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Forced expression of tropomyosin 2 or 3 in v-Ki-ras-transformed fibroblasts results in distinct phenotypic effects

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ABSTRACT **Transformation of cells in tissue culture results in a variety of cellular changes including alterations in cell growth, adhesiveness, motility, morphology, and organization of the cytoskeleton. Morphological and cytoskeletal changes are perhaps the most readily apparent features of transformed cells. Although a number of studies have documented a decrease in the expression of specific tropomyosin (TM) isoforms in transformed cells, it remains to be determined if the suppression of TM synthesis is essential in the establishment and maintenance of the transformed phenotype. To address the roles of different TM isoforms in transformed cells we have examined the effects of expressing specific TM isoforms in transformed cells using a Kirsten virus-transformed cell line (ATCC NRK 1569) as a model system. In contrast to normal fibroblasts, the NRK 1569 cells contain reduced levels of TM-1 and undetectable levels of TM-2 and TM-3. These cells have a rounded morphology and are devoid of stress fibers. Employing expression plasmids for TM-2 and TM-3, stable cell lines were established from the NRK 1569 cells that express these isoforms individually. We demonstrate that expression of TM-2 or TM-3 leads to increased cell spreading accompanied by the formation of identifiable microfilament bundles, as well as significant restoration of well-defined vinculin-containing focal adhesion plaques, although expression of each isoform exhibited distinct properties. In addition, cells expressing TM-2, but not TM-3, exhibited contact-inhibited cell growth and a requirement for serum.**

Malignant transformation of cells is commonly accompanied by a variety of morphological changes such as rounded cell shape, a poorly organized microfilament system, alterations in locomotory activity, and a reduced cell-substrate adhesion. Morphological changes are perhaps the most striking feature of transformed cells. These changes in cell shape are clearly associated with the cytoskeleton. Immunofluorescence and electron microscopic studies have shown that transformed cells exhibit profound changes in the organization of microfilaments. Such studies reveal that microfilament bundles exist in a more dispersed state and are reduced in size and number in transformed cells. In addition, changes in microfilament structure are highly related to both anchorage-independent growth and cellular tumorigenicity (1), suggesting a role for microfilament alteration in oncogenic transformation. The molecular, biochemical, and structural bases for these alterations of microfilament cables in transformed cells are poorly understood. The altered expression of a number of individual cytoskeletal proteins have been implicated in the transformed phenotype including actin, tropomyosin (TM), gelsolin, caldesmon, and myosin light chain (reviewed in ref. 2).

A number of studies have demonstrated that TM expression is selectively altered in several transformed cells (3–13). In general, these experiments demonstrate that in transformed cells one or more of the major TM isoforms of higher molecular weight are decreased or missing, while the levels of one or more of the lower M_r TM isoforms are increased. These changes in TM expression appear to correlate well with the rearrangement of microfilament bundles and morphological alterations observed in transformed cells. The perturbations in TM synthesis have been reported to occur in cells transformed by a variety of agents including chemical carcinogens, UV radiation, DNA and RNA tumor viruses. In addition, the changes in TM expression following transformation occur in cells of all species examined including chicken, rodents (mouse and rat), and human, indicating that modulations in TM expression are common features of the transformed phenotype and that TM genes may represent a target for oncogene action.

TMs are a family of actin-binding proteins that are expressed in all eukaryotic cells. Different forms are expressed in muscle (skeletal, cardiac, and smooth) and nonmuscle cells (reviewed in refs. 14 and 15). Multiple forms of TM have been detected in different cultures of nonmuscle cells, including rodent fibroblasts (6, 7, 16), human fibroblasts (17, 18), and chicken fibroblasts (4, 10, 19). Normal rat fibroblasts contain at least seven isoforms of TM: three TMs of major abundance termed 1, 2, and 4 (apparent M_r = 40,000, 36,500, and 32,400, respectively), and four relatively minor TMs termed 3, 5, 5a, and 5b (apparent M_r = 35,000, 32,000, 32,000, and 32,000, respectively) (6, 20, 21). The function of the multiple forms of TM in nonmuscle cells remains to be established. Structural differences however, do exist among the various protein isoforms. These divergent regions correspond to functional domains of the proteins including actin binding regions and head-to-tail binding domains (reviewed in ref. 15).

Since biochemical studies suggest that TM plays a role in stabilization of microfilaments *in vivo*, it is possible that the altered patterns of TMs observed in many transformed cells contribute to the alterations in actin filament assembly (reviewed in ref. 15). To determine if the suppression of TM synthesis is essential in the establishment and maintenance of the transformed phenotype we studied the effects of stably expressing specific TM isoforms in a Kirsten virus-transformed cell line. These cells do not express two high M_r TM isoforms, namely, TM-2 and TM-3. Forced expression of TM-2 or TM-3 in these cells lead to cell spreading and formation of identifiable microfilament bundles, although expression of each isoform resulted in distinct morphological properties. In addition, cells expressing TM-2, but not TM-3, exhibited contact inhibited cell growth and a requirement for serum. These studies provide new information concerning the

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Abbreviations: TM, tropomyosin; UTR, untranslated region; SV40, simian virus 40.

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role of individual TM isoforms in the assembly of actin-based suprastructures and their relationship to cell growth and the aberrant morphology of transformed cells.

MATERIALS AND METHODS

Construction of Expression Vectors. The coding regions derived from full-length cDNAs encoding rat fibroblast TM-2 and TM-3 (20) were modified with *Hin*dIII linkers and cloned into the unique *Hin*dIII site located 70 bp downstream of the transcriptional start site in the eukaryotic expression vector pXKH (D. Hanahan, personal communication). The cDNA contained the entire coding sequence and ≈20 bp of the 3′-untranslated region (UTR). The expression plasmid contained the simian virus 40 (SV40) early promoter and the small t intron and poly(A) site, respectively, flanking the TM cDNA.

Cell Culture, Transfection, and Clonal Selection. NRK 1570 and NRK 1569 cell lines were grown in DMEM supplemented with penicillin (50 unit/ml), streptomycin (50 μg/ml), and 5% fetal calf serum. Cells were grown at 37°C with 5% CO_2 content. Stable lines were derived from the NRK 1569 cells by cotransfection of TM constructs with pKoNeo (10:1 ratio), using the calcium phosphate coprecipitation procedure. The cells were incubated for 16–20 hr at 37°C, washed with PBS, refed with fresh media, and incubated an additional 24 hr. The transfected cells were then plated at various dilutions in media containing G-418 (400 μg/ml). Drug-resistant colonies were randomly selected after 7–10 days and cell lines were single cell cloned to assure homogeneity. The derived cell lines were maintained in medium containing G-418 (200 μg/ml).

Immunoblot Analysis. Cells were grown on 60-mm plates to ≈70% confluence, washed three times with PBS, and then harvested in 1 ml of PBS by scraping with a rubber policeman. The cells were recovered by centrifugation in a microcentrifuge and resuspended in 150 ml of Laemmli sample buffer, and the lysate was boiled for 3 min. Lysates were resolved on analytical SDS/12.5% polyacrylamide gels and analyzed by Western blot analysis.

Immunofluorescent Staining. For immunofluorescence cells were cultured on 12-mm glass coverslips. Cells were washed and fixed in 3% paraformaldehyde as described (22). Incubations with antibodies were performed in TBS (20 mM Tris/154 mM NaCl/2 mM $MgCl_2$/1 mM NaN_3, pH 7.5). Fluorescent images were photographed on a Zeiss Axiophot using a ×63 oil immersion lens and Kodak P3200 Tmax film.

Antibodies. Monoclonal anti-TM antibody (clone TM311), anti-vinculin (clone hVin1), and anti-α-tubulin (clone DM 1A) antibodies were from Sigma and monoclonal anti-pan-ras (AB-3) was from Oncogene Science. Fluorescent labeled secondary antibodies and phalloidin were purchased from Molecular Probes.

Anchorage-Independent Growth and Growth in Low Serum. Growth on soft agar assay was performed using 10^3, 10^4, and 10^5 cells essentially as described (1). To determine the ability of cells to grow in low serum 10^3, 10^4, and 10^5 cells were plated on 60-mm plates in reduced (0.5%) serum and refed every 3 days, and the cells were counted using Trypan blue.

Image Analysis. Random fields of subconfluent cultures were photographed by phase contrast microscopy, and prints were scanned into a Macintosh computer using the UMax UC 630 scanner. Areas of ≈100 cells from a minimum of five individual fields were measured for each cell line using the IMAGE 1 software (Universal Imaging, Media, PA). The mean area determined for each cell line in pixels squared for NRK 1570, 285.2 (SD = 12.7); NRK 1569, 21.5 (SD = 5.2); NRK 1569/TM-2, 208.3 (SD = 30.8); and NRK 1569/TM-3, 50.7 (SD = 5.5).

Two-Dimensional Gel Electrophoresis of Metabolically Labeled Cells. Cells grown to 70% confluence in 60-mm culture dishes were incubated for 16 hr in methionine-free DMEM (GIBCO) in the presence of 100 μCi (1 Ci = 37 GBq) of [^{35}S]Met-translabel (New England Nuclear). [^{35}S]Methionine-labeled cells were solubilized and extracted in 9 M urea (United States Biochemical), and soluble proteins were analyzed on isoelectric focusing gels (pH range 3–10) in the first dimension and on SDS/slab 10% polyacrylamide gels in the second dimension as described by Garrels (23) with the modifications given by Patterson and Latter (24). Gels were dried and exposed to x-ray films for 7–14 days.

RESULTS

Stable Expression of TM-2 and TM-3 in the Kirsten Virus-Transformed Cell Line (NRK 1569). The Kirsten virus-transformed cell line (NRK 1569) expresses no TM-2 and TM-3 protein or mRNA and has reduced levels of TM-1 protein (6, 20). These cells have a rounded morphology and are devoid of microfilament bundles. We have used constructs containing full-length cDNAs for TM-2 and TM-3 and driven by the SV40 early promoter to express them in these cells. These constructs contain the entire coding region, the authentic stop codon with 20 bp of 3′-untranslated sequence, and a functional splice site (small t intron) as well as a poly(A) addition site downstream of the TM cDNA sequences. Plasmids encoding TM-2 or TM-3 were cotransfected with a pKO-neo plasmid that contains a neomycin-resistant gene into NRK 1569 cells. Cells were selected based on resistance to G-418. Single colonies were expanded and analyzed for expression of TM, morphological changes, and cytoskeletal rearrangements. The expression of TM-2 or TM-3 proteins in the NRK cells was determined by Western blot analysis of cells expressing the neomycin-resistant plasmid alone, three independent clones expressing the TM-2 plasmid, and three independent clones expressing the TM-3 plasmid (Fig. 1). The only high M_r TM isoform detected by antibody 311 in NRK 1569 cells stably transfected with Neo alone is TM-1 (Fig. 1, lane H). As shown in lanes B–G the clones that were isolated have a range of expression levels of TM-2 or TM-3.

To confirm that the expressed isoforms represent authentic TM-2 and TM-3, the cells were labeled *in vivo* with [^{35}S]methionine for 16 hr, and total cell lysates were analyzed by two-dimensional gel electrophoresis (Fig. 2). New spots, corresponding to the positions of endogenous TM-2 (Fig. 2*b*) or TM-3 (Fig. 2*c*) appeared in the respective clones and were positively identified by Western blot analysis using a monoclonal TM antibody (data not shown). To confirm further that the stable lines still expressed the viral ras gene product, cell lysates were analyzed by Western blot analysis and the expression of v-ras was confirmed using a pan-ras antibody (data not shown).

Expression of TM-2 and TM-3 Results in Cell Spreading and Distinct Effects on the Actin Cytoskeleton. To determine if expression of TM-2 or TM-3 had an effect on cell morphology the parental cell line NRK 1569 was compared with cells

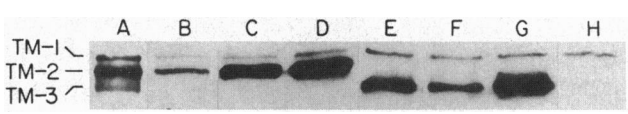

FIG. 1. Stable expression of TM-2 and TM-3 in NRK 1569 cells. Cell lines successfully cotransfected with the SV40-TM-2 or SV40-TM-3 and pKO-neo plasmids were grown. Whole cell lysates from the cell cultures were analyzed by Western blot analysis using mAb to TM (Sigma, clone TM311) that recognizes only the high M_r TM isoforms TM-1, TM-2, and TM-3 in fibroblasts. Lane A, mixture of purified TM-1, TM-2, and TM-3 for control; lanes B–D, whole cell lysates from clones 2.1, 2.6, and 2.10, respectively; lanes E–G, whole cell lysates from clones 3.4, 3.7, and 3.9, respectively; and lane H, lysate from clone neo.1 of NRK 1569 cell expressing the neomycin-resistance plasmid alone.

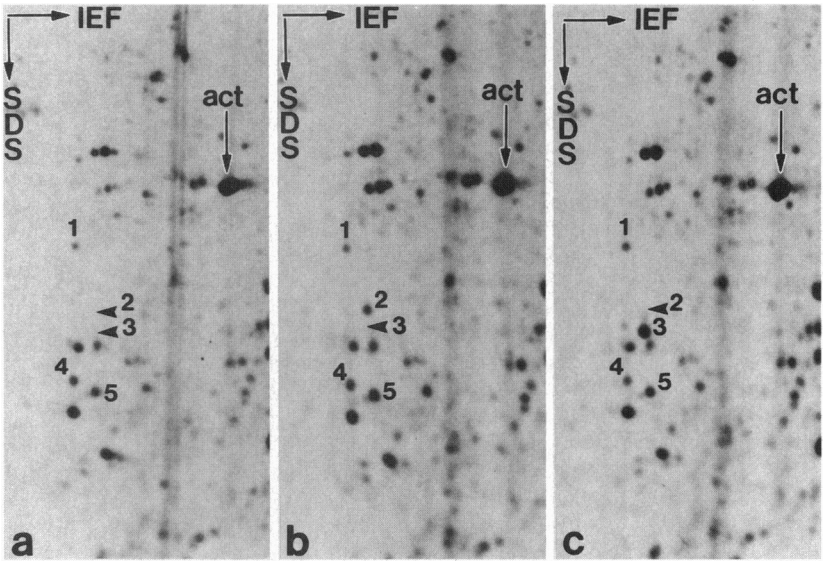

FIG. 2. Two-dimensional gel electrophoresis of cells expressing TM-2 or TM-3: Autoradiographs of parental NRK 1569 cells with no detectable TM-2 or TM-3 proteins (*a*), NRK 1569 cells expressing TM-2 (*b*), or NRK 1569 cells expressing TM-3 (*c*). Similar results were obtained for all three clones expressing TM-2 or TM-3. The positions of the TMs are indicated by numbers, and the position of the major actin spot is indicated by act.

expressing TM-2 or TM-3 and with the untransformed cell line NRK 1570. In contrast to the NRK 1570 cells that exhibit a classic, fibroblast-like phenotype (Fig. 3*B*), the NRK 1569 cells have adopted a rounded morphology (Fig. 3*A*) with cell spreading of <10% of the values obtained with untransfected 1570 cells. Cells expressing TM-3 spread to ≈18% of the value measured for the NRK 1570 cells (Fig. 3*C*). TM-2 expressing clones, by contrast, exhibited substantial cell spreading regaining on average ≈75% of the 1570 phenotype when cell spreading was used as a measure to define phenotypic reversion (Fig. 3*D*).

We next determined if cell spreading was accompanied by changes in the organization of actin. Actin filaments were visualized by fluorescence microscopy using rhodamine-labeled phalloidin. Individual actin filaments are noticeably absent from the parental 1569 cell line (Fig. 4*A*), compared with the well-developed stress fibers observed in the untransformed control NRK 1570 cells (Fig. 4*b*). NRK 1569 cells stably expressing TM-2 or TM-3 exhibited identifiable microfilament bundles, although they were more prominent in the cells expressing TM-2 compared with those expressing TM-3 (Fig. 4 *c* and *d*). In addition, the NRK 1569 cell clones expressing TM-3 also exhibited patches of actin-filaments localized to one region of the cell (Fig. 4*d*). Identical results were found for all independent cell lines expressing TM-3 (data not shown).

Since expression of TM-2 and TM-3 resulted in changes in cell shape and organization of actin, we also examined the distribution of vinculin and tubulin in these stable cell lines. In TM-2 expressing clones the formation of well-developed focal adhesions could be observed that colocalized with actin at the ends of stress fiber bundles (Fig. 5*C*). In the untransfected NRK 1569 cells vinculin was observed to be concentrated in the area of high actin concentration at the periphery of the cells (Fig. 5*A*) but did not get organized into prominent adhesion plaques. Similarly to NRK 1569 cells, TM-3 clones exhibited a reduced degree of actin stress fiber formation and contained areas of "actin-disorganization" resembling in part NRK 1569 phenotype. In agreement with the low level of stress fiber organization in TM-3 clones, the distribution of vinculin was less well-defined in these cells (Fig. 5*D*). Since cells expressing TM-2 and TM-3 exhibit a somewhat different distribution of actin, it was of interest to determine if differences would be observed for the distribution of microtubules. In contrast to the actin cytoskeleton, the microtubular apparatus appeared similar in both cell lines expressing TM-2 or TM-3 (Fig. 6). In the areas of "actin disorganization" present in the TM-3 clones (Fig. 6*c*), microtubules could be observed

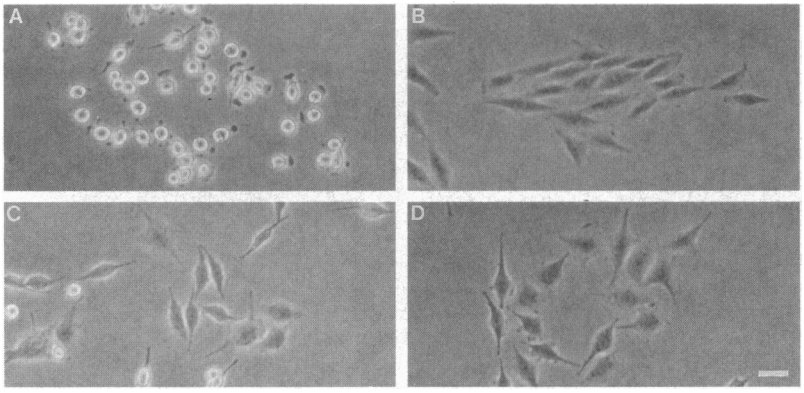

FIG. 3. Stable expression of TM-2 or TM-3 in NRK 1569 cells leads to cell spreading. Cell spreading was monitored by phase contrast microscopy 3 days after plating. (*A*) NRK 1569, (*B*) NRK 1570, (*C*) cells from clone 3.9 expressing the SV40-TM-3 plasmid, and (*D*) cells from clone 2.10 expressing the SV40-TM-2 plasmid. (Bar = 100 μm.)

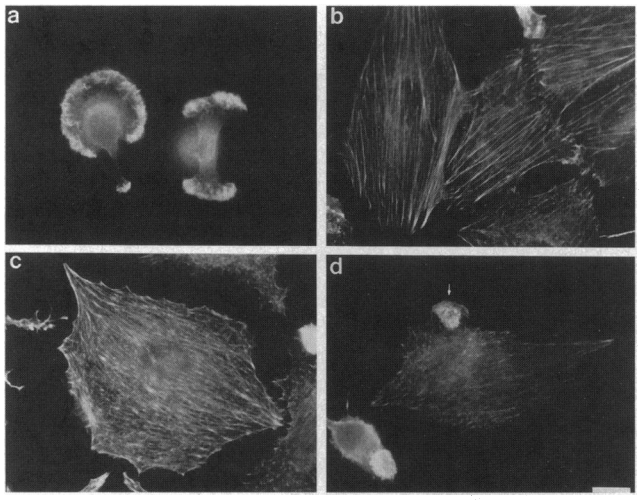

FIG. 4. Stable expression of TM-2 or TM-3 in NRK 1569 cells leads to the formation of microfilament bundles. Microfilaments were visualized using rhodamine-phalloidin. (*a*) NRK 1569 cells; (*b*) NRK 1570; (*c*) clone 2.6; and (*d*) clone 3.9. Note the similar degree of disorganization of the actin in the cell periphery of NRK 1569 cells and in areas of clone 3.9 (arrow in *d*). (Bar = 15 μm.)

to penetrate these regions without noticeable alterations (Fig. 6*d*).

Growth Properties. Since overexpression of TM-2 and TM-3 led to partial morphological reversion of the transformed phenotype it was of interest to determine whether these changes in cell shape and cytoarchitecture were accompanied by changes in other properties of the transformed cells including density and anchorage-independent growth and the ability to grow in low serum. As indicated in Table 1, all clones exhibited the same, unaltered ability to grow in soft agar, demonstrating the ability for anchorage-independent growth was retained in these cells even after TM transfection. We next determined if the cells expressing TM-2 or TM-3 would be able to grow in low serum. Interestingly, each of three independent cell lines expressing TM-2 was no longer able to grow in conditions of low serum (Table 1), whereas cells expressing TM-3 retained their ability to grow in low serum. Finally, we determined if the expression of TM-2 or TM-3 had effects on density-related growth. All clones expressing TM-2 were observed to grow to a confluent monolayer upon prolonged cultivation resulting in a subsequent decrease in cell growth. This is indicative of a restoration of contact inhibition as can be found in normal fibroblasts. In contrast, TM-3 clones did not form a monolayer and showed no signs of density regulated cell growth, thus exhibiting a growth characteristic comparable to the transformed NRK 1569 cell line.

DISCUSSION

We report in this study that the expression of TMs in cells transformed with viral oncogenic ras can rescue the normal fibroblastic phenotype and demonstrate the possibility of circumventing the effects of ras *in vivo* by expression of cytoskeletal proteins from a plasmid. We also show that TM-2 and TM-3 exhibit differential potential for this phenotypic reversion suggesting distinct cellular roles for these two isoforms. These results raise a number of questions including: (*i*) Do changes in expression of TM play a causal role in transformation? (*ii*) How does expression of TM lead to alterations in microfilament structure? (*iii*) Why do different isoforms exhibit different phenotypic effects?

Table 1. Growth characteristics of tropomyosin-expressing cell lines

Clone	Expressed exogenous genes	Growth in soft agar	Growth in low serum	Density-independent growth
0.1	Neo	+	+	+
2.1	Neo, TM-2	+	−	−
2.6	Neo, TM-2	+	−	−
2.10	Neo, TM-2	+	−	−
3.4	Neo, TM-3	+	+	+
3.7	Neo, TM-3	+	+	+
3.9	Neo, TM-3	+	+	+

We are only starting to understand the possible direct relationship between expression (or modulation) of actin-associated proteins and the acquisition of a transformed phenotype. With respect to the role of TM expression in transformation, a number of studies have reported that expression of TM coding or noncoding sequences can revert some of the properties of the transformed phenotype. In a recent study it was found that increased expression of fibroblast TM-1 in a v-Ki-ras-transformed 3T3 cell line was accompanied by cell spreading, induced anchorage-dependence cell growth, and inhibition of tumor-forming capabilities when compared with the parental cell line (25). Although the level of TM-1 expression is reduced by 50% in the transformed 3T3 cells and expression of TM-2 is below detectable levels, increased expression of TM-1 was able to revert some of the features of the transformed phenotype. In another study of a v-raf-transformed rat cell line that exhibits largely reduced levels of TM-1 and TM-2, expression of TM-2 resulted in the restoration of microfilament bundles, but did not affect the rate of cell growth or the ability to grow in soft agar (26). These data are similar to the results obtained in the present study where we observed that cells expressing TM-2 or TM-3 exhibited an increased formation of microfilament bundles but were still able to grow on soft agar. Collectively these results demonstrate that increased expression of any of the high M_r TM isoforms (TM-1, TM-2, and TM-3) is sufficient to induce cell spreading and actin filament organization, but exhibit differences in their ability to alter other properties of the transformed phenotype. Recently it was reported that expression of an antisense RNA to TM-1 led to a reduction of TM-1 protein and disorganization of microfilaments (27). Thus changes in the levels of one isoform could affect filament dynamics.

Since we could not detect any noticeable changes in the expression levels of other cytoskeletal components or of actin itself following the expression of TM-2 or TM-3, the reported effects on the restoration of stress fiber bundles might reflect alterations in the state of assembly or in the composition of the microfilaments. Rodriguez-Fernandez *et al.* (28) have reported a similar mechanism for the assembly of adhesion complexes following the modulation of vinculin expression in 3T3 cells and suggested a relation between the organization state of microfilaments and focal adhesion. Our results with high M_r TMs support this assumption and demonstrate that alterations in microfilament organization may influence the assembly of cell–substrate contact sites. It is worth noting that clones having spontaneously lost their transgene after a number of passages reverted to the original transformed phenotype and were indistinguishable from NRK 1569 cells in terms of their morphological as well as their growth characteristics (data not shown). Spontaneous reversion in phenotype with concomitant disorganization of microfilaments were observed by others in similar studies (28–30). These phenotypic reversions further support the conclusion that the effects seen in the TM clones are due to the presence of the TM proteins in the cell expressed from the transgene.

In addition to effects obtained following expression of coding sequences for TMs, a role for the 3′-untranslated sequences (3′-UTRs) in TM mRNAs has been reported (31). Recent work by Blau and coworkers (31) reported that a

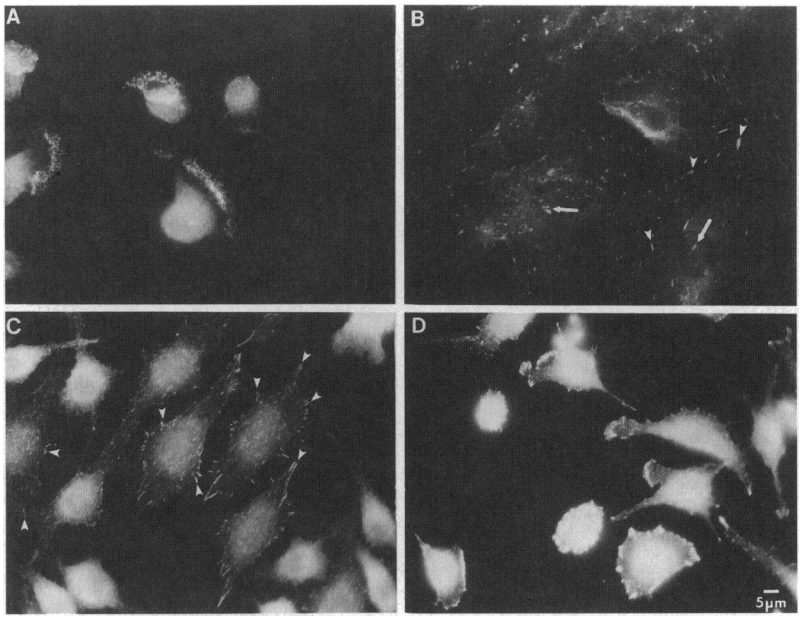

FIG. 5. Cells expressing TM-2 reform focal adhesion contacts. Focal adhesion contacts were visualized using a monoclonal anti-vinculin antibody. (*A*) NRK 1569 cells; (*B*) NRK 1570; (*C*) clone 2.6; and (*D*) clone 3.9. Note the well-defined vinculin-containing focal adhesion sites in *B* and *C* at the periphery of both cell lines (arrowheads) and anchorage sites at the basal surface of the 1570 cells (arrows in *B*).

portion of the 3′-UTR of the α-TM gene functions as a "riboregulator." When this region was stably expressed in a myogenic cell line (NMU2), this RNA was sufficient for the suppression of anchorage-independent growth and the suppression of tumor formation. The constructs used in experiments that demonstrate expression of TM-1 in the v-Ki-ras-transformed 3T3 cells do have the 3′-UTR of the mRNA (25). These results are in contrast to those obtained with the cDNA constructs used in the v-raf-transformed cells (26) and in the present study that do not contain this region in the mRNA. Thus, many of the phenotypic changes may be attributed to the expression of protein and not to sequences present in the 3′-UTRs. There are some questions about the general effects of 3′-UTRs in transformation since these sequences are common to more than a single mRNA for TM. For example, in the case of the v-Ki-ras-transformed cells used in this study, although these cells generate neither mRNA nor protein for TM-2 and TM-3, they still express detectable levels of TM-5a, which is also the product of the α-TM gene and contains the same 3′-UTR (20). In addition, a number of TM isoforms containing the same 3′-UTRs (TM-2, TM-3, TM-5a, and TM-5b) are sometimes up-regulated in transformation. An adenovirus transformed cell line has been characterized that expresses elevated levels of TM-2, TM-3, TM-5a, and TM-5b, all products of the α-TM gene. Therefore, down-regulation of sequences present within the 3′-UTR of TM mRNAs cannot fully explain the effects associated with TM in transformation.

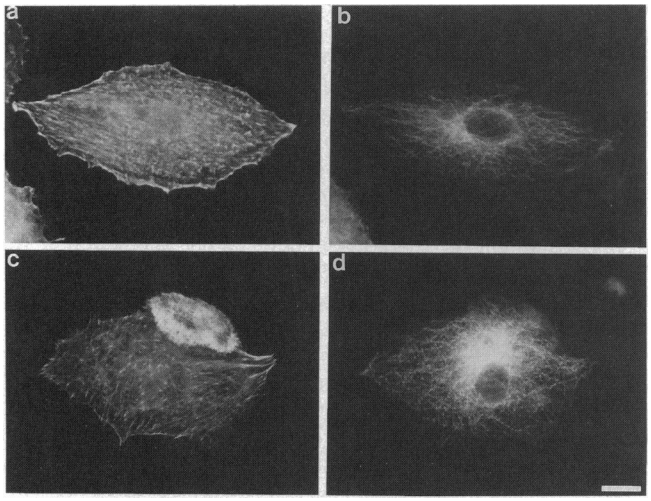

FIG. 6. Double-label immunofluorescence to analyze the distribution of microfilaments and microtubules in cells expressing TM-2 or TM-3. Microfilaments were visualized using rhodamine-phalloidin (*a* and *c*) and microtubules were visualized using a monoclonal anti-α-tubulin antibody (*b* and *d*). (*a* and *b*) Clone 2.6; (*c* and *d*) clone 3.4. (Bar = 15 μm.)

The ability of TM-2 and TM-3 to exhibit different effects on cytoarchitecture and growth properties shown in the present study are unlikely to be due to differential localization of these isoforms, since previous studies have demonstrated that these isoforms exhibit identical cellular localization patterns in fibroblasts (32). TM-2 and TM-3 are both expressed from the α-TM gene via alternative RNA splicing, and differ by the use of a single exon, exons 6b and 6a, respectively (20). How the use of these exons can contribute to the differences observed remains to be determined. The different effects observed are not simply due to differences in the levels of the expressed proteins since each isoform was expressed to similar levels in the cells lines established and the effects were seen regardless of the amount expressed. Previous studies of TM-2 and TM-3 indicated no significant differences in their relative affinities for F-actin (32).

Although expression of TM can result in cell spreading and the formation of prominent microfilaments, more work will be required to determine more precisely the role of TM in transformation. Perturbations in the integrity of the cytoskeleton could lead to global effects by interfering normal signaling pathways. Whether expression of TM-2 will suppress tumorigenicity will have to be determined in future studies. However, the ability of these cells to grow on soft suggests that will not be the case since this assay is a good indicator of tumorigenicity (1, 33). While changes in TM are common in many transformed cell lines, it is unresolved whether TM gene expression is a target of oncogene action or is an indirect consequence. In one study using a rat-1 cell line that contained a temperature sensitive src, changes in TM expression and cell shape were observed at the permissive temperature and returned to normal at the nonpermissive temperature (6). Thus,

at least in this case, it appears that TM expression is likely a target of oncogene action. How TM can overcome or circumvent the effects of ras or other oncogenes in the presence of their action is not known. Work currently in progress focuses on the elucidation of a possible direct interaction between oncogenic ras and TM and the involvement of components of the actin cytoskeleton in the complex events of signal transduction. In recent years a number of studies have demonstrated that expression of structural proteins such as vinculin (30), and α-actinin (34, 35) can suppress tumorigenicity in transformed cells, demonstrating the importance of structural proteins in the transformed phenotype. Further work will be required to address the relationship of alterations in cytoskeletal protein expression and transformation. Such studies will provide important information for the role that the cytoskeleton plays in normal and aberrant cell signaling.

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