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Universality of Hormonal Imprinting in Different Taxa of *Tetrahymena* and Inter-strain Variations in Its Intensity

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Synopsis. Seven *Tetrahymena* taxa preexposed to insulin showed a quantitative change in binding potency upon reexposure to the hormone. This suggests that hormonal imprinting can be induced in all tested taxa of *Tetrahymena*. Imprinting was most efficient in the taxon having the lowest growth rate, whereas least efficient in the taxon with the highest growth rate among all strains studied. Lectin (Concanavalin A) binding of the various *Tetrahymena* taxa was different, with tendencies similar to insulin. Although insulin imprinted the cells for lectin binding, too, this effect was lower than for insulin.

The first interaction of the hormone with the cell gives rise to hormonal imprinting, which accounts for an altered — usually increased — cellular response to reexposure (Csaba 1980, 1981). In higher (vertebrate) organisms, the cell — hormone interaction, including receptor formation under hormonal influence, is genetically predetermined (Lin and Becker 1983). In unicellular organisms, which do not possess an encoded receptor — hormone system, hormone receptors may arise either from receptors for other materials, which originally represent non-specific structures for the hormone, or by assembly of certain membrane-associated subunits (Koch et al. 1979). Thus hormonal imprinting can also take place at the unicellular level (Csaba 1980, 1981). For example, the *Tetrahymena* possesses certain membrane structures which

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act as adequate receptors for hormones and can usually also mediate a specific response to these. The *Tetrahymena* is therefore an ideal model organism for investigations into the mechanism of hormonal imprinting (Csaba 1984, 1985).

Our hormonal imprinting studies had formerly been based on *Tetrahymena pyriformis* GL strain. In the present study we examined seven taxa of *Tetrahymena* for response to a vertebrate hormone (insulin), to clarify whetre hormonal imprinting was a potential of the GL strain in particular, or of all *Tetrahymena* species in general. We also studied the possible impact of sexual and asexual development on lin), to clarify whether hormonal imprinting was a potential of the progeny generations. Finally we pursued a possible interrelationship between cellular (membrane) lipid composition and intensity of hormonal imprinting, since indications of that have merged from earlier experiments (Kovács et al. 1984) with *Tetrahymena pyriformis* GL cells.

Materials and Methods

The taxa *Tetrahymena pyriformis* WH 14, GL (Zeuthen, Copenhagen), GL (Budapest), W, ST and Saphiro, as well as *Tetrahymena thermophila* and *Tetrahymena pigmentosa* (from Dr. Nanney) were grown in enriched proteose-pepton medium at 28°C. One-day cultures were used in the logarithmic phase of growth. Each mass culture was divided into two lots. One lot was treated with 10^{-6} M insulin (0.144 IU/ml Insulin Semilente, Novo, Copenhagen) for 1 h, and was subsequently returned to plain medium for 1 day, whereas the other lot was transferred to plain medium for one day without insulin treatment. After 24 h, the cell cultures were fixed in 4% neutral formaline, washed, and incubated for 1 h in presence of fluorescein-isothiocyanate (FITC) labelled insulin (BDH, England, FITC protein ratio: 0,14). Certain taxa were incubated for 1 h in the presence of FITC-labelled Concanavalin A (Con-A, Serva, FITC-protein ratio: 1.32). After incubation, the cells were washed in three changes of PBS, spread on slides, and assayed for intensity of fluorescence with a Canon Canola SX 320 apparatus, coupled with an Olympus MMSP fluorescence microscope. Thirty cells were assayed in each lot, and 3 replica assays were performed to calculate mean values, which thus covered 90 cells. The inter-group differences were analyzed for significance with Student's t-test.

The growth rates of the strains were determined in subcultures obtained single-cell cloneing in capillary tubes.

Results and Discussion

The FITC-labelled insulin was also bound by the cultures not preexposed to insulin, (Fig. 1). The binding capacity varied between taxa, being lowest in *T. thermophila* and highest in *T. pigmentosa* cultures.

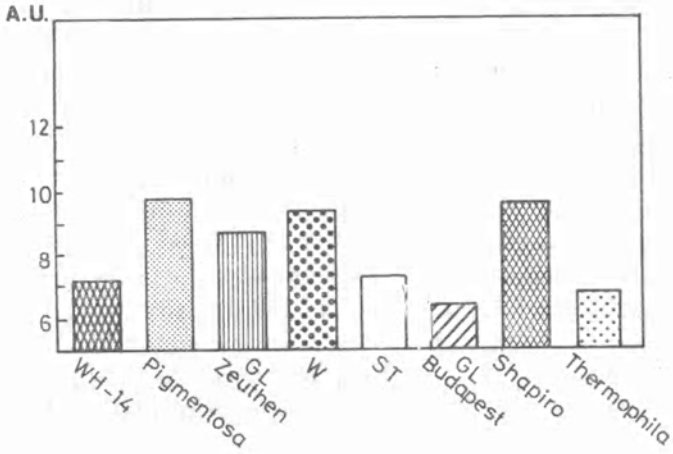

Fig. 1. Binding of FITC-labelled insulin to the different taxa of *Tetrahymena*

Although the difference between the two extremes approximated 50%, no parameter has been available for the quantitative evaluation of inter-strain variations in binding. No information emerged either on variations in the specificity of binding. These aspects were therefore disregarded, since the purpose of the study was *ab ovo* to assess the impact of the first interaction with the hormone (imprinting) from quantitative differences between binding relations on the first and second insulin exposure.

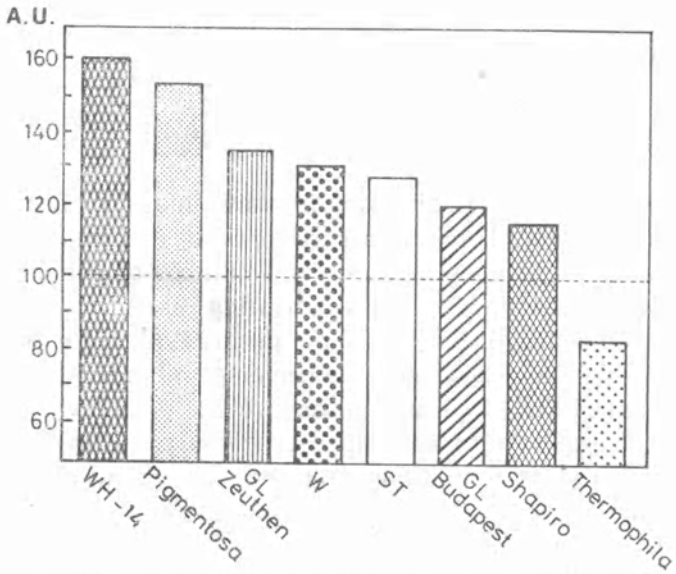

Fig. 2. Binding of FITC-labelled insulin to *Tetrahymena* pretreated (imprinted) with insulin. Values related to the not pretreated control as 100

Hormonal imprinting did in fact take place in all seven *Tetrahymena* species (Fig. 2), and accounted for an increase in the binding capacity of all strains except *T. thermophila,* in which it depressed affinity to insulin. It follows that hormonal imprinting is a general potential of the *Tetrahymena,* but its intensity varies between the taxa. The intensity of imprinting was unrelated to the quantitative relations of binding in the first interaction. This suggests that not so much the quantity of bound hormone, as the quality of the first interaction, which depends on the endogenous properties of the membrane, represents the decisive factor of hormonal imprinting.

The efficiency of imprinting (increase in binding capacity), as inferred from the difference between responses to the first and second exposure, was highest in the case of *T. pyriformis* WH14, and lowest in the case of the strain Saphiro. Interestingly, the former strain showed the lowest, whereas the latter the highest growth rate among all strains studied (except the negatively imprinted *T. thermophila*) (Table 1). There are two alternative explanations for the inverse relationship between the intensities of hormonal imprinting and growth rate: (1) The

Table 1

Growth rate of five (positively imprinted) *Tetrahymena* taxa related to the most slowly dividing WH-14 as 1

Taxon	Growth rate
WH-14	1
T. pigmentosa	2.5
GL (Copenhagen)	2.6
GL (Budapest)	2.7
Shapiro	5.6

greater the number of cell divisions in unit time, the more distant cell generations are involved in reexposure, and the "memory" of hormonal imprinting tends to decline after a certain number of generation changes, and (2) if the transmission of imprinting is membrane-associated, the "memory" of imprinting will be diminished with the increasing number of divisions. However, the inverse relationship between the intensity of imprinting and the number of cell divisions may as well as not be a chance coincidence, since other correlations have also been found. The membrane lipid composition of the strain WH 14, in which imprinting was most efficient, differs from that of the earlier studied *Tetrahymena* strains (Gl and W), inasmuch as the membrane of WH 14 contains a considerably greater amount of cardiolipin, linoleic acids

(n-18 : 2 $\Delta^{9,12}$; n-18 : 3 $\Delta^{6,9,12}$) and unsaturated fatty acids (Fukushima et al. 1978, Holz and Conner 1973). The membrane lipid composition has a considerable influence on membrane fluidity (Nozawa 1980) and membrane receptor formation (Dave and Witorsch 1983), and thereby on the imprinting mechanism as well (Kovács et al. 1984). It appears that differences in membrane lipid composition could account for quantitative differences in imprinting, and probably also in the mitotic rate.

The intensity of imprinting was unrelated to the sexual or asexual nature of the developmental cycle, i.e., to presence or absence of a micronucleus. Transmission of imprinting to the progeny generations did equally take place in sexually and asexually developing *Tetrahymena* strains.

Mention should be made of the experimental fact that the intensity of imprinting also differed between the Copenhagen and Budapest cultures of *Tetrahymena pyriformis* GL. It appears that the quantitative relations of imprinting may vary between different cultures of the same strain, depending on their origin, conditions of maintenance, and/or on the conditions of imprinting itself.

In addition the binding of Con-A to *Tetrahymena* was studied, as this lectin can occupy the binding sites of insulin (inhibiting the binding of this latter) without influencing the cells functionally (Csaba et al. 1983). In the present experiments Con-A bound to *Tetrahymena* in each taxa studied, and the sequence of binding capacity was very similar to those of insulin (Fig. 3). This support the earlier observations on the

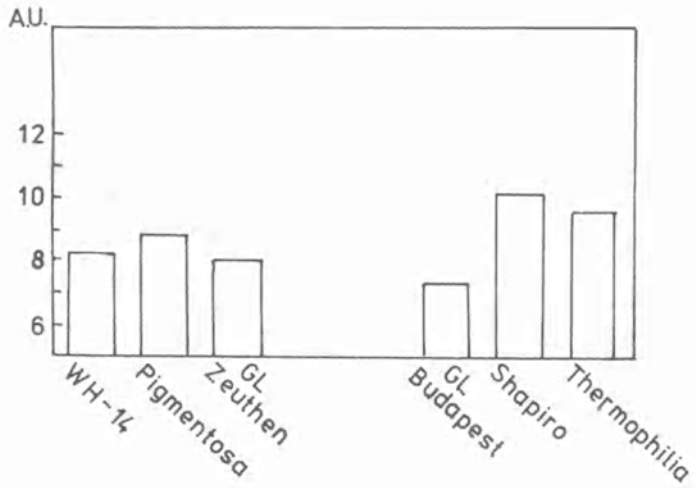

Fig. 3. Binding of FITC-labeled Con-A to the different taxa of *Tetrahymena*

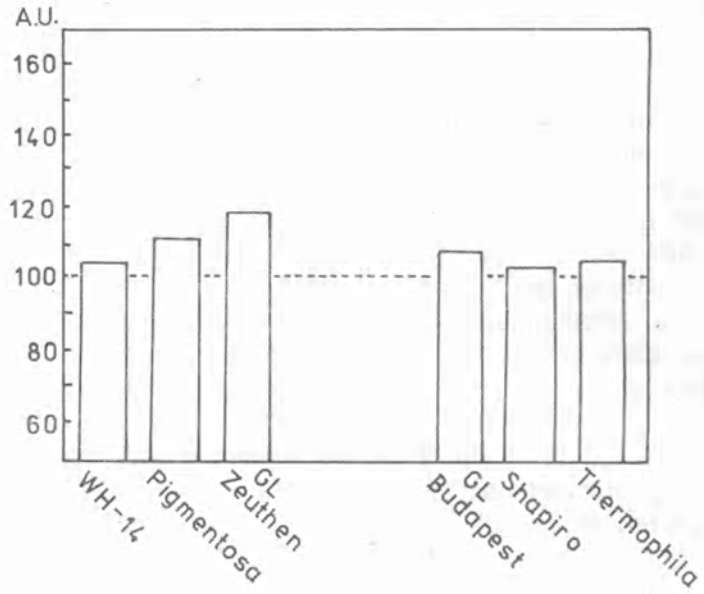

Fig. 4. Binding of FITC-labelled Con-A to *Tetrahymena* pretreated (imprinted) with insulin. Values related to the not pretreated control as 100

common binding site (Csaba and Kovács 1982, Sandra et al. 1979). Nevertheless, the imprinting for Con-A was much lower, than for insulin (Fig. 4), which shows the specificity of imprinting and demonstrates, that binding of Con-A is only a "side-product" of this latter. There was not similarity in the sequence of lectin binding capacity compared to insulin binding in the different taxa, demonstrating the development of sensitivity for insulin and Con-A after insulin imprinting.

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