Germ disk formation in the araneid spider, *Neoscona nautica* (L. Koch)

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Abstract

The early embryonic development of the araneid spider, *Neoscona nautica*, was examined under the electron microscope. Blastomeres were formed at the 8-nucleus stage, and the cleavage pattern represented a modified type of total cleavage. The large yolk granules were sequestered by the cell membranes, together with various organelles and glycogen granules, in the blastocoel. The embryo at the germ disk stage had spherical germ disk cells, and the region including large yolk granules was exposed directly to the perivitelline space.

Introduction

Embryos of many spiders have been examined in detail under the light microscope. According to Sekiguchi (1957), in many Araneidae, a rip appears in the blastoderm so that the yolk mass is exposed. Then all blastoderm cells take part in the formation of germ disk, and no cells are observed in the region in which the germ disk is not formed. A study of germ disk formation by electron microscopy was made for lycosid spiders (Kondo, 1969, 1970). In a theridiid spider, *Achaearanea tepidariorum*, the fine structure of embryo at the germ disk stage was described by Suzuki and Kondo (1991). In the present study, we examined the embryonic development of the araneid spider, *Neoscona nautica*, from the blastomere formation to the germ disk formation, under the electron microscope.

Materials and Methods

Mature females of *Neoscona nautica* (L. Koch), collected in Funabashi and Sakura, were reared and their eggs were used in this study. The live eggs were examined in liquid paraffin, in which the opaque chorion became transparent. The determination of cleavage stages was made using paraffin sections of developing eggs.

For fine-structural observations, the eggs were fixed for 3 hr in a mixture of 2% paraformaldehyde and 2.5% glutaraldehyde solution in 0.1M phosphate buffer, pH 7.4, that contained 0.2M sucrose. During fixation, the eggs were cut in half with a tungsten needle. After rinsing for more than one hour with same buffer plus 0.2M sucrose, the samples were postfixed for one hour in 2% osmic acid in 0.1M phosphate buffer, pH 7.4, without sucrose. After rinsing with same buffer without sucrose, samples were dehydrated in an ethanol series, transferred to propylene oxide, and embedded in Quetol-812. Ultrathin sections were cut on LKB-4800 ultra-microtome, stained with uranyl acetate and lead citrate, and examined under Hitachi HU-12A electron microscope. Semithin sections were prepared simultaneously, and these sections were stained with methylene blue for light microscopy.

Results

The eggs of *Neoscona nautica* are *ca.* 1.2mm × *ca.* 1mm in size.

They were syncytial during the first and second nuclear divisions. Blastomeres were formed for the first time at the 8-nucleus stage, 15 hr after oviposition at 23℃ (Fig. 1). At this stage, the vitelline membrane was *ca.* 1 μm in thickness. Each blastomere contained many large yolk granules, each of which was 15-30μm in diameter, and cleavage nuclei were 10-20μm. The cell membrane invaginated sporadically from the blastomere surface (Fig. 2). Desmosome-like structures were observed not only between the blastomeres but also in the regions between the invaginated cell membranes. The vesicles were arranged forward the tips of invaginations (Fig. 3).

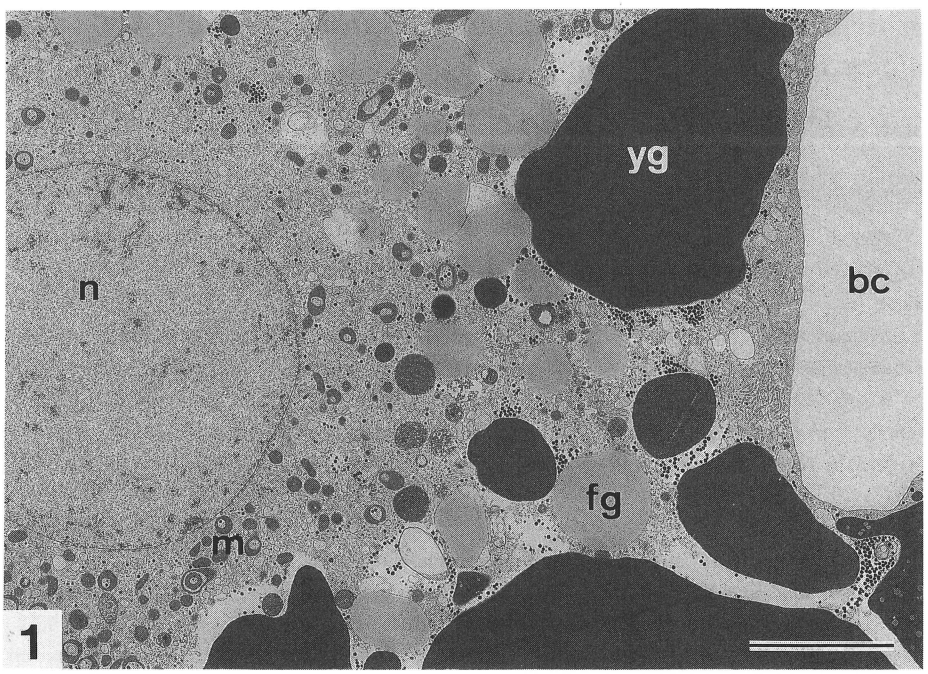

Fig. 1 Perinuclear zone at 8-nucleus stage. Scale = 5 μm. bc: blastocoel, fg: fatty granule, m: mitochondria, n: nucleus, yg: yolk granule.

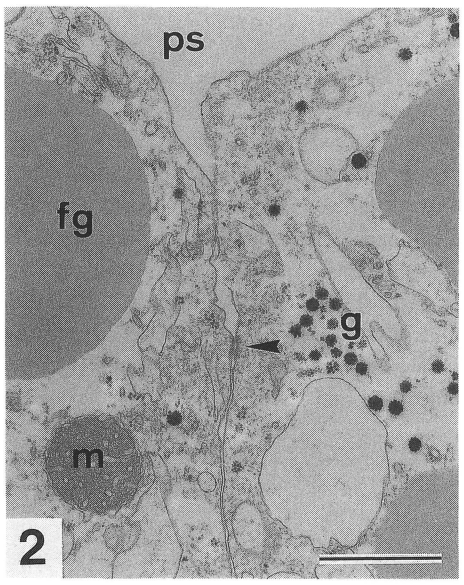

Fig. 2 Invagination of cell membrane. Scale = 1 μm. Arrowhead: desmosome-like structure, fg: fatty granule, g: glycogen granule, m: mitochondrion, ps: perivitelline space.

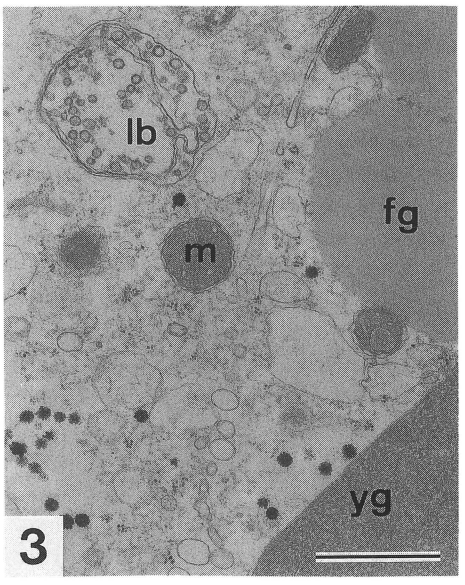

Fig. 3 Vesicles arranging forward tip of cell membrane invagination. A lysosome-like body (lb) contains small vesicles. Scale = 1 μm. fg: fatty granule, m: mitochondrion, yg: yolk granule.

The cytoplasm was distributed near the egg surface as the periplasm, which was about 10μm thick, and in the perinuclear zone. The main components of cytoplasm were fatty granules and vesicles. The fatty granules, 2-4.5μm in diameter, had a moderately electron-dense matrix, and the limiting membranes were often obscure. Some vesicles were empty, while others contained a slightly electron-opaque matrix. Many lysosome-like bodies were also observed, and some fine yolk granules of less than 5μm in diameter were observed. Mitochondria had a highly electron-dense matrix, and cristae were faintly visible. Many mitochondria were either oval or rod-shaped, but the others resembled cups or rings (Fig. 4). Smooth-surfaced endoplasmic reticula were seen to enclose the fatty granules, but rough-surfaced endoplasmic reticula were not observed. Several Golgi bodies were observed. The glycogen granules, which were about 0.1μm in diameter, were very electron-dense.

The nucleus of each blastomere migrated toward the egg surface as development proceeded. Mitochondria were found in abundance around cleavage nuclei after the 16-nucleus stage. The vitelline membrane increased in thickness to about 2.3μm by the end of 32-nucleus stage, 21 hr after oviposition. At one day after oviposition, about 90 nuclei were observed and some cleavage nuclei, accompanied by perinuclear cytoplasm, reached the periplasm.

At the 120-nucleus stage, 28 hr after oviposition, the cleavage nuclei had all reached the periplasm, and the blastoderm was completed. The blastoderm cells were about 100μm in length. Many of them, which were about 20μm in thickness, had almost no large yolk granules, but the others, which were about 60μm in thickness, contained several large yolk granules. Vesicles were sometimes arranged along the surface of large yolk granules (Fig. 5). The nuclei of blastoderm cells were about 15μm in diameter, and desmosome-like structures and invaginating cell membranes were observed. Many mitochondria were often found around the nucleus. At this stage, several large yolk granules were sequestered by the cell membranes, together with various organelles and glycogen granules, in the blastocoel.

Forty-five hours were needed from the oviposition to the completion of germ disk. The germ disk was formed as a single layer of spherical cells. The diameter of many cells in the germ disk was about 45μm, and that of nuclei was about 20μm. Although fine yolk granules of less than 2μm in diameter were observed, almost no large yolk granules were visible. Between these cells, desmosome-like structures were observed in the superficial region. Invaginating cell membranes were never observed, and mitochondria were often crowded round the nucleus.

At the egg surface in which the germ disk was not formed, large yolk granules packed by the cell membranes with various organelles and glycogen granules were exposed to the perivitelline space (Fig. 6). No blastoderm cells at all were observed in this region.

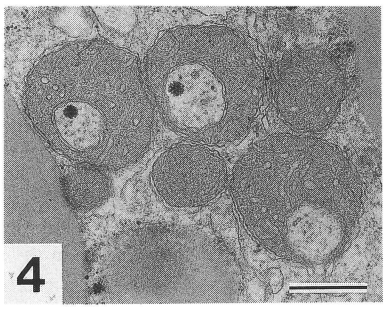

Fig. 4 Ring-shaped mitochondria. Scale=0.5 μm.

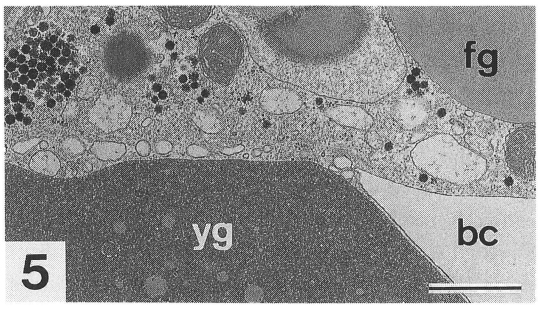

Fig. 5 Vesicles arranging along large yolk granule surface. Scale=1μm. bc: blastocoel, fg: fatty granule, yg: yolk granule.

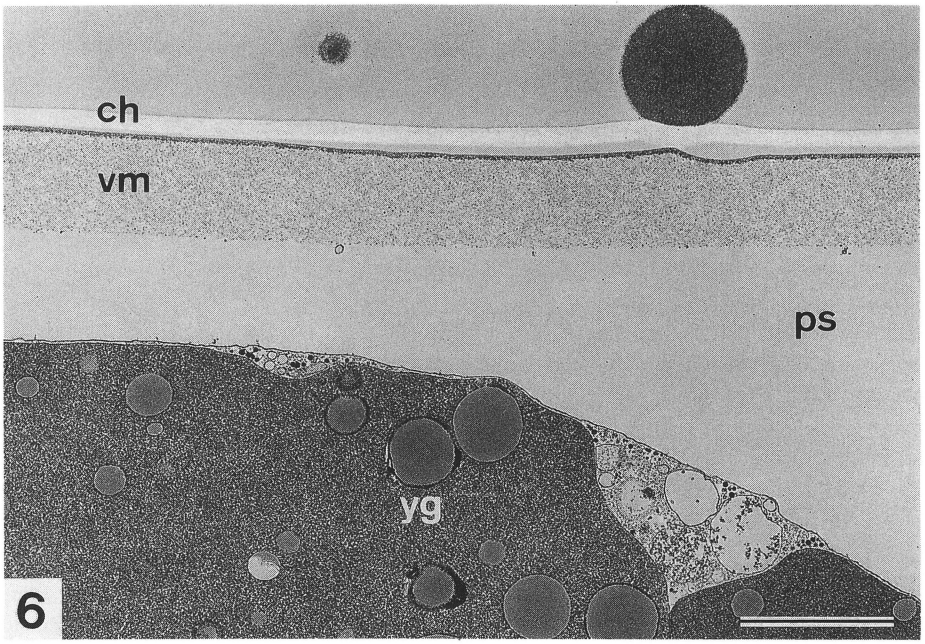

Fig. 6 Egg surface of extra-embryonic region. A structure including large yolk granules (yg) is exposed directly to the perivitelline space (ps). Scale = 3 μm. ch: chorion, vm: vitelline membrane.

Discussion

In the present study, some cup- or ring-shaped mitochondria were observed. They appeared for the first time 6 hr after oviposition in the present material (Suzuki and Kondo, unpublished). Such mitochondria were not found in the case of lycosid spider embryos (Kondo, 1969, 1970), but they were observed in the embryos of a theridiid spider, *Achaearanea tepidariorum* (Suzuki and Kondo, 1991). They also have been found during the spermatogenesis in scorpion (Andre, 1959) and grasshoppers (Tahmisian *et al.*, 1956).

In *Neoscona nautica*, many lysosome-like bodies were observed, however, histochemical studies are required for their final identification. They appeared 1.5 hr after oviposition (Suzuki and Kondo, unpublished).

In *N. nautica*, the formation of blastocoel and blastomeres occurred at the 8-nucleus stage. The cleavage nuclei had not reached the periplasm at that time. In each blastomere, the cytoplasmic segmentation by invaginating cell membranes preceded the nuclear divisions. Therefore, we conclude that the cleavage pattern of this spider represents a modified type of total cleavage, as the case in the lycosid spiders (Kondo, 1969). The elongation of invaginating cell membranes may be caused by the fusion of vesicles that arrange forward the tips of invaginations.

In the region in which the germ disk was not formed in *N. nautica*, several large yolk granules were packed by the cell membranes with various organelles and glycogen granules, as occurs in *A. tepidariorum* (Suzuki and Kondo, 1991). These structures, including large yolk granules, were described as yolk spheres in the lycosid spiders (Kondo, 1969). In the present study, the vesicles were arranged along the surface of large yolk granules in the blastoderm cells. We suggest that these large yolk granules may be excluded from the blastomeres or blastoderm cells through the fusion of these vesicles, and the limiting membranes of vesicles may be reconstructed as the envelopes for the structures which include the large yolk granules and the cell membranes of blastoderm cells. Since no desmosome-like structures were formed, the fusion of vesicles that participated in the exclusion of large yolk granules could be distinguished from the fusion of vesicles that led to the elongation

of invaginating cell membranes.

The embryonic rudiment at the germ disk stage of *N. nautica* had the spherical cells. However, there were no blastoderm cells in the extra-embryonic region. The region of large yolk granules were exposed directly to the perivitelline space. Further morphological studies are needed to explain how the structures including large yolk granules are maintained as a yolk mass in the eggs. Both in the lycosid spiders (Kondo, 1970) and the theridiid spider, *A. tepidariorum* (Suzuki and Kondo, 1991), there are spherical germ disk cells and flat cells in the surface of extra-embryonic region. Thus, we conclude that the formation of germ disk in the araneid type should be distinguished from those of the lycosid and theridiid types.

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