Differential Regulation of Tropomyosin Isoform Organization and Gene Expression in Response to Altered Actin Gene Expression

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Abstract. Phenotypically altered C2 myoblast cells, generated by the stable transfection of human nonmuscle actin genes (Schevzov, G., C. Lloyd, and P. Gunning. 1992. *J. Cell Biol.* 117:775–786), exhibit a differential pattern of tropomyosin cellular organization and isoform gene expression. The β-actin transfectants displaying a threefold increase in the cell surface area, showed no significant changes in the pattern of organization of the high M_r tropomyosin isoform, Tm 2, or the low M_r tropomyosin isoform, Tm 5. In contrast, the γ- and β_{sm}-actin gene transfectants, exhibiting a twofold decrease in the cell surface area, had an altered organization of Tm 2 but not Tm 5. In these actin transfectants, Tm 2 did not preferentially segregate into stress fiber-like structures and the intensity of staining was greatly diminished. Conversely, a well-defined stress fiber-like organization of Tm 5 was observed. The pattern of organization of these tropomyosin isoforms correlated with their expression such that a profound decrease in Tm 2 expression was observed both at the transcript and protein levels, whereas Tm 5 remained relatively unchanged. These results suggest that relative changes in nonmuscle actin gene expression can affect the organization and expression of tropomyosin in an isoform specific manner. Furthermore, this apparent direct link observed between actin and tropomyosin expression suggests that nonpharmacological signals originating in the cytoskeleton can regulate cytoarchitectural gene expression.

THE ability of eukaryotic cells to adopt a variety of shapes and to carry out coordinated and directed movements depends on the cytoskeleton. Many of the components that constitute the cytoskeleton of nonmuscle cells belong to a multigene family (Buckingham and Minty, 1982). The presence of distinct but yet similar cytoskeletal isoforms arising from different genes presumably enables a cell to carry out diverse cellular processes such as cell motility, organelle and chromosome movement, cytokinesis, and the generation of cell shape. At present, however, the biological significance of these multiple contractile protein isoforms is still not fully understood.

In an attempt to address the question of isoform function we have stably transfected human nonmuscle actin genes into a mouse myogenic cell line (Schevzov et al., 1992). We observed that elevated expression of either the β- or γ-actin genes resulted in cells which displayed reciprocal changes in the overall surface area of the cells. Correlated with these changes in cell morphology was a dramatic change in the organization of the actin microfilament network. Additionally, elevated expression of a mutant form of the β-actin gene, one that codes for a stable but aberrant actin protein (β_{sm}), resulted in cells which displayed a phenotype similar to that of the γ-actin transfectants.

The studies presented here were designed to elucidate the possible mechanisms by which β- and γ-actin may elicit the observed changes in cell shape and cytoarchitecture. Earlier studies have shown that different cell types have unique β:γ ratios (Otey et al., 1987) coupled with distinct cytoarchitectures. Analysis of our actin transfectants had revealed that changes in the relative expression of β- and γ-actin had occurred such that different β:γ mRNA and protein ratios were observed (Schevzov et al., 1992). Consequently, is an alteration in the composition of β- and γ-actin alone sufficient to alter cell shape or is a change in the composition of other cytoskeletal components also required? Previous observations have demonstrated that a correlation exists between morphological changes and the expression of tropomyosin, a cytoskeletal component that closely associates with actin microfilaments. For example, morphological alterations coupled with changes in the pattern of tropomyosin gene expression is a general feature of oncogenic transformation (Cooper et al., 1985; Hendricks and Weintraub, 1984; Matsumura et al., 1983). In addition, in vitro cellular differentiation has also been demonstrated to result in cell shape changes accompanied by changes in the expression of particular tropomyosin isoforms (Ben-Ze'ev et al., 1989).

Tropomyosins are rod-shaped dimeric molecules that lie

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head-to-tail along the groove of actin filaments (Smillie, 1979). At present it is known that in skeletal muscle tropomyosin mediates the calcium-ion dependent regulatory effect of the troponin complex on actomyosin ATPase activity, thereby bringing about contraction of the muscle (Huxley, 1972). The biological function of this protein in nonmuscle cells still remains unclear. The observation that tropomyosin can stabilize actin filaments suggests that tropomyosin may play a structural and/or regulatory role in the organization of microfilaments and may ultimately regulate cell shape (Wegner, 1982; Bernstein and Bamburg, 1982). The work of Liu and Bretscher (1989) strongly suggests that this may be the case. In yeast, disruption of a tropomyosin gene results in reduced growth rate, heterogeneity in cell size, and most importantly disruption of actin cables.

In mammals at least five different nonmuscle tropomyosin isoforms have been identified. The high M_r tropomyosin isoforms (Matsumura and Yamashiro-Matsumura, 1985) are 284 amino acids in length, have apparent molecular masses on SDS-polyacrylamide gels of 40, 36.5, and 35 kD and are commonly known as Tm 1, 2, and 3, respectively. The low M_r tropomyosins are 248 amino acids in length, have apparent molecular masses of 32.4 and 32 kD and are commonly known as Tm 4 and 5, respectively (Matsumura et al., 1983). Recently two more 248 amino acid tropomyosins, Tm 5a and 5b, have been found in rat fibroblasts (Lees-Miller et al., 1990). These different isoforms are encoded by a multigene family and in mammals four tropomyosin genes have been characterized. In nonmuscle cells the β-Tm gene encodes Tm 1 (MacLeod et al., 1985; Helfman et al., 1986); α-Tm_f gene encodes Tm 2, 3, 5a and 5b (Wieczorek et al., 1988; Goodwin et al., 1991); and the Tm 4 gene encodes Tm 4 (MacLeod et al., 1987; Yammawaki-Kataoka and Helfman, 1987). The fourth gene termed hTm_{nm} by Reinach and MacLeod (1986) also known as the αTm_s gene by Gunning et al. (1990) since the muscle product of this gene is preferentially expressed in slow twitch skeletal muscle, codes for Tm 5, also known as fibroblast $TM30_{nm}$ (Clayton et al., 1988). The Tm_{nm} gene has until recently only been characterized in humans, however, a recent report by Takenaga et al. (1990) demonstrates that the equivalent gene exists in rodents.

In an attempt to understand the morphological changes observed in our actin transfectant clones, we have investigated the cellular organization of two different tropomyosin isoforms by immunofluorescence. We also examined the expression of nonmuscle tropomyosin isoforms using isoform specific cDNA probes. We have found isoform specific changes to tropomyosin organization and gene expression in the different actin gene transfectant clones suggesting that a feedback regulatory mechanism exists for cytoskeletal organization and gene expression of its components.

Materials and Methods

Cell Culture and Transfection

Actin transfectants were generated by the stable transfection of human nonmuscle actin genes into mouse C2 myoblasts as previously described in Schevzov et al. (1992). In brief, ~20 clones from each transfection were randomly selected. All these clones were analyzed for expression of the exogenous nonmuscle actin genes and only those expressing the exogenous gene at a significant level were chosen for further morphological and biochemical assays.

Immunofluorescence Staining

Cells were seeded on collagen-(Calf Skin Collagen, Calbiochem., Alexandria, NSW, Australia) coated glass slides for 24 h before fixing and permeabilizing in 1% formaldehyde followed by −20°C methanol. Nonspecific binding on slides was blocked by incubation at 37°C in PBS containing 10% FCS. Antibody incubations were carried out at 37°C for 2 h. Slides were washed extensively in PBS between antibody incubations and finally mounted with 1,4-Diazabicyclo[2.2.2]octane (Sigma Chem. Co., St. Louis, MO). Cells were examined and photographed on a Zeiss epifluorescence microscope with a 40× objective 0.75 NA. Photographs were taken with Kodak T-Max 400 ASA film, developed with D-76, and printed on Agfa b/w paper. For matched pairs of double-labeled cells, exposure times for photography and printing were identical.

Antibodies

The Tm 5 mAb used at 1:1,000 dilution was provided by Drs. J. J.-C. and J. L.-C. Lin and characterized in chicken tissues in Lin et al. (1985*a*) and in human EJ bladder carcinoma cells in Lin et al. (1988). The TM311 mAb used at 1:400 dilution was purchased from Sigma Chem. Co., St Louis, MO. The rabbit antibody which specifically recognizes the B_{sm}-actin protein (anti-asp) used at 1:50 dilution was provided by Dr. U. Aebi (Leavitt et al., 1987*a*). The goat anti-mouse Ig's (G + L) FITC and goat anti-rabbit IgG rhodamine were purchased from Tago Inc. (Burlingame, CA).

One and Two-Dimensional Gel Electrophoresis and Western Transfer

Total cellular protein was isolated from 30% confluent cells grown in 100-mm plastic dishes. Samples were adjusted to equal protein concentrations by the method of Lowry et al. (1951) and electrophoresed on 12.5% SDS-PAGE gels according to Laemmli (1970) followed by Western blotting according to Towbin et al. (1979). Antibody binding was visualized by incubation with 1:250 dilution of a mouse Ig ^{35}S-labeled antibody (Amersham Australia, North Ryde, NSW, Australia) followed by exposure to Kodak XAR film (Kodak, North Ryde, NSW, Australia) for 1 d or goat anti-mouse alkaline phosphatase (Sigma Chem. Co.). The resulting autoradiographs were scanned using a Model 300 series computing densitometer (Molecular Dynamics, Inc., Sunnyvale, CA). To correct for any loading discrepancies, duplicate gels were run concurrently and one stained with Coomassie brilliant blue. The levels of protein of each tropomyosin isoform were expressed as a percentage of that seen in the control cells which was set to 100%. Two-dimensional gel electrophoresis was performed essentially as described (Lin et al., 1988) with the first dimension containing pH 4–6 ampholines and the second dimensional slabs containing 12.5% acrylamide and 0.104% bis-acrylamide.

DNA Probes

Human Nonmuscle Actin. Probes specific for the human β- and γ-actin mRNAs and which do not crosshybridize with the mouse mRNAs, pHFβA-3′UT-HH and pHFγA-3′UT-HX, have been characterized previously (Ng et al., 1985; Erba et al., 1986). These DNA fragments were used to measure the level of expression of the transfected human gene in all the mouse C2 clones relative to that in a human myoblast sample.

Tm 2 and 3. A probe specific for Tm's 2 and 3 (pRαTm-1A) was generated by PCR DNA amplification by Dr. R. Weinberger (Weinberger et al., 1992). The entire exon 1a and 79bp of 5′UTR from the rat α-Tm_f gene was amplified using the following oligonucleotides 5′GTG GAT CCG CTC GCA CTC CGG CTC TGC CAC 3′ and 5′ GGT AGA CGC AGA CCT GCT TGC 3′ (Lees-Miller et al., 1990). Amplification was carried out using rat genomic DNA. This probe detects only the 2.0-kb transcript and does not hybridize to the Tm 5a and 5b transcripts also produced by the same gene (α-Tm_f).

Tm 5a and 5b. A probe specific for Tm 5a and 5b (pRαTm-1B) was generated by PCR DNA amplification by Dr. R. Weinberger (Weinberger et al., 1992). The entire exon 1b and 78bp of 5′UTR from the rat α-Tm_f was amplified using oligonucleotides 5′ GGC TCC GTA ACT GCA GGA GCC 3′ and 5′ TGA TGG GTG GAT CCC TAA CGG 3′ (Lees-Miller et al., 1990). Amplification was carried out using rat genomic DNA. This probe detects a 2.0-kb transcript.

Tm 5. The Tm 5 nonmuscle cDNA (pMMTm5) was isolated from a mouse macrophage cDNA library (Clontech Laboratories Inc., Palo Alto, CA). The library was screened with a ^{32}P-labeled cDNA fragment contain-

ing the coding sequence for amino acids 213–284 and 48 bp of 3′UTR of the human α-Tm_5 cDNA. Hybridization was in 4× SSC (1× SSC is 0.15 M NaCl, 0.015 M sodium citrate), 50 mM NaH_2PO_4 (pH 7.0), 5× Denhardt's solution (Denhardt, 1966) at 65°C for 16 h, followed by three 30 min washes in 0.5× SSC, 50 mM NaH_2PO_4 (pH 7.0), 0.1% SDS at 65°C. Partial sequence analysis of an isolated positive clone revealed that it is the mouse homologue of the human Tm 5 (Fig. 1, *A* and *B*). Furthermore, the sequence obtained was identical to that of Takenaga et al. (Takenaga et al., 1990, accession number X53753) which unambiguously demonstrates that it is the mouse Tm 5 nonmuscle isoform. The probe used for Northern blot analysis was generated by PCR amplification using the 5′ primer 5′ CCC TGC TGG ACC TGA ACG AG 3′ and the 3′ primer 5′ ACC CCG CAG TGC AAC TGT TT 3′ (denoted in Fig. 1). The reaction was performed with 100 ng of pMMTm5 and 1 μg of each primer at an annealing temperature of 55°C. A 1,200-bp fragment was amplified corresponding to the entire 3′UTR of Tm 5 and which showed no identity to other Tm sequences (data not shown). This fragment was subcloned into the HincII site of pGEM3Zf, to give the plasmid pMMTm5-3′UT. This probe detects a 2.5-kb transcript in nonmuscle tissues.

DNA Sequence Analysis

DNA sequencing was carried out by the dideoxy chain termination method (Sanger et al., 1977). Double stranded plasmid DNA was denatured to give single stranded DNA using 200 mM sodium hydroxide and 2 mM EDTA, and precipitated from ethanol plus sodium acetate and resuspended in water.

Northern Blots

Total cellular RNA was isolated from 30% confluent cells by the method described by Chomczynski and Sacchi (1987). Total RNA was denatured and electrophoresed on 1% agarose gels containing 2.2 M formaldehyde and transferred to Hybond N+ (Amersham Australia, North Ryde, NSW, Australia) as described in Maniatis et al. (1982). Probes were labeled by the random priming method (Feinberg and Vogelstein, 1983) and hybridized to RNA blots at 10^9 dpm/μg of DNA in a solution containing 4× SSC, 50 mM NaH_2PO_4 (pH 7.0), 5× Denhardt's solution (Denhardt, 1966), and 10% (wt/vol) dextran sulfate at 65°C for 16 h. After hybridization, the blots were washed at 65°C for 2 h in 0.5× SSC, 0.1% SDS. Filters were exposed to Kodak XAR film for 1–3 d. To verify that equivalent amounts of RNA were transferred, the RNA blots were hybridized to an 18S specific ribosomal RNA oligonucleotide probe under conditions of probe excess and washed at 55°C in 4× SSC, 0.1% SDS. Levels of mRNA were quantitated by densitometry as described (Gunning et al., 1990).

Results

Specificity of Tropomyosin cDNA Probes and Antibodies

Even though the work of Helfman and coworkers (Goodwin et al., 1991; Lees-Miller and Helfman, 1991) suggests that only three tropomyosin genes exist in rodents, Takenaga et al. (1990) have in fact cloned and characterized the mouse equivalent of the fourth human tropomyosin gene, Tm_{nm}. This gene encodes the mouse nonmuscle Tm 5 protein which differs to the human Tm 5 (also known as TM30nm) by a single amino acid. In parallel, we also screened a mouse macrophage cDNA library with a cDNA fragment from the human αTm_5 cDNA (see Materials and Methods). Partial sequence analysis was performed on the 3′UTR of the clone obtained (Fig. 1 *A*). A comparison between our clone (pMMTm5) and that obtained by Takenaga et al. (Takenaga et al., 1990) unambiguously demonstrates that it is the mouse nonmuscle Tm 5 isoform. This clone which shares high homology to the human Tm 5 nonmuscle isoform (Takenaga et al., 1990) differs from human Tm 4 (MacLeod et al., 1987) and rat Tm 4 (Yamawaki-Kataoka and Helfman, 1987). The probe used for subsequent Northern blot analysis was generated by PCR

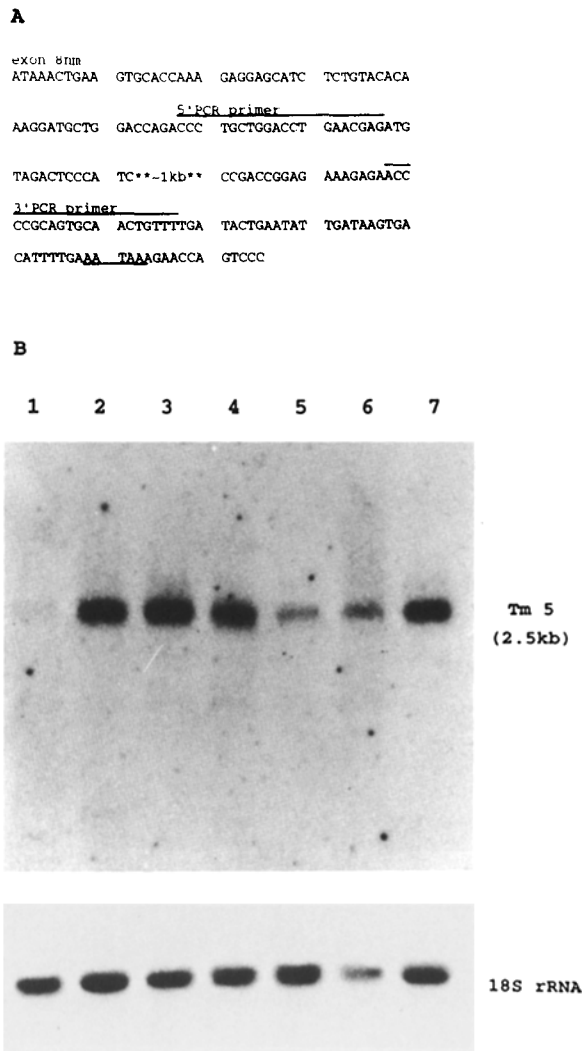

Figure 1. Partial nucleotide sequence and Northern blot showing the specificity of the pMMTm5-3′UT clone. (*A*) Nucleotide sequence at the 5′ and 3′ end of the nonmuscle exon VIII. The PCR primers used to generate a Tm 5 isoform specific probe are indicated. The polyadenylation site is underlined. Approximately 1,000 bp have not been sequenced yet and are denoted as ***~1kb***. (*B*) Northern blot indicating the specificity of the PCR generated Tm 5-specific probe. Total cellular RNA was isolated from nonmuscle mouse tissues and NIH-3T3 cells. 5 μg of RNA was size fractioned and hybridized with the Tm 5 probe (pMMTm5-3′UT) as described in Materials and Methods. The Northern blot was subsequently probed with 18S ribosomal RNA to correct for loading discrepancies. (Lane *1*) gastrocnemius; (lane *2*) brain; (lane *3*) kidney; (lane *4*) testes; (lane *5*) liver; (lane *6*) stomach; and (lane *7*) NIH-3T3.

as described in Materials and Methods and the primers used are denoted in Fig. 1 *A*. Northern blot analysis using adult mouse tissues confirms that mouse Tm 5 has the expression pattern characteristic of a nonmuscle tropomyosin (Fig. 1 *B*).

The specificity of the two tropomyosin antibodies used in this study was determined on total cell extract isolated from Rat-1, C2 parental cell line, NIH-3T3, and a pUC18 transfected C2 clone. As illustrated in Fig. 2 *A*, the TM311 antibody recognizes two bands of size 40 and 36.5 kD corre-

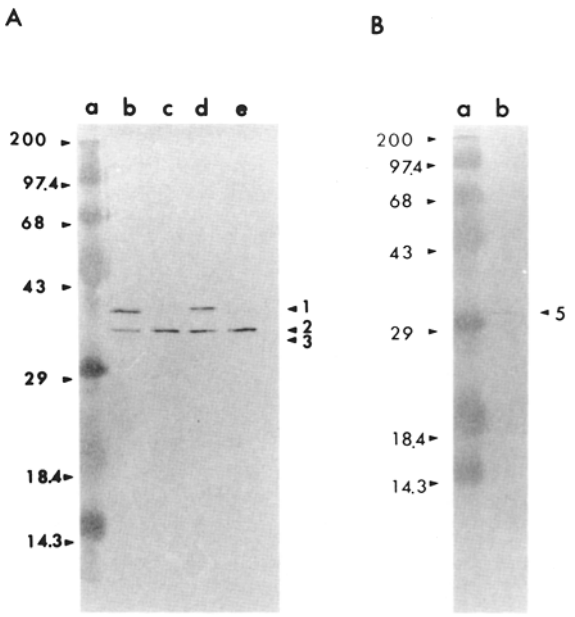

Figure 2. Western blot analysis showing the specificity of two tropomyosin mAbs. Total cell extracts were electrophoresed on a 12.5% polyacrylamide gel, transferred to nitrocellulose, and immunoblotted with a Sigma Chem. Co. tropomyosin mAb (TM311) (*A*) or a Tm 5 mAb (*B*). Antibody binding was visualized by incubating the blot with a mouse ^{35}S-labeled IgG. (*A*, lane *a*) prestained molecular weight standards; (lane *b*) Rat-1; (lane *c*) C2 parental cell line; (lane *d*) NIH-3T3; and (lane *e*) pUC18 transfected C2 clone. (*B*, lane *a*) prestained molecular weight standards and (lane *b*) pUC18 transfected C2 clone. The migration positions of the mouse tropomyosin isoforms are indicated by the numbers 1, 2, 3, and 5. Note that the Sigma Chem. Co. TM311 antibody primarily detects Tm 2 in C2 myoblasts and the Tm 5 antibody detects only one band.

sponding to Tm 1 and Tm 2 and a much weaker band of 35 kD (Tm 3) in the Rat-1 and NIH-3T3 cell extracts (lanes *b* and *d*). In the C2 and pUC18 transfected cells (Fig. 2 *A*, lanes *c* and *e*), the major protein recognized by the antibody is Tm 2. Two minor bands corresponding to Tm 1 and 3 are also detected in these samples if the immunoblot is overdeveloped or overloaded. No reaction is seen with Tm 4 and Tm 5 (32.4 and 32 kD) despite the expression of all these tropomyosin isoforms in C2 cells (Gunning et al., 1990). The Tm 5 antibody (CG3) previously characterized by Lin et al. (1988) and found to detect only the Tm 5 isoform in human EJ bladder carcinoma cells is shown to detect only one band of 32 kD in a pUC18 transfected cell extract (Fig. 2 *B*, lane *b*). Since Tm 4 and Tm 5 appear to migrate to the same position in SDS-PAGE gels, the cross-reactivity of this antibody to Tm 4 cannot be determined from one-dimensional gel electrophoresis. To address this issue, two-dimensional gel analysis was performed to unambiguously demonstrate that this antibody does not cross-react with Tm 4. As shown in Fig. 3 *B* the Tm 5 antibody detects only one isoform. To demonstrate that the different tropomyosin isoforms were effectively separated, the same Western blot was reprobed with the Sigma Chem. Co. TM311 antibody. As

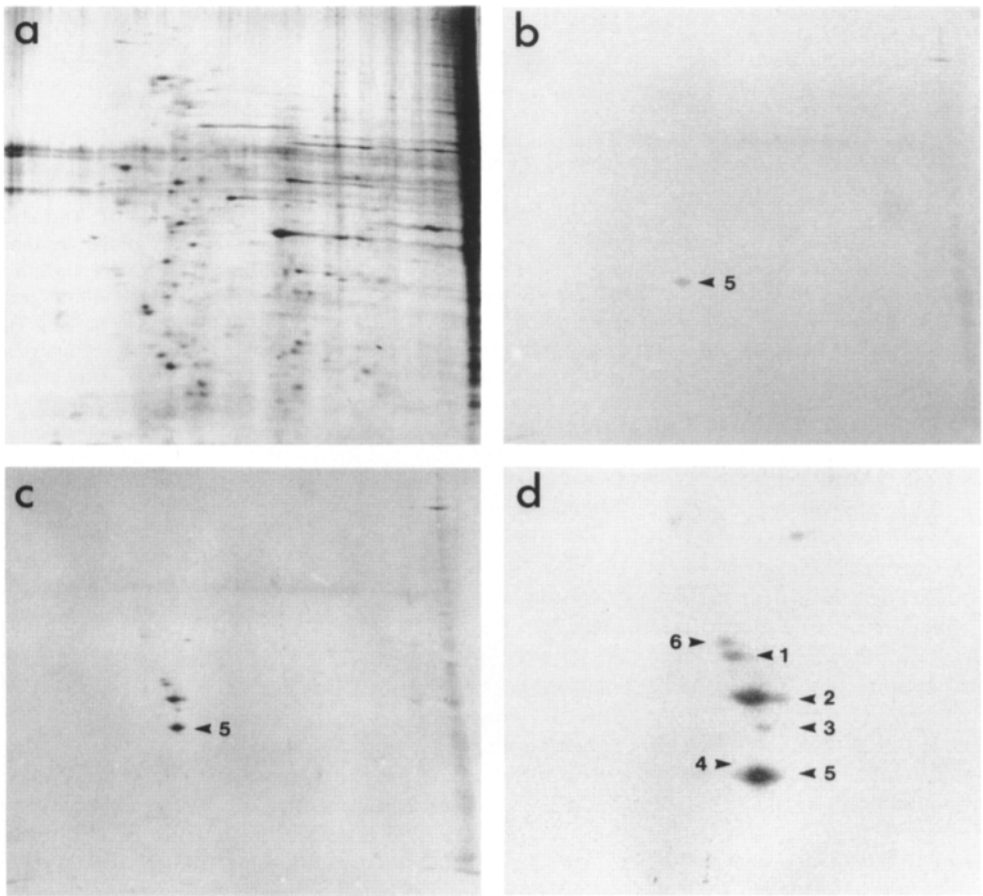

Figure 3. Two-dimensional gel electrophoresis showing specificity of the Tm 5 antibody. Total cellular protein from normal C2 cells was run with the first dimensional gel containing pH 4–6 ampholines and the second dimensional slab gel containing 12.5% acrylamide, 0.104% bisacrylamide. (*A*) Coomassie blue–stained gel, (*B*) immunoblot with the Tm 5 mAb, (*C*) the same immunoblot reacted with the Sigma Chem. Co. tropomyosin mAb (TM311) followed by goat anti–mouse alkaline phosphatase, and (*D*) an enlargement of *C*. Gels are shown with acidic ends to the left. Nonmuscle tropomyosin isoforms are indicated by the numbers 1–6. As shown, the Tm 5 mAb preferentially detects Tm 5.

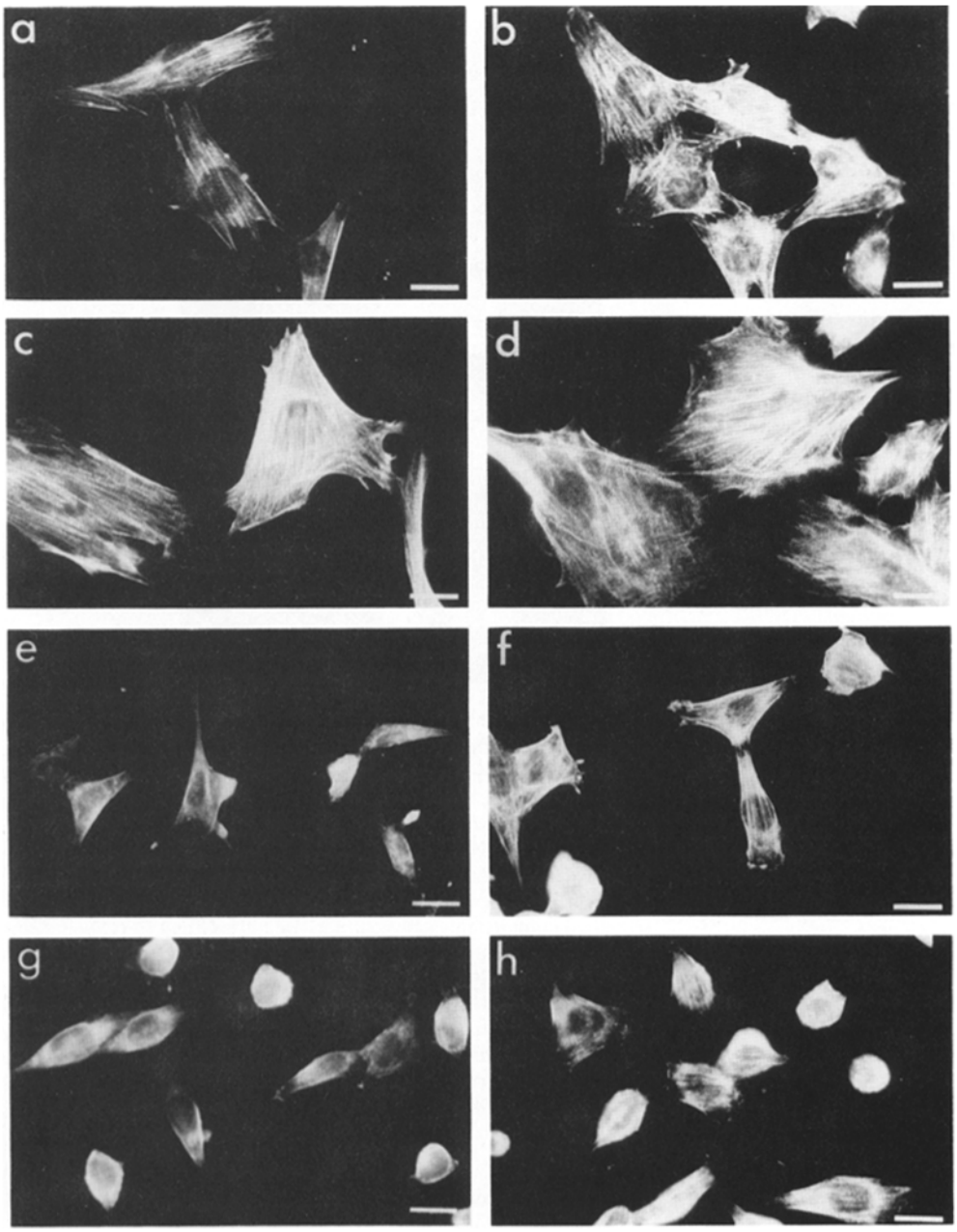

Figure 4. Cytoarchitectural localization of tropomyosin isoforms in the highest expressing actin transfectants. Actin transfectant clones with the highest level of expression of the exogenous genes, β_{tm}, β_{wt}, β_{sm}, and γ-actin genes were immunofluorescently stained with the Tm 2 or Tm 5 antibodies. (*a*, *c*, *e*, and *g*) Cells immunofluorescently stained with the Tm 2 mAb (TM311) followed by rhodamine-conjugated goat anti-mouse IgG. (*b*, *d*, *f*, and *h*) Cells immunofluorescently stained with the Tm 5 mAb followed by rhodamine-conjugated goat anti-mouse IgG. Cells were prepared and treated as described in Materials and Methods. (*a* and *b*) β_{tm}-33; (*c* and *d*) β_{wt}-11; (*e* and *f*) γ_{33}-B1; and (*g* and *h*) β_{sm}-22. Bar, 10 μm. Note that both the β_{sm}-22 and γ_{33}-B1 showed a diffuse organization of Tm 2 but displayed a stress fiber-like organization of Tm 5.

shown in Fig. 3, *C* and *D*, overexposure of the TM311 antibody reveals a clear separation of Tm's 1, 2, 3, and 6. Therefore, we can conclude that the Tm 5 antibody does not cross-react with Tm 4. There is, however, a formal possibility that this antibody may cross-react with Tm 5a and 5b. To assess the potential contribution of Tm 5a + 5b, we have hybridized the α-Tm_f gene exon 1a (see Tm 2 + 3) and exon 1b (see Tm 5a + 5b) probes in parallel to identical filters containing C2 cell RNA. We observed only trace hybridization of the 1B probe and estimate that the level of Tm 5a + 5b mRNA is <5% of that of Tm 2 + 3 (data not shown). Consequently, we expect that subsequent cellular organization studies performed with this antibody will give a pattern of tropomyosin organization that corresponds predominantly to Tm 5.

Cellular Organization of Tropomyosin Isoforms in the Actin Transfectants

The cellular organization of tropomyosin was investigated in the different actin transfectants using the two distinct tropomyosin antibodies described above. Actin transfectant clones exhibiting the highest level of expression of the exogenous actin genes were chosen for the following studies because these cells showed the most severe phenotypic changes (Schevzov et al., 1992). The control cells used are C2 myoblasts transfected with a triple mutant form of the human β-actin gene (β_{tm}), previously shown to cause no detectable changes in cell morphology or the actin microfilament network (Schevzov et al., 1992). Immunofluorescence staining

revealed that the highest expressing β_{tm} clone (β_{tm}-33) displayed a stress fiber-like organization of both Tm 2 and Tm 5 (Fig. 4, *a* and *b*). The observed organization of tropomyosin is the same as that seen by Lazarides (1975) and Lin et al. (1988) in other nonmuscle cell types. Similarly, the highest expressing β_{wt} clone (β_{wt}-11) also showed a stress fiber-like organization of Tm 2 and Tm 5 (Fig. 4, *c* and *d*). However, a diffuse organization of Tm 2 was consistently observed in the highest expressing γ-actin clone (γ_{33}-B1) (Fig. 4 *e*) as compared to the control cells (photo *a*). In contrast, the γ_{33}-B1 cells displayed highly visible stress fiber-like organization of Tm 5 (Fig. 4 *f*). This indicates that actin transfectants which display different morphologies may also show a differential pattern of tropomyosin organization.

Elevated expression of the β_{sm} gene has previously been found to elicit a similar phenotype to that of the γ-actin transfectants. This includes reduced surface area and diffuse organization of the actin cables (Schevzov et al., 1992). These similarities also extend to the pattern of expression of the endogenous actin genes (Lloyd et al., 1992). Do cells that exhibit similar alterations in their morphologies and actin stress fiber organization also show common changes in tropomyosin isoform cellular organization and expression?

The highest expressing β_{sm} clone (β_{sm}-22) displayed a diffuse organization of Tm 2 (Fig. 4 *g*) similar to that observed in the γ_{33}-B1 cells (Fig. 4 *e*). Conversely, as in the case of the high expressing γ-actin transfectants, γ_{33}-B1, a stress fiber-like organization of Tm 5 staining was observed in many β_{sm}-22 cells.

Thus, the impact of high level expression of the β_{sm}-actin gene on Tm 2 and Tm 5 organization parallels that seen with the introduced γ-actin gene, but differs from that in the high expressing β_{wt} transfectants and the control cells. Hence, elevated expression of two distinct actin genes (β_{sm} and γ-actin) which lead to similar changes in morphology and actin cable organization are also found to elicit similar changes in the cytoskeletal organization of Tm 2 but not Tm 5.

Expression of the β_{sm} and γ-actin Genes Leads to Significant Changes in the Intensity of Tropomyosin Staining

The β_{sm}-22 and γ_{33}-B1 cells displayed a substantial reduction in the intensity of Tm 2 but not Tm 5 immunofluorescent staining (Fig. 4). To visualize this differential staining intensity, C2 parental cells were cocultured with either the β_{sm}-22 or the γ_{33}-B1 cells. The β_{sm}-22 cells could be discriminated from the C2 parental cells by immunofluorescent staining with the antibody specific for the aberrant β_{sm}-actin protein (anti-asp, Leavitt et al., 1987*a*). Consequently, the cells were reacted with both the anti-asp and either the Tm 2 or the Tm 5 antibody. The use of two different secondary antibodies, goat anti-rabbit rhodamine to detect the anti-asp and anti-mouse FITC to detect the Tm 2 or the Tm 5 allowed us to make a direct comparison of staining intensity on the same microscopic field. As illustrated in Fig. 5, the cells which stained for the aberrant β_{sm} protein, photo *a*, show negligible staining for Tm 2, photo *b*, as compared to the control cells present in the same field. In contrast, Tm 5 staining intensity was equivalent in the β_{sm} expressing cells and control C2 cells (Fig. 5 compare *c* and *d*).

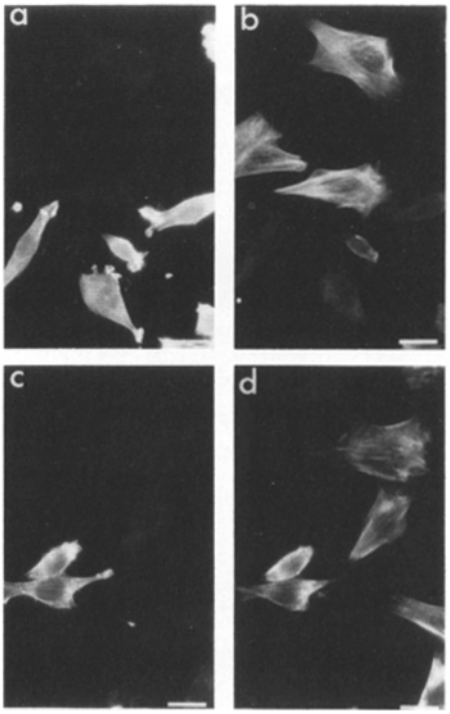

Figure 5. Elevated expression of the β_{sm} gene leads to significant changes in the intensity of the tropomyosin staining. Indirect double-label immunofluorescence of β_{sm}-22 cells cocultured with normal C2 cells. A total of 2,500 C2 parental cells were seeded together with 2,500 β_{sm}-22 cells on collagen-coated glass slides. Cells were prepared and treated as described in Materials and Methods. (*a* and *b*) Cells in the same microscopic field were reacted with both the anti-asp and the Tm 2 mAb (TM311), followed by rhodamine-conjugated goat anti-rabbit IgG and FITC-conjugated goat anti-mouse IgG, respectively. Similarly, (*c* and *d*) cells were reacted with both the anti-asp and the Tm 5 mAb. Photos *a* and *c* show the anti-asp localization and *b* and *d* show the localization of Tm 2 and Tm 5, respectively. Bar, 10 μm. Note the decrease in staining intensity of the β_{sm}-22 cells with the Tm 2 antibody as compared to control cells but no change in staining intensity with the Tm 5 antibody.

A similar observation was made with cocultures of C2 parental cells and γ_{33}-B1 cells. In this case the detection of the γ-actin transfectants was based on their morphology and cytoarchitecture (data not shown). In light of these results, we conclude that differential expression at the protein level of Tm 2 and Tm 5 may occur in these actin transfectants.

Distinct Expression of Tropomyosin Isoforms in the Actin Transfectants

It is conceivable that the decrease in intensity observed with the Tm 2 antibody might be due to epitope blocking in the β_{sm} and γ-actin cells. To eliminate this possibility, the steady-state level of Tm 2 protein was determined by SDS-PAGE gel electrophoresis followed by immunoblotting (Fig. 6). Densitometric scans of the immunoblot revealed that in the β_{sm}-22 and γ_{33}-B1 cells, Tm 2 was decreased by fivefold and fourfold, respectively, whereas the β_{wt}-11 cells showed no significant changes in Tm 2 levels as compared to the control cells (β_{tm}-33). We conclude, therefore, that the ob-

served decrease in the immunofluorescence staining intensity of Tm 2 in the β_{sm}-22 and γ_{33}-B1 cells corresponds to a decrease in the steady-state protein level.

The changes in protein content parallel changes in the level of mRNA accumulation. Since the highest expressing clone from each of the actin transfectants has been shown to elicit the most severe phenotypic changes, tropomyosin mRNA levels were initially analyzed in these clones. The levels of tropomyosin transcript accumulation in the β_{tm}-33, β_{wt}-11, γ_{33}-B1, and β_{sm}-22 cells (expressed as a percentage of that found in a pUC18 transfected cell), were determined by the statistical analysis of mRNA values obtained from multiple Northern blot determinations (Table I). In the β_{tm}-33 cells no change in the level of expression of Tm 2, 3 was observed (Table I). An unexpected result was the downregulation in Tm 5 transcript levels. It appears that the presence of an extra actin transcript coding for an unstable protein can impact on Tm 5 expression at the mRNA level. The highest expressing β_{wt} transfectant, β_{wt}-11, revealed an increase in mRNA levels for the tropomyosin isoforms studied (Table I). This is interesting since no changes in the protein levels of Tm 2 occurred (103 ± 15; $n = 2$). One possible explanation for this discrepancy between protein and mRNA levels could simply be due to the fact that the antibody only detects Tm 2 protein, whereas for the transcript data the probe used detects both Tm 2 and Tm 3 transcript. Hence, the increase in transcript may be due to the contribution from Tm 3. In contrast, the

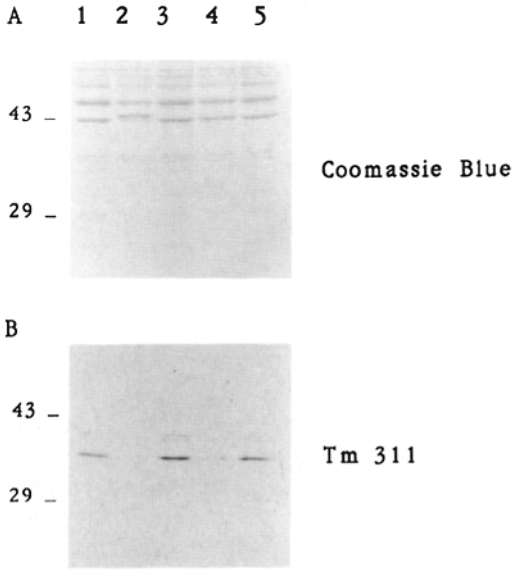

Figure 6. Western blot analysis showing the distinct expression of Tm 2 in the highest expressing actin transfectants. Total cell extract isolated from control cells and the actin transfectants with the highest level of expression of the exogenous genes, β_{wt}, β_{sm}, β_{tm}, and γ-actin genes were electrophoresed on a 12.5% polyacrylamide gel. Duplicate gels run concurrently were either Coomassie blue stained (*A*) or immunoblotted with the Sigma Chem. Co. tropomyosin mAb (TM 311) (*B*). Antibody binding was visualized by incubating the blot with the mouse-^{35}S labeled IgG. (*A* and *B*, lane *1*) β_{wt}-11; (lane *2*) β_{sm}-22; (lane *3*) β_{tm}-33; (lane *4*) γ_{33}-B1; and (lane *5*) pUC18 transfected clone. Migration of molecular mass standards are indicated by the numbers 43 and 29 kD. Note the decrease in Tm 2 protein in the β_{sm}-22 and γ_{33}-B1 cells.

Table I. Multiple Determinations of Tropomyosin Isoform Gene Expression in the Highest Expressing Actin Transfectants

Clone	Tm 2,3	Tm 5
pUC-A	100	100
βtm-33	112 ± 15 *$n = 5$	52 ± 8 $n = 5$
βwt-11	151 ± 18 $n = 2$	128 ± 3 $n = 2$
βsm-22	12 ± 1 $n = 4$	154 ± 31 $n = 4$
γ33-B1	11 ± 0.6 $n = 4$	82 ± 10 $n = 3$

The level of transcript accumulation of the different Tm isoforms was determined in the highest expressing actin transfectants from independent RNA isolations. The mRNA values are expressed as a percentage of that found in a pUC18 transfected clone (pUC-A) which was set to 100% and are represented as a mean ± SD.
* n denotes the number of RNA isolations.

γ_{33}-B1 and β_{sm}-22 cells revealed a decrease in transcript accumulation of Tm 2, 3 but not Tm 5 (Table I). For these transfectants, the immunofluorescence staining intensity and steady-state protein levels coincide with the observed changes in Tm 2 and Tm 5 mRNA levels.

Subsequent analysis of tropomyosin expression was performed on actin transfectant clones which expressed the exogenous actin genes at different levels. This enabled the evaluation of the relationship between tropomyosin and exogenous actin gene expression.

Northern blots were quantitated by densitometry and the results are depicted graphically. Linear regression analysis was performed on the data points to determine if significant linear relationships exist between the levels of expression of the transfected actin genes and that of the tropomyosin isoforms. Elevated expression of the β_{tm} gene resulted in no changes in the mRNA levels of Tm 2, 3 (Fig. 7 *A*), whereas a decrease in Tm 5 was observed (Fig. 7 *B*). These results were confirmed by regression analysis which demonstrated that no linear relationship was found to exist between the level of the human β_{tm} gene expression and Tm 2, 3 ($R = 0.57$, $p > 0.1$) mRNA levels. Surprisingly, a linear relationship was found to exist between β_{tm} gene expression and Tm 5 mRNA levels ($R = 0.95$, $p < 0.01$) suggesting that expression of the human β_{tm} gene progressively affected Tm 5 expression.

The impact of elevated expression of the human wild-type β-actin gene on the levels of tropomyosin isoform mRNAs is depicted graphically in Fig. 7, *C* and *D*. No linear relationship was found to exist between the levels of human β_{wt} gene expression and Tm 2, 3 ($R = 0.66$, $p < 0.1$). The low expressing β_{wt} transfectants showed a heterogeneous response in Tm 2, 3 transcript levels, and a substantial increase in Tm 2, 3 transcript levels was found to occur only in the highest expressing β_{wt} transfectant, β_{wt}-11 (Table I). In the case of the low M_r tropomyosin isoform (Tm 5) no linear relationship was found to exist between the levels of human β_{wt} gene expression and Tm 5 ($R = 0.67$, $p < 0.1$).

Impact of expression of the human γ-actin gene on the levels of tropomyosin isoform mRNAs is depicted graphically in Fig. 8, *A* and *B*. A linear relationship was found to exist between the levels of human γ-actin gene expression and Tm 2, 3 ($R = 0.89$, $p < 0.025$). Consequently, elevated expression of the human γ-actin gene leads to progressive down-

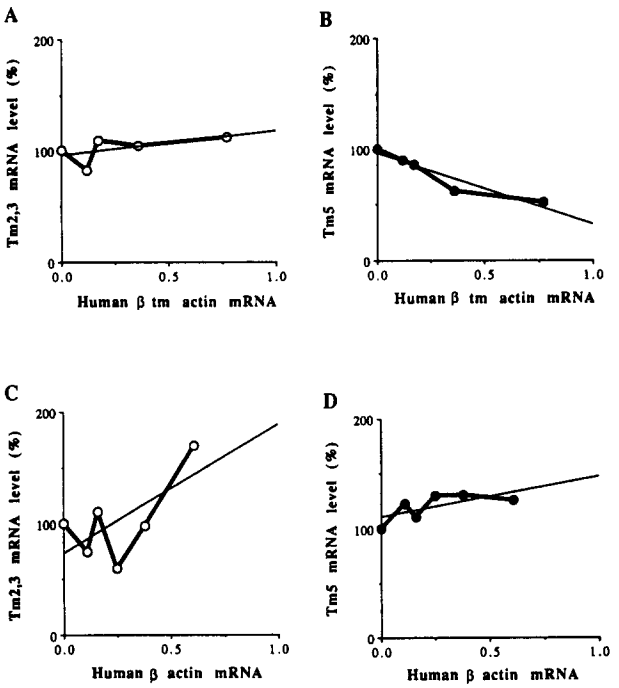

Figure 7. Expression of nonmuscle tropomyosin mRNAs in the β_{tm} and β_{wt}-actin transfectants. Total cellular RNA was isolated from four β_{tm} and six β_{wt}-actin transfectants, having different levels of expression of the exogenous gene. 3 μg of RNA from each clone was size fractionated and hybridized with the tropomyosin isoform specific probes Tm 2, 3 (pRαTm-1A) and Tm 5 (pMMTm5-3'UT) as described in Materials and Methods. The Northern blots were subsequently probed for 18S ribosomal RNA and corrected for loading discrepancies. The autoradiograms were quantitated by densitometry and the results are presented as graphs. The mRNA values for the different tropomyosin isoforms are expressed as a percentage of that seen in a control cell (pUC18 transfected C2 cells) which was set to 100%. The values for the human exogenous gene are expressed relative to that seen in a human myoblast RNA sample. Graphs *A* and *B* correspond to β_{tm} transfectants and graphs *C* and *D* correspond to β_{wt} transfectants. Linear regression analysis was performed on the data points and the resulting line is shown on each graph.

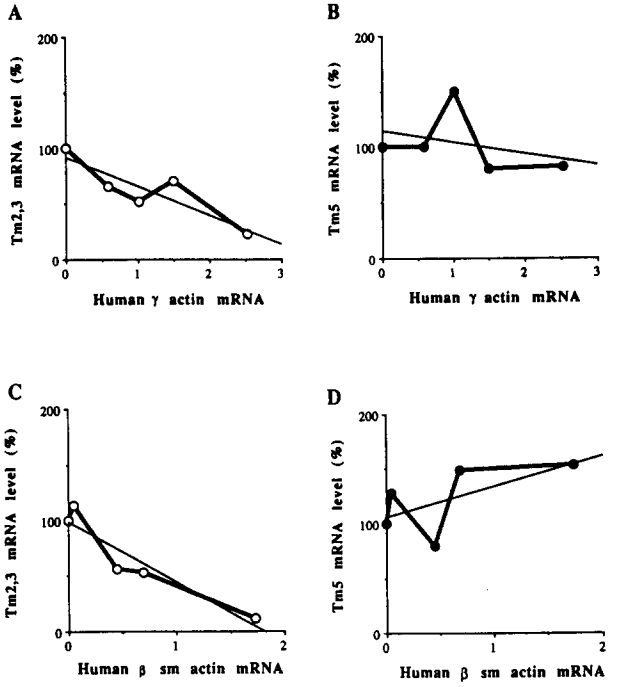

Figure 8. Expression of nonmuscle tropomyosin mRNAs in the γ- and β_{sm}-actin transfectants. Total cellular RNA was isolated from four γ- and four β_{sm}-actin transfectants and analyzed as described in Fig. 7. Graphs *A* and *B* correspond to γ-actin transfectants and graphs *C* and *D* to β_{sm}-actin transfectants. Linear regression analysis was performed on the data points and the resulting line is shown on each graph.

regulation of a tropomyosin gene that encodes the high M_r tropomyosin isoforms, Tms 2 and 3. These results are in agreement with the observed decrease in the staining intensity and steady-state protein levels of Tm 2 in the γ_{33}-B1 cells (Fig. 6, lane *4*). In terms of the low M_r tropomyosin isoform, no linear relationship occurred for Tm 5 ($R = 0.35$, $p > 0.1$). Thus, elevated expression of the γ-actin gene results in Tm 5 levels remaining relatively unchanged.

The observed morphological and cytoarchitectural similarities displayed after elevated expression of the γ-actin and the β_{sm} gene also extend to the pattern of mRNA expression of different tropomyosin isoforms. The impact of elevated expression of the β_{sm} gene on the levels of tropomyosin isoform mRNAs is illustrated in Fig. 8, *C* and *D*. While a decrease in the levels of Tm 2, 3 with increasing expression of the β_{sm} gene were observed, no such changes occurred to Tm 5 transcript levels. An increase in human β_{sm} gene expression resulted in the progressive downregulation of the tropomyosin gene encoding the high M_r tropomyosin isoforms, Tms 2 and 3 ($R = 0.94$, $p < 0.01$). However, linear regression analysis demonstrates that increasing β_{sm} gene expression has no impact on Tm 5 expression ($R = 0.61$, $p > 0.1$). Once again these results correlate with the immunofluorescence staining intensity (Fig. 5) and protein levels of Tm 2 (Fig. 6, lane *2*). In general, the pattern of tropomyosin gene expression observed in the β_{sm} transfectants corresponds to that observed in the γ-actin transfectants.

In summary, the results presented show that C2 myoblasts which display distinct morphologies and cytoarchitectures due to changes in the relative expression of nonmuscle actin genes, differentially regulate tropomyosin isoforms at the transcript and protein levels. Most importantly, different tropomyosin isoforms respond differently, strongly suggesting functional differences among the tropomyosin isoforms. Furthermore, elevated expression of two distinct actin genes previously shown to elicit (*a*) similar changes in morphology and actin cable organization (Schevzov et al., 1992), and (*b*) a similar pattern of expression of the endogenous actin genes (Lloyd et al., 1992) were also shown to have a similar impact on the pattern of expression of the tropomyosin isoforms. These observations suggest that a similar mechanism might be operating to regulate tropomyosin expression in response to morphological and cytoarchitectural changes arising from the impact of two different actin genes.

Discussion

Evidence for a Dynamic Equilibrium between Cytoskeletal Gene Expression and Changes in Cell Shape

The studies presented in this paper strongly suggest that al-

tering cell shape by directly targeting the expression of a major cytoskeletal component, actin, can ultimately lead to changes in the expression of other components that constitute the cell cytoskeleton. Hence, elevated expression of two distinct actin genes γ- and β_{sm}, previously shown to elicit similar changes in cell morphology and actin cable organization (Schevzov et al., 1992), were also shown to give rise to similar changes in tropomyosin organization and gene expression. Thus, at the transcript level, a decrease in the expression of the Tm 2, 3 gene products was observed, whereas the mRNA levels of Tm 5 remained relatively unchanged.

The distinct pattern of tropomyosin gene expression observed in these actin transfectants was also found to correlate with the distinct cellular organization of these two tropomyosin isoforms. The γ- and β_{sm}-actin transfectants were found to exhibit a decrease in the intensity of staining and diffuse organization of Tm 2 which parallels with the significant decrease in Tm 2 expression at both the protein and transcript levels. The well-defined stress fiber-like organization and similar staining intensity of Tm 5 is in agreement with no significant changes found in the expression of this isoform. Additionally, elevated expression of the β_{wt}-gene, known to alter morphology and actin cable organization in a manner distinct from elevated γ- and β_{sm} gene expression, was also found to result in a different pattern of tropomyosin gene expression to that observed in the γ- and β_{sm} transfectants. Additional evidence to support the observed coordinated regulation of cell shape and cytoskeletal gene expression is provided by the work of Leavitt et al. (1987*a*,*b*). In their experiment, the stable transfection of the β_{sm}-actin gene into human KD fibroblasts lead to cell shape changes coupled with a significant decrease in the protein synthesis of Tm 1, 2 and 3, whereas Tm 4 and 5 were less affected. Together these experiments provide evidence to support a dynamic equilibrium between changes in actin gene expression and tropomyosin organization and gene expression.

A Feedback Regulatory Mechanism May Control Tropomyosin Gene Expression

The present study together with that described in Schevzov et al. (1992) suggests that the observed changes in tropomyosin organization and gene expression described above may be influenced by the actin composition of the microfilaments. Previously reported studies have shown that the aberrant β_{sm} protein is impaired in its ability to form filaments. It does however form dimers that can assemble into bundle-like structures (Milloning et al., 1988). A total of 80% of the actin present in the β_{sm} transfectants consists of the β_{sm} protein (Schevzov et al., 1992). Therefore, the inability of the β_{sm} protein to form proper filaments may render the filaments incapable of interacting with the high M_r tropomyosin isoforms. The failure of Tm 2 to segregate into the preexisting stress fibers may result in the downregulation of Tm 2 both at the mRNA and protein levels. The similar observations made in the γ-actin transfectants in terms of the actin and tropomyosin organization strengthen this hypothesis. In this case, elevated expression of the γ-actin gene (0.3 β:γ mRNA and protein ratio, Schevzov et al., 1992) most likely leads to actin filaments consisting predominantly of γ-actin. These filaments may be dysfunctional like those present in the β_{sm} transfectants because similar changes in the expression and cellular organization of Tm 2 are observed. In contrast, no changes in the cellular organization and expression of Tm 5 were observed in these actin transfectants. It appears that Tm 5 preferentially segregates into filaments in all these cells suggesting that Tm 5 does not discriminate between different actin templates.

We propose two possible mechanisms by which the observed relationship between cytoarchitectural changes and cytoskeletal gene expression may take place. First, it is possible that different populations of actin filaments exist in the cell and some of these filaments have a better association with Tm 5 than others. In the β_{sm} and γ-actin transfectants the Tm 5-associated filaments may not have been altered, with no consequent alterations in Tm 5 organization. Rather, a second class of filaments containing mainly the Tm 2 isoform would be predicted to be largely eliminated in the γ- and β_{sm}-actin transfectants. Such a mechanism may be unlikely to occur considering the findings of Lin and coworker (1986). They report that immunoprecipitation of tropomyosin-containing actin filaments with isoform-specific tropomyosin antibodies precipitates all filaments. This suggests that different populations of actin filaments associated with specific tropomyosin isoforms are unlikely to be present. A second possible molecular mechanism that may account for the observed differential organization and expression of tropomyosin isoforms has been proposed to operate during myogenesis (Gunning et al., 1990). The model suggests that competition exists between isoforms of a particular cytoskeletal component for inclusion into cellular structures and that the level of gene expression is determined by the competitiveness of the individual isoforms. The observations presented in this study would support this model. In the case of the γ- and β_{sm}-actin transfectants, failure of Tm 2 to segregate into stress fibers is proposed to lead to the downregulation of this isoform. In contrast, the ability of Tm 5 to continue to segregate into stress fibers results in no change in the levels of Tm 5. These results are therefore consistent with a feedback regulatory mechanism where cellular demand for a particular structural protein can regulate the mRNA levels. For example, the findings by Weinberger et al. (1992) reveal that the induction and maintenance of the neuronal tropomyosins is dependent on morphological differentiation and the maintenance of the neuronal phenotype. Similar feedback regulatory mechanism have also been previously demonstrated for tubulin (Cleveland, 1989) and more recently for actin (Lloyd et al., 1992).

Similar models have been previously proposed for the global autoregulation of the cytoskeleton by Ben-Ze'ev (for review see Ben-Ze'ev, 1991). In these experiments cell shape changes induced by inhibiting cell–matrix and cell–cell contacts, stimulation with growth factors, cell differentiation, and transformation by either chemical carcinogens or tumor viruses were found to correlate with changes in the expression of a number of cytoskeletal components. Although such a correlation has been previously documented, the difference between those experiments and the ones presented in this manuscript is the manner in which the cytoskeletal alterations are induced. This is important in determining whether a direct causal relationship exists between changes in cell shape and the expression of cytoskeletal components or are the observed changes in gene expression initially induced by a different pathway that is independent of the cell cytoskeleton. Because of the complex manner in which information from the outside environment can be transmitted to the cell

nucleus it may well be possible that changes in gene expression induced by the above methods can initially by-pass the cell cytoskeleton. A mechanism to explain how this may occur could involve a second messenger system and/or regulatory molecules that could directly influence gene expression independent of the cytoskeleton. In contrast, the manner in which we have induced cytoskeletal changes in the C2 cells was by directly influencing the expression of a major cytoskeletal component. We therefore postulate that the observed changes in morphology and/or cytoskeletal gene expression are a direct result of altering actin gene expression. In summary, this apparent direct link between actin and tropomyosin expression presented in this manuscript suggests that nonpharmacological signals originating in the cytoskeleton can regulate cytoarchitectural gene expression.

Different Tropomyosin Isoforms May Discriminate between β- and γ-actin

Our initial aim has been to understand the functional significance of different cytoskeletal isoforms. In our previous study we showed that elevated expression of β- or γ-actin genes resulted in a reciprocal impact on myoblast shape and actin filament organization (Schevzov et al., 1992). In the present study we show that these actin transfectants display a differential organization and expression of tropomyosin isoforms. Hence, a possible mechanism by which β- and γ-actin may elicit the observed reciprocal changes in myoblast cytoarchitecture is by differential regulation of tropomyosin isoforms. In particular, β-actin may provide a better template for association with the high M_r tropomyosins than γ-actin. This would in turn promote stress fiber stability in β-actin transfectants and undermine stability in the γ-actin transfectants. Stress fiber stability would be seen as a major determinant of cell morphology which is consistent with the work of Liu and Bretscher (1989) in yeast.

The work of Leavitt et al. (Ng et al., 1988) further emphasizes the correlation between the decrease in the β:γ ratio and downregulation in the synthesis of the high M_r tropomyosin isoforms. In these experiments a decrease in the β:γ ratio induced by overexpression of the γ-actin gene in HuT-14T cells resulted in a 36–65% decrease in the protein synthesis of Tm 3 and to a lesser extent Tm 1 and 6. In contrast, increasing the β:γ ratio had no impact on tropomyosin synthesis (Ng et al., 1988). Thus, it may be suggested that in both myoblasts and transformed fibroblasts, the β:γ ratio can influence tropomyosin synthesis.

The differential expression of tropomyosin isoforms implies that they are independently regulated and thus may be functionally distinct. These studies are supported in part by the work of Matsumura and Yamashiro-Matsumura (1985) where in vitro studies have shown differential affinities of tropomyosin isoforms for the actin filaments. In addition, immunofluorescence studies on normal cultured fibroblast cells suggest a differential localization of tropomyosin isoforms (Lin et al., 1988). Moreover, the morphological and cytoarchitectural changes observed after the transformation of cells have also been correlated with a differential pattern of expression of tropomyosin isoforms (Cooper et al., 1985; Hendricks and Weintraub, 1984; Leavitt et al., 1986; Lin et al., 1985*b*; Matsumura et al., 1983). These observations are consistent with the proposal that the tropomyosin isoform composition of microfilaments may play a fundamental role in regulating cell cytoarchitecture.

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References

Ben-Ze'ev, A. 1991. Animal cell shape changes and gene expression. *Bioessays.* 13:207–212.

Ben-Ze'ev, A., G. Baum, and A. Amsterdam. 1989. Regulation of tropomyosin expression in the maturing ovary and in primary granulosa cell cultures. *Dev. Biol.* 135:191–201.

Bernstein, B. W., and J. R. Bamburg. 1982. Tropomyosin binding to F-actin protects the F-actin from disassembly by brain actin-depolymerizing factor (ADF). *Cell Motil.* 2:1–8.

Buckingham, M. E., and A. J. Minty. 1982. Contractile protein genes. *In* Eukaryotic Genes: Their Structure, Activity and Regulation. N. Maclean, S. P. Gregory, and R. A. Flavell, editors. Butterworth-Heinemann Ltd., London. 365–395.

Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal. Biochem.* 162:156–159.

Clayton, L., F. C. Reinach, G. M. Chumbley, and A. R. MacLeod. 1988. Organization of the hTm_{sm} gene: implications for the evolution of muscle and non-muscle tropomyosin. *J. Mol. Biol.* 201:507–515.

Cleveland, D. W. 1989. Autoregulated control of tubulin synthesis in animal cells. *Curr. Opin. Cell Biol.* 1:10–14.

Cooper, H. L., N. Feuerstein, M. Noda, and R. H. Bassin. 1985. Suppression of tropomyosin synthesis, a common biochemical feature of oncogenesis by structurally diverse retroviral oncogenes. *Mol. Cell. Biol.* 5:972–983.

Denhardt, D. T. 1966. A membrane-filter technique for the detection of complementary DNA. *Biochem. Biophys. Res. Commun.* 23:641–646.

Erba, H. P., P. Gunning, and L. 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.

Feinberg, A. P., and B. Vogelstein. 1983. A technique for radiolabeling DNA restriction endonuclease fragments to high specific activity. *Anal. Biochem.* 132:6–13.

Goodwin, L. O., J. P. Lees-Miller, M. A. Leonard, S. B. Cheley, and D. M. Helfman. 1991. Four fibroblast tropomyosin isoforms are expressed from the rat α-tropomyosin gene via alternative RNA splicing and the use of two promoters. *J. Biol. Chem.* 266:8408–8415.

Gunning, P., M. Gordon, R. Wade, R. Gahlmann, C.-S. Lin, and E. Hardeman. 1990. Differential control of tropomyosin mRNA levels during myogenesis suggests the existence of an isoform competition-autoregulatory compensation control mechanism. *Dev. Biol.* 138:443–453.

Helfman, D. M., S. Cheley, E. Kuismanen, L. A. Finn, and Y. Yamawaki-Kataoka. 1986. Nonmuscle and muscle tropomyosin isoforms are expressed from a single gene by alternative RNA splicing and polyadenylation. *Mol. Cell. Biol.* 6:3582–3595.

Hendricks, M., and H. Weintraub. 1984. Multiple tropomyosin polypeptides in chicken embryo fibroblasts: differential repression of transcription by Rous sarcoma virus transformation. *Mol. Cell. Biol.* 4:1823–1833.

Huxley, H. E. 1972. Structural changes in the actin- and myosin-containing filaments during contraction. *Cold Spring Harbor Symp. Quant. Biol.* 37: 361–376.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature (Lond.).* 227:680–685.

Lazarides, E. 1975. Tropomyosin antibody: the specific localization of tropomyosin in nonmuscle cells. *J. Cell Biol.* 65:549–561.

Leavitt, J., G. Latter, L. Lutomski, D. Goldstein, and S. Burbeck. 1986. Tropomyosin isoform switching in tumorigenic human fibroblasts. *Mol. Cell. Biol.* 6:2721–2726.

Leavitt, J., S.-Y. Ng, U. Aebi, M. Varma, G. Latter, S. Burbeck, L. Kedes, and P. Gunning. 1987*a*. Expression of transfected mutant β-actin genes: alterations of cell morphology and evidence for autoregulation in actin pools.

Mol. Cell. Biol. 7:2457–2466.

Leavitt, J., S.-Y. Ng, M. Varma, G. Latter, S. Burbeck, P. Gunning, and L. Kedes. 1987*b*. Expression of transfected mutant β-actin genes: transitions toward the stable tumorigenic state. *Mol. Cell. Biol.* 7:2467–2476.

Lees-Miller, J. P., L. O. Goodwin, and D. M. Helfman. 1990. Three novel brain tropomyosin isoforms are expressed from the rat α-tropomyosin gene through the use of alternative promoters and alternative RNA processing. *Mol. Cell. Biol.* 10:1729–1742.

Lees-Miller, J. P., and D. M. Helfman. 1991. The molecular basis for tropomyosin isoform diversity. *Bioessays.* 13:429–437.

Lin, J. J.-C., and J. L.-C. Lin. 1986. Assembly of different isoforms of actin and tropomyosin into the skeletal tropomyosin-enriched microfilaments during differentiation of muscle cells in vitro. *J. Cell Biol.* 103:2173–2183.

Lin, J. J.-C., C.-S. Chou, and J. L.-C. Lin. 1985*a*. Monoclonal antibodies against chicken tropomyosin isoforms: production, characterization and application. *Hybridoma.* 4:223–242.

Lin, J. J.-C., D. M. Helfman, S. H. Hughes, and C.-S. Cahou. 1985*b*. Tropomyosin isoforms in chicken embryo fibroblasts: purification, characterization and changes in Rous sarcoma virus-transformed cells. *J. Cell Biol.* 100:692–703.

Lin, J. J.-C., T. E. Hegmann, and J. L.-C. Lin. 1988. Differential localization of tropomyosin isoforms in cultured nonmuscle cells. *J. Cell Biol.* 107:563–572.

Liu, H., and A. Bretscher. 1989. Disruption of the single tropomyosin gene in yeast results in the disappearance of actin cables from the cytoskeleton. *Cell.* 57:233–242.

Lloyd, C., G. Schevzov, and P. Gunning. 1992. Transfection of non-muscle β- and γ-actin genes into myoblasts elicit different feedback regulatory responses from endogenous actin genes. *J. Cell Biol.* 117:787–798.

Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* 193:265–275.

MacLeod, A. R., C. Houlker, F. C. Reinach, L. B. Smillie, K. Talbot, G. Modi, and F. S. Walsh. 1985. A muscle-type tropomyosin in human fibroblasts: evidence for expression by an alternative RNA splicing mechanism. *Proc. Natl. Acad. Sci. USA.* 82:7835–7839.

MacLeod, A. R., K. Talbot, L. B. Smillie, and C. Houlker. 1987. Characterization of a cDNA defining a gene family encoding $TM30_{pl}$, a human fibroblast tropomyosin. *J. Mol. Biol.* 194:1–10.

Maniatis, T. E., F. Fritsch, and J. Sambrook. 1982. Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Matsumura, F., and S. Yamashiro-Matsumura. 1985. Purification and characterization of multiple isoforms of tropomyosin from rat cultured cells. *J. Biol. Chem.* 260:13851–13859.

Matsumura, F., J. J.-C. Lin, S. Yamashiro-Matsumura, G. P. Thomas, and W. C. Topp. 1983. Differential expression of tropomyosin forms in the microfilaments isolated from normal and transformed rat cultured cells. *J. Biol. Chem.* 258:13954–13964.

Milloning, R., H. Salvo, and U. Aebi. 1988. Probing actin polymerization by intermolecular cross-linking. *J. Cell Biol.* 106:785–796.

Ng, S.-Y., E. Erba, G. Latter, L. Kedes, and J. Leavitt. 1988. Modulation of microfilament protein composition by transfected cytoskeletal actin genes. *Mol. Cell. Biol.* 8:1790–1794.

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 multipseudogene family: conservation of noncoding regions and chromosomal dispersion of pseudogenes. *Mol. Cell. Biol.* 5:2720–2732.

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.* 34:113–124.

Reinach, F. C., and A. R. MacLeod. 1986. Tissue-specific expression of the human tropomyosin gene involved in the generation of the *trk* oncogene. *Nature (Lond.).* 322:648–650.

Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA.* 74:5463–5467.

Schevzov, G., C. Lloyd, and P. Gunning. 1992. High level expression of transfected β- and γ-actin genes differentially impact on myoblast cytoarchitecture. *J. Cell Biol.* 117:775–786.

Smillie, L. B. 1979. Structure and functions of tropomyosins from muscle and non-muscle sources. *Trends Biochem. Sci.* 4:151–155.

Takenaga, K., Y. Nakamura, H. Kageyama, and S. Sakiyama. 1990. Nucleotide sequence of cDNA for nonmuscle tropomyosin 5 of mouse fibroblast. *Biochim. Biophys. Acta.* 1087:101–103.

Towbin, H., T. Staehelin, and J. Gordon. 1979. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc. Natl. Acad. Sci. USA.* 76:4350–4354.

Wegner, A. 1982. Kinetics analysis of actin suggests that tropomyosin inhibits spontaneous fragmentation of actin filaments. *J. Mol. Biol.* 161:217–227.

Weinberger, R. P., R. C. Henke, O. Tolhurst, P. L. Jeffrey, and P. Gunning. 1992. Induction of neuron specific tropomyosin mRNAs by NGF is dependent on morphological differentiation. *J. Cell Biol.* 120:205–215.

Wieczorek, D. F., C. W. J. Smith, and B. Nadal-Ginard. 1988. The rat α-tropomyosin gene generates a minimum of six different mRNAs coding for striated, smooth, and nonmuscle isoforms by alternative splicing. *Mol. Cell. Biol.* 8:679–694.

Yamawaki-Kataoka, Y., and D. M. Helfman. 1987. Isolation and characterization of cDNA clones encoding a low molecular weight nonmuscle tropomyosin isoform. *J. Biol. Chem.* 262:10791-10800.