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EXPERIMENTAL ANALYSIS OF MEMBRANE TRAFFIC DURING SECRETION OF SCALES IN GREEN ALGAE

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INTRODUCTION

Many flagellated algae release cell surface-associated structures of discrete size and shape (scales) by exocytosis following assembly of these structures in the Golgi apparatus (GA). Studies on biogenesis and exocytosis of the large cellulosic scales of Pleurochrysis showed that these scales are transported through the GA by cisternal progression and are exocytosed still enclosed in a GA-cisterna (Brown 1974). In recent years it has become obvious that the GA-stack of some animal cells consists of at least three functionally and compositionally distinct compartments (cis-, medial-, trans-compartment; e.g. Dunphy and Rothman, 1985). These and other data have led to the now widely accepted hypothesis that secretory proteins are transported through the GA by a shuttling of vesicles between successive GA-cisternae (Rothman and Lenard, 1984) and not by cisternal progression (as proposed by the endomembrane flow hypothesis; Franke et al. 1971). Scale formation in Pleurochrysis is then regarded as "a rare formula connected with the unusual geometry and size of the product" (Farquhar and Palade, 1981). We have recently studied scale biogenesis in the green flagellate Scherffelia dubia (McFadden and Melkonian, 1986; McFadden et al. 1986; Melkonian et al. 1987). This organism synthesizes numerous small-sized scales (ca. 40 nm diameter) in the GA and should in this respect be more comparable to other secretory systems. Conclusive evidence has been presented that in this scale-secreting system transport of scales through the GA occurs

also by cisternal progression and not by shuttle vesicles (summarized in Melkonian et al. 1987).

Here we would like to examine briefly the advantages of the scale-secreting systems Scherffelia and Tetraselmis as model systems for studying exocytosis in plants and for the experimental dissection of membrane traffic.

RESULTS

Scherffelia (freshwater) and Tetraselmis (most species marine) are unicellular algae that can be grown to large cell densities in simple inorganic growth media. In addition the cell cycle can be synchronized by alternating light/dark regimes (Ricketts, 1979; Reize and Melkonian, 1987). In contrast to other scaly algae (e.g. Pleurochrysis) Tetraselmis and Scherffelia synthesize scales only during a limited time period (3 hrs) when cells divide. Scales are of two types: thecal scales (these form the cell wall or theca by extracellular self assembly) and flagellar scales (3 varieties: pentagonal scales, rod-scales, hair-scales). During cell division assembly of thecal and flagellar scales is temporally separated. Scale synthesis can also be induced experimentally by a) flagellar amputation (only flagellar scales are synthesized; McFadden and Melkonian, 1986) or by b) producing protoplasts (only thecal scales are synthesized; Melkonian et al. 1987). Induction of scale synthesis transforms the GA into a scale-synthesizing machinery within 15 min. The precise timing of the induction of scale synthesis allows pulse-chase experiments using radioactive precursors to be performed as well as the application of inhibitors of GA-function. Monensin (100 nM), for example, leads to a dilation of trans-GA cisternae and blocks scale biogenesis and flagellar regeneration in Scherffelia dubia (unpublished observations). Additionally one can trace the signals that are responsible for GA-activation. We know that deflagellation in the absence of external calcium does not lead to GA-activation, so an increase in cytosolic free calcium may be required to activate the GA for scale secretion.

Another advantage of the scale-secreting system Scherffelia/Tetraselmis is that scales are readily visible in the EM and there is no need to use cytochemical and/or immunocytochemical methods to localize the secretory product at the ultrastructural level. It is also easy to quantify the amount

of secretory product simply by counting the number of scales present in the cellular compartments and on the cell surface. Since the GA is inactive through most of the cell cycle and scales are always found in regular patterns on the surface of flagella and cell body, it follows that the number of scales present on the cell surfaces represents roughly the number of scales that are synthesized during cell division. When this number is compared with the number of scales present in the intracellular organelles during scale secretion, one can calculate scale production rates for the GA (McFadden and Melkonian, 1986; McFadden et al. 1986). Since it has been found that scales are not transported through the GA by shuttle vesicles, but through cisternal progression one can also estimate membrane flow rates through the GA (Melkonian et al. 1987). From these estimations we concluded that a surplus of membrane flow accompanies scale secretion and that a considerable proportion of the transported membrane is retrieved via unknown mechanisms (McFadden and Melkonian, 1986; McFadden et al. 1986).

The scale-secreting system Scherffelia/Tetraselmis offers also the possibility to investigate sorting mechanisms of secretory products during secretion. Flagellar scales are of three structurally different types. All types can be found within one GA-cisterna, but they are located in different domains of this cisterna. We found that the scale-type which after exocytosis is attached to the plasmamembrane is exclusively located at the cisternal rims suggesting that the newly synthesized flagellar membrane is also confined to this region of a GA-cisterna (McFadden and Melkonian, 1986). We are now attempting to immuno-localize the prospective flagellar membrane using an antibody against the major flagellar membrane protein (Mr 200000). Interestingly, all three flagellar scale types are abstracted from the trans-side of the GA into one secretory vesicle indicating that in this system sorting of scales does not occur in the GA but later in the secretory pathway. Consequently we have been unable to identify clathrin-coated membranes at the cisternal rims of the trans-GA-region.

The GA in these flagellates consists of 2-4 large Golgi bodies that are closely associated with the nucleus. It should therefore be possible to isolate the Golgi bodies together with the nucleus. If this becomes possible it would pave the way for in vitro studies of membrane traffic during scale secretion.

The most perplexing result of the previous investigation is that the transport of secretory products through the GA-stack occurs by cisternal progression. It may be argued that all except the three cis-most cisternae do not belong to the GA, but represent flattened "condensing vacuoles". Since nascent thecal scales can be traced back to the 2nd cis-most cisterna, this interpretation is unlikely. Additionally we note that the secretion-inactive GA consists of even more cisternae (19-21) than the scale-secreting GA. Alternatively it may be possible that the GA of scale-secreting algae is not biochemically compartmentalized into three subcompartments as is the GA of some animal cells. Cytochemical studies to localize marker enzymes for different GA-compartments are underway in this laboratory.

CONCLUSION

Secretion of scales in the green flagellates Scherffelia and Tetraselmis represents a unique model for studying membrane traffic in lower plants. The system is amenable to experimental manipulation and in vitro studies should be possible. Since secretory products are transported through the GA-stack by cisternal progression this system could represent an alternative type of GA-membrane traffic operative mainly in plant cells.

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