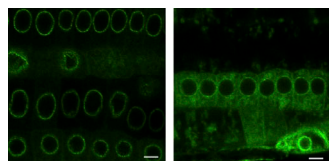


Harvesting a new KASH crop



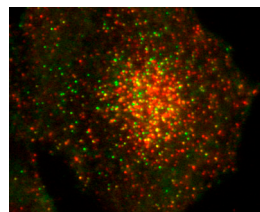
SINE2 (green) localizes to the nuclear envelope in a wild-type plant (left) but is mostly cytoplasmic in the absence of SUN proteins (right).

Zhou et al. reveal that plants express diverse KASH-like proteins that perform discrete functions at the outer nuclear membrane.

In animals and fungi, LINC complexes—composed of inner nuclear membrane SUN proteins and outer nuclear membrane KASH proteins—

connect the nucleus to the cytoskeleton to control a variety of processes including nuclear positioning and chromatin organization. Plants express SUN proteins, but their genomes lack KASH homologues. They do, however, encode at least one family of proteins that, like animal and fungal KASH proteins, bind to SUN family members and localize to the outer nuclear membrane.

Endocytosis in its natural state



Endocytic punctae decorate a cell expressing genomically tagged clathrin light chain (red) and dynamin2 (green).

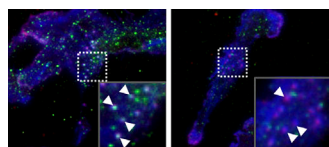
Graessart and Cheng et al. use genome editing and quantitative microscopy to examine the dynamics of actin and dynamin2 during clathrin-mediated endocytosis.

Clathrin coat proteins form invaginated pits at the plasma membrane, which are subsequently released by the GTPase dynamin into the cytoplasm as coated vesicles. The dynamics of clathrin and dynamin assembly are

incompletely understood, however, in part because overexpressing fluorescently tagged versions of these proteins might interfere with the endocytic process. Graessart and Cheng et al. therefore used genome-edited cell lines that express fluorescent versions of dynamin2 and clathrin light chain at wild-type levels uniformly across the cell population, allowing the researchers to quantitatively analyze their dynamics in an unbiased manner without perturbing endocytosis.

In the initial phase of endocytosis, which was highly variable

Talin's invasive side



Compared with a control cell (left), NHE-1 (green) isn't recruited into invadopodia containing cortactin (red) and F-actin (blue) in a cell lacking talin (right).

The integrin-binding protein talin stimulates invadopodia formation and tumor cell metastasis by recruiting the sodium/hydrogen exchanger NHE1, Beaty et al. reveal.

Tumor cells form actin-rich protrusions called invadopodia that degrade the extra-

cellular matrix and facilitate cell invasion and metastasis. The adhesion receptor $\beta 1$ integrin promotes invadopodial maturation, but whether integrin-associated proteins such as talin assist in this process is unknown. Beaty et al. found that talin localizes to invadopodial precursor structures and that knocking down talin prevented their maturation into matrix-degrading protrusions by

Zhou et al. devised a bioinformatic approach to identify additional SUN-binding proteins in *Arabidopsis* and other plant species, uncovering ten new families of potential KASH-like plant proteins. The researchers tested several of these new proteins and confirmed that they localize to the nuclear envelope by binding to members of the SUN family.

One new *Arabidopsis* KASH protein, which the researchers named SINE1, was strongly expressed in leaf guard cells, which control gas exchange by opening and closing stomata. SINE1 was required to anchor the nucleus in the center of each guard cell, probably by interacting with the actin cytoskeleton. Meanwhile, a paralogue of SINE1, SINE2, was expressed in the epidermal and mesophyll cells of *Arabidopsis* leaves and protected the plant from infection by an oomycete pathogen. Senior author Iris Meier now wants to investigate how SINE2 fights infection and to determine how defects in nuclear positioning affect the function of guard cells. Zhou, X., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201401138>.

in duration, clathrin accumulated in membrane punctae that transiently recruited small numbers of dynamin2 molecules. In a final, more regular phase lasting around 20 seconds, approximately 26 molecules of dynamin2—enough to form a single loop around the neck of the invaginated pit—stably associated with each clathrin puncta before it disappeared from the plasma membrane and internalized into the cell.

The researchers then examined genome-edited cells expressing fluorescently tagged actin. Whether actin is an integral component of the clathrin-mediated endocytosis machinery has been uncertain, but the researchers found that actin accumulated at almost every endocytic site, typically before the appearance of dynamin2. Treating cells with actin inhibitors such as jasplakinolide or cytochalasin D indicated that actin polymerization promotes dynamin2 recruitment and aids vesicle scission.

Senior author David Drubin now wants to analyze the many accessory factors that assist dynamin and clathrin in order to build a quantitative model of clathrin-mediated endocytosis.

Graessart, A., A.T. Cheng, et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201403041>.

inhibiting the recruitment of the sodium/hydrogen exchanger NHE-1.

NHE-1 promotes invadopodial maturation by increasing the local cytoplasmic pH, thereby activating the actin-severing protein cofilin to generate free barbed ends and stimulate actin polymerization. The researchers discovered that talin's C terminus binds directly to the FERM domain of the ERM protein moesin, which, in turn, recruited NHE-1 into invadopodial precursors. Tumor cells lacking talin thus formed fewer invasive protrusions and showed reduced migration through their surrounding tissue when injected into mouse mammary glands. Accordingly, loss of talin impaired the tumor cells' ability to enter the bloodstream and form metastases in the lung.

Talin localized to invadopodia independently of $\beta 1$ integrin, but the adhesion receptor was nevertheless required to recruit moesin and NHE-1 into the protrusions. Lead author Brian Beaty therefore wants to investigate how talin and $\beta 1$ integrin combine to regulate NHE-1 activity and cell invasion.

Beaty, B.T., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201312046>.