The Occurrence of an Extended Perinuclear Space in Androgenic Gland Cells of the Crayfish, *Procambarus clarki*

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After the physiological role of the androgenic gland of Crustacea was first established by Charniaux-Cotton (1960, 1962), several studies were carried out attempting to purify and identify the hormone or hormones secreted by this gland (Katakura et al. 1975) and to describe its morphology and the process of hormone secretion from the androgenic gland cells (Carpenter and DeRoos 1970, Hoffman 1969, King 1964, Payen et al. 1971). We have been carrying out some electron-microscopical observations of the androgenic gland cells of *Procambarus clarki*, and found a remarkable large space in the nuclear membrane of the androgenic gland cell, which is a subject to be dealt with this article.

Material and methods

Androgenic glands, attached to small pieces of the distal portion of the sperm duct, were dissected out from adult and juvenile male crayfish, *Procambarus clarki*. Thirty-seven adults used had a 50-60 mm carapace length and 6 juveniles used had a carapace length of less than 25 mm. The tissues were fixed immediately in one of the following three methods: double fixation with glutaraldehyde and osmium; 1% osmium alone; and 3% potassium permanganate, all of which were buffered (pH 7.4) and regulated osmotically by adding sucrose to 4.5%. The glands were then washed in buffer solution and distilled water, dehydrated through an acetone series and embedded in araldite resin. Ultrathin sections were prepared by using a Porter-Blum MT-1 or JUM-7 ultramicrotome, and stained with lead citrate or uranyl acetate/lead citrate. The cells were studied by JEM 100C electron microscope at 80 KV.

Observations

The gross anatomy, arrangement of the cells, and the cellular composition of the androgenic glands of *Procambarus clarki* are similar to what has been reported for other crustacean species, and will be presented elsewhere. In the following, we deal with only the unusual structure of the nuclear envelope of these cells.

The typical nuclear envelope of plant and animal cells consists of a double unit membrane and is continuous with the granular endoplasmic reticulum and Golgi
Figs. 1-3. All the figures (Figs. 1-6) are electron micrographs of the nuclear envelope indicating the perinuclear spaces and the inclusion bodies. Inner membrane (IM), outer membrane (OM), nucleous (NO), chromatin (CH), cell membrane (CM), mitochondria (M). Scales indicate 1 micron. 1, a slightly extended perinuclear space. 10,500 x. 2, a perinuclear space deeply extended into the cytoplasm. 11,100 x. 3, an extremely enlarged perinuclear space. 6,900 x.
membrane and is, furthermore, perforated by many small pores. In the cells of the androgenic gland of this species, however, the structure of the nuclear membrane seems to be quite different. As seen in the micrographs presented here, the space between the inner and outer layers of the nuclear envelope is, very often, widely extended, forming a roomy perinuclear space. The size or dimension of the perinuclear space is fairly variable, being small or narrow in some cells (Fig. 1), while in others it is large and extends deeply into the main body of the cytoplasm. In extreme cases, the perinuclear space occupies a considerable volume of the cell, reaching to the cell membrane (Figs. 2 and 3). But, no direct connection of the perinuclear space to the outside of the cell was found. Interestingly this space contains structural bodies, or inclusion bodies. The inclusion bodies found there are rod, branched rod, round, or spherical in shape, and variable in size. Inside the limiting membrane of bodies are particles or granules which resemble ordinary ribosomes. Some of these particles appear attached to the limiting membrane of the inclusion bodies. It seems likely, therefore, that the inclusion bodies in this
perinuclear space are actually an inverted, inside out, granular endoplasmic reticulum. One major difference between the inclusion bodies and the ordinary endoplasmic reticulum is that the former are separate bodies, not continuous as is the endoplasmic reticulum. As a matter of fact, one way that the ordinary granular endoplasmic reticulum could form is by external folding of the outer layer of the nuclear membrane concerned. If inward folding of the outer layer of the nuclear membrane occurred instead of external folding, the observed inclusion bodies would be formed. Unfortunately, the transitional phase of this inward folding is not frequently observed in the electron micrographs taken in this study, but nevertheless can be seen in Figs. 4 and 5. The possibility that the inclusion bodies rise from the inner portion of the nuclear membrane is suggested by inspection of Figs. 5 and 6 where it appears that the nuclear contents bulge out into the perinuclear space and are pinched off from the nucleus. However the latter possibility seems much more improbable than the former. But, because so many inclusion bodies were seen adhering closely to the inner portion of the nuclear membrane, the latter possibility can not be excluded for this picture.

Discussion

As mentioned above, the occurrence of an extended or elongated perinuclear space in the cells of androgenic glands of Procambarus clarki seems to be an extraordinary variation from the typical nuclear membrane structure. The occurrence of such an extended perinuclear space has not been reported for other crustacean species whose androgenic gland cells have been studied by electron microscopy (Hoffman 1969, King 1964, Payan et al. 1971). The presence of such a perinuclear space occurring in the present species was so unexpected that we at first assumed it as a fixation artefact. The temperature, pH, and osmotic condition of the fixative were carefully checked in every fixation. After repeated dissections and fixations of the material with great care, the space was shown as a structure of actual existence.

Possibility that the inclusion bodies occurring in the extended perinuclear space might be parasitic or symbiotic microbes in these cells was also considered. Though we do not have enough knowledge about such microbes, the fact that the extended perinuclear space with inclusion bodies was found in every animal that we examined, not only in the adult male specimens but also in very young ones, greatly reduced the probability that the inclusion bodies are microorganisms parasitic to the cells of these glands. And furthermore, it should be noted that neither such a space nor such inclusion bodies was seen in the cells of other tissues of the present species, such as the hepatopancreas, green gland, nerve, Y organ, and epidermis. It should also be noted that the perinuclear space and inclusion bodies were found in animals collected from nature all year round, indicating that their presence does not dependent on the season.

Palay (1960) reported the presence of fat droplets in the nuclear envelopes of the intestinal epithelial cells of fat-fed rats. He considered the evidence that the perinuclear cisternae are continuous with the lamina of the endoplasmic reticulum. Since the androgenic glands of Crustacea certainly secrete hormone(s) responsible
for the male sex characteristics (Charniaux-Cotton 1960, 1962), these glands should show morphological signs of secretory activity. In this connection, it is worth suggesting that the extended perinuclear space and its inclusion bodies represent a unique process of hormone secretion.

Summary

The cells of androgenic glands of the crayfish, *Procambarus clarki*, were examined by means of electron microscopy, and the occurrence of an enormously extended perinuclear space was indicated. In this space were a great number of inclusion bodies which have a limiting membrane. They contained fine ribosome-like particles. The inclusion bodies seem most likely to develop by inversion of portions of the granular endoplasmic reticulum.

References


