Microtubule-dependent, F-actin-independent Cytokinesis in Green Alga *Chlamydomonas* Involves Kinesins and Extracellular Matrix Proteins

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Land plants divide their cells by the expansion of cell plate, a process driven by trafficking along the phragmoplast microtubules that delivers vesicles containing extracellular matrix to the division plane. Similar associations of microtubules (or its homologous structure) and ECM in cell division are found in various organisms, such as the cleavage-furrow-associated microtubule array and ECM proteins in animals, the post-anaphase microtubules array and ECM proteins and polysaccharides in yeasts, and even the FtsZ ring and peptidoglycan in bacteria. However, the evolution and conservation of roles of microtubules and ECM remain unclear, partly due to a historical emphasis of cytokinesis mechanisms on the contractile actomyosin ring (CAR) for eukaryotes outside of land plants. The CAR was extensively studied in animals and yeasts but is not conserved in many other organisms (land plantes, algae, ciliates, etc.). Even some animal cells and yeasts can still divide without the CAR under some conditions. In the green alga *Chlamydomonas reinhardtii*, cytokinesis can occur even in the complete absence of F-actin, suggesting an alternative mechanism at play. Thus, to shed light on the evolution of cytokinesis in eukaryotes, we investigated the roles of microtubules and ECM in *Chlamydomonas* furrow ingression.

We found that microtubules are nucleated from the cleavage furrow by augmin complex independently of F-actin. Removing an augmin subunit significantly delayed furrowing and blocked furrow formation when F-actin was simultaneously depleted. Through BioID labeling and other approaches, we identified several kinesins that localized to the cleavage furrow in an augmin-dependent manner, including the sole *Chlamydomonas* kinesin-5 KIF11. A *kif11-TS* mutant showed significant delay in cytokinesis, and combining *kif11-TS* and F-actin loss completely blocked furrow formation. Thus, the furrow-associated microtubules and KIF11 are essential for furrowing without F-actin.

KIF11 Co-IP-MS experiment further identified more ECM components, including several hydroxyproline-rich proteins. One of the top hits is PKHD1/fibrocystin, a 500-kDa single-pass transmembrane protein with an extensive extracellular N-terminus. *pkhd1* mutations in humans cause the autosomal recessive polycystic kidney disease, which affects one in 20,000 newborns. We found that CrPKHD1 co-localized with KIF11 specifically in the furrow and determines post-division cell polarity. The PKHD1 furrow localization was lost in the *kif11-TS* mutant but persisted in F-actin depletion, suggesting that KIF11, but not F-actin, is involved in transporting PKHD1 to the furrow. TurboID labelling on PKHD1 N-terminus during cytokinesis identified more candidates in the protein-rich *Chlamydomonas* ECM. As a membrane-spanning protein, PKHD1 may bridge the microtubules cytoskeleton and the ECM during division.

Our results suggest that the microtubules-and-ECM-dependent division mechanism featured in land plants may be a basal mechanism that drives cytokinesis in other eukaryotes independently of a canonical contractile actomyosin ring.