

CYCLIC CHANGES IN LECTIN BINDING TO THE MEMBRANE OF *TETRAHYMENA*

SHORT COMMUNICATION

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Glycoconjugates of the cell membrane are involved in many important life functions of the cell. They undergo qualitative and quantitative changes during cell differentiation to cope with the altered functions [5]. Such changes can be easily followed up in the course of organ development [1]. Cycle-related changes in surface morphology are also demonstrable in cells in culture [1]. Such changes involve, among others, alteration of the membrane's saccharide components [4] which may be identified by exposure to sugar-specific lectins. During the mammalian cell cycle, considerable alterations have been observed in the availability of Concanavalin A (Con-A) binding sites; this prompted us to examine the membrane of *Tetrahymena* for similar cyclic changes in saccharide components.

Tetrahymena pyriformis GL cells, cultured in 0.1% yeast extract containing 1% Bacto Tryptone (Difco, Michigan) medium at 28 °C, were used in the experiment in the logarithmic phase of growth. The cells were synchronized by the method of Zeuthen [7], by applying two heat-shocks at 34 °C. Samples taken from the synchronized cultures 0, 30, 50, 60, 70, 90, 100, 110 and 140 min after the second heat shock were fixed for 5 min in 4% formalin (in PBS of pH 7.2), washed in three changes of PBS, and incubated for 1 h at room temperature in the presence of 50 µg/ml FITC-labeled lectin of the following types: Con-A (Serva, Heidelberg), *Phaseolus vulgaris* type S. (bean) lectin (Serva), *Pisum sativum* (pea) lectin [2], and *Lycopersicon esculentum* (tomato) lectin [3]. After incubation, the preparations were washed in three changes of PBS, mounted on slides, dried, and examined for the intensity of fluorescence in a Zeiss Fluoval cytofluorimeter. The results were recorded in a Zeiss K 100 linear compensograph. The intensity of lectin binding, was expressed in terms of integral of the area beneath the fluorescence curve constructed from the mean values of measurements on 100 cells in each group. Repeated experiments had the same results.

The binding of Con-A and pea lectin, which are of similar sugar specificity, followed a similar course during the cell cycle (Fig. 1). Both had a binding peak at 60 min, i. e. in the synthetic (S) phase of the cycle [7]. The curves for tomato and bean lectin practically

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represented negative images of the Con-A and pea lectin curves, with binding-minima around 60 min (Fig. 2).

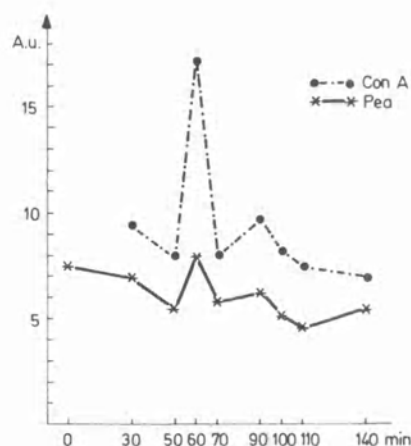


Fig. 1. Binding of Concanavalin-A and pea lectin to the membrane of the *Tetrahymena* in different phases of the cell cycle in a synchronized culture. Note binding peak in phase S

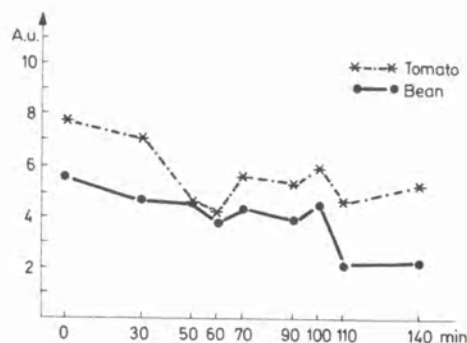


Fig. 2. Binding of tomato lectin and bean lectin to the membrane of the *Tetrahymena* in different phases of the cell cycle in a synchronized culture. Note decline of the initially notable binding during phase S

The experimental observations suggest that the lectin-binding capacity of *Tetrahymena* changes during the cell cycle, owing in all probability to cycle-related alterations in the saccharide composition and hence in the surface pattern of the membrane. Con-A and pea lectin, which bind primarily to simple sugars, had binding peaks, while tomato and bean lectins had binding minima, in phase S, indicating prevalence of simple sugars and relative deficiency of the tomato and bean-lectin-binding oligomers in that particular phase of the cell cycle.

It has been learned from Collard's [1] experiments that mammalian cells of different types bind Con-A in different phases of the cell cycle. In view of this, the prevalence

of Con-A binding and diminution of oligomeric binding in phase S of *Tetrahymena* in particular, does in all probability not apply to all animal cells in general. This is supported by the experiments of Smets and De Ley [6], demonstrating the decreased binding of Con-A in the S phase of 3T3 cells.

The fact nevertheless remains that the actual phase of the cell cycle should be taken into consideration in lectin-binding studies. The assumption also lies close at hand that accumulation of binding sites of one or another type in given phases of the cell cycle can considerably influence the interaction of the cell with its environment.

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