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Girl power: NORTIA polarization seals pollen tube fate

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How protein dynamics contribute to developmental processes is a critical biological question. In this issue of *Developmental Cell*, Ju et al. show that subcellular localization of NORTIA in the female gametophyte is required for pollen reception. NORTIA redistribution boosts cues that drive pollen tube bursting, thus promoting male gamete release and fertilization.

During pollination, cells from genetically distinct individuals must communicate in order to direct the cellular processes that lead to fertilization. In flowering plants, fertilization involves the delivery of two sperm cells to the female gametophyte (reviewed by Johnson et al., 2019). The two sperm cells are carried by a pollen tube (PT) that grows through floral tissues, guided by female cues toward a target ovule where it ultimately enters an aperture called the "micropyle." After arrival and reception, the PT stops growing and bursts at one of two specialized cells of the female gametophyte, which are called "synergid cells," and the receptive synergid cell then undergoes programmed cell death. PT burst releases the sperm cells, one fuses with the egg cell to generate the embryo, and the other fuses with the central cell to form the nutritive endosperm.

Synergid cells are reproductive accessory cells with a central role in PT guidance, reception, and bursting (reviewed by Johnson et al., 2019 and others). Evidence that links synergid cells to early events in the intercellular communication in pollination comes from mutants in the receptor-like kinase FERONIA (FER) and a glycosylphosphatidylinositol (GPI)-anchored protein, LORELEI (LRE), which exhibit similar PT reception phenotypes (Capron et al., 2008). In *fer* and *lre*, PTs are attracted to ovules, but they are not properly received, and so they fail to stop growing; this results in one or more PTs curling around inside the ovule. In these mutants, PTs do not rupture, and male gametes are not released (Huck et al., 2003; Escobar-Restrepo et al., 2007). FER and LRE proteins accumulate asymmetrically within synergid cells, localizing at a highly invaginated plasma-membrane-rich region, called the filiform apparatus (FA), that is covered by a thickened cell wall (Rotman et al., 2003; Escobar-Restrepo et al., 2007). Before PT arrival, LRE functions as a FER chaperone to enable FER movement from the endoplasmic reticulum to the FA (Li et al., 2015), where together, LRE and FER function in PT reception.

Mutants in another synergid-specific protein, the mildew resistance locus O (MLO)-like protein NORTIA (NTA), exhibit *fer* and/or *lre*-like phenotypes (Kessler et al., 2010). Prior to PT arrival, NTA fused to green fluorescent protein (NTA-GFP) is homogeneously distributed in synergid cells in a compartment that colocalizes with a *cis*-Golgi marker (Jones et al., 2017; Ju et al., 2021). After PT arrival, NTA-GFP is only detected at the FA plasma membrane. In *fer* and *lre* mutants, NTA-GFP is not re-distributed to the FA,

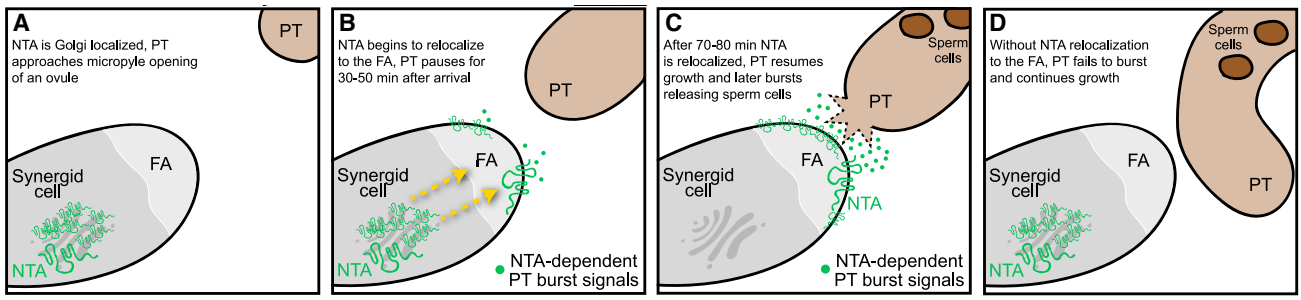

Figure 1. Schematic summary of NTA localization and function from live imaging of protein dynamics and polarization during pollen tube reception

(A) Prior to arrival of the pollen tube (PT) at the micropyle aperture of the ovule, NTA-GFP (green) is localized to the Golgi (dark gray).

(B) After the PT reaches the micropyle, it pauses growth for 30–50 min, and NTA begins to redistribute to the filiform apparatus (FA).

(C) It takes ~70–80 min for NTA-GFP to complete its redistribution to the FA, and this time coincides with resumption of PT growth. Then the PT will burst and release the sperm cells for fertilization. This suggests that NTA localization is responsible for a female-derived cue that promotes PT bursting and sperm cell release.

(D) Failure of NTA to redistribute to the FA disrupts PT bursting, and this results in PT overgrowth and reduced fertilization.

and this indicates that NTA accumulation at the FA depends on FER and/or LRE signaling. However, the precise timing and functional relevance of NTA redistribution after PT arrival and during PT reception has been unknown.

Using live imaging combined with a semi-*in vivo* pollination system, Ju et al., (2021) determined that redistribution of NTA from the Golgi to the FA starts at the time when the PT reaches the micropylar opening (t = 0 min) and continues during the pause in growth of the PT (t = 30–50 min). Then, as the PT resumes growth, NTA-GFP is only detectable at the FA (t = 70–80 min). Importantly, PT arrival is required for this redistribution, and PT burst was observed in ovules where NTA-GFP was redistributed to the FA (Figure 1). Finally, no general reorganization of organelles (Golgi, trans-Golgi network, or endoplasmic reticulum) toward the FA was observed, and a version of NTA that was confined to the Golgi failed to rescue the *nta* PT reception phenotype. This suggests that PT arrival likely triggers NTA-GFP trafficking from the *cis*-Golgi to the FA plasma membrane, and its accumulation there promotes PT reception and burst.

To further explore the functional relevance of differential NTA-GFP subcellular accumulation, Ju et al., 2021 generated a chimeric version of the protein that was constitutively localized to the FA (faNTA). This important tool showed that premature FA accumulation of NTA-GFP does not appear to affect synergid cell function but does rescue the *nta* PT reception phenotype. Additionally, faNTA expressed in *fer* or *lre* mutants suppresses their PT perception phenotypes, indicating that FA-localized NTA can circumvent the FER/LRE signal transduction cascade. The identification of the faNTA variant and its Golgi-retained counterpart provide a clear and elegant demonstration of NTA function at the FA and its role downstream of the FER/LRE pathway in PT reception.

PT reception constitutes a significant prezygotic barrier to interspecific hybridization because in interspecific crosses, PTs do not burst and sperm cells are not released (reviewed by Johnson et al., 2019 and others). Remarkably, Ju et al. (2021) show that polarized accumulation of faNTA in *A. thaliana* synergid cells bypasses this barrier, thus allowing reception of interspecific PT from *Arabidopsis lyrata*. Altogether, the authors provide key insights into the molecular mechanisms that underly reproductive specificity in plant systems and their findings lead to the hypothesis that modulation of NTA subcellular distribution serves as a safety mechanism in reproductive control by the female gametophyte, which is important for full fertility through promotion of PT reception and bursting and the release of correct (intraspecific) sperm cells.

These findings raise several critical questions, including: How does the FER signal transduction cascade lead to polar redistribution of NTA at the FA? What is the NTA mode of action at the FA? Identification of NTA partners on the male and female sides will be central to answering these questions. Interestingly, the NTA C terminus contains a calmodulin domain suggesting that NTA may be able to detect Ca^{2+} dynamics, and in synergid cells, Ca^{2+} oscillations are connected with PT approach (Iwano et al., 2012). It would be fascinating to determine whether NTA redistribution is linked to Ca^{2+} oscillations or if, once NTA is localized to the FA, its function is needed to perceive Ca^{2+} oscillations and facilitate PT reception. Finally, investigating the relative importance of the spatiotemporal subcellular redistribution of NTA as a conserved safety mechanism to maintain reproductive isolation between plant species, particularly among and between self-crossing species, such as *Arabidopsis thaliana*, and out-crossing species, such as *Arabidopsis lyrata*, could lead to important insights into plant breeding and evolution.

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DECLARATION OF INTERESTS

The authors declare no competing interests.

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The importance of long-lived proteins: Not just nuclear anymore

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The significance of mitochondrial long-lived proteins (mitoLLPs) to tissue health has remained mysterious for over a decade. In this issue of *Developmental Cell*, Krishna et al. demonstrate that mitochondrial lifetimes are highly heterogeneous and that mitoLLPs promote respiratory capacity by facilitating supercomplex assembly within the electron transport chain.

Age-related changes in protein homeostasis (proteostasis) underlie the dysfunction of multiple tissues across species. Therefore, determining precisely how changes in proteome integrity throughout life contribute to tissue dysfunction with age is crucial for our ability to promote long-term health (Hipp et al., 2019). One area of emerging significance for our understanding of the relationship between proteostasis and aging is that of protein lifetimes. During the last decade, several studies have demonstrated that the turnover rates of individual proteins can vary substantially, with some long-lived proteins (LLPs) persisting for weeks or even months in the nucleus and mitochondria of mouse and rat brain tissues *in vivo* (Price et al., 2010; Toyama et al., 2013; Fornasiero et al., 2018). Although LLPs in the nucleus have been shown to exhibit a high degree of lifetime mosaicism, both across and within cells, and to act as key scaffold proteins for the formation of nuclear pore complexes, the importance of mitochondrial long-lived proteins (mitoLLPs) for mitochondrial function and tissue health, and the degree of lifetime heterogeneity that exists in mitochondria within cells and between tissues, remains unknown.

To determine the degree of lifetime heterogeneity within mitochondria *in vivo*, Krishna et al. (2021) took advantage of MIMS-EM imaging (a technique that facilitates visualization of protein, organelle, and cellular turnover *in vivo*) to establish that aged mouse muscle and brain tissue have long-lived mitochondria with limited turnover. Interestingly, the authors also found that even within the same cells and tissues, mitochondria can exhibit different longevity and turnover rates. This finding indicates substantial mitochondrial age mosaicism. This phenomenon was also observed by the authors using human neurons *in vitro*, where the protein turnover of a mitochondrial ATP synthase subunit, ATP5C1, was monitored.

Next, the authors performed an unbiased *in vitro* analysis of proteome turnover using SILAC, a heavy isotope labeling method, to determine whether mitochondria contain LLPs in neurons and myotubes derived from differentiated human embryonic stem cells and mouse C2C12 myoblasts, respectively. These experiments revealed the presence of many LLPs in both neuronal and myotube mitochondria, and the overall protein turnover observed in neurons was slower than in myotubes. Remarkably, neuronal and myotube mitochondrial proteins have longer lifetimes than do proteins within other organelles, such as the endoplasmic reticulum and lysosomes. The authors noted that mitochondrial proteins are longer lived than the average