

Horizontal Transfer of Functional Nuclear Genes Between Multicellular Organisms

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The horizontal transfer of functional genes between organisms is the theoretical foundation of the endosymbiotic origin of cellular organelles, as well as the basis of genetic therapies and the technology of genetic modification. Without doubt, transfer of functional genes is routine between prokaryotes (1), has occurred between both mitochondria (2) and chloroplasts (3), and the cell nucleus. In addition, DNA has been transferred from endosymbiotic bacteria into insect host cell nuclei (4). However, no direct evidence exists for the natural transfer of nuclear genes between multicellular organisms. We have recently presented circumstantial and pharmacological evidence that nuclear genes encoding for chloroplast proteins are transferred from an alga to an ascoglossan sea slug (5, 6). We now demonstrate, using molecular techniques, that such a gene is present in the genomic DNA of the slug.

Elysia (= Tridachia) crispata is one of a few species of elysiid sea slugs that has an intracellular symbiosis of several months' duration with chloroplasts acquired from specific, siphonaceous algal food. The slug slits open the algal filament with its radula and sucks the contents into its digestive system. As digestion proceeds, certain cells lining the digestive diverticula phagocytize the plastids into intracellular vacuoles. In some species, the chloroplasts reside in their vacuole for as long as 8–9 months (5, 6, 7), 3–4 months in *E. crispata* (8, 9). In several elysiid slugs, including *E. crispata*, the plastids remain photosynthetically active, and photosynthetic carbon fixation contributes to a variety of molecules that participate in the slug's energy metabolism and mucus production (9, 10, 11).

Maintenance of a chloroplast's photosynthetic functions

requires that a variety of proteins associated with the photosystems turn over, but chloroplast genomes only code for a small fraction of the proteins needed for plastid function (e.g., 11, 12). For example, in chromophytic algae—the food source of some species of elysiids—the chloroplast genome encodes only 13% of the plastid proteins (13). In higher plants, the genes for as many as 90% of plastid proteins, including many of the photosystem components, are located in the cell nucleus (3). Therefore, the persistence of photosynthesis in the endosymbiotic plastids indicates that protein turnover must be occurring, and that support from the nuclear genome of the slug must be necessary.

The algal species providing the plastids in *E. crispata* (and many other species of elysiid slugs) is unknown and controversial. Some reports indicate that *E. crispata* eats, primarily, species of *Caulerpa*, especially *C. verticellata* (14). Others (9) report that *E. crispata* does not consume *Caulerpa* spp. at all, but rather eats other genera, such as *Batophora*, *Bryopsis*, *Halimeda*, and *Penicillus*. These conflicting results suggest that *E. crispata* eats a variety of ulvophytic, coenocytic algae; but whether it retains chloroplasts from multiple algal species is unknown and is a matter that we are currently investigating. Regardless of their origin, the endosymbiotic chloroplasts in *E. crispata* are unexceptional in that they require substantial protein synthesis support from the nucleus. When slugs are incubated in ³⁵S-methionine (methods in 6, 15), radioactivity is incorporated into many plastid proteins (Fig. 1). In *E. crispata*, some of this protein synthesis is inhibited by chloramphenicol, a well-studied blocker of organelle-encoded protein synthesis on organellar ribosomes. The synthesis of the remaining chloroplast proteins is blocked by cycloheximide, an inhibitor of nuclear-encoded protein synthesis on cytosolic ribosomes (Fig. 1). These results, to-

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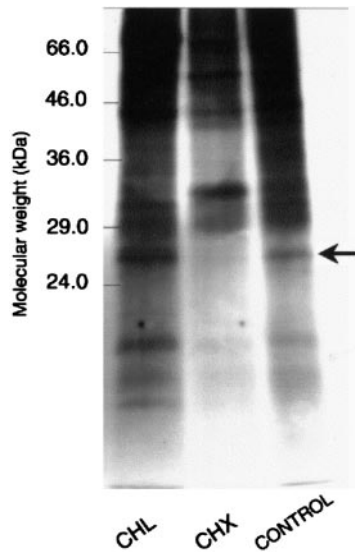


Figure 1. Fluor-enhanced autoradiogram of a 12.5% SDS-polyacrylamide gel electrophoresis (PAGE), showing the differential effects, of chloramphenicol (CHL) and cycloheximide (CHX) on the synthesis of *E. crispata* chloroplast proteins. After dose-response curves determined effective concentrations, slugs were preincubated, for 2 h under intense artificial light, in one of the inhibitors (chloramphenicol, 2.5 mg ml⁻¹; cycloheximide, 2.0 mg ml⁻¹) or (as a control) in DMSO carrier (0.024 %). The solutions were made in artificial seawater. After this pre-incubation, ³⁵S-methionine (40 μCi/ml) was added, and the slugs were incubated for an additional 6 h. A chloroplast fraction was then prepared, and the proteins were extracted (6) and electrophoresed. The gels were stained with Coomassie Brilliant Blue, dried, and exposed to X-ray film. The arrow indicates the position of a protein, which we have identified as FCP (see text); note that its synthesis is inhibited by CHX.

gether with similar findings we have reported from a closely related species, *Elysia chlorotica* (6), indicate that a variety of chloroplast proteins are being synthesized while the plastids reside in the cytoplasm of the slug's digestive cells. More important, the cycloheximide inhibition clearly demonstrates that several of the proteins are being translated on cytoplasmic ribosomes, indicating, in turn, that the genes encoding those proteins are located in the animal cell nucleus.

We used antibodies on Western blots and immunoprecipitations to identify the proteins in *E. crispata* whose synthesis was blocked by cycloheximide. Antibody screening, based on both molecular weights and available antibodies, was done on Western blots of protein extracts from radiolabeled isolated chloroplasts. A polyclonal antibody raised against fucoxanthin-chlorophyll binding protein (FCP) from *Pavlova gyra*ns was kindly made available to us by Dr. Marvin Fawley at North Dakota State University. This antibody binds to a Western blot (Fig. 2) at a position corresponding to the molecular weight of a prominent, cycloheximide-inhibited band (Fig. 1)—a result similar to those with *E. chlorotica* (6, 7). The FCP antibody also precipitates radioactivity from ³⁵S-labeled chloroplast pro-

teins (using standard protein A Sepharose procedures), and the amount of radioactive immunoprecipitate is reduced by 71% in the presence of cycloheximide. These immunoprecipitation results clearly indicate that synthesis of FCP is blocked by cycloheximide.

To ensure that the immunolabeled protein in the Western blot was indeed FCP, we purified it from *E. chlorotica* chloroplasts and determined the N-terminal amino acid sequence and three internal sequences. BLAST searching indicated that the N-terminal 30 amino acids had a 66% identity to those of the FCP protein of the chromophyte, *Cylindrotheca fusiformis* (AAN08832); and one of the internal sequences (11 amino acids) had an 81% identity with the FCP protein (Q40300) of *Macrocystis pyrifera*, another chromophyte. The remaining two internal sequences showed no significant similarity to known FCP sequences, but few algal FCP sequences are available in the databases, and sequence variation is high in some regions of the protein. Together, therefore, the immunological results and amino acid sequence similarities show that a chloroplast protein in *E. crispata*, whose synthesis is blocked by cycloheximide, is FCP.

Degenerate primers, based on the N-terminal and internal amino acid sequences of the native FCP, were used in PCR to amplify the gene encoding FCP from *Vaucheria litorea*, an alga eaten by *E. chlorotica* (5) and, according to our feeding experiments, by juveniles of *E. crispata*. A 350 bp PCR product was generated and sequenced. BLAST searching of the translated sequence confirmed that the PCR

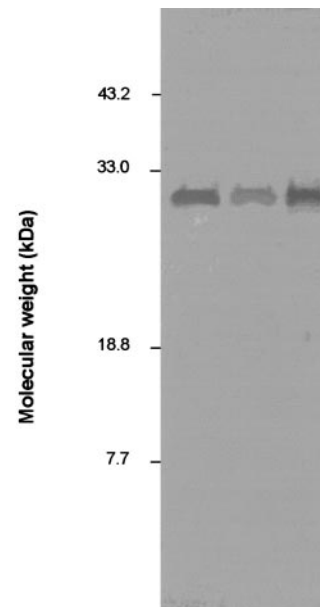


Figure 2. Western blot of a PAGE of chloroplast proteins extracted from *E. crispata* exposed to chloramphenicol (left lane), cycloheximide (center lane), or DMSO carrier (right lane), as described in Figure 1. The blot was exposed to a primary antibody (anti-FCP, see text), and then labeled with secondary antibody (HRP-conjugated, anti-rabbit IgG).

product is the gene encoding FCP, with a 51% amino acid sequence identity with *Laminaria saccharina* FCP (*lhcf7*) (AF226863). Moreover, the two unidentified internal amino acid sequences mentioned above were identical with portions of the translated gene sequence.

The most conserved region of our native FCP amino acid sequence was used to design a 100 bp probe for Southern blotting of *E. crispata* genomic DNA, which had been prepared by differential centrifugation on cesium chloride gradients (16). A prominent *fcp* homolog was revealed on blots of *E. crispata* DNA (Fig. 3), but not on the negative control—genomic DNA from *Aplysia brasiliana*, another herbivorous opisthobranch sea slug, which does not retain chloroplasts. The *fcp* probe also labeled Southern blots of genomic DNA from *Vaucheria litorea* (not shown), not surprising since FCP's are always encoded by the algal nuclear genome (17).

Together, the pharmacological, immunological, and molecular results establish that a gene for an algal chloroplast protein is present in the genomic DNA of *E. crispata*, where it waits for the acquisition of new plastids in each generation of slugs. Similar pharmacological and immunological results suggested that genes for another group of chloroplast proteins, light harvesting complex (LHC), have been trans-

ferred from the nuclear genome of *V. litorea* to that of *E. chlorotica* (6).

Always lurking in the background, casting doubt on these conclusions, is the concern that, somehow, algal nuclear DNA, either from nuclei remaining in the gut lumen, or taken up with the chloroplasts, has contaminated the slug genomic DNA. This is most improbable for several reasons. First, the slugs were starved for at least 7 days before any of the experiments were done. Thus, the guts were empty, and contamination from that source was therefore unlikely. Second, we used primers designed from conserved regions of the 18S rRNA gene to generate a PCR product from *E. crispata* DNA. This product (accession number AY292202) was 98% identical to the 18S rRNA gene sequence of the elysioid slug *Thuridilla bayeri*, and 96% similar to that of *Limapontia nigra*, also an ascoglossan species. Therefore, the DNA we used in the Southern blots and PCR experiments was molluscan, not algal. Third, neither other molecular probing (18, 19) nor extensive electron microscopy (5, 20) has ever revealed any evidence of algal nuclear material in the slugs. Finally, it seems extremely unlikely that that algal nuclei could be taken up by all the chloroplast-containing cells in every animal.

If the gene-encoding FCP is located in the slug DNA, a horizontal gene transfer between algal and slug nuclei must have occurred. The means by which such a transfer of genes between multicellular organisms might occur is presently unknown. However, every specimen of *E. chlorotica* that we have examined for it over the last decade has contained an endogenous virus with several characteristics of the Retroviridae. The virus is present in hemocytes, digestive cells, and plastids and is expressed just before the annual synchronous death of the population (5). A comparable virus has not yet been demonstrated in *E. crispata*, nor have other transferred genes been reported in other species of chloroplast-retaining elysioids. However, a retroviral mechanism of gene transfer offers an interesting explanation of our results, and may be an important, even common, mechanism of evolution.

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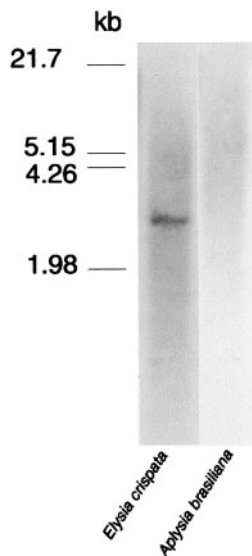


Figure 3. Autoradiogram of a Southern blot of genomic DNA from *E. crispata* and *A. brasiliana* labeled with a ^{32}P -labeled 100 bp nucleotide sequence constructed from the native FCP amino acid sequence. Genomic DNA was purified from the two species using cesium chloride density centrifugation (16). *Hind*III-digested genomic DNA from each species (5 μg) was electrophoresed on a 0.8% agarose gel. The DNA was transferred and UV crosslinked to a Pall Biodyne Plus nylon membrane. The membrane was hybridized with the radiolabeled probe in buffer (6 \times SSPE, 0.5% SDS, and 50 $\mu\text{g ml}^{-1}$ heparin) (16) overnight at 42°C. The blot was washed at 50°C with 0.1 \times SSC, 0.1% SDS and exposed overnight to X-ray film (16).

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