SYNTHESIS OF *DICTYOSTELIUM DISCOIDEUM* SECRETORY PROTEINS IN *XENOPUS LAEVIS* OOCYTES

E. DICOU, P. BRACHET, G. HUEZ* and G. MARBAIX*

Unité de Différenciation cellulaire, Institut Pasteur, 25 rue du Dr Roux, 75024 Paris Cedex 15, France and **Laboratoire de Chimie Biologique, Université de Bruxelles, 67 rue des Chevaux, 1640 Rhode StGenèse, Belgium*

Received 6 June 1979
Revised version received 21 June 1979

1. Introduction

The cellular slime mould *Dictyostelium discoideum* presents a model system for the study of the developmental regulation of gene expression. Development is initiated by nutrient deprivation. After a few hours of starvation, cells develop new properties which allow them to emit and respond to pulses of cyclic AMP, the chemotactic agent, and to move toward aggregation centers [1]. During this stage of their development toward aggregation competence, several proteins are secreted in the extracellular medium. Among them a cyclic AMP phosphodiesterase and its specific inhibitor play a regulatory role in the aggregation process [2,3].

In the present work, we have used an antiserum against *Dictyostelium* secretory proteins to monitor the expression of the genes coding for proteins released into the extracellular medium until the amoebae reach the aggregation phase. For this purpose, poly(A)-containing RNAs from growth-phase cells and from aggregation competent cells were microinjected into *Xenopus* oocytes and the translation products were analysed for proteins cross-reacting with this antiserum.

2. Experimental

2.1. *Cells*

The axenic strain AX2 was grown in HL5-glc medium [4] to a cell density of $2–3 \times 10^6$ cells/ml. Cells were pelleted, washed in 17 mM potassium phosphate buffer, pH 6.2 and an aliquot of 7×10^8 cells was taken for RNA extraction. The remaining cells were gently shaken in the same buffer at a density of 10^7 cells/ml, and aliquots of about 7×10^8 cells were harvested 3 h and 8 h after initiation of starvation. For the test of aggregation competence, cells were placed on a Falcon tissue culture dish at a cell density of $1–2 \times 10^5$ amoebae per cm^2.

2.2. *RNA extraction*

The cell pellets were taken up in 5 ml of a solution of 20 mM NaCl–20 mM Tris–HCl, pH 7.5, 0.5% sodium dodecyl sulfate. The RNAs were extracted twice with a 1 : 1 mixture of phenol : chloroform, and the resulting aqueous layers were precipitated with ethanol, as described previously [5]. Polyadenylated RNA was isolated by affinity chromatography on oligo dT-cellulose [6], precipitated with ethanol and resuspended in 100 μl of water. The poly(A)-containing RNA preparation from growing cells and from cells starved for 3 h and 8 h were, respectively, 8.4, 7.2 and 6.0 mg/ml, as determined by OD_{260}.

2.3. *Translation assay of mRNAs*

Poly(A)-RNAs were tested in a cell-free system from wheat germ, for their capacity to direct in vitro translation. 2 μg of each RNA was used in a 100 μl reaction mixture in the presence of [^{3}H]leucine, as previously described [7].

2.4. *Antiserum*

The serum [8] was prepared against the extracellular proteins released by amoebae starved in shaken suspension as described above. For the characterization of the serum the extracellular proteins were radioactively labeled as follows. *Aerobacter aerogenes* bacteria were grown in 20 ml of a synthetic medium, depleted of sulfur and containing glucose, all amino-acids except cysteine and methionine, and 0.5 mCi of [^{35}S]sulfate (2.8 mCi/ml, Amersham, England). At the end of the growth phase bacteria were harvested and resuspended in 5 ml of 17 mM phosphate buffer, pH 6.2 together with 4×10^6 amoebae per ml. Amoebae were allowed to feed on the labeled bacteria under gentle agitation. The supernatant was collected about 8 h after exhaustion of bacteria. The 100 μl of supernatant, in 0.1 M NaCl, 0.02 M Tris, pH 7.5 and 0.5% Triton was treated with 2 μl of serum and 20 μl of settled protein A-Sepharose CL4B (Pharmacia). A control aliquot of 100 μl of supernatant was treated with 2 μl of normal rabbit serum. After overnight incubation at 4°C with constant agitation, the Sepharose beads were washed and the immune complexes eluted [9] and electrophoresed as previously described [10]. Figure 1 shows a fluorogram [11] of the *Dictyostelium* proteins treated with the normal serum (lane 1), and the serum prepared against *Dictyostelium* extracellular proteins (lane 2).

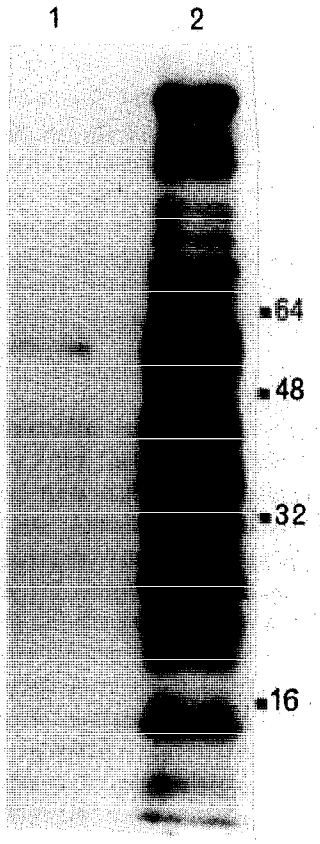

Fig.1. Fluorogram of the ^{35}S-labeled extracellular, *Dictyostelium* proteins immunoprecipitated by a normal rabbit serum (lane 1) and a serum prepared against *Dictyostelium* secretory proteins. Electrophoresis was performed in a 15% polyacrylamide gel. Radioactive labeling of the extracellular proteins and analysis of the immune complexes were performed as described in section 2. Apparent molecular weights $\times 10^{-3}$ are given.

2.5. *Translation in* Xenopus *oocytes and immunoprecipitations*

Oocytes from *Xenopus laevis* were each injected with about 50 nl of a solution of poly(A)-containing RNA at a concentration of 6 mg/ml. Batches of 10 oocytes were incubated at 19°C for about 20 h in 100 μl of Barth medium containing 1 mCi/ml [^{3}H]-leucine [12]. The oocytes were washed and homogenised in 80–100 μl buffer A per oocyte (buffer A: 20 mM Tris–HCl, pH 7.5, 50 mM NaCl, 0.5% Triton X-100, 0.5% sodium deoxycholate, 0.1% leucine). After centrifugation at 10 000 × *g* for 10 min, an amount of supernatant containing about 8×10^5 cpm was used for the indirect immunoprecipitation. 2 μl of antiserum were added and the incubation was carried out for 2 h at 37°C and then at 4°C overnight. Then 100 μl of a *Staphylococcus aureus* suspension [13] were added and the incubation continued at 4°C for about 4 h. The immunoprecipitates were washed three times by centrifugation through a cushion of 400 μl of 1 M sucrose in buffer A.

The precipitates were finally dissolved in electrophoresis sample buffer. Sodium dodecyl sulfate electrophoresis was carried out according to Laemmli [10] with a 5% stacking gel and a 15% acrylamide running gel. The gels were stained with Coomassie blue and prepared for fluorography as previously described [11].

3. Results and discussion

The poly(A)-containing RNAs from wild type, axenic cells in the growth phase and from cells starved in liquid suspensions for 3 h and 8 h were obtained from total RNA by oligo dT-cellulose chromatography. Cells starved during 3 h were in the so-called 'interphase' stage and were not competent for aggregation while cells starved for 8 h were competent for aggregation, as seen by their capacity to orient toward centers when placed onto a solid support. Prior to injection into the *Xenopus* oocytes the capacity of poly(A)-containing RNAs to direct in vitro translation was tested in a wheat germ cell-free system as described in section 2. About 50 nl of each of these RNA preparations were subsequently micro-injected into separate batches of oocytes. The translation products from ten injected oocytes, for each preparation, were analysed by indirect immunoprecipitation with a serum prepared against *Dictyostelium* proteins secreted during starvation. As seen in fig.2, little or no cross-reacting material was synthesized in the control, non-injected oocytes (lane 4), while at least six proteins of molecular weight ranging between 30 000 and 60 000 were synthesized in the oocytes injected with mRNAs from growing *Dictyostelium* cells (lane 1). These proteins were not immunoprecipitable when a normal rabbit serum was employed. A decrease in the relative rate of synthesis of these proteins was observed in the oocytes injected with mRNAs from cells in the interphase stage (lane 2, results confirmed in two independent experiments) while the rates of synthesis of most of them showed a certain increase in oocytes injected with mRNAs from aggregation-competent cells (lane 3). Apart from these variations in their relative rates of synthesis no major differences were found between the immunoprecipitated *Dictyostelium* proteins synthesized in oocytes injected with the various RNA preparations examined.

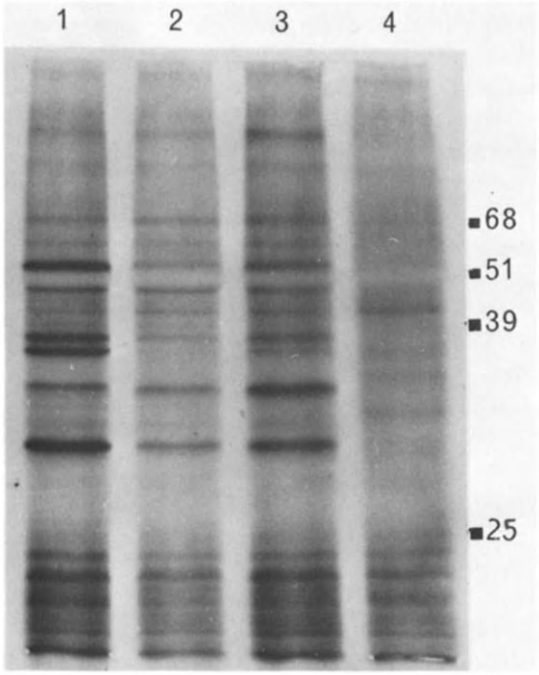

Fig.2. Fluorogram of the translation products of poly(A)-containing RNAs of *Dictyostelium discoideum* micro-injected in *Xenopus* oocytes and immunoprecipitated with a serum against *Dictyostelium* secretory proteins. Electrophoresis was performed in 15% polyacrylamide gels. Apparent molecular weights $\times 10^{-3}$ are given. *Lane 1*: oocytes injected with RNAs from growth phase AX2 cells. *Lane 2 and 3*: oocytes injected with RNAs from cells after 3 h and 8 h of development, respectively. *Lane 4*: control, non-injected oocytes.

The above results show that *Xenopus* oocytes programmed with *Dictyostelium* mRNA synthesize proteins specific to *Dictyostelium*. They further indicate that the mRNAs coding for the extracellular proteins detected with this serum are already present in growing cells, before the beginning of the developmental cycle. The similarity of the profiles of the immunoprecipitates suggests that no major changes in the species of mRNAs coding for extracellular protein that can be detected by this method occur until cells start to aggregate. Assuming that the different RNAs are translated with equal efficiency in the oocytes, the differences in the relative rates of synthesis may reflect differences in the availability of these mRNAs.

Acknowledgements

This work was supported by grants from the NATO, the French CNRS and the Belgian State (Action de Recherche concertée). E.D. is supported by an EMBO long term fellowship. G.H. and G.M. are fellows of the Belgian FNRS.

References

[1] Darmon, M. and Brachet, P. (1979) in: Taxis and Behaviour, series B, vol. 5, Chapman and Hall, London.
[2] Riedel, V., Malchow, D., Gerisch, G. and Nägele, B. (1972) Biochem. Biophys. Res. Commun. 46, 279–287.
[3] Klein, C. and Darmon, M. (1975) Biochem. Biophys. Res. Commun. 67, 440–447.
[4] Watts, D. J. and Ashworth, J. M. (1970) Biochem. J. 119, 171–174.
[5] Alton, T. H. and Lodish, H. F. (1977) Develop. Biol. 60, 180–206.
[6] Aviv, H. and Leder, P. (1972) Proc. Natl. Acad. Sci. USA 68, 1408–1412.
[7] Roberts, B. E. and Paterson, B. M. (1973) Proc. Natl. Acad. Sci. USA 70, 2330–2334.
[8] Nivet, C. (1977) FEBS Lett. 84, 174–178.
[9] Feunteun, J., Kress, M., Gardes, M. and Monier, R. (1978) Proc. Natl. Acad. Sci. USA 75, 4455–4459.
[10] Laemmli, U. K. (1970) Nature New Biol. 227, 680–685.
[11] Laskey, R. A. and Mills, A. D. (1975) Eur. J. Biochem. 56, 335–341.
[12] Gurdon, J. B. (1974) The control of Gene Expression in Animal Development, Clarendon Press, Oxford.
[13] Kessler, G. (1975) J. Immunol. 115, 1617–1624.