ANTISERA AGAINST ECDYSTEROID-INDUCED PROTEINS IN AN ESTABLISHED LINE AND A CLONE OF *DROSOPHILA MELANOGASTER* CELLS

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1. Introduction

The development of insects is under the control of the steroid hormones ecdysone and ecdysterone [1]. For in vitro studies on the mode of action of these hormones, established cell lines of *Drosophila melanogaster* [2] and in particular the homogeneous, stable and diploid cells obtained by cloning [3,4] supply an interesting experimental model. We have previously shown that ecdysterone, perhaps through saturable 'receptors' [5], acts by promoting cellular morphological modifications and by terminating cell division, while ecdysone does not provoke these effects [6,7]. After ecdysone or ecdysterone treatment of the cells of clone FC [4], the electrophoretic analysis of the protein pattern of crude extracts permitted the demonstration of a specific time and concentration-dependent appearance of a protein band (rel. migr. 0.29: '0.29 proteins') [8,9]. This band was cut off and injected into rabbits. In this paper, we report the preparation of antisera directed against the '0.29 proteins'. These antisera allowed us to demonstrate that the protein induction requires a treatment by ecdysone or ecdysterone, and is independent of the cell morphological modifications and termination of cell division promoted by ecdysterone, and of the presence of fetal calf serum in the culture medium.

2. Materials and methods

2.1. *Cells*

All the cells used were derived from one of the diploid lines of *Drosophila melanogaster,* line Kc [2]. Clone FC, diploid, cultured in D22 medium supplemented with 10% Fetal calf serum, was selected for its sensitivity to ecdysterone [4]. Subline Kc 0%, heteroploid, was adapted to grow without fetal calf serum [10].

2.2. *Hormone source*

Ecdysone and Ecdysterone were gifts of Simes, Milano.

2.3. *Antisera*

2.3.1. Preparation of antigens

'0.29 proteins' were isolated by electrophoresis of extracts of 0.1 μM ecdysterone-treated FC clone cells, on non denaturing and unstained polyacrylamide gels, as in [8]. Crude extracts containing 0.7–1.5 mg protein were deposited on each gel. After electrophoresis, a 4 mm gel slice was cut off at the 0.29 position. All the slices were ground together with 1 ml Freund's complete adjuvant. Ten slices were used for each injection.

2.3.2. Preparation of antisera

The above mixture was used to immunize two rabbits. Three injections were given at 3 week intervals; the rabbits were bled 4 weeks after the last injection.

2.3.3. Double immunodiffusion tests

The antisera were tested by immunodiffusion on agarose (1%). The two antisera were allowed to diffuse

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for 48 h against crude extracts [8], of FC or Kc 0% cells, untreated or treated for 4 days with 0.1 μM ecdysone or ecdysterone, and adjusted to the same protein concentration (4–11 mg protein/ml). The slides were then washed, dried and stained with amido black.

3. Results

Figure 1 shows the precipitin arcs obtained by immunodiffusion with the extracts of untreated (left) and ecdysterone treated FC clone cells (right), both extracts being adjusted to 4 mg protein/ml. Only the ecdysterone-treated cells produce two precipitin lines. The major line is clearly visible and a minor one can also be distinguished. This result may be related to the observation that '0.29 proteins' often appear on electrophoretic gels as a double band. On the other hand, a precipitin line is formed by both treated and untreated cells. This line is probably due to a common antigen; in fact, all the gels contain a band located at 0.31 point.

Figure 2 shows the coalescence between the major precipitin lines formed by 0.1 μM ecdysone (C) and by 0.1 μM ecdysterone-treated (D) FC cells. Ecdysone (0.1 μM), in contrast to ecdysterone, does not promote morphological modification and termination

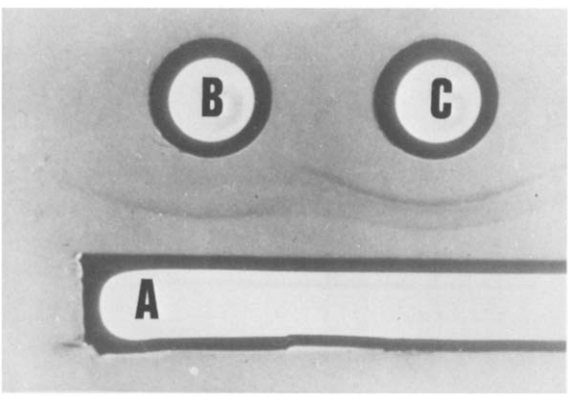

Fig.1. Immunodiffusion analysis of crude extracts of FC clone cells, untreated or ecdysterone-treated (0.1 μM, 4 days). The slide was prepared as in section 2 but was not stained. (A) 400 μl anti-'0.29 proteins' serum. (B) 100 μl extract of untreated cells (4 mg/ml). (C) 100 μl extract of treated cells (4 mg/ml).

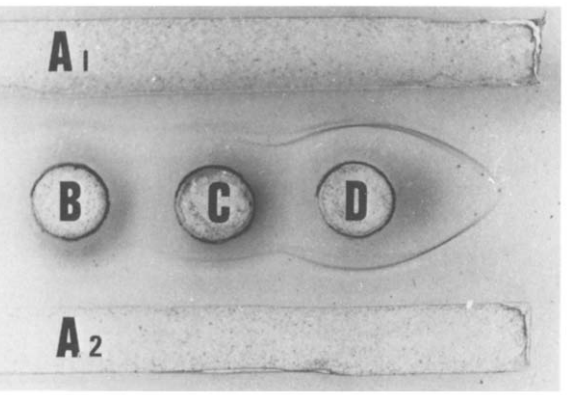

Fig.2. Immunodiffusion analysis of crude extracts of FC clone cells, untreated, ecdysone-treated (0.1 μM, 4 days) and ecdysterone-treated (0.1 μM, 4 days). The slide was prepared as in section 2.
(A_1) 600 μl of anti-'0.29 proteins' serum from rabbit 1.
(A_2) 600 μl of anti-'0.29 proteins' serum from rabbit 2.
(B) 100 μl of extracts of untreated cells (4 mg/ml).
(C) 100 μl of extract of ecdysone-treated cells (4 mg/ml).
(D) 100 μl of extract of ecdysterone-treated cells (4 mg/ml).

of cell division [6,7]. However, similarly to ecdysterone, it modifies the protein pattern of treated cells [9] causing the appearance of proteins. Such proteins seem to present common immunological properties with the ecdysterone induced proteins.

The antisera obtained against the ecdysterone-induced '0.29 proteins' of treated FC clone cells were also tested against cell extracts of another cell line: Kc %, untreated or treated by ecdysone or ecdysterone (0.1 μM). The results were the same as for the extracts of FC cell, i.e., the formation of precipitin arcs was seen only in the case of ecdysone- or ecdysterone-treated Kc % cells.

4. Discussion

We have seen that, in *Drosophila melanogaster* cells cultured *in vitro*, both ecdysone and ecdysterone are capable of inducing the formation of proteins which show the same immunological properties. Such protein induction is independent of:

1. The presence of fetal calf serum in the culture medium since Kc % cells respond as well as FC cells.
2. The morphological modifications.

3. The termination of cell division promoted by ecdysterone.

However, not all the established cell lines of *Drosophila* respond to ecdysterone as FC clone or Kc % subline do, according to the immunological criteria. Therefore, we have to consider the existence of inducible and non-inducible cell lines [11]. Although we do not know if these induced proteins are synthesized de novo, and what their physiological function is. The availability of the antisera will help to clarify these remaining questions and also facilitate analysis of the hormonal response either in vitro or in vivo.

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