

Synthesis of Cytoskeletal and Contractile Proteins by Cultured IMR-90 Fibroblasts

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ABSTRACT Models of the assembly of cytoskeletal and contractile proteins of eukaryotic cells require quantitative information about the rates of synthesis of individual component proteins. We applied the dual isotope technique of Clark and Zak (1981, *J. Biol. Chem.*, 256:4863–4870) to measure the synthesis rates of cytoskeletal and contractile proteins in stationary and growing cultures of IMR-90 fibroblasts. Fibroblast proteins were labeled to equilibrium with [^{14}C]leucine over several days, at the end of which there was a 4-h pulse with [^3H]leucine. Fractional synthesis rates (percent per hour) were calculated from the $^3\text{H}/^{14}\text{C}$ ratio of cell protein extracts or protein purified by one- or two-dimensional polyacrylamide gel electrophoresis and the $^3\text{H}/^{14}\text{C}$ ratio of medium-free leucine. The average fractional synthesis rate for total, SDS- or urea-soluble; Triton-soluble; and cytoskeletal protein extracts in stationary cells each was $\sim 4.0\%/h$. The range of values for the synthesis of individual proteins from total cell extracts or cytoskeletal extracts sliced from one-dimensional gels was similar, though this range was greater than that for major proteins of Triton-soluble protein extracts. Three specific cytoskeletal proteins—actin, vimentin, and tubulin—were synthesized at similar rates that were significantly slower than the average fractional synthesis rate for total protein. Myosin, on the other hand, was synthesized faster than average. Synthesis rates were the same for β - and γ -actin and polymerized (cytoskeletal extract) vs. Triton-soluble actin. The same was true for α - and β -tubulin and two different forms of vimentin. Synthesis rates were uniformly higher in growing cells, though the same pattern of differential rates was observed as for stationary cells. Synthesis rates in growing cells were higher than the rate necessary to maintain the growth rate, even for those cytoskeletal proteins being synthesized slowly. Therefore, there appears to be some turnover of these cytoskeletal elements even during growth. We conclude that proteins in cytoskeletal extracts may have nonuniform rates of synthesis, but at least one important subclass of cytoskeletal proteins that comprise filament subunits have the same synthesis rates.

Much attention recently has focused on understanding the organization and function of cytoskeletal and contractile elements of nonmuscle cells. It is now clear that an extensive, highly organized though complicated three-dimensional structural network, variously referred to as the cytoskeleton or skeletal framework, exists in the cytoplasm of nonmuscle cells (3, 21, 51,–53, see reference 41 for a comprehensive review). This cytoskeleton is composed of a number of different structural elements such as microtubules, microfilaments, intermediate filaments, and microtrabeculae; and is composed in part of proteins such as the tubulins, actins and vimentin,

desmin or keratins, depending on the cell type (3, 9, 31, 41, 51–53). At the same time, other elements, many with a regulatory role, remain uncharacterized. It has been proposed that these elements may constitute not only a functional, but also a distinct, biochemical “compartment” of the cell (e.g., reference 21). The cytoskeleton appears to be responsible for maintenance of cellular three-dimensional architecture as well as involved in changes in that structure that occur throughout the life of the cell, such as during cell division, spreading, migration, etc. (see reference 41). The cytoskeleton may also provide an essential scaffolding on which metabolic processes

such as protein synthesis occur (3, 7, 22, 32, 51–53).

Little is known about the turnover of cytoskeletal and contractile proteins that comprise the cytoskeleton. Most studies of synthesis have involved the use of tracer quantities of [³⁵S]methionine and have addressed questions about relative rates and patterns of synthesis of specific cytoskeletal proteins in dividing (e.g., references 8, 35, and 45), differentiating (e.g., references 11, 24, 44, 48, and 49), and attaching or spreading cells (e.g., references 4, 5, and 20). Such studies have led to specific models for the synthesis of these classes of proteins (21, 22, 32). An impression that these studies give is that the turnover of cytoskeletal and contractile proteins is slow (e.g., reference 6). The nature and design of most experiments to date, however, have precluded accurate assessment of fractional and absolute synthesis rates, principally because of lack of measurement of the specific activity of the precursor pool of isotopic amino acid (see reference 42) and only semiquantitative measurements of incorporated isotopic amino acid.

An understanding of the assembly and function of cytoskeletal and contractile elements, however, requires quantitative information about the synthesis and turnover of individual proteins, and of other elements. The purpose of our studies was to measure the fractional synthesis rates of specific cytoskeletal and contractile proteins of growing and stationary cultured human lung fibroblasts under more rigorous conditions. A preliminary report of these results appeared elsewhere (34).

MATERIALS AND METHODS

Materials: IMR-90 human embryonic lung fibroblasts were obtained from the Institute for Medical Research (Camden, NJ). All culture medium components were purchased from Gibco Laboratories (Grand Island, NY); isotopes and NCS tissue solubilizer from Amersham Corp. (Arlington Heights, IL); Econofluor from New England Nuclear (Boston, MA); and electrophoresis supplies from Bio-Rad Laboratories (Richmond, CA) and LKB (Rockville, MD). General laboratory reagents were from Sigma Chemical Co. (St. Louis, MO) or J. T. Baker Chemical Co. (VWR Scientific, San Francisco, CA).

Cell Culture: IMR-90 human embryonic lung fibroblasts (39) were cultured as described previously (27, 28) in Eagle's minimal essential medium containing 10% fetal bovine serum and L-glutamine (2 mM). All experiments involved cultures between the population doubling levels of 23 and 31. The cells were plated at different seeding densities, depending on the experiment, and grown for the periods and under the conditions specified for each experiment (see below).

Isotopic Labeling: We used the methods described by Clark and Zak (14). Basically, cells were grown over several days to isotopic equilibrium in the presence of one isotope of leucine (usually ¹⁴C) and then pulsed for several hours with another leucine isotope (usually ³H) in the continued presence of the first isotope. Comparison of the isotope ratio (³H/¹⁴C) in protein(s) with that in the medium allows calculation of fractional synthesis rates (14), since it has been shown that leucine in the precursor pool for protein synthesis equilibrates isotopically with free leucine in the culture medium (14, 34).

In control experiments to determine the time course of isotopic equilibration, cells were plated at 3×10^5 cells/60-mm dish and grown for 5 d in the presence of 1.0 μ Ci/ml of [³H]leucine in Eagle's minimum essential medium containing 0.1 mM nonradioactive leucine (final specific activity, ~ 10 mCi/mmol). In experiments designed to measure fractional synthesis rates in stationary cells, the cells were plated at 3×10^5 cells/60-mm dish and grown for 5 d in the presence of 0.1 μ Ci/ml of [¹⁴C]leucine (final concentration, 0.1 mM; final specific activity, 1.0 mCi/mmol) beginning 1 d after plating. Medium that contained the [¹⁴C]leucine was then changed every other day. On the fifth day the cells were given a 4-h pulse of [³H]leucine (2.5 μ Ci/ml; final specific activity, 25 mCi/mmol) which was added to the [¹⁴C]containing medium. The medium and cells were then processed as described below. In experiments to measure synthesis in growing cells, the cells were plated at 5×10^5 cells/60-mm dish and grown with [¹⁴C]leucine as described above for 4 d, when they were replated in the presence of [¹⁴C]leucine at $2.5\text{--}3.0 \times 10^5$ cells/60-mm dish. The 4-h [³H]leucine pulse was then begun 3 d later.

Sample Preparation: Medium was removed from each dish at the end of the 4-h pulse with [³H]leucine. We determined the ³H/¹⁴C ratio by adding 10 μ l medium to 150 μ l NCS + 3.0 ml Econofluor and counted the samples by conventional liquid scintillation spectrometry (³H efficiency, 25%; ¹⁴C efficiency, 49%; ¹⁴C spill, 4.7%) in a liquid scintillation counter (model 3320; Packard Instrument Co., Downers Grove, IL). Control experiments in which we determined the ³H/¹⁴C ratio of dansyl-leucine derivatives of medium free leucine separated by two-dimensional thin layer chromatography (2) validated use of the direct measurement.

Cell monolayers were rinsed through five beakers, each of which contained 200 ml phosphate-buffered saline, before being recovered and extracted according to the protocols used for separating classes and individual proteins. We prepared total cell extracts by solubilizing the cells, with scraping, in 1% SDS in 0.05 M sodium cacodylate, pH 6.0, containing 1.0 mM phenylmethylsulfonyl fluoride. Extracts for isoelectric focusing and two-dimensional gel electrophoresis were made in sample buffer of 9.0 M urea, 0.025 M Tris-(hydroxymethyl)aminomethane (Tris) pH 7.4, 0.01 M dithiothreitol (18) that also contained 1.0 mM phenylmethylsulfonyl fluoride. Separation of cytoskeletal from other cellular proteins was accomplished by extracting cells in buffer containing 0.140 M NaCl, 0.005 M MgCl₂, 1% Triton X-100, and 0.01 M Tris, pH 7.4, at 0–4°C, plus 1.0 mM phenylmethylsulfonyl fluoride. After homogenization, the two protein fractions were separated by centrifugation at 8,000 g for 1 min in a Beckman microfuge (Beckman Instruments, Inc., Fullerton, CA). The pelleted cytoskeletal protein extract was then washed twice with NaCl-MgCl₂-Tris buffer without Triton and suspended in 0.2 ml of urea two-dimensional sample buffer.

The isotope content and ratio of total, cytoskeletal, and Triton-soluble extracts were determined by precipitating proteins in cold trichloroacetic acid (final concentration, 8%), filtering and counting as described previously (2).

Gel Electrophoresis: One-dimensional SDS PAGE was performed according to Laemmli (30). Two-dimensional electrophoresis (isoelectric focusing followed by SDS) was according to O'Farrell (40) and Garrels (26). In some experiments, individual protein bands (actin and vimentin) were sliced out of one-dimensional SDS gels and reelectrophoresed in isoelectric focusing slab gels to achieve better separation of actin and vimentin isoforms (33). In brief, bands containing actin or vimentin were cut from one-dimensional unstained 8.5% acrylamide SDS gels and placed in the wells of isoelectric focusing slab gels (4% acrylamide, containing pH 4–6 and pH 3–10 ampholines). These gels were then focused for 20 h at 350 V. The gels were placed in 10% trichloroacetic acid and then washed three times in 50% methanol before staining with either Coomassie Brilliant Blue or silver (54).

Counting of Proteins in Polyacrylamide: Gels were stained, destained, and the bands of interest (see Results) were sliced out with a surgical blade and placed in 20-ml glass scintillation bottles. 1 ml of NCS/water (9:1) was added, and the bottles, were tightly capped and then heated at 55°C for 2 h. The samples were then cooled and counted in 10 ml Econofluor. We removed the slices before counting to preclude the problem of differential counting of ³H and ¹⁴C remaining in protein still in the gel.

Protein Standards: Cardiac myosin, prepared as described previously (33), was the kind gift of Dr. Raye Z. Litten, Department of Physiology and Biophysics, University of Vermont. Actin was prepared from liver acetone powders according to Vandekerckhove and Weber (50), vimentin from IMR-90 fibroblasts according to Franke et al. (19), and tubulin from calf brain according to Shelanski et al. (47).

Statistical Analyses: Data from different cell extracts or individual proteins were analyzed for significant differences by standard *t*-tests (15) using multiple comparisons tests when appropriate (36).

RESULTS

Separation and Identification of Proteins

Measurement of synthesis rates of individual, specific proteins requires their purification and identification before analysis for isotopic content. The methods we used to accomplish this depended on a combination of cell fractionation, followed by one- or two-dimensional electrophoresis with the proper identification of bands or spots by co-electrophoresis with purified proteins. The latter was of major importance, given the variability with regard to the relative positions of proteins such as vimentin and the tubulins in two-dimensional gels that has been reported in the literature. Thus, for example, α - and β -tubulin identified by Bravo and Celis (8) are in a

distinctly different pattern relative to vimentin than are the same two proteins as identified by Lazarides et al. (e.g., reference 23).

We have defined cytoskeletal proteins on the basis of their insolubility in our Triton-containing extraction buffer. It is the resistance of the *in vivo* cytoskeletal complex to neutral detergent disruption that is responsible for this. For purposes of discussion, we describe proteins not part of this complex as Triton soluble.

As shown in Fig. 1, α -tubulin migrates very close to vimentin in both the SDS and isoelectric focusing direction in our gel system. This necessitated their separation from one another by a cell fractionation procedure. To effect clean protein separations, we carried out the extractions at 0–4°C so that the tubulins, normally part of the cytoskeletal complex, are found in the Triton-soluble fraction (Fig. 1D) with vimentin remaining in the cytoskeletal fraction (Fig. 1B). Our measurements of the fractional synthesis rates for the tubulins isolated on one-dimensional SDS gels of Triton-soluble ex-

tracts were significantly higher than when measurements were based on analysis of spots on two-dimensional gels (not shown). This appeared not to be a problem for actin and vimentin, at least based on measurement of similar synthesis rates for one- and two-dimensional gel separations of appropriate extracts. Similarly, the same rates of synthesis were obtained for myosin whether it was analyzed in total cell extracts or cytoskeletal extracts.

Another aspect of this problem is that individual proteins (e.g., actin) exist in different physical states in the cell and are fractionated differentially on that basis. Interpretation of the data would, in this case, also have to contend with possible precursor-product relationships (see below).

Our synthesis measurements required that one isotope of leucine be present long enough for cellular proteins to achieve the same specific activity as medium free leucine. A control experiment showed that this was accomplished by a protocol in which cells were labeled, in this case with [³H]leucine, for 5 d with a medium change every other day. The specific

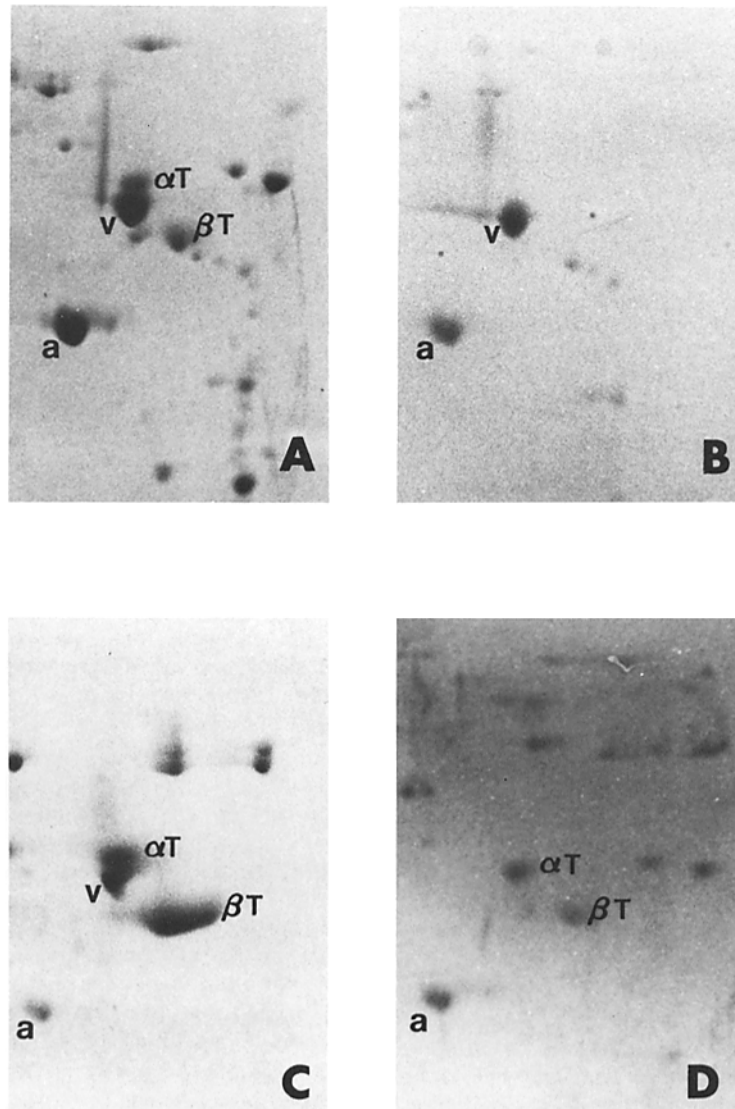


FIGURE 1 Two-dimensional PAGE of protein extracts of cultured IMR-90 fibroblasts. Extraction procedures were as described in Materials and Methods. Only the pertinent region of the gel is shown. A, total cell extract; B, cytoskeletal extract; C, cytoskeletal extract to which purified alpha- and beta-tubulin had been added; D, Triton-soluble extract. a, actin; α T, alpha-tubulin; β T, beta tubulin; v, vimentin.

TABLE I. IMR-90 Fibroblast Protein Synthesis

Cell fraction	Fractional synthesis rate		Fractional synthesis rate normalized to that for total protein	
	Growing	Stationary	Growing	Stationary
	%/h			
Total cellular proteins	4.8 ± 0.2 (8)	4.0 ± 0.2* (11)	1.0	1.0
Cytoskeletal extract	4.8 ± 0.3 (6)	4.1 ± 0.2* (9)	0.98 ± 0.05 (6)	1.00 ± 0.04* (9)
Triton-soluble extract	4.7 ± 0.1 (9)	3.9 ± 0.1* (6)	0.93 ± 0.02 (9)	0.87 ± 0.03* (6)
Myosin	5.6 ± 0.4 ^{§§} (7)	4.9 ± 0.3 ^{§§} (11)	1.15 ± 0.07 ^{§§} (11)	1.21 ± 0.04 ^{§§} (11)
Actin	3.9 ± 0.2 [‡] (7)	2.7 ± 0.1 ^{**} (11)	0.80 ± 0.03 [‡] (7)	0.69 ± 0.02 ^{**} (11)
Vimentin	3.8 ± 0.1 [‡] (6)	2.6 ± 0.1 ^{**} (9)	0.79 ± 0.01 [‡] (6)	0.63 ± 0.02 ^{**} (9)
α-Tubulin	3.4 ± 0.1 [‡] (4)	2.2 ± 0.3 ^{**} (4)	0.72 ± 0.03 [‡] (4)	0.59 ± 0.04 ^{**} (4)
β-Tubulin	3.4 ± 0.2 [‡] (3)	2.2 ± 0.2 ^{**} (4)	0.71 ± 0.02 [‡] (3)	0.61 ± 0.04 ^{**} (4)

Cellular fractionation, separation of individual proteins by one- and two-dimensional PAGE, and counting procedures were as described in Materials and Methods. Myosin and actin were isolated for analysis by one-dimensional PAGE of whole cell extracts, vimentin by one-dimensional PAGE of cytoskeletal extracts, and tubulins by two-dimensional PAGE of Triton-soluble extracts. Data are expressed as mean ± SE (n).

* $P < 0.01$ vs. growing cells by multiple comparisons test.

† $P < 0.01$ vs. total cellular proteins by paired multiple comparisons test.

‡ $P < 0.01$ vs. actin, vimentin, and tubulin by paired multiple comparisons test.

activity of [³H]leucine relative to that of extracellular free leucine was 0.95 ± 0.05 (mean ± SE; $n = 6$) for total cellular protein; 0.92 ± 0.03 ($n = 3$) for actin; 0.94 ± 0.03 ($n = 3$) for vimentin; and 0.94 ± 0.02 ($n = 3$) for myosin. All experiments, therefore, were done using a protocol that included a minimum of 5 d for equilibration of the first isotope. We confirmed the achievement of equilibration in each individual experiment by comparing the specific activity of the equilibration label in protein with the specific activity of radiolabeled free leucine in the medium.

Our experiments were designed to have [¹⁴C]leucine as the equilibrated isotope, as did Clark and Zak (14). A pilot experiment showed, however, that $0.4 \mu\text{Ci/ml}$ of [¹⁴C]leucine, necessary to achieve a sufficient amount of labeling in the presence of 0.4 mM unlabeled leucine normally present in Eagle's minimum essential medium, inhibited cell growth observed 5 d later (not shown). This was not true when [¹⁴C]leucine was present at $0.1 \mu\text{Ci/ml}$ (not shown), the concentration we used subsequently. This necessitated reducing the leucine concentration in the culture medium from 0.4 to 0.1 mM to ensure that sufficient ¹⁴C radioactivity would be present in individual proteins isolated by gel electrophoresis.

Table I summarizes the results of our measurements of the fractional synthesis rates of total cellular proteins, proteins in the cytoskeletal and soluble fraction, and individual proteins purified by gel electrophoresis. The extracts used, as well as the gel methods employed to separate each protein, are described in the legend. Our first experiments were with cells in the stationary phase of growth in which the cells were accumulating little or no protein. The fractional rate of synthesis of total cellular proteins was $\sim 4.0\%/h$. The overall synthesis rates for cytoskeletal and soluble proteins as groups were not significantly different ($P > 0.10$ by paired t -test). We calculated from the total ¹⁴C-radioactivity in the two extracts that 10–15% of total cellular protein was in the cytoskeletal extract, and the rest was in the Triton-soluble fraction.

At the same time, a wide spectrum of rates were calculated for individual proteins in each of these fractions. The data in Table II show the range of values for isotope ratio obtained when proteins in these extracts were separated by electrophoresis in one-dimensional gels and major stained bands with sufficient radioactivity sliced out and counted. The variance (mean/SD) for each extract was approximately the same for

TABLE II. Synthesis of Electrophoretically Separated Proteins of IMR-90 Cell Extracts

	Gel band ³ H/ ¹⁴ C ratio			
	Number of bands	Mean	SE	Range
Total extract				
Growing	20	4.6	0.2	3.3–7.3
Stationary	15	3.6	0.3	2.2–6.0
Cytoskeletal extract				
Growing	15	5.1	0.3	3.4–8.5
Stationary	15	3.8	0.2	2.4–5.8
Soluble extract				
Growing	14	4.2	0.1	3.6–4.9
Stationary	10	3.7	0.1	3.1–4.5

Extracts described in Materials and Methods were electrophoresed in one-dimensional SDS gels, stained, and bands were sliced out and counted as described. SE, standard error.

the bands sliced from gels of total and cytoskeletal extracts, whereas it was substantially less for the bands from gels of soluble extracts.

The calculated fractional synthesis rates of several major individual contractile and cytoskeletal proteins in stationary cells also are shown in Table I. Myosin was synthesized at a rate significantly higher than for total cellular proteins. Actin, vimentin, and α- and β-tubulin, however, were synthesized at a significantly lower rate than average. It is also important to note that the latter three proteins were being synthesized at approximately the same rate (see Discussion).

The corresponding data for growing cells also are shown in Tables I and II. Synthesis rates were higher for each fraction and individual protein analyzed than were the corresponding rates in stationary cells. The difference between the fractional rates of synthesis for proteins with a relatively fast (e.g., myosin) and relatively slow (e.g., actin) synthesis rate was less, however, as is expected for growing vs. stationary cells (37; see Discussion).

We also compared the synthesis rates of actin in cytoskeletal and soluble extracts, as well as of β- and γ-actin, the two isoforms present in fibroblasts (25). The rates were the same in all cases, as shown in Table III. We also calculated that β-actin was $\sim 60\%$ of total actin present on the basis of the total

TABLE III. *Synthesis of Actin and Vimentin*

	Fractional synthesis rate	
	Growing	Stationary
Actin		
Soluble	3.7 ± 0.1 (11)	2.9 ± 0.1 (6)
Cytoskeletal	3.5 ± 0.2 (6)	2.6 ± 0.1 (7)
β	3.4 ± 0.1 (8)	2.5 ± 0.2 (11)
γ	3.6 ± 0.1 (9)	2.6 ± 0.2 (11)
Vimentin		
Spot 1	3.8 ± 0.1 (9)	2.6 ± 0.2 (11)
Spot 2	3.3 ± 0.2 (9)	2.4 ± 0.2 (11)

Data shown are mean ± SE (*n*). Vimentin appeared as a major spot (spot 1) and a minor, more acidic one (spot 2), as discussed in Results.

amount of ¹⁴C radioactivity (equilibration label) in each actin spot (not shown).

Vimentin bands cut from one-dimensional SDS gels of cytoskeletal extract migrated as two proteins of slightly different isoelectric point in isoelectric focusing slabs (not shown). Others have found this and showed that the more acidic form (spot 2) is phosphorylated (17). These two putative forms of vimentin were synthesized at approximately the same rates in growing and stationary cells (Table III).

DISCUSSION

Our results for the first time provide quantitative information about the fractional synthesis rates for several prominent proteins associated with the cytoskeleton. This has been possible through use of the elegant dual isotope methodologies described by Clark and Zak (14), which doubtless will prove a most useful tool for future similar studies.

We have confirmed on a more quantitative basis the general impression reached by others (e.g., reference 6) that the synthesis rates of many cytoskeletal proteins are slow relative to that of cellular proteins in general. At the same time, the synthesis rate of one component protein, myosin, is higher than average. The difference in relative synthesis rates of actin and myosin is similar to that reported for skeletal and cardiac muscle (see reference 42) and raises important questions about the turnover of the complex contractile machinery of muscle and nonmuscle cells alike (see below).

The mean fractional synthesis rate for total extractable cellular proteins of stationary cells was ~4.0%/h. Cells in this culture stage are approximately in a steady state condition of cell growth. Hence, the synthesis rates we measured are also an approximation of turnover rates. Thus, proteins on the average are degraded at a rate of ~4.0%/h. Proteins such as myosin are degraded more rapidly, whereas proteins such as actin, vimentin, and tubulin are replaced more slowly.

The synthesis rate of groups of proteins as well as of specific ones was higher in growing cells, as expected. The smaller difference between the rates of synthesis of myosin and actin, vimentin, or tubulin in growing as compared with stationary cells should not be construed to indicate the proportion of these proteins is changing. Rather it is simply a result of imposing a uniform rate of increase in protein content (percent per hour) on top of the steady state rate needed to maintain protein content, as Millward (37) has discussed.

The number of growing cells we used doubled every 24 h, as based on direct cell counts made on additional dishes of cells cultured with each experiment (not shown). The fractional synthesis rate necessary to support this growth rate is 2.9%/h. It is noteworthy that the synthesis rates we measured

for actin, vimentin, and tubulin were significantly higher. This indicates that there must be some degradation of these elements even in rapidly dividing cells.

Our data about the synthesis of the specific proteins we analyzed would be complicated if there were precursor pools of the proteins from which polymers were assembled. Such a precursor pool has been proposed for vimentin (17, 38, 46). In this case, however, there appears to be rapid entry of newly synthesized soluble vimentin into the insoluble cellular pool. There appears also to be a rapid rate of exchange of G- and F-actin with a half-time on the order of a few minutes (10, 13, 29). We chose a 4-h pulse at least in part to avoid this potential problem. Time-course studies that involved brief isotope pulses (e.g., references 13 and 22) would provide important new information bearing on this important problem.

The recent results of Rechsteiner et al. (43) may indicate that the rate of turnover of cellular proteins is a function of the extent of their association with the structural elements of the cell. A complete understanding of cytoskeletal protein turnover will not come easily if this is true for cytoskeletal proteins themselves, as may be the case for elements of the striated muscle sarcomere (16, 37, 55). Most of the methods used to measure protein turnover assume that the degradative process is random (37, 55). The possibility this is not true may require use or development of alternative strategies as the problem is investigated further.

Our data provide an interesting insight into the model proposed by Fulton et al. (22) by suggesting that cytoskeletal proteins are assembled directly upon synthesis by polyribosomes attached to the cytoskeleton. It has been known for some time that polysomes are associated with the three-dimensional microtrabecular lattice seen in the cytoplasm of most cells (51–53). Lenk et al. (32) found polysomes to be associated with nonionic detergent-insoluble cytoskeletal extracts of HeLa cells. Polysomes were associated with nonfilamentous portions of the cytoskeleton (but see reference 1) and could be released by treatment with ribonuclease, whereas ribosomes but not mRNA were released when polysomes were disaggregated. This suggests an attachment through mRNA. The finding of major changes in protein synthesis when anchorage-dependent cells are forced into a suspension (4, 20) condition that also profoundly affects the cytoskeleton, suggests a functional association of major importance. This is supported by the findings of Cervera et al. (12) that the binding of mRNA to cytoskeletal elements may be obligatory for proper translation.

Fulton et al. (22) used acridine fluorescence to localize polyribosomes on the cytoskeleton of 3T3 fibroblasts and [³⁵S]methionine autoradiography to locate the synthesis of newly made proteins. Pulse-labeled, nonionic detergent (Triton)-insoluble proteins were located near polysomes near the center of the cell and migrated outwards only if protein synthesis was allowed to continue. The data also suggested that there was little protein exchange within the cytoskeleton. Their model suggests that cytoskeletal proteins are associated with skeletal framework before they are released from ribosomes. The studies and model have been extended by Fey et al. (21) to include Madin-Darby canine kidney cells.

The model above would be satisfied most easily if the involved cytoskeletal proteins had the same fractional synthesis rates. Our data on the spectrum of synthesis rates for proteins in our cytoskeletal extracts (Table III) suggest that this is not true. It is striking, however, that the fractional

synthesis rates of three major components—actin, vimentin, and tubulin—are not significantly different from one another. Bear in mind, however, that though we isolated tubulin as a soluble unpolymerized protein at low temperature, normally it is an important component of the cytoskeleton.

Such data would tend to support the model of Fulton et al. (22). At the same time, models can be constructed that still allow assembly of cytoskeletal structures as nascent chains even if there are dissimilar turnover rates for the individual elements. This would be possible if nascent chains elongation rates are dissimilar. It would also be possible if a portion of newly synthesized components are degraded immediately or otherwise not incorporated directly into the cytoskeleton. Finally, turnover rates could be dissimilar if there were no stable interactions between individual elements, such that each could enter and exit the cytoskeletal framework at different rates. The aforementioned studies of Rechsteiner et al. (43) add the further complication that the turnover of individual cytoskeletal proteins may partly be determined by the degree of association with the cytoskeleton.

In summary, we have defined fractional synthesis rates for several prominent cytoskeletal and contractile proteins in growing and stationary cultures of IMR-90 fibroblasts. The spectrum of synthesis rates for the major protein in cytoskeletal extracts is as great as for major proteins in total extracts. At the same time, the similar, relatively slow rates of synthesis of actin, vimentin, and tubulin are consistent with a model in which the cytoskeleton is assembled as nascent chains.

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