Chloroplast Autonomy in Pigment Synthesis

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With 4 Figures

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Summary
An experimental design developed using Acetabularia permits a novel approach to studies of cytoplasmic genetic functions. The site of the genes for the enzymes of the plastid pigment pathways were examined by 1. determining pigment content per cell and per chloroplast in intact cells and during long periods of enucleate cell growth, 2. comparing pigment synthetic activities of plastids isolated from intact and enucleate cells at various times post-enucleation and 3. comparing the ability of intact and enucleate cells to modulate pigment content in response to various light regimens.

Intact cells grow and increase their pigment content exponentially. Enucleate cells grow at the control rate for several weeks and increase their chloroplast number and pigment content proportionally. Pigment content per plastid remains constant in both intact and enucleate cells. Pigment synthesis in isolated chloroplasts from enucleate cells is normal up to 65 days post-enucleation when compared with isolated chloroplasts from intact control cells. Enucleate cells differentially modulate their pigment content in response to various light regimens in a manner indistinguishable from normal cells.

The problems in interpreting these and other results are discussed and it is concluded that plastid autonomy in pigment synthesis is the simplest explanation.

1. Introduction
In the presence of the nucleus and the translation system of the cytosol it has been difficult to obtain definitive information on the encoding of chloroplast proteins. Unlike the situation in free living organisms, the simple presence of an m-RNA or a protein within the chloroplast is not adequate evidence for its coding by the chloroplast genome (SAGER 1972). The giant alga Acetabularia provides a system that circumvents many of the obstacles to study of the chloroplast genome. Reasonable quantities of cells may be surgically enucleated, instantly and completely "arresting" nuclear transcription. Chloroplasts

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multiply and grow in the enucleate cell (Shephard 1965 b, Clauss et al. 1970, Vanden Driessche et al. 1973) and the chloroplast genome seems to continue normal activity for many weeks (Shephard 1965 a) while nuclear products are decaying and being diluted by growth. The enucleate system thus approaches the situation of a free living organism or a cultured organelle population. Under these conditions the synthesis of a chloroplast RNA species would necessitate its transcription from the chloroplast genome. A continued normal content and synthetic rate of a plastid protein strongly suggests in situ transcription and translation, as therefore does the normal functioning of an enzymatic pathway. There is no absolute need to obtain mutations, and drugs need not, although they may, be used. Moreover, the Acetabularia system has yielded a highly intact and relatively uncontaminated isolated chloroplast fraction (Shephard and Bidwell 1973) that is active in photosynthesis (Shephard et al. 1968) and biosyntheses (Shephard and Levin 1972, Moore and Tschismadia 1977, Tschismadia and Moore 1978) and provides a standard preparation lacking in cytosolic activities. This fraction is capable of forming the plastid pigments at normal rates in vitro (Moore and Shephard 1977).

We have used this system in an attempt to examine whether or not the enzymes of the pigment pathways are encoded in the plastid genome. Three approaches have been used. The first was a determination of the pigment content per cell and per chloroplast in intact cells and during long periods of enucleate cell growth. The second was a comparison of the pigment synthetic activities in chloroplasts isolated from intact and enucleate cells at various time intervals post-enucleation. The third compared adaptive changes in the pigment content of intact and enucleate cells subjected to various light regimens. The results of these studies are presented here.

2. Materials and Methods

Stocks of Acetabularia mediterranea and Acetabularia (Acicularia) schenckii are maintained in continuous laboratory culture by methods previously reported (Shephard 1970). Cells for enucleation were selected during the log phase of growth. All manipulations were carried out under a sterile hood. Cells were carefully matched for size and condition and half were enucleated by ligation with 7-0 surgical nylon thread at a point just above the rhizoid. When the rhizoid containing the nucleus was amputated no cytoplasmic loss occurred and the resulting enucleate cells were only slightly smaller than the intact controls (Shephard 1970). Enucleate and intact control cells were grown under identical conditions. Enucleate cells which had lost the ligatures were not used since they frequently had also lost cytoplasm.

When individual cells were to be assayed for pigment content, they were randomly selected from enucleate or control groups, blotted dry and ground in a small volume of 80% acetone. The cell debris was centrifuged from the extract before photometric measurement.

Chloroplast isolation was carried out using mannitol based media as previously described (Shephard and Levin 1972). The preparations were sheared by passage through 5 or 8 μ Nuclepore filters (General Electric Co., Pleasanton, Calif.) to remove adhering cytosol, and centrifuged through step gradients to eliminate cytosolic and bacterial contamination.