nucleotide 2832.

of the gene sequence, exactly 21 bp downstream of the AATAAA polyadenylation signal (43).

The locations of the five introns were determined by comparing the sequence of the γ-actin gene with that of the full-length cDNA, pHFγA-1 (15). The exon/intron boundaries all conform to the GT-AG rule for a potential splicing site (7). IVS I, the largest of the introns, is located within the 5′-UTR, 6 bp upstream of the translation start codon. The remaining four introns occur in the coding region at codons 41/42, 121/122, 267, and 327/328. Therefore, the human γ-cytoskeletal actin gene is interrupted by introns at the same positions as in the vertebrate β-actin genes. However, these introns are shorter than their counterparts in the human β-actin gene (35, 37). The intron lengths in the human β-and γ-actin genes are, respectively, 832 and 373 bp (IVS I), 134 and 89 bp (IVS II), 441 and 277 bp (IVS III), 95 and 79 bp (IVS IV), and 112 and 93 bp (IVS V). These data provide a formal demonstration that the human β- and γ-actin genes arose by gene duplication of a common ancestral gene.

Although the actin-coding region of pgHγNMA-1 predicts an amino acid sequence identical to that of the γ-actin isoform, the nucleotide sequence of this human γ-cytoskeletal actin gene is not identical to that of the cDNA, pHFγA-1. There are 11 nucleotide substitutions in the last exon: two silent base substitutions in the coding region and nine in the 3′-UTR. The 5′-UTRs of the cDNA and genomic clone differ by an additional single-base change. The existence of these sequence differences between the mRNA obtained from simian virus 40-transformed human fibroblasts and the gene obtained from HeLa cells may reflect neutral mutations which have occurred during culture of the cell lines or polymorphisms in the human population or both. Below, we demonstrate that polymorphisms are readily detected in the human γ-actin locus.

Identification of transcription initiation site. The location of the cap site was determined by S1 nuclease analysis (59). A uniformly labeled, antisense, single-stranded S1 probe (see Fig. 5B and Materials and Methods) was isolated by electrophoresis on a denaturing acrylamide gel. This probe was annealed to 10 μg of either total RNA from HeLa cells or yeast tRNA. The RNA-DNA hybrids were digested with nuclease S1, denatured, and analyzed on a denaturing 8% acrylamide gel.

HeLa RNA protects a 70-nucleotide region of the probe (Fig. 5A, arrow). Since the first two nucleotides of intron I are identical to the first two nucleotides of exon 2, the protected fragment overestimates the size of exon 1 by two nucleotides. Therefore, exon 1 is 68 bp, and the entire 5′-UTR of the γ-cytoskeletal actin mRNA is 74 nucleotides in length. The identification of the cap site has been confirmed by extension of an anti-sense oligonucleotide (nucleotides +49 to +68; Fig. 4), following hybridization of the primer to total RNA from a human fibroblast cell line, MRC-5 (data not shown).

The assignment of the cap site to the G residue at position +1 is supported by the following two observations. A recently isolated processed human γ-actin pseudogene is flanked by direct repeats (26). The junction of this repeat sequence and the 5′-UTR is within 2 bp of the cap site. Therefore, this reverse transcript-like pseudogene appears to be full length. The longest human γ-actin cDNA we have characterized, pHFγA-1, also begins at this cap site (15).

Conservation of sequences in the 5′-flanking region. Nucleotide sequence comparison of this human γ-actin gene with that of β-actin genes and the *Xenopus* type 1 nonmuscle actin gene indicates the existence of regions which have been highly conserved for over 400 million years. Two consensus sequences associated with eucaryotic RNA polymerase II promoters, TATA and CAAT, are located at positions −29 and −122, respectively (Fig. 4). Although the sequence TTAAATA does not conform exactly to the Goldberg-Hogness box consensus sequence (8), it is identical in both sequence and location to the TATA box of the *X. borealis* type 1 actin gene (Cross et al., unpublished data).

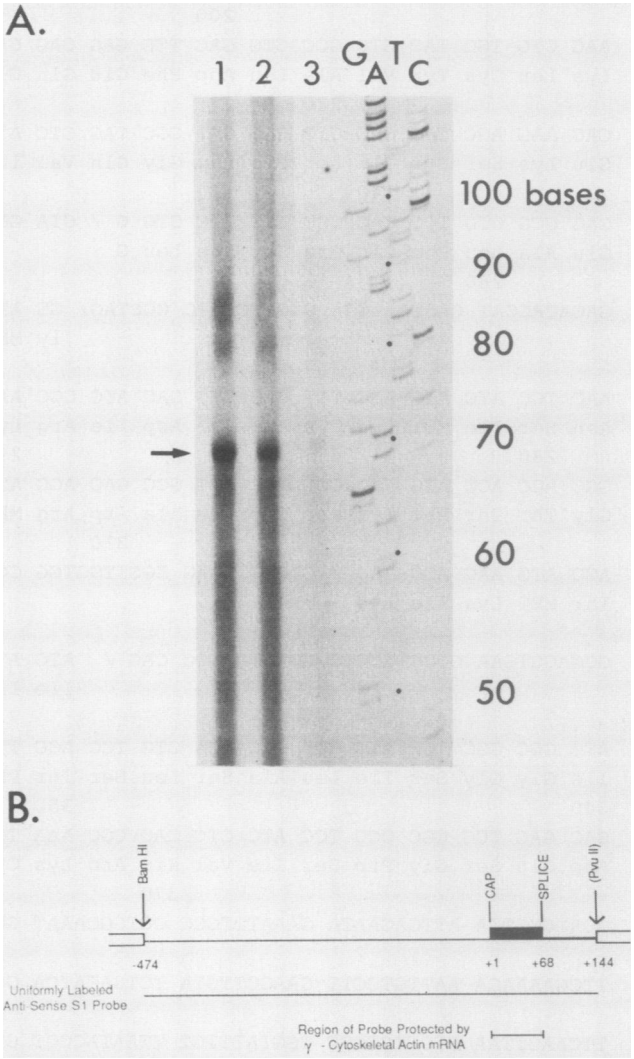

FIG. 5. Nuclease S1 analysis of the transcription initiation site of the human γ-cytoskeletal actin gene. (A) A 10-μg portion of total HeLa cell RNA was hybridized with an antisense probe at either 52°C (lane 1) or 57°C (lane 2). The antisense probe was also incubated with 10 μg of yeast tRNA at 52°C under identical conditions (lane 3). The hybrids were treated with nuclease S1, and the protected DNA fragments were analyzed on an 8% acrylamide–urea gel. M13mp8 single-stranded DNA was sequenced by the dideoxy chain terminator method and coelectrophoresed with the S1 digestion products as a size standard (lanes G, A, T, and C). The 70-nucleotide protected DNA fragment is indicated by an arrow. (B) The uniformly labeled antisense S1 probe was derived from an M13 recombinant, which contains 474 bp of 5′-flanking sequence, the entire first exon, and 76 bp of intron I of the human γ-cytoskeletal actin gene. The region of the probe protected by the γ-cytoskeletal actin mRNA from S1 digestion corresponds to the first exon. Since the location of the intron I donor splice site is known, the size of the protected fragment indicates the location of the cap site.

```
H γ  GGCCAATGG-GTGCCCGGCTTTCGGAAAGATCGCCATATATGG  -83
     ::::::: : : :  ::  :::: :::::::  :::::: :::
X 1  GGCCAATCGCGAG-GCGTGTTTCTGAAAGATGCCCATATTTGG  -68

H γ  GGCCAAT-GGGTGCCCGGCTTTCGGAAAGATCGCCATATATGG  -83
     ::::::: : :::: : : :: : ::::: : ::: : :::::
H β  GGCCAATCGCGTGCGCCG-TTCC-GAAAGTT-GCCTTTTATGG  -52

H γ  GGCCAATGGGTGCCCGGCTTTCGGAAAGATCGCCATATATGG  -83
       ::::::  : ::::: : ::: ::::  : ::: : :::::
R β  AGCCAATCAGCGCCCGCCGTTCCGAAA--TTGCCTTTTATGG  -51

H γ  GGCCAATGGGTGC-CCG-GCTTTCGGAAAGATCGCCATATATGG  -83
       ::::::  : ::  :: :::  : ::::: :  :: : :::::
C β  AGCCAATCAGAGCGGCGCGCT-CC-GAAAGTT-TCCTTTTATGG  -52
```

FIG. 6. Alignment of the 5'-flanking regions of vertebrate nonmuscle actin genes. The region of the human γ-cytoskeletal actin gene (Hγ) between nucleotides −124 and −83 was aligned with a corresponding region from the 5'-flanking sequences of the *X. borealis* type 1 (X1) (Cross et al., unpublished data), human β (Hβ) (37), rat β (Rβ) (38), and chicken β (Cβ) (24) nonmuscle actin genes. The nucleotides listed to the right of the sequences represent the distance of the last nucleotide from the cap site of the mRNA.

The CCAAT sequence of the human γ-actin gene closely resembles the consensus (3) but occurs 30 bp further away from the cap site than it does in the vertebrate β-actin genes (24, 35, 37, 38). However, the unusual position of the CCAAT box relative to the start of transcription has also been observed for the *X. borealis* type 1 actin gene (Cross et al., unpublished data).

The 5'-flanking regions of the vertebrate sarcomeric- and β-actin genes have been shown to contain a sequence motif which has been conserved during evolution (33). The sequence element $CC(A/T)_6GG$ (CCArGG) is located 61 to 63 bp upstream of the cap site in the human, rat, and chicken β-cytoskeletal actin genes (24, 35, 37, 38). A single CCArGG element is found in the 474 bp of 5'-flanking sequence of the human γ-cytoskeletal actin gene. However, this sequence is located at −92 (Fig. 4), 30 bp further upstream than the CCArGG element in the β-actin genes. A CCArGG sequence also occurs in the *X. borealis* type 1 actin gene at −87 bp (Cross et al., unpublished data). The location of the CCAAT and CCArGG elements 30 bp further upstream from the cap site in the γ-actin gene relative to the β-actin genes appears to be due to an expansion of an extremely G+C-rich region (85% G+C) located between TATA and CCArGG sequences (Fig. 4) since the divergence of these two gene families.

Despite the difference in the positions of the CCAAT and CCArGG sequences between the β- and γ-actin genes, the region between these two elements has been conserved. The alignment of this region of the human γ-cytoskeletal actin gene with that of the vertebrate β and *Xenopus* type 1 actin genes is shown in Fig. 6. The degree of similarity, $K_N(1)$, of these sequences was calculated by the method of Miyata et al. (34). Comparison of the human γ-cytoskeletal actin gene with the human β and *Xenopus* type 1 actin genes in the 42-bp region of the CCAAT and CCArGG elements reveals a $K_N(1)$ value of 0.26 for both alignments. Since the β- and γ-actin genes most likely arose by gene duplication during amphibian evolution, the similarity of this region between the human cytoskeletal actin genes may reflect the conservation of sequences involved in the expression of these housekeeping genes.

Sequence conservation has also been observed in the promoter regions of the human c-*fos* proto-oncogene, the *Xenopus* type 1 actin gene (51), and the human γ-cytoskeletal actin gene. Transcription of this oncogene and the cellular actin genes increases following stimulation of quiescent mammalian tissue culture cells with purified growth factors (13, 20). The promoter element responsible for this transcriptional response to serum has been mapped by deletion analysis of the human c-*fos* and *Xenopus* type 1 actin 5'-flanking region (51). This serum response factor protects a region of dyad symmetry from DNase I digestion and dimethyl sulfoxide-mediated methylation. Although the factor-binding site (serum response element) is located 260 bp upstream of the c-*fos* TATA box, the serum response element sequence is similar to a region including the CCArGG element of the human γ-cytoskeletal actin gene and corresponds to the CCArGG element in the *Xenopus* type 1 actin gene (Cross et al., unpublished data).

Finally, the human γ-actin gene contains five copies of the promoter-specific factor Sp1-binding sequence GGGCGG (12, 18) in its 5'-flanking region. This GC box occurs at positions −196, −164, and −159 and its complement at −368 and −241 relative to the cap site (Fig. 4). The *Xenopus* type 1 actin gene also contains an Sp1-binding site, but this is located closer to the TATA box at −57 relative to the cap site (Cross et al., unpublished data). In contrast, the human β-actin gene contains no Sp1-binding sites in the 200 bp of 5'-flanking region sequenced (37).

We conclude that there is significant sequence similarity between the human γ-actin gene and the human, rat, and chicken β-actin genes which may contribute to the coexpression of β- and γ-actin. However, it is apparent that stronger conservation exists between the γ-actin gene and the *Xenopus* type 1 actin gene including the serum response element and Sp1-binding sites. The differential expression of γ- and β-actin may in part reflect the contribution of these γ-specific promoter elements.

Functional γ-cytoskeletal actin gene is single copy in the human genome. The 3'-UTR of the γ-cytoskeletal actin cDNA hybridizes to 10 to 14 restriction endonuclease-generated DNA fragments in the human genome (15, 42). Several of these sequences are known to represent processed pseudogenes as determined by molecular cloning and restriction enzyme mapping (Erba, unpublished data) and DNA sequence analysis (26). However, such analysis has not excluded the possibility that there may be more than one intron-containing γ-actin gene in the human genome. Therefore, we prepared a DNA fragment from IVS III of the functional gene as a hybridization probe. The 230-bp probe, γ-IVSIII-HD, extends from position 925 in an *Hgi*AI site to position 1154 in the *Dde*I site (Fig. 4).

HeLa genomic DNA was digested with six restriction endonucleases, none of which cuts within the sequence of γ-IVSIII-HD. The DNA was size fractionated by agarose gel electrophoresis, blot transferred to a nitrocellulose filter, and hybridized with the γ-IVSIII-HD probe. The autoradiogram of this filter is shown in Fig. 7. Only a single DNA fragment from each restriction enzyme digest hybridizes with the sequences of IVS III. The 2.7-kbp *Bgl*II-, 2.8-kbp *Pst*I-, and 3.4 kbp *Pvu*II-generated fragments are identical in size to the fragments predicted from the restriction enzyme map of pgHγNMA-1. Therefore, we conclude that there is only one intron-containing γ-cytoskeletal actin gene in the human

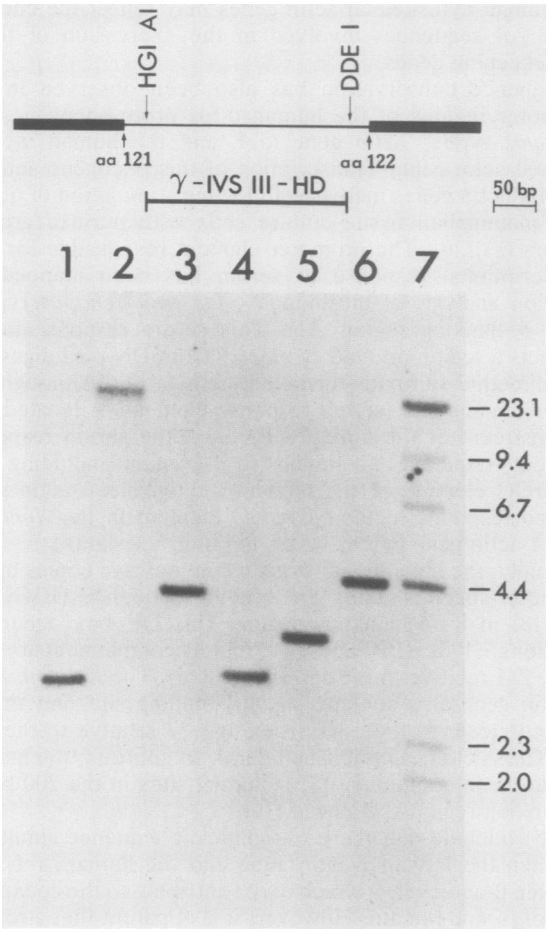

FIG. 7. Evidence that the functional human γ-cytoskeletal actin gene is single copy in the human genome. A 10-μg amount of HeLa cell genomic DNA was restricted with *Bgl*II (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), *Pst*I (lane 4), *Pvu*II (lane 5), and *Sac*I (lane 6). An end-labeled λ phage *Hin*dIII size standard (lane 7) was coelectrophoresed with the genomic digests on a 0.7% agarose gel and blot transferred to a nitrocellulose filter. The filter was hybridized with the radiolabeled probe γ-IVSIII-HD. This 230-bp probe was derived from intron III of the human γ-cytoskeletal actin gene by restriction with *Hgi*AI and *Dde*I. Following three washes in 0.5× SSC–0.1% SDS at 65°C, the filter was autoradiographed. The sizes of the λ *Hin*dIII DNA fragments are indicated in kilobase pairs at the right.

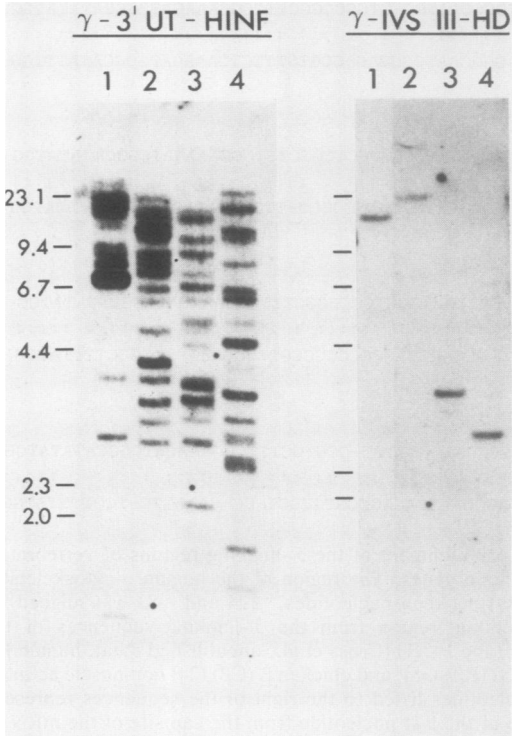

FIG. 8. Conservation of intron III sequences between the human and mouse γ-cytoskeletal actin genes. Genomic DNA from a single female mouse was a gift of Robert Wade. An 8-μg portion of genomic DNA was digested with the restriction enzymes *Bam*HI (lane 1), *Eco*RI (lane 2), *Hin*dIII (lane 3), and *Pst*I (lane 4). The restricted DNAs were size fractionated on an agarose gel and transferred in duplicate to nitrocellulose. The filters were hybridized with a 3'-UTR probe (γ-3'UT-HINF) and an intron III probe (γ-IVSIII-HD) prepared from the functional human γ-cytoskeletal actin gene. The panels were washed and autoradiographed. The sizes of λ *Hin*dIII marker fragments are indicated in kilobase pairs at the left.

genome and the other members of this multigene family are most likely nonfunctional processed pseudogenes.

Conservation of intron III sequences. The sequence of IVS III of the β-cytoskeletal actin gene is conserved between human and rat (35, 37). Furthermore, a 68-bp subsegment of this intron has been conserved since the divergence of mammals and birds (37). Since a functional γ-actin gene has not yet been characterized from any other vertebrate species, we could not analyze the intron III sequence of the γ-actin gene by direct sequence comparison. However, we have found that γ-IVSIII-HD does hybridize to a single-copy sequence in the mouse genome under stringent conditions. Genomic DNA from a single female mouse was digested with four restriction enzymes, size fractionated on an agarose gel, and blot transferred to a nitrocellulose filter. Across-species, γ-specific probe (γ-3'UT-HINF) was hybridized to one such filter; the IVS III probe was hybridized to a duplicate panel. The autoradiographs are presented in Fig. 8.

As expected (15, 42), the probe derived from the 3'-UTR of the γ-actin cDNA hybridizes to multiple restriction enzyme-generated fragments in the mouse genome. Only one fragment in each restriction digest, however, is detected with the IVS III probe. Since the T_m of this fragment in 0.5× SSC is 84°C, the mouse-human hybrid is stable at a wash stringency of T_m −20°C. Cross-hybridization of the human IVS III probe to the mouse γ-cytoskeletal actin gene can still be observed at a wash stringency of T_m 12°C (see Fig. 10). Furthermore, this result suggests that most of the γ-actin-related sequences in the mouse genome, like those of the human genome, appear to be intronless processed pseudogenes.

The 5'-flanking region and the 3'-UTR of the human γ-cytoskeletal actin and *X. borealis* type 1 actin genes are similar. Most of IVS III of this amphibian cytoskeletal actin gene has been sequenced. Surprisingly, a 57-bp subsegment of intron III has been conserved between the human γ and *Xenopus* type 1 actin genes. The K_N(1) (34) of this conserved element is 0.18 (Fig. 9A). This extraordinary degree of sequence conservation since the amphibian radiation implies selection for some unknown function.

Intron III of the human γ-cytoskeletal actin gene also shares some of the features of the β-actin IVS III sequence.

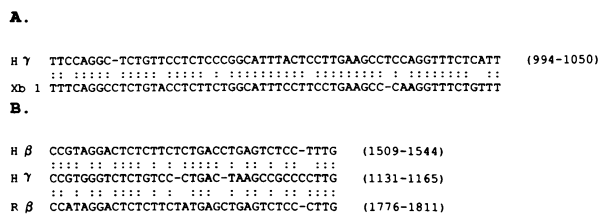

FIG. 9. Alignment of IVS III sequences of vertebrate nonmuscle actin genes. (A) A region of intron III of the human γ-cytoskeletal actin gene (H γ; nucleotides 994 to 1050 in Fig. 4) is similar to a region of intron III of the *X. borealis* type 1 actin gene (Xb1) (Cross et al., unpublished data). (B) A region of intron III of the human γ-cytoskeletal actin gene (H γ; nucleotides 1131 to 1165 in Fig. 4) is similar to a region of intron III of the human (H β; nucleotides 1509 to 1544) and rat (R β; nucleotides 1776 to 1811) β-cytoskeletal actin genes (35, 37, 38).

IVS III of the γ-actin gene is composed of 67% pyrimidine with four stretches of 10 or more C/T. This is not a feature of any of the other introns interrupting the γ gene, but is remarkably similar to the sequence composition of IVS III of the human β-actin gene (35, 37). A 35-bp region near the 3′ end of the γ intron III appears to be conserved because it is similar to a region of both the human and rat β-actin IVS III (Fig. 9B). The $K_N(1)$ (34) of the comparison of the human β and γ sequences is 0.31. Interestingly, the region of the β-actin IVS III which is similar to intron III of the human γ-actin gene has been shown to be conserved among the human, rat, and chicken β-actin genes (37). Unfortunately, we cannot compare this with the *Xenopus* type 1 actin gene because no sequence data are available for this particular region of the *Xenopus* gene (Cross et al., unpublished data). Nevertheless, it appears that a region of IVS III may contribute to common regulation of β- and γ-actin and that a second region may contribute unique regulation to the type 1 and γ-actin genes.

Chromosome location of human γ-cytoskeletal actin gene. One possible explanation for the coexpression of β- and γ-actin would be chromosomal linkage. Since the human β-actin gene is located on chromosome 7 (37), we determined the location of the γ-actin gene to evaluate the possible contribution of chromosome linkage. Thirty-four human-mouse somatic cell hybrids were tested for the presence of both the human γ-cytoskeletal actin gene and a specific human chromosome. Genomic DNA from each of the hybrid cell lines was digested with *Eco*RI, size fractionated by agarose gel electrophoresis, and transferred to a nitrocellulose filter. The immobilized genomic DNA was probed with the gene-specific fragment γ-IVSIII-HD and autoradiographed. The results from 16 of the hybrids are illustrated in Fig. 10.

The IVS III probe hybridizes to two *Eco*RI-generated DNA fragments in the genomes of the mouse parental cell line and each of the human-mouse cell hybrids. The 20- and 12-kbp mouse DNA fragments are indicated by open arrowheads. Since hybridization of γ-HD to the genome of a single female mouse (Fig. 8) only demonstrates the 20-kbp *Eco*RI fragment, it appears that the mouse γ-actin gene locus may be polymorphic. Only a subset of the human-mouse cell hybrids contain the >23-kbp *Eco*RI-generated fragment from the human γ-actin gene locus (closed arrowhead).

Each cell hybrid was analyzed for its content of human chromosomes by Giemsa staining, mapped enzyme markers, and previously mapped DNA probes (Table 2). Concordant

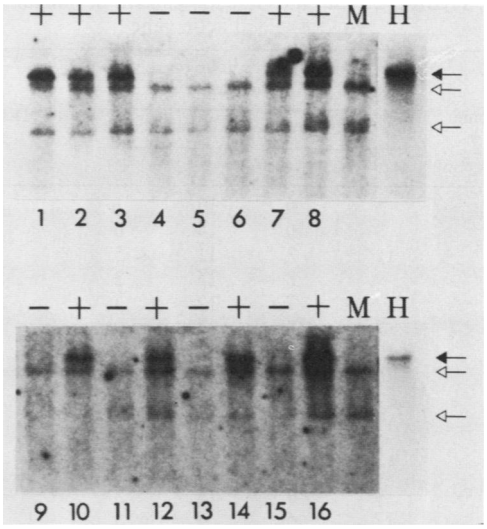

FIG. 10. Detection of the functional human γ-cytoskeletal actin gene in human-mouse somatic cell hybrids. DNA was isolated from somatic cell hybrids (lanes 1 to 16) and the parental mouse (lane M) and human (lane H) cell lines. After digestion with *Eco*RI, the DNA was electrophoresed on a 0.8% agarose gel, transferred to nitrocellulose, and hybridized with the functional human γ-actin gene-specific probe γ-IVSIII-HD. The expressed human γ-cytoskeletal actin gene is located on a >23-kbp *Eco*RI-generated fragment (closed arrowhead). Two mouse DNA fragments (20 and 12 kbp) also hybridize with this probe (open arrowheads). The presence (+) or absence (−) of the >23-kbp *Eco*RI-generated band from the human γ-actin gene locus was scored for each cell hybrid.

hybrids have either retained or lost the functional human γ-actin gene together with a specific human chromosome. Discordant hybrids either retained the gene but not the chromosome or lost the gene but retained the chromosome. All human chromosomes, except chromosome 17, segregate discordantly with the γ-cytoskeletal actin gene locus. The absence of discordancy between chromosome 17 and the human γ-cytoskeletal actin gene is the basis of assignment to this chromosome. Furthermore, hybridization of the γ-specific probe to the genome of the human-mouse cell hybrid NSL-5 (data not shown) with a 17/9 translocation (Table 2) indicates that the functional human γ-cytoskeletal actin gene is located in the region of chromosome 17 between p11 and qter. Thus, we conclude that the functional human β- and γ-actin genes are located on different chromosomes and that linkage cannot be contributing to their coexpression.

Genetic polymorphism of the human γ-cytoskeletal actin gene. A number of our observations have suggested that the functional γ-actin gene locus is highly polymorphic. Originally, we detected three sequence differences between two γ-actin cDNAs isolated from a simian virus 40-transformed human cDNA library (15). We have further noted differences between our cDNAs and the HeLa cell gene (Fig. 4) and also detected an apparent *Eco*RI polymorphism in the mouse (Fig. 10). We therefore decided to evaluate whether the γ-actin gene is truly polymorphic in the human population. Genomic DNA from the sperm of five nonconsanguineous men was digested with the restriction enzymes *Bam*HI and *Ava*I. The digestion products were electrophoresed on an agarose gel, transferred to a nitrocellulose filter, and probed with γ-IVSIII-HD. The autoradiograph is shown in Fig. 11. Two individuals (B and D) are homozygous for the 15-kbp *Bam*HI allele and individual E is homozygous for the 11-kbp

TABLE 2. Chromosome assignment of human γ-cytoskeletal actin gene[a]

Chromosome	Hybridization of probe/presence of chromosome				% Discordancy
	Concordant		Discordant		
	+/+	−/−	+/−	−/+	
1	5	9	19	1	59
2	9	10	15	0	44
3	12	3	11	5	52
4	9	8	15	2	50
5	12	7	12	3	44
6	7	10	17	0	50
7	10	8	14	2	47
8	16	7	8	3	32
9	3	8	18	2	65
10	15	5	9	5	41
11	9	10	15	0	44
12	14	5	10	5	44
13	9	7	15	3	53
14	17	5	7	5	35
15	9	8	14	2	48
16	8	9	16	1	50
17	22	10	0	0	0
18	14	7	10	3	38
19	5	10	19	0	56
20	13	5	11	5	47
21	19	4	5	6	32
22	7	7	17	2	58
X	11	3	11	3	50

[a] Cell hybrids were characterized for both human chromosomes and specific enzyme markers. Concordant results were obtained when γ-actin sequences were present together with a specific chromosome and its enzyme markers or when the γ-actin gene and a specific chromosome and its enzyme markers were both absent. Discordant data refer to cell hybrids that retain the human γ-actin gene but not a specific chromosome or the reverse. Percentage discordancy identifies the total discordancy; no discordancy indicates chromosome assignment.

*Bam*HI allele. *Ava*I digestion also generates two fragments (16 and 14 kbp). The 16-kbp *Ava*I allele cosegregates with the 11-kbp *Bam*HI allele; the 14-kbp *Ava*I allele segregates with the 15-kbp *Bam*HI allele.

Neither the 11- nor the 15-kbp *Bam*HI allele of the human γ-actin gene locus corresponds in size to the 9.2-kbp *Bam*HI-generated fragment isolated from the HeLa genome as λgHγNMA-1. There are two likely explanations for this inconsistency. The limited number of individuals tested for restriction fragment-length polymorphism involving the γ-actin gene locus do not possess the 9.2-kbp *Bam*HI allele. Alternatively, this allele may not occur naturally in the human population, but may have been created by mutation since the initial isolation of the HeLa cell line. In addition, the sizes of the *Ava*I-generated fragments observed in digests of normal human DNA are not consistent with those predicted by the DNA sequence of pgHγNMA-1. We therefore conclude that the human γ-actin gene is truly polymorphic in the human population and that this locus will be useful in linkage analysis of loci mapped to the p11-qter region of chromosome 17.

Human γ-actin gene is expressed and regulated in mouse tissue culture cells. The detection of mutations in the HeLa cell γ-actin gene which may not be present in the human population raises the question of whether the gene is fully functional in its isolated form. Proof that pgHγNMA-1 is fully functional requires the demonstration that this gene is capable of being expressed and regulated. We first introduced this gene into mouse Ltk$^-$ fibroblasts and examined the production of human γ-actin mRNA. We then introduced the gene into the mouse myogenic C2 cell line and examined its regulation when the C2 cells are induced to differentiate and form myotubes.

The two mouse cell lines were cotransfected with plasmid pgHγNMA-1 and the selectable marker pSV2neo (49) in a ratio of 10:1, respectively. Transfectants resistant to the antibiotic G418 were expanded in culture as a total pool. Clones derived from single-cell transfection events were isolated from the pool by dilution cloning and also expanded in culture. Cytoplasmic RNA was isolated from the pooled L-cell clones and from 10 individual cell clones. The RNA was electrophoresed on a denaturing agarose gel, transferred to nitrocellulose, and hybridized with the human γ-actin 3′-UTR probe pHFγA-3′UT-HX which we have found to hybridize to human, but not to mouse, γ-actin mRNA (Erba and Gunning, unpublished data). Figure 12 shows an autoradiogram of this hybridization. Authentic human γ-actin mRNA is detected in the RNA isolated from the pooled clones as well as in all but one (Fig. 12, E-10) of the individual clones. As expected, no signal is observed in RNA from the parental L cells. The level of expression observed in the individual clones shows wide variation. We have measured human γ-actin gene copy number in these clones and have found no obvious correlation between copy number and level of expression (Erba, unpublished observations). Thus, we conclude that the human γ-actin gene we have isolated is capable of generating γ-actin mRNA.

Finally, we demonstrated that the human γ-actin gene is appropriately regulated in the myogenic C2 cells. Pooled C2 transfectants were allowed to differentiate and cytoplasmic RNA was prepared from proliferating myoblasts and 24- and 48-h myotubes. The RNA was electrophoresed on a denaturing agarose gel and blot transferred to nitrocellulose. Three identical blots were produced. Hybridization of the actin-coding region probe α-PX (15) to these RNA preparations reveals that the steady-state level of the endogenous 16S sarcomeric actin transcripts increases 7.5-fold during fusion (Fig. 13, α-PX). Most of this increase is due to

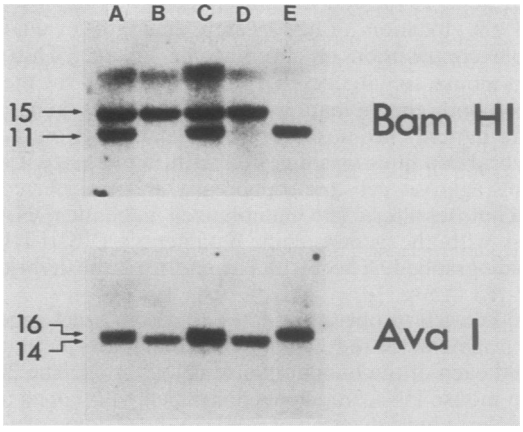

FIG. 11. Restriction fragment length polymorphism of the human γ-cytoskeletal actin gene locus. Genomic DNA, isolated from the sperm of five unrelated men (A, B, C, D, and E), was a gift of Jeremy Nathans. A 6-μg portion of DNA from each individual was restricted with *Bam*HI and *Ava*I, size fractionated on an agarose gel, and transferred to a nitrocellulose filter. The filter was hybridized with the functional γ-gene-specific probe γ-IVSIII-HD, washed, and autoradiographed. The sizes of the gene alleles are indicated in kilobase pairs at the left.

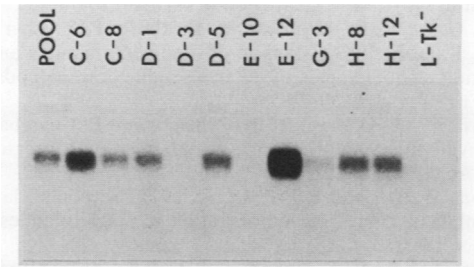

FIG. 12. Expression of the functional human γ-cytoskeletal actin gene in mouse Ltk⁻ cells. Ltk⁻ cells, stably transfected with pgHγNMA-1 and pSV2neo, were selected by growth in G418. Cytoplasmic RNA was prepared from the pool of stable L-cell transfectants, 10 single-cell clones, and the parental L-cell line. The RNA was size fractionated by electrophoresis on a denaturing agarose gel and transferred to nitrocellulose. The filter was hybridized with γ-3'UT-HX, washed, and autoradiographed.

accumulation of the α-cardiac actin mRNA (1). Furthermore, expression of the endogenous 20S β-actin mRNA decreases 2.5-fold during fusion (Fig. 13, β-3'UT-HF), as expected. The source of the nonmuscle actin transcripts in the fusion cultures, whether from unfused myoblasts or the continued low-level expression of these genes in the myotubes, remains uncertain.

The exogenous human γ-cytoskeletal actin gene is expressed in the proliferating transfected myoblasts (Fig. 13, γ-3'UT-HX, lane 1). However, by 48 h in differentiation medium (lane 3), the steady-state level of the human γ-gene transcript has decreased by 7.5-fold. The steady-state level of the exogenous γ-actin gene transcripts, like that of the endogenous γ-actin mRNA, decreases more rapidly than the level of the mouse β-actin gene (1). Therefore, the expression of the exogenous human γ-actin gene parallels the expression of the mouse gene in C2 cells. This establishes that our isolate of the human γ-actin gene contains the information necessary for its appropriate regulation during myogenesis.

DISCUSSION

Differential modulation of β- and γ-actin mRNA levels. As expected, we find that the β- and γ-actin mRNAs are truly coexpressed in the mouse. However, the relative expression of these two mRNAs varies over 60-fold between different tissues. This suggests that the mechanism responsible for determining whether a gene is expressed or silent may be common for both β- and γ-actin but that the level of β- and γ-actin transcript accumulation may be controlled by different mechanisms for each. Indeed, the finding that β-actin mRNA is more constant per microgram of DNA whereas γ-actin mRNA is more constant per microgram of RNA (except liver) indicates that quite separate mechanisms may be determining transcript accumulation of the two. In particular, it is possible that γ-actin, but not β-actin, mRNA levels are regulated in part by total RNA synthesis rates. Elucidation of the mechanisms responsible for this differential expression will require direct measurements of β- and γ-actin transcription rates.

The variability of the steady-state ratio of β-/γ-actin mRNA in mouse tissues supports the argument that actin isoforms may be functionally distinct. In fact, both this study and protein steady-state analysis in the rat indicate that both sarcomeric and aortic smooth muscles have very high levels of β-relative to γ-actin mRNA and protein (39). This suggests a possible specific role for β-actin in some muscle tissues. However, the relationship between steady-state mRNA and protein levels may not be the same in all tissues. For example, the ratio of β-/γ-actin protein is approximately 2 in both rat brain and lung (39), but the ratios of these mRNAs are 2.2 and 5.0 in mouse brain and lung. Furthermore, in mouse liver the β-actin transcript is 100 times more abundant than that of γ-actin, whereas the β/γ protein ratio in rat liver is about 3 (39). Three possible explanations suggest themselves. First, the rat and the mouse may not accumulate β- and γ-actin in the same ratios. Second, there may be differential regulation of translation or protein turnover in different tissues. Alternatively, other actin genes may be contributing β- or γ-like proteins which are included in the protein steady-state measurements. It is clear that the best candidate for the third possibility is mouse liver.

Functional γ-actin gene is probably single copy. The human γ-actin gene family consists of at least 16 members as defined by hybridization with the γ-actin-specific 3'-UTR of our cDNA (15, 42). It is therefore of importance to establish whether the gene we have isolated is functional and single copy in the human genome. The virtual identity of the gene and our γ-actin cDNA together with the expression and regulation of the gene in mouse cells unambiguously demonstrate that we have isolated a functional γ-actin gene. However, this does not rule out the possibility that other γ-actin gene sequences are also expressed. We have isolated 12 other human γ-actin gene sequences from the human genome and by restriction mapping and hybridization demonstrated that none of them contain introns between the codon for amino acid 84 and the termination codon (Erba, unpublished data). Further, we find that none of the γ-actin genes recognized by the 3'-UTR probe hybridize to the IVS

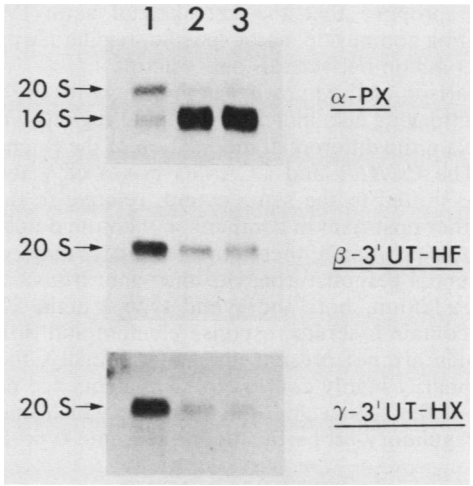

FIG. 13. Regulation of the human γ-cytoskeletal actin gene in the mouse myogenic cell line C2. A pool of C2 cells, stably transfected with pgHγNMA-1 and pSV2neo, were allowed to differentiate in culture. Cytoplasmic RNA was prepared from cells at different stages of in vitro differentiation. The RNA was electrophoresed on a denaturing agarose gel and transferred to nitrocellulose filters in triplicate. The filters were hybridized with an actin-coding region probe (α-PX), a β-specific probe (β-3'UT-HF), and a human-specific, γ-actin-specific probe (γ-3'UT-HX). The panels were washed and autoradiographed. The sizes of the actin transcripts were determined relative to the positions of the 28S and 18S rRNAs. Lane 1, Proliferating myoblasts; lane 2, +24-h myotubes; lane 3, +48-h myotubes.

III sequence derived from the functional gene whereas this same IVS III probe recognizes the mouse γ-actin gene. Therefore, we suggest that the human γ-actin gene family, like that of β-actin (37), consists of a single functional gene and a family of nonfunctional intronless pseudogenes.

γ-Actin gene structure and expression. The precise conservation of intron positions and the similarity of coding, 5′-flanking, and IVS III sequences between the β- and γ-actin genes unambiguously demonstrate that these two genes arose from a gene duplication. Furthermore, the similarity between the human γ and *Xenopus* type 1 actin genes places the time of duplication during early amphibian evolution about 450 million years ago. It is therefore likely that the duplication which generated β- and γ-actin involved duplication of regulatory elements which have resulted in the persistent coexpression of β- and γ-actin. Coexpression is clearly not due to chromosome linkage since the human β-actin gene lies on chromosome 7 (37) whereas that of γ-actin is located on chromosome 17. Sequence elements which determine coexpression of β- and γ-actin are likely held in common between these two genes. Indeed, a subsegment of the 5′-flanking region has been conserved between the β- and γ-actin genes since their origin during amphibian evolution. This region between the CCAAT and CCArGG boxes may be responsible for the coexpression of the cytoskeletal actin genes.

The 5′-flanking regions are not the only noncoding sequences conserved between the cytoskeletal actin genes. All of IVS III has been subject to strong sequence conservation between the human and rat β-actin genes (35, 37). A 68-bp region of IVS III is also conserved between the mammalian and chicken γ-actin genes (37). It is therefore notable that within this 68-bp region lies a 36-bp sequence which is also conserved between the three β-actin genes and the human γ-actin gene. Furthermore, it is only the cytoskeletal actin genes which are interrupted by an intron at codon 121 (IVS III). We propose that the cytoskeletal actin IVS III is contributing nonmuscle, actin-specific, regulatory properties held in common between β- and γ-actin.

Comparison of the human γ-actin gene with the *Xenopus* type 1 actin gene also indicates potential regions which may help explain the differential modulation of the β- and γ-actin genes. The CCAAT and CCArGG boxes of γ and type 1 actin are shifted to the same extent, relative to the TATA box, further upstream in comparison with the β-actin genes. A change in position of these core sequences may contribute to differential responsiveness to the same *trans*-acting factors. In addition, both the γ and type 1 actin 5′-flanking regions contain a serum response element and Sp1-binding sites which are not present in β-actin. Finally, there is an extraordinarily highly conserved 57-bp sequence present in IVS III of the type 1 and γ-actin genes which may confer unique regulatory properties upon the γ and type 1 genes.

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LITERATURE CITED

1. **Bains, W., P. Ponte, H. Blau, and L. Kedes.** 1984. Cardiac actin is the major actin gene product in skeletal muscle cell differentiation in vitro. Mol. Cell. Biol. **4**:1449–1453.
2. **Beltz, G. A., K. A. Jacobs, T. H. Eickbush, P. T. Cherbas, and K. C. Kafatos.** 1983. Isolation of multigene families and determination of homologies by filter hybridization methods. Methods Enzymol. **100**:266–285.
3. **Benoist, C., K. O'Hare, R. Breathnach, and P. Chambon.** 1980. The ovalbumin gene-sequence of putative control regions. Nucleic Acids Res. **8**:127–142.
4. **Benton, W. D., and R. W. Davis.** 1977. Screening lambda gt recombinant clones by hybridization to single plaques in situ. Science **196**:180–182.
5. **Bergsma, D. J., K. S. Chang, and R. J. Schwartz.** 1985. Novel chicken actin gene: third cytoplasmic isoform. Mol. Cell. Biol. **5**:1151–1162.
6. **Blau, H., C. P. Chiu, and C. Webster.** 1983. Cytoplasmic activation of human nuclear genes in stable heterokaryons. Cell **32**:1171–1180.
7. **Breathnach, R., C. Benoist, K. O'Hare, F. Gannon, and P. Chambon.** 1978. Ovalbulmin gene: evidence for a leader sequence in mRNA and DNA sequences at the exon-intron boundaries. Proc. Natl. Acad. Sci. USA **75**:4853–4857.
8. **Breathnach, R., and P. Chambon.** 1981. Organization and expression of eukaryotic split genes coding for proteins. Annu. Rev. Biochem. **50**:349–383.
9. **Chang, K. S., W. E. Zimmer, D. J. Bergsma, J. B. Dodgson, and R. J. Schwartz.** 1984. Isolation and characterization of six different chicken actin genes. Mol. Cell. Biol. **4**:2498–2508.
10. **Childs, G., R. Maxson, and L. Kedes.** 1979. Histone gene expression during sea urchin embryogenesis: isolation and characterization of early and late messenger RNAs of Strongylocentrotus purpuratus by gene specific hybridization and template activity. Dev. Biol. **73**:153–173.
11. **Denhardt, D. T.** 1966. A membrane filter technique for the detection of complementary DNA. Biochem. Biophys. Res. Commun. **23**:641–646.
12. **Dynan, W. S., and R. Tjian.** 1985. Control of eukaryotic messenger RNA synthesis by sequence-specific DNA-binding proteins. Nature (London) **316**:774–778.
13. **Elder, P. K., L. J. Schmidt, T. Ono, and M. J. Getz.** 1984. Specific stimulation of actin gene transcription by epidermal growth factor and cyclohexamide. Proc. Natl. Acad. Sci. USA **81**:7476–7480.
14. **Engel, J. N., P. W. Gunning, and L. H. Kedes.** 1981. Isolation and characterization of human actin genes. Proc. Natl. Acad. Sci. USA **78**:4674–4678.
15. **Erba, H. P., P. Gunning, and L. H. Kedes.** 1986. Nucleotide sequence of the human γ cytoskeletal actin mRNA: anomalous evolution of vertebrate non-muscle actin genes. Nucleic Acids Res. **14**:5275–5294.
16. **Frischauf, A., H. Lehrach, A. Poustka, and N. Murray.** 1983. Lambda replacement vectors carrying polylinker sequences. J. Mol. Biol. **170**:827–842.
17. **Garrels, J. I., and W. Gibson.** 1976. Identification and characterization of multiple forms of actin. Cell **9**:793–805.
18. **Gidoni, D., W. S. Dynan, and R. Tjian.** 1984. Multiple specific contacts between a mammalian transcription factor and its cognate promoters. Nature (London) **312**:409–413.
19. **Graham, F. L., and A. J. van der Eb.** 1973. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology **52**:456–467.
20. **Greenberg, M. E., and E. B. Ziff.** 1984. Stimulation of 3T3 cells induces transcription of the c-*fos*. Nature (London) **311**:433–438.

20a. **Gunning, P. W., G. E. Landreth, M. A. Bothwell, and E. M. Shooter.** 1981. Differential and synergistic actions of nerve growth factor and cyclic AMP in PC 12 cells. J. Cell Biol. **89**:240–245.

21. **Gunning, P., P. Ponte, H. Blau, and L. Kedes.** 1983. Alpha-skeletal and alpha-cardiac actin genes are co-expressed in adult human skeletal muscle and heart. Mol. Cell. Biol. **3**:1985–1995.
22. **Gunning, P., P. Ponte, L. Kedes, R. Eddy, and T. Shows.** 1984. Chromosomal location of the co-expressed human skeletal and cardiac actin genes. Proc. Natl. Acad. Sci. USA **81**:1813–1817.
23. **Gunning, P., P. Ponte, H. Okayama, J. Engel, H. Blau, and L.**

Kedes. 1983. Isolation and characterization of full-length cDNA clones for human alpha-, beta-, and gamma-actin mRNAs: skeletal but not cytoplasmic actins have an amino-terminal cysteine that is subsequently removed. Mol. Cell. Biol. **3:** 787–795.

24. **Kost, T. A., N. Theodorakis, and S. H. Hughes.** 1983. The nucleotide sequence of the chick cytoplasmic actin gene. Nucleic Acids Res. **11:**8287–8301.
25. **Leader, D. P., I. Gall, and H. Lehrach.** 1985. The structure of a cloned mouse γ-actin processed pseudogene. Gene **36:**369–374.
26. **Leube, R., and D. Gallwitz.** 1986. Structure of a processed gene of human cytoplasmic gamma-actin. Nucleic Acids Res. **14:**6339.
27. **Lu, R. C., and M. Elzinga.** 1977. Partial amino acid sequence of brain actin and its homology with muscle actin. Biochemistry **16:**5801–5806.
28. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1982. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
29. **Maxam, A. M., and W. Gilbert.** 1980. Sequencing end-labelled DNA with base-specific cleavage reactions. Methods Enzymol. **65:**499–560.
30. **Melluol, D. D., B. Aloni, J. Calvo, D. Yaffe, and U. Nudel.** 1984. Developmentally regulated expression of chimeric actin genes containing muscle actin DNA sequences in transfected myogenic cells. EMBO J. **3:**983–990.
31. **Messing, J.** 1983. New M13 vectors for cloning. Methods Enzymol. **101:**28–78.
32. **Minty, A. J., and F. Gros.** 1980. Coding potential of non-polyadenylated mRNA in mouse Friend cells. J. Mol. Biol. **139:**61–83.
33. **Minty, A. J., and L. H. Kedes.** 1986. Upstream regions of the human cardiac actin gene that modulate its transcription in muscle cells: presence of an evolutionarily conserved repeated motif. Mol. Cell. Biol. **6:**2125–2136.
34. **Miyata, T., T. Yasunaga, and T. Nishida.** 1980. Nucleotide sequence divergence and functional constraint in mRNA evolution. Proc. Natl. Acad. Sci. USA **77:**7328–7332.
35. **Nakajima-Iijima, S., H. Hamada, P. Reddy, and T. Kakunaga.** 1985. Molecular structure of the human cytoplasmic β-actin gene. Interspecies homology of sequences in the introns. Proc. Natl. Acad. Sci. USA **82:**6133–6137.
36. **Naylor, S. L., A. Y. Sakaguchi, T. Shows, M. L. Law, D. V. Goeddel, and P. W. Gray.** 1983. J. Exp. Med. **157:**1020–1027.
37. **Ng, S.-Y., P. Gunning, R. Eddy, P. Ponte, J. Leavitt, T. Shows, and L. Kedes.** 1985. Evolution of the functional human β-actin gene and its multi-pseudogene family: conservation of the noncoding regions and chromosomal dispersion of pseudogenes. Mol. Cell. Biol. **5:**2720–2732.
38. **Nudel, U., R. Zakut, M. Shani, S. Neuman, Z. Levy, and D. Yaffe.** 1983. The nucleotide sequence of the rat cytoplasmic β-actin gene. Nucleic Acids Res. **11:**1759–1771.
39. **Otey, C. A., M. H. Kalnoski, and J. C. Bulinski.** 1987. Identification and quantification of actin isoforms in vertebrate cells and tissues. J. Cell. Biochem. **13:**113–124.
40. **Palmiter, R. D.** 1974. Magnesium precipitation of ribonucleoprotein complexes: expedient techniques for the isolation of undegraded polysomes and messenger ribonucleic acid. Biochemistry **13:**3606–3615.
41. **Pollard, T. D., and R. R. Weihing.** 1974. Actin and myosin and cell movement. Crit. Rev. Biochem. **2:**1–65.
42. **Ponte, P., P. Gunning, H. Blau, and L. Kedes.** 1983. Human actin genes are single copy for alpha-skeletal and alpha-cardiac actin, but multicopy for beta- and gamma-cytoskeletal genes: 3′-untranslated regions are isotype specific, but are conserved in evolution. Mol. Cell. Biol. **3:**1783–1791.
43. **Proudfoot, N. J., and G. G. Brownlee.** 1976. 3′ Non-coding region sequences in eukaryotic messenger RNA. Nature (London) **263:**211–214.
44. **Rigby, P. W., M. Dieckmann, C. Rhodes, and P. Berg.** 1977. Labeling DNA to high specific activity in vitro by nick-translation with DNA polymerase I. J. Mol. Biol. **113:**237–251.
45. **Sanger, F., S. Nicklen, and A. R. Coulson.** 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. USA **74:**5463–5467.
46. **Shows, T. B.** 1972. Genetics of human-mouse somatic cell hybrids: linkage of human genes for isocitrate dehydrogenase and malate dehydrogenase. Biochem. Genet. **7:**193–204.
47. **Shows, T. B., J. A. Brown, L. L. Haley, M. G. Byers, R. L. Eddy, E. S. Cooper, and A. P. Goggin.** 1978. Assignment of the beta-glucuronidase structural gene to the pter→q22 region of chromosome 7 in man. Cytogenet. Cell Genet. **21:**99–104.
48. **Southern, E. M.** 1975. Detection of specific sequences among DNA fragments separated by gel electrophoresis. J. Mol. Biol. **98:**503–517.
49. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:**327–341.
50. **Tokunaga, K., K. Yoda, and S. Sakiyama.** 1985. Structure of a processed gene of mouse cytoplasmic γ-actin transposed into a BAM5 sequence: insertion has created 13 base-pair direct repeats. Nucleic Acids. Res. **13:**3031–3042.
51. **Treisman, R.** 1986. Identification of a protein-binding site that mediates transcriptional response of the c-*fos*. Cell **46:**567–574.
52. **Vandekerckhove, J., W. Franke, and K. Weber.** 1981. Diversity of expression of non-muscle actin in amphibia. J. Mol. Biol. **152:**413–426.
53. **Vandekerckhove, J., J. Leavitt, R. Kakunaga, and K. Weber.** 1980. Co-expression of a mutant β-actin and the two normal β- and γ-cytoplasmic actins in a stably transformed human cell line. Cell **22:**893–899.
54. **Vandekerckhove, J., and K. Weber.** 1978. Mammalian cytoplasmic actins are the products of at least two genes and differ in primary structure in at least 25 identified positions from skeletal muscle actins. Proc. Natl. Acad. Sci. USA **75:**1106–1110.
55. **Vandekerckhove, J., and K. Weber.** 1979. The amino acid sequence of actin from chicken skeletal muscle actin and chicken gizzard smooth muscle actin. FEBS Lett. **102:**219–222.
56. **Vandekerckhove, J., and K. Weber.** 1979. The complete amino acid sequence of actins from bovine aorta, bovine heart, bovine fast skeletal muscle, and rabbit slow skeletal muscle. Differentiation **14:**123–133.
57. **Vandekerckhove, J., and K. Weber.** 1981. Actin typing on total cellular extracts. A highly sensitive protein-chemical procedure able to distinguish different actins. Eur. J. Biochem. **113:** 595–603.
58. **Vandekerckhove, J., and K. Weber.** 1984. Chordate muscle actins differ distinctly from invertebrate muscle actins. J. Mol. Biol. **179:**391–413.
59. **Weaver, R. F., and C. Weissmann.** 1979. Mapping of RNA by a modification of the Berk-Sharp procedure: the 5′ termini of 15S β-globin mRNA precursor and mature 10S β-globin mRNA have identical map coordinates. Nucleic Acids Res. **7:**1175–1193.
60. **Wilbur, W. J., and D. J. Lipman.** 1983. Rapid similarity searches of nucleic acids and protein data banks. Proc. Natl. Acad. Sci. USA **80:**726–739.
61. **Yaffe, D., U. Nudel, Y. Mayer, and S. Neuman.** 1985. Highly conserved sequences in the 3′ untranslated region of mRNAs coding for homologous proteins in distantly related species. Nucleic Acids. Res. **13:**3723–3737.
62. **Yaffe, D., and O. Saxel.** 1977. Serial passaging and differentiation of myogenic cells isolated from dystrophic mouse muscle. Nature (London) **270:**725–727.