CYTOLOGICAL PHENOMENA CONNECTED WITH SPERMATOGENESIS IN LIVERWORTS AND MOSSES

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The liverworts and mosses (Bryophytes) are, for the most part, land plants which, however, complete a certain part of their life history under aquatic conditions. Very distinct changes in protoplasmic structure occur during the adaptation to the aquatic condition. Cells once non-motile and stationary, which are adapted to life and growth in an approximately fixed position, change to form and structure suitable to a very actively motile existence. These changes occur not in tissues or organs as a whole, in which case the cells or protoplasts might function en masse, but separately and co-ordinately in individual cells. Consequently the history of one of these cells is the approximate parallel of the history of any other similar cell.

The cytological phenomena, which will be under brief discussion in this paper, occur in each individual of one particular cell generation of the male sexual organ, the antheridium. This generation is the one in which each cell, when mature, constitutes a male sexual cell or gamete, commonly known as a spermatozoid or sperm. In order that it may function in the life history of the plant bearing it, the sperm finds and unites with the female sexual cell known as the egg or egg cell. To reach the egg the sperm must traverse a longer or shorter distance through an aquatic medium. This medium is supplied at more or less irregular intervals by rain and dew, or standing or flowing surface water. Our discussion concerns the changes occurring in the sperm cell which result in an admirable adaptation to the actively motile life which the sperm leads at this stage in the plant's life history. Although of much consequence, in fact, of absolute importance and necessity, the period of motility lasts but a very short time.

It might be well first to describe and locate the cells which give rise to the sperms. At certain places on the plant are formed multicellular globular bodies, just large enough to be detected by the unaided eye, known as the antherida. Each of these develops by growth and cell division from a single cell of the plant body. When near maturity each antheridium consists of a more or less globular mass of cubical cells, surrounded by a wall layer and united with the main body of the plant by a relatively short thick stalk. In the majority of the liverworts, at least, if not also in the mosses, the protoplast of each cubical cell gives rise by oblique division to two cells which when viewed from one side present a triangular outline. The two triangular protoplasts, Allen of Wisconsin has termed androcytes. Each androcyte becomes transformed into a sperm. Hence two sperms are eventually developed from each of the cubical cells. The occurrence of the last division in an oblique plane seems at least not so constant and prominent in the mosses as in the liverworts.

The protoplast, or organized protoplasm of one of these cubical cells, consists of a nucleus and cytoplasm. The outer boundary of the cytoplasm next to the cell wall forms a very delicate membrane, the plasma membrane. The remainder of the cytoplasm is more or less finely granular or lumpy when fixed and stained. Allen, however, finds in one of the mosses, Polytrichum, a certain part of the cytoplasm, the kinoplasm, organized into definite granular plates or membranes. The individual granules of the plates he calls kinetosomes. These kinoplasmic plates are present in the resting stage of the cell and seem to actively function in spindle formation as the cell prepares to divide.

The nucleus of this cubical cell, or androcyte mother cell, is spherical and sharply delimited by a distinct membrane, the nuclear membrane. The most prominent content of the nucleus is chromatin material variously arranged. Either a very distinct densely staining nucleolus may be present with surrounding chromatin granules in a more or less clearly defined, often sparse net work, or the chromatic material may be almost entirely included in one densely staining central nucleolar like mass. It seems quite evident that during a certain prophase of division whether a distinct nucleolus may be observed or not, the chromatin becomes largely collected into a mass in the center of the nuclear cavity. This mass, sometimes presenting an irregular, sometimes a smooth outline, is resolved into a more or less closely wound or irregularly gathered spireme. From this spireme the chromosomes are differentiated. Six chromosomes have been counted in the dividing spermatogenous cells of Mnium and Polytrichum, two common mosses, six is probably the number in Blasia and Porella two of the liverworts, while three other liverworts, Ricca, Marchantia and Fegatella have eight chromosomes each.

The androcyte, that is the cell destined to be transformed into the sperm, whether it is triangular in outline as the result of an oblique division or not, is constructed on the same general plan as the androcyte mother-cell just described. We find a definitely organized protoplast consisting of a nucleus and cytoplasm, the latter bounded apparently by a very delicate plasma membrane. Somewhat conflicting reports have been made recently as to the nature of the processes occuring as this androcyte becomes transformed into the mature sperm. In addition to the presence of nucleus and cytoplasm, there is quite general agreement as to the early appearance somewhere within the cytoplasm of a conspicuous dark staining body, the blepharoplast. The origin and nature of this body is as yet a matter of dispute. Whether it functions in the growth processes of the cilia or has to do with the change in form of the androcyte, or possesses other entirely distinct functions, it certainly forms the base of attachment of the cilia.

Briefly the transformation of the androcyte as the writer has observed it in Mnium, one of the common mosses, is as follows: (I find practically similar processes occurring in Marchantia, Blasia, Porella and Fegatella.) The protoplast of the androcyte rounds off slightly from the cell wall. The blepharoplast appears in the cytoplasm near the plasma membrane apparently as a cytoplasmic differentiation in the androcyte in which it is to function. The blepharoplast grows as a more or less radially flattened band in a course closely applied to the plasma membrane. The nucleus becomes closely applied to the blepharoplast, the chromatin network and nucleolus, if the latter is present, changing meanwhile in structure so that eventually the entire nucleus stains quite homogeneously. The nucleus lengthens parallel with and becomes more and more closely applied to the blepharoplast, so that the two form first a crescent and then a coil of one or two turns. The development of the blepharoplast precedes the change in form of the nucleus. During this process the nucleus and cytoplasm do not seem to be sharply separated by the nuclear membrane, but there are indications of diffusion from one to the other. The blepharoplast and nucleus continue to lengthen and fuse more closely, at last becoming indistinguishable, and eventually forming a long slender coiled almost filiform band or cord pointed at both extremities. The blepharoplast may be seen protruding for a short distance as a delicate filiform thread from one extremity of the sperm which we may call the anterior end. Attached near the tip of the blepharoplast are two long very delicate cilia. A vesicle, of granular cytoplasm and perhaps some nuclear material, within the coiled body of the sperm, disappears as the sperm reaches maturity, doubtless being absorbed by the main body or used up in protoplasmic activity, perhaps both.

To secure a common answer to the question of the origin and nature of the blepharoplast seems quite difficult. Two investigators, Ikeno and Bolleter, have described the blepharoplast as originating in the nucleus, and passing out into the cytoplasm through the nuclear membrane in the following manner. Prior to the last division of the spermatogenous tissue in the androcyte mother cell referred to above, a body separates from the chromatin structure and passes through the nuclear membrane into the cytoplasm. Here it divides and the two daughter bodies move to opposite sides of the nucleus and function as centrosome like bodies during spindle formation and the division of the nucleus. Each of these two daughter bodies persists in its respective cell, or androcyte, and functions as the blepharoplast. The figures of Ikeno and Bolleter scarcely present convincing evidence that any particular body which is first shown within the nucleus is identical with the particular body later seen outside the nucleus.

Others have described the appearance of the centrosome like bodies at the poles of the last division and the persistence of these bodies, each one in its respective androcyte, where it functions as a blepharoplast. Still others report centrosome like bodies during the last as well as earlier divisions of the antheridium, but fail to discover genetic continuity of these bodies from one cell generation to the next.

These polar bodies, according to the writer's observation, are more frequently found during the last division of the spermatogenous tissue, but even then seem to disappear during the telophase. Miss Black agrees with the writer in believing that the blepharoplast originates as a sharply differentiated cytoplasmic body in the androcyte in which it is to function and not from a previously formed polar or centrosome like body.

Wilson, writing in 1911, describes three distinct structures, originating from the nucleolus in the androcyte. One division results in the separation from the nucleolus of bodies which pass through the nuclear membrane into the cytoplasm, where they are resolved first into rod-like structures and then built into a more or less hollow spherical structure, termed a limosphere. The second division separates the nucleolus into two parts, one of which forms an accessory body and the other becomes the blepharoplast. The function of the blepharoplast has been described. The accessory body, doubtless similar to the Nebenkörper of Ilkeno, and the limosphere perhaps become part of the vesicle.

It seems safe to make the following statements referring in general to the liverworts and mosses:

The slender, flexible, more or less coiled bi-cilate sperm represents the metamorphosed protoplast of the androcyte.

In the androcyte two distinct parts are distinguishable, the nucleus and the cytoplasm.

In the sperm, besides the main body, or nuclear portion, which stains densely and homogenously and certainly contains the chromatin, there are present two cilia and a slender thread, the blepharoplast connecting these with the main body.

A more or less clearly defined vesicle, which contains remains of the cytoplasm, disappears as the sperm approaches maturity.

During the last as well as the earlier divisions of the spermatogenous tissue polar bodies are often present.

The facts recently brought to light leads the writer to venture also the following statements:

1. There is not at present satisfactory evidence that true centrosomes occur in the spermatogenous tissue of the Bryophytes.

2. Polar bodies, which often occur, do not seem to be identical with the blepharoplasts.

3. There is strong evidence, on the other hand, that the blepharoplast is of cytoplasmic origin.

4. It seems extremely doubtful if the various structures termed respectively "limosphere," "nebenkörper," and "kinoplasmic plates" occur regularly and constantly in the majority of the members of the Byrophytles.

5. In the observation and description of protoplasmic phenomena in this particular field it is not only wise, but quite necessary to bear in mind the small size of the cells, the dense protoplasmic contents with relatively little cell and nuclear sap, resulting in slow and difficult penetration of the killing fluids, and especially the extreme plasticity of the cells during this period of marked and rapid transformation of the androcyte into the mature and actively motile sperm.