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Review

What triggers grass endophytes to switch from mutualism to pathogenism?

Carla J. Eaton[a,d], Murray P. Cox[a,b,c], Barry Scott[a,b,*]

[a] Institute of Molecular BioSciences, Massey University, Palmerston North, New Zealand
[b] The Bio-Protection Research Centre, New Zealand
[c] The Allan Wilson Centre for Molecular Ecology and Evolution, New Zealand
[d] Department of Plant Pathology and Microbiology, University of California, Riverside, USA

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ABSTRACT

Symbioses between cool season grasses and fungi of the family Clavicipitaceae are an integral component of both natural and agricultural ecosystems. An excellent experimental model is the association between the biotrophic fungus *Epichloë festucae* and *Lolium perenne* (perennial ryegrass). The fungal partner produces a suite of secondary metabolites that protect the host from various biotic and abiotic stresses. The plant host provides a source of nutrients and a mechanism of dissemination via seed transmission. Crucial mechanisms that maintain a stable mutualistic association include signaling through the stress activated MAP kinase pathway and production of reactive oxygen species by the fungal NADPH oxidase (Nox) complex. Disruption of components of the Nox complex (NoxA, NoxR and RacA), or the stress-activated MAP kinase (SakA), leads to a breakdown in this finely balanced association, resulting in pathogenic infection instead of mutualism. Hosts infected with fungi lacking a functional Nox complex, or the stress-activated MAP kinase, display a stunted phenotype and undergo premature senescence, while the fungus switches from restricted to proliferative growth. To gain insight into the mechanisms that underlie these physiological changes, high throughput mRNA sequencing has been used to analyze the transcriptomes of both host and symbiont in wild-type and a mutant association. In the Δ*sakA* mutant association, a dramatic upregulation of fungal hydrolases and transporters was observed, changes consistent with a switch from restricted symbiotic to proliferative pathogenic growth. Analysis of the plant transcriptome revealed dramatic changes in expression of host genes involved in pathogen defense, transposon activation and hormone biosynthesis and response. This review highlights how finely tuned grass-endophyte associations are, and how interfering with the signaling pathways involved in maintenance of these associations can trigger a change from mutualistic to pathogenic interaction.

Contents

* Corresponding author at: Institute of Molecular BioSciences, Massey University, Private Bag 11222, Palmerston North 4442, New Zealand. Tel.: +64 6 350 5543; fax: +64 6 350 2267.
E-mail address: d.b.scott@massey.ac.nz (B. Scott).

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1. The biology of grass–endophyte interactions

Interactions between plants and fungi play a crucial role in terrestrial ecosystems. These associations span a broad continuum from detrimental pathogens to beneficial symbionts. One of the most ecologically important plant–fungal associations is the mutualistic interaction between fungal endophytes of the *Epichloë* and *Neotyphodium* species (henceforth referred to as epichloë endophytes) and cool season grasses. In these associations, the fungus obtains host nutrients, protection, and a means of transmission via the colonization of host seeds [1]. In return, endophyte infection dramatically enhances plant survival through increased drought tolerance, and protection against insect and mammalian herbivory through the production of bioprotective secondary metabolites (reviewed in Ref. [1]). However, fungal synthesis of these secondary metabolites has important consequences for grazing animals in agricultural ecosystems. Epichloë endophytes produce at least four classes of bioprotective secondary metabolites; lolines, peramine, indole diterpenes and ergot alkaloids. While endophyte synthesis of lolines and peramine is beneficial in agricultural ecosystems because of their insect deterrence properties, ergot alkaloids (despite also having insect deterrence properties) and indole diterpenes are detrimental because of their physiological effects on livestock, including ryegrass staggers and ergot alkaloid toxicoses respectively (reviewed in Ref. [2]).

Interestingly, although epichloë endophyte associations are commonly referred to as mutualistic, this is not entirely accurate. In some *Epichloë* spp. the onset of host flowering induces the fungal sexual cycle, which causes these fungi to switch from a mutualistic asexual lifecycle to an antagonistic pathogenic sexual lifecycle. Hyphae proliferate over the surface of the flag leaf surrounding the host inflorescence and form a stroma that prevents emergence of the inflorescence, a phenomenon known as 'choke' [2]. This begs the question: are epichloë endophytes really mutualistic symbionts, or pathogens whose growth is modulated by the host? Under normal conditions the host is able to suppress or restrict fungal growth. However, when the host begins flowering, resources are mobilized to the inflorescence for reproduction. This change in resource distribution triggers a change in fungal physiology, which in some cases leads to proliferative growth and choke of the host inflorescence. Identifying the genes that trigger this change would be very difficult in natural isolates, but the pathogenic potential of epichloë endophytes can be systematically reconstructed by generating mutants that disrupt the symbiotic interaction. *Epichloë festucae* mutants that cause a stunting of the grass host invariably display a proliferative growth phenotype within the grass leaves. These mutational changes are discussed in more detail below.

Maintenance of endophyte–grass associations requires tightly regulated responses from both host and symbiont, including suppression of host defenses, strict control of fungal growth, and inhibition of fungal production of toxic proteins or metabolites that might elicit a host defense response. In wild-type associations, hyphae systemically colonize the intercellular spaces of host aerial tissues (Fig. 1), are aligned parallel to the leaf axis and seldom branch [3]. Hyphae are rarely found within host vascular tissues and they never penetrate host cells or produce specialized feeding structures such as haustoria, as found in other fungi [2]. Fungal growth in these associations is tightly coordinated with host growth, such that hyphae only grow during periods of leaf growth, resulting in comparable hyphal mass in old and young leaves [3,4]. However, this pattern of growth is inconsistent with the dogma that fungi grow mainly by polarized tip growth [5]. Instead, it has been proposed that epichloë endophytes grow in the leaves by intercalary division and extension [6]. We propose that hyphae initially spread by tip growth in the host shoot apical meristem and form a highly branched hyphal network. The hyphae then enter the developing leaf primordia, where they adhere to host cells undergoing division and extension, thus causing the hyphae to stretch. This stretching is thought to trigger a switch from tip growth to intercalary extension and cell division, thereby avoiding hyphal shear from the rapid leaf growth of >10 mm a day [6]. Above the leaf expansion zone, hyphae stop expanding but remain metabolically active [6,7].

2. Breakdown of mutualism in mutant associations

Given the highly regulated and coordinated nature of endophyte growth *in planta*, signaling between the fungus and its host must control fungal growth and maintain a balanced symbiotic interaction. To gain insight into the genes underlying the signaling required to maintain these associations, a synthetic association between *E. festucae* strain Fl1 and *Lolium perenne* (perennial ryegrass) was developed as a model experimental system [8]. This association provides an excellent framework for studying the symbiotic interaction. *E. festucae* is haploid, grows relatively fast in culture compared to other epichloë endophytes, and has high rates of homologous recombination [8]. The two partners form a stable symbiosis, and it is also relatively easy to inoculate *E. festucae* into perennial ryegrass seedlings, with infection rates of 80–90% for the wild-type strain [8]. In addition, draft genome sequences are now available for *E. festucae* strains E2368 (http://www.endophyte.uky.edu/) and Fl1 (http://csbio-l.csr.uky.edu/ef894/gbrowse/ef/). In a first step to identify fungal genes involved in the signaling required to maintain symbiosis with perennial ryegrass, plasmid insertional mutagenesis was used to create fungal mutants that were then screened for any change in their interaction with perennial ryegrass [9]. This resulted in identification of a mutant that switched from mutualistic to pathogenic growth. In this association, the fungus grew in an unrestricted manner with colonization of host vascular bundles, and dramatically increased biomass. Infected hosts were severely stunted, and precociously senescenced. The mutated gene encoded NoxA, a component of the multi-subunit NADPH oxidase complex, which produces the reactive oxygen species (ROS), superoxide, from molecular oxygen. Using a candidate gene approach, an additional two components of the *E. festucae* Nox complex, NoxR [10] and RacA [11], were found to be essential for maintaining a mutualistic association with perennial ryegrass. This highlights an interesting conundrum, as in the rye endophyte *Claviceps purpurea* deletion of *Cpnox1* [12] or *racA* [13] leads to a loss of pathogenicity. This reduction in pathogenicity is also seen in *Magnaporthe grisea NOX1* and *NOX2* mutants [14], and *Botrytis cinerea bcnoxA* and *bcnoxB* mutants [15], suggesting the Nox complex has evolved to play a role in pathogenicity of phytopathogenic fungi. So why does loss of ROS production induce a switch to pathogenicity in *E. festucae*, whereas pathogenicity is lost in phytopathogenic fungi? One possible explanation arises from the fact that ROS are required for polarized growth and cellular differentiation events (reviewed in Ref. [16]). In phytopathogenic fungi, the loss of pathogenicity is, at least in some systems, linked to either non-differentiation of infection structures such as penetration pegs and/or an inability to establish the polarized growth required for infection [16]. So what is the differentiation event that requires ROS in *E. festucae?* We propose that in *E. festucae*, ROS is needed for the endophyte to switch from proliferative, polarized tip growth in the host shoot apical meristem to intercalary extension in the expanding leaf primordium. Thus, mutants defective in ROS production do not switch to intercalary growth but instead continue proliferating in an unrestricted manner.

However, this hypothesis is complicated by the discovery that the switch from mutualism to pathogenicity is not restricted to *E. festucae* Nox mutants. An iron siderophore, encoded by *sidN*, is