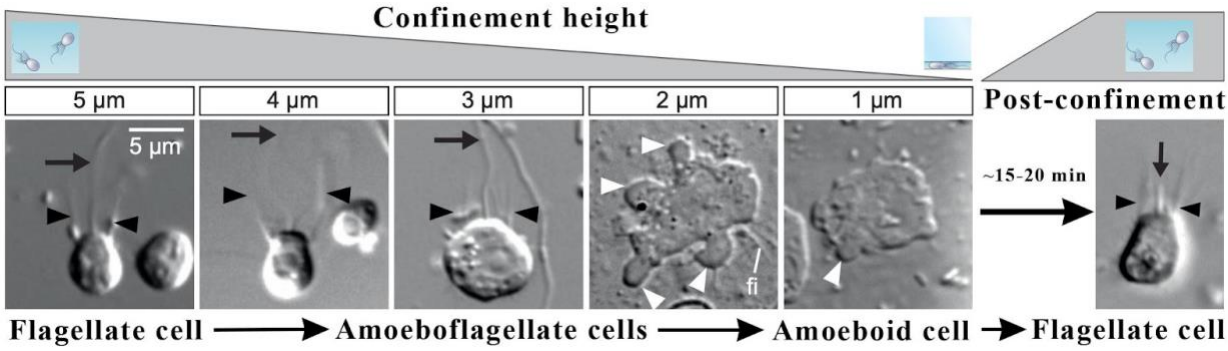
<start FURTHER DEVELOPMENT 8.2 ONLINE>

Confinement of the choanoflagellate *S. rosetta*

While *C. flexa* appears to use actin-myosin contractions to bend a sheet, these choanoflagellates do not exhibit any behaviors like ingression. However, it was curiously observed that when the volume of media used to maintain a different choanoflagellate species (*Salpingoeca rosetta)* was significantly reduced, they exhibited a dramatic cell shape change. Using controlled confinement methods, *S. rosetta* shifts from a free-swimming flagellated protist into a migrating amoeba-like cell with no flagella or microvillus structures (Brunet, et al. 2021). This cell shape change is reminiscent of the kinds of cell shape changes that define epithelial to mesenchymal transitions in gastrulating metazoans. In addition, it's important to also understand the fact that the last common ancestor of both choanoflagellates and metazoans possessed representatives of the cadherin cell adhesion protein families, which are well-known to mediate EMTs in animals. These discoveries suggest that choanoflagellate protozoa, sister group to all gastrulating animals, has the molecular and cellular abilities to carry out both apical constriction and EMT types of behaviors.



<CAPTION>**FIGURE 1@@**Confinement of the choanoflagellate *S. rosetta* induces an amoeboid

phenotype. *S.rosetta* is shown here in decreasing volumes of confinement, which trigger a progression from a flagellate cell to an amoeboflagellate and then into a full amoeba-like cell phenotype. When these amoeboid cells are returned to the larger dimension of confinement so is returned the flagellate phenotype.

Watch Development 8.1 Choanoflagellate colony @@Watch how *C. flexa* inverts the curvature of its colony when the light conditions are changed.

SCIENTISTS SPEAK 8.1@@Choanoflagellates and the origin of multicellularity with Dr. Nicole King, 2021.

Brunet T, Albert M, Roman W, Coyle MC, Spitzer DC, King N. A flagellate-to-amoeboid switch in the closest living relatives of animals. Elife. 2021 Jan 15;10:e61037. doi: 10.7554/eLife.61037. PMID: 33448265; PMCID: PMC7895527.