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Local Change of an Exogastrula-Inducing Peptide (EGIP) in the Pluteus Larva of the Sea Urchin *Anthocidaris crassispina*

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ABSTRACT—Immunofluorescence staining of cryosections of pluteus larvae of the sea urchin *Anthocidaris crassispina* was performed with an antiserum raised in rabbit against exogastrula-inducing peptide D (EGIP-D). The apical side of the ectoderm and gut of pluteus larvae that had been cultured for 36 hr was stained. However, the gut of pluteus larvae that had been cultured for 38 hr was only partially stained and the gut of larvae that had been culltured for 40 hr was not stained at all. By contrast, the ectoderm of the 38-hr and 40-hr pluteus larvae remained stainable. These results suggest that EGIP-D is present in the gut of pluteus larvae 36 hr after fertilization, but it begins to disappear from the gut at 38 hr and is lost at all from the gut at 40 hr. The significance of the disappearance of EGIP-D from the gut of the pluteus larva is discussed in relation to the differentiation of the gut.

INTRODUCTION

Exogastrula-inducing peptides (EGIPs) are intrinsic factors that are present in mesenchyme blastulae of the sea urchin *Anthocidaris crassispina*. When added exogenously to embryos, they induce the extrusion of the archenteron toward the outside of the embryo, which leads to exogastrulation [2, 6].

In previous studies [6–8], four EGIPs, designated EGIPs A-D, were purified from mesenchyme blastulae of the sea urchin, and the amino acid sequences of all four peptides and the positions of the disulfide bonds in EGIP-D were determined [5]. The results suggest that EGIPs are homologous to epidermal growth factor (EGF), as indicated by the similarities between EGIPs and EGF in terms of the amino acid sequences and the positions of the disulfide bonds [1].

Recently, we reported the presence and quantitative changes in levels of maternal EGIPs during early development [3] and suggested that EGIP-D is present in acidic vesicles [4]. However, during early development, every type of blastomere in embryos of the sea urchin *A. crassispina* contains EGIP-vesicles, which are identical to acidic vesicles [4]. The biological role of EGIPs during the normal development of sea urchin embryos is not clear.

In the present report, we describe the disappearance of EGIP-D from the gut of the pluteus larva of the sea urchin and we discuss the significance of the local change of EGIP-D in relation to the differentiation of this organ.

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MATERIALS AND METHODS

The eggs of *Anthocidaris crassispina* were obtained by introducing 0.5 M KCl into the coelom and they were then cultured after fertilization at 24°C for 36–40 hr in normal seawater, at a concentration of 3×10^6 embryos per liter, with gentle agitation. The pluteus larvae of the sea urchin were fixed for 24 hr in seawater that contained 3.7% formaldehyde at room temperature.

After fixation, larvae were embedded in O.T.C. compound (Miles Inc., Elkhardt, IN, USA) and sectioned on a Cryocut microtome (Reichert-Jung, Nussloch, F.R.G.), ad described previously [4]. The cryosections were treated with an antiserm raised in rabbit against EGIP-D and fluorescein isothiocyanate-conjugated antibodies raised in goat against rabbit IgG (Cappel, Malvern, PA, USA). Specimens were observed under UV illumination, as described previously [4].

RESULTS AND DISCUSSION

Immunofluorescence staining of cryosections of pluteus larvae of the sea urchin *Anthocidaris crassispina*, using a polyclonal antiserum raised in rabbit against EGIP-D, was performed here as described in Materials and Methods. Figure 1 shows the results of immunofluorescence staining of cryosections of pluteus larvae that had been cultured at 24°C for 36 hr after fertilization. The apical side of the ectoderm and the gut of 36-hr pluteus larvae was strongly stained, as shown in Figures 1C and E.

The immunofluorescence staining of cryosections was also carried out using larvae that had been cultured for 38 hr after fertilization (Fig. 2). The gut of the 38-hr pluteus larvae was only barely stained, as shown in Figure 2C, or it was partially stained, as shown in Figure 2E. The apical side of the ectoderm of the 38-hr pluteus larvae remained strongly

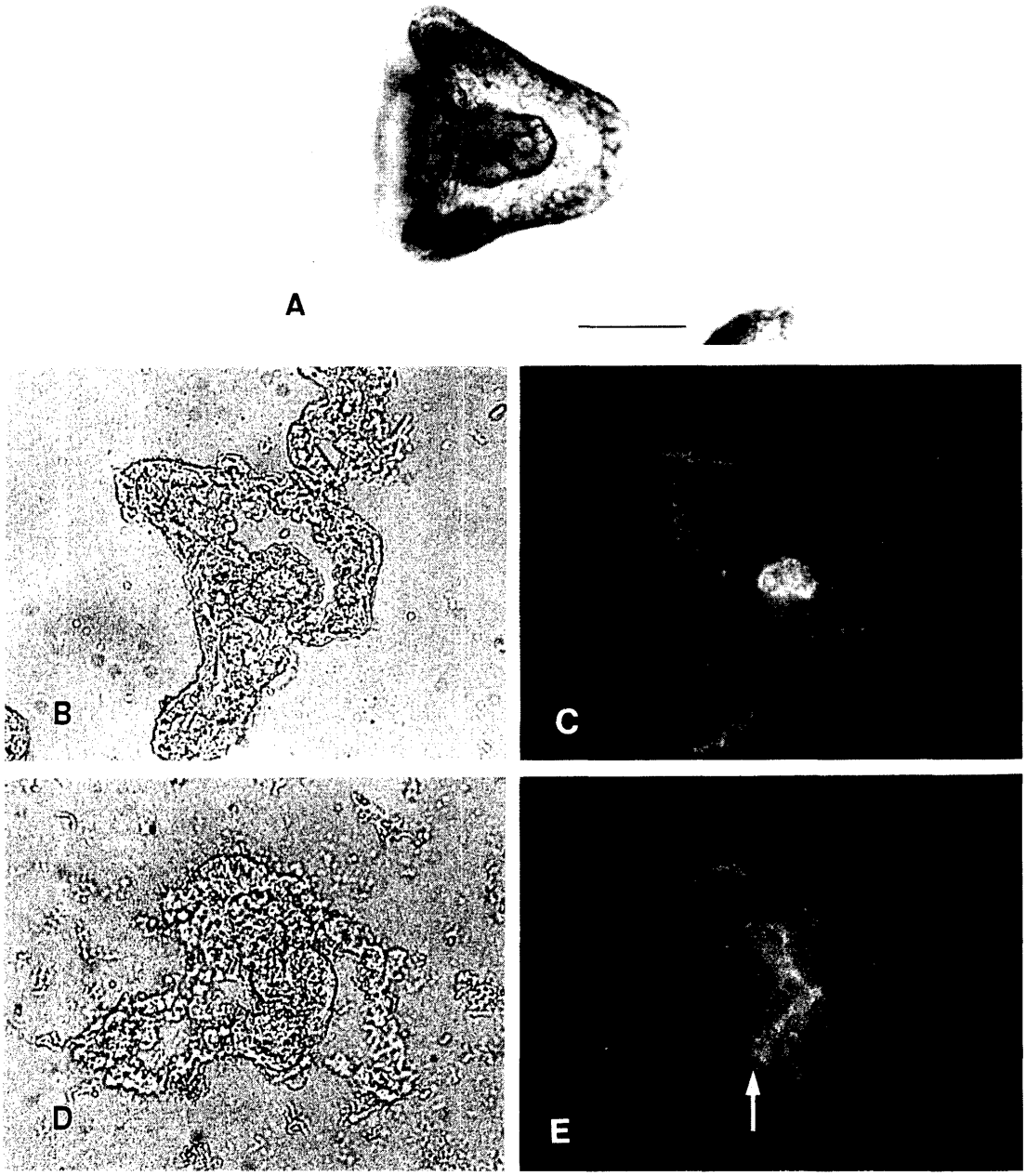

FIG. 1. Localization of EGIP-D in pluteus larvae that had been cultured at 24°C for 36 hr after fertilization. Cryosections of larvae were stained for indirect immunofluorescence with polyclonal antiserum against EGIP-D. (A) Light micrograph of an intact larva. (B, C) A matched pair of a light micrograph and an epifluorescence micrograph of a horizontal section of a larva. (D, E) A matched pair of a light micrograph and an epifluorescence micrograph of a longitudinal section of a larva. The arrow shows the position of the larval anus. Bar, 50 μm.

stainable.

Figure 3 shows immunofluorescence staining of cryosections of pluteus larvae that had been cultured for 40 hr after fertilization. The gut of the 40-hr pluteus larvae was not stained at all, while the apical side of the ectoderm of the 40-hr pluteus larvae was still stained, as shown in Figures 3C and E.

The results described above indicate that EGIP-D is present in the gut of the 36-hr pluteus larvae, begins to disappear from the gut of the 38-hr pluteus larvae and is lost at all from the gut of the 40-hr pluteus larvae. By contrast, EGIP-D remains in the ectoderm of the 40-hr pluteus larvae. The beginning of the desappearance of EGIP-D from the gut of the 38-hr pluteus larvae may be related to the differentiation of the gut of the larva, because the 38-hr pluteus larvae has a distinct oesophagus, stomach and intestine, as compared with the 36-hr pluteus larvae as shown in Figures 1 and 2. However, it remains to be determined whether the gut of

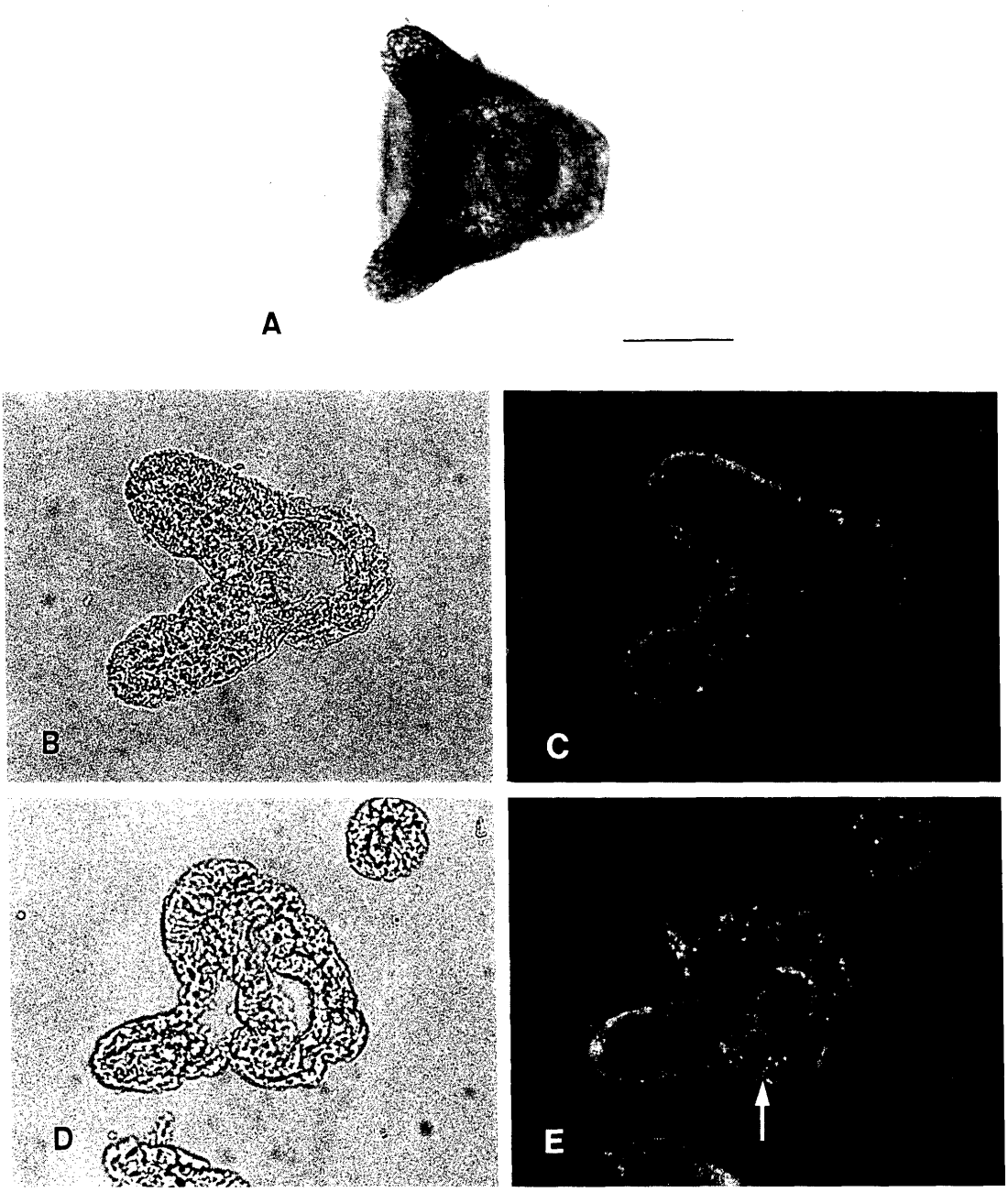

FIG. 2. Localization of EGIP-D in pluteus larvae that had been cultured at 24°C for 38 hr after fertilization. Cryosections of larvae were stained for indirect immunofluorescence in the same way as those in Figure 1. (A) Light micrograph of an intact larva. (B, C) A matched pair of a light micrograph and an epifluorescence micrograph of a horizontal section of a larva. (D, E) A matched pair of a light micrograph and an epifluorescence micrograph of a longitudinal section of a larva. The arrow shows the position of the larval anus. Bar, 50 μm.

the pluteus larva requires the degradation of EGIP-D for further differentiation or whether the differentiated gut of the pluteus larva no longer requires and secretes it.

In exogastrulated larvae of plutei under the influence of EGIPs, EGIP-D was also lost from the extruded gut after differentiation. These changes will be reported later in detail with electron microscopic studies.

Recently, we suggested that EGIP-D is contained in acidic vesicles [4]. Therefore, we are now examining whether acidic vesicles disappear during later development from the gut of the pluteus larvae simultaneously with the disappearance of EGIP-D from the gut.

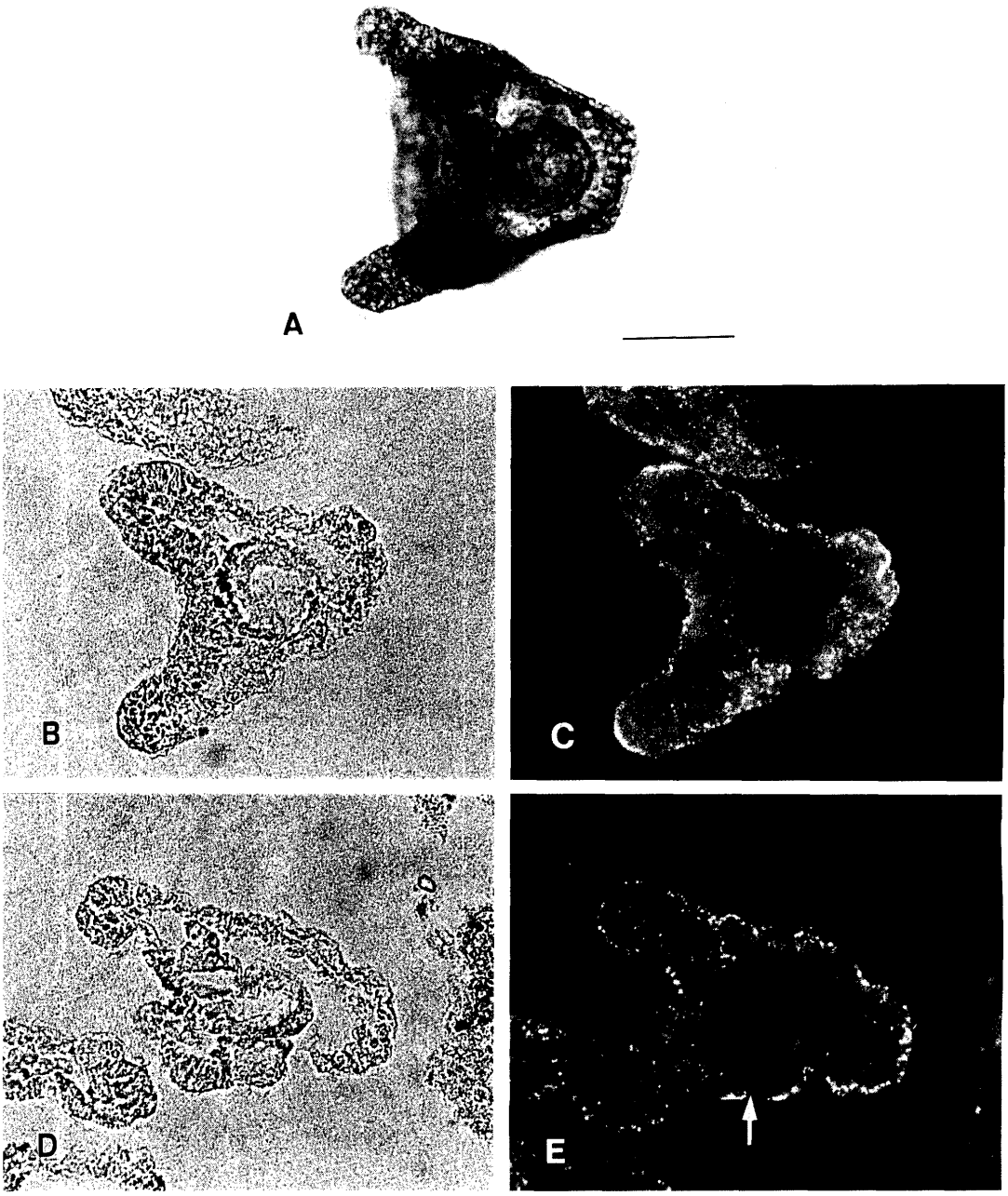

FIG. 3. Localization of EGIP-D in pluteus larvae that had been cultured at 24°C for 40 hr after fertilization. Cryosections of larvae were stained for indirect immunofluorescence in the same way as those in Figure 1. (A) light micrograph of an intact larva. (B, C) A matched pair of a light micrograph and an epifluorescence micrograph of a horizontal section of a larva. (D, E) A matched pair of a light micrograph and an epifluorescence micrograph of a longitudinal section of a larva. The arrow shows the position of the larval anus. Bar, 50 μm.

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