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TRANSLATION OF HOMOLOGOUS RNA INJECTED INTO THE CILIATE STYLONYCHIA MYTILUS

J.K.C. Knowles [1], H.J. Lipps [2] and A. Nock

Institut für Biologie III, Abt. Zellbiologie, Universität Tübingen, D - 74 Tübingen, W - Germany

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Abstract: Evidence is presented that RNA isolated from Stylonychia becomes translated when injected back into Stylonychia cells and that the RNA, synthesized about 1 hour after interaction of Stylonychia with Con A contains sequences specifically concerned with the first regeneration steps after Con A damage. The use of microinjection combined with a homologous system is discussed.

Translation of isolated messenger RNAs by in vitro translation systems such as the wheat germ (1) or the reticulocyte system (2) has proved to be a powerful technique providing evidence of the information content of these messenger RNAs. However, while this technique may permit the synthesis and subsequent identification of the proteins coded for by a particular message, it does not usually give any information with respect to the biological function of these proteins. It has been demonstrated that many messenger RNAs are translated very efficiently when injected into oocytes (3) and, more recently, that human and avian tissue culture cells are capable of translating duck globin messenger RNA (4) as well as viral envelope messenger RNA (5). In these experiments

1) Present address: Institute of Animal Genetics, Edinburgh, Scotland
2) To whom correspondence should be send

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the information content of the RNAs was already known. We have used the technique of injection of RNA into living cells in an attempt to identify the biological function of new classes of RNA synthesized by the ciliate Stylonychia mytilus after treatment with the plant lectin Concanavalin A (Con A).

It has been shown that about one hour after the binding of Con A to the pellicle of Stylonychia an increase in RNA synthesis occurs. Inhibition studies with actinomycin D suggested that these newly synthesized RNAs might be involved in the repair of Con A damage to the cell surface (6). To test this hypothesis we have prepared whole cell RNA from Con A treated cells (Con A RNA) and untreated control cells (control RNA) and injected these RNAs into Stylonychia treated with Con A and in which RNA synthesis had been strongly inhibited.

In this paper we present evidence that RNAs synthesized by Con A treated cells become translated when injected into other cells and that these RNAs provide specific information required for the first regeneration steps after Con A damage.

MATERIAL AND METHODS

RNA from whole cells was prepared essentially by the methods of Parish and Kirby (7) and Loening (8). Control RNA was isolated from logarithmically growing cells and Con A RNA from similar cells treated for 45 to 60 min. with 4 µg / ml Con A. The RNA was separated on 2.4 % polyacrylamide gels according to the method of Loening (8). As shown in Figure 1 the densitometer tracings of both RNA preparations are very similar.

RNA for injection was dissolved in neutral Pringsheim solution (9) at a concentration of 2.5 mg / ml. Cells for injection were treated with 75 µg / ml actinomycin D for several hours. Pulse label experiments showed that this treatment is sufficient to inhibit more than

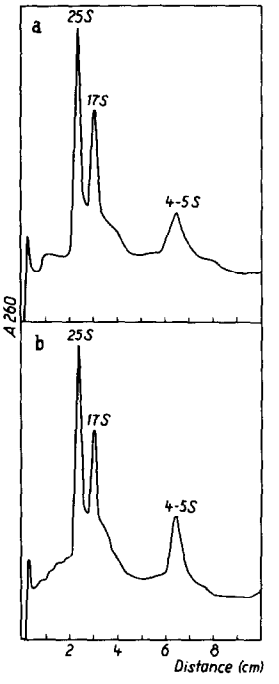

Figure 1: Densitometer tracings of control RNA (Fig. 1 a) and RNA from Con A treated cells (Con A RNA) (Fig. a b). RNA was prepared and separated on polyacrylamide gels as described in material and methods.

90 % of RNA synthesis. Stylonychia survives at this concentration without apparent ill effects for at least 48 hours. Just prior to injection cells were treated with 10 µg / ml Con A for 30 min. to make sure that all Con A receptors are saturated after that time. Each cell (cell volume about 100 μm^3) was then injected with 2.5 x 10^{-8} mg RNA of either Con A RNA (three different preparations) or control RNA (three different preparations) using the microinjection technique of Knowles (10). Surviving of cells after injection was observed in Pringsheim medium containing 25 µg / ml actinomycin D.

RESULTS AND DISCUSSION

Con A treated cells, in which RNA synthesis has been inhibited, die after some hours depending on the concentration of Con A and actinomycin D. If it is assumed that 1) the injected RNA is translated and 2) the RNA synthe-

sized 30 to 60 minutes after Con A treatment is concerned with recovery from Con A damage, treated cells injected with Con A RNA would be expected to survive longer than similar cells injected with control RNA.

The results of three individual sets of experiments are summarized in the figures 2 and 3. There is no significant difference between the time of survival of cells injected with control RNA and treated but uninjected cells (Fig. 2 b), or cells injected with buffer (Fig. 2 c). There is, however, a significant difference between the time of survival of cells injected with control RNA and those injected with Con A RNA (Fig. 2 a - c). The greatest difference (26 %) is observed 6 hours after injection, but 12 hours after injection there is still a difference of 11 % (Fig. 3). In addition, 4 to 5 % of cells injected with Con A RNA survived for more than 20 hours but all control cells, both injected and uninjected were dead after 15 hours. This difference was dependent on the amount of RNA injected; below 0.5×10^{-8} mg per cell the difference between the two different RNA preparations was no longer significant (Fig. 2 d). The maximum death rate of cells injected with control RNA occured at 6 hours after injection but that of cells injected with Con A RNA was about 2 hours later (Fig. 2 a - c, Fig. 3).

The following morphological correlation to this difference in survival rate was noted: Almost all control cells maintained their natural form for some hours after injection (Fig. 4 a) before they lysed whereas about half of the cells injected with Con A RNA became

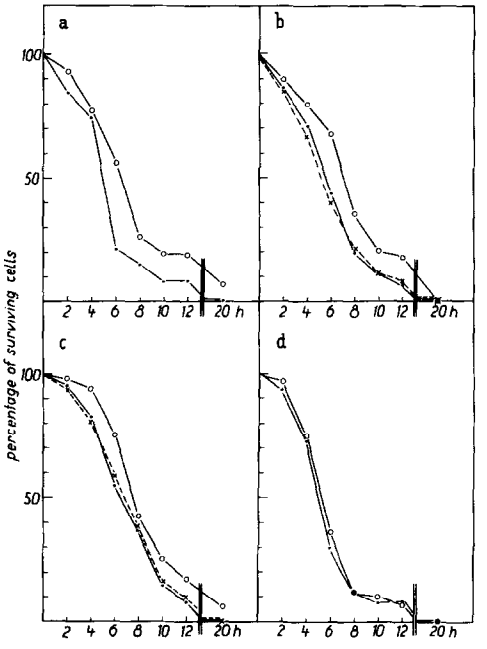

Figure 2: Survival rate of injected cells. Cells for injection were treated as described in material and methods. Fig. 2 a - c show the survival rate of cells injected either with three different Con A RNA preparations (o———o) or injected with three different control RNA preparations (•———•). To exclude the possibility that cells die because of injection treated but not injected cells were included (Fig. 2 b x-----x) as well as cells injected with buffer alone (Fig. 2 c, x-----x). Fig. 2 d shows the survival rate of cells injected with about 0.5×10^{-8} mg of either Con A RNA (the same preparation as in Fig. 2 c) or control RNA. The number of injected cells was: Fig. 2 a: 92 cells injected with Con A RNA and 96 cells injected with control RNA, Fig. 2 b: 80 cells injected with Con A RNA and 90 cells injected with control RNA, 48 cells were treated with Con A and actinomycin D but were not injected, Fig. 2 c: 104 cells injected with Con A RNA and 120 cells injected with control RNA, 60 cells were treated as described and injected with buffer alone, Fig. 2 d: 82 cells injected with Con A RNA, 78 cells injected with control RNA.

rounded and shed their cirrae (Fig. 4 b). The rounded cells survived much longer than those which remained normal. In the course of regeneration after Con A damage, cells able to synthesize RNA shed their Con A loaded pellicle and become round, so reducing external cell

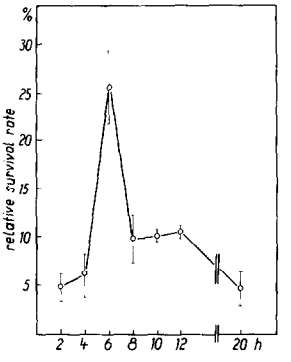

Figure 3 shows the increased survival rate of cells injected with Con A RNA relative to cells injected with control RNA. The standard deviation is calculated from the experiments summarized in Fig. 2 a - c.

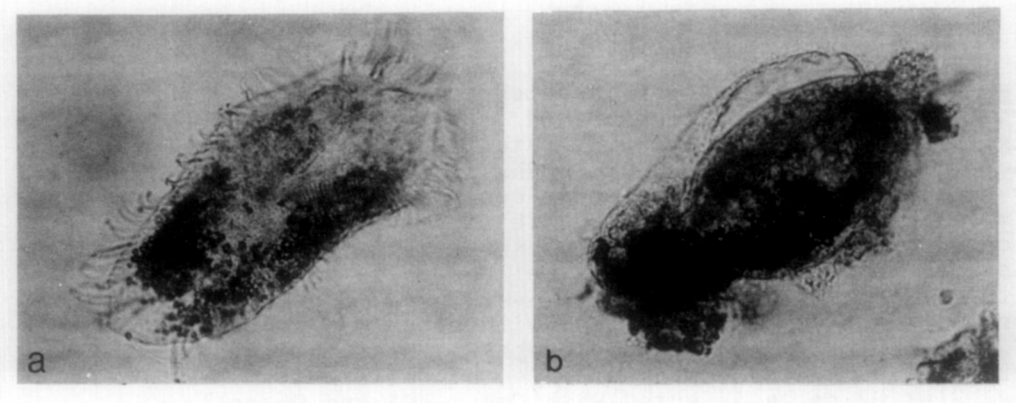

Figure 4: a) A micrograph of a cell 2 hours after injection with control RNA. This cell shows still a "normal" form (x 425), b) A micrograph of a cell 2 hours after injection with Con A RNA. The cell has shed most of its cirrae and become rounded as do cells treated with Con A but not with actinomycin D. A new membrane is clearly visible at this stage (x 425).

surface (11). In this respect cells injected with Con A RNA behave in an analogous manner to regenerating cells. Analysis of the RNA preparations showed only traces of DNA and no detectable amounts of protein. It is therefore likely that the agent responsible for the increa-

sed resistance to Con A damage is indeed the injected RNA. Although the densitometer pattern of Con A RNA and control RNA are very similar, our results can not just be explained by the addition of new r - RNA to Con A treated cells since the injected RNA concentration was identical in cells injected with Con A RNA and cells injected with control RNA.

Cells injected with Con A RNA are not expected to regenerate their pellicle entirely since resynthesis of a complete new pellicle takes more than 20 hours in non-inhibited cells (11). Furthermore, cells inhibited with actinomycin D one hour after Con A application survive this treatment but are also not able to regenerate a complete new pellicle (6).

Our results suggest that some RNA synthesized by the ciliate Stylonychia mytilus early after Con A treatment codes for proteins which are required in the early stages of regeneration of the cell from Con A damage. We feel that the technique of microinjection combined with a homologous system as described here can serve as a useful tool in the identification of the biological function of messenger RNAs in particular when, as in this case, the individual RNA species have not yet been isolated.

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REFERENCES

1) Marcus, A. (1974) Methods Enzym. 30, 749 - 761
2) Pelham, H.R.G. and Jackson, R.J. (1976) Eur. J. Biochem. 67, 247 - 256.
3) Gurdon, J.B., Lane, C.D., Woodland, H.R. and Marbaix, G. (1971) Nature 233, 177 - 182.

4) Stacey, D.W. and Allfrey, V.G. (1976) Cell 9, 725 - 732.

5) Stacey, D.W., Allfrey, V.G. and Hanafusa, H. (1977) Proc. Nat. Acad. Sci. (Wash.) 74, 1614 - 1618.

6) Lipps, H.J. and Bessler, W.G. (1977) Cell Diff. 6, 187 - 197.

7) Parish, J.H. and Kirby, K.S. (1966) Biochim. Biophys. Acta 129, 554 - 562.

8) Loening, U.E. (1967) Biochem. J. 102, 251 - 257.

9) Ammermann, D., Steinbrück, G., v. Berger, L. and Hennig, W. (1974) Chromosoma (Berl.) 45, 401 - 429.

10) Knowles, J.K.C. (1974) Exp. Cell Res. 88, 79 - 87.

11) Frisch, A., Bessler, W., Lipps, H.J. and Ammermann, D. (1976) J. Protozool. 23, 427 - 430.