Efficient Mammalian Protein Synthesis Requires an Intact F-Actin System*

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The mammalian protein synthesizing system is highly organized in vivo, and its substrate, tRNA, is channeled throughout the translation process. However, the cellular components responsible for this organization are not known. To examine this question a series of studies was carried out using intact and permeabilized Chinese hamster ovary cells. We show that cold shock dramatically reduces the protein synthetic capacity of these cells by as much as 95%. The loss of activity can be reversed by a short recovery period under conditions that allow energy metabolism to occur; transcription and translation during the recovery period are not needed. While individual components of the translation apparatus are not inactivated by the cold shock, the supramolecular organization of the system appears to be altered and F-actin levels are found to decrease. Resumption of protein synthesis during the recovery period coincides closely with the restoration of F-actin to normal levels. Moreover, disruption of actin filaments, but not microtubules, also leads to a major reduction in translation. These data support the conclusion that the cellular microfilament network plays an important role in the structure and function of the translation system and that perturbations of this network can have profound effects on protein synthesis.

Considerable evidence has now accumulated showing that the mammalian protein synthetic machinery is highly organized in vivo, both structurally and functionally (1–3). For example, recent work with a permeabilized Chinese hamster ovary cell (CHO)1 system, that closely mimics the living cell, demonstrated that a channeled tRNA cycle functions during the translation process (3), i.e. aminoacyl-tRNA and tRNA, the intermediates in the process, are directly transferred from the aminoacyl-tRNA synthetases, to the elongation factor, to the ribosome, and back to the synthetases, without dissociation into the cellular fluid. Such studies suggest that the various parts of the translation apparatus must associate as tRNA is transferred from one component to the next. In fact, since only small amounts of RNA and protein leak out of permeabilized cells under conditions in which large, exogenously-added macromolecules can enter, it is likely that the components of the translation machinery are permanently organized into a supramolecular structure (2). Additional evidence for an organized translation machinery comes from the isolation of a multienzyme aminoacyl-tRNA synthetase complex and other assemblies which contain multiple translation components (4–8). Interactions among various individual components of the translation system also have been demonstrated in vitro, supporting the idea that similar associations occur in vivo (9–12). Thus, elucidating the components responsible for this supramolecular organization and how they influence protein synthesis is essential for a complete understanding of the translation process in vivo.

Several pieces of information suggest that the cytoskeletal elements of the cell serve this organizing function for the eukaryotic protein synthesizing system. For example, microscopic analyses showed that a number of translation components colocalize with cytoskeletal structures (13–15). Other studies demonstrated that polyribosomes (16–18), mRNA (19, 20), aminoacyl-tRNA synthetases (21, 22), initiation factors (17), and elongation factors (23, 24) all can associate with the cytoskeleton. However, very little is understood about the physiological significance of such interactions between the cytoskeleton and components of the translation apparatus and how these interactions might actually affect protein synthesis.

In this paper we show, using intact and permeabilized CHO cells, that rapidly reversible, relatively minor perturbations of the cytoskeleton can have profound effects on protein synthesis and on the structural organization of the translation system. These effects have been localized to the actin filaments. Our data suggest that the microfilament network plays an important role in maintaining the structural integrity and function of the protein synthesizing machinery of the mammalian cell.

EXPERIMENTAL PROCEDURES

Materials—A mixture of five 3H-labeled amino acids (leucine, lysine, phenylalanine, proline, and tyrosine) and 3H-labeled uridine was purchased from Amersham. Unlabeled amino acids, ATP, GTP, creatine phosphokinase, phosphocreatine, saponin, trypsin inhibitor, trypsin blue, oligo(dT)-cellulose, cytochalasin D, colchicine, TRITC-phalloidin, phallolidin, cycloheximide, actinomycin D, DNase I, and RNase A were obtained from Sigma. Latrunculin B was from ICN. Cell culture reagents were from Life Technologies, Inc. Rabbit liver tRNA was prepared as described (2). Goat polyclonal antibodies against rabbit EF-1a were kindly provided by W. C. Merrick (Case Western Reserve University, Cleveland, OH).

Growth and Treatment of Cells—CHO cells were cultured as described (25) and used for experiments 1 day after reaching confluency. Cells were washed once with warm phosphate-buffered saline (PBS) (137 mM NaCl, 5 mM KCl, 1.8 mM KH2PO4, 8 mM Na2HPO4) and harvested from plates by incubation with 0.05% trypsin, 0.53 mM EDTA solution (Life Technologies, Inc.) for 4 min at 37 °C. Approximately 1 ml of ice-cold PBS containing 0.45 mg/ml trypsin inhibitor was added per 75-cm² flask and left for several minutes. After centrifugation, released cells were washed twice with ice-cold PBS, and suspended in ice-cold PBS supplemented with 50 μM of each of the 20 amino acids, 25 mM

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glucose, 2 mM CaCl₂, and 1 mM MgCl₂. These cells were used for measurement of protein synthesis either after staying on ice for 20 min (non-recovered cells) or after incubation at 28 °C for 20 min (recovered cells).

Permeabilization of CHO Cells—Cells were washed once with ice-cold permeabilization buffer S (130 mM sucrose, 50 mM KCl, 50 mM potassium acetate, 20 mM Hepes, pH 7.4) and were suspended in the same buffer using ~60 μl of buffer per 10⁷ cells. The volume of the total cell suspension was measured by pipetting, and an equal volume of saponin solution (150 μg/ml) in buffer S was added to give ~1.2 μg of saponin per 10⁷ cells. The suspension was mixed, incubated at 37 °C for 6 min on ice, and centrifuged for 1 min at 420 × g at 4 °C. In experiments in which permeabilized cells were used to determine leakage of translation components, the separated cell pellet and supernatant fraction were each used to measure the component of interest. When the permeabilized cells were used for other studies, they were further washed with PSW buffer (150 mM sucrose, 50 mM KCl, 50 mM potassium acetate, 20 mM Hepes, pH 7.4, 5 mM ATP, 13 mM phosphocreatine, 6.1 mM MgCl₂, 2.6 mM CaCl₂, 5.3 mM EDTA, 5 mM glucose), and were suspended in 4 to 5 volumes of PSW buffer for immediate use. A small portion was taken to measure cell permeability by trypan blue exclusion and for cell counting.

Measurement of Protein Synthesis—Protein synthesis in intact cells was carried out in PBS containing 250 μg of each of the 20 amino acids, a mixture of [H]-labeled leucine, lysine, phenylalanine, proline, and tyrosine (~25 dpm/μg each), 25 μM of each of the 20 amino acids, and 1% Triton X-100. Incubation was at 28 °C. At various times, portions were taken, precipitated with 10% trichloroacetic acid containing 0.5% casamino acids, and boiled for 5 min. After at least 10 min on ice, the precipitate was collected on Whatman GF/C filters. The hot trichloroacetic acid-insoluble radioactivity was considered to represent newly-synthesized protein.

Measurement of Aminoacyl-tRNA—Intact cells were incubated for 5 min under the same conditions as for protein synthesis to presynthesize aminoacyl-tRNA. After cooling on ice, cells were spun down, washed once in buffer S, and permeabilized with saponin, as above. Cell and supernatant fractions were separated by centrifugation. The cell pellet was resuspended in buffer S. Both fractions were made 0.3M in sodium acetate (pH 6.8), and were extracted with an equal volume of phenol. The aqueous phases from each sample were divided into two equal portions. One portion was used to measure total trichloroacetic acid-precipitable radioactivity, and the second was first incubated with RNase A (2 μg/ml) for 10 min at 30 °C before acid precipitation. The difference in radioactivity between these two portions was considered to represent aminoacyl-tRNA.

Measurement of Aminoacyl-tRNA Synthetase Activity—After permeabilization, cell and supernatant fractions were sonicated. Fractions were assayed at 28 °C for 5 min in 10 μl of reaction mixture containing the same components as for protein synthesis in the presence or absence of 1 mg/ml rabbit liver tRNA, and precipitated with 10% trichloroacetic acid. Since exogenous tRNA is known not to increase the level of protein synthesis in each preparation as described under “Experimental Procedures.” Protein synthesis was carried out under optimal conditions for each system, but the overall concentration of cellular components was kept identical in each case. What differed, and what can be seen to affect translation as much as 100-fold, is how these cellular components remained unchanged. F-actin measurements were highly reproducible, varying <10% in different experiments.

RESULTS

Relation of Protein Synthesis and Cell Organization—Previously, we suggested that the structural organization of the cell influences channeling of aminoacyl-tRNA and the efficiency of protein synthesis (2). To re-enforce this point, we directly compared the rate of protein synthesis in intact, permeabilized, and sonicated CHO cells (Table I). Protein synthesis was carried out under optimal conditions for each system, but the overall concentration of cellular components was kept identical in each case. What differed, and what can be seen to affect translation as much as 100-fold, is how these cellular components are organized in each system. For example, rendering cells permeable reduced protein synthesis 2–3-fold. This value is slightly different from that reported earlier (2) primarily because of the use of more optimal conditions for the intact cells. However, a much more dramatic 40-fold reduction in translation was observed when the permeabilized cells were sonicated, or likewise, when homogenates were used (not shown). These data emphasize the importance of the organization of the protein synthesis machinery for efficient translation and they raise the important question of what cellular component(s) is responsible for maintaining this organization.

Recovered and Non-recovered Cells—To address this question, we made use of an earlier observation (3) in which it was found that CHO cells after trypsin/EDTA treatment to remove them from the substratum transiently lost most of their translation capacity. Protein synthesis could be restored by preincubating the suspended cells in 10% calf serum in PBS for 20 min at 28 °C (3). More detailed investigation has shown that the

| Preparation       | Protein synthesis % |
|-------------------|---------------------|
| Intact cells      | 100                 |
| Permeabilized cells| 40                  |
| Sonicated cells   | 1                   |

Buffer using the same staining mixture except that the Nonidet P-40 was omitted. To determine the level of nonsaturable staining with TRITC-phalloidin (27), unlabeled phalloidin (45 μM) was added as a competitor to some samples. All of the samples were stained for 2 h at 4 °C, pelleted for 20 min at 15,000 rpm in a microcentrifuge, and the pellet extracted with methanol for at least 48 h. Fluorescence of the extracted TRITC-phalloidin was read in a Perkin-Elmer LS-50 fluorometer (excitation 540 nm, emission 575 nm). Non-saturable staining was subtracted from all data.

Measurement of Poly(A) RNA—Cells were prelabeled with [3H]uridine for 12 h and treated to prepare recovered or non-recovered cells. Total RNA was isolated from each. Chromatography on oligo(dT)-cellulose was carried out as described (28). Fractions from the oligo(dT)-cellulose column containing poly(A) RNA were precipitated with 10% trichloroacetic acid, and the precipitate was collected on Whatman GF/C filters. Acid-insoluble radioactivity was considered to represent poly(A) RNA.

All the experiments presented were carried out at least twice, and in most cases, multiple times with completely reproducible results. Representative experiments are presented. Although the absolute level of protein synthesis may vary slightly among experiments due to differences in batches of CHO cells, the relative activities of recovered and non-recovered cells, the effects of inhibitors and the leakage of components remained unchanged. F-actin measurements were highly reproducible, varying <10% in different experiments.
Procedures."

One portion of the cells was preincubated with glucose, Ca\(^{2+}\) and Mg\(^{2+}\) at 28 °C for 20 min (recovered cells), and one portion was left in ice for the same length of time (non-recovered cells). Protein synthesis in both populations was subsequently measured for the indicated times using the procedure under "Experimental Procedures." Presented in Fig. 1 is a comparison of the rate of protein synthesis in cells that were treated with trypsin/EDTA/cold PBS without a subsequent recovery period and identically treated cells that were allowed to recover at 28 °C. In the absence of the recovery period (non-recovered cells), there is an almost complete shut-down of protein synthesis, especially during the first several minutes of incubation. However, as incubation of these cells continues, the rate of protein synthesis begins to accelerate because the recovery process also occurs during the conditions of protein synthesis. In contrast, cells allowed a prior 20-min recovery period at 28 °C (recovered cells) immediately synthesize protein at a rapid rate which is comparable to that of cells remaining in a monolayer. These dramatic effects are not due to differences in amino acid uptake which was found to be identical for both recovered and non-recovered cells. Moreover, non-recovered cells were fully recoverable and were able to grow normally after plating on DMEM. Additional studies revealed that the loss of protein synthetic capacity was not due to removal of cells from the substratum nor to the trypsin/EDTA treatment, but rather to the cold shock engendered by placing the suspended cells in ice and washing them with cold PBS. This was shown by the major reduction in protein synthesis observed when cells still present in a monolayer were cooled on ice and washed with cold PBS (Table II).

The fact that protein synthesis in cold-shocked cells could be restored completely by a brief incubation with glucose and cations suggested that the reversible rapid loss and subsequent recovery of translation capacity might simply represent subtle changes in organizational states of the cells, and that these changes might be used to investigate the importance of cellular organization for the functioning of the translation machinery. This idea was examined in more detail in the studies to follow.

Comparison of Components of the Translation Machinery from Recovered and Non-recovered Cells—The low level of protein synthesis in non-recovered cells suggested that whatever perturbation of the translation machinery had occurred in these cells, its effects on protein synthesis were major. As a first step to ascertain what these alterations might be, we compared the level of a number of the obvious, key components of the translation machinery in recovered and non-recovered cells.

The amount of aminoacyl-tRNA was the same in both groups of cells (1382 versus 1379 cpm/10\(^6\) cells). Since it is known that most of the tRNA in the cell is in the aminoacylated state (29), these data suggest that the amount of total tRNA in the cell is unaltered. Likewise, aminoacyl-tRNA synthetase activity in non-recovered cells (432 cpm/1.5 min/10\(^6\) cells) was as high as that in their recovered counterparts (428 cpm/1.5 min/10\(^6\) cells), indicating that this component of translation machinery also was not damaged.

The level of elongation factor 1a (EF-1a), was measured by Western blot analysis and densitometric scanning of the resulting films. No significant difference was found in the amount of this translation component between recovered (100%) and non-recovered cells (95%).

The most vulnerable and short-lived component of the protein synthetic machinery is the mRNA, the vast majority of which is polyadenylated at its 3' end. mRNA would be a likely component to be affected when protein synthetic capacity is diminished. However, as with the other components, there was no decrease in the amount of radioactive poly(A) RNA in non-recovered cells (120% of recovered cells), suggesting that mRNA degradation during trypsin/EDTA-cold PBS treatment was not responsible for the loss in protein synthetic capacity. Taken together, these data suggest that important elements of the translation machinery have not been destroyed or lost in non-recovered cells.

Effect of Inhibitors on the Recovery Process—As an additional approach to understanding the translation defect in non-recovered cells, and the recovery process that corrects it, a series of inhibitors that affect various metabolic processes were examined for their ability to interfere with recovery. Inhibitors were added during the recovery period at a concentration that was shown in control experiments to dramatically reduce the specific metabolic process under study, but which could be washed out after the recovery period so that subsequent protein synthesis would be unaffected. Using such a protocol, inhibitors that are known to affect RNA synthesis, protein synthesis, or energy metabolism were examined.

As shown in Table III, neither the transcription inhibitor, actinomycin D, nor the translation inhibitor, cycloheximide, affected the recovery process as determined by the subsequent initial rate of protein synthesis. At the concentrations used actinomycin D inhibited RNA synthesis >95% and cycloheximide inhibited translation >80% during the recovery period.
The measurement of the leakage of certain of its components after cells are permeabilized with saponin (2, 30). Permeabilization generates cells able to synthesize proteins at rates similar to that of the intact cell rate despite the fact that there are openings in the cell membrane large enough for macromolecules to enter (2). Since the macromolecular components required for protein synthesis are organized into a supramolecular structure, they are largely retained within the permeabilized cells (2, 30). However, if this structure were disturbed, one might expect increased leakage of at least some macromolecular components of the translation machinery. Accordingly, we examined whether there was any change in leakage from permeabilized, non-recovered cells compared with permeabilized, recovered cells.

The results of these experiments are shown in Table IV. Reproducibly, a significantly greater percentage of the total aminoacyl-tRNA and EF-1a populations leaks out of non-recovered cells compared with recovered cells. These data are consistent with what might be expected if there were damage to the supramolecular organization of the translation machinery resulting in release of aminoacyl-tRNA and EF-1a. It should also be noted that the values shown probably represent minimum values for leakage from non-recovered cells. During the permeabilization procedure cells are incubated at 37 °C for 6 min and some recovery can occur during this period. Thus, the full extent of leakage of components may not be realized. In contrast to aminoacyl-tRNA and EF-1a, only a small difference in leakage between recovered and non-recovered cells was observed for aminoacyl- tRNA synthetases (Table IV), suggesting that these enzymes are tightly sequestered even within non-recovered cells. Likewise, using a different procedure involving Triton X-100, no difference was found in the extractability of total RNA or poly(A) RNA from the two cell populations (data not shown). Overall, these data suggest that non-recovered cells have been altered with respect to their supramolecular organization. As a consequence, some of the translation components are more weakly, or no longer, associated with other parts of the system, resulting in their leakage from permeable cells.

Relation between Protein Synthesis and the Cytoskeleton—Since it is known that various components of the translation apparatus interact and co-localize with cytoskeletal elements of the cell (13–23), we focused our attention on the cytoskeleton to determine whether changes in its structure might be related to the loss and recovery of translation capabilities. We first examined whether the microtubule network might be affected during the course of re-acquisition of protein synthetic capacity during recovery. Our data indicated that the exposure of cells to cold shock was what initiated the decrease in translation activity, and such treatment is known to depolymerize a major portion of the cell’s microtubules (31). However, as shown in Fig. 3A, addition of the microtubule depolymerizing agent, colchicine, to non-recovered cells has no effect on their ability to recover and to resume protein synthesis despite the fact that the concentration of colchicine used has dramatic effects on cell division and on the morphology of cells. Thus, these observations suggest that the recovery process is not dependent on the re-formation of microtubules.

In contrast, F-actin levels were found to correlate with protein synthetic activity, decreasing with cold shock and increasing upon recovery (Table II). Moreover, the time course of the increase in F-actin coincides closely with the restoration of protein synthesis (Fig. 4). Thus, at 10 min of recovery, when the F-actin levels reach their maximum, the initiation of the upswing in protein synthesis rate begins. These data indicate that non-recovered cells have an altered F-actin system that results in decreased phalloidin binding, and that restoration of a fully

| Inhibitor | Effect on recovery |
|-----------|-------------------|
| Transcription |
| Actinomycin D, 50 µg/ml | No effect |
| Translation |
| Cycloheximide, 0.3 µg/ml | No effect |
| Energy metabolism |
| 2-Deoxyglucose, 10 mM | Inhibits |
| Fluoride, 5 mM | Inhibits |
| Dinitrophenol 0.4 mM | Inhibits |
| Cyanide, 1.5 mM | Inhibits |
| Low temperature (0 °C) | Inhibits |

The lack of an effect by actinomycin or cycloheximide on recovery supports the conclusion that the resumption of translation after recovery does not involve re-synthesis of any of the RNA or protein components of the translation apparatus. From this information and that presented above, it is likely that the depressed protein synthetic capability of non-recovered cells is not a consequence of the destruction of a translation component.

On the other hand, the fact that incubation at 28 °C in the presence of glucose is necessary to obtain recovery suggests that this process might require energy. Indeed, as shown in Table III, a variety of inhibitors of energy metabolism interfere with the recovery process. As mentioned, in control experiments all of these inhibitors were shown to be without effect on protein synthesis after the cells are washed. Thus, the effect of the energy inhibitors is not on protein synthesis per se, but on the recovery process. The importance of energy metabolism is supported by the experiment presented in Fig. 2A in which the recovery process was carried out at temperatures below 28 °C. At 10 and 19 °C, metabolic processes would be expected to occur at a slower rate, and as can be seen, there is a concomitant reduction in the rate of recovery of protein synthesis at these temperatures. The higher the temperature during the recovery period, the more rapid the attainment of the maximum translation rate. From these data we conclude that a continued and sufficient energy supply is required for the restoration of translation capacity.

Organization of the Translation Machinery in Recovered and Non-recovered Cells—Since the data presented above failed to show any significant damage or reduction in individual components of the translation apparatus in non-recovered cells, it appeared that the components needed for protein synthesis, although present, might not be functioning optimally. A hallmark of protein synthesis in eukaryotic cells is that the process is carried out by a highly organized translation apparatus (2, 3), and as shown in Table I, damage to this organization can lead to major reductions in efficiency of the process. This knowledge, coupled with the data presented above, raised the possibility that the diminished protein synthesis in non-recovered cells might be due to an effect on the supramolecular organization of the translation machinery, rather than to a reduction in the amount of individual components. A useful tool to test the organization of the translation machinery is measurement of the leakage of certain of its components after cells...
FIG. 2. Effect of temperature on recovery of protein synthesis and F-actin levels. **A**, protein synthesis. Preparation of cells and protein synthesis were carried out as in Fig. 1 except that the preincubation temperature was as indicated. **B**, F-actin levels. Cells after the washes with ice-cold PBS were incubated at the indicated temperatures. Samples were withdrawn at the indicated times for the determination of F-actin as described under “Experimental Procedures.” The level of F-actin after incubation at 28 °C for 20 min was set at 100%.

![Diagram](image)

TABLE IV
Leakage of translation components by permeabilized, recovered, and non-recovered cells

Recovered and non-recovered cells were prepared and permeabilized with saponin as described under “Experimental Procedures.” After permeabilization, cell and supernatant fractions were separated by centrifugation, and the amounts of individual components of translation were determined in each fraction. Each component was measured in a separate experiment.

| Component                      | Present in supernatant fraction |
|--------------------------------|---------------------------------|
|                                | Recovered | Non-recovered |
| Aminoacyl-tRNA                 | 28        | 51            |
| EF-1α                          | 35        | 61            |
| Aminoacyl-tRNA synthetase      | 31        | 38            |

Interestingly, the presence of cytochalasin D (100 µg/ml) during the recovery period does not prevent the restoration of F-actin to maximum levels or the resumption of protein synthesis (Fig. 3B). However, because cytochalasin inhibits protein synthesis in CHO cells (data not shown), as previously observed in HeLa cells (20), an effect on translation becomes evident at the 40-min time point. The concentration of cytochalasin used in these experiments is much higher than that needed to induce major changes in the morphology of CHO cells (10 µg/ml). From these data it is apparent that the changes in the F-actin system induced by cold shock and its subsequent restoration by the recovery process are directly related to changes occurring in the microfilament system.

Effect of Disruption of the F-actin Network by DNase I or Latrunculin—Additional evidence for a relation between the cellular F-actin network and translation comes from studies of the effects of DNase I and latrunculin. It is known that these agents promote depolymerization of actin filaments (32, 33). Since DNase I cannot cross the cell membrane, studies with this agent used the permeabilized cell system. In addition, studies with DNase I were carried out in the presence of protease inhibitors to negate any effect due to proteases that might be present in the DNase I preparation.

DNase I inhibits protein synthesis in permeabilized, recovered cells, but has essentially no effect on the translation rate in permeabilized, non-recovered cells (data not shown). This observation supports the idea that an intact actin filament system is required for optimal protein synthesis. Thus, in non-recovered cells, the protein synthesis apparatus already has been altered, and DNase I has no additional effect. Recovered cells, on the other hand, presumably have a restored microfilament system, maintain an active, organized translation system, and consequently, their protein synthetic machinery is susceptible to the effects of DNase I. Support for this conclusion comes from the simultaneous measurement of F-actin levels and protein synthesis in permeabilized, recovered cells (Fig. 3B).
DNase I causes a decrease in F-actin levels concomitant with a dramatic inhibitory effect on protein synthesis. Addition of the same amount of the nonspecific protein, bovine serum albumin, has no effect on either F-actin or protein synthesis. Latrunculin B also is a potent inhibitor of protein synthesis (Fig. 5B). Destruction of the F-actin network by this agent is accompanied by a dramatic decrease in the protein synthetic capacity of the CHO cells. As might be expected from its effect on F-actin, the presence of latrunculin B completely prevents the recovery process in non-recovered cells and destroys the residual F-actin present (data not shown). These data confirm that an intact F-actin system is required for efficient protein synthesis.

**DISCUSSION**

The data presented here support and extend our knowledge about the supramolecular organization of the mammalian protein synthetic machinery and how this organization affects translation. In earlier studies it was shown that the components of the translation apparatus are highly organized (2), and that aminoacyl-tRNA is sequestered in cells (30); moreover, it was shown that functionally, translation is a channeled process in which the aminoacyl-tRNA substrate is directly transferred from one translation component to the next (25) leading to a completely channeled tRNA cycle (3). What was not clear from the earlier work was what was responsible for maintaining this highly organized system and what would be the consequences of disrupting it.

The findings reported in this paper demonstrate that (a) cold shock dramatically reduces the protein synthetic capacity of CHO cells; (b) the defect can be completely reversed by a short incubation that allows energy metabolism to resume; (c) individual components of the translation machinery are not damaged by the treatment, but the supramolecular organization of the system is altered and F-actin levels decrease; (d) recovery of protein synthetic capability coincides closely with a restoration of F-actin to normal levels; and (e) disruption of actin filaments, but not microtubules, leads to a major reduction in protein synthesis. Although correlative, taken together, these data strongly support the conclusion that the cellular microfilament network plays an important role in the structure and function of the mammalian translation system, and that readily reversible perturbations of this network can have profound effects on translation.

An involvement of microfilaments in protein synthesis is not unexpected. Various studies have shown that components of the translation apparatus associate and co-localize with this element of the cytoskeleton (13, 15, 23, 34). The microfilaments have also been shown to participate in mRNA localization (34–37). It has been estimated that “two-thirds of the newly synthesized proteins in the cell are made within a few tenths of a micron of an actin filament” (35). Our data go further in suggesting that the actin filaments are directly required for optimal protein synthesis.

It was surprising that the presence of cytochalasin D at relatively high concentrations (100 μg/ml) during the recovery period does not inhibit restoration of F-actin to normal levels. Clearly, the effects on the actin cytoskeleton induced by cold shock can be reversed even in the presence of this drug. The
effects of cytochalasin on microfilaments in vivo are complex. While it binds and inhibits elongation, at least in some cases, neither short nor long-term exposure to the drug produces net depolymerization of actin filaments (38, 39). Ornelles et al. (20) had previously shown that cytochalasin D inhibits protein synthesis in HeLa cells, and we have confirmed these observations with CHO cells. However, the concentrations of drug needed to obtain effects on translation are much higher than those found to induce changes in the cytoskeleton (20). So, even though the cytochalasin data (20) support the conclusion that the actin network is important for translation, it is likely that the effects of cytochalasin on translation differ from those due to cold shock.

While cold treatment is known to disassemble microtubules (31), its effects on microfilaments are less clear (40). Under our cold shock conditions, the amount of F-actin measured by phalloidin binding decreases approximately 30% both in cells in suspension and in monolayers. However, what is responsible for the decreased phalloidin binding is not known. While it may be due to some depolymerization of filaments, an effect on F-actin accessibility to phalloidin seems a more likely possibility, particularly in light of the lack of an effect of cytochalasin on recovery. In either case, it is reasonable to assume that only a portion of the microfilament network is involved in organization of the translation machinery, and that it is this part of the network that is affected by cold shock. Further work will be necessary to answer this question. On the other hand, microtubules, which are known to be depolymerized by cold treatment, do not appear to be directly required for translation as colchicine had no effect on either the recovery process or on subsequent translation. Yet, microtubules may play a role in certain aspects of localization of components of the translation machinery (41).

The sequence of events leading from cold treatment of cells to an ultimate effect on protein synthesis is not yet known. However, we can speculate on a possible scenario. Cold shock may lead to an efflux of K\(^+\) ions, an import of Na\(^+\) and H\(^+\), and a slight reduction in cellular pH. In fact, in high K\(^+\), cold shock has much less of an effect on translation.\(^2\) As recently suggested by Liu et al. (42), these changes can modulate the interaction of EF-1a with the actin cytoskeleton and can affect translation. At lower pH values, EF-1a increases the bundling of actin filaments. These events might be related to the decreased phalloidin binding that is observed as well as to the disorganization of the translation machinery and the concomitant reduction in protein synthesis. Incubation under conditions that allow energy metabolism would result in re-adjustment of the intracellular pH and reversal of the process. Further work will be needed to determine whether this scenario is correct and to unravel all the details of these events.

\(^2\) R. Stapulionis and M. P. Deutscher, unpublished observation.

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