

# 1

## ***Actin Organization as an *in Vitro* Assay for Tumorigenicity***

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### ***1. Introduction***

The endpoints of *in vivo* and *in vitro* assays applied to cells after exposure to a potential oncogenic transforming agent are cellular tumorigenicity and transformation. Tumors are failures of *in vivo* growth control; transformations are failures of *in vitro* growth control. Many agents cause tumors *in vivo*, many agents transform normal cultured cells, and some agents do both. However, even when caused by a single agent, the *in vivo* and *in vitro* endpoint assays show only a partial overlap. That is, some but not all tumors will grow as transformed cells in culture, and some but not all *in vitro* transformants will be tumorigenic on injection into susceptible animals (Shin *et al.*, 1975). Recently, we have described a subset of *in vitro* phenotypic changes that correlate with *in vivo* tumorigenicity (Steinberg *et al.*, 1979; Barrett *et al.*, 1979; Pollack, 1981). In this chapter, we will describe recent studies on one of the *in vitro* changes linked to tumorigenicity, the disruption in organization of cytoskeletal actin.

### ***1.1. Actin Organization and Oncogenic Transformation***

The major intracellular protein of both normal and transformed cells is actin (Pollard and Weihing, 1974). The actin of a normal spread cell is par-

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tioned by cofactors into a low-molecular-weight globular (G)-actin complex and a set of macromolecular arrays that include long chains of filamentous (F)-actin (Spudich *et al.*, 1977; Heuser and Kirschner, 1980). These large arrays contain several components each, and are found in the cell as a microfilament gel, a set of actin-containing stress fibers or cables, and an isotropic matrix of single microfilaments that fills the cytoplasm, interacting with microtubules and intermediate filaments (Heuser and Kirschner, 1980). The gel is located just under the membrane. Microfilament cables contain polyactin, myosin,  $\alpha$ -actinin, and tropomyosin. In normal cells, cables are located primarily at the adherent side of the cells just under the surface (Goldman *et al.*, 1975; Geiger, 1979). Thus, they share a localization with that part of the microfilament gel that also lies under the adherent surface.

In tumorigenic anchorage-transformed cells, the actin is repartitioned among these different macromolecular arrays. More of the actin is found in the microfilament gel and less of it in the cables (McNutt *et al.*, 1973; Heuser and Kirschner, 1980). This last event is most dramatic because the cables, mostly in one plane of focus, are easily visualized by immunofluorescence microscopy (Pollack *et al.*, 1975; Edelman and Yahara, 1976). Heuser and Kirschner (1980) have shown that this transition is accomplished by a shift of actin filaments in the cytoskeleton from bundles to a crudely interwoven structure. Apparently, then, transformation reweaves actin filaments.

Phalloidin is a compound derived from poisonous mushrooms that binds very tightly to F-actin (Lengsfeld *et al.*, 1974). To quantitate the pattern changes in actin-filament organization at the level of light microscopy, we have stained cells with fluorescent (Fl)-phalloidin (Wulf *et al.*, 1979; Verderame *et al.*, 1980). We have found two different changes in actin-cable size and number accompanying transformation and a human mutation leading to development of colonic neoplasms.

### **1.2. Actin Organization and Human Cancer**

Most human cancers are of unknown etiology. The relative weight of environmental factors and host genetic susceptibility is thought usually to be tipped toward the environment. In rare cases, however, the disease occurs as the result of a host mutation. In such cases, it is reasonable to hope that all cells of an affected person might reveal an altered phenotype when appropriately examined. Detection of such an abnormality in easily cultured cells might permit prognosis of asymptomatic children of individuals affected by an inherited cancer.

Patients with the autosomal dominant mutation adenomatosis of the colon and rectum (ACR) develop colonic polyps and adenocarcinomas of the lower intestine by middle age. One half of their children carry the mutation as well. In 1977, we reported that cytoskeletal actin patterns in forearm-skin-biopsy fibroblasts from ACR patients and in some of their children were abnormal. Bundles of actin were replaced in many cells by a diffuse actin distribution (Kopelovich *et al.*, 1977). Recently, we reported that this change

in actin patterns does not occur in skin fibroblasts from patients with other non-ACR, familial colonic neoplasms (Kopelovich *et al.*, 1980). We will consider here the quantitative distribution of F-actin patterns in skin cultures from patients with ACR and other inherited neoplasms and compare the distributions seen to those of normal and oncogenic transformed cell lines (Nicholson *et al.*, 1981).

## **2. Methods**

### **2.1. Cells and Culture**

A large number of cells from different sources were used in these studies. Established cells have been previously described (Steinberg and Pollack, 1979; Steinberg *et al.*, 1978; Pollack *et al.*, 1968). Human precrisis cells were received from Dr. M. Lipkin at the Memorial Sloan-Kettering Cancer Institute. All human and rat cells had been grown on plastic dishes in Dubecco's Modified Eagle medium (DME) (GIBCO H21) and 10% fetal calf serum (FCS) (Reheis) previous to assay. Mouse cells had been grown in DME and 10% calf serum (GIBCO). Culture conditions and procedures for passaging cells have been described (Steinberg and Pollack, 1979).

### **2.2. Anchorage Independence**

Cells were plated in triplicate at  $10^5$ ,  $10^4$ , and  $10^3$  cells/60-mm dish in 3 ml DME plus 10% FCS containing 0.33% agarose (Difco) over a 2-ml layer of 0.9% agarose in the same medium. Cultures were fed twice weekly with an additional 2 ml of the soft agarose-DME-10% FCS and cultured for 3 weeks. Large colonies greater than 0.2 mm in diameter were scored using a dissecting microscope. Total colony-volume increase in agar was determined as described by Steinberg and Pollack (1979).

### **2.3. Fixation and Staining for Localization of Actin by Antibody Immunofluorescence\***

Cells were plated on coverslips at a density of  $2-4 \times 10^3$  cells/cm<sup>2</sup> in 10% FCS. At 1 day later, medium was switched to 1% serum. After an additional day, coverslips were fixed in 10% formalin in phosphate-buffered saline (PBS), pH 7.1, kept in formalin for 4-8 days at 4°C, and then acetone-postfixed, stained sequentially with rabbit antiactin (1 : 80 in the PBS) and fluorescein-isothiocyanate-conjugated goat antiserum to rabbit IgG (1 : 20 in PBS), and mounted cell-side down in Aquamount (Pollack and Rifkin, 1975). Cells were scanned by fluorescence microscopy and were scored as positive for actin cables if fluorescent bands were seen to run the length of the

\*The method described in this section is that of Kopelovich *et al.* (1977, 1980).

cell when the edge of the cell was in focus. More than 100 cells were scored on each coverslip, and all experiments were scored in ignorance of the origin of the cells examined.

#### **2.4. Fixation and Staining for Localization of Actin by Fluorescent Phalloidin**

Cells were plated on coverslips at a density of  $2-4 \times 10^3$  cells/cm<sup>2</sup> in 10% FCS. At 1 day later, medium was switched to 1% serum. After an additional day, cells on coverslips were fixed in 10% formalin in PBS, for 20 min, rinsed with PBS, and extracted with 1% Nonidet P-40 in PBS for 20 min. Phalloidin concentrations were determined by absorbance spectroscopic measurements of concentrated stock solutions at 300 nm, which were corrected for the fluorescein absorbance by measurements at 492 nm. For FI-phalloidin staining, 10  $\mu$ l FI-phalloidin (1  $\mu$ g/ml in PBS, gift of T. Wieland, Göttingen West Germany) were incubated with cells at 37° C for 30 min. Coverslips were then rinsed three times in PBS and mounted on microscope slides with Aquamount.

#### **2.5. Fluorescence Microscopy**

Stained coverslips were examined with a Leitz Orthoplan microscope. A Zeiss Planapo 63 $\times$  oil immersion objective was coupled with a Letiz L2 exciter-barrier filter cube to visualize fluorescein.

#### **2.6. Specificity of Fluorescent Phalloidin Stain**

FI-phalloidin specificity for F-actin was determined in the following preincubation controls (Verderame *et al.*, 1980). G-actin, purified from acetone powder of chicken gizzard (J. Feramisco, personal communication), was converted to F-actin by the addition of KCl to a final concentration of 0.1 M. Actin preparations were mixed with FI-phalloidin for 20 min at room temperature, then applied to coverslips of fixed 3T3 cells for 20 min at 37°C. FI-phalloidin staining was completely blocked by 1 mg/ml of F-actin, not blocked by 2 mg/ml of G-actin, and only partially blocked by 10 mg/ml of G-actin. At this high concentration of G-actin, approximately 1%, or 0.1 mg/ml, is expected to be polymerized. Thus, staining with FI-phalloidin was sensitive to the polymerized state of the actin. Staining by antibody to actin was completely blocked by 45-min preincubation with 2 mg/ml of G-actin.

FI-phalloidin staining was blocked by preincubation of fixed cells with 1 mg/ml of unlabeled phalloidin solution or by coincubation with a mixture of 1  $\mu$ g/ml of FI-phalloidin and 1 mg/ml of phalloidin. Fixed cells preincubated with phalloidin at concentrations up to 100  $\mu$ g/ml showed normal antibody staining with antiactin. Thus, phalloidin blocked specific binding of FI-phalloidin, but not of actin antibody.

### 2.7. Photography

The photographs were taken by a Leitz Orthomat camera on Kodak Tri-X film developed in Microdol-X (1:3) at 22°C for 13 min. The prints were made on Kodak Polycontrast SC paper.

## 3. Results and Discussion

### 3.1. Fluorescent Phalloidin Permits Quantitation of Actin Patterns

Fl-phalloidin binds specifically to actin, and preferentially to F-actin (Wulf *et al.*, 1979; Verderame *et al.*, 1980). Well-spread fibroblasts stained with 1  $\mu\text{g/ml}$  of Fl-phalloidin show many patterns of actin organization. In some cells, large cables predominate, while in others, fine cables fill the cytoplasm at the plane of focus of cell-substrate adhesion, but large cables are few or entirely lacking. Finally, some spread cells do not contain any cables, but rather show a diffuse fluorescence (Fig. 1).

To quantitate the overall degree of organization of actin in a cell population, we scored 200 random well-spread stained cells by placing each into one of the four categories typified by the cells in Fig. 1. For each line, some cells fell into each category. However, the distributions were reproducibly different for different cell lines (Verderame *et al.*, 1980; Nicholson *et al.*, 1981).

### 3.2. Tumorigenicity and Fluorescent-Phalloidin Patterns

To quantitate a possible relationship between actin organization and tumorigenicity, we scored phalloidin patterns in cultures from 15 rat and mouse cell lines with widely varying phenotypes of growth control (Table 1). These lines included precrisis mouse and rat fibroblasts (MEF, REF), post-crisis growth-controlled cell lines (3T3, Rat 1), fully transformed cell lines (SV101, SVR85, SVR87, 14B, MCA), and a set of cell lines of intermediate growth control isolated as revertants (FL SV101, 1-4, 3-8) and intermediate transformants (SVR42, SVR63, SVR13). Cellular tumorigenicity of cell lines was assayed in *nude* mice (Shin *et al.*, 1975). Anchorage independence correlated well quantitatively and qualitatively with tumorigenicity in these lines (Table 1), as had been seen previously for other sets of rodent cell lines (Barrett *et al.*, 1979; Kahn and Shin, 1979; Shin *et al.*, 1975).

In earlier studies using antibodies to actin, data on actin organization of a cell line were presented as the percentage of cells containing detectable cables of any sort (Pollack *et al.*, 1975; Pollack and Rifkin, 1975; Rifkin *et al.*, 1979). With Fl-phalloidin, distribution of cells into four categories permits us to characterize populations according to three different criteria: the percentage of cells filled with large cables (I), the percentage of cells containing at least two large cables (I+II), and the percentage of cells containing any detectable

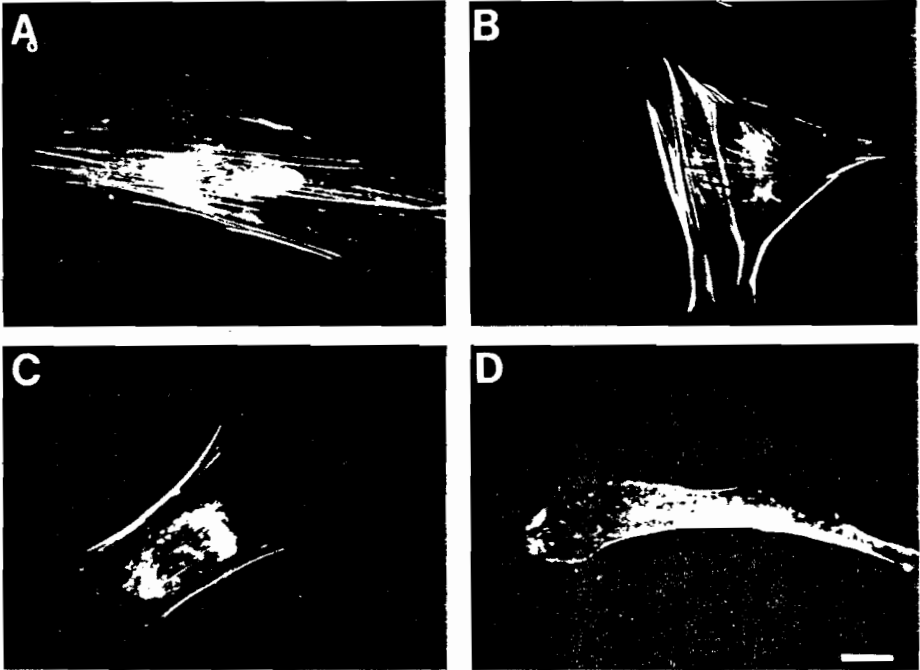


Figure 1. Four categories of F-actin distribution used for scoring cultures stained with Fl-phalloidin. (A) Category I: more than 90% of cell area filled with thick cables; (B) category II: at least two thick cables running under nucleus, rest of cell area filled with fine cables; (C) category III: no thick cables, but some fine cables present; (D) category IV: no cables visible in the central area of the cell. Scale bar: 20  $\mu\text{m}$ . Adapted from Verderame *et al.* (1980).

Table 1. Properties of Rodent Fibroblastic Cells<sup>a</sup>

Cell	Pre-/post-crisis	Transformed by	Subcloned from	Anchorage independence <sup>b,d</sup>		Tumorigenicity in <i>nude</i> mice <sup>c,d</sup>	
				RPE (%)	CVI		
Mouse	MEF	Pre-	—	—	≤0.001	ND	0/7
	3T3	Post-	—	—	≤0.001	ND	0/4
	SV101	Post-	SV40	3T3	27	ND	5/16
	FL SV101	Post-	—	SV101	0.01	ND	0/2
	SVR42	Post-	SV40	3T3	≤0.001	ND	ND
	SVR63	Post-	SV40	3T3	≤0.001	ND	0/2
	SVR13	Post-	SV40	3T3	0.2	ND	0/2
	SVR85	Post-	SV40	3T3	10.6	ND	ND
	SVR87	Post-	SV40	3T3	38.6	ND	3/4
Rat	REF	Pre-	—	—	<1	1.6	0/6
	Rat 1	Post-	—	—	$7 \times 10^{-3}$	13	2/3
	14B	Post-	SV40	Rat 1	15	380	3/3
	1-4	Post-	—	14B	$3 \times 10^{-3}$	21	0/4
	3-8	Post-	—	14B	$4 \times 10^{-3}$	1	0/4
	MCA	Post-	—	1-4	5.4	4200	3/3

<sup>a</sup> Adapted from Verderame *et al.* (1980).

<sup>b</sup> Growth without anchorage is measured in two ways: Relative plating efficiency (RPE) measures colonies greater than 0.2 mm diameter in agar as a percentage of colonies on a plastic dish. Colony-volume increase (CVI) measures the total increase in cell number in agar culture. The latter measure is more sensitive to slow anchorage-independent cell growth (Steinberg and Pollack, 1979).

<sup>c</sup> *Nude* mice with tumors at 6 months/total animals injected with  $10^7$  cells (Kahn and Shin, 1979).

<sup>d</sup> (ND) Not done.

cables, whether large or fine (I+II+III). In Fig. 2, tumorigenicity of 15 cell lines (Table 1) is compared with the fraction of cells in categories I+II and with the fraction of cells in categories I+II+III.

According to both criteria of actin organization, actin structures are reduced in size and number in transformed cells compared with normal cells. Many normal cells, however, do not have large cables, but only fine ones. As a result, the fraction of cells in categories I+II is as low in these nontumorigenic lines as it is in the tumorigenic lines (Fig. 2). On the other hand, the percentage of cells with any detectable cables (I+II+III) is reproducibly higher for nontumorigenic lines than for tumorigenic ones, so that the fraction of cells in categories I+II+III is roughly inversely proportional to the tumorigenicity of a cell line (Fig. 2). The one "nontumorigenic line" with a low value for this measure of actin organization in Fig. 2 is the revertant rat line 1-4 (Steinberg

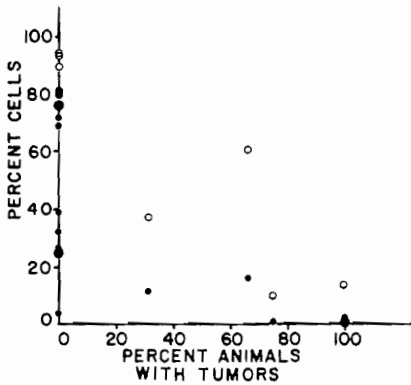


Figure 2. Correlation of disappearance of fine cables of F-actin in rodent cells with cellular tumorigenicity in *nude* mice. Rat and mouse cell lines with varying degrees of growth control (Table 1) were analyzed for F-actin organization by Fl-phalloidin stain. For a given cell line, the plots are of the fraction of cells in categories I+II (Fig. 1) vs. the tumorigenicity of that line (●) and of the fraction of cells in categories I+II+III vs. tumorigenicity (○). Tumorigenicity was measured by injection of cells into *nude* mice at  $10^7$  cells/mouse (Shin *et al.*, 1975; Steinberg *et al.*, 1979; Kahn and Shin, 1979).

*et al.*, 1978, 1979). This line forms long-lasting benign nodules in *nude* mice (Steinberg *et al.*, 1979).

Thus, according to this last criterion, all tumorigenic lines fell below 65%, suggesting that a quantitative threshold for predicting tumorigenicity may be available with the assay. Apparently, the loss of all detectable cables is more directly related to the acquisition of tumorigenicity than is the shift from large to fine cables.

### 3.3. Organization of Actin in Human Skin Fibroblasts

Since 1977, we have been studying the actin organization of human skin fibroblasts from patients with inherited propensities to develop cancer. Table 2 summarizes the results obtained by scan of these cells with antibody to actin (Kopelovich *et al.*, 1977, 1980).

Six groups of subjects were examined for their actin cable patterns: (1) normal subjects, either from the general population of spouses from the cancer families; (2) patients diagnosed with adenomatosis of the colon and rectum (ACR); (3) asymptomatic children of ACR patients, who have a 50%

Table 2. Actin Organization in Human Skin Fibroblasts as Determined with Antibody to Actin<sup>a</sup>

Phenotype	Cells positive for actin cables			
	Kopelovich <i>et al.</i> (1977)		Kopelovich <i>et al.</i> (1980)	
	Number of subjects	Positive (%) <sup>b</sup>	Number of subjects	Positive (%) <sup>b</sup>
Normal	9	77 (9.9)	16	76 (25)
ACR	11	32 (8.9)	7	37 (68.7)
CCP-	—	ND	7	81 (14.3)
CCP+	—	ND	6	67 (47.9)

<sup>a</sup> Adapted from Kopelovich *et al.* (1977, 1980).

<sup>b</sup> Positive cells contain at least two cables running the length of the cell. Results are expressed as mean percentage of cells (S.D.). (ND) Not done.

probability of developing the disease; (4) persons from colon-cancer-prone families who have colon cancer (CCP+); (5) persons from colon-cancer-prone families who have no symptoms but a 50% risk of developing colon cancer (CCP-); and (6) one patient with multiple primary colonic tumors. These published data with antiactin are based on a single threshold (+/-) score for the presence of cables (e.g., Pollack *et al.*, 1975).

In both earlier studies with antibody, we had found an ACR-specific reduction in the percentage of cells with cables (Table 2). However, since the percentage did not fall to zero, the possibility remained that cells from ACR patients had not undergone a change in actin similar to that in tumorigenic transformed cells, but rather had undergone a shift from larger to smaller cables.

Since we had already found that such a shift could occur in murine fibroblasts without acquisition of tumorigenicity (Fig. 2), we reexamined these human skin fibroblasts with F1-phalloidin (Nicholson *et al.*, 1981). Cells from ACR patients clearly show a dramatic loss of cells in categories I and II, compared with cells from other subjects (Table 3). In addition, a fraction of these cells lose all detectable cables (Table 3). Both changes are statistically significant for ACR by Student's *t* test (Table 4).

### 3.4. Asymptomatic Children of Patients with Adenomatosis of the Colon and Rectum

We examined cells from four children of ACR patients (Table 5). While these children were all free of symptoms of ACR at the time of their skin biopsies, we found that cells from one child (subject 4) had an F1-phalloidin actin distribution much like that of cells from ACR patients (See Table 3). That is, cells at the category I threshold were not different, but subject 4 had fewer cells with sufficient cables for the I+II threshold or the I+II+III

Table 3. Actin Organization in Human Skin Fibroblasts Determined with Fluorescent Phalloidin

Phenotype	Number of subjects	Cells in categories (%) <sup>a</sup>		
		I	I+II	I+II+III
Normal	5	25 (28)	48 (26)	89 (10)
ACR <sup>b</sup>	7	4 (4)	15 (9)	68 (19)
CCP- <sup>c</sup>	2	54 (14)	66 (11)	88 (6)
CCP+ <sup>d</sup>	2	26 (32)	53 (15)	88 (7)
MPT <sup>e</sup>	1	1 (2)	19 (21)	73 (9)

<sup>a</sup> Skin-fibroblast cultures were examined between the 5th and 12th passages. Categories of F-actin distribution in fixed cells are shown in Fig. 1. Briefly, category I cells have large F-actin cables, category II cells have a mixture of large and fine cables, category III cells have only fine cables, and category IV cells lack cables detectable with F1-phalloidin. Results are expressed as mean percentage of cells (S.D.).

<sup>b</sup> Skin fibroblasts from patients with adenomatosis of the colon and rectum (Kopelovich *et al.*, 1980).

<sup>c</sup> Skin fibroblasts from asymptomatic colon-cancer-prone subjects.

<sup>d</sup> Skin fibroblasts from symptomatic colon-cancer-prone subjects.

<sup>e</sup> Skin fibroblasts from a subject with multiple primary colonic tumors.

Table 4. Differences among Human-Fibroblast Filamentous-Actin Distributions Significant by Student's T Test

Comparison	Cells in categories (Fl-phalloidin)		
	I	I+II	I+II+III
Normal vs. ACR	0.2 > P > 0.1	0.01 > P > 0.001	0.01 > P > 0.001
Normal vs. CCP-	P > 0.9	P > 0.9	0.8 > P > 0.7
Normal vs. CCP+	P > 0.9	P > 0.9	P > 0.9
Normal vs. MPT	0.6 > P > 0.5	0.1 > P > 0.05	0.2 > P > 0.1
CCP- vs. CCP+	0.2 > P > 0.1	0.9 > P > 0.8	P > 0.9

threshold than did the other three subjects. This distribution is significantly different from normal and also different from that of other non-ACR subjects or the other three asymptomatic children (Table 6).

All four children are under clinical observation, so it will be known in time whether any of them develop symptoms of ACR. Insofar as the Fl-phalloidin pattern distribution of subject 4 is not significantly different from the average distribution found in the cells of ACR patients (Table 6), it is reasonable to predict that subject 4 carries the ACR mutation and in time will develop symptoms of the disease.

### 3.5. Actin Organization vs. Age of Patients

Most patients with ACR present symptoms by early adulthood. Thus, an age bias may have arisen in the production of data of the sort presented above. If actin organization in fibroblasts increased with age of the individual, then our results might be explained as a consequence of the younger age of ACR patients and their children as compared with our normal control subjects.

To test this, we examined the skin fibroblasts of a group of normal children ranging in age from 3 months to 18 years. In Figs. 3 and 4, we show the actin organization of all persons examined so far, vs. their ages. Figure 3 shows the percentage of cells in categories I+II, while Fig. 4 shows the percentage of

Table 5. Percentages of Cells in Fluorescent-Phalloidin Categories from Asymptomatic Children of ACR Patients

Subject	Age	Sex	Cells in categories (%) <sup>a</sup>		
			I	I+II	I+II+III
1	20	F	13 (5)	48 (8)	91 (3)
2	10	F	7 (7)	51 (17)	92 (8)
3	19	M	33 (27)	64 (12)	97 (1)
4	8	M	10 (11)	20 (12)	40 (11)

<sup>a</sup> Results are expressed as mean percentage of cells (S.D.).

Table 6. Differences among Fluorescent-Actin Distributions in Asymptomatic Children of ACR Patients Significant by Student's *t* Test<sup>a</sup>

Comparison	Cells in categories (F1-phalloidin)		
	I	I+II	I+II+III
Subjects 1-3 avg. vs. Normal avg.	0.7 > <i>P</i> > 0.6	0.9 > <i>P</i> > 0.7	<i>P</i> > 0.9
Subjects 1-3 avg. vs. ACR avg.	0.2 > <i>P</i> > 0.1	0.3 > <i>P</i> > 0.2	0.01 > <i>P</i> > 0.001
Subject 4 vs. Normal avg.	0.7 > <i>P</i> > 0.6	0.4 > <i>P</i> > 0.3	0.01 > <i>P</i> > 0.001
Subject 4 vs. ACR avg.	0.5 > <i>P</i> > 0.4	0.6 > <i>P</i> > 0.5	0.05 > <i>P</i> > 0.02
Subjects 1-3 avg. vs. Subject 4	0.9 > <i>P</i> > 0.8	0.1 > <i>P</i> > 0.05	0.01 > <i>P</i> > 0.001

cells in categories I+II+III. Comparison of the two figures reveals that age does indeed have an effect on actin organization, but that this effect is largely on the fraction of cells with large cables (I+II) (Fig. 3). This fraction is quite low in young normal people under age 10, as low as it is in ACR patients.

However, the fraction of cells with any detectable cables (I+II+III) remains high at all ages (Fig. 4). By this criterion, actin cables in ACR cells are slightly but consistently less well organized, at all ages. In no case does the percentage of ACR cells in categories I+II+III fall below the threshold of 60%, which separates tumorigenic from nontumorigenic rodent fibroblasts (cf. Figs. 2 and 4).

#### 4. Conclusions

From these data, we may reasonably conclude that:

1. The actin cytoskeleton of a normal cell can be disrupted in at least two distinct ways: loss of all detectable cables or loss of only large cables.

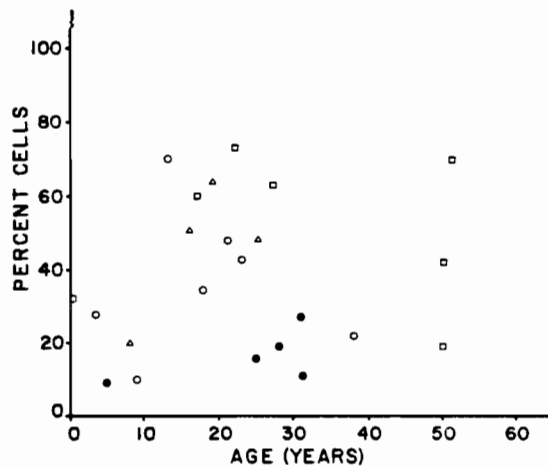


Figure 3. Percentage of human skin fibroblasts with large cables (categories I+II) from subjects of different ages. Skin fibroblasts from subjects with different diagnoses were stained with F1-phalloidin and scored for the appearance of large cables (categories I+II in Fig. 1). For each culture, the fraction of cells in categories I+II is plotted vs. the age of the subjects. (●) patients with ACR; (○) normal subjects; (△) children of ACR patients; (□) CCP subjects.

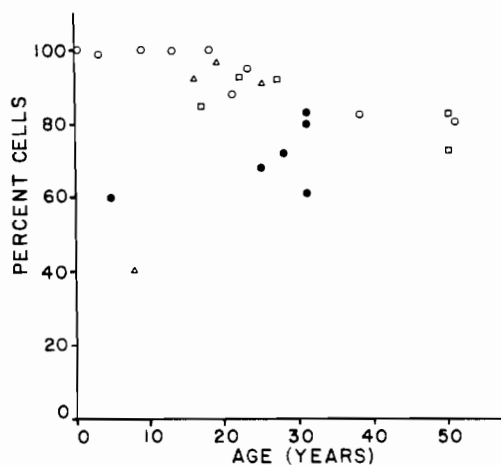


Figure 4. Percentage of human skin fibroblasts with detectable cables (categories I+II+III) from subjects of different ages. Skin fibroblasts from subjects with different diagnoses were stained with Fl-phalloidin and scored for the appearance of large and fine cables (categories I+II+III in Fig. 1). For each culture, the fraction of cells in categories I+II+III is plotted vs. the age of the subjects. For the key to the symbols, see the Fig. 3 caption.

2. Only the greater of these disruptions correlates with tumorigenicity.
3. The lesser disruption may be predictive for at least one inherited propensity to develop cancer.

Finally, Fl-phalloidin has permitted the detection of two different alterations in actin organization, and there is no reason to exclude the possibility of even finer distinctions existing among cell lines in culture. However, assaying the differences by eye has proven quite tedious. We plan in the near future to couple Fl-phalloidin fluorescence images to a vidicon-based digitalized image analyzer (Macagno *et al.*, 1979) to yield signals that can be analyzed and compared by computer. This will make possible detailed prospective studies of cultured skin-biopsy cells from persons at risk for cancer.

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