Requirement for protein synthesis for the transfer of meiotic induction in a multicellular complex of *Blepharisma*[1]

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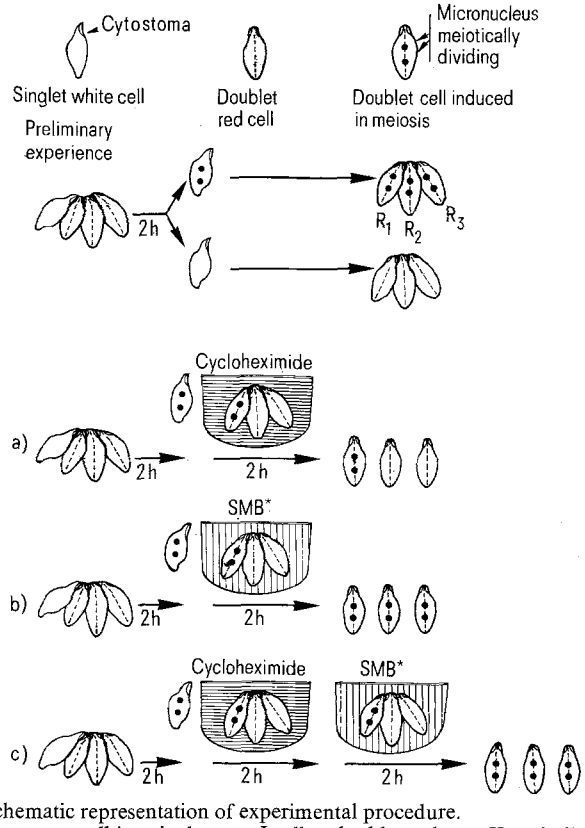
Summary. In the ciliate *Blepharisma japonicum* it is possible to induce meiosis in multicellular homotypic chains. In this work we demonstrate that protein synthesis is required to transfer meiotic activation from one cell to another in a chain.

The population of the ciliate *Blepharisma japonicum* consists of 2 complementary mating types each of which excretes a soluble substance (gamone) that serves as a messenger for cell pairing. The cell-gamone interaction brings about the formation of 2 types of pairs; homotypic pairs between cells of the same sexual type, and heterotypic pairs between cells of complementary types. Meiosis and nuclear exchanges occur solely in heterotypic pairs[2]. *Blepharisma* may be induced to form symmetrical doublet cells capable of persistence through cell division. By treating doublets of one mating-type with the complementary gamone, linear arrays (chains) of doublets may develop. Meiosis however does not occur in these homotypic chains unless a single cell of the complementary type attaches to one end of the chain. This heterotypic union generates the meiotic message that sequentially propagates from the 1st up to the 5th doublet of the chain[3]. The message is produced at the heterotypic union site within 1-2 h after pair formation, unless its production is reversibly inhibited by cycloheximide[4]. All these facts indicate that the meiotic induction message is a diffusible substance, strictly associated with protein synthesis occurring during the first 1-2 h of heterotypic union, and which can remain active for at least a few hours. Moreover, a cell activated to meiosis can induce another cell of the same type with which it conjugates to undergo meiosis[5].

On this basis we asked ourselves whether the propagation of the meiotic activation factor must be sustained by protein synthesis at each cell component of the chain or whether it can diffuse passively from cell to cell even in the presence of a protein-synthesis inhibitor.

Materials and methods. To answer the question, doublets of the red mating-type II, clone DR-10, and singlet albinos of mating type I, clone A5-3, were used. The cultivation and the experimental techniques used to obtain homotypic chains were those described by Miyake et al.[3]. Only heterotypic chains consisting of 3 doublets (R1, R2, R3, numbered from the closest doublet to the albino cell) and an attached albino, were used in the experiments described below. The experiments, performed at 24 ± 1 °C, are schematically reported in the figure. In all experiments the albino cell was carefully removed by a thin glass needle, after 2 h of union, from the heterotypic chains. If the chains were damaged or a piece of albino cell remained attached to an R_1 doublet during surgical separation, they were discarded. In the upper part of the figure, the preliminary experiment is illustrated. The removal of the albino cell after 2 h of heterotypic union left the chains either capable or incapable of undergoing meiosis, i.e., the results for 16 chains were of the all-or-nothing type. Meiotic activation was ascertained through the differential pigmentation occurring in activated cells 14-18 h after heterotypic union[3]. Three different kinds of experiments then followed. In experiment type a (fig.) many chains, after cutting away the albino cell, were separately incubated in SMB (salt medium for *Blepharisma*) to which 10 µg/ml of cycloheximide was added. The incubation period in the protein inhibitor[6] was 2 h. Afterwards each chain was washed with and suspended in SMB, where the 3 doublets of a chain were surgically separated and singly isolated in a 3-depression slide so that



Schematic representation of experimental procedure.
1st row: an albino singlet type I cell, a doublet red type II, a similar doublet after meiotic activation.
2nd row: after 2 h of heterotypic union the albino singlet was cut away and all the cells in the chain were either activated or not activated.
Row a: after the operation, chains were kept for 2 h in cycloheximide and then immediately each component was cut apart, singly isolated and inspected for meiotic activation. No R_3 doublet was activated. The number of activated doublets is reported in table 1.
Row b: control chains, operated as above, kept in SMB* (physiological medium) for 2 h before cutting. Meiotic activation progressed up to the 3rd cell.
Row c: chains were treated as in A and maintained for 2 h in SMB before cutting. The activation proceeded through the chain as in b.

Number of activated/operated doublets of 30 chains. R_1, R_2, R_3 indicate the position of doublets in the chain with R_1 the closest to the albino cell. a, b, c are related to the operational scheme presented in the figure

	a	b	c	X^2	
R_1	30/30	30/30	30/30		
R_2	9/30	24/30	20/30	a vs c	6.67, $p < 0.01\%$
				a vs b	13.2, $p < 0.01\%$
				b vs c	0.77, not significant
R_3	0/30	7/30	4/30	b vs c	0.44, not significant

the R_2 doublet occupied the middle well. The separated doublets were examined under the dissecting microscope in the following 24 h for meiotic activation. If only 1 doublet became activated, it was considered the R_1 cell of the chain. When 2 doublets resulted in being activated the second one was always the R_2 cell of the chain. In experiment type b (fig.), after the removal of the albino cell, the chains were kept for 2 more hours in SMB without cycloheximide and then cut apart into their single components. The same procedure of isolation and inspection described above was followed. These chains served as a control. In experiment type c (fig.) the chains were treated as in experiment a, and left united in SMB for additional 2 h over the above incubation period in cycloheximide. Finally they were surgically separated, singly isolated as in experiment a, and inspected for meiotic induction. In all 3 types of experiments, chains with no activated doublet were discarded and not included in the results of the table. Thus, as the 1st doublets (R_1) were always activated attention was focused on the R_2 and R_3 doublets.

Results. As reported in the table, out of 30 chains considered, 9 R_2s were activated in experiment type a vs 24 R_2s and 20 R_2s in b and c respectively. Using the χ^2-test, a highly significant difference ($p < 0.01$) was found for R_2 cells between the a vs b and a vs c experiments. No significant difference was observed for R_2 and R_3 cells in b vs c.

Conclusions. 1. In a chain system of 3 doublets, when the R_1 cell is induced to undergo meiosis after removing the complementary singlet, induction always propagates into the other 2 doublets provided they remain united. 2. In similar chain systems the propagation of meiotic induction is reduced by cutting apart the doublets 2 h after the interruption of heterotypic union (experiment b). The number of activated doublets is lower than that observed by Miyake et al.[3] in analogous experiments. The discrepancy is most likely due to the elimination of the heterotypic effect from the chain after 2 h of union, which did not occur in Miyake's experiment. 3. The 2-h incubation period in cycloheximide soon after the cutting apart of the complementary cell (experiment a) significantly reduced the number of R_2 and R_3 cells induced to undergo meiosis when compared to the cells of the chains in experiment b. Protein synthesis, blocked by the inhibitor, does appear to be an indispensable process for the propagation of meiotic activation factor along the chain. This assumption is confirmed by the lack of a significant difference between the chains of experiments b and c, in experiment c, the resumed protein synthesis, after washing, permits an almost regular propagation (throughout the chain) although with a slight delay in the transference of meiotic activation factor. The reversible effect of the protein inhibitor cycloheximide, was similar to that observed in pairs of *Blepharisma* by Miyake et al.[4].

To conclude, these results strongly indicate the need for protein synthesis for the transfer of meiotic induction to other cells of chains in which one cell is already activated. As was previously found[4], the heterotypic cell union induces and maintains the synthesis of a protein required for meiotic activation. Our results do not exclude the possibility that the protein synthesized within the homotypic chain might be the same as that produced at the heterotypic cell union.

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Neutron activation analysis of saliva from the tick *Amblyomma variegatum*[1]

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Summary. The hygroscopic saliva produced by fasting *Amblyomma variegatum* adults during prolonged exposure to dry air was found to contain sodium, potassium and chlorine in molar proportions of 1.1–1.7–3.8 respectively, or proportions by dry weight of 8, 21 and 41%. These values are quite different from those reported previously for saliva from recently fed ixodid ticks. The *A. variegatum* saliva contained, by dry weight, less than 5% sulphur and less than 1.5% each of phosphorus, calcium, magnesium and iron.

Certain terrestrial arthropods have the remarkable ability to obtain much of their water requirement by absorbing water from air having a relative humidity well below saturation[2]. Evidently water is collected from vapor by a hygroscopic secretion in some mites. In ixodid ticks this secretion is produced by the salivary glands[3,4]; in certain mites among the Acaridida a secretion of the supracoxal glands appears to be involved[5].

Analyses of these fluids are important in regard to their hygroscopic properties, but analysis is impeded by the minute amounts of secretion available. The elements sodium, potassium, chlorine and sulphur were identified in the hygroscopic salivary secretion of ticks[3,6]. The solids precipitated in the supracoxal gland ducts of *Dermatophagoides farinae* and *Tyrophagus putrescentiae* were found to contain potassium and chlorine[5,7].

In ixodid ticks only the copiously secreted saliva of recently-fed individuals has been analyzed quantitatively; it was found to contain sodium, potassium and chlorine at approximately 180, 10 and 140 µM/l respectively[8]. The salivary secretion of recently-fed ticks (which excrete water, ions and certain other components of their blood meal) might be expected to differ in composition from the hygroscopic salivary secretion associated with vapor uptake by nonfeeding ticks.

Materials and methods. During exposure to dry air after several weeks of fasting, *Amblyomma variegatum* adults produced a saliva from which the solids accumulated on the mouthparts. This solid residue was collected on an aluminium pan, weighed, and then kept in polyethylene vials. A 770 µg sample of the saliva solids was examined by X-ray fluorescence spectroscopy and then, after a brief