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DIFFERENTIATION OF NUCLEAR AND CYTOPLASMIC FINE STRUCTURE DURING SPOROGONIC DEVELOPMENT OF *PLASMODIUM BERGHEI*

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INTRODUCTION

The value of protozoa in investigations of nucleocytoplasmic relationships has long been appreciated, as indicated by reviews (1, 3, 7) of the extensive literature on this subject. Though the malarial parasite apparently has not previously been employed in such studies, we have found it to be a potentially useful organism in this regard. The elaborate differentiation that the parasite undergoes during its complex life cycle makes it possible for us to study nucleocytoplasmic relationships during the process of cellular development.

After a suitable vector mosquito has ingested blood from an animal infected with malaria, parasite macrogametes are fertilized by microgametes in the midgut of the mosquito. The resulting zygote, known as the ookinete, then penetrates the midgut epithelium of the mosquito and continues its sporogonic development as an encapsulated oocyst attached to the epithelium.

In the course of electron microscopical studies which we conducted with the rodent malarial parasite, *Plasmodium berghei*, we observed a simultaneous differentiation of nuclei and of regions of cytoplasm in their immediate vicinity during this developing sporogonic stage of the parasite. The changes in fine structure appear to represent interactions between the nuclei and cytoplasm during development.

The development of the uninuclear oocyst into the mature oocyst containing many infective sporozoites has already been described by us (11). The work reported here focuses on one aspect of this differentiation process: a morphological indication of nucleocytoplasmic relationships resulting in the initiation of sporozoite formation.

MATERIALS AND METHODS

Electron microscopical and histochemical observations were made on various stages of development of the KSP 11 strain (12) of *P. berghei* in experimentally infected, laboratory-reared *Anopheles quadrimaculatus*. Techniques utilized for maintenance of the infected mosquitoes and for the electron microscopical and histochemical procedures were previously described (11).

RESULTS

The differentiating oocyst just prior to the onset of sporozoite formation is a spherical or ovoid

structure about 35 μ in diameter (Fig. 1). It is surrounded by a capsule which varies from 0.1 to 0.2 μ in thickness. The differentiating material within the oocyst is known as the sporoblastoid body. This material is surrounded by a thin trilaminar plasma membrane, the outer sporoblastoid membrane. Large sporoblastoid nuclei, which may reach 7 μ in diameter, result from growth and repeated division of the original zygote nucleus.

Changes in these sporoblastoid nuclei, accompanied by the formation of fragments of a new cytoplasmic membrane, mark the onset of sporozoite development. The large nuclei around the periphery of the sporoblastoid become flattened along one side where they come close to the outer sporoblastoid membrane (Fig. 1). At the same time, electron-opaque nuclear material appears at the periphery of the nucleus along its flattened side (Fig. 1, arrows). This peripheral nuclear material takes the form of cross-striated fibers, each about 500 A thick. Several of these fibers merge at the site of the nuclear envelope (Fig. 2). The periphery of each nucleus is Feulgen positive at this time.

In conjunction with the appearance of these nuclear fibers, fragments of a second and more dense membrane are condensed immediately under the outer sporoblastoid membrane. These portions of a new membrane, which we may term the inner sporoblastoid membrane, have a multilaminate structure. They appear to be formed initially along sites close to the nuclear fibers and are separated from the fibers by a distance of

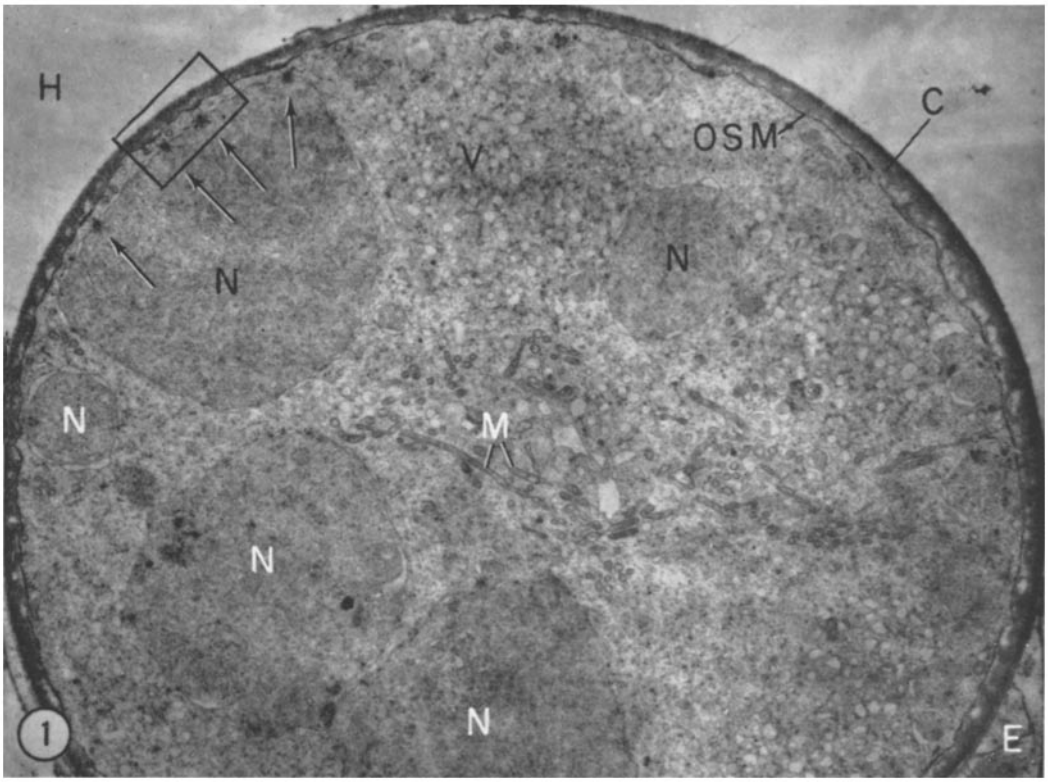

FIGURE 1 This micrograph shows a developing oocyst attached to the midgut epithelium (*E*) of the mosquito and projecting into the mosquito hemocoele (*H*). The oocyst is covered by a thick capsule (*C*). The material within the oocyst is known as the sporoblastoid body. This material is surrounded by the outer sporoblastoid membrane (*OSM*). The cytoplasm of the sporoblastoid body contains mitochondria (*M*) and circular vesicles (*V*). A nucleus (*N*) in the upper left portion of the oocyst has moved close to the oocyst periphery and has become flattened along one side. The flattened side of this nucleus shows nuclear material which has condensed (arrows). × 6,000.

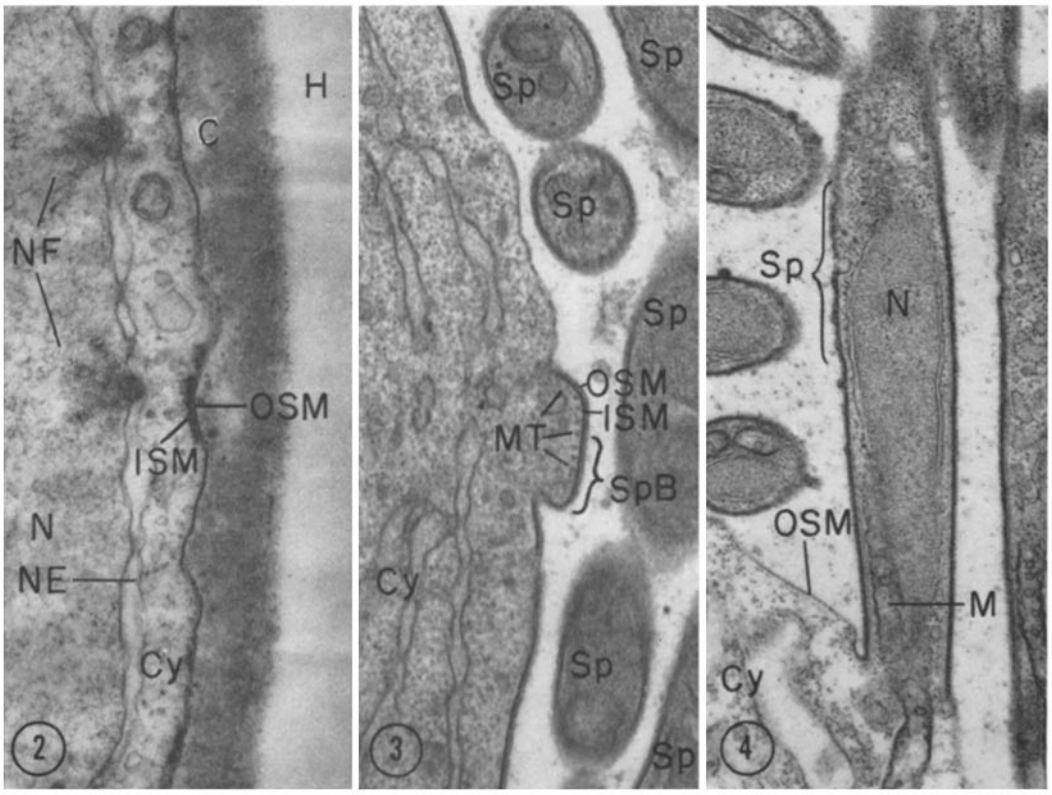

FIGURE 2 The upper left portion of the oocyst in Fig. 1 is shown enlarged in this micrograph. The condensed nuclear material is seen to consist of nuclear fibers (*NF*) which are cross-striated. Portions of a new membrane, the inner sporoblastoid membrane (*ISM*), have formed just under the outer sporoblastoid membrane (*OSM*), at sites close to the nuclear fibers. Seen in high magnification are the nucleus (*N*), nuclear envelope (*NE*), cytoplasm of the sporoblastoid body (*Cy*), oocyst capsule (*C*), and mosquito hemocoele (*H*). × 38,000.

FIGURE 3 A sporozoite bud (*SpB*) has formed as the result of the evagination of a portion of a double-membraned site, i.e. outer sporoblastoid membrane (*OSM*) and inner sporoblastoid membrane (*ISM*). A row of microtubules (*MT*) has formed just under the two membranes. The cytoplasm of the sporoblastoid body (*Cy*) is seen, as well as sporozoites (*Sp*) which have already budded off from the sporoblastoid body. × 33,000.

FIGURE 4 Further elongation of the sporozoite bud produces a sporozoite (*Sp*). Note the transition from the outer sporoblastoid membrane (*OSM*) to the two membranes of the sporozoite. Passing into the sporozoite from the cytoplasm of the sporoblastoid body (*Cy*) are mitochondria (*M*) and a nucleus (*N*). × 18,750.

about 1600 A of sporoblastoid cytoplasm. The inner and outer sporoblastoid membranes together average about 300 A in thickness.

The appearance of a row of microtubules, each about 200 A in diameter immediately inside and along the fragments of newly condensed membrane, is the next visible event in the process of sporozoite differentiation (Fig. 3). Each of these newly differentiated double-membrane regions then evaginates to form a sporozoite bud. The further elongation of this bud and the transfer of nuclear and cytoplasmic components into it from the sporoblastoid body result in the formation of the sporozoite (Fig. 4). We have described in detail elsewhere (11) the entire process of sporozoite development.

DISCUSSION

The formation of fragments of a new cytoplasmic membrane, within the oocyst, at sites close to the peripheral nuclear fibrils suggests an interaction between the nucleus and the cytoplasm. That the nuclear fibrils may represent condensed chromatin material is indicated by their presence in the Feulgen-positive portion of the nucleus. Nuclear fibrils in the form of clusters of helices have been found in the amebae, *Amoeba proteus* and *Pelomyxa carolinensis*, by several investigators (3). The cross-sectional diameter of these helices is similar to the diameter of the peripheral fibrils which we have described in *Plasmodium*.

The first recognizable structures to appear within the budding sporozoite are the subpellicular microtubules. These microtubules continue to elongate as the sporozoite lengthens. Similar microtubules have been found in a diverse assortment of plants and animals (4–6, 8, 10). Byers and Porter (2) have suggested that such microtubules may represent a framework associated with morphogenetic changes. During differentiation, the tubules are oriented parallel to the direction of cell elongation. Microtubule formation has been reported in association with morphogenetic changes during the process of spermatogenesis in cestodes (9), plant cell wall formation (5), and lens formation in the avian embryo (2). Our observations on microtubule formation during sporozoite elongation are in accord with the hypothesis of Byers and Porter (2).

SUMMARY

The development of the oocyst of the rodent malarial parasite, *Plasmodium berghei*, in the mosquito was studied by electron microscopy. During this development, a condensation of cross-striated fibers occurs in the Feulgen-positive periphery of nuclei which are close to the surface of the oocyst. Concomitant with the appearance of these fibers, fragments of a new cytoplasmic membrane are formed a short distance away. These changes appear to represent nucleocytoplasmic interactions during development.

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