J. Membrane Biol. 87, 217–224 (1985)

The Journal of
Membrane Biology

Uptake of Valine and Alanine by a Neutral Amino-Acid Carrier in Sea Urchin Eggs: Cyclic Variations in the Early Cleavage Stage

Denis Allemand, Guy De Renzis, Corrinne Maistre, Jean-Pierre Girard, and Patrick Payan
Laboratoire de Physiologie Cellulaire et Comparée, Université de Nice, Parc Valrose, 06034 Nice Cedex, France

Summary. The characteristics of valine and alanine uptake (respectively, preferential substrates of the L and A or ASC systems in mammalian cells) were studied in sea urchin eggs before and 40 min after fertilization. Substrate concentration dependence showed that in unfertilized eggs alanine is absorbed linearly up to an external concentration of 20 mM, whereas valine uptake presented a saturable kinetic with a K_m of 6 μM. Competition experiments showed that valine is absorbed by a carrier-mediated transport resembling the L system. Fertilization develops a new Na-dependent system, resembling the ASC system which is specific for neutral amino acids but does not discriminate between them. This system is superimposed on that of the unfertilized egg. In fertilized eggs, amino-acid transport displayed cyclic variations which have been previously associated with cell cleavage. We have found that eggs prevented from cleavage by treatment with antimitotic undergo a sequence of periodic amino-acid uptake timed with the mitotic cycle of untreated eggs. In addition, artificially activated eggs (A23187) which failed to divide showed a time course of amino-acid uptake similar to that observed in fertilized eggs. Furthermore, these variations are independent of protein synthesis. These results suggest to us that a biological clock exists in the cytoplasm or cortex of sea urchin eggs, which may be involved in timing the cell cycle.

Key Words sea urchin eggs · amino-acid uptake · fertilization · cyclic variations · Ca ionophore · amtimitotic drugs

Introduction

The regulation of amino-acid transport by cells is a fascinating example of an adaptive phenomenon. Indeed, in most cells, nutritional requirements are related to the activity of cellular metabolism. When cells are activated by an external agent such as a hormone or growth factor, they develop a specific and efficient mechanism of uptake for several substances (amino acids, glucose, nucleosides, etc.). In this case, numerous studies performed with mammalian cells pointed out that the mechanism of amino acid transport was switched from a Na-independent to a Na-dependent system which appears to be a more efficient pathway (Guidotti et al., 1978).

On the basis of competitive experiments using specific analogs, Oxender and Christensen (1963) initially proposed in Ehrlich cells the existence of two main transport mechanisms: the L system which is Na-independent and transports mainly large neutral amino acids (leucine, phenylalanine, valine, etc.) and the A and ASC systems which absorb small neutral amino acids (alanine, serine, glycine, etc.) more efficiently with the help of the electrochemical gradient of Na.

In 1972, Epel showed that fertilization of sea urchin eggs triggered the development of Na-dependent amino-acid transport. More recently, Allemand et al. (1984), by studying valine absorption, proposed that in fertilized eggs the mechanism of valine uptake closely resembles the ASC systems described in mammalian cells.

The present study extends information on valine transport and compares valine and alanine uptake in both unfertilized and fertilized eggs. Alanine is used as a model substrate of the A or ASC systems. We demonstrate that in zygotes uptake of these two amino acids exhibits similar characteristics in regard to dependence upon external Na, Li and the effect of competitors, including acidic and basic amino acids. This suggests that, when fertilized, eggs develop a mechanism of transport for amino acid with a broad specificity for neutral amino acids.

Numerous cellular events in fertilized eggs (membrane properties, respiration, macromolecule synthesis, etc.) have been shown to undergo cyclic variations synchronous with the mitotic cycle. In particular, amino-acid absorption was found to be timed with the cleavage cycle by Mano (1970) and Allemand et al. (1984) in sea urchin eggs or by Sander and Pardee (1972) in somatic cells.

In the second part of this work, we studied the

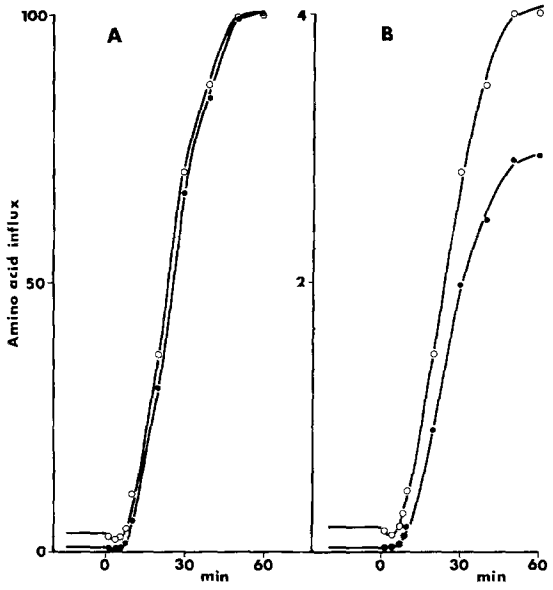

Fig. 1. Time course of alanine (●) and valine (○) absorption at fertilization for an external concentration of amino acids of 0.5 μM. Sperm is added at time zero. (*A*) Results are expressed as percent of maximal influx of each amino acid observed 40 min after fertilization. (*B*) Results are expressed as nmol/hr · mg protein

amino-acid uptake in the absence of mitosis obtained either by treating the fertilized eggs with antimitotic drugs (colchicine, cytochalasin B) or in eggs parthenogenetically activated with the Ca^{2+} ionophore A23187. We have found that antimitotic drugs did not prevent the pattern of amino-acid uptake and that, in addition, activation of eggs by A23187 yields periodic waves similar to controls. The origin and significance of such cyclic variations are discussed.

Materials and Methods

Biological Materials

Sea urchins *Paracentrotus lividus*, *Arbacia lixula*, or *Sphaerechinus granularis* were collected in the bay of Villefranche-sur-Mer and gametes handled by procedures previously described (Payan et al., 1981). Egg concentration was adjusted to 4% per volume and maintained in suspension at 20°C by stirring with a three-blade propeller. Unless otherwise specified, experiments were carried out on *Paracentrotous lividus*.

Measurement of Amino-Acid Transport

Measurement was made by the pulse technique which allows initial velocity to be measured as described earlier (Allemand et al., 1984). Unless otherwise specified, 1-min pulse experiments were carried out with external concentration of amino acids of 0.5 μM. As in our preceding work, the characteristics of amino-acid transport were determined between 40 and 50 min post fertilization. Results are expressed as nmol/hr · mg protein. The labeled precursors were 3H-valine (40 Ci/mmol) and 14C-alanine (160 mCi/mmol) obtained from CEA, Saclay.

Media and Chemicals

All experiments were performed as described by Allemand et al. (1984), using artificial seawater (ASW) in order to avoid any contamination by exogenous amino acids, thus abolishing any competition or trans effect. Na-free (0 Na) ASW was made using choline chloride as substitute for NaCl and $KHCO_3$ as substitute for $NaHCO_3$. Measurement of internal Na was made using a flame photometer (Eppendorf). Proteins were measured by the Lowry method with the help of a Technicon autoanalyzer.

Results

Time Course of Alanine and Valine Transport at Fertilization

In unfertilized eggs, alanine transport is sixfold lower than valine uptake when measured at an external amino-acid concentration of 0.5 μM [0.040 ± 0.004 nmol/hr · mg protein (n = 12), valine uptake in the same conditions being of 0.220 ± 0.021 nmol/hr · mg protein (n = 10)].

Fertilization induces a dramatic increase in both alanine and valine transport (Fig. 1). When the results are expressed for each amino acid as percent of the maximal influx measured 40 min after sperm addition (Fig. 1*A*), the same course of the stimulation of alanine and valine transport follows similar kinetics except during the first minutes, when alanine influx is not reduced below the unfertilized level as with valine. On the other hand, when valine and alanine uptake are expressed as nmol/hr · mg protein, we observed (Fig. 1*B*) that the uptake of alanine is lower than that of valine.

Substrate Concentration Dependence

Alanine influx was measured in the presence of external concentrations of this amino acid, ranging from 0.2 to 50 μM.

In unfertilized eggs, the relationship between the initial velocity of alanine uptake and the external concentration is linear (Fig. 2). This linearity which has been tested up to 20 mM suggests that alanine enters the egg by a diffusional process. However, the permeability coefficient PD = 4×10^{-6} cm/sec calculated by assuming the egg to be a sphere with a diameter of 90 μm is about 1000-

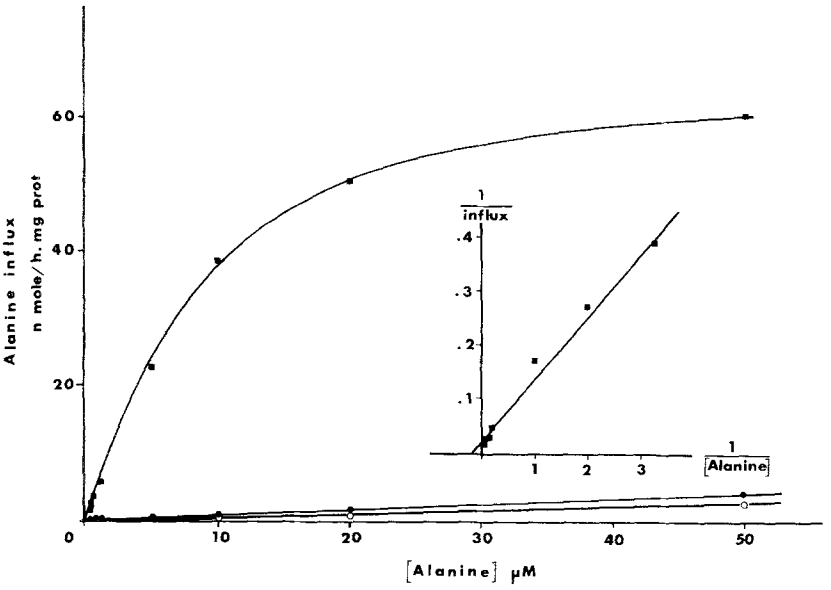

Fig. 2. Concentration dependence of alanine influx in: unfertilized eggs in SW (●), fertilized eggs in SW (■) and fertilized eggs transferred in 0 Na ASW (○). Inset: Lineweaver-Burk plot of the data from fertilized eggs. V_{max} = 59 nmol/hr · mg protein, K_m = 6.7 μM

fold higher than that characterizing diffusional process (Young & Ellory, 1977). This value would fit better with a carrier-mediated mechanism characterized by an extremely low affinity. Present results do not permit discrimination between these two hypotheses.

In fertilized eggs, the alanine influx presents saturable kinetics of a Michaelis-Menten type with a maximum at about 20 μM. This result suggests that in this case alanine is absorbed like valine (Allemand et al., 1984) via a carrier-mediated mechanism. In fertilized eggs transferred in 0 Na ASW the uptake of alanine is reduced to the level in unfertilized eggs and depends linearly on external concentrations of substrate as in unfertilized eggs (Fig. 2). In the Lineweaver-Burk plot (insert, Fig. 2), this absorption has been subtracted from the total uptake for the calculation of the maximal influx and apparent affinity of alanine for its carrier (V_m = 59 nmol/hr · mg protein; K_m = 6.7 μM).

Competition Experiments

Reciprocal Competition

These experiments were carried out in order to determine if, after fertilization, alanine and valine shared the same carrier. These two amino acids present the same affinity for their transport systems (respectively, 6.7 μM, present study; 6 μM, Allemand et al., 1984). Figure 3 shows a Dixon plot of the results obtained for external concentrations of amino-acid competitors ranging from 0.5 to 20 μM. It appears that the two amino acids compete for a single transport system, as the inhibition constants (K_i) for alanine and valine were, respectively, 6.2 and 5.7 μM, values comparable to the affinity constant presented above.

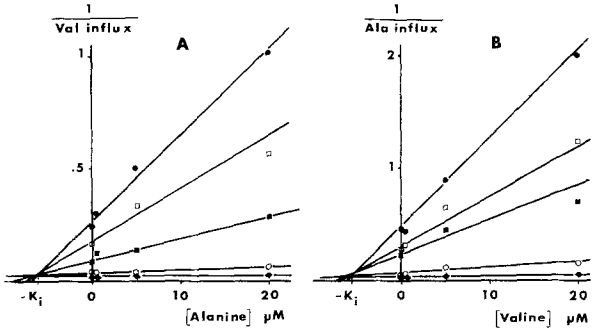

Fig. 3. Dixon plots showing reciprocal competitions between valine and alanine. Valine (*A*) and alanine (*B*) absorptions have been determined at different concentrations (●: 0.3 μM; □: 0.5 μM; ■: 1 μM; ○: 5 μM; ◆: 20 μM) in presence of different concentrations of competitor (respectively, alanine (*A*) and valine (*B*)) ranging from 0.5 to 20 μM

Competition by Other Neutral Amino Acids

We have tested the effects of different amino acids and analogs over a wide range of concentrations from 0 to 100 μM on the valine uptake by unfertilized eggs and on alanine influx in fertilized eggs, unlabeled valine and alanine being used as standard.

Figure 4*A* shows that in unfertilized eggs, the constant of inhibition (K_i) of valine for its own transporter was 12 μM, comparable to the K_m reported by Allemand et al. (1984). The amino acids

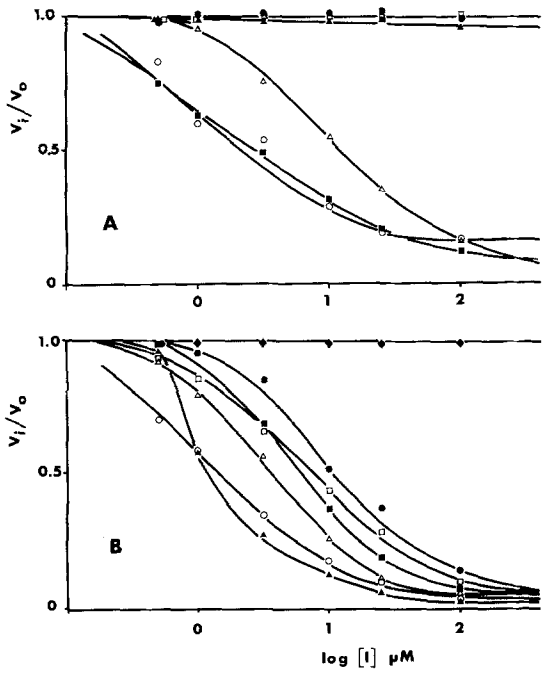

Fig. 4. Inhibition by neutral amino acid analogs of valine uptake in unfertilized eggs (*A*) and of alanine uptake in zygotes (*B*). The ratio of the amino acid uptake in the presence and in the absence of competitor was plotted against the logarithm of competitor concentration. The values of the constants of inhibition (K_i) were graphically determined. ▲: glycine; □: alanine; ●: serine; △: valine; ■: phenylalanine; ○: leucine; ◆: N-Met AIB. Valine and alanine external concentration was of 0.5 μM

which characterize the L system (phenylalanine, leucine) have a high apparent affinity for the valine carrier (K_i = 2.6 and 2.2 μM, respectively). On the contrary, alanine, glycine and serine, which are the preferential substrates of the A or ASC system did not compete, even at high concentrations, with valine uptake. These results are in agreement with the existence in unfertilized eggs of a single transport system resembling the L-system.

In fertilized eggs, the same type of experiment shows (Fig. 4*B*) that all the amino acids tested compete with alanine for transport. The constants of inhibition are of the same order of magnitude as the K_m of alanine (values ranging between 2 μM for glycine and 10 μM for serine). A nonmetabolizable substrate, N-methyl AIB specific to the A-system does not compete at all with alanine uptake, whatever the concentration used. These results suggest that the system of transport developed at fertilization insures the indiscriminate transport of all neutral amino acids tested and closely resembles the mammalian ASC system.

Competition experiments performed on valine uptake in fertilized eggs transferred extemporaneously in 0 Na ASW gave results similar to those obtained in unfertilized eggs in ASW (*compare* Fig. 4*A* and Fig. 5). This result clearly demonstrates the persistence of the L-amino-acid uptake system.

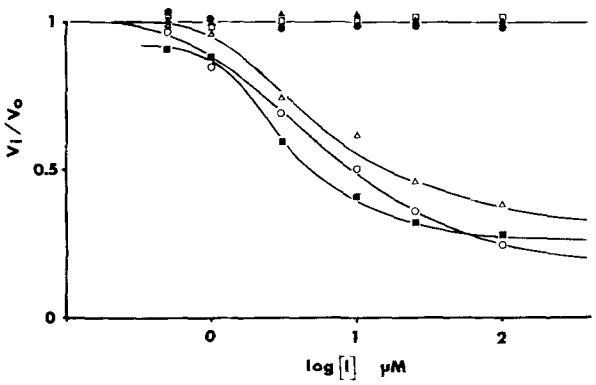

Fig. 5. Inhibition of valine transport by neutral amino acids in fertilized eggs measured in Na^+-free ASW. Same symbols as in Fig. 4

Competition by Amino Acids with Net Charge and by β-Alanine

The inhibition constants of histidine, lysine, glutamic acid and aspartic acid were measured by the same method on valine uptake in unfertilized eggs and on alanine uptake in fertilized eggs. We also tested the competition by β-alanine. The Table shows that β-alanine and amino acids with net charge except histidine did not compete with valine or alanine uptake.

Effects of Lithium on Amino-Acid Transport

Fertilized eggs were transferred to ASW containing 500 mM LiCl instead of NaCl. Alanine influx was thus reduced to the unfertilized level (Li-ASW: 0.058 nmol/hr · mg protein; unfertilized eggs in normal ASW: 0.047 nmol/hr · mg protein). This intolerance of the alanine transport mechanism for lithium suggests a parallel with the ASC system described in mammals (Guidotti et al., 1978).

Cyclic Variations of Amino-Acid Uptake

Figure 6*A* shows that 60 min after fertilization alanine influx follows cyclic variations whichever the sea urchin species (*Paracentrotous lividus:* Figure 6*A*; *Sphaerechinus granularis*: Fig. 6*B*; and *Arbacia lixula*: Fig. 8*C*). These variations have been previously associated with the cell by Mano (1970),

Table Constant of inhibition by amino acids with net charge and by β-alanine in unfertilized and fertilized eggs[a]

	Unfertilized	Fertilized
Anionic amino acid:		
Glutamic acid	>1,000	360
Aspartic acid	>1,000	>1,000
Cationic amino acid:		
Histidine	7.9	15
Lysine	>1,000	550
β-Alanine:	>1,000	>1,000

[a] Constant of inhibition is expressed as μM and is determined by competition experiments as described in the text.

and Allemand et al. (1984). However, from the result described in Fig. 7, it appears that the calcium ionophore A23187, which initiates most of the metabolic events normally occurring at fertilization in sea urchin eggs (Steinhardt & Epel, 1974) increases both the valine and alanine uptake by *Paracentrotus lividus* eggs. The kinetics and magnitude of this stimulation are comparable to those obtained after sperm contact (Fig. 6*A*). Furthermore, the uptake of these amino acids undergoes cyclic variations. The above results become striking since A23187 does not provoke any cytokinesis, suggesting that the membrane parameters and particularly those responsible for amino-acid transport could vary in a cyclical manner even though no cell division is observed.

Moreover, the effect of antimitotic drugs, such as cytochalasin B (CB 10 μM) and colchicine (250 μM), has been tested on amino-acid transport. These inhibitors were added 30 min after fertilization and amino-acid uptake was measured during 1 hr. Under these conditions, there was no cell division and the eggs remained blocked at the streak stage. Figure 8 shows that neither CB nor colchicine significantly modified the pattern of cyclic variations of alanine uptake. However, it can be noted that CB induces a short delay in the first cycle and that colchicine slightly decreases the transport of amino acids.

These results clearly demonstrate that cyclic variations of amino-acid uptake do not depend on the rearrangement of the cytoskeleton of the eggs during cell division.

Mano (1970) reported that protein synthesis underwent variations synchronous with the cell cycle. In order to test if the cyclic variations of protein synthesis and amino-acid uptake are linked, we measured alanine transport in presence of emetine (10^{-4} M) which completely blocks the incorporation of 3H-leucine into proteins (Wagenaar, 1983).

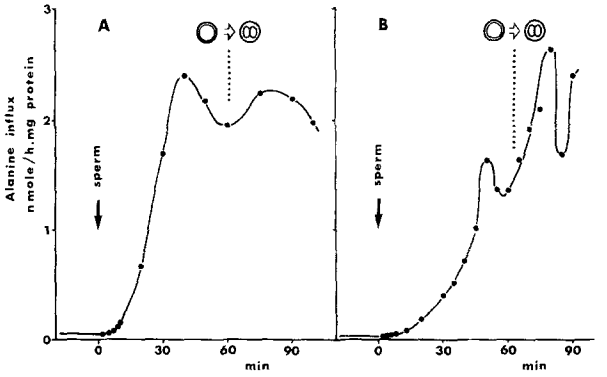

Fig. 6. Cyclic variations of alanine uptake after fertilization in *Paracentrotus lividus* (*A*) and *Sphaerechinus granularis* (*B*) eggs. In both cases. first division occurred about 70 min after fertilization

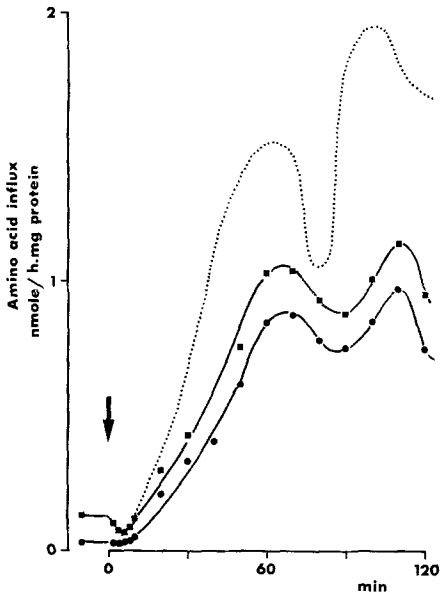

Fig. 7. Cyclic variations of alanine (●) and valine (■) uptake after activation by the Ca^{2+} ionophore A23187 (50 μM) in *Paracentrotus*. Dotted line: valine absorption in fertilized eggs taken as controls

Figure 8*C* shows that emetine added 5 min before fertilization does not alter at all the cyclic variations in alanine uptake. In our experiment, cell cleavage is totally abolished, in accord with Wagenaar's results (1983).

Discussion

These results and those previously reported (Epel, 1972; Allemand et al., 1984) point out that alanine, like valine, is transported by a carrier-mediated pro-

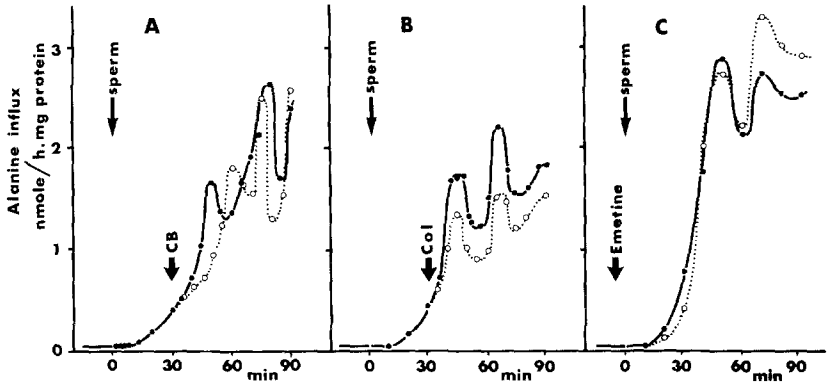

Fig. 8. Effect of cytochalasin B (CB) (part *A*), colchicine (Col) (part *B*) and emetine (part *C*) on alanine uptake by fertilized eggs of *Sphaerechinus granularis* (parts *A* and *B*) or of *Arbacia lixula* (part *C*). Cytochalasin B and colchicine were added 30 min after sperm contact (arrow); emetine was added 5 min before fertilization. ●: control; ○: inhibitor-treated eggs

cess in fertilized eggs, while, before fertilization, only valine shows clear saturability of uptake. By studying the transport of valine and alanine, which are, respectively, the model substrates of the L and A/ASC systems, we have pointed out that fertilization triggers the development of a new transport mechanism.

Comparison Between Alanine and Valine Uptake at Fertilization

The time courses of the stimulation of valine and alanine transport are comparable and reach a maximum at about 40 min after fertilization (Fig. 1). Furthermore, it can be noted that these two amino acids have indistinguishable affinities for their carriers.

Meanwhile, the evolution of the influx of these two amino acids during the first minutes following sperm contact is different: alanine influx is quickly stimulated and does not present any transient decrease as previously described for valine uptake by Allemand et al. (1984) (*see also* Fig. 1*A*). We may hypothesize that this difference between the pattern of transport of these amino acids could be related to drastic modifications of the plasma membrane soon after fertilization (exocytosis of cortical granules, elongation of microvillosities, endocytosis, etc.; *see* review by Vacquier, 1981). It has been shown in mouse eggs by Johnson and Edidin (1978) that fertilization provokes a large decrease in the mobility of membrane proteins due to a reduction in the lateral diffusion rate of lipids. Such a phenomenon occurring in sea urchin eggs at fertilization could explain the decrease in valine uptake which is mediated by a carrier mechanism. The absence of such a decrease in alanine uptake suggests that this amino acid does not enter unfertilized eggs via a carrier-mediated mechanism, which would be affected by the membrane reorganization.

Specificity of the Amino-Acid Uptake

Competition experiments using a wide range of amino acids have been carried out to study the specificity of amino-acid transport in unfertilized and fertilized eggs.

In unfertilized eggs, valine uptake is reduced in the presence of amino acids with branches or a ring (leucine, phenylalanine, valine) which characterize the L system. Linear amino acids (alanine, glycine, serine) which are specific for A and ASC systems did not interfere with valine uptake. These results and other features previously reported, such as the Na independence indicate that unfertilized eggs possess a single transport mechanism which closely resembles the L system described in mammalian cells.

In fertilized eggs, competition experiments (carried out with alanine, serine, glycine and N-methyl AIB), Na dependence and intolerance by alanine uptake of Li suggest that zygotes displayed a transport system for amino acids resembling the ASC system. However, this mechanism did not discriminate between any of the neutral amino acids studied.

Furthermore, in unfertilized and fertilized eggs, the transport systems are specific for the neutral amino acids tested since no competition was observed in presence of charged amino acids. Indeed the inhibition obtained with histidine (*see* Table) may be explained by the pK value (6.0) of the imidazole group, describing a dissociation that transforms this basic amino acid almost entirely into a neutral form at pH 8.0.

In conclusion, fertilization triggers the development of a new transport system of a much higher rate which is superimposed on the unfertilized one. Indeed, the latter continues to function after fertilization, as demonstrated by competition experiments in the absence of Na.

Cyclic Variations in Amino-Acid Uptake

Several years ago, Mano (1970) reported that amino-acid absorption by eggs of many species of sea urchin oscillated with the cell cycle. Allemand et al. (1984) came to identical conclusions on *Paracentrotus lividus* and, as the system was Na dependent, have associated these oscillators to the cyclic variations of Na permeability, itself being synchronous to cell division (Payan et al., 1981).

In the present study we have reported that artificially activated eggs (A23187) which failed to cleave, presumably for lack of a functioning centriole, undergo a sequence of amino-acid uptake waves timed with the cleavage cycle in control eggs. These observations demonstrate that certain experimental conditions disconnect a part of the cellular events from the mitotic cycle to which both are apparently bound under control conditions. Thus, activation switches on an autonomous oscillatory regulator which can be other than nuclear: cortical or cytoplasmic.

Numerous cellular events in fertilized eggs have been shown to undergo cyclic variations synchronous with the cell cycle: respiration (Zeuthen, 1953), macromolecule synthesis (Mano, 1970), state of protein SH (Sakai, 1960), tubulin polymerization and depolymerization (Coffe et al., 1983), surface stiffness (Yoneda et al., 1978), Na permeability (Payan et al., 1981) and amino-acid concentration (Kavanau, 1954). Cyclic modifications have also been described after parthenogenetic activation of eggs. They mainly concern mechanical properties such as cytoplasmic resistance (Coffe et al., 1982), tubulin polymerization and depolymerization (Coffe et al., 1983), surface stiffness in sea urchin eggs (Yoneda et al., 1978), surface contraction waves in *Xenopus* eggs (Hara et al., 1980) or surface tension in starfish oocytes (Yamamoto & Yoneda, 1983).

The present study extends the description of independence between cyclic variations and cell division to transport mechanisms, in particular amino-acid uptake by activated sea urchin eggs.

Experiments carried out in the presence of emetine clearly demonstrate that the cyclic variations of protein synthesis do not induce variations in amino-acid uptake either directly or through the intermediary of a newly synthesized specific protein such as cyclin (Celis et al., 1984). They also confirm the results of Epel (1972) which showed that protein synthesis is not required for the increase in amino-acid transport after sperm activation.

Experiments performed in the presence of cytochalasin B or colchicine strongly suggest that this phenomenon is independent of the state of polymerization of both actin and tubulin. These results invalidate the hypothesis of a cyclic modification of the number of amino-acid transport systems in the plasma membrane, as proposed by Everhardt and Rubin (1974) concerning thymidine transport in CHO cells and Suzuki and Kono (1980) for recycling glucose carriers in fat cells. Therefore, it can be postulated that in sea urchin eggs, regulation of these cyclic variations is mediated by biochemical modification of the carrier *in situ*: a phosphorylation-dephoshorylation cycle of a protein component of the transport system, for example, as suggested by Shotwell et al. (1983). Another possibility that can be entertained concerns the modification of environment of the carrier, i.e., the membrane fluidity (Sanderman, 1978). Indeed, the timing of the variation in amino-acid uptake is related to the variation in egg surface tension. According to these results, an increase in surface stiffness would correspond to a decrease in amino-acid absorption. In mammalian cells, it has been postulated that cyclic changes in uptake of critical molecules, such as essential amino acids could regulate cell growth (Everhardt & Presscott, 1972; Holley, 1972). Moreover, the control of cell proliferation seems to be related to changes in ionic permeability (Lubin, 1980; Rozengurt & Mendoza, 1980). In this case, the membrane could be considered as the key to the biological clock which controls, among other parameters, the transport systems and, therefore, cell growth and division, as previously suggested by Njus et al. (1976). An understanding of the mechanism of timing of these cyclic changes may be useful for the understanding of the overall control of the cell cycle in somatic cells.

This research was partially funded by the CNRS (UA 651) DGRST (81E 1231). We also wish to thank the CEA for facilitating the purchase of radioactive products and Dr. C. Ellory and C. Ungar for their comments and suggestions on the manuscript.

References

Allemand, D., De Renzis, G., Ciapa, B., Girard, J.P., Payan, P. 1984. Characterization of valine transport in sea urchin eggs. *Biohchim. Biophys. Acta* **722**:337–346

Celis, J.E., Bravo, R., Larsen, P.M., Fey, S.J. 1984. Cyclin: A nuclear protein whose level correlates directly with the proliferative state of normal as well as transformed cells. *Leukem. Res.* **8**:143–157

Coffe, G., Foucault, G., Raymond, M.N., Pudles, J. 1983. Tubulin dynamics during the cytoplasmic cohesiveness cycle in artificially activated sea urchin eggs. *Exp. Cell Res.* **149**:409–418

Coffe, G., Rola, F.H., Soyer, M.O.., Pudles, J. 1982. Parthenogenetic activation of sea urchin eggs induces a cyclical varia-

tion of the cytoplasmic resistance to hexylene glycol-Triton X-100 treatment. *Exp. Cell Res.* **137**:63–72

Epel, D. 1972. Activation of an Na^+-dependent amino acid transport system upon fertilization of sea urchin eggs. *Exp. Cell Res.* **72**:74–89

Everhardt, L.P., Presscott, D.M. 1972. Reversible arrest of Chinese hamster cells in G1 by partial deprivation of leucine. *Exp. Cell Res.* **75**:170–179

Everhardt, L.P., Rubin, R.W. 1974. Cyclic changes in the cell surface. 1. Changes in thymidine transport and its inhibition by cytochalasin B in Chinese hamster ovary cells. *J. Cell Biol.* **60**:431–441

Guidotti, G.G., Borghetti, A.F., Gazzola, G.C. 1978. The regulation of amino acid transport in animal cells. *Biochim. Biophys. Acta* **515**:329–366

Hara, K., Tydeman, P., Kirschner, M. 1980. A cytoplasmic clock with the same period as the division cycle in *Xenopus* eggs. *Proc. Natl. Acad. Sci. USA* **77**:462–466

Holley, R.W. 1972. A unifying hypothesis concerning the nature of malignant growth. *Proc. Natl. Acad. Sci. USA* **69**:2840–2846

Johnson, M.H., Edidin, M. 1978. Lateral diffusion in plasma membrane of mouse egg is restricted after fertilization. *Nature (London)* **272**:448–450

Kavanau, J.L. 1954. Amino acid metabolism in the early development of the sea urchin *Paracentrotus lividus*. *Exp. Cell Res.* **7**:530–557

Lubin, M. 1980. Control of growth by intracellular potassium and sodium concentrations is relaxed in transformed 3T3 cells. *Biochem. Biophys. Res. Commun.* **97**:1060–1067

Mano, Y. 1970. Cytoplasmic regulation and cyclic variation in protein synthesis in the early cleavage stage of the sea urchin embryo. *Dev. Biol.* **22**:433–460

Njus, D., Gooch, V.D., Mergenhagen, D., Sulzman, F., Hastings, J.W. 1976. Membranes and molecules in circadian systems. *Fed. Proc.* **35**:2353–2357

Oxender, D.L., Christensen, H.N. 1963. Distinct mediating systems for the transport of neutral amino acids by the Ehrlich cell. *J. Bio. Chem.* **238**:3638–3699

Payan, P., Girard, J.P., Christen, R., Sardet, C. 1981. Na^+ movements and their oscillations during fertilization and the cell cycle in sea urchin eggs. *Exp. Cell Res.* **134**:339–344

Rozengurt, E., Mendoza, S. 1980. Monovalent ion fluxes and the control of cell proliferation in cultured fibroblasts. *Ann. N.Y. Acad. Sci.* **339**:175–190

Sakai, H. 1960. Studies on sulfhydryl groups during cell division of sea urchin egg. *J. Biophys. Biochem. Cytol.* **8**:609–615

Sander, G., Pardee, A.B. 1972. Transport changes in synchronously growing CHO and L cells. *J. Cell. Physiol.* **80**:267–271

Sanderman, H. 1978. Regulation of membrane enzymes by lipids. *Biochim. Biophys. Acta.* **515**:209–237

Shortwell, M.A., Kilberg, M.S., Oxender, D.L. 1983. The regulation of neutral amino acid transport in mammalian cells. *Biochim. Biophys. Acta* **737**:267–284

Steinhardt, R.A., Epel, D. 1974. Activation of sea urchin eggs by a calcium ionophore. *Proc. Natl. Acad. Sci. USA* **71**:1915–1919

Suzuki, K., Kono, T. 1980. Evidence that insulin causes translocation of glucose transport activity to the plasma membrane from an intracellular storage site. *Proc. Natl. Acad. Sci. USA* **77**:2542–2545

Vacquier, V.D. 1981. Dynamic changes of the egg cortex. *Dev. Biol.* **84**:1–26

Wagenaar, E.B. 1983. The timing of synthesis of proteins required for mitosis in the cell cycle of sea urchin embryo. *Exp. Cell Res.* **144**:393–403

Yamamoto, K., Yoneda, M. 1983. Cytoplasmic cycle in meiotic division of starfish oocytes. *Dev. Biol.* **96**:166–172

Yoneda, M., Ikeda, M., Washitani, S. 1978. Periodic change in the tension at the surface of activated non-nucleate fragments of sea urchin eggs. *Dev. Growth Differ.* **20**:329–336

Young, J.D., Ellory, J.C. 1977. Red cell amino acid transport. *In:* Membrane Transport in Red Cells. J.C. Ellory and V.L. Lew, editors. pp. 301–326. Academic, New York

Zeuthen, E. 1953. Biochemistry and metabolism of cleavage in the sea urchin egg, as resolved into its mitotic steps. *Arch. Neerl. Zool.* **10**:31–37

Received 4 March 1985; revised 28 May 1985