The Origin of the Metazoa and the Stigma of the Phytoflagellates

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Summary

It is well known that in various phytoflagellates one of the flagella is connected with the eye-spot or stigma. In the case of *Chromulina psammobia* the more differentiated structure of the eye-spot + flagellum complex seems analogous to that of a retinal rod with its connective cilium binding the external segment and the cell-body.

It is tempting to draw a parallel between this finding of comparative cytology and a hypothesis on the origin of Metazoa proposed first by J. R. Baker, and subsequently by A. C. Hardy.

On the Origin of the Metazoa

IT seems reasonable to suppose that in the course of biological evolution, the unicellular organisms, or Protista, preceded the multicellular plants and animals. In such a case, the development of multicellular organization from the unicellular state would imply:

(*a*) that the cells which make up the lineage of a protist have remained united during the course of their successive fissions;

(*b*) that of these cells, some were able to differentiate with respect to the others—as much structurally as functionally—their ensemble constituting a new, organized system endowed with a higher degree of complexity.

In accord with these suppositions we know that the lower plants offer broad potentialities for the transition from the unicellular organization. Thus, a number of phytoflagellates are able to aggregate temporarily in palmelloid form; moreover, multicellularity manifests itself in each of their groups by colonial or coenobial structures, which are achieved in many species before they become really established in the true Algae of the corresponding type.

The case of the Zooflagellata is opposed to that of the Phytoflagellata, as Baker (1948) has pointed out, since the former group shows few indications of the possible origins of the Metazoa. Among the Protozoa, only the Choanoflagellata can, through their aggregates, lead to a group of Metazoa—but to a very specialized group, the sponges; and if one examines the case of the Cnidosporidea with their pluricellular, differentiated states, the question arises as to whether they actually do represent Protozoa, and not Metazoa extremely degenerated by parasitism (see Poisson, 1953).

Baker believes that the attainment of a multicellular state is, to a large extent, conditioned by the particular mode of nutrition of the aggregated cells. Among the protophytes, exchanges take place across the whole surface

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of the body, and this process is not greatly modified when several cells are closely joined to each other. It is otherwise for the Protozoa furnished with a buccal differentiation serving for the ingestion of food, since the close association of such cells would presume that a new and collective manner of nutrition had been realized. Baker concludes from this that only the very primitive Protozoa lacking localized, definite alimentary organs could unite to give rise to the first Metazoa. In fact, the most highly differentiated Protozoa are able to form colonies; but their high degree of organization precludes the integration of the different zooids, which accept only a relative interdependence; take, for example, the colonies of heteromorphic *Zoothamnium*, or the remarkable planktonic ciliate, *Ctenoctophrys chattoni*, described by R. Weill (1946) (see Fauré-Fremiet, 1954).

Hardy (1953) has re-examined Baker's arguments, but, carrying the deductions to the end and leaving aside the case of the Choanoflagellata and the sponges, he infers protophytic origin for the Metazoa. Collective phagotrophic nutrition would have established itself among very simple metaphytes after the loss of their chromoplasts; and we know that the evolution of the phytoflagellates offers, without any doubt, rather numerous examples of such an accident, leading to a change of nutrition in the holozoic direction (see Fritsch, 1935; Pascher, 1942).

These hypothetical considerations form a preface to the following remarks.

The Stigma of the Phytoflagellata

Among a large number of phytoflagellates there is an organelle in the form of a granule or a platelet, which is coloured orange-red by a pigment of the carotene type (Wolken, 1956*b*); this is the 'stigma' or 'eye-spot', considered to be a sensory organelle and, in particular, a photoreceptor (Franzé, 1893, 1908; Rothert, 1914; Mast, 1927, 1938; Wolken, 1956 *a*, *b*, &c.). Rothert (1914) showed that the stigma of a phytoflagellate is a derivative of the chromoplasts; Mangenot (1920), Chadefaud (1931, 1935), and Hollande (1938*b*) have verified this fact by demonstrating precisely that the eye-spot can be a delimited part, a localized differentiation of a plast, as with the Chlorophyceae or the Xanthophyceae for example; or else, as with the Euglenidae, a small chloroplast entirely transformed into a stigma.

The electron microscope has revealed a certain structural complexity of the stigma. In *Euglena gracilis* (Wolken and Palade, 1952, 1953; Wolken, 1956 *a*, *b*), *Chlamydomonas reinhardi* (Sager and Palade, 1954, 1957), the spermatozoid of *Fucus* (Manton and Clarke, 1956), *Chromulina psammobia* (Fauré-Fremiet and Rouiller, 1957), &c., the eye-spot is formed by the regular juxtaposition, underneath the membrane of the chromoplast, of a series of elements. These are spheroid, oval, or cylindrical 'chambers' measuring from 100 to 430 mμ. These 'chambers' are limited by double membranes comparable to those which subdivide the plastid; they contain a pigment of the carotene group.

Another remarkable characteristic of the stigma is shown in its connexions with a flagellum. One recognizes, among the Euglenidae, the presence of a paraflagellar body located in the vicinity of the stigma and presumed to be a photoreceptor; the electron microscope permitted the establishment of this fact (Wolken and Palade, 1952, 1953) and the demonstration of other examples of a flagello-stigmatic coaptation.

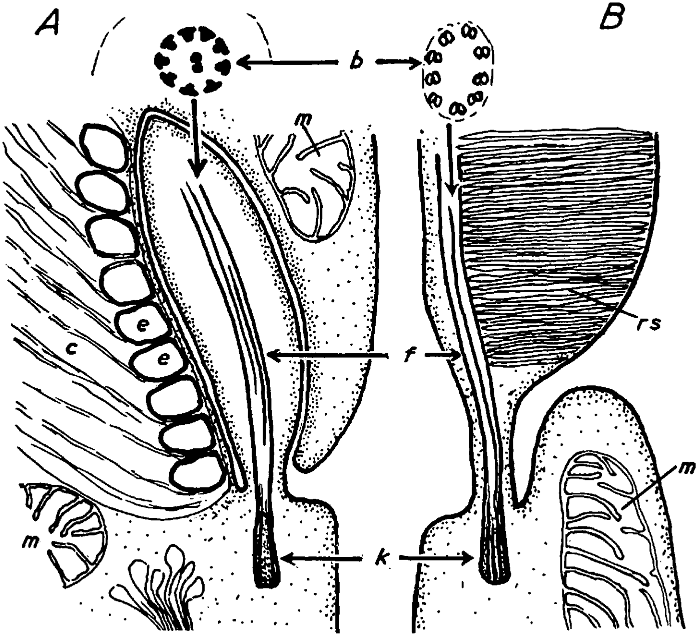

FIG. 1. Composite diagrams based on several electron micrographs. A, eye-spot of *Chromulina psammobia*, according to Fauré-Fremiet and Rouiller (1957). B, junction of outer and inner segments of a retinal rod cell, redrawn after de Robertis (1957). *b*, transverse sections of a fibrillar bundle of *Chromulina* (on the left) and of a retinal rod cell (on the right) (these sections are at a higher magnification than the rest of the diagram); *c*, chromoplast of *Chromulina*; *e*, eye-spot chambers of *Chromulina*; *f*, fibrillar bundle; *k*, kinetosomes; *m*, mitochondria; *rs*, flattened rod sacs of the retinal cell (both the eye-spot chambers and the rod sacs contain carotene pigment)

Thus, in the spermatozoid of *Fucus* (Manton and Clarke, 1956), the recurrent flagellum turns back and touches the surface of the body at the level of the eye-spot; it becomes flat and adheres closely to the cell-membrane along the whole width of the eye-spot, while beyond it becomes free again and cylindrical in shape.

In *C. psammobia* (Fauré-Fremiet and Rouiller, 1957), next to the external flagellum there is a very short internal flagellum, not visible in the living organism, because it is developed in an invagination of the cell-membrane. It displays the classical structure of a ciliary organelle, with a tubular basal granule and a sheath of 9 double, peripheral fibrils surrounding a double, central fibril; but this sheath is surrounded by an appreciable mass of amorphous matrix. This curious internal flagellum is tightly lodged in a depression of the membrane of the chromoplast, in contact with the stigma (fig. 1, A), thus realizing a coaptation comparable to the one already known in the spermatozoid of *Fucus*, in comparison with which the flagello-stigmatic complex of *Chromulina* seems a highly differentiated organelle.

The knowledge of such a complex photo-receptive apparatus in a phytoflagellate suggests a comparison with the retinal cells of the vertebrates.

The External Segment of the Retinal Cells of Vertebrates

Long ago the cytological techniques utilized by Fürst (1904) and Leboucq (1909) showed that a ciliary organelle participates in the morphogenesis of the external segment of the retinal rods and cones; and the studies pursued by Schmidt (1935, 1937) on some of the physical properties of the visual cells, indicated that this same external segment is formed by the layering of lipoprotein lamellae to which the pigment carotene is bound. We are indebted to the electron microscope, through the recent works of Sjöstrand (1953, 1956) and of Robertis (1956*a*, *b*) on mammals, and of N. Carasso (unpublished) on the toad, for additional information which is especially important.

The external and internal segments of a rod, for example, are bound together by a fibrillar bundle surrounded by a membrane and corresponding to a cilium, of which the basal granule is enclosed in the internal segment. This is the 'connective cilium' of which the double axial fibril is missing, whereas the 9 double peripheral fibrils cross the intersegmentary neck and spread out along the side of the proximal part of the external segment, in contact with its stack of bilamellar elements, or flattened sacculi, containing the red pigment (fig. 1, B).

The validity of a comparison between this structure and that of the flagello-stigmatic complex of *Chromulina* is evident, as Wilmer (1955), on the evidence of some less significant data, has already pointed out. In one case as in the other, the photosensitive pigment, carotene, is distributed through a series of compartments—vesicles or flat sacculi—to the length of which there is closely applied a cilium, whose basal granule is situated farther on, within the cytoplasm. Let us, however, point out some differences:

(*a*) In the phytoflagellates the fibrillar infrastructure of the cilium is complete, while in the vertebrates it is reduced to the external bundle.

(*b*) In the phytoflagellates there is a close coaptation of the two distinct organelles: the flagellum, on the one hand, and on the other, the 'carotenophore' or pigmented structure (stigma) differentiated within a plastid; whereas in the vertebrates the pigmented structure is differentiated, according to Robertis, from the 'primitive cilium' at the expense of a 'morphogenic material' contained in its matrix.

These differences do not diminish the value of the suggestions formulated by Wilmer and apropos of which Wolken (1956*a*) added, concerning the case of *Euglena*: 'We can look upon the *eye-spot* + *flagellum* as really a "primitive eye" or the most "elementary nervous system".'

The Case of the Protozoa

The Protozoa, lacking chromoplasts, do not seem to have developed the equivalent of the eye-spot of the Protophyta. This fact does not exclude the possibility of a certain sensitivity to light.

Among the most highly organized Protozoa, such as the ciliates, a certain number of species are positively or negatively phototactic: *Stentor coeruleus*, provided with a diffuse, blue pigment, is photophobic; *S. niger*, provided with a very peculiar black pigment (Barbier, Fauré-Fremiet, and Lederer, 1956), is phototropic; *Chlamydodon mnemosyne*, a psammic photophobic species, bears a diffuse spot composed of an orange-yellow, liposoluble pigment. We know of still other species possessing a pigmented spot without definite structure; the physiological role of the spot has not been determined.

A much more curious case is that of *Strombidium oculatum* Gruber: this marine ciliate lives in symbiosis with a *Chlamydomonadina* (Fauré-Fremiet, 1948) which multiplies more rapidly than its host; the latter digests the excess of that population, but the chlamydomonadian stigmata are conserved and accumulate in the apical part of the ciliate body, forming the 'eye-spot' well described by Gruber. Now, it is remarkable to verify that this ciliate manifests a very marked positive phototropism, during the entire active phase of its existence at least. This fact confirms that Protozoa such as the ciliates are unable, by themselves, to realize the differentiation of a photoreceptive organelle.

Questions

The facts and considerations examined in the preceding pages do not justify the formulation of a conclusion; they permit us, however, to trace a parallel between an hypothesis on evolution and a fact of comparative cytology.

The hypothesis, advanced by Hardy, upsets a current opinion by assuming that the Metazoa more probably evolved from the Protophyta than from the Protozoa.

The fact is that the external segment of a visual cell of the vertebrates and the eye-spot of several phytoflagellates are comparable in the sense that they both realize the close coaptation of a ciliary organelle and a vesicular system containing the carotene.

The parallel is obviously suggestive, but, before admitting that the fact can support the hypothesis, one would have to know if it reflects something other than a fortuitous resemblance or some superficial analogy. And if these cilio-carotenophore complexes that had developed in the chrysomonad cell and in the retinal cell could be considered as physiologically and morphologically homologous, other questions would arise at once. One would have to know if such a cellular differentiation, presumed to be photoreceptive, could have developed several times in independent ways and by diverse mechanisms, or if it implies some causal relationship; if the idea of 'plast' is necessarily tied to that of a carotene-bearing organelle, or if other cytoplasmic structures, of the Golgi type, for example, could play the same role. Those are the problems of comparative cytology which the electron microscope will perhaps permit us to resolve.

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