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A cryptic, bacteria-dependent collective invasion in *Fonticula alba* reveals a new multicellularity in fungi-animal tree

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Multicellularity emerged in Opisthokonta from its amoeboid ancestor and resulted in the distinct fungal and animal organizations. However, the evolutionary history of these multicellularities is challenging to unravel given their striking mechanistic differences. *Fonticula alba* is an understudied, early-diverging Opisthokont, which uniquely retains a social amoeba lifestyle. *F. alba* grows as unicellular amoebae that aggregate to build multicellular, spore-generating fruiting bodies. We aim to establish *F. alba* as a new model organism to explore the evolutionary cell biology of fungi and animals. *F. alba* is a non-axenic cellular slime mold and only grows in co-culture with feeder bacteria. Greatly-improved culture methods for the organism revealed a unique bacterial age-dependent germination for *F. alba* and a cryptic invasive social behavior distinct from fruit formation. The amoebae coalesced into dynamic collectives, which invaded virgin bacterial resources. Collectivity allowed groups of cells to migrate in a highly directional manner and thereby invade large distances more rapidly than isolated amoeba. Invasion collectivity emerges from amoeba cell density and is dependent on the bacterial environment. These features are undescribed for other cellular slime molds and offer clues into the changes that occurred during Opisthokont diversification. Specifically the surprising discovery of a new bacteria-dependent, animal-like invasion collectives in addition to spore-generating collectives reshapes our understanding of the origins of both Metazoan and fungal multicellularity.

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Study of *Mob1* in the apicomplexan *Toxoplasma gondii*: at the crossroads of asexual and sexual development

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Toxoplasma gondii, the causative agent of toxoplasmosis, is the uttermost member of apicomplexan parasites due to its zoonotic character. To achieve a sustainable infection, parasites must avoid overgrowth given that uncontrolled parasite growth may lead to the death of both the host and the parasite. Indeed, understanding how parasites regulate its replication rate, namely balancing proliferation versus cell death, is essential to understand and control parasitic diseases. MOB1 is a conserved protein among eukaryotes, present in signaling pathways regulating cytokinesis in unicellular eukaryotes (mitotic exit network, MEN) and apoptosis, cell proliferation, cell differentiation, and organ size in metazoans (Hippo). In *T. gondii* we found one *Mob1* gene whose expression decreases 94% during tachyzoite replication inside the host cell. *T. gondii* RH tachyzoites overexpressing MOB1 had a significant delay in replication rate and *Mob1* knockout led to an increase in replication rate. In tachyzoites, MOB1 accumulates between the two newly formed nuclei. Although this localization is compatible with a function in MEN, no effect on cytokinesis was observed (a hallmark of MEN in MOB1 depleted unicellular eukaryotes). This phenotype led us to investigate the function of *Mob1* at different phases of the *T. gondii* life cycle. Open access phenotypic data confirm that this gene is not essential in

tachyzoites while RNA-seq data shows it is more expressed in *T. gondii* sexual stages. *T. gondii* only completes sexual development in the feline intestinal epithelium where naturally occurring high concentrations of linoleic acid are present due to lack of delta-6-desaturase. This recent discovery by the Knoll Lab opens the possibility of reproducing the *T. gondii* sexual phase *in vitro*. We have developed *in vitro* culture of small intestinal epithelium as 3D enteroids, supplemented with linoleic acid, establishing a tissue culture model to study the role of *Mob1* in the *T. gondii* sexual development.

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Molecular mechanism of wound healing in *Stentor coeruleus*

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Wound healing is a fundamental property of living systems and understanding its biology is important for reconstituting cellular healing in vitro or in the building of artificial cell systems that are robust against external injury. An organism that is extremely good at healing wounds is the freshwater ciliate Stentor coeruleus, which can regenerate a new cell from a fragment 1/27th its original size. The significance of the study of wound healing mechanisms in Stentor is therefore two-fold, first to discover potentially novel mechanisms of wound healing and second, to adapt them in building artificial cell systems. We investigate whether Stentor uses mechanisms of wound healing that are akin to those utilized in other eukaryotic systems, such as membrane patching or actomyosin-based purse-string contraction, or if it employs divergent strategies to heal wounds. In order to answer this question, we carried out RNAi of candidate molecules that have a proven role in wound healing in other eukaryotic systems, including the cytoskeletal protein tubulin. We then asked how this influenced Stentor's ability to recover from manually and microfluidically generated wounds. We find that S. coeruleus has a remarkable tolerance for molecular perturbation in its capacity for wound healing and remains viable despite perturbation of key cytoskeletal molecules.

Cell Death

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Membrane-bound LGR4 and its soluble form (LGR4-ECD) as novel regulators of ß-cell survival and proliferation

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Our lab has shown that RANK (Receptor activator of the NF-κB) by interacting with its ligand, RANKL, inhibits ß-cell proliferation and survival, which can be reversed by Osteoprotegerin. Recently, the G protein-coupled receptor LGR4 (leucine-rich repeat-containing G protein-coupled receptor 4), which classically binds R-spondin (RSPO), was identified as a novel receptor for RANKL in osteoclast precursor cells. Thus, RANKL can bind two distinct receptors, RANK and LGR4 in osteoclasts, leading to opposite effects on osteoclastogenesis. LGR4 is expressed in rodent and human ß-cells, but the role of this receptor in ß-cells remains unknown. We postulated that LGR4, through its stoichiometry with RANKL and RANK, is involved in regulating ß-cell survival and proliferation. Our data indicate expression of specific LGR4 family members, *Lgr4, Rank, Rankl*, is modulated by stressors, such as cytokines, in a ß-cell line (INS1), rodent and human islets. Knocking down *Lgr4 in vitro*, in INS1 cells or rodent islets is