Chapter 2
Mammalian Sperm Head

In mammals, spermatozoa complete meiosis before spermiation, while oocytes do not complete meiosis before ovulation. A mature spermatozoon weighs only picograms, and its head length is approximately 5 μm. On the other hand, a mature oocyte weighs 20–40 ng and is approximately 100 μm in width. Since spermatozoa have to cover a large distance to reach the oocyte, the sperm nucleus (containing the genome) must remain in a protected and safe condition; in fact, it is thoroughly embedded in the nuclear matrix. Approximately $2.5 \times 10^7$ of such structurally complex spermatozoa are produced daily in the human testes; in comparison, only a few oocytes (generally 1 cell per menstrual cycle in humans and about 10 per estrous cycle in rodents) develop in the ovaries and are ovulated into the fallopian tube.

2.1 Structures, Domains, and Related Functions

Mammalian sperm heads are divided into two major regions (domains): the acrosomal region and the postacrosomal region (PAR); these regions can be clearly visualized by using specific antibodies. In my studies, I have used the antiacrosomal antibody MN9 (antiEquatorin antibody) and the anti-PAR antibody MN13 for this purpose (Fig. 2.1). These regions are further divided into substructures and subdomains. The acrosome region contains two subdomains: the anterior acrosome and the posterior acrosome or the ES. The anterior acrosome is involved in the acrosome reaction, one of the most intriguing events of which is the release of acrosomal enzymes and matrix proteins. The ES appears to be involved in gamete membrane fusion. The PAR extends from the end of the ES and the posterior ring located at the distal-most end of the head, and it forms the border between the head and the flagellum or neck region (connecting piece). The posterior ring exhibits a belt-like constricted zone of plasma membrane that fuses with the underlying nuclear envelope. The proximal part of the PAR is also presumed to be involved in egg activation.
Cytoplasmic layers are the spaces formed between the membrane systems in the sperm head (Fig. 1.3). The layers develop as flat and narrow structures and exhibit dense accumulation of cytoskeletal substances and functional molecules. In mature spermatozoa, the acrosome, which is a membrane-bound organelle, is interposed between the plasma membrane and the underlying nucleus at the anterior head. Thus, the molecules accumulated in the cytoplasmic layers are likely to play important roles in the fertilization processes, and it is important to analyze these molecules. The cytoplasm can be differentiated into at least three layers (spaces): the periacrosomal, subacrosomal, and postacrosomal layers.

2.2.1 Periacrosomal and Subacrosomal Layers

The periacrosomal layer is the cytoplasmic layer present between the outer acrosomal membrane and the overlying periacrosomal plasma membrane, while the subacrosomal layer is the narrow layer of cytoplasm that lies between the inner acrosomal membrane and the nuclear envelope. The periacrosomal layer is reported to contain actin filaments (Hernández-González et al. 2000) and the actin-regulatory protein gelsolin (Cabello-Agüeros et al. 2003). These components are considered to be involved in the initial step of the acrosome reaction and subsequently in sperm–egg fusion. The outer surface of the apical segment of the subacrosomal layer forms a prominent cytoskeletal structure around the nucleus; this structure is called the perforatorium, and it protrudes toward the

Fig. 2.1 IIF image showing sperm subdomains; acrosome (red) and postacrosomal region (green). Acrosome is specifically recognized by antiEquatorin (MN9) antibody, while the postacrosomal region is recognized by antipostacrosome substance (MN13) antibody
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rostral region in a triangular conformation. Ultrastructurally, the perforatorium is composed of an electron-dense substance (perinuclear theca substance). The peri- and subacrosomal layers are lost during the acrosome reaction, but the cytoplasm that extends to the ES remains structurally unchanged even after the acrosome reaction.

2.2.2 Postacrosomal Layer

The plasma membrane at the PAR is tightly bound to the underlying postacrosomal sheath (PAS) via a paracrystalline sheet (Longo et al. 1987; Toshimori et al. 1991b). The paracrystalline sheet consists of regularly arranged crossbridges (filaments) with a diameter of 10–14 nm, and the MN13 antigen is localized on these cross-bridges (Figs. 2.2 and 2.3). The paracrystalline sheet can be visualized only after treatment with a mild detergent such as 0.1% Triton X-100. The postacrosomal layer can be further divided into the peri-PAS layer and the sub-PAS layer. The peri-PAS layer corresponds to the cytoplasm between the PAS and the overlying peri-PAS plasma membrane, while the sub-PAS layer corresponds to that between the PAS and the underlying nuclear envelope.

Since the substances in the sub- and postacrosomal layers can only be isolated collectively, these combined layers are referred to as the perinuclear theca or postnuclear cap (Bellvé et al. 1992). The part of the perinuclear theca at the PAR is also known as the calyx (Longo et al. 1987). The perinuclear theca is presumed to play a role in maintaining the conformation of the sperm nucleus or that of the sperm head per se and in storing many molecules required for egg activation, as discussed later.

![Fig. 2.2 TEM image showing sperm structural components at the border between the equatorial segment (ES) and the postacrosomal region (PAR). Tannic acid fixation. Note the periodic ladder-like striations (arrows with asterisks) between the postacrosomal sheath and the overlying plasma membrane, and the glycoalyx-like substance on the plasma membrane covering the postacrosomal region (asterisks)
Membrane System

Many functional molecules necessary for spermatogenesis and fertilization are organized into the sperm membrane system. Here, I demonstrate the distinct nature of the membrane system, which has been clarified using the freeze-fracture (FFR) method. FFR performed using the antibiotic filipin as a specific marker is particularly helpful for studying the differences in the nature of the membrane components, since filipin has an affinity for membrane sterol. Cholesterol is present in abundance on the equatorial plasma membrane and the membrane at the anterior acrosome region, but it appears to be deficient or absent in the nuclear envelope and the outer and inner acrosomal membranes (Fig. 2.4) (Toshimori et al. 1987). The distal part of the redundant nuclear envelope is also rich in cholesterol.

Plasma membrane: The sperm plasma membrane is effectively modified (Flesch and Gadella 2000). Structurally, the plasma membrane covers the whole surface of the sperm head and tail prior to the acrosome reaction. The postacrosomal plasma membrane expresses a glycocalyx-like substance on its surface (Fig. 2.2); this substance is presumed to contain various unknown molecules that are involved in the events induced by capacitation and the acrosome reaction until the spermatozoon fuses with the oolemma.

As mentioned above, the periacrosomal plasma membrane can fuse with the outer acrosomal membrane during the acrosome reaction, while the equatorial plasma membrane can fuse with the oolemma during gamete membrane fusion. This suggests that after the acrosome reaction, the fusogenic molecule(s) is present in both the heterogeneous membranes (i.e., the periacrosomal plasma membrane and the outer acrosomal membrane). Similar fusion-related molecules can be identified on the equatorial plasma membrane and the microvilli of the oolemma during gamete membrane fusion. Recent evidence has demonstrated the importance of raft formation on the sperm plasma membrane prior to gamete membrane fusion. It is of particular interest to investigate how variations in the cholesterol
Content of the plasma membrane are related to the raft formation induced by the acrosome reaction.

**Acrosomal membrane:** The outer and inner acrosomal membranes are differentiated from the proacrosomal granule-derived membrane that originates from the Golgi apparatus (Fig. 5.2). This differentiation appears to occur at the stage of late elongating spermatids, which approximately corresponds to step 14 in mice and the late Sd step in humans.
Outer acrosomal membrane: Peanut agglutinin (PNA), a plant lectin isolated from *Arachis hypogaea*, specifically binds to the β-galactose (1–3)-N-acetyl-galactosamine (β-Gal(1–3)-GalNAc) linkage of O-linked glycoproteins expressed on the outer acrosomal membrane (Huang and Yanagimachi 1985). Accumulated evidence has proven that the nature of the outer acrosomal membrane is more complex than thought previously.

The equatorial outer acrosomal membrane is not involved in the acrosome reaction; it is strongly attached to the inner acrosomal membrane via crossbridges separated from each other by a distance of approximately 7 nm. Since these bridges are embedded deep in the matrix, they are not clearly visible in mature spermatozoa but become distinct with the gradual release of the matrix substances during sperm–egg interaction (Manandhar and Toshimori 2003). Thus, the equatorial acrosomal membrane matrix contains tenacious cytoskeleton element(s) in its outer and inner membranes.

Inner acrosomal membrane: The inner acrosomal membrane is remarkably resilient, exhibiting resistance to damage induced by detergents and sonication, and it is not involved in the acrosome reaction. After the acrosome reaction, the inner acrosomal membrane remains intact and can be recognized by the plant lectin concanavalin A (Con-A), which has a specific affinity for glycoproteins with a high mannose content (Holden et al. 1990). Upon fertilization, the inner acrosomal membrane is internalized by the ooplasm via phagocytosis, as described later. The intramembranous particles are densely packed and resemble paracrystalline arrays (Fig. 2.4). Many molecules are localized in the inner acrosomal membrane, presumably in the form of a functional molecular complex, as discussed later.

Nuclear envelope: The sperm nucleus is dormant in terms of DNA synthesis. In fact, the nuclear envelope lacks pores, and the head cytoplasm lacks the machinery required for protein synthesis.