Review

PREFORMED ACROSOME FILAMENTS. A CHRONICLE

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ABSTRACT

The growth in knowledge on 'preformed acrosomal filaments' is treated here from a start at the beginning of the last century until today. The acrosome is the only organelle that is restricted to spermatozoa; on the other hand it is present in sperm cells from most - but not all - animal species. The description of an 'acrosome reaction' by Jean Dan in 1952 [23], was an important and inspiring event. Within short, this reaction was shown to be nearly universal in the animal kingdom and to be required for fertilization to occur. Most marine invertebrates and many other animals have an acrosome that consists of two components: a vesicle containing lytic enzymes and a subacrosomal space containing an amorphous actin derivative. The acrosome reaction then means a rapid rearrangment of actin molecules to form a filament and the opening of the vesicle. In a few animal groups the formation of an acrosomal filament takes place during spermiogenesis rather than when approaching the egg; this is true of the horseshoe crab and arachnids and further of lampreys, sturgeons, a lungfish and Latimeria, thus species close to the line leading to 'higher vertebrates' (and thus to us).

Key words: acrosome vesicle, subacrosomal substance, acrosome reaction, profilactin, triplet acrosome filaments

INTRODUCTION

One hundred years ago the Swedish scientist Gustaf Retzius [41] started an ambitious project, namely to investigate spermatozoa of all animal groups of some importance. The spermatids and spermatozoa of nearly 200 vertebrate species were thus investigated as well as spermatozoa from more than 200 invertebrates. His findings are documented in accurate and artistic illustrations in several large, folio-format volumes printed at his own cost and entitled Biologische Untersuchungen, Neue Folge. He could draw several conclusions from this rare material; others can be drawn today from studying his figures and text (see e.g. Afzelius [3]).

One of the very last animal groups to be investigated by Retzius was the cyclostomes, represented by *Myxine glutinosa* and *Lampetra* (or *Petromyzon*) *fluviatilis*. These studies were actually published only after his death and by his friend and colleague Carl M. Fürst. The spermatozoon of *Lampetra* has a 10 μm long, cylindrical sperm-head, and a 50 μm long flagellum of a common type. To his surprise he could also discern a long and exceedingly thin filament, which projects anteriorly from the apical tip of the nucleus.

The lamprey spermatozoon with its barely visible, anterior filament had actually been described some years previously by the German biologist Emil Ballowitz [12] who also saw the filament and named it 'Kopf-borste'; it was declared to be a peculiar and unique sperm component. The filament could not be found on all spermatozoa and, when seen, its length seemed to vary. One spermatozoon seen by Retzius [41] had a filament, seven times longer than the sperm head. Due to the thinness of the filament, it could not be decided whether, for instance, it had a flagellar motion near the lamprey egg, nor whether it is the first sperm component to hit the egg. Retzius [41] could not clarify these questions in spite of several brave attempts. He concluded his paper with the words "Möge es anderen Forschern besser gelingen" (in translation: May other researchers be more successful). And about the 'Kopf-borste', his judgement was: "Es ist ein Unicum."

Now, a century later, the thin thread on the lamprey sperm appears less enigmatic. No more can the filament be regarded a unique sperm ornament – a unicum. For reasons that will be clarified in

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the following, its filament will be called 'acrosome filament' and specifically 'preformed acrosome filament'. This paper will deal with the gradual growth in understanding of the preformed acrosome filament, its distribution in the animal kingdom, its mode of formation and chemical nature.

Acrosome reaction in echinoderms

In 1952 Jean Dan [23] published a paper that has had a decisive importance for the understanding of the fertilization process. The goal of her study was simple and straightforward: to observe and describe the fertilizing spermatozoon under conditions that, as much as possible, resemble natural conditions. For this reason she chose to work with spermatozoa of animals that shed their gametes in sea water for external fertilization, such as sea urchins and other echinoderms. She exposed the spermatozoa to 'egg water' which means sea water that contains extracts of the jelly coat surrounding the egg and she studied the spermatozoa in the living state by phase contrast microscopy or after formaldehyde fixation by electron microscopy.

She found that the spermatozoon had ejected a small droplet 5 seconds after addition of egg water, a droplet that appeared to make the spermatozoon sticky. Electron microscopy was made by a whole-mount shadowcast technique rather than from ultrathin sections and hence could not reveal any details of the sperm interior. Yet the specimen preparations were instructive in that they clearly show that the spermatozoon had ejected a droplet and that this droplet was situated at the tip of a filament with a length a little less than 1 μm [23]. Jean Dan named this transformation 'the acrosome reaction'.

A second paper dealt with acrosome reaction of starfish [24]. The same technique was used and the same results obtained, except that the length of the acrosome filament was 25 μm rather than 1 μm and hence also several times longer than the sperm head. A survey of the literature showed that a very thin filament actually had been discerned on inseminated

Figure 1. Five persons with an interest in the acrosome reaction. From left to right: Jean André (University of Paris). In 1965 he described preformed acrosome filament, Björn Afzelius (University of Stockholm). In 1955 he noted the sub-acrosomal space, Jean Dan (Ochanomizu University Tokyo). In 1952 she described acrosome reaction, Laura H. Colwin (University of Miami) and Arthur Colwin (University of Miami). In 1963 they explained the exocytotic opening of the acrosome vesicle. Photograph taken on August 31, 1973 in Stockholm.

starfish eggs but had been interpreted as a filopodium extending from a reception cone of the egg [29].

The 1950s was the decade when specimen peparation techniques for biological electron microscopy were elaborated, and I had the luck to be accepted in the laboratory of Professor Fritiof Sjöstrand who had created a laboratory at the Department of Anatomy, Karolinska Institute, Stockholm that was the leading laboratory in the field of biological electron microscopy. My task was to study sea urchin gametes and I could show [1] that the acrosome consists of two parts, namely a round 'particle' or 'globule' and a cave in the nucleus containing a less dense material. The two parts are now referred to as the acrosomal vesicle and the subacrosomal space.

In a second study [5], I examined acrosome-reacted spermatozoa, which either had been treated with egg-water or else had been added to eggs. I found that the acrosomal globule had been expelled and was situated on the outside of the cell membrane and that the substance of the cave was in continuity with the expanded acrosome filament. How the globule could pass through an apparently intact membrane remained a mystery.

The mystery was solved by Laura H. Colwin and Arthur Colwin [16-18] who interpreted the acrosomal 'globule' as a membrane-bound vesicle capable of exocytosis in that its membrane will fuse with the sperm plasma membrane, whereupon the sperm apex is opened, the vesicular content is exposed, and a continuous mosaic membrane is formed, made of the acrosomal membrane and the sperm plasma membrane. The posterior part of the acrosomal membrane then everts and the subacrosomal material changes into a fibrous material that forms the content of the acrosome filament. The filament retains its cover of mosaic membrane.

The mechanism of filament formation was studied by Lewis Tilney and co-workers who concluded that the subacrosomal material contains actin bound to two more protein species [44,45]. They named this form of actin 'profilamentous actin', later abbreviated to 'profil.actin' and later still with the period sign omitted 'profilactin'.

The acrosome reaction, triggered by the ionophore A23187, starts with the opening of the acrosome vesicle followed by cleavage of profilactin to its two main components profilin and actin, whereupon the actin polymerizes and aligns to form the actin fiber bundle. This is a spectacular, explosive event; in maximally 10 seconds the 25 μm long filament is formed with a well organized actin bundle and covered by a membrane – it seems that membrane precursor material is stored near the base of the forming acrosome filament.

Spermatozoa from echinoderm classes Crinoidea, Holothuroidea, Asteroidea, and Ophiuroidea are roundish cells with a sunken acrosomal vesicle surrounded by a profuse periacrosomal material capable of producing a long acrosome filament [46]. In the fifth echinoderm class, Echinoidea, the spermatozoa are conical and have a protruding acrosome vesicle on top of a relatively small amount of subacrosomal material. The sea urchin species *Echinocardium cordatum* is exceptional in that its acrosomal vesicle rests on a 2 μm long shaft containing a relatively large amount of subacrosomal material [1]. The length of its acrosomal filament has not been determined.

Acrosome reaction in other animal species

The acrosome is the only organelle that is restricted to spermatozoa; it is found in spermatozoa of most - but not all - animal species. Characteristically it consists of two components: the acrosome vesicle and a subacrosomal (or periacrosomal) material. The acrosome vesicle contains lytic enzymes, which enable the spermatozoon to make a path to the egg and fuse with it. The subacrosomal material contains profilactin, which in the acrosome reaction will form the acrosome filament and thereby open the vesicle and expose its contents. The needle-like shape of the filament is advantageous for mechanical penetration of the egg's covers and plasma membrane. The term 'perforatorium' is used as a synonym of 'subacrosomal substance'. It is used by many authors as a descriptive term, which reflects its presumed or actual function [11].

Spermatozoa from teleost fishes have no acrosome [31] nor have the spermatozoa of several other animal taxa. The teleost spermatozoa enter the egg in a specialized area of the egg's plasma membrane, the so called micropyle, which has the functions of recognition, reception and uptake of one or several spermatozoa.

The chitonid spermatozoa have a conical sperm head, which apically appears as a long, needle-like filament capped by a minute acrosome vesicle. There is no subacrosomal material and hence no acrosome filament will be formed. The nuclear filament will act as a substitute for an acrosome filament. Upon fertilization the nuclear chromatin is injected into

the egg as a long thread, but organelles such as mitochondria and centrioles are left outside the egg – evidently because they are too bulky for the narrow hole made by the nuclear filament [14].

A subacrosomal (or periacrosomal) material is lacking in spermatozoa from other animal species, for instance the ascidians [26,27], the inarticulate brachiopod *Terebratulina caput-serpentis* [4], and most dipteran flies [22]. The acrosome vesicle opens to the exterior but no acrosome filament is formed by the ascidian spermatozoa (which usually are fairly slender cells). Whether the round-headed spermatozoa of the inarticulate brachiopods are able to form acrosome filaments seems not to be investigated – the odds are against it.

The acrosome of some 'primitive' dipterans is complete, in that it consists of an acrosome vesicle as well as subacrosomal substance, whereas flies in the majority of Diptera lack a subacrosomal material and hence presumably cannot form an acrosome filament. Both vesicle and subacrosomal substance are missing altogether in dipteran family Cecidomyiidae [22]. What adaptations there are in the egg or in the mating behavior of the dipteran species remains to be investigated.

The *Limulus* spermatozoon

Next significant step was taken in the mid-60s. The French biologist Jean André investigated spermatozoa from many animals, among them those of the horseshoe crab, *Limulus polyphemus* [10]. It is a 'living fossil' closer related to scorpions and trilobites than to crabs. At light microscopical magnifications the spermatozoon was found to be rather ordinary; specifically it is of a kind that is termed 'primitive spermatozoon' by Retzius and others: a roundish nucleus with a surmounted acrosome, a short midpiece and a long (around 50 μm) flagellum.

By electron microscopy a prominent acrosome filament was noted to be present even in the unreacted *Limulus* spermatozoa; prior to mating it stays inside the cell rather than ejected to the outside [25,45]. The length of the filament is several-fold longer than that of the sperm head - about 35 – 50 μm versus about 3 μm for the head diameter [43]. The preformed acrosome filament is lodged in an intranuclear canal whose walls are made of the greatly extended nuclear membrane; posteriorly it continues into the sperm midpiece, which is encircled by it in about six loops [44]. When added to the egg, the spermatozoon undergoes an acrosome reaction, in which the long acrosome filament is ejected [13,43].

As in the various types of acrosome-reacted spermatozoa, described from other animal species, the acrosome filament consists of a bundle of actin microfilaments. The crystalline order of these is, however, more strict than is the order in corresponding filaments from other species, such as the mussel [39]. The crystalline arrangement of actin microfilaments is evident also in the cross-sectioned acrosome filament [43,44]. The high degree of order in the acrosome filament of *Limulus* has permitted a crystallographic analysis, which led to the proposal that "the assembly of a stable bundle with a defined maximum diameter can be controlled by the crystallographic packing of the twisted filaments" [42].

Preformed acrosome filaments in Chelicerata

Limulus polyphemus, together with only three other extant horseshoe crab species, constitute order Xiphosura (or Limulida) and class Merostomata in the large phylum Arthropoda. Alternatively, Merostomata are joined to Arachnida to constitute the arthropod class (or subphylum) Chelicerata (or Arachnomorpha) with its 75 000 species. A major difference between the two classes (or subclasses) is that the limulids live in sea water and have retained external fertilization, whereas arachnids live a terrestrial life and have internal fertilization.

Spermatozoa from the various arachnid order differ in outer shape and inner composition, but one trait seems common to all, or most, of them: there is a preformed acrosome filament [47]. The filiform spermatozoon of the scorpion thus has a preformed filament that penetrates the nucleus to about a third or a fourth of its length [7,20]. Spermatozoa from Uropygi, Amblypygi and Aranae appear as round balls with a rolled up flagellum and a very long preformed acrosomal filament, which penetrates part of the nucleus and continues as a helix surrounding the nucleus [8,34].

In the order Opiliones the spermatozoon may have the shape of a baton or of a boat with the acrosomal vesicle in the concave side and with an acrosomal filament that emerges from the vesicle [36,37]. Spermatozoa in order Acari are fusiform and have a long preformed acrosomal filament that extends from

the acrosome vesicle to the cytoplasm and further into the nucleus where it runs in an intranuclear canal [6-9]. In conclusion: the preformed acrosomal filament seems to be a useful autapomorph of Chelicerata (or synapomorph of Merostomata+Arachnida).

Search for preformed acrosomal filaments in other animals

A search for preformed acrosome filaments in other invertebrate groups has been without result. For instance, in the largest of all animal classes, Insecta, sperm diversity is nearly endless, but no clear-cut examples of preformed acrosomal filaments have been reported [32]. Depending on the species, the acrosome may be one-layered (vesicle only), two-layered (with vesicle and subacrosomal material), or three-layered acrosome (with vesicle, subacrosomal material and a cytoplasmic layer between the vesicle and cell membrane) or may lack an acrosome. Most insect species have spermatozoa with a two-layered acrosome.

The subacrosomal material in insect spermatozoa may reside in a posterior inpocketing of the acrosome vesicle, as in *Machilis* [21] or may fill a (relatively short) intranuclear canal as in the honey-bee [19]; then it resembles the preformed acrosomal filaments in some arachnid spermatozoa, but the subacrosomal material does not form a slender filament of uniform diameter, nor does it have a periodic substructure. For this reason the material is named 'acrosomal rod' rather than 'acrosomal filament'. It would be interesting to know whether the acrosomal rod contains actin and whether it can be activated with an ionophore.

A somewhat similar situation can be found in class Crustacea with its 40 000 species. The aflagellate spermatozoa of the rather primitive, malacostracan species *Anaspides tasmaniae* has an acrosome vesicle and a highly developed subacrosomal filament, or rather rod, that extends from the vesicle throughout the sperm cell [35]. This filament or rod is much thicker than the filament in *Petromyzon* or Chelicerata , it makes no contact with the nucleus at any stage of development, and it shows no periodic substructure. Whether it is homologous or only analogous to the true subacrosomal filaments remains to be investigated.

Sperm diversity in Vertebrata (about 45 000 species) and related sub-phyla is likewise enormous. Some species in each subphylum have 'primitive spermatozoa' with an acrosome that contains both acrosomal vesicle and a subacrosomal space with amorphous contents:

In Hemichordata, Enteropneusta, the spermatozoon of *Saccoglosssus kowalewski* has an acrosome that undergoes a normal acrosome reaction.

In Urochordata, Appendicularia, the spermatozoon of *Oikopleura dioica*, has acrosome and acrosome reaction of the same kind [28].

In Cephalochordata, the lancelet, *Branchiostoma lanceolatum* has acrosome with vesicle and subacrosomal space, but its acrosome reaction seems not to be studied [48].

In Vertebrata, Cyclostomata Myxiniformes, the hagfish *Eptatretus burgeri* has an acrosome reaction of common kind; it thus forms a short acrosome filament, which contains filamentous actin [40].

It is of interest that these four Chordate species are considered to be the most 'primitive' ones in respective subphyla - a finding in agreement with the notion that close to the base of the phylogenetic tree there are many plesiomorphic traits.

In the second cyclostome order, Petromyzontiformes (lampreys), the spermatozoon of *Petromyzon fluviatilis* shows a novel feature, not found in primitive spermatozoa (except *Limulus)*, namely the acquisition of a preformed acrosome filament running in an endonuclear canal. The acrosome filament seems not to undergo any observable changes upon ejection in an acrosome reaction [30].

In cartilagenous fish (class Chondrictyes) the spermatozoa have acrosomes of a rather normal kind: an acrosome vesicle and a pocket with some subacrosomal material, as in so many invertebrates. The acrosome reaction has not ben studied.

In bony fish (Class Osteichthyes) the situation is more diverse: thus spermatozoa in subclass Cladistia (bichirs) *Polypterus senegalus*, have spermatozoa of aberrant shape but with an apical acrosomal vesicle and a long endonuclear canal. Probably it contains a preformed filament [38].

In subclass Dipnoi the Australian lungfish, *Neoceratodus forsteri*, has a very long sperm head, 70 μm, with an apical acrosome vesicle and two preformed filaments. These run partly inside the nucleus and partly outside and along it [33]. They have been named perforatoria but are regarded as true preformed acrosomal filaments [33]. The African lungfish does not have such filaments.

In subclass Crossopterygii, *Latimeria chalumnae* has spermatozoa with a long and slender nucleus that is penetrated by an endonuclear canal that contains three preformed acrosome filaments [38].

In subclass Actinopterygii, the sturgeons (Chondrostei) have spermatozoa that are remarkable only by having three endonuclear canals, each with a preformed acrosomal filament which runs through most of the length of the nucleus. Acrosome reacted spermatozoon with one, two or three extruded filaments can be found [15]. Other actinopterygians including the teleosts have lost the acrosome [2,31].

The tetrapod line has branched from the 'fish-line' probably near the lines leading to lungfishes and crossopterygians. It can hence be asked: Did our ancesters about 360 million years ago come from an egg that was fertilized with a spermatozoon that carried preformed acrosomal filaments? And further: Did these spermatozoa carry one, two or three filaments?

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