

The Pyrenoid: ITS STRUCTURE, DISTRIBUTION, AND FUNCTION. Panel chairman: **R. Malcolm Brown**, University of Texas, Austin. Panel members: **H. J. Arnott**, University of Texas, Austin; **T. Bisalputra**, University of British Columbia, Vancouver, Canada; **L. R. Hoffman**, University of Illinois, Urbana.

The pyrenoid was observed and described in 1803 (49) by Vaucher. The term pyrenoid (NL fr GK *pyren*-stone of a fruit) was introduced by Schmitz in 1882 (40). He described the pyrenoid as a dense, highly refractive, spherical body in the chromatophore, now usually called the chloroplast, which is frequently surrounded by one or several layers of tightly packed starch granules. The similarity of the pyrenoid to the nucleolus of the nucleus was noted. Pyrenoid activities were recognized and reported as early as 1850 by Braun (9). Boubier in 1899 showed that starch grains are formed in a special layer on the surface of the pyrenoid (6,7). Timberlake (45) believed that the proteinaceous pyrenoid body itself was transformed into starch, a view which was based entirely on fixed material and was later questioned by Czurda (15) in 1928 and Bold (3) in 1933. In 1928, Czurda published the most complete account of the algal pyrenoid up to that time (15). Subsequent major publications dealing with light-microscopic observations of the pyrenoid include the work of Bold, 1951 (4); Smith, 1950 (43); Simon, 1954 (41); Fritsch, 1935 (19). Some of the earliest electron micrographs of algal pyrenoids were made by Steinmann in 1952 (44) and by Wolken & Palade in 1953 (50). For convenience, the following groups of notable recent publications on algal pyrenoids will be listed under a given major algal division. Only in the Cyanophycophyta are pyrenoids never present:

Chlorophycophyta:

Leyon, 1954 (29); Sager & Palade, 1957 (39); Ueda, 1961 (47); Gibbs, 1962 (24); Lang, 1963 (27); Manton, Oates, & Parke, 1963 (32); Brown & Bold, 1964 (11); Bisalputra & Weier, 1964 (2); Lembi & Lang, 1965 (28); Manton & Parke, 1965 (33); Parke & Manton, 1965 (36).

Chrysophycophyta:

Bose, 1941 (5); Hovasse & Joyon, 1960 (26); Manton & Lee-dale, 1961 (31); Gibbs, 1962 (22,23); Drum, 1963 (16); Drum & Pankratz, 1964 (17); Manton & von Stosch, 1965 (34).

Pyrrophyphyta:

Gibbs, 1962 (22,23).

Phaeophycophyta:

Simon, 1954 (41); Manton, 1957 (30); Gibbs, 1962 (22); Bouck, 1965 (8); Evans, 1966 (18).

Rhodophycophyta:

Brody & Vatter, 1959 (10); Mitrakos, 1960 (35); Gibbs, 1962 (22); Gantt, 1965 (20).

Euglenophycophyta:

Gibbs, 1960 (21); Ueda, 1960 (46); Gibbs, 1962 (22).

On the basis of light microscopy, pyrenoid structure has not been adequately defined except to indicate that its ground substance is colorless in some instances (12) and exhibits a green color in others (29). In the Phaeophycophyta, Simon (41) has reported the density of the pyrenoid to be intermediate between that of the cell chloroplast and cell hyaloplasm. This observation was based on the relative movement of cell organelles in a centrifugal field. Micromanipulation experiments have revealed that the pyrenoid in the Phaeophycophyta undergoes dissolution following puncture of the chloroplast (41).

Our knowledge of the ultrastructure of the pyrenoid ground substance has been very limited due to a lack of adequate preservation with permanganate fixation. Our best evidence for structural organization of the pyrenoid ground substance comes from the work of Gibbs (24) who reported the presence of fibrils or granular substances 60 Å in diameter in certain green algae. This work was based on osmium fixation. Our present knowledge of the exact structure of the ground substance is still limited because of an absence of the combined application of high-resolution electron microscopy and the newer glutaraldehyde procedures (38). Recent investigations of Dr. H. J. Arnott and the author (1) have revealed the presence of several types of fibrillar particles in addition to those described by Gibbs (24). The structure of these particles has been made evident by examining various stages of dissolution of the ground substance during zoospore formation in *Tetracystis*. If the structure of the pyrenoid ground substance is constant in all representatives examined, as our present state of knowledge implies, then the pyrenoid is not a variable organelle insofar as its organization is concerned. The observed variation is a manifestation of the associated structures, namely the assimilatory products (carbohydrates [47,24,22,11] and lipids [17,18]), the nature of the associated chloroplast lamellae (47, 22,24,11,20), and the shape of the ground substance itself (11).

The formation of new pyrenoids has been frequently observed. The pyrenoid may divide giving rise to two or more daughter pyrenoids (11,41,18,15,48). In this case, pyrenoid division precedes chloroplast division and cleavage, nuclear division, and cytokinesis. In other instances, the pyrenoid disappears and new pyrenoid bodies arise seemingly *de novo* in the progeny (25,1,11,15). Recent evidence (12) indicates that new pyrenoids may be formed adjacent to or in the DNA-containing areas of the chloroplast, as described by Ris & Plaut (37).

Although the literature repeatedly states that the pyrenoid ground substance is composed of protein, the specific methods to detect proteins are usually not mentioned. One of the most

recent and authoritative studies on the chemical composition of the pyrenoid was published by Simon (41) who reported inconclusive or negative data with respect to the presence of glucosides, lipids, general proteins, ribonucleoproteins, and deoxyribonucleoproteins in pyrenoids of the Phaeophycophyta. Of interest was the positive report for alkaline phosphatase and negative data for oxidase. Leyon (29) reported the presence of chlorophyll in pyrenoids of *Closterium*, and this is not surprising in view of the quantity of chloroplast lamellae which penetrate the pyrenoid ground substance. In those pyrenoids which are not traversed by lamellae, the pyrenoid appears colorless (12) thus suggesting that the chlorophyll is associated with the chloroplast lamellae and not the ground substance. On the basis of acridine-orange fluorescence and methyl green pyronine, the author has suggested that the pyrenoid may contain quantities of RNA (12,11). On this basis, and considering the fact that pyrenoids do divide and are located in proximity to the DNA-containing areas of the chloroplast, it seems likely that the pyrenoid may have a genetic role in the chloroplast analogous to that of the nucleus in the cytoplasm. A better understanding of the chemical composition and the possible function of the pyrenoid as a genetic constituent awaits further investigation. It must not be forgotten, however, that certain algae lack pyrenoids.

The function of the pyrenoid in the formation of starch has long been recognized (5,4); however, the pyrenoid does not necessarily contribute to all of the starch which may exist because starch may be present in cells which lack pyrenoids (27). On the other hand, Bisalputra & Weier (2) have shown that so-called stroma starch in *Scenedesmus quadricauda* originates from the pyrenoid thus agreeing with the contention of Timberlake (45) and Smith (42) that all stroma starch is derived from the pyrenoid. In connection with pyrenoidal starch synthesis, it is believed that the pyrenoid ground substance is a center of condensing enzymes, but this remains to be unequivocally demonstrated. Certainly it now seems clear that the pyrenoid ground substance itself is not transformed into starch as suggested by Timberlake (45) and questioned by Bold (3). It has been suggested that the pyrenoid may function in the formation of lipids (5,17) in the Chrysophycophyta. The exact nature of the formation of paramylon in the Euglenophycophyta is unknown, although it exists in the cytoplasm adjacent to the surface of the chloroplast pyrenoid (21).

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