Table 2. Cells and cell lines of various origins classified according to their centromeric chromatin condensation status in prematurely condensed chromosomes of G_1 phase after 50-min condensation time

Cell type	Cell line	Reference	Condensed centromeric chromatin
Fibroblast	NIH/3T3	24	−
Fibroblast	ANN-1	5	+
Spleen			−
Thymus			−
Macrophage	$P388D_1$	25	−
Mastocytoma	P815	26, 27	−
Monocytoma	WEHI-3	28	−
Melanoma	B_{16}	29	−
Lymphoblast (stimulated by lipopolysaccharide)			+
Pre-B cell	18-81	30	+
Pre-B cell	18-48	30	+
Pre-B cell	PD31	31	+
Pre-B cell	K	32	+
Pre-B cell	70Z/3	33	−
B lymphoma	WEHI 279	34	+
B lymphoma	38C-13	35	+
Plasmacytoma	NS1 and Ag8.653	36	+
Plasmacytoma	J558	37	+
Plasmacytoma	MOPC-315	38	+

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Possible involvement of protein kinase C in the stimulation of amino acid transport by phorbol ester, platelet-derived growth factor and A23187 in Swiss 3T3 cells[1]

K. Kitagawa, H. Nishino and A. Iwashima

Department of Biochemistry, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602 (Japan), 10 December 1985

Summary. Stimulation of amino acid transport induced by phorbol-12,13-dibutyrate, platelet-derived growth factor or A23187 was not observed in cells lacking protein kinase C. On the other hand, stimulation of transport by epidermal growth factor or insulin was not affected. These results suggested that the stimulation of amino acid transport is mediated by at least two separate pathways.
Key words. Amino acid transport; protein kinase C; calcium ion; mouse fibroblast.

Among the early events associated with initiation of growth in quiescent cultured cells are alterations in the activity of a number of membrane transport systems[2-4]. Increased rates of amino acid transport, as well as hexose transport, have been demonstrated in a variety of cell types upon initiation of cell proliferation induced be several growth factors[5-7], hormones[7,8] and phorbol ester[9].

Recently it has been suggested[10,11] that the hexose transport system is regulated by the Ca^{2+}-activated, phospholipid-dependent protein kinase (protein kinase C), which appears to be a key enzyme in many transmembrane control systems. Therefore, it is of interest to elucidate the involvement of protein kinase C in the control mechanism of amino acid transport activity. In this study, we examined the role of protein kinase C in the stimulation of amino acid transport by phorbol-12,13-dibutyrate, platelet-derived growth factor, epidermal growth factor, insulin and A23187, and found that there may be at least two mechanisms of stimulation by these mitogens, only one of which is dependent on the activation of protein kinase C.

Methods. Cell culture. Mouse embryo fibrablast Swiss 3T3 cells were cultured in Dulbecco's modified Eagle medium supplemented with 10% fetal calf serum at 37 °C in a humidified CO_2 incubator. Three days after seeding at a density of 3×10^5 cells/2 ml in plastic Petri dishes (35 mm in diameter), the culture became confluent and these dishes were used for experiments.

Measurement of amino acid uptake. Cells were incubated for a designated period of time, and rinsed twice with 2 ml of Krebs-Ringer bicarbonate buffer. The uptake was initiated by addition of 1 ml of Krebs-Ringer bicarbonate buffer containing [^{3}H]-α-aminoisobutyric acid (500 μM, 50 μCi) at 20 °C. Choline chloride

and choline bicarbonate replaced NaCl and $NaHCO_3$, respectively, when Na^+-free medium was used. It was possible to discriminate operationally between the individual contributions of the two systems of mediation to the total transport by carrying out the uptake experiments in the presence or absence of Na^+ and unlabeled transport model substrates. The transport activity of each system was measured separately in parallel cultures. The Na^+-dependent A and ASC systems were rendered inoperative in Na^+-free medium. The presence of excess phenylalanine (5 mM) specifically blocks the L system while excess methyl-α-aminoisobutyric acid (5 mM) specifically blocks the A system[12]. After 5 min of incubation, the uptake was stopped by washing the plates twice with 2 ml of ice-cold Krebs-Ringer bicarbonate buffer. Aliquots of the cells were taken for assay of radioactivity and protein determination[13]. The linearity of uptake was maintained for at least 10 min. Triplicate dishes were used for all experiments. Data are expressed as mean ± SE. Significant differences were assessed by the Student's two-tailed t-test.

Results and discussion. Transport activity of α-aminoisobutyric acid was determined after incubation of cells with phorbol-12,13-dibutyrate, platelet-derived growth factor, A23187, epidermal growth factor and insulin, using serum-deprived cultured cells. The table shows that the addition of each agent markedly enhanced the transport activity for α-aminoisobutyric acid by the A system. On the other hand, no substantial changes in the activities of the ASC system and the L system could be detected (data not shown). Although each agent stimulated the rate of influx of α-aminoisobutyric acid transport by means of the A system, the rate of efflux from preloaded cells was not affected (data not shown). These results indicate that the Na^+-dependent A system, but not the Na^+-dependent ASC system or the Na^+-independent L system, is sensitive to the addition of hormone, growth factor, phorbol ester and A23187 in quiescent Swiss 3T3 cells to increase the amino acid transport.

It has been reported that prolonged pretreatment of Swiss 3T3 cells with phorbol ester causes a decrease in the number of phorbol ester binding sites[14], and in the activity of protein kinase C[15]. Furthermore, associated with this lack of protein kinase C activity, it was found that the cells became unresponsive to the biological effects elicited by phorbol esters and other mitogens that stimulate protein kinase C[15–17]. In our cell culture system, protein kinase C activity measured by the method of Nishizuka et al.[18] was decreased by pretreatment with 200 nM phorbol ester for 30 h (80.2 vs 6.8 pmol/10^6 cells/min at control and phorbol ester-pretreated cells, respectively). As shown in the figure, prolonged pretreatment with phorbol ester abolished the increase in α-aminoisobutyric acid uptake induced by a subsequent addition of phorbol-12,13-dibutyrate and platelet-derived growth factor. Our finding indicates that activation of protein kinase C is essential to trigger the stimulation of amino acid transport by phorbol-12,13-dibutyrate and platelet-derived growth factor. In contrast, addition of epidermal growth factor or/and insulin increased the α-aminoisobutyric acid uptake in phorbol ester-pretreated cells. This result suggested that epidermal growth factor and insulin modulate the amino acid transport activity through a pathway independent of protein kinase C.

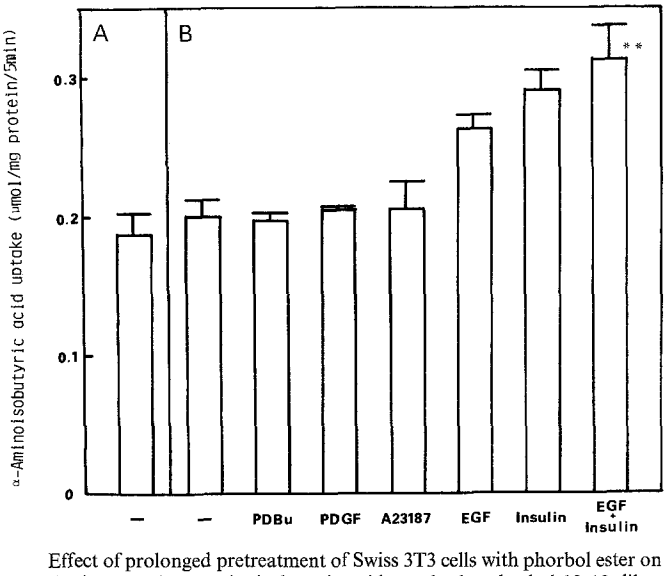
It has been proposed that not only protein kinase C, but also the level of free Ca^{2+} in cytoplasm plays an important role in transmembrane control[19]. Therefore, we examined the role of Ca^{2+} in the regulation of amino acid transport activity. The Ca^{2+} ionophore A23187 and platelet-derived growth factor, which increases cytosolic free Ca^{2+} in phorbol ester-pretreated and untreated cells (data not shown), show a stimulatory effect on α-aminoisobutyric acid transport in phorbol ester-untreated cells (table) but no stimulatory effect on α-aminoisobutyric acid transport in phorbol ester-pretreated cells (fig.). These findings imply that an increase in the intracellular Ca^{2+} alone is not sufficient to stimulate the amino acid transport; the concurrent presence of protein kinase C is essential for the stimulation of the transport.

The results indicate that the activity of the amino acid transport system may be regulated by at least two separate pathways, one of which is dependent on the presence of protein kinase C.

Effect of phorbol-12,13-dibutyrate, platelet-derived growth factor, A23187, epidermal growth factor and insulin on α-aminoisobutyric acid uptake

Addition	α-Aminoisobutyric acid uptake (μmol/mg protein/10 min) A system	(% of control)	
Control (none)		0.19 ± 0.02	(100)
Phorbol-12,13-dibutyrate	(100 nM)	0.34 ± 0.02*	(179)
Platelet-derived growth factor	(100 ng/ml)	0.36 ± 0.04*	(189)
A23187	(5 μM)	0.33 ± 0.02*	(174)
Epidermal growth factor	(5 ng/ml)	0.27 ± 0.03*	(142)
Insulin	(1μg/ml)	0.27 ± 0.01*	(142)

Confluent cultures were preincubated with serum-free Dulbecco's modified Eagle medium for 2 h at 37 °C and then each agent was added. After 3 h, the transport activity of A system was measured as decribed in Methods. Data are expressed as mean ± SE for triplicate dishes in each experiment. * $p < 0.05$ (vs control value).



Effect of prolonged pretreatment of Swiss 3T3 cells with phorbol ester on the increase in α-aminoisobutyric acid uptake by phorbol-12,13-dibutyrate, platelet-derived growth factor, A23187, epidermal growth factor and insulin. Confluent cultures were incubated in Dulbecco's modified Eagle medium supplemented with 10% calf serum in the absence (A) or presence of (B) 200 nM phorbol-12,13-dibutyrate at 37 °C. After 30 h, cells were preincubated with serum-free Dulbecco's modified Eagle medium for 2 h and then phorbol-12,13-dibutyrate, platelet-derived growth factor, A23187, epidermal growth factor or insulin was added. After 3 h, the α-aminoisobutyric acid uptake was measured as described in Methods. PDBu, phorbol-12,13-dibutyrate; PDGF, platelet-derived growth factor; EGF, epidermal growth factor. ** $p < 0.05$ (vs control value).

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Ammonia photoproduction by *Cyanospira rippkae* cells 'entrapped' in dialysis tube

M. Vincenzini, R. De Philippis, A. Ena and G. Florenzano

Istituto di Microbiologia Agraria e Tecnica dell'Università degli Studi, Piazzale delle Cascine 27, I-50144 Florence (Italy), and Centro di Studio dei Microrganismi Autotrofi del C.N.R., Florence (Italy), 29 November 1985

Summary. The photoproduction of ammonia by cells of the heterocystous cyanobacterium *Cyanospira rippkae* in the presence of the glutamine synthetase inhibitor, L-methionine D,L-sulfoximine (MSX), was investigated. The time course of changes in protein, pigment and carbohydrate concentrations and the C_2H_2-reducing activity of nitrogenase in MSX treated and untreated filament suspensions was also determined. The results show that nearly 40 h after MSX addition the cells are able to recover from the nitrogen starvation induced by the inhibitor by themselves, without the removal of MSX or the addition of nitrogenous compounds. Biliproteins, mobilized as a consequence of MSX addition, seem to play a key role in the process of cell recovery. These findings were exploited in a semicontinuous ammonia producing process with cells 'immobilized' in a dialysis tube photobioreactor.
Key words. Heterocystous cyanobacteria; nitrogenase; nitrogen fixation; MSX-inhibition; ammonia photoproduction.

Photobiological ammonia production by nitrogen fixing cyanobacteria, treated with the glutamine synthetase inhibitor L-methionine D,L-sulfoximine (MSX), is under investigation in different laboratories employing free living[1-3] and immobilized cells[4,5]. From these studies it appears that the ammonia production rate is generally lower in immobilized cells, probably because of diffusional limitations. Nevertheless, cell immobilization stabilizes the NH_3-producing activity for a longer time and gives many operational advantages over free cells.

In our study we have utilized a special 'immobilization' technique consisting of an 'entrapment' of the microbial cells inside a dialysis tube. In this way the high substrate diffusion typical of free living cells, and the operational benefits of working with immobilized cells, were both obtained.

Certainly, this 'entrapment' technique does not achieve all the specific advantages of the classical immobilization methods[6] but it constitutes an effective system for evaluating the ability of microbial cells to produce low molecular weight extracellular metabolites.

Cyanospira rippkae strain Mag II 702, the type strain of the new genus *Cyanospira* described in this Research Centre[7], was the N_2-fixing organism selected for this study. The choice was suggested by the high nitrogenase activity of the organism and by the high pH required for its optimal growth, both properties being suitable for the ammonia production process.

Materials and methods. Organism and culture conditions. Cyanospira rippkae strain Mag II 702 was grown in 2-l Erlenmeyer flasks, containing 800 ml of the synthetic mineral medium previously described[7], under continuous white fluorescent illumination (80 μ E/m^{2}s on the liquid surface) at 28 °C. Air was bubbled through the culture at a flow rate of 500 ml/min in order to avoid cell stratification on the liquid surface.

Cells from 2-day-old cultures (late exponential phase) were used for the experiments on NH_3-photoproduction.

Description of the dialysis tube photobioreactor. The reactor was derived from the cooling jacket of an LKB 2137 chromatography column (16 mm i.d.) by replacing the glass column with dialysis tubing (Visking size 2-18/32″) as shown in figure 1. A volume of 125 ml of cyanobacterial trichome suspension was poured into the dialysis tube and immediately air was bubbled through the culture. Outside the dialysis membrane 150 ml of growth medium were introduced. When necessary, the outer medium was replaced without washout of the cells.

The bioreactor containing the cell suspension was incubated under continuous white fluorescent light (70 μ E/m^{2}s) at 28 °C.

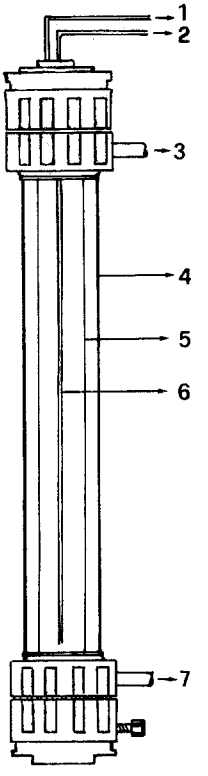

Figure 1. Dialysis tube photobioreactor. 1, air outlet; 2, air inlet; 3, inlet for outer medium; 4, cooling jacket of LKB chromatography column; 5, dialysis tube containing cell suspension; 6, connection for air bubbling; 7, outlet for outer medium.