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Molecular Biology of Lichens: A Look to the Future

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Abstract

The rapidly developing field of genetic engineering provides mycologists with an excellent opportunity to study the lichen symbiosis at the molecular level. It is now possible to isolate genes of lichen-forming fungi, make libraries of these genes, and use them to transform plants or fast-growing fungi with the best features of the lichen symbiosis. Combining the techniques of molecular biology with those of artificial synthesis makes it possible to devise studies to determine if gene transfer takes place, by means of plasmids, between bionts and whether the mycobiont genetically controls the photobiont. This paper considers possible directions in lichenological research along molecular lines.

1. Introduction

Although lichens have great economic potential, they have not been suitable for commercial exploitation because of their slow growth and inability to grow in the laboratory. Molecular biology, however, allows us to circumvent these obstacles and gain greater access to lichens and their unusual characteristics, some of which are as follows: (1) Lichens synthesize several hundred different and unique organic compounds many of which inhibit the growth of bacteria, fungi, viruses, protozoa, and plants. This characteristic alone merits detailed study since these compounds represent an entirely new class of antibiotics. In nature, these compounds appear to protect the long-lived thalli from microbial decay. (2) Lichens withstand desiccation and often grow in habitats such as bare, exposed rock surfaces where other life forms cannot survive. (3) Lichens grow very slowly — there does not appear to be a rapid turnover of cells inside a thallus. Can DNA molecules remain intact

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and unchanged during the long periods of a lichen's lifetime? What protects these molecules? (4) Lichens absorb and concentrate metals and radionuclides from the outside environment. Such efficient mechanisms of uptake have made lichens vulnerable to pollutants such as sulfur dioxide and thus valuable pollution indicators. (5) Lichen fungi have shown biological ice nucleation activity which may be involved in moisture uptake and/or frost protection (Kieft and Ahmadjian, 1989).

Some of the qualities exhibited by lichens must be genetically determined. If so, then the genes which code for these traits could be incorporated into suitable vectors that might be used to transform other organisms. Although very little is known about the genetics of lichen bionts, gene flow has been demonstrated to occur in a natural lichen population (Culberson et al., 1988).

Some of the goals that could be pursued in molecular lichenology include the following: (1) To search for genetic messages (on plasmids) that pass between the bionts of a lichen. (2) To make a gene library of the genomes of selected mycobionts and photobionts and clone their genes in suitable vectors. (3) To determine if any of the genes can express themselves in other organisms such as bacteria. (4) To see if there is a genetic basis for the morphological transformation of a fungus into a lichen. (5) To apply the techniques of molecular taxonomy to lichens.

2. Protoplast and DNA Isolation From Mycobionts

Methods for isolating protoplasts and DNA from cultured mycobionts of lichens have been described in Ahmadjian et al. (1987). Protoplast isolations from lichen mycobionts have also been accomplished by Japanese investigators (unpublished). Separation of mycobiont DNA into mtDNA, nDNA and plasmid DNA was carried out by cesium chloride/bisbenzimide density gradient centrifugation (unpublished). Armaleo and Clerc (1990) have developed a new method for isolating nucleic acids from lichen mycobionts.

Protoplast release from lichen fungi depends on the age of the cultures. Fungi in young cultures release more protoplasts than those in old cultures. This reflects the thinner walls of the younger hyphae. In culture, mycobiont hyphae have thick bands of polysaccharide material (Ahmadjian, 1982), which in the natural thallus functions in water storage. One of the interesting aspects that was discovered about mycobiont protoplasts has been their remarkable durability. Protoplasts of the mycobiont *Cladonia cristatella* Tuck. remained intact for more than 2 weeks in a citrate phosphate buffer and then regenerated to form hyphae in a medium containing the osmotic stabilizer sorbitol at 8°C. The durability of the protoplasts of a lichen fungus contrasted greatly with the fragility of *Microsporum gypseum* (dermatophyte) protoplasts that burst within a day or two if they had not regenerated (Chadegani et al., 1989). The toughness of the mycobiont protoplasts is not surprising considering the extreme environments under which most lichens grow.

The ability to isolate protoplasts from lichen mycobionts, the durability of these protoplasts, and the probability that the protoplasts can regenerate to form hyphae opens the door to transformation experiments as well as to hybridization studies between different strains of mycobionts. In order to obtain the large percentage of protoplasts necessary for such studies, however, a method must be found to remove the extrahyphal matrix formed by mycobionts in culture in order to expose their walls to digestive enzymes.

3. Gene Transfer Between Bionts

The movement of genes between cell organelles such as mitochondria, chloroplasts, and even the cell nucleus, appears to be a common phenomenon. Promiscuous DNA, the name given to the mobile DNA, has been demonstrated for a wide variety of species, including ascomycetous fungi from which lichen fungi evolved (Gellissen and Michaelis, 1987). Since mitochondria and chloroplasts (and some would argue nuclei as well) represent ancient and transformed symbiotic bacteria (Taylor, 1987), we are dealing basically with genetic exchange between symbiotic partners. One could speculate that if gene transfer occurs in one symbiotic system, such as that inside eukaryotic cell, and is also common in fungi (Mishra, 1985), then it most likely occurs in other symbiotic systems as well.

There are two possible ways of looking at gene exchange in lichens. One way is to consider the long evolutionary history of lichens and assume that genetic exchange occurred during that period of time. The other way is to consider the possibility of short-term exchange during the development of a lichen. There is evidence to support both possibilities.

With regard to evolutionary exchange, lichens are ancient (according to Hallbauer et al., 1977, fossil specimens found in some precambrian conglomerates in South Africa resemble certain types of lichens), they are highly coevolved (Ahmadjian, 1987), and they consist of an autotroph (i.e. the photobiont, which is either an alga or a cyanobacterium and could be considered as analogous to a chloroplast) and a heterotroph (i.e. the fungus, which is analogous to the mitochondrion). The discovery in a lichen of a cholinergic-like system that regulates a developmental process also indicates an ancient origin for lichens (Raineri and Modenesi, 1986). Another point that argues in favor of evolutionary gene transfer between lichen bionts is that the age of the individual cells of the algae and fungi may be very old. Lichens live for hundreds and even thousands of years. The oldest reported age of an individual lichen that I am aware of was made by Denton and Karlen (1973) who stated: "The largest thallus of *Rhizocarpon alpicola* measured 480 mm in diameter. If the

extrapolated portion of the lichen growth curve is approximately correct, the large thallus near Sjangeli may be as much as 9000 years old". Since there is little evidence of cell turnover inside of a thallus, the individual biont cells may be many years old. According to Hill (1985), "The balance of the codevelopment (of lichen symbionts) involves the coordination of the expression of the two separate genomes. These features suggest that the photobiont population increases rapidly at and near the growing point but, with a certain amount of cell turnover, remains more or less constant in the rest of the thallus. It appears, therefore, that the photobiont cell population must be under some kind of limiting control involving changed rates of progress through the cycles of cell growth and division".

It is difficult to imagine that individual cells of the lichen symbionts have remained genetically isolated from each other, i.e. that communication, perhaps through gene exchange, has not occurred between them over such long periods of time.

Evidence for possible short-term genetic exchange between bionts comes from the results of artificial lichen synthesis studies (Ahmadjian, 1973; Ahmadjian and Jacobs, 1983). These studies have revealed that lichen fungi are virulent parasites on algae and that during lichenization both bionts undergo various types of transformation. Ahmadjian and Jacobs (1983) concluded that in the lichen symbiosis some type of regulatory process, which they called controlled parasitism, is involved.

I believe that we are now in a position to determine if short-term gene transfer occurs between lichen symbionts. We have developed techniques to grow algal, cyanobacterial, and fungal symbionts in the laboratory under axenic conditions and in large quantities. We have a collection of clonal cultures of lichen fungi and algae and can thereby work with only specific genetic types. We also may have found at least one mutant strain of the mycobiont *C. cristatella* which will not synthesize fully with its algal symbiont. Lastly, we can recombine symbionts in different combinations to form synthetic lichens. In effect, we can take a mycobiont clone, combine it with a phycobiont clone and develop an axenic lichen that consists of only two genomes, with no interfering contaminant microorganisms such as bacteria, fungi, and yeasts.

If gene transfer does occur between lichen symbionts, does it occur from the fungus to the alga or from the alga to the fungus or is there a reciprocal exchange? One place to look is from the alga to the fungus because it is the fungus that undergoes the remarkable transformation during lichen formation that results in a distinct morphological unit called the thallus. On the other hand, the fungus undoubtedly exerts control over the algal metabolism and carbohydrate release and it is possible that it does this by means of mobile

genetic elements. The symbionts of a lichen are in very close contact with each other and in many lichens the fungus penetrates the algal cells by means of haustoria and the symbionts are separated by only thin cell walls. In symbiosis the photobiont cells are extremely permeable and photosynthetic products such as ribitol, in the case of the green algae, and glucose, in the case of the cyanobacteria, readily pass out of the cell. In a lichen, the photobiont excretes 90% of the carbon that it fixes photosynthetically, but when the photobiont is isolated and grown separately from the fungus, excretion of carbon products either stops or is extremely low (1–2% of the total fixed C) (Smith and Douglas, 1987). The high degree of permeability of the photobiont cells in a lichen thallus suggests that different types of products pass out of the photobiont cells and are taken up by the fungus. It is conceivable that such products might include segments of DNA or mRNA.

One possible experiment is to analyze separately the mitochondrial genomes of the fungus and the alga (*Trebouxia erici*) of the lichen *C. cristatella.* This is a lichen with which we have had much experience and success with. We have isolated clones of both bionts, over 100 of the mycobiont, and can recombine the symbionts to form synthetic lichens (Ahmadjian, 1982). After different periods of time, the symbionts from the synthetic lichens could be reisolated into culture and their mtDNA analyzed and compared with the DNA patterns obtained before synthesis. Synthetic lichens can be maintained under laboratory controlled conditions for several years or longer, thus allowing plenty of time for gene transfer to occur.

A molecular view of lichens will reveal many surprises that will help to place these long-neglected associations into the mainstream of mycological research.

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