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POST-TRANSLATIONAL CONTROL BY CARRIER AVAILABILITY OF AMINO ACID TRANSPORT IN FETAL HUMAN FIBROBLASTS

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SUMMARY The action of serum on the expression of the starvation-enhanced amino acid transport by System A (as a part of the adaptive regulation mechanism) has been studied in cultured fetal human fibroblasts. Serum enhanced L-proline uptake of cells starved in serum-free medium. This effect was rapid,proportional to the amount of pre-existing transporters, insensitive to cycloheximide and kinetically characterized by an increase of transport V_{max}. These results can be interpreted to indicate that serum is essential for a vectorial post-translational event leading to insertion of transport proteins into the cell membrane.

In human fibroblasts, as in a large variety of animal tissues and cells, the transport of amino acids by System A is subject to adaptive regulation (1,2). The activity of this system increases when the cells are incubated under conditions of amino acid shortage (derepression phase) and decreases when the cells are exposed to a medium supplied with site A-reactive amino acids (repression phase). Evidence has been presented indicating that the starvation-induced derepression of System A involves an active synthesis of mRNA species, whose translation increases the availability of specific transport proteins (2). A consistent enhancement of the activity of System A upon starvation requires serum in the incubation medium (cf. Ref. 2), suggesting that some step between gene translation and carrier function at the cell membrane is serum-dependent. The results to be reported indicate that serum is essential for a vectorial post-translational event leading to exposure of newly synthesized transport proteins on the cell surface.

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MATERIALS AND METHODS

Cell Culture. Fetal human fibroblasts, obtained from a 10-week gestational age, karyotypically normal male abortus, were routinely grown in 10-cm diameter dishes in Medium 199 containing 15% fetal calf serum. The conditions of culturing were: pH 7.4; atmosphere, 5% CO_2 in air; temperature, 37°C. For uptake experiments cells were seeded in 24-well plates and used when cell density reached 25 ± 3 µg of protein/cm^2. The culture medium was always renewed 48 h before the experiment.

Experimental Techniques. Incubation and pretreatment of cells with the agents specified in the "Results" were all performed in Earle's balanced salt solution (EBSS) at 37°C.

L-Proline uptake was measured under conditions approaching initial entry rates (1 min) at 37°C in EBSS. Details of the transport assay and the equipment used have been published (3).

Protein content and intracellular fluid volume were measured as described previously (4). All uptake data (corrected for unsaturable components) are expressed as µmoles/ml of intracellular water/min. Kinetic parameters were determined by a computer (HP 9845A) using the Marquardt's algorithm (5).

Materials. Fetal calf serum and growth medium were purchased from Gibco. L-[5-^{3}H]Proline (specific activity 30 Ci/mmol) was obtained from Amersham. Sigma was the source of cycloheximide and all other chemicals.

RESULTS

L-Proline has been used as a tracer amino acid to evaluate the transport activity by System A in cultured human fibroblasts (4).

Fig. 1 shows that L-proline uptake increases with time upon amino acid starvation. The increase was more marked in the presence than in the absence of dialyzed serum in the incubation medium. The addition of serum after 6-h starvation in its absence caused a rapid increase of L-proline uptake. In less than 30 min, the activity of System A reached values comparable with those observed in cells starved for 6 h in the presence of dialyzed serum. This effect was 2/3 complete within 5 min of the addition of serum. The addition of cycloheximide at a concentration (18 µM) which inhibited protein synthesis almost completely (2) did not affect substantially the fast serum-mediated effect.

When serum and cycloheximide were added at subsequent time intervals to cells starved for 3,6 or 9 h in serum-free medium, L-proline uptake raised quickly, its increment being roughly proportional to the time elapsed since the beginning of cell starvation in the absence of serum (Fig. 2).

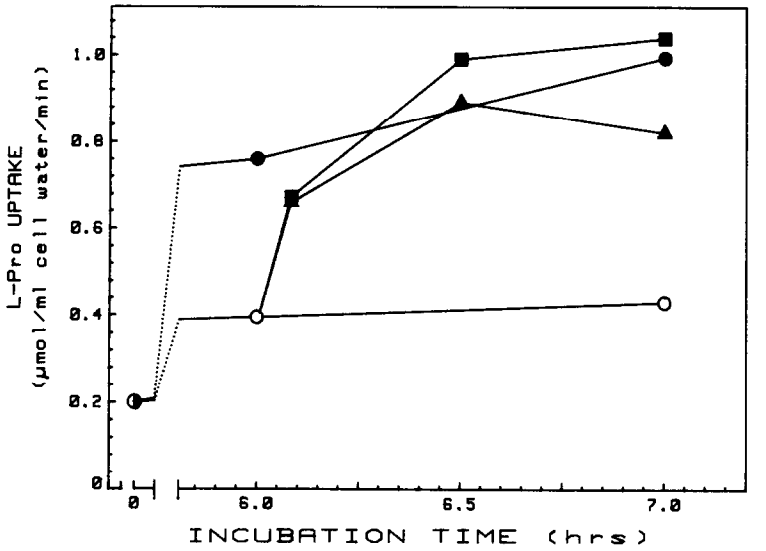

Fig.1 Changes in L-proline inward transport by System A in cultured fetal human fibroblasts incubated in amino acid-free medium. Effect of serum addition. Cells were incubated for 7 h in Earle's balanced salt solution (EBSS) in the absence (○) or in the presence of 15% dialyzed fetal calf serum (●). Serum (15%, final concn.) was added to cells previously starved for 6 h in EBSS (■) or in EBSS to which 18 μM (final concn.) cycloheximide was added 15 min before serum addition (▲). At the times indicated, cell monolayers were washed and uptake of 0.1 mM labeled L-proline was measured in 1-min experiments (initial velocity).

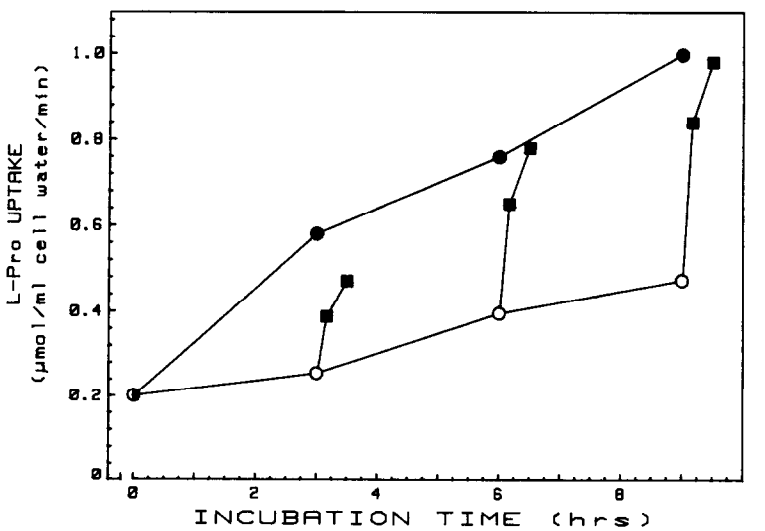

Fig.2 Changes in L-proline uptake by cultured fetal human fibroblasts incubated in amino acid-free medium. Effect of the addition of serum at successive time intervals during starvation. Cells were incubated for 9 h in EBSS in the absence (○) or in the presence of 15% dialyzed fetal calf serum (●). Serum (15%, final concn.) was added (■) to cells incubated for 3, 6 and 9 h in serum-free EBSS (always supplemented with cycloheximide 15 min before serum addition). Incubation was continued for additional 20 min. Points represent transport activity, assayed as described in Fig.1.

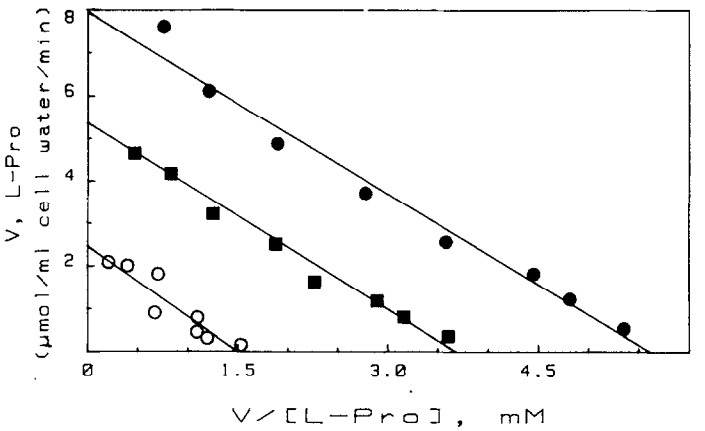

Fig.3 Plot of v versus v/[S] for L-proline uptake (saturable component) by cultured fetal human fibroblasts starved for 6 h in EBSS in the absence (○) or in the presence of 15% fetal calf serum (●). In (■), transport measurements have been performed 10 min after the addition of serum to cells previously incubated for 6 h in serum-free EBSS. In all conditions, transport assay was made over a range of 0.1 - 10 mM labeled L-proline. The regression analysis of the data yelded the following values of K_m (mM) and V_{max} (µmol/ml cell water/min):(○),1.6 and 2.5; (●),1.4 and 7.9; (■),1.5 and 5.4.

The change induced by serum addition to cells previously starved in serum-free medium was dependent upon an increased transport V_{max}, with no appreciable alteration of K_m (Fig.3). Similar changes were observed in cells incubated for the entire period of time in an amino acid-free, serum-containing medium.

Cultured human fibroblasts starved for 6 h in a serum-free medium containing cycloheximide (18 µM) failed to show the changes in transport activity observed in cells incubated without cycloheximide either in the same medium (slight increase) or in a medium supplemented with serum (marked increase,Fig.4,panel a). The addition of serum to cells starved in serum-free medium elicited an increase of L-proline uptake during the subsequent 30 min in fibroblasts previously incubated under conditions allowing active protein synthesis (absence of cycloheximide), but not in cells whose protein synthesis had been suppressed by cycloheximide during the starvation period. The addition of 5 mM L-proline (Fig. 4, panel b), completely prevented the increase in transport activity of cells incubated for 6 h in serum-free

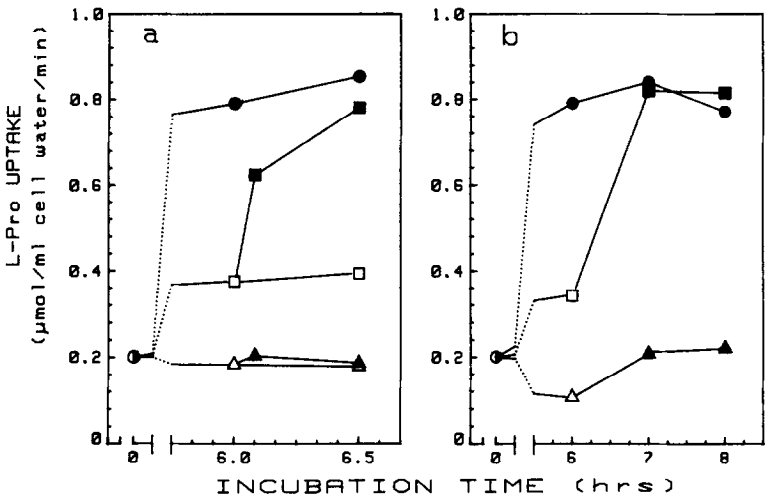

Fig.4 Changes in L-proline uptake induced by serum in human fibroblasts previously incubated in EBSS containing cycloheximide (panel a) or a repressive concentration of L-proline (panel b).
Panel a. Cells were incubated for 6 h in: EBSS (□); EBSS containing 15% dialyzed fetal calf serum (●) or EBSS containing 18 µM cycloheximide (△). Serum (15%, final concn.) was added to cells incubated in EBSS (■) or in EBSS containing cycloheximide (▲) and the incubation was continued for additional 30 min.
Panel b. Cells were incubated for 6 h in: EBSS (□); EBSS containing 15% dialyzed fetal calf serum (●) or EBSS containing 5 mM L-proline (△). Cell monolayers were washed and the incubation was continued for additional 2 h in EBSS containing 15% dialyzed fetal calf serum in the presence of 18 µM cycloheximide (●, ■, ▲).
In both panels, points represent transport activity assayed as described in Fig.1.

medium and actually lowered it as a consequence of trans--inhibition by the pre-accumulated amino acid (cf. Ref. 4). Again, a slight or a marked increase in transport activity occurred upon a 6-h starvation of the cells in serum-free or serum-containing media, respectively. When these cells were exhaustively washed (a treatment introduced to remove the external unlabeled L-proline from the medium containing it) and subsequently incubated for 2 h in an amino acid-free medium supplemented with serum (in the presence of cycloheximide to prevent the synthesis of transport proteins, cf. Ref. 4) a serum-induced fast increase of transport activity occurred only in fibroblasts previously incubated in L-proline-free, serum--free medium. No substantial deviation from a steady increase in

transport activity occurred in cells starved in the presence of serum and a nearly constant low transport activity (except for a transient increase due to a release from trans-inhibition, cf. Ref.4) was exhibited by cells formerly incubated in the presence of L-proline.

DISCUSSION

The starvation-induced increase of amino acid transport by System A in cultured human fibroblasts is a rather slow, long--lasting process which is suppressed by inhibitors of the macromolecular synthesis, including cycloheximide (2,4). As shown in Fig. 2 the rate of change in transport was low in the absence of serum and a full expression of the adaptive response could be attained only in its presence. The addition of serum to cells previously starved in serum-free medium (Fig. 1) allowed, in minutes, a much higher expression of this response and its effect was not suppressed by cycloheximide. These results indicate that serum acts at some post-translational step. Serum enhanced transport V_{max} (Fig. 3), thus suggesting an increased availability of functional carrier proteins at the cell membrane. The effect of serum was roughly proportional to the amount of transport proteins generated by derepressive stimuli (starvation) of increasing duration (Fig. 2). It vanished when the formation of transport proteins was shut off (Fig. 4) either by a specific inhibition of adaptive derepression at the transcription level (as expected in the presence of L-proline, a site A-reactive amino acid, cf. Refs. 2,6) or by a suppression of mRNA translation (as expected in the presence of cycloheximide). These features are consistent with the hypothesis that serum exploits a vectorial effect by causing a net translocation of pre-existing functional transporters from an intracellular location to the plasma membrane. Preliminary experiments in which colchicine and

cytochalasine B, used as inhibitors of tubuline and actine polymerization, prevented the effect of serum almost completely are in accord with the hypotesis. A hormone-dependent regulation of glucose transport, hydrochloric acid secretion and water permeability by exocytotic insertion into the plasma membrane and endocytic retrieval of pertinent transporters or channels has been recently proposed (7).

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