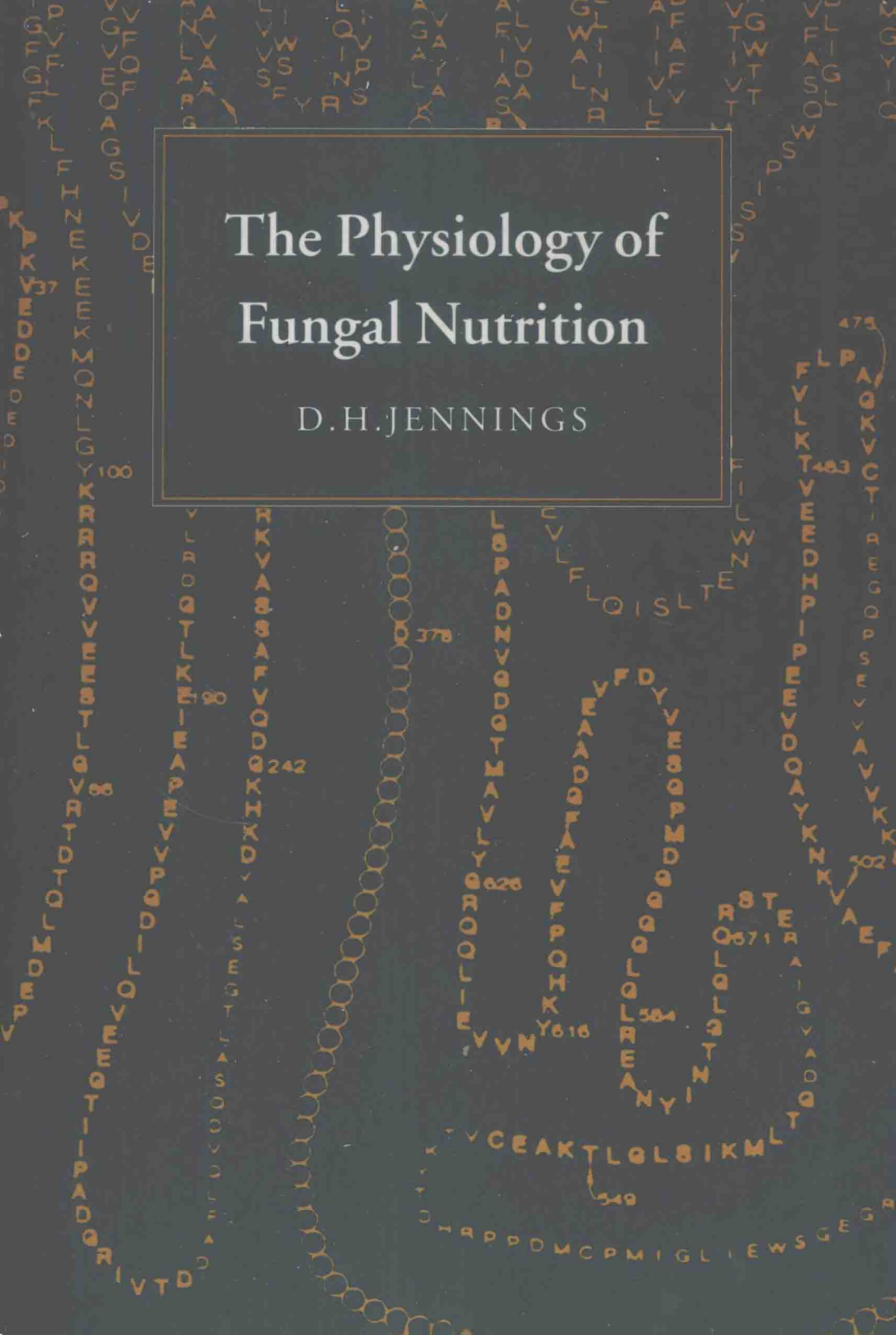

The Physiology of Fungal Nutrition

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The nutrition of a vegetative fungal colony can be viewed as a web of interconnected processes. In this volume, the author provides a mechanistic basis to the subject, focusing on processes at the plasma membrane, considering the modulating effects of the fungal wall and describing the fate of nutrients entering the fungus. The emphasis is physiological, but biochemical and molecular biological information has been drawn upon as appropriate to reflect the power of the multi-faceted approach and encourage such study further. A comprehensive review of what is known for the more commonly studied fungal species is complemented by information on other fungi to provide an indication of the diversity of nutritional processes that exist in the Fungal Kingdom.

THE PHYSIOLOGY OF FUNGAL NUTRITION

Introduction

As far as I am aware, there has only been one other book about fungal nutrition. This contrasts very markedly with the large number of books devoted to the nutrition of higher plants. Here, we have a coherent field of study, well established through the need to understand how plant yield might be increased through the provision of inorganic nutrients. The economic benefits of an effective fertiliser regime for an agricultural or horticultural crop have been an important driving force for establishing plant nutrition as an identifiable discipline in plant physiology. But this identity for plant nutrition is aided in general germs by the fact that the higher plant has a specific organ, the root, for absorbing nutrients and the effectiveness of that organ can be determined by observable responses in other parts of the plant, such as change in shape and colour.

Fungal nutrition is clearly about what kinds and amounts of nutrients will support growth or bring about differentiation, whether it be secondary metabolism on reproductive structures. However, to focus on fungal nutrition thus described would be to produce recipes and little else. This book is about the nutrition of fungi as a web of processes, attempting to provide a mechanistic basis for the subject. For the most part fungi do not produce specialised organs of nutrition, nor, because of their great physiological plasticity, which is much greater than that of higher plants, can one observe in such a clear manner the consequences of changed nutrient conditions for the vegetative colony. Usually, any observable response, apart from a change in growth rate, is differentiation.

It should be apparent that nutrition of the vegetative colony is not easily defined as a topic. The fungus responds to a change in nutrient conditions homeostatically, with changes both qualitatively and quantitatively in a plethora of processes. My focus in this book is on how the fungus interfaces with the external medium, both in terms of acquiring

nutrients from it and of avoiding the deleterious effects of constituents within it that might be toxic to the fungus. So the emphasis of the volume is for the most part on processes at the plasma membrane as well as a consideration of the wall as a structure modulating the interactions between the external medium and the protoplasm of the fungus. Inevitably one cannot isolate processes occurring at the plasma membrane from other processes occurring within the protoplasm. Here, my decisions as to what or what not to include have been to a certain extent arbitrary. But as a rough rule, I have indicated how a nutrient once entering a fungus interacts with primary metabolism or might have its concentration regulated by internal processes.

In this volume, the external medium is essentially the non-living medium/substratum inhabited for the most part by saprotrophic fungi. I have resisted the temptation to consider parasitic fungi because of the complexity of the interactions between fungus and host but also because my assessment of present knowledge is that our *physiological* understanding of what may be occurring is somewhat limited. To introduce too much speculation would run counter to the general philosophy underlying the text, namely that the emphasis should be on the facts about any process under consideration. Although the book is very much concerned with saprotrophic fungi, I have included, where appropriate, information about parasitic fungi when they are grown in culture and mycorrhizal fungi when they are behaving saprotrophically.

One aim in writing this book has been to indicate for those processes coming within its preview the diversity that exists within the Fungal Kingdom. Mycological research is dominated by studies on *Saccharomyces cerevisiae* and to a lesser extent on *Aspergillus* sp., *Neurospora crassa*, *Penicillium* and *Schizosaccharomyces pombe*. One must not ignore the very important work on these fungi and I have attempted to give adequate coverage of the information relevant to this volume. However, wherever possible, I have tried to give a sensible picture of what is known for other fungi for three reasons. First, I believe it is important that we do not, without good reason, try to extrapolate from what we know about processes occurring in the above-named species to equivalent processes occurring in species in other parts of the Fungal Kingdom. Second, by referring to the little that is known for much less-studied species I hope to encourage more research on those species. Third, I am very anxious that we avoid more positively the tendency to see the fungi as a physiologically homogeneous group of organisms. Though species possess similar physiological features, there are nevertheless many striking

differences. A stumbling block often to the appreciation of those differences is our ignorance of what a fungus might be doing in its natural environment. The need to attempt to draw together physiology and ecology of fungi is a theme underlying the volume.

Essentially this book has been written for those involved in fungal physiology, as teachers or students, but particularly those are active in research. For that reason I have concentrated on known facts and eschewed speculation, unless it points up a clear line of research. I have almost punctiliously avoided referring to what might be occurring in other eukaryotic organisms to avoid clouding the readers' judgement as to what might be happening in fungi when present knowledge seems inadequate. While there is a considerable amount of information in the volume, I cannot claim that it is encyclopaedic. Nevertheless, in spite of the rate of advance of the subject, I hope the volume will be a useful source of information for some period for those with interests in fungal nutrition. For those areas where coverage has been to an extent superficial, I hope the literature to which I have referred will allow the reader to probe deeper.

Finally, though the major emphasis of the volume is physiological, biochemical and molecular biological information has been drawn upon wherever appropriate. Not one of those approaches can give more than a partial picture of what might be taking place in a particular process. I stress this, because today's emphasis on molecular biology, the undoubted power of which must be acknowledged, has tended to lead to much less reliance on other approaches, particularly the physiological. Wherever possible, I have tried to show the success of the multi-faceted approach to the study of fungal nutrition. If this volume encourages future research also to be more multi-faceted then I shall be pleased.

On a more personal note. I wish to thank Karen McGowan for her great skill in transferring my poor handwriting onto computer disc. Also, I am particularly grateful to Sandi Irvine of Cambridge University Press for her meticulous editing of the manuscript. Any mistakes that are still present are my responsibility! But an especial word of thanks is due to all those many fellow scientists who over the years so kindly responded to my many requests for reprints. Without them, my job in preparing the book would have been immeasurably harder.

David Jennings

Contents

1

Primary active transport

Introduction

A living cell must do work to maintain the composition of its internal medium different from that of the external. If that cell is growing, work has also to be done to generate the small but necessary osmotic gradient for the inwardly directed influx of water to take place. In a cell with a wall, such as a fungus, the osmotic gradient is much more significant, in order to maintain the necessary internal hydrostatic pressure for turgor (see Chapter 13). Two important loci for the above work are the plasma and the vacuolar membranes. When work is carried out in moving solutes across these two or indeed any other membranes, we can speak of active transport, i.e. the movement of solutes against their electrochemical potential gradient. Of course, there may also be diffusion of solutes across the membrane, albeit almost always, if the solute is polar, aided by the presence of carrier proteins that overcome the activation energy required by such a solute to enter through the lipid portion of the membrane. When diffusion occurs, it must be down the electrochemical potential gradient. However, it needs to be remembered that if the affinity of such a carrier for the solute on one side of the membrane were to be much higher than on the other or the mobility of solute transfer were to be faster in one direction than in the other – both of which changes would depend on the cell doing work – then active transport would occur (Jennings, 1974).

Active transport may be classified as primary or secondary. Primary active transport or translocation is brought about by reactions that involve the exchange of primary bonds between different chemical groups or the donation or acceptance of electrons. These reactions lead to the translocation of a chemical group or solute across the membrane. Thus, chemical energy is used to generate a vectorial process. Secondary

transport or translocation does not involve primary bond exchange between different chemical groups or donation or acceptance of electrons. Thus, the involvement of proteins in secondary active transport is different from that in primary active transport. In the latter process, the protein is acting like an enzyme, catalysing a vectorial rather than a scalar process. Proteins involved in secondary active transport do not function like the classical concept of an enzyme. It is for this reason that they are called 'porters' (Mitchell, 1967). A consequence of the mode of action of porters is that they are able to catalyse vectorial processes in a reversible manner.

The reactions bringing about primary active transport can be described as chemiosmotic (Mitchell, 1979). It is these reactions that ultimately drive secondary active transport or purely osmotic reactions (Figure 1.1). These latter reactions can be described either as uniport, namely the bringing about of exchange diffusion (Ussing, 1947), or sym- or antiport, in which there are coupled flows (Figure 1.2). A uniport will lead only to accumulation of a solute if it is charged and there is an appropriate electrical potential difference across the membrane to provide the driving force for such accumulation. Where there are coupled flows, the flow of one solute, i.e. either protons or sodium (Figure 1.1), down its electrochemical potential gradient can bring about the movement of

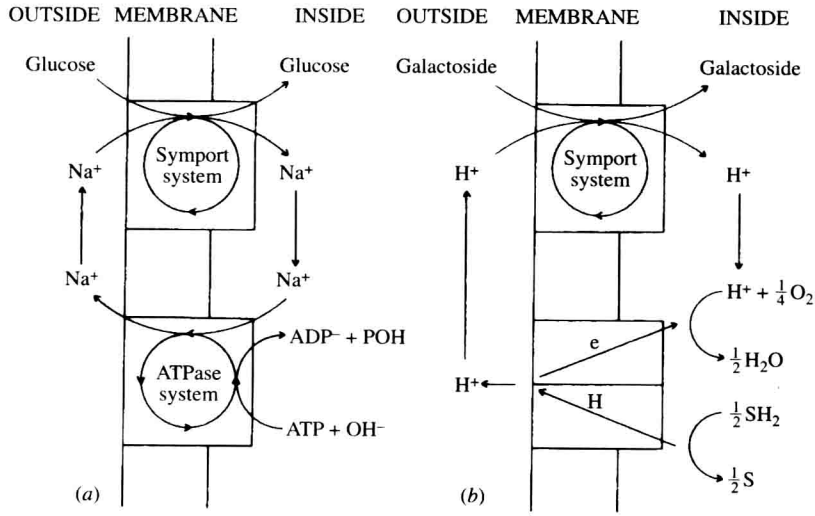

Figure 1.1. Coupling in two well-studied transport systems: (*a*) between the sodium-transporting ATPase and the sodium/glucose symporter in mammalian intestinal mucosa; (*b*) between a proton-motive respiratory chain system and an H^+/galactoside symporter in *Escherichia coli*. S, sulphur. (From Mitchell, 1979.)

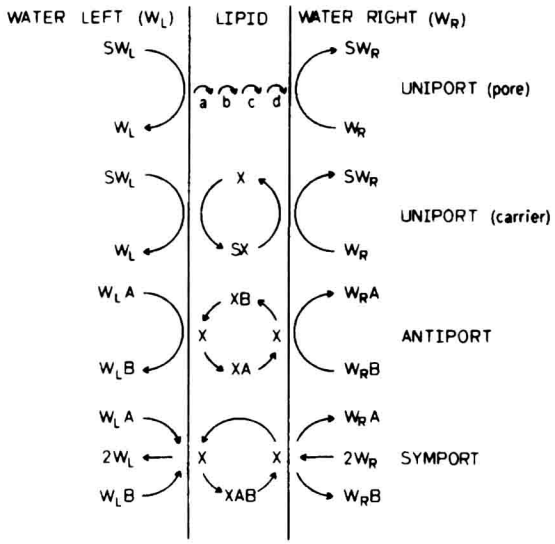

Figure 1.2. Diagrams of porter-catalysed translocation of solutes (S, A and B) across a lipid membrane between aqueous phases in which S, A and B exist as hydrates SW, WA and WB. Left and right aqueous phases are denoted by suffixes L and R. a–d and X represent a chemically specific pore and many carriers. (From Mitchell, 1979.)

another solute against its electrochemical potential gradient. Thus, there is active transport of one solute across the membrane brought about by the free energy in the gradient of the other solute across the membrane. The latter gradient of course is maintained by primary active transport. In considering secondary translocation, movement of charge is important, as well as any ensuing changes in concentrations on either side of the membrane. Figure 6.3 (p. 209) shows possible movements of charge that can occur as a result of secondary translocation.

As is discussed more fully below, primary active transport at the plasma membrane in fungi is predominantly the extrusion of protons using the free energy of hydrolysis of ATP. The evidence for any other primary active transport process is tenuous, a matter that also is considered below. As far as we know, therefore, fungi interact with the external medium almost entirely through a proton economy. There are indications that sodium can accompany solutes such as phosphate (see Chapter 7) in translocation processes but essentially it is protons that cotransport with other solutes requiring entry into or expulsion from a fungus. Secondary active transport is not considered as a particular topic. There is now such a

plethora of secondary active transport processes occurring at the plasma membrane that they are considered as appropriate in the relevant sections of the text dealing with utilisation of different nutrients.

The plasma membrane H^+-ATPase of *Neurospora crassa*

We know more about this particular primary active system than any other because it has been possible to probe it electrically, as well as biochemically and by use of molecular biological techniques. Details about the system have been reviewed elsewhere (Slayman, 1987; Sanders, 1988).

The reason there is greater knowledge about the H^+-ATPase of *Neurospora crassa* than that of other fungi is because this enzyme has been probed more extensively electrically. One cannot stress too strongly the importance of electrical studies for our understanding of active transport of charged atoms or molecules. In the case of primary active transport in *N. crassa*, electrophysiological studies have allowed a kinetic description of the process of proton extrusion, taking into account not only the characteristics of the ATPase *in vitro* and in membrane vesicles but also the membrane electrical field in which the enzyme resides.

The success of electrophysiological studies with *N. crassa* is due to the fact that, within mycelium growing on cellophane overlaying agar, there are hyphae 10–20 μm in diameter that are found 7–9 mm behind the growing margin (Slayman, 1965). The diameter is sufficiently large for insertion of electrodes for measuring the voltage and for either injecting current or measuring pH.

The functioning H^+-ATPase leads to the extrusion of protons electrogenically from the hyphae. Thus, we can speak of the enzyme acting physiologically as a proton pump. If the pump is non-functional, the membrane potential difference is *c.* -25 mV, inside negative (Slayman, 1970; Slayman, Long & Lu, 1973). When the pump is operational, the potential difference under normal physiological conditions is *c.* -270 mV, thus something like -200 mV of potential is generated by pump activity. When a mycelium is in 100 mM potassium chloride in a calcium-free medium, the potential difference is as low as -60 mV (Slayman, 1965); on the other hand, in the absence of chloride (which tends to depolarise the membrane) the potential difference can be as great as -300 mV (Blatt & Slayman, 1983).

The evidence that ATP is the substrate for the pump comes from the equivalence of the decay of the potential with the decay of ATP in the mycelium upon the addition of sodium azide. Both decay in an exponential