Comparative Study of Ion Channels in Filamentous Fungi Following the Novel Development of Laser Nanosurgery for Cell Wall Removal

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The patch-clamp technique has been a standard method for the functional characterization of ion channels in living cells for several decades. However, despite the fungal kingdom's vast diversity and significance, the study of ion channels in filamentous fungi remains very limited due to the difficulty of electrically isolating membrane patches by creating a GΩ seal with a glass pipette. Cytoplasmic droplets (CDs) are a model system used in our research group that has so far yielded the most data on novel channels in the native membrane, but this approach is limited to the use of giant sporangiophores of *P. blakesleeanus*. CDs have been shown to be metabolically active, enclosed by a plasma membrane, and to contain many nuclei, making them suitable for studying membrane channels in their native physiological state [1].

The development of laser nanosurgery for precise cell wall removal enabled the first ion current recordings from *P. blakesleeanus* mycelium [2]. Results show differences in dominant activities compared to sporangiophore membrane, as well as the spatial distribution of distinct single ion channel activities along the hyphae. The osmotically sensitive, outwardly rectifying inactivating current (ORIC)—the dominant current of the CD membrane and the most thoroughly studied to date [3]—is absent in recordings from both the whole protoplast membrane and excised membrane regions, suggesting that its expression may be sporangiophore-specific. A dominant current in the protoplast membrane seen in all whole-protoplast recordings is an inward inactivating anionic current that resembles a rarely observed activity in CDs, that remains to be fully characterized. Various single channel activities were recorded under the same recording conditions on both CDs and mycelium protoplast in parallel. Besides ORIC, a 70 pS potassium current- one of the first single-channel currents described in the CD membrane [4]- has also not been observed in the mycelium membrane, while several activities suggest a novel type of ion channels specific for the mycelium.

The greatest advantage of using laser nanosurgery to obtain mycelial protoplasts is that the method can be further optimized for a wide range of filamentous fungi species. One ongoing project is focused on adjusting the protocol for phytopathogenic fungal species, to be followed by measurements of the currents induced by the insertion of peptides from biocontrol species of the genus Trichoderma. We have recently studied their effects on the growth inhibition of autochthonous phytopathogens [5], and it was further observed that peptaibol-containing extracts of *T. harzianum* exert an acute lethal effect on microscopic specimens of *R. solani* when applied extracellularly.

Overall, this strategy represents a significant step toward accelerating the discovery of novel ion channels and enabling the systematic exploration of their diversity and physiological roles in filamentous fungi.

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