Mechanical stress initiates intercalary growth in Epichloëfungal symbionts of grasses.

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Colonization of aerial grass tissues by seed-transmitted Epichloëendo-symbionts initially occurs through ramification of hyphal tips between cells of the host shoot apical meristem (SAM). Uniquely, when hyphae in the SAM start to invade developing leaves, growth ceases at apices, and hyphae extend via intercalary growth (division and extension in non-apical compartments). We hypothesise that intercalary growth is stimulated by mechanical stretch imposed on hyphae by their attachment to elongating host cells, and that this stress is sensed by mechano-sensors located on hyphal membranes. Deletion of *E. festucae* mid1, a putative orthologue of the mid1 yeast mechano-sensor, and a component of the Mid1/Cch1 calcium channel, reduced E. festucae radial growth rate in culture, caused aberrations in hyphal cell walls, and greatly restricted intercalary growth in infected plants. A technique to mimic the hyphal stretching proposed to occur in planta has been developed and tested on wild type E. festucae growing in culture. Intercalary compartments remained viable despite being stretched to 20% of their original length, and stretching also initiated de novo mitosis and septation in intercalary compartments. Calcium imaging experiments on *E. festucae* growing in culture have revealed that the Mid1 protein is responsible for calcium pulses at the hyphal tip during growth, and that the calcium originates from the exterior of the hypha and not from calcium stores. Studies are underway to characterise calcium signalling in intercalary compartments in wild type and the Δ mid1 deletion mutant when subjected to mechanical stress.