MOLECULAR AND CELLULAR BIOLOGY, Apr. 1988, p. 1790–1794
0270-7306/88/041790-05$02.00/0

Vol. 8, No. 4

Modulation of Microfilament Protein Composition by Transfected Cytoskeletal Actin Genes

SUN-YU NG,[1]† HARRY ERBA,[2] GERALD LATTER,[1] LARRY KEDES,[2] AND JOHN LEAVITT[1]*

Armand Hammer Cancer Research Center, Linus Pauling Institute of Science and Medicine, Palo Alto, California 94306,[1] and MEDIGEN Project, Department of Medicine, Stanford University Medical School, and Veterans Administration Medical Center, Palo Alto, California 94304[2]

Received 13 July 1987/Accepted 24 December 1987

HuT-14T is a highly tumorigenic fibroblast cell line which exhibits a reduced steady-state level of β-actin due to coding mutations in one of two β-actin alleles. The normal rate of total actin synthesis could be restored in some clones of cells following transfection of the functional β-actin gene but not following transfection of the functional γ-actin gene. In γ-actin gene-transfected substrains that have increased rates of γ-actin synthesis, β-actin synthesis is further reduced in a manner consistent with an autoregulatory mechanism, resulting in abnormal ratios of actin isoforms. Thus, both β- and γ-actin proteins can apparently regulate the synthesis of their coexpressed isoforms. In addition, decreased synthesis of normal β-actin seems to correlate with a concomitant down-regulation of tropomyosin isoforms.

In all nonmuscle cells of higher eucaryotes, cytoskeletal actins and tropomyosins form a complex and dynamic network of microfilaments (19). The two major cytoskeletal actin isoforms in human fibroblasts, β- and γ-actins, differ by only four amino acids near the amino terminus (20), and no unique functional role is known for either actin isoform (17). These facts raise the following questions: first, since the ratios of the co-expressed β- and γ-actins are similar in most fibroblast cell lines (8, 20), would drastic alterations of this ratio have an impact on cellular phenotypes? For example, would an induced imbalance of actin isoforms affect tropomyosin expression? Second, is there a unique functional role for either the γ-actin or the β-actin protein? For example, we recently demonstrated that the β-actin protein autoregulates the synthesis of both β- and γ-actin isoforms (12, 13); does the γ-actin protein have the same autoregulatory function?

Previously, we introduced cloned mutant (Gly244→Asp244) and wild-type human β-actin genes into normal and transformed human fibroblasts to examine the effects of synthesis of exogenous actin on endogenous actin gene expression, the total cellular program of protein synthesis, and cellular phenotypes (12, 13). We succeeded in identifying transfected substrains that expressed the exogenous mutant β-actin gene in excess of the combined amount of β- and γ-actin synthesized from the highly active endogenous actin genes. These transfected substrains consistently exhibited reduced levels of synthesis of endogenous actin in apparent compensation for elevated exogenous actin synthesis to maintain the normal cellular concentration of actin. The expression of both endogenous β- and γ-actin decreased proportionately in response to expression of exogenous mutant β-actin, resulting in an unnatural imbalance in the two actin isoforms in individual transfected substrains.

This abundant expression of the transfected mutant β-actin gene also resulted in changes in cell morphology and concomitant reduction of rates of synthesis of five of six tropomyosin isoforms (12, 13). Such modulations in tropomyosin synthesis are characteristically seen in neoplastic transformation of avian, rodent, and human fibroblasts (1, 2, 5, 11, 15). Furthermore, abundant expression of mutant β-actin in immortalized, nontumorigenic human HuT-12 fibroblasts led to stable tumorigenic conversion of a subpopulation of transfected cells which exhibited enhanced mutant β-actin synthesis (13). These results from these previous studies left unanswered the question of whether it is the mutant β-actin itself or an imbalance in actin isoforms that contributes to the neoplastic phenotype of immortalized human fibroblasts by imposing cytoarchitectural defects or inducing abnormal expression of cytoskeletal tropomyosins or both.

In an attempt to distinguish these two possibilities and to address the other questions posed above, we have expressed exogenous wild-type β- and γ-actin genes in HuT-14T cells and examined the effect on actin and tropomyosin syntheses. The HuT-14T cell line has less β-actin, 70% that of KD, HuT-12, and HuT-14 cells (11), due to three coding mutations in one β-actin allele (7, 14) which encodes a labile variant protein (7). Another consequence of the mutant β-actin gene in HuT-14T cells, is a slightly higher rate of γ-actin synthesis relative to that of β-actin. In KD, HuT-12, and HuT-14 cells, the relative ratio of β-/γ-actin synthesis rates is 1.65 (8, 12). In contrast, the relative ratio of β-/γ-actin synthesis rates is about 0.82 in HuT-14T cells. These cells also exhibit abnormal expression of the tropomyosin isoforms (11). We have now found that, after introduction and expression of functional β- or γ-actin gene clones in HuT-14T cells, we can alter the β/γ actin synthesis ratios to greater degrees than in the cell lines previously studied, which have all of their normal actin alleles. Analysis of several such derived cell lines has allowed us to correlate alterations in expression of both β- and γ-actin with synthesis of microfilament proteins.

Wild-type actin gene expression in transfected HuT-14T cells. HuT-14T cells were transfected with either a wild-type human β-actin gene, 14Tβ-17 (10), or a wild-type human γ-actin gene, γNMA-1 (1a) in SV2neo vectors (18) and selected by resistance to G418 (12, 13). Eighteen and 22 subclonal isolates of HuT-14T cells transfected with either wild-type human β-actin or γ-actin genes, respectively, were picked and screened by examination of their relative rates of

* Corresponding author.
† Present address: Institute of Molecular Biology, Academica Simica, Taipei, Taiwan 11529.

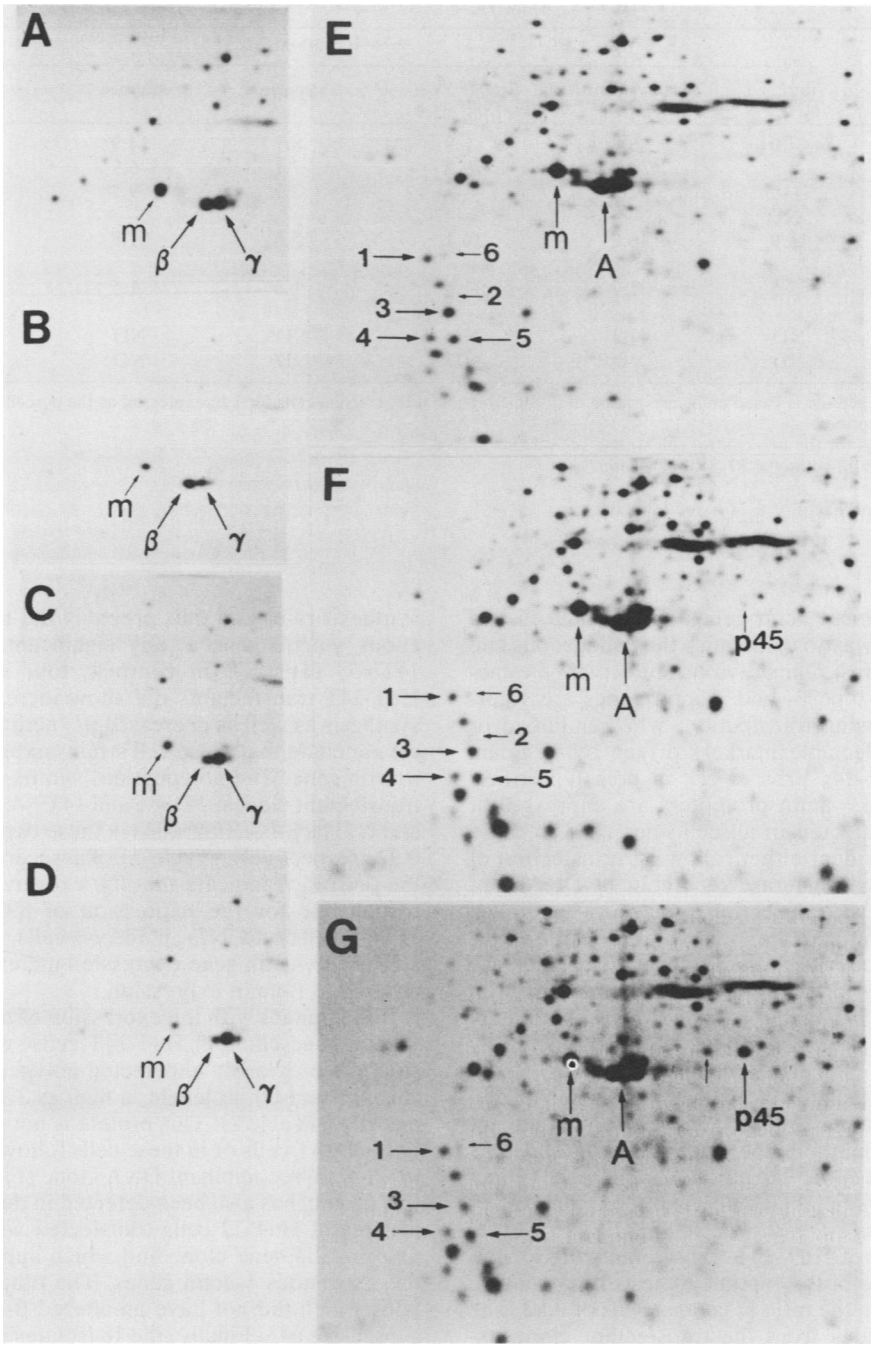

FIG. 1. Autoradiograms of [^{35}S]methionine-labeled polypeptides separated by two-dimensional polyacrylamide gel electrophoresis from total unfractionated HuT-14T (7) fibroblast G418-resistant subclones. (A) Unsaturated autoradiograms of actin polypeptides from 14Tβ8 (control, Table 1); (B) from 14Tβ9; (C) from 14Tγ4; (D) from 14Tγ6; (E) long-exposure autoradiograms of the abundant acidic polypeptides including the actin and tropomyosin isoforms and p45 from 14Tβ9; (F) from 14Tγ4; and (G) from 14Tγ6. Numbers 1 through 6 refer to the six tropomyosin isoforms (11), A indicates β- and γ-actin combined in saturated autoradiograms (E, F, G), β and γ indicated β-actin and γ-actin, respectively, in unsaturated autoradiograms (A, B, C, D), m indicates the triple mutant β-actin (7, 14), p45 indicates the 45-kilodalton polypeptide whose expression is induced accompanying expression of the exogenous γ-actin gene (Table 1). The small arrow indicates a polypeptide next to p45 (p45′, Table 1) that is coinduced with p45.

actin synthesis, using two-dimensional gel electrophoresis and computerized microdensitometry (11–13).

Figure 1 shows autoradiograms of [^{35}S]methionine-labeled total proteins from subclonal cells transfected with the wild-type β-actin (Fig. 1A, B, and E) or γ-actin (Fig. 1C, D, F, and G) gene clone. The relative rates of actin synthesis, determined by microdensitometry (11), are listed in Table 1. In our previous experiments, a mutant β-actin gene with a distinguishing amino acid mutation was introduced into these cell types (12, 13). In the experiments described here,

TABLE 1. Relative rates of protein synthesis[a]

Polypeptide	Protein synthesis rate (%)						
	14Tγ-5	14Tβ-8	Avg of controls[b]	14Tβ-10	14Tβ-9	14Tγ-4	14Tγ-6
β-Actin (wild type)	7.0	6.8	6.9	8.7	14.9	2.8	1.5
γ-Actin (wild type)	8.9	9.4	9.2	7.1	6.9	12.9	16.5
Exogenous β-actin[c]	—[d]	ND	—	2.9	9.2	—	—
Exogenous γ-actin[e]	ND[f]	—	—	—	—	9.5	14.7
Total stable actin	15.9	16.2	16.1	15.8	21.8	15.7	18.0
Total Tm isoforms	1.5	1.7	1.6	1.6	1.5	1.2	1.0
p45	ND	ND	ND	ND	ND	0.055	0.522
p45′	ND	ND	ND	ND	ND	0.012	0.184

[a] The amount of each polypeptide is based on incorporation of [^{35}S]methionine after labeling cells for 2 h, expressed as the percentage of each polypeptide in 700 abundant cellular polypeptides.
[b] Average of measurements for the two control cell strains 14Tγ-5 and 14Tβ-8.
[c] Exogenous β-actin = total β-actin − 0.82 × total γ-actin.
[d] —, Not present.
[e] Exogenous γ-actin = total γ-actin − 1.22 × total β-actin.
[f] ND, Not detectable.

in which wild-type human actin genes are introduced and expressed, there is no way to distinguish the endogenous and exogenous actin proteins. Our previous studies have established that these wild-type β- and γ-actin genes are vigorously expressed following transfection, whether linked or cotransfected with selectable markers driven by a simian virus 40 promoter (12–14; Erba et al., in press). Furthermore, both the β- and γ-actin promoters are expressed at high levels when transfected in all cell lines used in these experiments: this is evident either following transfection of chloramphenicol acetyltransferase recombinant DNA constructs tested in transient transfection assays or when linked to SV40neo vectors and stably expressed in cloned isolates (4; S.-Y. Ng and J. Leavitt, unpublished data). Thus, we assume that alterations of cytoskeletal actin expression in the transfected HuT-14T cells reported here is directly related to the accumulation of actin proteins engendered by the transfected genes.

Of the 18 subclonal isolates of HuT-14T cells transfected with the wild-type human β-actin gene clone, 14 did not show any apparent change in the β/γ-actin ratio and thus probably did not express the exogenous β-actin gene at any significant level. The radiolabeled polypeptides from one of these transfectant clones are shown in Fig. 1A. However, four subclonal isolates of HuT-14T transfectants did exhibit an altered relative ratio of β-/γ-actin synthesis rates ranging from 1.2 to 2.2, up from the ratio in control cells of 0.82. The radiolabeled polypeptides from the transfectant clone expressing the exogenous β-actin gene at the highest level, 14Tβ-9, are shown in Fig. 1B. We infer from this increase in the ratio of β/γ-actin synthesis rates that the exogenous β-actin gene expresses 62% of the cellular β-actin in the 14Tβ-9 substrain. A β/γ ratio as high as 2.2 has also been found in a subclonal isolate of KD cells transfected with the same wild-type human β-actin gene clone (12). Furthermore, 14Tβ-9 has an increased rate of total actin synthesis (Table 1), comparable to that of KD and HuT-12 cells. Therefore, expression of exogenous wild-type human β-actin genes can restore the normal rate of total actin synthesis in HuT-14T cells.

Of 22 subclonal isolates of HuT-14T cells transfected with a wild-type human γ-actin gene clone, 18 did not show any apparent change in the relative ratio of β- and γ-actin synthesis rates and thus probably did not express the exogenous γ-actin gene at any significant level (for example, 14Tγ-5, Table 1). In contrast, four subclonal isolates of HuT-14T transfectants did show increased rates of γ-actin synthesis as well as decreased β/γ actin synthesis ratios, and we conclude that these cell strains expressed the exogenous γ-actin gene. The polypeptides synthesized by two of these transfectant clones, 14Tγ-6 and 14Tγ-4, are shown in Fig. 1D and C. The β-/γ-actin ratios in these two isolates are 0.09 and 0.22, respectively (Table 1). These dramatic reductions in the β/γ ratios indicate that the exogenous γ-actin gene was responsible for the expression of 89% of the γ-actin in 14Tγ-6 cells and 74% in 14Tγ-4 cells. Thus, in the 14Tγ-6 substrain γ-actin gene expression increased almost eightfold relative to β-actin expression.

Concomitant with the expression of the exogenous human γ-actin gene clone in HuT-14T cells, we found the expression of a previously undetected polypeptide, designated p45 (Fig. 1F and G), at levels as high as 3% of total actin levels in 14Tγ-6 (Table 1). This protein is not detectable in HuT-12 or HuT-14T cells or in these cells following transfection with the β-actin recombinant DNA clone (Fig. 1E). Furthermore, this protein has also been detected in three of eight subclonal isolates of HuT-12 cells transfected with the wild-type human γ-actin gene clone and which appear to be expressing the exogenous γ-actin genes. The other five subclonal isolates which did not have an altered β-/γ-actin ratio did not synthesize p45. Finally, the 18 isolates of HuT-14T cells that did not express the exogenous γ-actin gene clone also did not have detectable p45 synthesis. Thus, there is a direct correlation between increased γ-actin accumulation due to expression of the exogenous γ-actin gene and the expression of p45. However, we cannot distinguish, based on our present data, whether p45 is induced by abnormal levels of γ-actin expression or is in some way encoded by the transfected γ-actin genomic segment. Analysis of the p45 peptide will be required to distinguish these alternatives.

Regulation of endogenous actin synthesis. After transfection of a human β-actin gene clone encoding a mutant β-actin into both diploid KD and immortalized HuT-12 cells, our previous work demonstrated that the steady-state level of β-actin appears to autoregulate its own synthesis to maintain a presumably optimal homeostatic cellular concentration of

actin (12, 13). Furthermore, the autoregulation mechanism does not seem to discriminate between β- and γ-actin since the relative rates of endogenous β- and γ-actin syntheses are each diminished to the same degree.

One of the goals of the experiments described here was to determine whether exogenous expression of γ-actin leads to a similar autoregulation or whether this function is peculiar to the β-actin isoform. Our analysis of HuT-14T cell clones expressing the transfected wild-type β- or γ-actin genes demonstrates that expression of either isoform leads to a down-regulation of endogenous actin synthesis and that the autoregulatory function does not distinguish β- from γ-actin isoforms. For example, in the two positive β-actin gene transfectant clones, 14Tβ-10 and 14Tβ-9, there is an apparent decrease in the rate of γ-actin synthesis concomitant with an increase in the rate of β-actin synthesis (Table 1). Since this increase in β-actin synthesis must be due to expression of the transfected β-actin gene, the corresponding decrease in the rate of γ-actin synthesis would most likely be due to the autoregulation of actin synthesis. Similarly, we also observe a down-regulation of endogenous actin synthesis in HuT-14T cells concomitant with the expression of the transfected wild-type γ-actin gene: in the two positive γ-actin gene transfectant clones, 14Tγ-4 and 14Tγ-6, there is an apparent decrease in the rate of β-actin synthesis (Table 1). Thus, although the expression of the transfected γ-actin gene in HuT-14T cells does not appear to restore total actin synthesis to normal levels, it does appear to down-regulate endogenous β-actin synthesis. We conclude that since the overproduction of either β-actin or γ-actin proteins from exogenous genes down-regulates the expression of endogenous β- and γ-actin genes, autoregulation of actin synthesis is a common characteristic of both isoforms and may involve common functional domains of the β- and γ-actin proteins or a common effector molecule(s).

In our previous studies on the effects of expression of transfected mutant β-actin genes (12, 13), we determined that it was the accumulation of β-actin proteins that effected the accumulation of endogenous γ-actin mRNA. Our conclusions in this report are based on the measurements of the relative rates of actin protein synthesis and assume that no major changes in the turnover of the usually long-lived actin proteins (>30 h) are induced by overexpression of the exogenously introduced genes. In support of this assumption we note that the ratios of the actin isoforms in the stained gels parallel the autoradiographic data (not shown). A more precise examination of the mechanism of this autoregulation is currently under study.

Regulation of tropomyosin synthesis. The alterations of accumulation of the actin proteins by expression of transfected genes also offers the opportunity to examine the impact on the synthesis rates of other proteins associated with the microfilament network. In particular, down-regulation of tropomyosin isoforms has been associated with neoplastic transformation (1, 2, 5, 11, 15). For example, the synthesis of tropomyosin isoforms is reduced in HuT-14T cells to levels half that of diploid KD and nontumorigenic HuT-12 cells (11). In HuT-14T cells, this decreased level of tropomyosin synthesis can be mostly accounted for by reduction in levels of tropomyosin isoforms Tm1, -2, and -6 (11). Furthermore, the expression of transfected mutant β-actin genes in KD and Hut-12 cells also led to down-regulation of the synthesis of the various tropomyosin isoforms (13). Analysis of total tropomyosin synthesis in the two clones, 14Tβ-10 and 14Tβ-9, which overexpress the wild-type β-actin appears normal (Table 1). In addition, a more detailed examination of the six tropomyosin isoforms in these two clones demonstrated no significant alterations among their relative rates of synthesis (e.g., Fig. 1E).

In contrast, in the two γ-actin transfectant subclones that synthesize significant levels of γ-actin, 14Tγ-4 and 14Tγ-6, the total synthesis of tropomyosin was down-regulated (Table 1). Analysis of the synthesis of the individual tropomyosin isoforms of these two clones (Fig. 1F and G) reveals that much of the alteration is related to down-regulation of Tm3 (decreased 36% in 14Tγ-4 and 65% in 14Tγ-6) and to a lesser extent to down-regulation of Tm1 and Tm6. Although the effect of overproduction of γ-actin on overall rates of tropomyosin synthesis is clear, additional clones must be examined to verify and confirm the more subtle changes we observed in the patterns of relative levels of individual tropomyosin isoforms.

Our experiments have successfully reversed the low β-actin phenotypes unique to HuT-14T cells by introduction of wild-type actin genes into these cells. Despite this, but not surprisingly, the transfectant clone 14Tβ-9 retains its tumorigenicity and still produces tumors after inoculation of 2×10^5 cells per mouse (7). Nor is the expression of the transfected wild-type β-actin gene in these cells accompanied by reversal of the suppressed rate of synthesis of tropomyosin isoforms seen in HuT-14T cells. This results in an even further increase in the molar ratio of actin to tropomyosin in 14Tβ-9 relative to HuT-14T cells. A high actin/tropomyosin molar ratio is associated with, and may have an important role in, the maintenance of the neoplastic state. Notably, mutant β-actin expression (13) and, at least in the case of the two clones 14Tγ-4 and 14Tγ-6, excessive γ-actin expression is associated with a reduction in synthesis of the four transformation-sensitive tropomyosin isoforms (Tm1, Tm2, Tm3, and Tm6). It will be of interest to learn whether a reduction in β-/γ-actin ratio (other examples are HuT-14T and FocT [9]) may be directly or indirectly (for example, via down-regulation of tropomyosin synthesis) involved in oncogenic transformation.

In regard to the questions posed at the outset, we can tentatively conclude the following: the autoregulatory function is common to both β- and γ-actin proteins and does not distinguish these two isoforms on a functional basis. Second, at least in the case of clone 14Tβ-9, a rise in the β/γ ratio does not affect tumorigenicity. Finally, a rise in the β/γ ratio by overproduction of β-actin does not appear to effect the synthesis of the microfilament-associated tropomyosins. However, levels of tropomyosin do fall in those cases in which the wild-type β/γ ratio is lowered, by either production of a mutant β-actin (12, 13) or overproduction of γ-actin (this study). One caveat to this conclusion is that the degrees of overproduction of β-actin in the clones we examined did not match those of γ-actin. With this reservation in mind, it remains tempting to speculate that tropomyosin levels may be sensitive to decreased levels of normal β-actin in cells and that this role of β-actin may prove to distinguish it from the function of the γ-actin isoform. Attempts to evaluate this hypothesis by direct alteration of synthesis rates of individual actins and tropomyosins are in progress.

The skillful technical assistance of Mary E. Ross and Margit Schlez is highly appreciated. We also thank Sandra Schwoebel for preparation of this manuscript.

H.E. was supported by the National Institutes of Health Medical Scientist Training Program at Stanford Medical School. This work was supported in part by a grant from the Public Health Service National Cancer Institute (CA 34763) to J.L., by grants from NIH (HD 17031) and the Veterans Administration to L.K., and by funds

donated to the Linus Pauling Institute from the Japan Shipbuilding Foundation and to J.L. by the Ripple Foundation.

LITERATURE CITED

1. **Cooper, H. L., N. Feurerstein, 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.

1a. **Erba, H. P., R. Eddy, T. Shows, K. Dedes, and P. Gunning.** 1988. Structure, chromosome location, and expression of the human γ-actin gene: differential evolution, location, and expression of the cytoskeletal β- and γ-actin genes. Mol. Cell. Biol. **8**: 1775–1789.

2. **Franza, B. R., and J. I. Garrels.** 1984. Transformation-sensitive proteins of REF52 cells detected by computer-analyzed two-dimensional gel electrophoresis. Cancer Cells **1**:137–146.

3. **Goldstein, D., and J. Leavitt.** 1985. Expression of neoplasia-related proteins of chemically transformed HuT fibroblasts in human osteosarcoma HOS fibroblasts and modulation of actin expression upon elevation of tumorigenic potential. Cancer Res. **45**:3256–3261.

4. **Gunning, P., J. Leavitt, G. Muscat, S.-Y. Ng, and L. Kedes.** 1987. A human β-actin expression vector system directs high level accumulation of antisense transcripts. Proc. Natl. Acad. Sci. USA **87**:4831–4835.

5. **Hendricks, M., and H. Weintraub.** 1981. Tropomyosin is decreased in transformed cells. Proc. Natl. Acad. Sci. USA **75**: 1334–1338.

6. **Kakunaga, T.** 1978. Neoplastic transformation of human diploid fibroblasts cells by chemical carcinogens. Proc. Natl. Acad. Sci. USA **78**:5633–5637.

7. **Leavitt, J., G. Bushar, T. Kakunaga, H. Hamada, T. Hirakawa, D. Goldman, and C. Merril.** 1982. Variations in expression of mutant β-actin accompanying incremental increases in human fibroblast tumorigenicity. Cell **28**:259–268.

8. **Leavitt, J., D. Goldman, C. Merril, and T. Kakunaga.** 1982. Changes in gene expression accompanying chemically-induced malignant transformation of human fibroblasts. Carcinogenesis (London) **3**:61–70.

9. **Leavitt, J., P. Gunning, L. Kedes, and R. Jariwalla.** 1985. Smooth muscle α-actin is a transformation-sensitive marker for mouse NIH 3T3 and Rat-2 cells. Nature (London) **316**:840–842.

10. **Leavitt, J., P. Gunning, P. Porreca, S.-Y. Ng, C.-S. Lin, and L. Kedes.** 1984. Molecular cloning and characterization of mutant and wild-type human β-actin genes. Mol. Cell. Biol. **4**:1961–1969.

11. **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.

12. **Leavitt, J., S.-Y. Ng, U. Aebi, M. Varma, G. Latter, S. Burbeck, L. Kedes, and P. Gunning.** 1987. Expression of transfected mutant β-actin genes: alterations of cell morphology and evidence for autoregulation in actin pools. Mol. Cell. Biol. **7**:2457–2466.

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

14. **Lin, C.-S., S.-Y. Ng, P. Gunning, L. Kedes, and J. Leavitt.** 1985. Identification and order of sequential mutations in β-actin genes isolated from increasingly tumorigenic human fibroblast strains. Proc. Natl. Acad. Sci. USA **82**:6995–6999.

15. **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.

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

17. **Otey, C. A., M. H. Kalnoski, J. L. Lessard, and J. C. Bulinski.** 1986. Immunolocalization of the gamma isoform of non-muscle actin in cultured cells. J. Cell Biol. **102**:1726–1737.

18. **Southern, P. J., and P. Berg.** 1981. 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.

19. **Stossel, T. P., C. Chaponnier, R. M. Ezzell, J. H. Hartwig, P. A. Janmey, D. J. Kwiatkowski, S. E. Lind, D. B. Smith, F. S. Southwick, H. L. Yin, and K. S. Zaner.** 1985. Non-muscle actin-binding proteins. Annu. Rev. Cell Biol. **1**:353–402.

20. **Vandekerckhove, J., J. Leavitt, T. Kakunaga, and K. Weber.** 1980. Coexpression 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.