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ACTIVATION, AFTER MEDIUM REPLACEMENT, OF ^{32}P-PHOSPHATE INCORPORATION INTO PHOSPHOLIPIDS OF SUCCINYL-CONCANAVALIN A ARRESTED FIBROBLASTS

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SUMMARY

When succinyl-concanavalin A arrested fibroblasts are incubated in culture medium lacking succinyl-concanavalin A, the cell cycle is triggered, and S phase occurs 12-14 hours later. In pulse experiments, 32P-phosphate incorporation into cell phospholipids is shown to fastly increase. When succinyl-concanavalin A arrested cells are incubated in succinyl-concanavalin A containing culture medium, the cells remain arrested, and yet an initial and transient activation of 32P-phosphate incorporation into phospholipids is observed.

INTRODUCTION

Phospholipid metabolism has been studied under various conditions of cell arrest and stimulation (1-7). We have ourselves observed variations in phospholipid metabolism of 3T3 fibroblasts after arrest in serum free medium and stimulation by serum addition (8). Thus, we have noted that, during the first phases of the cell cycle, G_1 and S, ^{32}P-phosphate incorporation increases initially very quickly, principally into phosphatidyl inositol, but also into phosphatidyl ethanolamine and phosphatidyl choline. Subsequently, incorporation increases more slowly into phosphatidyl inositol and decreases into phosphatidyl ethanolamine and phosphatidyl choline.

Burger an col. (9) have observed the arrest of 3T3 fibroblasts on incubation with succinyl-concanavalin A (succinyl-con A) (10). When subsequently the medium is replaced by culture medium without succinyl-con A, the cells divide again. In this work, we have attempted to investigate whether this new

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system of cell arrest and stimulation triggers similar variations in ^{32}P-phosphate incorporation into phospholipids as those we have previously observed after serum deprivation and addition.

METHODS

3T3 Mouse fibroblasts were grown in Dulbecco's modified Eagle's medium, to which 10 per cent calf serum, 10^5 I.U. of penicillin and 0,1g of streptomycin per liter were added. Cell culture was performed in 25 cm^2 sterile plastic flasks (Falcon), in 5 per cent CO2. The cells were seeded at 10,000 cells per cm^2.

After attachment, 500 µg/ml of succinyl-con A (Industries Biologiques Françaises) were added to the medium, and the cells were incubated for 48 hours. The arrested cells thus obtained were then incubated (time 0) with culture medium either without or with succinyl-con A (1,000 µg/ml).

At various times after this medium replacement, 3H-thymidine incorporation into DNA was studied by pulse experiments. 1 µCi/ml of 3H-thymidine (CEA, Saclay) was then added to the medium. 1 hour later, the cells were detached with trypsin. The cell suspension was filtered using a Whatman GF/C filter, which was then washed, and the radioactivity of the filter was counted directly in a scintillation counter (Packard). Cell number was simultaneously measured on a aliquot of the cell suspension.

Likewise, ^{32}P-phosphate incorporation into phospholipids was studied, by pulsing, at various times after medium replacement. 40 µCi/ml of ^{32}P-phosphate (CEA, Saclay) were then added to the medium. 1 hour later, the radioactive medium was discarded, the monolayers washed with chilled phosphate buffer saline (P.B.S.), and the cells solubilized in sodium dodecyl sulfate (Sigma), 2 mM, pH 7.2 (2). 3 ml were then poured into 1/1 (vol/vol) methanol-chloroform. Phospholipid extraction (11-12), two dimensional chromatography (13) and radioactivity determination techniques have been described previously (8).

RESULTS AND DISCUSSION

When succinyl-con A arrested cells are incubated in culture medium without succinyl-con A, it can be observed, Fig.1, that the cell cycle is triggered, G1 phase finishes within 8 hours and S phase is reached synchronously (3H-thymidine incorporation peak at 12-14 hours). Cell division occurs after about 20 hours. Under these conditions, Fig.2 indicates that ^{32}P-phosphate incorporation into cell phospholipids increases initially very quickly into phosphatidyl inositol, and more slowly into phosphatidyl ethanolamine and phosphatidyl choline. Subsequently, until the end of G1 phase and then in S phase, ^{32}P-phosphate incorporation remains at a high level, a slight increase for phosphatidyl inositol and a

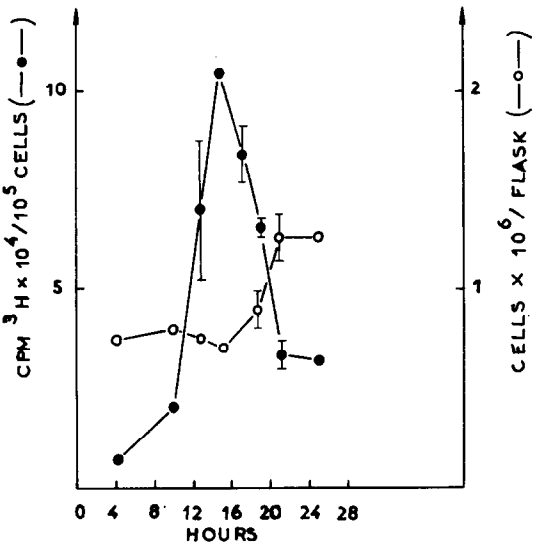

Fig. 1 - Succinyl-con A arrested cells were incubated (time 0) in culture medium without succinyl-con A. At various times after this medium replacement, ^{3}H-thymidine incorporation, in 1 hour pulse experiment, and cell number were measured. Abscissa : time in hours. Ordinate : cpm ^{3}H-thymidine per 10^5 cells (●-●) and cell number (O-O).

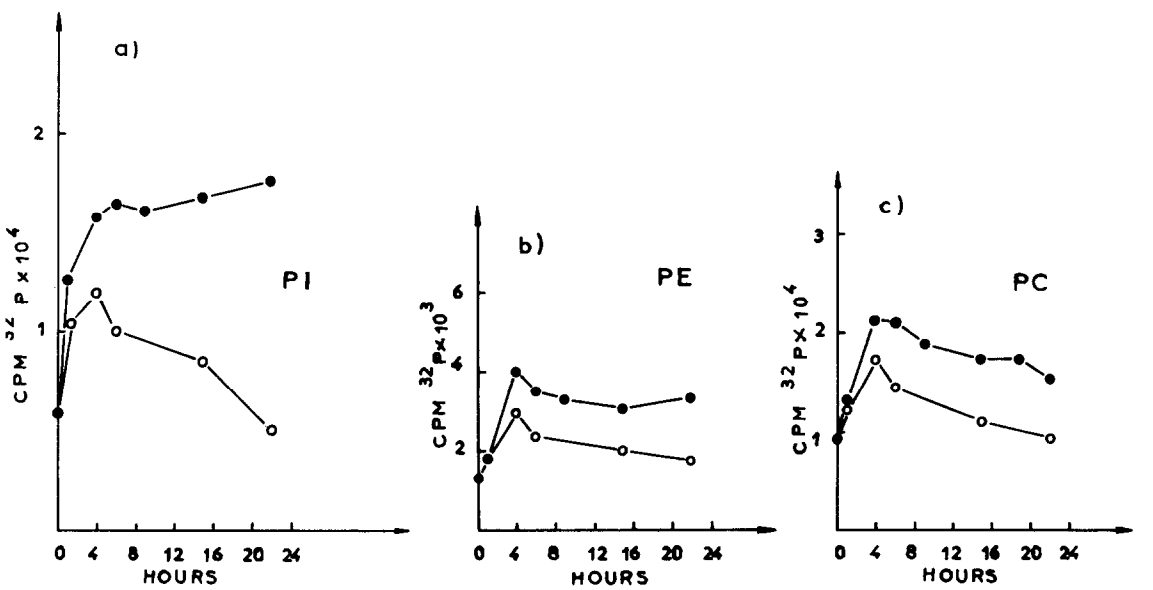

Fig. 2 a) b) c) Succinyl-con.A arrested cells were incubated (time 0) in culture medium either without (●—●) or with (o—o) succinyl-con.A. At various times after this medium replacement, ^{32}P-phosphate incorporation, in 1 hour pulse experiment, into cell phospholipid is measured. Abscissa: time in hours. Ordinate : cpm ^{32}P per flask. a) phosphatidyl inositol (PI) ; b) phosphatidyl ethanolamine (PE) ; c) phosphatidyl choline (PC) .

slight decrease for phosphatidyl ethanolamine and phosphatidyl choline being seen. These variations are similar to those observed when cells are arrested by serum deprivation and then stimulated by serum addition (8). Nevertheless, in this last case, the end of G1 phase occurs later, 14 hours post-stimulation rather than 8, suggesting that entry into the cell cycle is different.

When succinyl-con A-arrested cells are incubated in succinyl-con A-containing culture medium, we have verified that 3H-thymidine incorporation remains very low : 12 hours after medium replacement, time corresponding to the above described peak, the incorporation is the same as that observed at time 0. Thus, in this case, no efficient stimulation is seen. However, although the cells remain arrested, an initial activation of ^{32}P-phosphate incorporation into phosphatidyl inositol, phosphatidyl ethanolamine and phosphatidyl choline is still observed (Fig.2). This initial activation, which is lower than in the absence of succinyl-con A, is reversible and transient, and tends to disappear completely.

It can be concluded that, after medium replacement, there are two distinct and successive stages in ^{32}P-phosphate incorporation into phospholipids :

A primary stage, in which an initial activation in triggered by medium replacement. This activation is observed even when the cells remain arrested, i.e. when the cells are not engaged in a transition to S phase. The activation is then transient. Succinyl-con A acts on this stage, by lowering this initial activation of ^{32}P-phosphate incorporation into phospholipids, emphasizing the interaction between membrane stimuli and cell phospholipid metabolism.

A second stage, only observed when cell stimulation by medium replacement is efficient (in the absence of succinyl-con A), i.e. when the cells are definitively engaged in transition to S phase : this stage is marked by ^{32}P-phosphate incorporation into phospholipids remaining at a high value, principally for phosphatidyl inositol.

The hypothesis of the existence, between these two stages, of a restriction point, as previously defined by Pardee (14), must be considered.

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