C-24 substituent. When the accumulation and fate of these 7-dehydrosterols is considered, the following possibilities come to mind: 1) prothoracic glands, like guts[13], are able to dealkylate these 7-dehydrosterols such as **2b** and **2c** into 7-dehydrocholesterol, the latter being metabolized into normal ecdysone; 2) these dehydrosterols are converted into a modified ecdysone (24-alkylecdysones, without dealkylation of the C-24 substituents; 3) **2a** is selectively metabolized into ecdysone and the remaining **2b** and **2c** are equilibrated with **1b** and **1c**. Detailed analysis of the ecdysone of *B. mori* will shed some light on these questions. It is of interest to note that makisterone, an ecdysteroid with a 24-methyl group, has been isolated from *Oncopeltus fasciatus*[14,15], *Dysdercus cingulatus*[16], *D. fasciatus*[17], *Apis mellifera*[18], and *Drosophila melanogaster*[19], although, in the cases of *Oncopeltus* and *Dysdercus*, the insect do not convert their dietary plant sterol to cholesterol[15,17].

1 Present address: Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27514, USA.
2 *Tribolium confusum* exceptionally has the large amount of 7-dehydrocholesterol; Svoboda, J.A., Robbins, W.E., Cohen, C.F., and Shortins, T.J., in: Insect and Mite Nutrition, p. 505. Ed. J.G. Rodriguez. North Holland, Amsterdam 1972.
3 Robbins, W.E., Kaplanis, J.N., Svoboda, J.A., and Thompson, M.J., A. Rev. Ent. *16* (1971) 53.
4 Thompson, M.J., Kaplanis, J.N., Robbins, W.E., and Svoboda, J.A., Adv. Lipid Res. *11* (1973) 219.
5 Horn, D.H.S., Middleton, E.J., Thompson, J.A., and Wilkie, J.S., J. Insect Physiol. *20* (1974) 2433.
6 Morisaki, M., Ikekawa, N., and Ying, B., Experientia *37* (1981) 336.
7 Fujimoto, Y., Kimura, M., Khalifa, F.A.M., and Ikekawa, N., Chem. Pharm. Bull. *32* (1984) 4372.
8 Ikekawa, N., Fujimoto, Y., Takasu, A., and Morisaki, M., J. Chem. Soc. chem. Commun. (1980) 709.
9 MS *m/z* (as TMS ether): 456, 441, 366, 351, 325, 73 (base peak) for 2a; 470, 455, 380, 365, 339, 73 (base peak) for 2b; 484, 469, 394, 379, 353, 73 (base peak) for 2c.
10 Calvez, B., Hirn, M., and De Reggi, M., FEBS Lett. *71* (1976) 57.
11 Okuda, M., Sakurai, S., and Ohtaki, T., J. Insect Physiol. *31* (1985) 455.
12 Occurrence of 7-dehydro-24-methylenecholesterol as a principal sterol in the brain and whole body of a leaf-cutting ant, *Atta cephalotas isthmicola* was described, Ritter, K.S., Weiss, B.A., Norrborn, A.L., and Nes, W.R., Comp. Biochem. Physiol. *71B* (1982) 345.
13 Awata, N., Morisaki, M., and Ikekawa, N., Biochem. biophys. Res. Commun. *64* (1975) 157.
14 Kaplanis, J.N., Dutky, S.R., Robbins, W.E., Thompson, M.J., Lindquist, E.L., Horn, D.H.S., and Galbraith, M.N., Science *190* (1975) 681.
15 Kelly, Y.J., Woods, C.W., Redfern, R.E., and Borkovec, A.B., J. expl Zool. *218* (1981) 127.
16 Aldrich, J.R., Kelly, T.J., and Woods, C.W., J. Insect Physiol. *28* (1982) 857.
17 Gibson, J.M., Majumder, M.S.I., Mendis, A.H.W., and Rees, H.H., Archs Insect Biochem. Physiol. (1983) 105.
18 Feldlaufer, M.F., Herbert, E.W. Jr, Svoboda, J.A., Thompson, M.J., and Lusby, W.R., Insect Biochem. *15* (1985) 597.
19 Redfern, C.P.F., Proc. natn. Acad. Sci. USA *81* (1984) 5643.

0014-4754/86/091034-03$1.50 + 0.20/0

Possible target of Abelson virus phosphokinase in cell transformation[1]

G. Pantelias[2], H.-M. Jäck and M. Wabl[3]

Laboratory of Radiobiology and Environmental Health, University of California, San Francisco (California 94143, USA), 19 November 1985

Summary. By fusing interphase cells to cells undergoing mitosis, the interphase chromosomes can be visualized. When analyzed in this way, chromosomes of normal mouse cells show characteristic undercondensed centromeric regions. We have found that the centromeric regions of chromosomes from Abelson virus-transformed cells are fully condensed. Abelson virus transforms mouse cells by introducing into them a virally encoded phosphokinase that is expressed constitutively. Thus, we propose that the condensation of centromeric chromatin is a result of overphosphorylation by the Abelson virus phosphokinase, and that the centromeric region is the relevant target of overphosphorylation in transformed cell growth.
Key words. Abelson virus; centromere; chromosome condensation; premature chromosome condensation; transformation.

Interphase chromosomes, unlike metaphase chromosomes, cannot be directly visualized in situ by light microscopy because they are not condensed. During mitosis, the nuclear membrane disappears, allowing the condensed metaphase chromosomes to be spread on a microscope slide after hypotonic treatment of the cells. When interphase cells are fused to metaphase cells, the interphase nuclear membrane rapidly dissolves, and the interphase chromosomes condense and can be prepared in the same way as metaphase chromosomes[4]. By varying the time between fusion and chromosome preparation, we can vary somewhat the degree of condensation of the interphase chromatin, because the factors introduced by the metaphase cell take some time to act. We fused mitotic Chinese hamster ovary (CHO-B11) cells to untransformed interphase NIH/3T3 mouse fibroblasts and to the Abelson virus-transformed NIH/3T3 cell line ANN-1[5] (fig. 1). The mitotic condensation factors from the hamster cells were allowed to act on the interphase mouse chromosomes for 30, 40, or 50 min before chromosome spreads were prepared (fig. 2). Since the hamster metaphase chromosomes are metacentric with the two sister chromatids connected, they can be easily distinguished from the single acrocentric mouse chromatids of G_1 phase cells. On chromatids of the NIH/3T3 cells (fig. 2A), the centromeric regions characteristically appear as lightly stained elongated chromosomal stretches, as is characteristic of normal mouse cells[6,7]. At each of the sampling times, about 90% of the chromosome spreads from NIH/3T3 cells showed this undercondensation of the centromeric chromatin (table 1). In the transformed fibroblast line ANN-1, on the other hand, only 52% of the cells had undercondensed centromeric chromatin 30 min after fusion, and this decreased to 35% after 40 min and 15% after 50 min (table 1). The condensed centromere (fig. 2B) could be visualized only by using the C-banding technique[8] (not shown). In contrast to the centromeric region, the condensation rate for the noncentromeric chromatin was not markedly different in the two cell types.

At 50 min after fusion, the centromeric chromatin was fully condensed in four independent Abelson virus-transformed pre-B-cell lines, whereas 70Z/3, a chemically transformed pre-B-cell line, was similar to the NIH/3T3 cell line (table 2). Interestingly, other dividing cells of lymphocyte origin, including lipopolysaccharide-stimulated B lymphoblasts, exhibited fully condensed centromeric regions after fusion; normal spleen cells, thymus cells, and transformed cells of macrophage, mast cell, monocyte, melanocyte, and mammary carcinoma origin did not.

Although we cannot be sure of the proximate cause of the phenomenon described here, it is known that premature chro-

Table 1. Dependence of condensation of centromeric and noncentromeric chromatin on the time elapsed between cell fusion and chromosome preparation. Preparation of metaphase hamster cells, cell fusion, and chromosome spreading were performed as previously described[4,22]. Over 100 cells were scored for each time point

Time of chromosome condensation (min)	% of cells with chromosome condensation values* of 1 and 2		3 and 4		% of cells with undercondensed centromeric chromatin	
	NIH/3T3	ANN-1	NIH/3T3	ANN-1	NIH/3T3	ANN-1
30	49	55	51	45	92	52
40	62	70	38	30	89	35
50	70	78	30	22	89	15

*Numbers 1–4 represent decreasing degrees of chromosome condensation as classified by Hittelman and Rao[23] for cells in G_1 phase. On their scale of 1 through 6, value 1 represents the most highly condensed G_1 chromosomes, and 6 represents the most diffused. Chromosome spreads showing the degree of condensation for each value of the scale are shown in Hittelman and Rao[23].

mosome condensation[9,10], as well as chromosome condensation in metaphase[9,11–15], is correlated with, and perhaps caused by, phosphorylation of histone and nonhistone proteins. Of the five pre-B-cell lines, the four that showed fully condensed centromeric regions were transformed by Abelson virus, the oncogene of which encodes a phosphokinase[16]. On the other hand, the other lines of B cell origin and the B lymphoblasts showing fully condensed centromeric regions did not contain detectable amounts of messenger RNA of the v-*abl* or c-*abl* gene (examples are given in fig. 1). Thus, if overphosphorylation of the centromeric region is the reason for its complete condensation, another phosphokinase would have to cause that effect in these cells. Indeed, recent evidence suggests that lipopolysaccharide activates a protein kinase in B cells[17,18].

The centromeric region contains satellite DNA[19], the function of which is not known. However, as it is clear that the centromeric region is involved in cell division, we suggest that the packed state of satellite DNA is a factor in the proliferation of both activated B cells and cells transformed by Abelson virus.

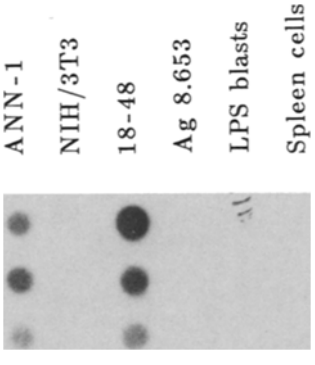

Figure 1. Titration of steady-state level of mRNA specific for v-*abl* by cytoplasmic dot hybridization. Serial 1:1 dilution steps were carried out, starting with the denatured cytoplasm[20] from 1.5×10^5 cells. The hybridization probe is the 1.6 kb Hind II fragment of v-*abl*[21], supplied by Oncor Inc., Gaithersburg, MD. The cell types listed are described in table 2. LPS blasts are lipopolysaccharide-stimulated spleen cells at an initial cell density of 2.5×10^5 cells, harvested at day 3 of culture.

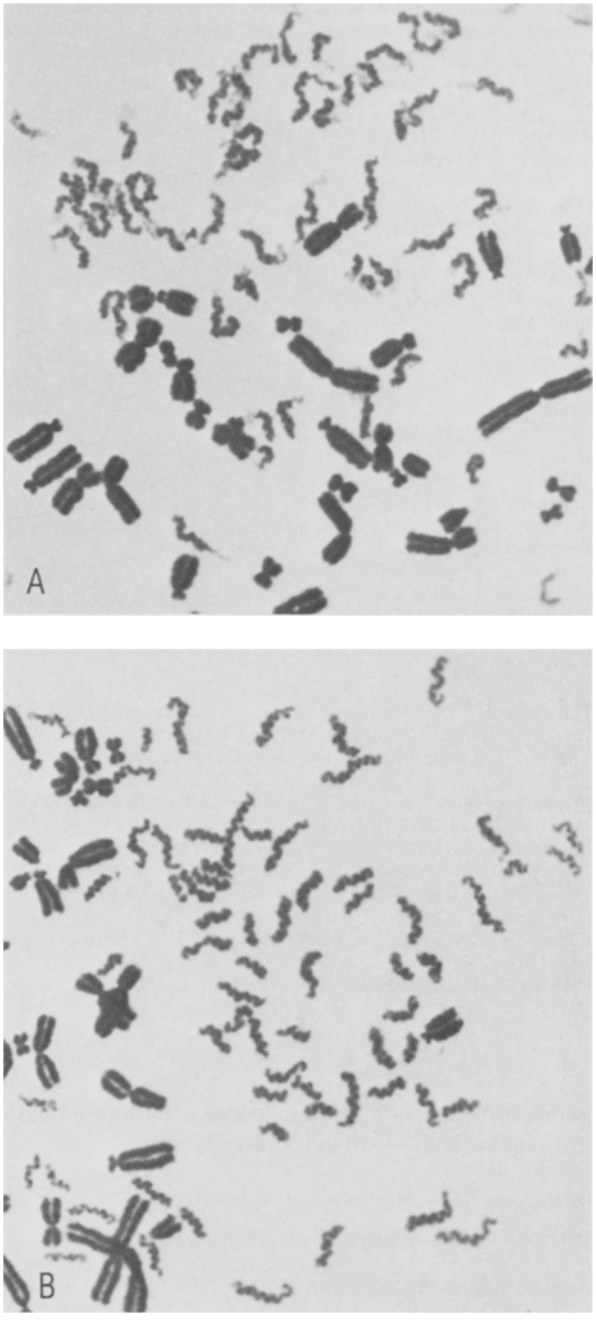

Figure 2. Photomicrographs of prematurely condensed chromosomes of cells derived from the fusion of metaphase hamster cells and G_1 phase mouse fibroblasts. *A* NIH/3T3 cells, *B* ANN-1 cells. The micrographs are matched for equal mouse chromosome length, i.e., equal chromatin condensation stage.

1 Acknowledgment. We thank J. Meyer and G. Beck-Engeser for technical assistance, O. Witte for advice and for providing the cell lines NIH/3T3 and ANN-1 and the plasmid pAB3sub3 containing v-*abl*, C. Steinberg and M. McKenney for critical reading of the manuscript, and S. Brekhus for typing it. This work was supported by the U.S. Department of Energy, contract No. DE-AC03-76-SF01012.

2 Present address: Nuclear Research Center 'Demokritos', Aghia Paraskevi Attikis, Athens, Greece.

3 To whom reprint requests should be addressed.

4 Johnson, R. T., and Rao, P. N., Nature, Lond. *226* (1970) 717.

5 Scher, C. D., and Siegler, R., Nature, Lond. *253* (1975) 729.

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	+

6 Drwinga, H.L., in: Premature Chromosome Condensation: Application in Basic, Clinical, and Mutation Research, p. 99. Eds P.N. Rao, R.T. Johnson and K. Sperling. Academic Press, New York 1982.
7 Hittelman, W.N., in: Cytogenetic Assays of Environmental Mutagens, p. 353. Ed. T.C. Hsu. Allanheld, Osmun, N.J., 1982.
8 Sumner, A.T., Expl Cell Res. *75* (1972) 304.
9 Krystal, G.W., and Poccia, D.L., Expl Cell Res. *134* (1981) 41.
10 Hanks, S.K., Rodriguez, L.V., and Rao, P.N., Expl Cell Res. *148* (1983) 293.
11 Bradbury, E.M., Inglis, R.J., and Matthews, H.R., Nature, Lond. *247* (1974) 257.
12 Fischer, S.G., and Laemmli, U.K., Biochemistry *19* (1980) 2240.
13 Ajiro, K., Borun, T.W., and Cohen, L.H., Biochemistry *20* (1981) 1445.
14 Allis, C.D., and Gorovsky, M.A., Biochemistry *20* (1981) 3828.
15 Sahasrabuddhe, C.G., Adlakha, R.C., and Rao, P.N., Expl Cell Res. *153* (1984) 439.
16 Witte, O.N., Curr. Topics Microbiol. Immun. *103* (1983) 127.
17 Coggeshall, K.M., Ransom, J., and Cambier, J.C., Fedn Proc. *44* (1985) 1294.
18 Wightman, P.D., and Raetz, C.R.H., J. biol. Chem. *259* (1984) 10048.
19 Pardue, M.L., and Gall, J.G., Science *168* (1970) 1356.
20 White, B.A., and Bancroft, F.C., J. biol. Chem. *257* (1982) 8569.
21 Goff, S.P., Gilboa, E., Witte, O.N., and Baltimore, D., Cell *22* (1980) 777.
22 Pantelias, G.E., and Maillie, H.D., Somat. Cell Genet. *9* (1983) 533.
23 Hittelman, W.N., and Rao, P.N., J. cell. Physiol. *95* (1978) 333.
24 Jainchill, J.L., Aaronson, S.A., and Todaro, G.J., J. Virol. *4* (1969) 549.
25 Koren, H.S., Handwerger, B.S., and Wunderlich, J.R., J. Immun. *114* (1975) 894.
26 Plaut, M., Lichtenstein, L.M., Gillespie, E., and Henney, C.S., J. Immun. *111* (1973) 389.
27 Lundak, R.L., and Raidt, D.J., Cell. Immun. *9* (1973) 60.
28 Ralph, P., Moore, M.A.S., and Nilsson, K., J. expl Med. *143* (1976) 1528.
29 Fidler, I.J., Nature, New Biol. *242* (1973) 148.
30 Siden, E.J., Baltimore, D., Clark, D., and Rosenberg, N.E., Cell *16* (1979) 389.
31 Lewis, S., Rosenberg, N., Alt, F., and Baltimore, D., Cell *30* (1982) 807.
32 Weimann, B.J., Cold Spr. Harb. Symp. quant. Biol. *41* (1976) 163.
33 Paige, C.J., Kincade, P.W., and Ralph, P., Nature, Lond. *292* (1981) 631.
34 Harris, A.W., in: Protides of the Biological Fluids, vol. 25, p. 601. Ed. H. Peeters. Pergamon Press, New York 1977.
35 Bergman, Y., and Haimovich, J., Eur. J. Immun. *7* (1977) 413.
36 Kearney, J.F., Radbruch, A., Liesegang, B., and Rajewsky, K., J. Immun. *123* (1979) 1548.
37 Weigert, M.G., Cesari, I.M., Yonkovich, S.J., and Cohn, M., Nature Lond. *228* (1970) 1045.
38 Schubert, D., Jobe, A., and Cohn, M., Nature, Lond. *220* (1968) 882.

0014-4754/86/091036-03$1.50 + 0.20/0

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