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Topic 4: Cytochemistry of Cellular Membranes: Biosynthesis and Membrane Specialization

BIOSYNTHESIS OF CHLOROPLAST MEMBRANES

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The membrane of the chloroplast discs (thylakoids) is expected to have a relatively small number of components since it appears to be highly and restrictively specialized in carrying out the initial steps of photosynthesis (photophosphorylation, photoreduction of NADP). This feature, together with its abundance and biological imporance, makes the thylakoid membrane suitable and interesting for an investigation of membrane biogenesis.

The process has been studied in a green alga, *Chlamydomonas reinhardi,* whose growth can be easily synchronized and for which are available a number of convenient mutants deficient in chlorophyll and membrane synthesis. One of them (Sager's yl) cannot synthesize chlorophyll and does not produce thylakoid membranes in the dark. Grown auxotrophically (on acetate) in the dark, it undergoes degreening and turns yellow. The chloroplast and many of its enclosed structures are retained, but the thylakoids and the stacks they form (grana) are progressively lost, until only a few single disorganized discs are left. Exposed to light, such yellow cells synthesize chlorophyll, by a photoreaction which bypasses the genetic defect, and produce membranes in large amount and at high rate. Full regreening is achieved in 9 to 12 hr, and during this period there is little cell growth and no cell division.

Results obtained in experiments with greening yl cells show that the algae accumulate chlorophyll at a rate comparable to that of their increase in photosystem II and photosystem I activity (Ohad, Siekevitz and Palade, *J. Cell. Biol.* 35; 531, 1967). The finding is compatible with the view that the membrane is assembled in one step, i.e. all components are introduced simultaneously and–as a result– the membrane is fully functional from the beginning. This concept, which implies an invariant biochemical composition for the membrane, was put to test by isolating the thylakoids at different times during greening and by measuring their content of a number of key components (cytochrome 553, chlorophyll, carotenoids). The relative concentrations of these components were found to vary continuously during greening, which ruled out one step assembly and suggested that this membrane –like others so far investigated– is assembled in a multistep operation (de Petrocellis, Siekevitz and Palade, *J. Cell Biol.* 44; 628, 1970).

Chlamydomonas, like all plant cells, has two ribosomal populations: small ribosomes (70S) in the chloroplast, and large ribosomes (80S) in the rest of the

cytoplasm (Hoober and Blobel, *J. Mol. Biol.* 41; 121, 1969). Experiments with specific inhibitors (cycloheximide for 80S, and chloramphenicol for 70S) suggested that proteins produced by both systems are required for thylakoid membrane assembly and that proteins synthesized by 70S ribosomes are needed for full activity and for the fusion of thylakoids into grana (Hoober, Siekevitz and Palade, *J. Biol. Chem.* 244; 2621, 1969). The proteins of isolated thylakoid membranes have been solubilized and resolved by electrophoresis on SDS gels in ~20 bands, and experiments using specific inhibitors, each in the presence of a different label (^{3}H-arginine or ^{14}C-arginine), have shown that a large number of the polypeptides of their membrane including its 2 major components, are produced in the cytoplasm (Hoober, *J. Biol. Chem.* 245; 4327, 1970; Eytan and Ohad, *J. Biol. Chem.* 245; 4297, 1970) and that, by comparison, the locally produced polypeptides represent quantitatively minor components. The finding implies that there is continuous transport of membrane proteins from cytoplasmic polysomes to the chloroplast, and recently suggestive evidence on the existence of a soluble precursor of one of the major membrane polypeptides was obtained (Hoober, *J. Cell Biol.* 52; 84, 1972).

Multistep assembly appears well established for thylakoid membranes produced during greening, but since this is a special stituation it may be argued that the cell takes care of it by a special formula. To find out whether multistep assembly is the general formula, rather than a special one, thylakoid membrane production was studied in wild strain Chlamydomonas whose growth was satisfactorily synchronized by exposure to alternative 12 hr light-12 hr dark periods. In such cells, key components of the membrane (cytochromes 553, 559, 563, chlorophyll) were found to accumulate at different and specific times during the light period of the cycle. Since in addition some of these components appear to have a rapid turnover, the biochemical composition of the membranes varies continuously and reproducibly throughout the cycle. These results (Schor, Siekevitz and Palade, *Proc. Nat. Acad. Sci.* 66; 174, 1970) in corroboration with others (Armstrong, Surzycki, Moll and Levine, *Biochemistry* 10; 1971) finally establish that under physiological conditions the cells use a multistep assembly procedure for the production of these membranes.

An additional important aspect in membrane biosynthesis concerns the topography of the assembly process. Many of the speculations of the past have considered the production of membranes *de novo* or have made the distinction between new and old membrane. All new molecules could be introduced in a distinct region of the membrane to produce a localized growth area, or could be inserted - apparently at random - throughout the membrane. In the latter alternative, the membrane expands in all directions; there are no areas of growth, no new membrane distinguishable from old, the membrane containing throughout its extent a mixture of old and new molecules.

Topography of assembly can be studied by localization procedures like radioautography (Goldberg and Ohad, *J. Cell Biol.* 44; 572, 1970), or by trying to separate old from new membranes (in y-1 greening experiments), taking advantage of the fact that the membranes become denser as greening progresses (Eytan and Ohad, *J. Biol. Chem.* 247; 112, 1972). The results of such studies show that at the time of their localization the new components appear fully randomized throughout the membranes. Randomization can be obtained by insertion of

new components according to a disperse formula, or by lateral diffusion within the plane of the membrane after regional insertion. A choice between these two alternative is not possible with the evidence available at present. It is noteworthy, however, that the cell dismantles regularly the thylakoid stacks to minimal dimensions (2 thylakoids), and creates numerous areas of focal separation between these thylakoids at the time (2 to 6 hr in the light period) when new components (cytochromes, chlorophyll) are inserted in the membrane. This suggests that the cell makes available a large part of the thylakoid membrane surface for the insertion of new components. Conversely, dismantling of the grana may facilitate lateral diffusion of newly inserted membrane proteins.