Control by Serum of Amino Acid Transport and Deoxyribonucleic Acid Synthesis in Cultured Avian Fibroblasts

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Serum has been reported to contain factors that seem to display a physiological role in the control of cell proliferation (Temin, 1971). When cells in culture are deprived of serum they cease growing and enter a quiescent state, often referred to as G0-phase. On restoration of serum, the cells return to the proliferative state as revealed by a quasi-synchronous initiation of DNA synthesis (S-phase) after a definite lag period. Internal mechanisms by which the cell re-entry into the S-phase is triggered are not clear and many factors may converge at some common site. It has been suggested that alterations in transport activities for small nutrients may be of central importance in the control of cell growth and in the performance of the cell cycle (Holley & Kiernan, 1974; Pardee, 1975). Indeed, changes in transport activity for sugars, amino acids and nucleosides are among the earliest events associated with reinitiation of cell growth in serum-deprived cell cultures when serum is added (Baserga, 1976). In particular, Isselbacher (1972) reported that a stimulation of γ-aminoisobutyric acid (2-amino-2-methylpropanoic acid) uptake took place within 90min after serum addition to serum-deprived cultured BHK cells. This model amino acid is a preferential substrate of the transport A-system, a Na+-dependent agency formally characterized in a variety of epithelial and mesenchymal cells (Christensen, 1969; Gazzola et al., 1972).

The experiments in the present paper were conducted: (a) to explore the temporal relationship between occurrence of amino acid-transport changes and initiation of DNA synthesis after serum re-addition to serum-deprived cultured cells (chick-embryo fibroblasts); (b) to identify the system(s) of mediation involved in serum-stimulated amino acid transport; (c) to define the kinetic parameter(s) of the rate of change in amino acid transport and their sensitivity to inhibitors of protein synthesis.

Fibroblasts were obtained from 11-day chick embryos. All experiments were performed with secondary cultures grown in plastic culture flasks in medium 199 (Gibco, Grand Island, NY, U.S.A.) (with Hanks salts) containing 2% (v/v) tryptose phosphate broth and 2% (v/v) chicken serum. Enough NaHCO3 was added to the medium to maintain the pH at 7.5 in an atmosphere of CO2/air (1:19) at 37°C. Exponentially growing cultures were stopped by serum deprivation for 20h. The resulting quiescent cultures were induced to grow by re-addition of serum (2% chicken serum). The relative rates and the pattern of synchronous DNA synthesis were measured by [3H]thymidine incorporation into acid-insoluble material and by autoradiography. Initial rates of amino acid uptake were measured at the desired intervals by incubating the cell monolayers (thoroughly rinsed) for 2-3min at 37°C in Krebs–Ringer bicarbonate buffer containing the labelled amino acid under study. The means for determining intracellular accumulation of the tracer amino acid and for evaluating the proper corrections to be introduced were essentially similar to those described previously (Guidotti et al., 1971).

Serum-deprived cultured avian fibroblasts (20h of deprivation) had a very low rate of DNA synthesis. This rate increased abruptly 4–6h after serum exposure reaching a maximum at 10h (Fig. 1). At the latter time, approx. 55% of the cells showed labelled nuclei (as measured by conventional autoradiography) when exposed to a 20min [3H]thymidine pulse. Fig. 2 shows that amino acid-transport activity, assayed by measurements of L-proline uptake (under conditions of initial velocity for this amino acid substrate of the A-system mediation), doubles between 30 and 90min after serum addition. This change in transport activity appears to be dependent on a definite increase in transport maximum (Vmax) without substantial changes in substrate concentration for half-maximal transport velocity (Km) and it is prevented by continuous exposure to cycloheximide. Definite increases in transport activity upon serum restoration were also observed with α-aminoisobutyric acid and, when transported by the A-system.
Fig. 1. *Time-dependent effect of serum re-addition to serum-deprived cultured fibroblasts on DNA synthesis*

The effect of serum re-addition was measured by $[^3]$H$[^3]$Hthymidine incorporation into acid-insoluble material (○) and into cell nuclei after radioautographic counting (●).

Fig. 2. *Time-dependent effects of serum re-addition to serum-deprived cultured fibroblasts on DNA synthesis and amino acid transport*

The effect of serum re-addition was measured by $[^3]$H$[^3]$Hthymidine incorporation into acid-insoluble material (empty bars) and on amino acid transport (black bars) as measured by the initial velocity of $[^14]$C$[^14]$Cproline uptake.
mediation (after proper discrimination), with glycine, L-alanine and L-serine. Under the same conditions, no substantial changes in transport activity were detected with L-leucine, L-phenylalanine and L-lysine, which are preferential substrates of transport systems L and Ly⁺ (Christensen, 1969). Discrimination from interfering agencies by transport-specific substrates indicated that the ASC system (Christensen, 1969) does not contribute to the stimulation of amino acid-transport activity by serum.

These results provide evidence that changes in transport activity for amino acids occur much earlier than the onset of DNA synthesis in cell populations induced by serum to enter the S-phase of the cell cycle. These changes consist of a cycloheximide-inhibitable increase in transport $V_{\text{max}}$, for amino acid substrates of the A-system mediation. Interestingly enough, the transport A-system appears to be the target mediation on which widely different (nutritional and hormonal) factors converge to control the amino acid-transport process (Guidotti et al., 1975, 1976; Robinson, 1976).

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**Nuclear-Protein Changes in the First Cleavage Cycle in Fertilized Sea-Urchin Eggs**

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Evidence is accumulating for structural differences between the histones of sperm and adult cells, especially for histone I. In echinoderm the switch from histone I₅ to I₆ occurs at gastrulation, but little is known about egg histones in early cleavage stages. This is primarily due to the difficulties inherent in obtaining good yields of pure nuclei when the nuclear/cytoplasmic ratio is low and a relatively large amount of extranuclear DNA and basic protein is present. Here nuclei were prepared by a simplification of earlier procedures (Loeb et al., 1969) and the phosphorylation of histone and non-histone proteins was followed during the first cleavage cycle.

Eggs were obtained in the usual way and the final preparations (up to $3 \times 3$ litres) contained 0.1–0.5 ml of packed eggs/10 ml of suspension. Pulse-labelling with $[^{32}\text{P}]{\text{P}}$ over several time periods in the first cycle was possible, since it was found that development could be arrested by the addition of 300 g of crushed ice at $-10^\circ\text{C}$/litre of suspension, which with stirring decreased the temperature to 0°C in less than 1 min. This treatment had no deleterious effect, and if the chilled eggs were restored to the original temperature development was resumed in the normal way, at least to hatching. Cleavage stages were followed microscopically.

*Echinus esculentus* eggs were sedimented for 5 min at 2000 g and washed three times with sea water. The packed eggs were then mixed with 1.5 vol. of 2.5M-sucrose/0.1% NaHSO₃ and homogenized in a Teflon–glass homogenizer. The material was then