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Supramolecular organization of the mammalian translation system

(permeable cells/substrate channeling)

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ABSTRACT **Although evidence suggests that the protein synthetic machinery is organized within cells, this point has been difficult to prove because any organization that might exist is lost upon preparation of the cell-free systems usually used to study translation *in vitro*. To examine this process under conditions more representative of the intact cell, we have developed an active protein-synthesizing system using Chinese hamster ovary (CHO) cells permeabilized with the plant glycoside saponin. This procedure renders cells permeable to trypan blue and exogenous tRNA, but there is little release of endogenous macromolecules. Protein synthesis in this system proceeds at the same rate as that in intact cells and is about 40-fold faster than that in a cell-free system prepared from the same cells. Active protein synthesis in this system requires the addition of only Mg^{2+}, K^+, and creatine phosphate, with a small further stimulation by ATP and an amino acid mixture; no exogenous macromolecules are necessary. The proteins synthesized in this system are indistinguishable from those made by the intact cell, and the channeling of aminoacyl-tRNA observed *in vivo* is maintained. Our data suggest that the permeabilized cell system retains the protein-synthesizing capabilities of the intact cell and presumably its internal structure as well. Studies with this system demonstrate that the protein-synthesizing apparatus is highly organized and that its macromolecular components are not freely diffusible in mammalian cells.**

The protein biosynthetic machinery, consisting of a large number of protein and nucleic acid components, is among the most complex systems in the mammalian cell. Evidence suggests that these components may be highly organized within the cell (1, 2); however, only remnants of this organization are thought to survive the procedures used to prepare cell-free translation systems. Thus, although it has been shown that ribosomes and other translation components can interact with the cytoskeletal framework of the cell (3–8), and with each other (9–12), presumably reflecting associations that normally occur *in vivo*, an intact protein-synthesizing system has not been isolated. Moreover, it is likely that the relatively low rates of protein synthesis usually obtained with cell-free systems (refs. 13 and 14 and references therein) are a consequence of disrupting the structural organization of the translation components that normally exists within the cell.

These conclusions were reinforced by recent evidence suggesting that protein biosynthesis in mammalian cells is a channeled pathway—i.e., aminoacyl-tRNAs, the intermediates in the process, are directly transferred from the aminoacyl-tRNA synthetases, to the elongation factor, and then to the ribosomes, without dissociation into the cellular fluid (15, 16). Additional support for channeling came from observations that endogenous aminoacyl-tRNA is sequestered within permeable cells and is resistant to the action of RNase A (17), as might be expected for a direct transfer process. Furthermore, while channeling could be observed in intact cells, it was lost upon preparation of cell extracts. All of these findings are consistent with the idea that it is the organized assembly of the protein synthetic apparatus in the cell that is responsible for the channeling phenomenon and consequently the high rates of protein synthesis *in vivo*.

To obtain additional support for these ideas, we have been examining protein synthesis under conditions that preserve as much as possible the structural integrity of the intact cell but nevertheless allow access to the internal environment for external manipulation. To do this we have developed a permeable Chinese hamster ovary (CHO) cell system that synthesizes proteins at rates comparable to intact cells. Studies with this system have shown that all the protein synthesis components are highly organized *in vivo* and that they require the addition of only ions and an energy source to maintain high rates of translation. Moreover, the channeling of aminoacyl-tRNA that previously could only be demonstrated in intact cells (15) occurs in this system and is now amenable to detailed study.

MATERIALS AND METHODS

Materials. ^{14}C-labeled amino acids and Tran^{35}S-label (containing ^{35}S-labeled L-methionine and L-cysteine) were obtained from ICN. A mixture of five ^3H-labeled amino acids (leucine, lysine, phenylalanine, proline, and tyrosine) was purchased from Amersham. [^3H]Uridine was from NEN. Unlabeled amino acids, cycloheximide, phosphocreatine, creatine phosphokinase, ATP, GTP, saponin, and trypan blue were obtained from Sigma. Fluorescein thiosemicarbazide was obtained from Molecular Probes. Cell culture reagents were from GIBCO. Rabbit liver tRNA and aminoacyl-tRNA were prepared as described (17, 18).

Cell Culturing and Permeabilization. CHO cells were obtained and cultured as described (15). Confluent cells were washed once with Dulbecco's phosphate-buffered saline (PBS) and harvested with trypsin. Approximately 20 ml of serum-supplemented Dulbecco's modified Eagle's medium per 75-cm^2 flask was added to inhibit the trypsin. The released cells were washed twice with 10 ml of PBS and once with SU buffer [130 mM sucrose/50 mM potassium acetate/50 mM KCl/20 mM Hepes, pH 7.4/0.5 mM dithiothreitol (DTT)] and suspended in 60 μl of SU buffer per 10^7 cells. The total volume of cell suspension was measured by pipetting, and an equal volume of saponin solution (150 μg/ml) in SU buffer was added. The suspension was gently mixed, incubated for 6 min at 37°C, cooled on ice, and centrifuged for 1 min at 420 × *g* (Sorvall) at 4°C, and the permeabilized cell pellet was resuspended in SU buffer.

Measurement of Protein Synthesis in Permeabilized Cells. One-half volume of a reagent mixture was added to the cell

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Abbreviation: DTT, dithiothreitol.

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suspension to give the following final concentrations: 0.8 mM ATP, 0.07 mM GTP, 30 μg of creatine phosphokinase per ml, 7.5 mM phosphocreatine, 1.3 mM magnesium acetate, 50 mM KCl, 50 mM potassium acetate, each of 20 amino acids at 250 μM, radiolabeled amino acids (as indicated in the legends), 0.3 mM DTT, and 20 mM Hepes (pH 7.4). The packed cell volume was one-fifth the total volume of liquid added (SU buffer and reagent mixture). Cells were incubated for 10 min on ice, and a portion was taken to measure cell permeability by trypan blue exclusion and for cell counting. Incubation of the remaining material for protein synthesis was at 28°C for the times indicated. Protein synthesis was determined as hot acid-precipitable radioactivity (15).

Determination of Specific Radioactivity of [^{14}C]Phenylalanine in Intact Cells. Cells (3.2×10^7) were resuspended in PBS, and 190 μM [^{14}C]phenylalanine with known specific radioactivity was added to the cell suspension. Cells were incubated for 2 min on ice and divided into three aliquots. One portion was centrifuged immediately, and the cells were resuspended in 40 μl of PBS. An equal volume of 30% (wt/vol) 5-sulfosalicylic acid was added to precipitate protein; the supernatant fraction obtained upon centrifugation was used for determination of amino acid concentration and radioactivity. The two remaining portions were incubated on ice for 10 and 20 min, respectively, and treated exactly as the first sample. Phenylalanine was quantitated using a Beckman amino acid analyzer, and radioactivity was determined by scintillation counting.

Measurement of Release of RNA and Protein from Permeabilized Cells. Cells were grown for 3 days under the usual conditions except that 100 μCi (1 Ci = 37 GBq) of [^{3}H]uridine was added to each flask. Cells were washed four times with PBS, harvested with trypsin, washed once with serum-supplemented Dulbecco's modified Eagle's medium, washed four times with PBS, and resuspended in 150 μl of SU buffer per flask for saponin permeabilization. After centrifugation, the supernatant fraction, which contained material released from the cells, was removed and precipitated with 10% (wt/vol) trichloracetic acid. The cell pellet was resuspended in 150 μl of SU buffer, an aliquot was taken for cell counting, and the remaining cells were spun down. The cell pellet fraction and the supernatant wash fraction were each precipitated with 10% trichloracetic acid, and radioactive RNA was determined (15).

For prelabeling protein, cells were grown with 20 μCi of a mixture of five ^{3}H-labeled amino acids for 2 days. Harvesting, permeabilization, and washing were carried out as described above. Protein was determined in each of the fractions as acid-precipitable radioactivity, as for RNA.

^{35}S Labeling of Permeabilized Cells. Cells were harvested and divided into two fractions, and one was permeabilized with saponin. The permeabilized and intact cells were centrifuged and resuspended in either SU buffer or PBS plus vitamins and glucose (PBS+) (19), respectively. A portion was taken from each for cell counting, and a one-half volume of either SU buffer with the components for protein synthesis (see above) or PBS+, both containing an amino acid mixture and Tran^{35}S-label, was added to the permeabilized or intact cells, respectively. Cells were incubated 10 min on ice and 20 min at 28°C. A one-fifth volume of sample buffer (250 mM Tris Cl, pH 6.8/500 mM DTT/10% SDS/0.5% bromphenol blue/50% glycerol) was added to each tube to terminate the reaction. The samples were boiled for 10 min and the ^{35}S-labeled proteins were subjected to SDS/PAGE (20).

RESULTS

Development of the Permeable Cell System. This procedure is an outgrowth of our earlier work in which the plant glycoside, saponin, was used to permeabilize CHO cells for studies of the sequestration of endogenous aminoacyl-tRNA (15). Under the conditions used in those experiments, however, a considerable amount of cell damage and leakage of macromolecular components was observed. Nevertheless, because the rate of protein synthesis in the permeable cells still was relatively high compared to the usual *in vitro* systems (13, 14), we thought it would be worthwhile to examine the saponin permeabilization procedure in more detail with the aim of finding conditions that would lead to less damage.

It was found that a major problem with the earlier procedure was the harvesting of the permeable cells by scraping them from the flask surface. To avoid this harsh procedure, cells were harvested by trypsin treatment and then exposed to saponin while in suspension. A second modification that was introduced was the inclusion of 130 mM sucrose during the saponin treatment and subsequent incubations. These two changes in the original procedure served to minimize cell destruction (see below) and also avoided the formation of cell clumps during the treatment.

Using these modified conditions, CHO cells could be rendered completely permeable (>98%) to trypan blue at a saponin concentration of 75 μg/ml by incubating at 37°C for 6 min (see *Materials and Methods*). Although the amount of saponin needed to permeabilize the cells under these conditions was higher than in the previous work (75 versus 30 μg/ml), the cells appeared less damaged upon microscopic observation (data not shown). Moreover, there was less leakage of RNA from the permeable cells. As shown in Table 1, only 13% of the RNA in prelabeled cells was released during the saponin treatment, with an additional 6% released in the subsequent wash. The total loss of RNA correlated well with the loss in cell number (≈20%), suggesting that most of the apparent leakage of RNA was actually from lysed cells and that the surviving cells were essentially intact.

The release of protein upon saponin permeabilization was also determined (Table 1). Again, the amount of material released into the supernatant fraction during the permeabilization procedure, and in the additional wash, was small and only slightly greater than the amount of lysed cells, suggesting that only a small percentage (<20%) of total cell protein

Table 1. Release of RNA and protein from saponin-permeabilized CHO cells

Fraction	Cell number, %	RNA, %	Cell number, %	Protein, %
Untreated cells	100	100	100	100
Supernatant after saponin treatment	—	13	—	19
Cells after saponin treatment	78	—	90	75
Supernatant after wash	—	6	—	8
Cells after wash	81	81	84	66

Cells were prelabeled with either [^{3}H]uridine or a mixture of five ^{3}H-labeled amino acids and then harvested and permeabilized as described in *Materials and Methods*. Cell number for each experiment was determined by direct counting in a hemacytometer. The initial cell number and RNA and protein, measured as acid-precipitable radioactivity, were set at 100%. —, The value was not determined in that fraction.

is removed by these procedures. Based on these data, we conclude that a fraction of the cells (≈20%) are sensitive to saponin and are destroyed by the treatment, but that the surviving cells are relatively stable and lose little of their macromolecular components, even upon washing.

Although relatively little leakage of endogenous macromolecules was observed, the interior of the permeable cells was accessible to exogenously supplied macromolecules. Thus, when cells were incubated with fluorescein-labeled tRNA and examined by confocal microscopy, tRNA was found to readily enter the permeabilized cells and to distribute throughout the cytoplasm (Fig. 1). Likewise, ^{14}C-labeled *Escherichia coli* 16S and 23S rRNAs were found to associate with the permeable cells (data not shown), suggesting that even large macromolecules can enter.

Efficiency of Protein Synthesis in Permeable Cells. An important consideration in evaluating the intactness of permeabilized cells is the rate with which they incorporate amino acids into protein compared to intact cells and to the usual cell-free systems. In initial experiments to examine this point, untreated cells, permeabilized cells, and homogenates were compared for their ability to utilize [^{14}C]phenylalanine for the synthesis of protein. A separate experiment, described in *Materials and Methods*, showed that the specific radioactivity of the total internal phenylalanine pool was identical to that of the external medium and did not change over a 20-min incubation period (data not shown). Thus, the external [^{14}C]phenylalanine-specific radioactivity value was also used for calculating the rate of incorporation in intact cells.

In these experiments (data not shown), saponin-permeabilized cells were able to maintain a rate of protein synthesis comparable to that of intact cells for a period of ≈10 min and this rate was ≈40-fold greater than that observed in a cell-free extract. However, after 10–15 min, the permeable cell system slowed down dramatically. The slowdown could not be prevented by the addition of RNasin, by a mixture of protease inhibitors, or by the addition of more ATP, phosphocreatine, or glutathione after 15 min; however, it could be eliminated entirely by increasing the concentrations of all the amino acids 5-fold. Under these conditions, protein synthesis in permeable cells and intact cells is essentially identical and displays linear incorporation for at least 30 min (Fig. 2). These data demonstrate that the saponin-permeabilized cells are highly active for protein synthesis and much more efficient than the usual cell extracts.

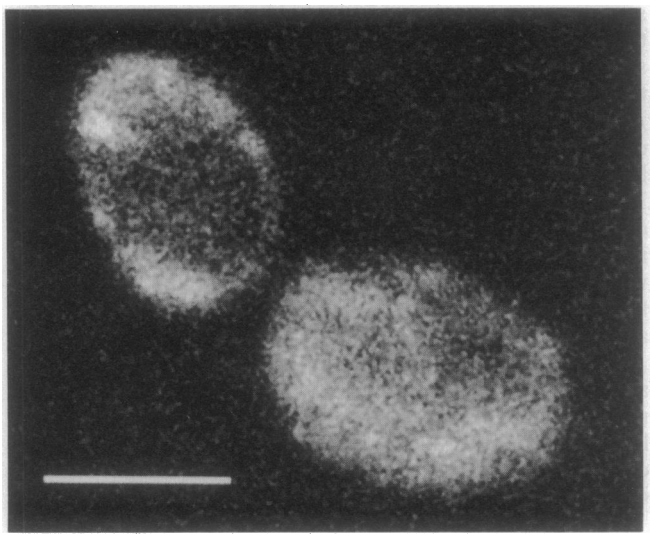

FIG. 1. Entry of tRNA into permeable cells. CHO cells were harvested and permeabilized as described in *Materials and Methods* and resuspended in SU buffer. One-fifth volume of tRNA (5 mg/ml), labeled with fluorescein thiosemicarbazide at the 3′ end and free of unreacted fluorescent label, was added, and the cells were incubated for 10 min on ice and 1 min at 28°C. One milliliter of a 3.7% (vol/vol) solution of formaldehyde in PBS was added, and the cells were left for 30 min. The cells were washed with 1 ml of PBS and gently resuspended in 30 μl of solution containing 10 mM Tris·HCl (pH 7.4), 0.9% NaCl, and 50% (vol/vol) glycerol. The suspension was transferred onto a glass slide, covered with a coverslip, and sealed with nail polish. Fluorescence was analyzed using a Bio-Rad MRC-600 confocal microscope. The figure shows an optical section through the cells at the level of the nucleus. (Bar = 10 μm.)

Requirements for Protein Synthesis in Permeable Cells. The high rate of protein synthesis supports the idea that a relatively intact protein-synthesizing system remains in the permeable cells and is consistent with the low level of release of macromolecular components from these cells. Despite the maintenance of cell organization, it might be expected that many low molecular weight cofactors required for protein synthesis would leak out of the permeable cells. Accordingly, an analysis of the agents necessary to restore optimal rates of amino acid incorporation in this system would be expected to provide information on the components sequestered within the cell and those that are diffusible.

The data presented in Table 2 examine the effect of removal of a variety of components usually used to supplement protein-synthesizing systems. The concentrations present in the complete reaction mixture (detailed in *Materials and Methods*) were determined to be optimal in separate experiments in which each of their levels was varied. Removal of ATP or of ATP and GTP had at most a 2-fold effect on protein synthesis, and the absence of GTP alone had essentially no effect, as long as the ATP-regenerating system using creatine phosphate was present. Removal of creatine phosphate, but not creatine phosphokinase, depressed incorporation to background levels. These data suggest that any ATP left in the cell after permeabilization is rapidly depleted but that it can be efficiently recycled by the regenerating system. Interestingly, the lack of a requirement for either creatine phosphokinase or for GTP indicates that the former enzyme and also nucleoside diphosphokinase must be retained in the permeabilized cell. Also, the optimal concentration of creatine phosphate for protein synthesis in the permeabilized cells, 7.5 mM, is considerably lower than that

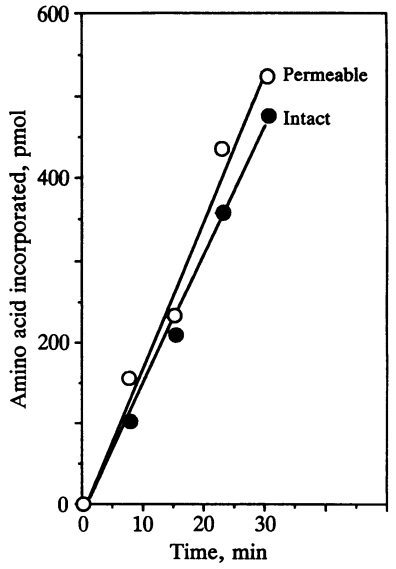

FIG. 2. Kinetics of protein synthesis in intact and saponin-permeabilized CHO cells. Cells (9.4×10^7) were harvested, permeabilized, and incubated for protein synthesis as described in *Materials and Methods*. The time course of amino acid incorporation into protein was determined with the mixture of five ^{3}H-labeled amino acids.

Table 2. Requirements for protein synthesis in saponin-permeabilized CHO cells

Component(s) omitted	[^{14}C]Phenylalanine incorporation, %
None (complete mixture)	100
ATP	54
GTP	90
ATP and GTP	49
Creatine phosphate	4
Creatine phosphokinase	89
Amino acid mixture	56
Mg^{2+}	5
K^+ (Na^+ added)	13
DTT	85

Cells were permeabilized and incubated for protein synthesis as described in *Materials and Methods*, except that the indicated component was omitted. The complete mixture, which incorporated 17.2 pmol of [^{14}C]phenylalanine per 2×10^6 cells in 15 min at 28°C, was set to 100.

recommended for efficient synthesis in cell-free systems, 20–30 mM (14, 19). Moreover, addition of extra creatine phosphate during the incubation did not stimulate the permeabilized cell system, whereas it did affect a cell-free system (14). These findings suggest that uncontrolled, rapid hydrolysis of ATP probably is not occurring in the permeabilized cells.

Removal of amino acids (other than phenylalanine) decreased protein synthesis ≈50%, as expected from the slowdown in rate at later times described above. On the other hand, the fact that protein synthesis proceeds at 50% of the maximum rate despite dilution and washing of the cells supports the idea that some sequestering of amino acids may take place in this system (21). Further work to investigate this possibility will be of interest.

Mg^{2+} and K^+ ions are essential components of the permeabilized cell system (Table 2), and in contrast to some cell-free systems (13, 19), either the acetate or chloride salts could be used (data not shown). The addition of Ca^{2+} had no stimulatory effect on the system and slightly inhibited at a concentration of 5 μM. Addition of 5 mM EGTA did not affect amino acid incorporation. As shown in Table 2, the addition of DTT was unnecessary, and reduced glutathione in the range of 5–50 μM also had no effect and was inhibitory at higher concentrations (data not shown).

Of most interest was the finding that addition of exogenous tRNA was unnecessary to maintain the high rate of protein synthesis in permeabilized cells. Concentrations of liver tRNA up to 1 mg/ml had little effect on protein synthesis. This indicates that sufficient tRNA is retained in the permeabilized cells to obtain maximal rates of amino acid incorporation. These data are completely consistent with the earlier suggestion (17) that tRNA is sequestered within cells and leaks out only slowly, if at all, upon their permeabilization.

Properties of Protein Synthesis in Permeable Cells. In agreement with other systems (19), protein synthesis in the saponin-permeabilized cells is very sensitive to the inhibitor of chain elongation, cycloheximide. A concentration of cycloheximide of 20 μg/ml was sufficient to completely abolish amino acid incorporation (data not shown). Inhibitors of translation initiation, aurintricarboxylic acid and poly(vinylsulfate), also inhibited protein synthesis in the permeable cell system, with 50% inhibition obtained at 250 μM and 150 μg/ml, respectively (data not shown). Inasmuch as these concentrations are somewhat higher than those found in other systems (13, 14), it is not yet certain whether initiation takes place in this system, although the linearity of incorporation for 30 min suggests that it may.

To compare the spectrum of proteins synthesized in the permeable cells with those in intact cells, both preparations were incubated with Tran^{35}S-label for 20 min at 28°C, and the labeled proteins were analyzed by SDS/PAGE. As shown in Fig. 3, there was no noticeable difference in the patterns of proteins synthesized in the two systems. These results support the idea that the protein-synthesizing apparatus, including the mRNAs, is retained within the permeabilized cells. Similar findings were obtained with another protein-synthesizing system that used a combination of detergent-extracted cells and a reticulocyte lysate (22).

Channeling of Aminoacyl-tRNA for Protein Synthesis in Permeable Cells. An important test of the intactness of saponin-permeabilized cells is whether this system is able to channel aminoacyl-tRNA for protein synthesis. This property, which is observed in intact cells, is lost when the cells are homogenized (15). Thus, retention of channeling in the permeable cells would be strong evidence that their organized structure has been preserved.

Channeling by the permeabilized cells was examined using the same protocol described for studies with intact cells (15). In this procedure cells are incubated with a mixture of ^{14}C-labeled amino acids and the corresponding ^{3}H-labeled aminoacyl-tRNAs, and the time course of incorporation of each label into protein is determined, as is the level of aminoacyl-tRNA. The rate of incorporation into protein relative to the level of each aminoacyl-tRNA (^{14}C or ^{3}H) serves as a measure of the utilization of each of these precursor substrates. Pool sizes or dilution of radioactivity are not problems because the amount of radioactivity incorporated into protein by each label is always compared to the level of the immediate precursor, aminoacyl-tRNA. If the ^{14}C-labeled aminoacyl-tRNAs, generated endogenously, mixed freely with the ^{3}H-labeled aminoacyl-tRNAs supplied from the outside, they would be utilized for protein synthesis with the same efficiency, dependent solely on their relative levels. If, on the other hand, the endogenously synthesized ^{14}C-labeled aminoacyl-tRNAs were transferred directly to elongation factor without mixing, then their preferential utilization for protein synthesis would be observed.

The data in Fig. 4 show that the ratio of protein synthesis to aminoacyl-tRNA differs greatly for the two isotopes and favors the ^{14}C label by >15-fold. This finding indicates that the endogenous ^{14}C-labeled aminoacyl-tRNA is a much more efficient precursor for protein synthesis than is the exogenous ^{3}H-labeled aminoacyl-tRNA, despite the fact that exogenous aminoacyl-tRNA can enter and distribute throughout permeable cells (Fig. 1). These results support the idea that the ^{14}C-labeled aminoacyl-tRNA, generated endogenously, and

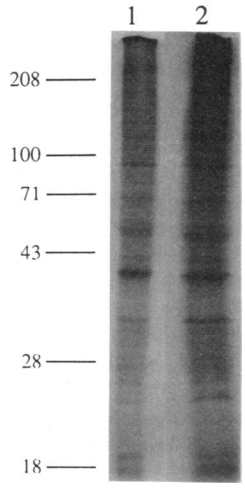

FIG. 3. Comparison of proteins synthesized in intact and permeable CHO cells. Cells were harvested, permeabilized, and labeled with Tran^{35}S-label as described in *Materials and Methods*. After incubation the ^{35}S-labeled proteins from each sample were separated by SDS/10% PAGE; the gel was run until labeled amino acid was removed from the gel. Lane 1, intact cells; lane 2, permeabilized cells. The migration positions of size markers are shown on the left.

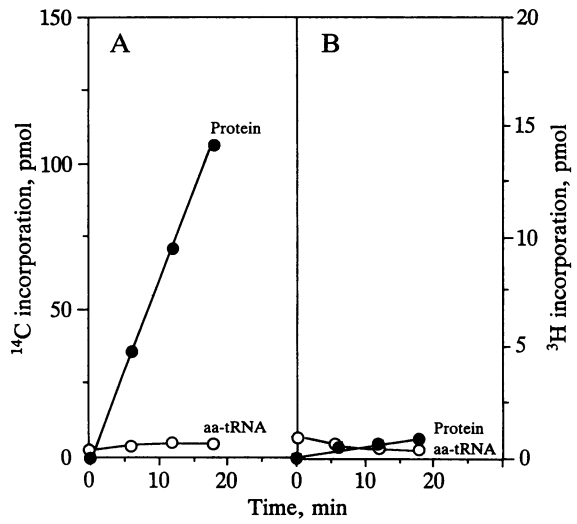

FIG. 4. Test for channeling of aminoacyl-tRNA (aa-tRNA) in permeable CHO cells. Cells were harvested, permeabilized, and incubated for protein synthesis as described in *Materials and Methods* except that four ^{14}C-labeled amino acids (leucine, phenylalanine, proline, tyrosine) and five ^{3}H-labeled aminoacyl-tRNAs (leucine, phenylalanine, proline, tyrosine, lysine) were present in the incubation mixture. The mixture was kept on ice for 10 min to allow equilibration of components between the cell and the medium. Samples were then incubated at 28°C, and at indicated time points samples were taken for measurement of aminoacyl-tRNA and protein synthesis. Two portions of $\approx 3.8 \times 10^6$ cells each and one of $\approx 7.6 \times 10^6$ were taken; the three portions were cooled on ice, and centrifuged immediately for 1 min at $420 \times g$ to separate cells and the supernatant fraction. The first two portions were used to determine total and hot acid-precipitable radioactivity for measurement of protein synthesis and aminoacyl-tRNA, as described (ref. 15; *Materials and Methods*). Since the hot-acid soluble material overestimated the actual amount of ^{14}C-labeled aminoacyl-tRNA, the third sample was subjected to phenol extraction in 0.3 M sodium acetate (pH 6.8). The aqueous phase was divided into two equal portions. One was used to measure total acid-precipitable radioactivity, and the second was first incubated with RNase A (2 μg/ml) for 10 min at 28°C prior to acid precipitation. Essentially all the phenol-extractable radioactivity was RNase A sensitive and was considered to represent ^{14}C-labeled aminoacyl-tRNA. Comparison of the phenol extraction and hot acid-soluble methods for ^{3}H indicated that the phenol method recovered 80% of the aminoacyl-tRNA. Control experiments with intact cells were carried out to determine the amount of ^{3}H-labeled aminoacyl-tRNA that was trapped in the cell pellet, rather than being present in the cell. This value (50% of total) was subtracted from the ^{3}H-labeled aminoacyl-tRNA considered to be present in the permeable cells. The ^{3}H-labeled aminoacyl-tRNA preparation was oxidized with $NaIO_4$ to eliminate any uncharged tRNA that could become aminoacylated with ^{14}C-labeled amino acids. (*A*) ^{14}C-labeled material. (*B*) ^{3}H-labeled material.

the ^{3}H-labeled aminoacyl-tRNA, supplied from the outside, do not freely mix in permeable cells.

DISCUSSION

Although considerable information is available about protein synthesis in cell-free systems, we still know very little about the process in intact cells. However, it is precisely under the conditions of the intact cell that one wants to study protein synthesis, particularly since it is becoming increasingly clear that the structural organization of the cell is a major factor in the process (23). Unfortunately, intact cells cannot be easily manipulated and studied to get at the details of the reactions and molecular associations that make protein synthesis so specific and efficient *in vivo*. Nevertheless, to approach this problem, we have evaluated the use of a permeable cell system as a close approximation of the intact cell, but one that would be amenable to experimental manipulation.

Several pieces of evidence demonstrate that the use of saponin for cell permeabilization leads to a system that maintains the properties of the intact cell with regard to protein synthesis: (*i*) only small amounts of macromolecules are released from the permeabilized cells and readdition of macromolecules is not necessary to obtain active protein synthesis; (*ii*) rates of protein synthesis in the permeable cells are comparable to those in intact cells; (*iii*) the spectrum of proteins made in the permeable cells is very similar to that in intact cells; and (*iv*) channeling of aminoacyl-tRNA is found in this system. All of these properties suggest that protein synthesis in these cells is representative of the *in vivo* situation. Moreover, the specificity of saponin for the plasma membrane (24, 25) spares destruction of internal membranes, which might be important for synthesis *in vivo* (26). In this regard, saponin is preferable to detergent treatment.

The permeable cell system should prove useful for studying the molecular details of protein synthesis. The system is highly efficient and very easy to prepare and should be readily applicable to most mammalian cells. Most importantly, the system has already provided evidence that all the macromolecular components of the protein-synthesizing machinery are highly organized and not freely diffusible in the cell fluid, which would allow for their leakage upon permeabilization of the cell membrane. In fact, washing the permeable cells as much as three times did not decrease translation (unpublished observation), suggesting that the protein-synthesizing machinery is tightly bound to cell structures. Moreover, confocal microscopy has shown that components of the translation machinery are colocalized in cells (J. Carson, F. Morgan, and M.P.D., unpublished data). This work has also shown that high rates of protein synthesis are attainable in a system accessible to external manipulation. Thus, an intact plasma membrane is not essential for the process.

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