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CYCLIC CHANGES OF CONCANAVALIN-A BINDING SITES IN TETRAHYMENA CELL MEMBRANE

L. Kőhidai, P. Kovács and G. Csaba

DEPARTMENT OF BIOLOGY, SEMMELWEIS UNIVERSITY OF MEDICINE, BUDAPEST, HUNGARY

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Tetrahymena pyriformis GL cells were incubated with Con-A and HRP to demonstrate the localization of cell membrane sugar-components. Membrane sugar-components were found to be mobile and to change their localization periodically during the cell cycle. Highest Con-A binding occurred during division at the transition between G_1 and S phases. The cap formation shown by the sugar components is a physiological phenomenon in Tetrahymena occurring mostly periorally and to a lesser extent aborally.

Introduction

The cell membrane shows characteristic changes associated with biological processes, and so the spatial and temporal alterations of membrane components may be utilized for modelling cellular functions [2]. This holds true also for unicellular organisms such as Tetrahymena [3].

To study changes in membrane structure, demonstration of protein-linked carbohydrates appeared to be suitable as their dynamic changes reflect the functional state of the membrane. A widely used method for the demonstration of changes is the study of carbohydrates with plant or animal proteins, lectins, of defined ligand-specificity [1, 6].

In the present work the well known phenomenon of lectin-binding to the membrane of unicellular organisms [4, 5, 10] was used to study changes in the localization of sugar components of membrane receptors during the cell cycle in *Tetrahymena pyriformis* GL.

Materials and methods

In the experiments *Tetrahymena pyriformis* GL cells were used. They were cultured at 28 °C on 1% Bacto Trypton (Difco, Michigan) containing 0.1% yeast extract. Sample were taken during the logarithmic phase of growth. Cells were incubated with Concanavalin-A (Con-A, Serva, Heidelberg) followed by a 1 h incubation with horseradish peroxidase (HRP-

Send offprint requests to dr. L. Kőhidai, Semmelweis Orvostudományi Egyetem, Budapest, Pf. 370, H-1445 Hungary

Reanal, Budapest). Controls were incubated without Concanavalin-A. Preparations were developed with diamino-benzidine (DAB, Sigma, St. Louis) for 15 min. In another experimental group after Con-A a 1 mg/ml mannose treatment (1 h) was performed prior to HRP-reaction.

Experiments were carried out in both synchronized and non-synchronized cultures. In synchronized cultures the cycle was adjusted by two 30 min 38 °C thermal shocks with a 158 min interval. The cycle was started after the second shock and samples were taken after 5, 10, 30, 45, 50, 55, 60, 70, 80, 85, 90, 95, 100, 110, 130 and 140 minutes.

Cells were fixed in PBS containing 4% formaldehyde. For washing, PBS and to obtain a better HRP reaction, TRIS-HCl containing 1 mM $CaCl_2$ were used.

Results and discussion

The principle underlying our experiments was the binding of Con-A to the simple sugars (to mannose and glucose and their derivatives) of the membrane and the subsequent binding of HRP to Con-A presumably due to its mannose content [7, 8, 9]. As earlier experiments have shown [5] that Con-A binds to Tetrahymena membranes, we assumed that HRP may indicate changes in sugar-labelled receptors.

Control cells stained with HRP only, showed a pale, homogeneous pale yellow staining. Lectin-incubation brought about a typical dark-brown peroxidase reaction. Some cells bound HRP on their entire surface others only periorally or aborally (cap formation). All Con-A-treated samples contained sporadic negative—pale yellow—cells (Figs 1*a–d*).

All the above suggest that Con-A and HRP bind to each other most likely because not all of the four sugar-binding sites of Con-A bind to the membrane sugar-component and free binding sites may react with HRP. This is supported by the saturation with mannose of free binding sites resulting in a change of the staining pattern: most cells were negative and only few contained diffuse dots of HRP-deposit (Fig. 1). This latter may have been due to incomplete saturation. Thus, HRP under our conditions can be regarded as demonstrating Con-A. The localization of Con-A, on the other hand, differed from cell to cell: it was either homogeneous or patchy at various parts of the cell. As in our non-synchronized cultures cells were in various phases of division, Con-A localization could be expected to depend on the actual cell cycle phase. Therefore, synchronized cultures were prepared in which five staining patterns (negative, homogeneous, rounded, periorally patchy, aborally patchy) were distinguished. The occurrence of these patterns was studied comparatively during the cell cycle (Fig. 2).

At 28 °C the cell cycle time of *Tetrahymena pyriformis* GL was 140–150 min. The interphase lasted about 90 min, while division occurred in 50–60 min. Peroxidase positive cells were observed during the whole cycle but their peak number was seen at the end of G_1 phase and the beginning of S phase. If only cellular "caps" were taken into consideration, it appeared that

the number of cells decreased to a minimum, while their maximum was found where the peak of peroxidase positive cells occurred. In spite of this, the two sets of data were not parallel because the peroxidase positive cells showed a second peak at 110 min, i.e. during mitosis whereas "cap" cells had a second

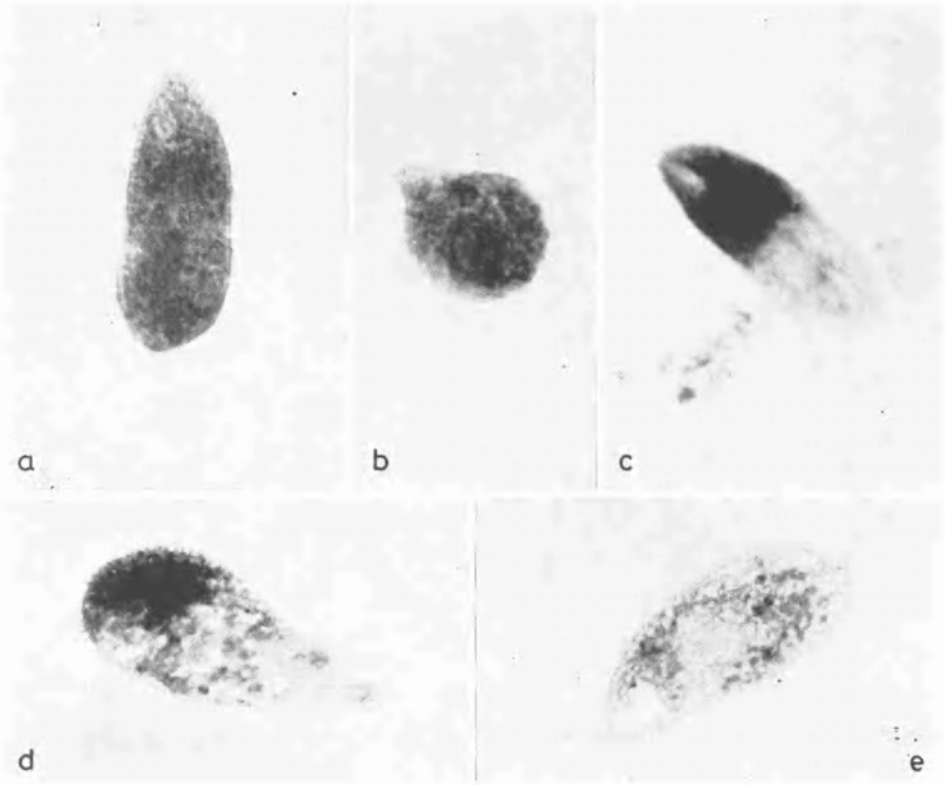

Fig. 1. Concanavalin-A pretreated, HRP-stained Tetrahymena cells. *a* HRP-binding on entire surface, *b* rounded with whole-surface binding, *c* perioral (cap) staining, *d* aboral (cap) staining, *e* mannose pretreatment

peak, smaller than the first one, around 80 min in the G_2 phase, wherefrom a gradual decrease occurred till the minimum at division.

From a comparison of the various forms of HRP-positivity (Fig. 3), it was evident that the homogeneous, rounded cells were the most frequent. Their distribution curve showed two peaks, one at the beginning of the S phase and the other during mitosis. Obviously, such cells should be present in high numbers during mitosis due to their rounding off before division. In the case of Tetrahymena cultures the cells were rounding off also in the S phase

which may explain their peak at this period. Next in number were the cells with perioral cap the peak of which fell to the transition between G_1 and S phases, and in G_2 another peak appeared. The peak of homogeneous, strongly positive cells was found at the beginning of the S phase and the next peak

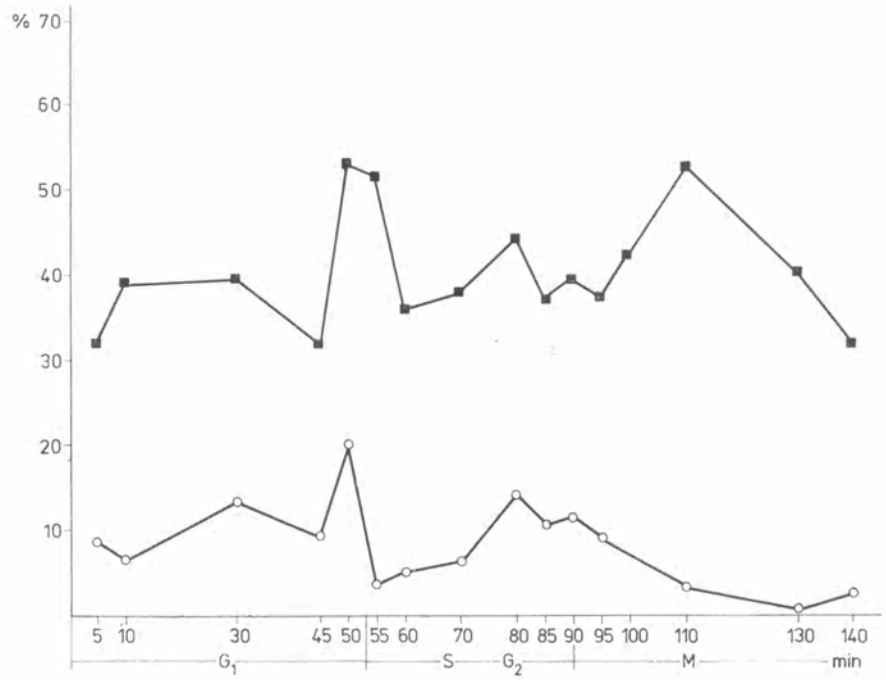

Fig. 2. Distribution of Con-A binding sites during cell cycle as shown by HRP. ■——■ HRP-positive cells/total cell number, ○——○ perioral cap cells/total cell number

was observed during division. In the lowest number the aboral cap cells were present whose peak occurred early in the G_1 phase, then the curve remained flat.

Our experiments suggest that sugars, and presumably receptors linked to them, detected by Con-A are located at various sites with changing cell cycle phases. As Con-A was added to fixed cells, cap formation could not be attributed to its effect. Thus the mobility (aggregation) of sugar-components of the Tetrahymena membrane seems to be a physiological phenomenon occurring in a given period of the cell cycle.

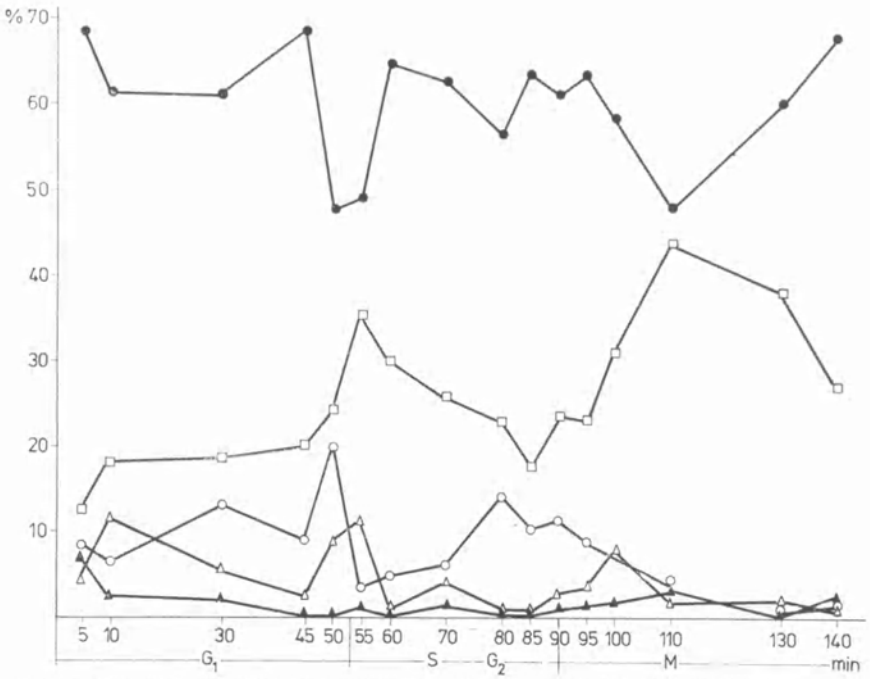

Fig. 3. Con-A binding patterns during cell cycle in percentage of total cell number. ○——○ perioral cap cells, △——△ cells stained on their whole surface, □——□ whole-surface binding in rounded cells, ▲——▲ aboral cap cells, ●——● HRP-negative cells

REFERENCES

1. Brown JC, Hunt RC: Lectins. Int Rev Cytol 52: 277, 1978
2. Collard JC, Temmink JHM: Binding and cytochemical detection of cell-bound Concanavalin-A. Exp Cell Res 86: 81, 1974
3. Csaba G: Ontogeny and Phylogeny of Hormone Receptors. Karger, Basel–New York 1981
4. Frisch A, Bessler W, Lipp HJ, Ammermann D: Immobilization of ciliates by Concanavalin A. J Protozool 23: 427, 1976
5. Kovács P, Csaba G: Studies on the lectin binding capacity of the Tetrahymena. Acta Protozool 21: 69, 1982
6. Monsigny M, Kieda C, Roche AC: Membrane lectins. Biol Cell 36: 289, 1979
7. Straus W: Cytochemical observations on mannose specific binding sites for horseradish peroxidase in liver sinusoidal cells. Histochemistry 77: 25, 1983
8. Straus W: Competition between glycoprotein hormones, and horseradish peroxidase for mannose-specific binding sites in cells of endocrine organism. Histochemistry 78: 289, 1983
9. Temmink JHM: Application of cytochemical methods to electron microscope investigations of cell surface receptors. Biol Cell 36: 227, 1979
10. Wise GE: Ultrastructural localization of surface Concanavalin-A binding sites in a unicellular organism *Amoeba proteus*. Cell Tissue Res 150: 161, 1975