Ionic Mechanism Underlying Fungal Tip Growth

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Abstract

Fungal organisms normally invade new territory by linear extensions of hyphae in a process generically called tip growth. This is a complex phenomenon that involves tightly regulated structural and physiological components. Of these, ion transport mediated by active pumps, co-transport carriers, and ion channels is studied in my laboratory. Our research focuses on the roles that ion transport plays in tip growth. The self-referencing ion-selective probes at the BioCurrents Research Center offered an effective, non-invasive way to determine the potential role of these ion channels in the process of tip growth, and 'map' the location of ion fluxes, if anywhere observed, at the growing tip. It is worthwhile to note that these measurements were intended to extend the considerable literature [e.g. 1-3] describing ionic currents at hyphal tips using the platinum black vibrating probe. The experimental plan was as comprehensive as possible an examination of ionic fluxes and other parameters. The intent was to measure calcium, proton, potassium and chloride fluxes and correlate their magnitude with growth rates of Saprolegnia ferax, Neurospora crassa, and Saccharomyces cerevisiae induced to grow as pseudo-hyphae. Mapping of the sides of the hyphae to identify ion fluxes associated with the initiation of bud formation. However, in the end, time constraints made it possible to examine only calcium and protons. The results are quite novel and to a certain extent unexpected. Saprolegnia ferax has a well-defined calcium current located within the first 10 microns behind the tip. The calcium current was either inward or outward. This was an unexpected result, because activation of the stretch-activated channels known to exist in a tip-high gradient should result in inward calcium flux. Clearly, our initial simple hypothesis is inadequate. It is possible, even likely, that the net flux is due to an interplay between calcium efflux via secretion and calcium uptake via the stretch-activated channels. Saprolegnia also has an inward proton current, as expected, which is maximal about 10-20 microns behind the tip. The inward proton current is likely due to extensive proton/glucose and proton/amino acid co-transport required to fuel the metabolic demands of tip growth[2]. Overall, the results were quite exciting for two reasons: First, they revealed a level of complexity (particularly observations of either inward or outward calcium fluxes) which was unexpected. Se condly, the calcium and proton fluxes were observed in extremely well-defined locations relative to the growing apex of the hyphal tip. It is very likely that the transporters causing the net calcium and proton fluxes are maintained at specific locations via interactions with cytoskeletal elements. By contrast, Neurospora crassa exhibits on average, a zero net calcium flux

at the tip or within the first 50 microns behind the tip. Although net flux was close to zero, many of the data sets showed a periodic shift in calcium flux at the tip, with a period of about 25 seconds. This does not match known pulsations in Neurospora tip growth, reported to have a period of about 4.5 seconds[4]. Proton fluxes were negatively correlated with growth rate. That is, the greater the inward proton current, the faster the growth rate. This is consistent with the potential role of proton/glucose and proton/amino acid co-transport [3], and explains the depolarized potentials we observe in the growing tips. Proton fluxes were not localized to specific regions of the growing hyphae in direct contrast to the results obtained for Saprolegnia ferax. Overall, it is clear that Neurospora crassa has markedly different transport properties compared to Saprolegnia. It was possible to obtain measurements of fluxes in the pseudo-hyphal, actively budding cells of Saccharomyces cerevisiae, in which measurements of the cell were significantly different from background controls, (measurements 10 microns away from the cell). Calcium flux was outward (1 to 2.5 micro Volt magnitude; Proton flux was inward (-12 +/4 micro Volt magnitude). It was not possible to 'map' the ion fluxes at budding versus non-budding ends of the cell. This is not too unexpected because the normal probe tip size is only slightly smaller than the diameter of the yeast cell. These preliminary experiments do indicate the feasibility of direct measurements on small fungal cells, which include the human pathogen Candida. I would characterize the visit as very successful. Specifically we confirmed some expectations, but will be forced to rethink other hypotheses/interpretations which were much too simplistic. References 1. Gow, N.A. 1984. Transhyphal electrical currents in fungi. J. Gen. Microbiol. 130: 3313-3318. 2. Schreurs, W.J. and Harold, F.M. 1988. Transcellular proton current in Achyla bisexualis hyphae: relationship to polarized growth. Proc. Natl. Acad. Sci. USA 85: 1534-1538. 3.Takeuchi, Y., Schmid, J., Caldwell, J.H. and Harold, F.M. 1988. Transcellular ion currents and extension of Neurospora crassa hyphae. J. Memb. Biol. 101: 33-41. 4. Lopez-Franco, R., Bartnicki-Garcia, S. and Bracker, C.E. 1994. Pulsed growth of fungal hyphal tips. Proc. Natl. Acad. Sci. USA 91: 12228-12232.

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