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CELL CYCLE RELATED CHANGES IN POLYAMINE CONTENT IN *EUGLENA*

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INTRODUCTION

In recent years, there has been an increasing interest in polyamines, which are ubiquitously present in animals, microorganisms and plants [1–3]. Their probable roles and physiological functions have been reviewed by several authors [4–6]. Although the precise mechanism of action of polyamines at the cellular level is still not elucidated, recent experiments using specific inhibitors of polyamine synthesis [7–12] have lent further support to the importance of polyamines in cell growth and division. Most studies on polyamine biosynthesis to date have been concerned with changes in the activity of L-ornithine decarboxylase (ODC), the rate-limiting enzyme in polyamine biosynthesis [13]. Experiments performed on mammalian cells [14–18] and *Euglena gracilis* Z [19] suggest that changes in ODC activity occur at specific phases of the cell cycle. As a number of discrepancies exist between ODC activity and putrescine synthesis, it has not yet been well established whether estimations of ODC are an accurate reflection of polyamine biosynthesis [20, 21].

In this paper, we have determined the composition of polyamines in *E. gracilis* by a modified HPLC method, recently developed by us. The variations in the levels of polyamines during different phases of the cell cycle in synchronous cultures of heterotrophically grown *Euglena* in total darkness have been studied. Biosynthetic pathways of polyamines in *Euglena* were also investigated by this method and the results are presented in another paper [22].

RESULTS AND DISCUSSION

Polyamine analysis in Euglena gracilis *Z*

Putrescine, *sym*-norspermidine (Nor SPD), spermidine and *sym*-norspermine (Nor SPN) were the major polyamines present. Carbamyl putrescine, *sym*-homospermidine and spermine were also present in small amounts. These results agree well with those reported by others recently [23, 24]. Nor SPD and Nor SPN which occur in *Euglena* have been found in thermophilic microorganisms [25, 26] and in other microorganisms [27, 28] which are not thermophiles. The automatic ion-exchange chromatographic method previously described [29] was improved to include the separation of usual as well as 'unusual' polyamines such as *N*-carbamylputrescine, Nor SPD, homospermidine, aminopropyl cadaverine and Nor SPN. Elution times of these 'unusual' polyamines were 32.1, 70.7, 76.3, 86.8 and 99.8 min, respectively.

Polyamines during the cell cycle in synchronous cultures of Euglena

We have previously reported [30, 31] that in synchronous cultures of *Euglena* in lactate medium, the *S* phase occurs *ca* 2 hr before mitosis (*M*) and at the end of the period of non-dividing cells. It was also observed at the same time that during the *S* phase the peak of DNA synthesis was superimposed with one of the peaks of RNA transcription. Fig. 1 illustrates the pattern of changes of major polyamines during the cell cycle in synchronous cultures of *Euglena*, grown on a lactate medium in total darkness. The level of Nor SPN, spermidine and putrescine increased as the cells start to divide and all 3 reached a maximum at 5 hr during the *M* phase, while the concentration of Nor SPD decreased during this period probably because of its utilization in the synthesis of Nor SPN. The amounts of most of the polyamines during the G_1 phase of non-dividing cells (interphase) remained almost constant except for Nor SPD whose concentration increased. In the *S* phase the level of Nor SPN declined drastically whereas there were only small changes in the concentration of putrescine, Nor SPD and spermidine. The enhancement in the synthesis of putrescine, spermidine and Nor SPN during the *M* phase and the sharp decline in the concentration of Nor SPN during the *S* phase suggest the possible involvement of these polyamines in DNA synthesis and cell division processes.

EXPERIMENTAL

Euglena gracilis (strain Z) was grown in inorganic medium supplemented with 33 mM DL-lactate, pH 3.5 at 27° in total darkness; the generation times were of 12 hr [32]. Dark-grown synchronous cultures were obtained as previously described [33]. Cell number was determined with a Mallassez counting chamber after dilutions in 10% KI. Cell vol. was determined by centrifugation in thrombohematocrit tubes.

Extraction of amines. Samples (50 ml) of cell culture ($1–10 \times 10^5$ cells) were collected by centrifugation at 5000 g for 15 min and the pellet was washed once with fresh medium and recentrifuged. The pellet was extracted with 1 ml 5% TCA in 0.05 M HCl at 4°. After homogenization followed by centrifugation the pellet was re-extracted with 0.5 ml 5% TCA in HCl and recentrifuged. Supernatants were pooled and a 50 μl aliquot was used for analysis.

Hydrolysis of samples. All samples were hydrolysed by the procedure of ref. [34].

Polyamine analysis. An amino acid analyser, Liquimat-Labotron, equipped with a fluorometer Labotron FFM-31

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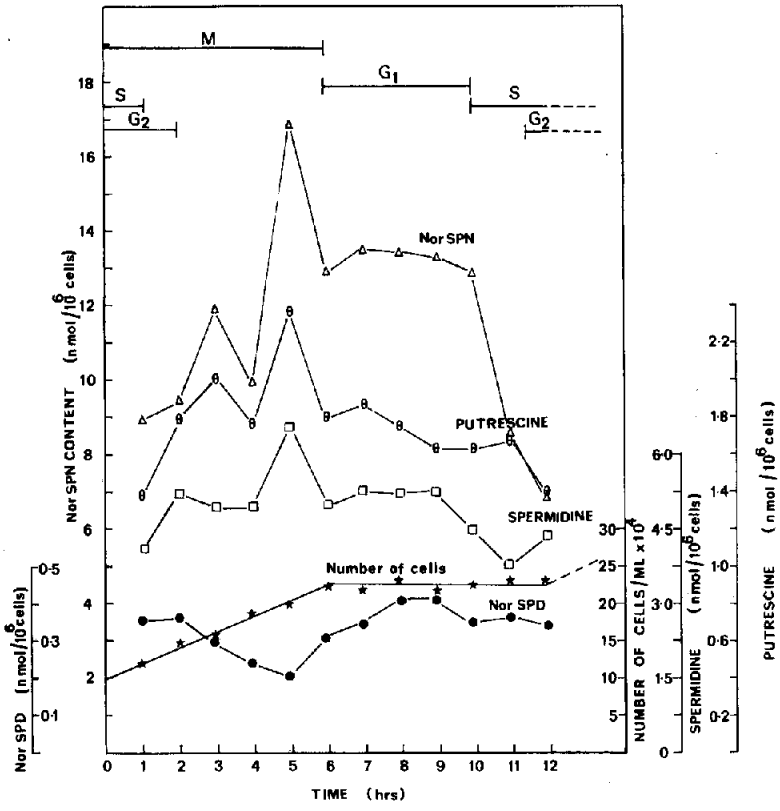

Fig. 1. Changes in the concentration of major polyamines during the different phases of the cell cycle in synchronous cultures of *Euglena gracilis* Z, Nor SPN (*sym*-norspermine) and Nor SPD (*sym*-norspermidine). *M*, *S*, G_1, and G_2 represent the approximate duration of the different phases. Note the sharp increase in the polyamine concentrations at 5 hr during the mitosis phase.

Table 1. Composition and conditions of eluting buffers

	Buffers	
	1st	2nd
Sodium citrate*	0.2 N	0.2 N
Sodium chloride*	0.3 N	2.5 N
Ethanol added	4%	6%
Final pH	5.60 ± 0.01	5.65 ± 0.01
Time (min)	44	76

* Molarity of Na^+. Two temperatures were used, 61° during the first 48 min and 78° thereafter.

(Kontron, Vélizy-Villacoublay), using a 50 μl flow cell, was employed. An integrator ICAP-10 (LTT, Saint-Honorine) was coupled to the fluorometer for quantification of the amines by the int. standard method. The recorder was set at 100 mV for 100% relative fluorescence. Separation of the amines was achieved on a 0.45 × 11.5 cm column of DC-4A cation exchange resin (Durrum Chemical Corporation). The composition of the eluting buffers and elution program are indicated in Table 1. Preparation of the buffers and other conditions were as described earlier [29, 35, 36].

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REFERENCES

1. Cohen, S. S. (1971) *Introduction to Polyamines*. Prentice-Hall, Englewood Cliffs, New Jersey.
2. Bachrach, U. (1973) in *Function of Naturally Occurring Polyamines*. Academic Press, New York.
3. Smith, T. A. (1977) in *Progress in Phytochemistry* (Reinhold, L., Harborne, J. B. and Swain, T., eds.) Vol. 4, p. 27. Pergamon Press, New York.
4. Tabor, C. W. and Tabor, H. (1976) *Annu. Rev. Biochem.* **45,** 285.
5. Campbell, R. A., Morris, D. R., Bartos, D., Daves, G. D. and Bartos, F. (1978) in *Advances in Polyamine Research*, Vols. 1 and 2. Raven Press, New York.
6. Russell, D. H. and Durie, B. G. M. (1978) in *Polyamines as Biochemical Markers of Normal and Malignant Growth*. Raven Press, New York.
7. Heby, O., Marton, L. J., Wilson, C. B. and Martinez, H. M., (1975) *J. Cell Physiol.* **86,** 511.
8. Anderson, G. and Heby, O. (1977) *Cancer Res.* **37,** 4361.
9. Anderson, G. and Heby, O. (1977) *Cancer Letters* **3,** 59.
10. Fillingame, R. H., Jorstad, C. M. and Morris, D. R. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72,** 4042.
11. Heby, O., Marton, L. J., Wilson, C. B. and Gray, J. W. (1977) *Eur. J. Cancer* **13,** 1009.
12. Mamont, P. S., Böhlen, P., McCann, P. P., Bey, P.,

Schuber, F. and Tardif, C. (1976) *Proc. Natl. Acad. Sci. U.S.A.* **73,** 1626.
13. Russell, D. H. and Snyder, S. H. (1968) *Proc. Natl. Acad. Sci. U.S.A.* **61,** 1420.
14. Friedman, S. J., Bellantone, R. A. and Canellakis, E. S. (1972) *Biochim. Biophys. Acta* **261,** 188.
15. Gerner, E. W. and Russell, D. H. (1977) *Cancer Res.* **37,** 482.
16. Heby, O., Marton, L. J., Zardi, L., Russell, D. H. and Basserga, R. (1975) *Exp. Cell Res.* **90,** 8.
17. Heby, O., Gray, L. W., Lindl, P. A., Marton, L. J. and Wilson, C. B. (1976) *Biochem. Biophys. Res. Commun.* **71,** 99.
18. Russell, D. H. and Stambrook, P. J. (1975) *Proc. Natl. Acad. Sci. U.S.A.* **72,** 1482.
19. Frayssinet, L. C., Bertaux, O., Valencia, R. and Frayssinet, C. (1978) *Biochim. Biophys. Acta* **539,** 435.
20. Harik, S. I., Hollenberg, M. D. and Snyder, S. H. (1974) *Nature* **249,** 250.
21. McCann, P. P., Tardif, C., Mamont, P. S. and Schuber, F. (1975) *Biochem. Biophys. Res. Commun.* **64,** 336.
22. Villanueva, V. R., Adlakha, R. C. and Calvayrac, R. (1980) *Phytochemistry* **19,** 787.
23. Kneifel, H. (1977) *Chem. Ztg.* **101,** 165.
24. Kneifel, H., Schuber, F., Aleksijevic, A. and Grove, J. (1979) *Biochem. Biophys. Res. Commun.* **85,** 42.
25. Oshima, T. (1975) *Biochem. Biophys. Res. Commun.* **63,** 1093.
26. De Rosa, M., De Rosa, S., Gambacorta, A., Carteni-Farina, M. and Zappia, V. (1976) *Biochem. Biophys. Res. Commun.* **69,** 253.
27. Stillway, L. W. and Walle, T. (1977) *Biochem. Biophys. Res. Commun.* **77,** 1103.
28. Rolle, I., Hobucher, H. E., Kneifel, H., Paschold, B., Riepe, W. and Soeder, C. J. (1977) *Analyt. Biochem.* **77,** 103.
29. Villanueva, V. R. and Adlakha, R. C. (1978) *Analyt. Biochem.* **91,** 264.
30. Calvayrac, R. and Lefor-Tran, M. (1973) *Soc. Phycol. Fr.* **18,** 1.
31. Ledoigt, G., Calvayrac, R., Orcival-Lafont, A. M. and Pineau, B. (1972) *Planta* **103,** 254.
32. Calvayrac, R. and Douce, R. (1970) *FEBS Letters* **7,** 759.
33. Calvayrac, R., Butow, R. A. and Lefor-Tran, M. (1972) *Exp. Cell Res.* **71,** 422.
34. Gehrke, C. H., Kuo, K. C. and Ellis, R. L. (1977) *J. Chromatogr.* **143,** 345.
35. Villanueva, V. R., Adlakha, R. C. and Cantera-Soler, A. M. (1977) *J. Chromatogr.* **139,** 381.
36. Villanueva, V. R., Adlakha, R. C. and Cantera-Soler, A. M. (1978) *Phytochemistry* **17,** 1244.