SHORT COMMUNICATIONS

Concanavalin A-induced Nuclear Division in *Stylonychia mytilus*

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INTRODUCTION

Protozoa provide a good system for the study of cytoplasmic-nuclear interactions (Tartar, 1961, 1969; De Terra, 1974). Alterations of the cortical structure can influence the nuclear behaviour of these cells (De Terra, 1974; Sapra & Ammermann, 1974). Lectins are suitable for the study of surface-induced events, because they bind to specific carbohydrate groups on the cell (Burger, 1973; Lis & Sharon, 1973). The motility of the protozoan *Stylonychia mytilus* can be specifically inhibited by interaction with the lectin concanavalin A (con A); the ciliate is able to recover from this immobilization process by shedding off the lectin-loaded surface components (A. Frisch, W. G. Bessler and D. Ammermann, unpublished observations). Here we report that the nuclear behaviour of *S. mytilus* is changed after the treatment with con A.

METHODS

Stylonychia mytilus, syngen I was grown in neutral Pringsheim medium (Ammermann *et al.* 1974). Con A was purchased from Calbiochem. Fluorescein-labelled con A was prepared using fluorescein-isothiocyanate (Serva), and the product was purified by gel filtration on a Biogel P-2 column (Biorad) and by affinity chromatography on Sephadex G100 (Pharmacia) using methyl α -D-mannopyranoside (Roth) as an eluant. Ciliate-lectin interaction experiments were performed in Pringsheim medium at 25 °C, using con A at a concentration of 16 μ g/ml. After lectin application to the cultures, nuclear events were followed over a 10 day period by staining the nuclei with Feulgen reagent. For autoradiographic experiments, [3 H]thymidine (Amersham-Buchler) was included in the culture medium (25 μ Ci/ml; 2×10^3 cells/ml). After incubation at 25 °C for 6 h, an autoradiographic procedure was followed as described by Prescott (1964). For label incorporation experiments, 10 μ Ci [3 H]-thymidine were applied to 5 ml cultures containing 2×10^3 cells/ml. After the addition of con A, cells were exposed to [3 H]thymidine for 90 min, beginning at 0, 1½, 3, 4½, 6, 9 and 24 h in separate cultures, which were precipitated with ice-cold 10 % (w/v) trichloroacetic acid (TCA) immediately following the pulse. Acid-precipitable material was washed with 10 ml TCA and 5 ml methanol, digested with 0.5 ml Soluene (Packard) and counted in a liquid scintillation spectrometer (Packard). All experiments were done in triplicate. RNA synthesis was inhibited with actinomycin D (Serva; 50 μ g/ml); macronuclear DNA synthesis was inhibited with hydroxyurea (1 mg/ml) according to Sapra & Ammermann (1974).

Fig. 1. Effect of con A treatment on *S. mytilus*. (a) *Stylonychia mytilus* treated for 30 min with fluorescein-labelled con A ($16 \mu\text{g/ml}$). (b) Feulgen-stained nuclei of *S. mytilus* after treatment for 4 h with Con A ($16 \mu\text{g/ml}$). Phase contrast.

RESULTS AND DISCUSSION

Cultures of *S. mytilus* were immobilized within 10 min after the addition of con A. By treating the cells with fluorescein-labelled con A we confirmed our earlier unpublished results that the lectin binds to the surface of the ciliate and prevents cell motion by clumping the cirri together (Fig. 1a). The lack of detectable fluorescence within the cell suggests that con A does not penetrate the cell surface. The relatively synchronous recovery of the cells 6 to 8 h after con A treatment was achieved by the quantitative shedding of the fluorescent pellicle and the synthesis of a new one. Inhibition of RNA synthesis with actinomycin D prevented this recovery, demonstrating that RNA synthesis is necessary. Inhibition of the macronuclear DNA synthesis with hydroxyurea had no effect on the regeneration process.

One hour after treatment with Con A, divisions of micro- and macronuclei could be observed in some animals. These divisions, which led to cells with an abnormal number of nuclei (Fig. 1b), could be detected after 5 to 6 h in all cells. In some cells up to seven micronuclei and up to three macronuclei, often atypically round-shaped, could be seen at this time. The nuclear events were not accompanied by cell division, which started only about 12 h after the shedding of the con A-loaded pellicle. Further observation of the animals showed that after 10 days about 50 % of the cells still had abnormal nuclei. The form and number of the nuclei became normal during succeeding cell divisions.

By autoradiography we observed thymidine incorporation in many micronuclei within 6 h of con A treatment, which indicated that DNA synthesis was a consequence of lectin treatment. Thymidine incorporation could be quantitated by pulse-labelling a culture of animals (Fig. 2). We found maximal incorporation 6 h after treatment with con A, corresponding with the observation that 5 to 6 h after lectin application a division of micro-

Fig. 2. Kinetics of [^3H]thymidine incorporation in cultures of *Stylonychia mytilus* treated with con A (16 $\mu\text{g/ml}$).

nuclei could be observed in all cells. Macronuclei, however, did not incorporate thymidine during the first 6 h after con A treatment, which suggests the fragmentation of these nuclei without DNA synthesis. This finding corresponds with the observation that the inhibition of macronuclear DNA synthesis did not affect the division of this nucleus.

There are several systems in which perturbations of the cell surface can influence nuclear behaviour; for example, in the ciliate *Stentor*, it is possible to induce cell divisions by micro-surgical removal of certain parts of the pellicle (Tartar, 1969), and in the egg cell of *Xenopus*, cortical injuries lead to abnormal mitosis (Brachet & Hubert, 1972). In *S. mytilus*, excision of parts of the pellicle can induce a regeneration process in which DNA synthesis in one or more micronuclei is triggered (Sapra & Ammermann, 1974). It is possible that the binding of con A to the surface of this ciliate induces similar events. Lectin-cell surface interaction could destroy the function of the pellicle, and thus either induce the formation of a new pellicle or give a signal for the nuclear events; alternatively, involvement of specific lectin-receptors could initiate these processes. It is tempting to compare these findings with the interaction of lectins with mammalian lymphocytes, by which cell differentiation and proliferation is induced (Burger, 1973).

The induction of nuclear divisions in *S. mytilus* by con A shows that lectins are useful tools for the study of cortical-nuclear interactions and may even provide a system for studying the activation of certain gene loci in this ciliate.

REFERENCES

- AMMERMAN, D., STEINBRÜCK, G., v. BERGER, L. & HENNIG, W. (1974). The development of the macro-nucleus in the ciliated protozoan *Stylonychia mytilus*. *Chromosoma* **45**, 401-429.
- BRACHET, J. & HUBERT, E. (1972). Studies on nucleocytoplasmic interactions during early amphibian development. I. Localized destruction of the egg cortex. *Journal of Embryology and Experimental Morphology* **27**, 121-145.
- BURGER, M. M. (1973). Surface changes in transformed cells detected by lectins. *Federation Proceedings* **32**, 91-101.
- DE TERRA, N. (1974). Cortical control of cell division. *Science, New York* **184**, 530-537.
- LIS, H. & SHARON, N. (1973). The biochemistry of plant lectins (phytohemagglutinins). *Annual Review of Biochemistry* **42**, 541-574.
- PRESCOTT, D. M. (1964). Autoradiography with liquid emulsion. In *Methods in Cell Physiology*, vol. 1, pp. 365-370. Edited by D. M. Prescott. New York: Academic Press.
- SAPRA, G. R. & AMMERMAN, D. (1974). An analysis of the development program in relation to RNA metabolism in the ciliate *Stylonychia mytilus*. *Developmental Biology* **36**, 105-112.
- TARTAR, V. (1961). *The Biology of Stentor*. Oxford: Pergamon.
- TARTAR, V. (1969). Morphogenesis in protozoa. In *Research in Protozoology*, vol. 2, pp. 1-116. Edited by T. Chen. Oxford: Pergamon.