ELSEVIER

FEBS Letters 344 (1994) 47–49

FEBS 13959

FEBS LETTERS

Changes in phospholipids during the cell cycle of myxomycete *Physarum Polycephalum*

Peter R. Makarov*, Victor K. Rotaru, Nina A. Polteva, Yuri V. Evtodienko

Institute of Theoretical and Experimental Biophysics, Russian Academy of Sciences, Pushchino, Moscow region, 142292, Russian Federation

Received 11 March 1994

Abstract

The rate of $^{32}P_i$ incorporation into the main membrane phospholipid fractions, i.e. phosphatidylcholine (PC), phosphatidylethanolamine (PE) and phosphatidylinositol (PI), as well as their contents in the cells during synchronous growth of the myxomycete, *Physarum polycephalum*, have been studied. It has been found that both the phospholipid levels and the rates of $^{32}P_i$ incorporation increase during the S phase till the early G_2 phase, remain nearly constant during the G_2 phase and fall to the initial level at the end of the G_2 phase and in mitosis. It has been revealed that the rate of $^{32}P_i$ incorporation into PC is very low compared to PE and PI, in spite of the fact that the PC level is the highest. The possible reasons of this phenomenon are discussed.

Key words: Cell cycle; Phospholipid; Physarum polycephalum

1. Introduction

One of the key problems of modern functional biochemistry is the understanding of temporal organization and interactions of intracellular processes occurring during the cell cycle. Membrane biogenesis plays an important role in these processes. It has been shown that during the cell cycle the volume of the cell and, consequently, the membrane area, increase non-monotonously and mitosis is controlled by the cell size [1,2]. It has also been shown that the rate of incorporation of the labelled precursors into the phospholipids periodically changes during the cell cycle of bacterial [3,4,] and higher eukaryotic cells [5,6].

On the other hand, phospholipids act as vital elements in transmembrane signaling. Stimuli, including growth factors, have been established to induce hydrolysis of phosphatidylinositides [7] and choline-containing phospholipids (phosphatidylcholine, sphingomyelin, and their metabolites) [8,9], which is a major mechanism for transmitting messages into the cells via protein phosphorylation, ultimately regulating gene transcription [10].

The myxomycete *Physarum polycephalum*, is a promising object for studying the cell cycle since it exhibits natural synchrony of nuclear division which excludes the possible artefacts occurring during synchronization of the cell culture. There are some investigations dedicated to phospholipid metabolism of *P. polycephalum*. Comes and Kleinig [11] were the first to report the composition of phospholipids and their fatty acid patterns. Minova et al. [12] have analyzed the changes in phospholipid composition during differentiation of *P. polycephalum*. However, metabolism of phospholipids during the cell cycle of *P. polycephalum* has not yet been investigated. The present study is concerned with the content changes of the main phospholipids and the rates of $^{32}P_i$ incorporation into them during the cell cycle of the myxomycete, *P. polycephalum*.

2. Materials and methods

The myxomycete, *Physarum polycephalum*, strain M_3C, was cultured as microplasmodia and macroplasmodia according to [13,14] except that the P_i level in the medium was decreased to 25% of the recommended one. The duration of the S phase was determined from an increase in methyl-[^{3}H]thymidine uptake [15]. The time of mitosis was determined by phase-contrast microscopic examination.

All investigations were carried out during the second cell cycle of macroplasmodia after fusion. The inter-division time was approximately 12 h in these experiments. Pieces of macroplasmodia (8 mm in diameter) were used for experiments.

The rates of $^{32}P_i$ incorporation into phospholipids were determined by incubation of the macroplasmodia pieces in the same medium containing 3.7 MBq/ml [^{32}P]orthophosphoric acid (carrier free) for 75 min at 26°C. Incubation was stopped by placing the pieces into 0.2 ml of boiling water for 2 min as Comes and Kleinig recommended [11]. After cooling, the lipids were extracted twice, first with 0.75 ml of chloroform/methanol (1:2, v/v), then the pellet was extracted with 0.95 ml of chloroform/methanol/water/conc. HCl (100:200:80:2, by vol.) [16,17]. The chloroform fractions were combined and evaporated to dryness under a stream of nitrogen, dissolved in 10–15 μl of chloroform/methanol/ water (75:25:2, by vol.) and separated by thin layer chromatog-

* Corresponding author. E-mail: Makarov@venus.iteb.serpukhov.su

Abbreviations: PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; $^{32}P_i$, [^{32}P]orthophosphoric acid.

SSDI 0014-5793(94)00344-U

raphy on silica gel plates impregnated with 1% potassium oxalate/2 mM EDTA in 20% methanol using chloroform/methanol/conc. NH_3 (9:7:2, by vol.). For lipid identification and evalution of separation quality they were also separated by two-dimensional thin layer chromatography with the same solvent in the first direction and using chloroform/acetone/methanol/acetic acid/water (6:2:2:2:1, by vol.) as a developing solvent in the second dimension. Individual phospholipids were identified by co-chromatography with standard phospholipids and located by autoradiography. Each located area was scraped into a counting vial, mixed with 5 ml of LS-8 liquid scintillator (5 g 2-(4-biphenyline)-5-phenyloxazole/150 ml 1-methylnaphtalene in 850 ml dioxane) and the radioactivity was determined with a SL-4000 (Intertechnics, France) liquid scintillation spectrometer.

The individual phospholipids to be quantified were visualized with iodine vapor to identify them and were scraped from the thin-layer plates and extracted. Phosphorous determination was performed by the method of van Veldhoven et al. [18].

Data are expressed as phospholipid P_i content (nmol P_i/mm^2) and the rate of $^{32}P_i$ incorporation (cpm/mm^2 × min) per square mm of the cell area.

All chemicals were of the purest grade commercially available.

3. Results and discussion

The contents (nmol P_i/mm^2) of the main phospholipids, i.e. phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylinositol (PI) during the cell cycle of macroplasmodium of *P. polycephalum* are shown in Fig. 1. During the S phase, the contents of the three investigated phospholipids increased up until the early G_2 phase. This period lasted for 5–6 h and about a two-fold increase in phospholipid levels was observed. Then for 4–5 h of the G_2 phase the levels of the phospholipids changed insignificantly. At the end of the G_2 phase, before nuclear division, a sharp decrease in the levels of all three phospholipids occured. These decreases continued in mitosis and ended only at the beginning of the following S phase. The changes of the total phospholipid content during the cell cycle are similar to the level changes of each of them. During the S phase and at the early G_2 phase the total level of phospholipids increased almost twice within the time consisting of only 0.4 of the cell cycle. It should be noted that individual phospholipids vary in a different manner. At the beginning of the S phase, the PE level increases more rapidly than PC, whereas at the end of the S phase to the beginning of the G_2 phase, the PC level increases quicker. The increase in the rate of PI content occurs later to those of PC and PE.

In order to estimate the rates of phospholipid synthesis during the cell cycle incorporation of a radioactive label into phospholipids was determined (Fig. 2). The rates of label incorporation into PE and PI can be seen to increase abruptly at the beginning of the S phase, then slow down until the middle of the G_2 phase. The rate of label incorporation decreased in the second part of the G_2 phase and during mitosis. These data on the whole satisfactorily explain the changes of PE and PI levels shown in Fig. 1.

The plot of $^{32}P_i$ incorporation into PC is different. During the S phase the rate of label incorporation into

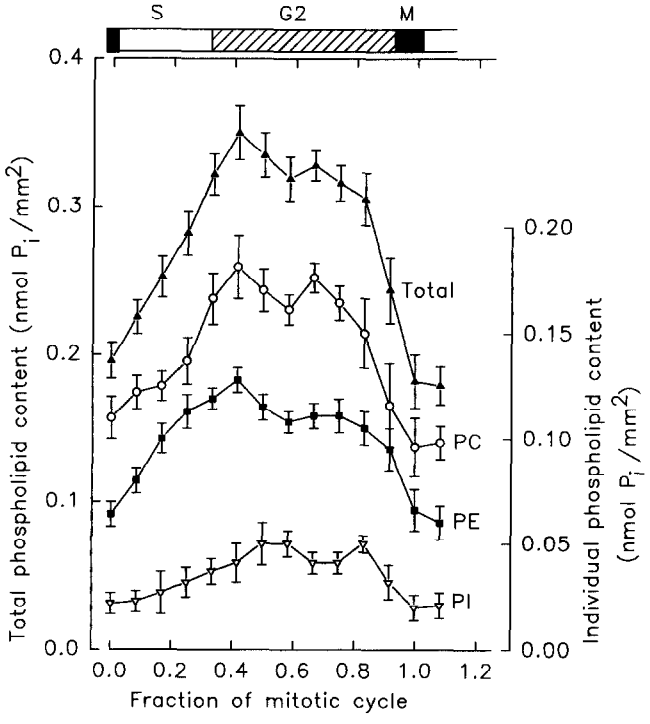

Fig. 1. Changes in the content of individual and total phospholipids of *P. polycephalum* during the cell cycle. The symbols show the means ± S.E.M. of four experiments; horizontal bars on the top indicate the cell cycle phases.

PC increases slightly and then decreases until the beginning of the next cell cycle.

The ratio of the average values of the phospholipid levels PC/PE/PI was found to be 1.0:0.7:0.3, which is in good agreement with the literature data obtained for asynchronous microplasmodium of *P. polycephalum* [8,9]. An unexpected finding was the ratio of the average specific rates of phospholipid labelling of PC/PE/PI which was equal to 1:6:15. This is a surprise because the mass of each phospholipid doubles during the cell cycle and it is sensible to expect this ratio to be similar to that of their contents. We believe that the reasons for such a large difference in these ratios may be the following: (i) PE is the major precursor for PC, indirectly supported by Comes and Kleinig who reported the similarity of fatty acid patterns of PE and PC [11]. So, the specific activity of label for PC synthesis is very reduced with a large pool of unlabelled PE. (ii) PI has a very high turnover because it is involved in processes of transmembrane signaling [19,20] and/or other metabolic processes. Work in this direction is continuing.

It is of interest to establish the reasons for the periodic changes of the phospholipid levels during the cell cycle, i.e. an increase at the beginning and a decrease at the end of the cell cycle. As we have already shown, the growth of macroplasmodium detected by the increase in cell area occurs non-monotonously during the cell cycle [21]. At

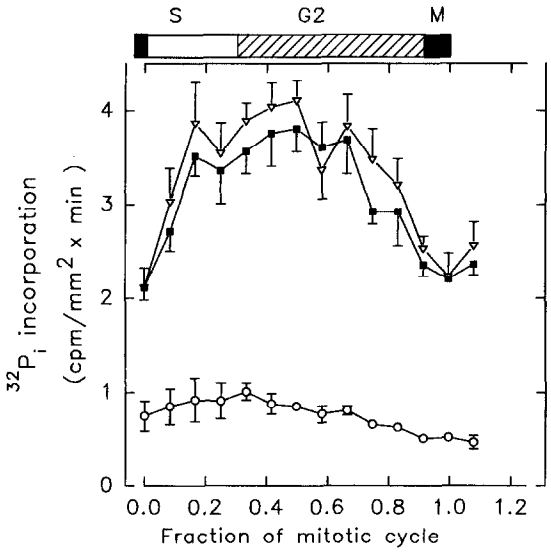

Fig. 2. The changes in $^{32}P_i$ incorporation rates into phospholipids of *P. polycephalum* during the cell cycle. The symbols are as in Fig. 1.

the beginning of the cell cycle a relatively low growth rate of the cell area can lead to an increase in cell thickness and it can be a reason for the observed increase in phospholipid levels per unit area. In the middle of the cell cycle the increase in the area is balanced with mass growth and the phospholipid levels do not change significantly. At the end of the cell cycle an abrupt increase in the cell area is observed which can induce a decrease in the cell thickness and lead to a decrease in the obtained phospholipid levels per unit area.

References

[1] Cameron, I.L. and Prescott, D.M. (1961) Exp. Cell Res. 23, 354–360.
[2] Fantes, P.A., Gravit, W.D., Pritchard, R.H., Sudbery, P.E. and Whels, A.B. (1975) J. Theor. Biol. 50, 213–244.
[3] Hakenbeck, R. and Messer, W. (1977) J. Bacteriol. 129, 1234–1238.
[4] Joseleau-Petit, D., Kepes, F. and Kepes, A. (1984) Eur. J. Biochem. 139, 605–611.
[5] Bosmann, H.B. and Winston, R.A. (1970) J. Cell Biol. 45, 23–33.
[6] Gerner, E.W., Glick, M.C. and Warren, L. (1970) J. Cell Physiol. 75, 275–280.
[7] Meldrum, E., Parker, P.J. and Corozzi, A. (1991) Biochim. Biophys. Acta 1092, 49–71.
[8] Zeisel, S.H. (1993) FASEB J. 7, 551–557.
[9] Asaoka, Y., Nakamura, S., Yoshida, K. and Nishizuka, Y. (1992) Trends Biochem. Sci. 17, 414–417.
[10] Stabel, S. and Parker, P.J. (1991) Pharmacol. Ther. 51, 71–95.
[11] Comes, P. and Kleinig, H. (1973) Biochim. Biophys. Acta 316, 13–18.
[12] Minova, A., Kobayashi, T., Shimada, J., Maeda, H., Murakami-Murofushi, K., Ohta, J. and Jnoue, K. (1990) Biochim. Biophys. Acta 1043, 129–133.
[13] Daniel, J.W. and Baldwin, H.M. (1964) in: Methods in Cell Physiology, vol. 1 (Prescott D.M. ed.), pp. 9–41, Academic Press, New York.
[14] Guttes, E. and Guttes, S., ibid., pp. 43–54.
[15] Braun, R., Mittermayer, C. and Rusch, H.P. (1965) Proc. Natl. Acad. Sci. USA 53, 924–931.
[16] Bligh, E.G. and Dyer, W.J. (1959) Can. J. Biochem. Physiol. 37, 911–917.
[17] Hauser, G., Eichberg, J. and Gonzales-Sastre, F. (1971) Biochim. Biophys. Acta 248, 87–95.
[18] Van Veldhoven, P.P. and Mannaerts, G.P. (1987) Anal. Biochem. 161, 45–48.
[19] Crews, F.T. (1985) in: Phospholipids and Cellular Regulation vol 1 (Kuo J.F. ed.) pp. 131–158, CRC Press, Boca Raton, FL.
[20] Nishizuka, Y., Kaibuchi, K., Kikkawa, U. and Takai Y., ibid., vol. 2, pp. 111–126.
[21] Vishnivetskii, S.A., Rotaru, V.K. and Evtodienko, Yu.V. (1992) Biochemistry (Rus.) 52, 891–899.