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On the Relation Between the Induction of Pseudoselfing Pairing and RNA Contents in *Paramecium bursaria*

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(*With 2 Text-figures*)

Working on the mechanism of the conjugation of paramecia, Metz (1947) reported in *Paramecium aurelia* that the pseudoselfing pairing was induced by formalin killed animals of the opposite mating type of the same group. In *Paramecium caudatum* Hiwatashi (1949) reached a similar conclusion that the mating reaction of living animals to the formalin killed animals of the opposite mating type of the same groups was successful.

On the basis of the fact that the dilute aqueous solution of victoria blue 4R stains the superficial layer, ectoplasm, of fixed paramecia, Hayashi (1956) has considered that the reaction to victoria blue 4R suggests the presence of ribonucleic acid in the superficial layer. By using some diaminoacridine dyes, Nagatani and Morihara (1958) have shown that ribonucleic acid is richly accumulated in the ectoplasm, since the ectoplasm emits selectively orange fluorescence on a black ground by progressive staining with acridine yellow. Further, it has been shown by some investigators that the surface layer of paramecia seems to play an important role in conjugation (Boell and Woodruff 1942, Metz 1946, 1947).

Here, special attention is directed toward the relationship of the existence of RNA in the ectoplasm to the mechanism of conjugation in paramecia. In the present study some experimental observations were made in an attempt to induce the pseudoselfing pairing by formalin killed animals following the depolymerization of RNA ; use was made of *Paramecium bursaria* as material.

This work was done under the direction of Professor Sajiro Makino to whom the author's cordial thanks are due for his keen interest in the subject. The author is also grateful to Dr. Ryo Katashima, Hiroshima University, for kindness in supplying the material.

Material and Method

Paramecium stocks : For the study *Paramecium bursaria* was kindly supplied by Dr. R. Katashima, Zoological Institute, Hiroshima University. They are the following eight clones : T, W (type I), F, P (type II), A, 25 (type III) and G, Z (type IV).

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Preparations of formalin killed animals : The preparations of formalin killed animals were essentially the same as those described by Metz (1947) and Hiwatashi (1949).

Degradation of RNA : The degradation of RNA was made with RNase. The speci mens were killed with 5 % formalin and then immersed in 0.05 %, 0.1 % RNase solution warmed previously at 20°, 37°, 60°C, respectively ; they were kept in RNase solution for 20, 40, 60 minutes and 3, 8 ,12, 24 hours in groups.

Victoria blue staining : Victoria blue staining followed the method described by Hayashi (1956).

Toluidine blue staining : For confirmation of the RNA, the technique developed by Brachet was adopted.

Results

1. Induction of pseudoselfing pairing by formalin killed animals after the treatment with RNase

Many strongly reactive living animals of one mating type of *Paramecium bursaria* were killed with 5 % formalin and washed thoroughly with culture medium. The killed individuals were placed in the same depression slide with strongly reactive living animals of the opposite mating type. The next reaction was that the living animals showed a rapid rotation in association with the killed animals. This indicates the induction of pseudoselfing pairing.

From the results of the above experiment, it was inferred that ribonucleic acid in ectoplasm still remains without degradation even after the treatment with formalin. Then, formalin killed animals were exposed to RNase for the purpose of removing RNA from their surface layer. The experiment has resulted in showing that the induction of pseudoselfing pairing was entirely unsuccessful in spite of the use of the most reactive living specimens for the experiment.

Next, the staining reaction with victoria blue 4R was examined in two sets of experiments: as shown in Figure 1, the formalin killed animals showed a peculiar reaction to the stain as has been already reported by Hayashi (1956), whereas the RNase-treated animals did not react to the stain at all. It is evident

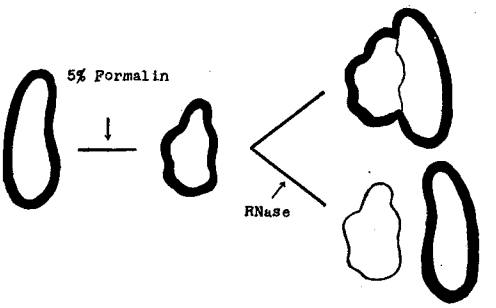

Fig. 1. Scheme showing that pseudoselfing pairs are induced in formalin killed animals, but not after the RNase treatment. Black surface layer indicates staining ability of victoria blue 4R.

from the results of the two sets of experiments that the animals which do not take victoria blue 4R never exhibit pseudoselfing pairing.

Experimental conditions with regard to concentrations of RNase and temperature are as shown in Table 1. After the RNase treatment, the agglutination of living animals with the treated individuals was disturbed with the passage of time and with the rise of temperature. The data presented in Table 1 indicate that according to the increase of concentration of RNase and to the rise of temperature, the organisms are inhibited from becoming agglutinated into clumps with each other. All specimens treated with RNase became non-reactive to the living individuals of the opposite mating type within at least 24 hours after the treatment. This seems to imply that the induction of pseudoselfing pairing never occurs in the individuals treated with RNase.

For the purpose of supplementing the results of victoria blue staining, an experiment with toluidine blue was carried out : the observations have revealed that the diminution of RNA in the ectoplasm occurred at the time when toluidine blue disappeared from the ectoplasm.

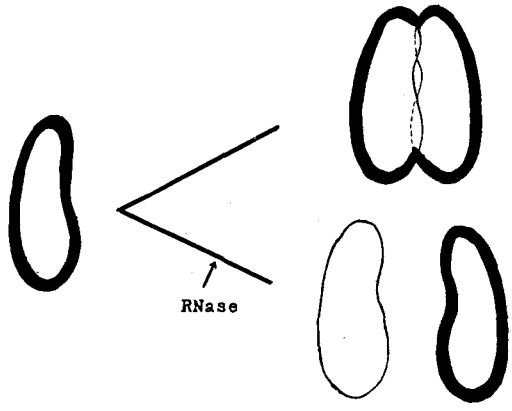

Fig. 2. Scheme showing that pseudoselfing pairs are never induced in living animals after the RNase treatment.

2. Induction of pseudoselfing pairing in living animals following the RNase treatment

In order to test the induction of pairing by living animals after the RNase treatment, experiments were carried out on the same schedule as above. The living animals of one mating type were exposed to RNase at several concentrations. When they were placed with living animals of the opposite mating type, there occurred no agglutination nor conjugation at all (Fig. 2). The results in detail are as follows; when RNase was applied at concentrations of 0.025% 0.03% and 0.05 %, the agglutination was inhibited at 18, 20 and 24 hours, respectively,

Table 1. Time-relation in inhibition of pseudoselfing pairing after the exposure to different temperatures and RNase concentrations

	Non-treatment (Control)			RNase-treatment						
				0.05%			0.1%			
	20	37	60	20	37	60	20	37	60	(°C)
After										
1	++	++	++	++	++	−*	++	++	−**	
4	++	++	−	++	+		+	−		
8	++	+		+	−		−			
12	++	−		+						
24 hours	++			−						

++ = heavy agglutination
\+ = one or more agglutination
− = non-agglutination
*= inhibited within 40 min.
**= inhibited within 20 min.

at room temperature (20–22°C). The experiment with victoria blue 4R indicated that the individuals treated with RNase showed a negative pattern as described in the preceding experiment.

In the light of the above findings, so far obtained, the following conclusion may be possible: that *Paramecium bursaria* do not show mating reaction when RNA has been degraded by means of the RNase treatment. On this basis it appears that RNA occurring in the ectoplasm may be indispensable for the conjugation of paramecia.

Discussion

The mechanism of the induction of pseudoselfing pairing has not been made clear up to the present. Metz (1947) expressed the view that the conjugation of paramecia might be induced by the interaction of some mating type substance on the cell surface of animals. Hiwatashi (1949) is of opinion that the conjugation of *Paramecium caudatum* may be induced by some a non-living substance which is produced by the mature individual; further that this substance is insoluble in culture medium, and reserved in the cell surface.

The present author has shown in a previous experiment with victoria blue 4R that RNA may exist in the surface layer of paramecia (Hayashi 1956). Nagatani and Morihara (1958) have also noted the existence of RNA in the surface of paramecia on the basis of their fluorescent microscope study.

As given in the foregoing pages the formalin treatment has shown that RNA occurring in the ectoplasm of paramecia is still preserved without degradation. Therefore, it was possible to induce pseudoselfing pairing in even formalin killed animals. But, such an induction capacity was entirely removed after the degradation of RNA by RNase treatment. In the light of the above facts, it is evident that the existence of RNA in the surface layer of paramecia may be

associated with the induction of pseudoselfing pairing.

Here, it should be considered that RNase may react to chemical components other than ribonucleic acid. It seems probable that the cytoplasmic complements to be dissolved by RNase are not RNA only. According to Metz and Butterfield (1951), mating reactivity of paramecia is probably associated with proteins. Hiwatashi (1955) observed that the conjugation of paramecia was inhibited after exposure to Tween 80, Tween 85 and Span 85. The latter chemicals are a series of nonionic surface-active agents having both hydrophilic and lipophilic properties. The inhibiting effect of these chemicals seems to suggest the degradation of RNA and therefore their function closely resembles that of RNase.

Although the results of the present study show that the RNA seems to be indispensable for conjugation of paramecia, there may be present several other causes connected with conjugation mechanism. But it is most probable that RNA may be one of the agents which induce mating of paramecia. The factor (or factors) remains entirely unknown which induces attraction and repulsion between different mating types.

Summary

The results of the present study have revealed that the induction of pseudoselfing pairing never occurs in formalin killed *Paramecium bursaria* when they were treated with RNase. This seems to be due to the degradation of RNA occurring in the surface layer of paramecia. The same results were obtained in living animals by a similar series of experiments.

It is concluded that RNA occurring in the surface layer of paramecia may play a significant role for the induction of agglutination and conjugation.

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