In eukaryotes, active import of large signaling molecules and proteins over 40 kDa in the nucleus is mediated by aptly named "importin" proteins. As there are considerably fewer importins than there are cargos, determining how cargo-transporter specificity is mediated constitutes a challenging issue in the field.

In Arabidopsis (*Arabidopsis thaliana*), *KETCH1* (*KARYOPHERIN ENABLING TRANSPORT OF CYTOPLASMIC HYL1*) encodes an essential importin responsible for transporting HYL1, a microRNA processor, to the nucleus (Zhang et al., 2017). However, fertility defects resulting from the loss of *KETCH1* suggested additional functions. Xiong and colleagues (2020) now report on new functions of *KETCH1*. Reexamining the loss-of-function allele *ketch1-2*/1, they found that seed abortion was close to 50%.

In flowering plants, meiosis produces haploid spores that continue to divide mitotically to produce multicellular organs called gametophytes in which gametes are ultimately formed. The female gametophyte is the embryo sac, and the male gametophyte is the pollen grain. The aborted seeds ratio in *ketch1-2*/1 plants strongly suggested gametophytic lethality. Indeed, most mutant embryo sacs and pollen grains were unable to develop to maturity and arrested at the first postmeiotic mitotic division. Expression analysis using a *KETCH1* promoter to drive a nucleus-localized yellow fluorescent protein (YFP) fusion protein revealed that *KETCH1* was expressed throughout embryo sac development and during early stages of pollen grain development.

An attractive hypothesis was that *KETCH1* was also responsible for the nuclear import of HYL1 during gametophyte development. Loss of *HYL1* results in a low level of sterility (Zhang et al., 2017). However, Xiong and colleagues (2020) did not find evidence of gametophytic sterility in a *hyl1* mutant, ruling out that hypothesis.

The ribosomal protein (RP) RPL27a fused to GFP (GFP-RPL27a, green) accumulates only when coexpressed with *KETCH1*-mRFP (magenta) in pollen grains from *ketch1-2*+/+ plants. Here, Xiong and colleagues (2020) identify a number of additional RPs that require *KETCH1* for accumulation. *KETCH1*-dependent RP stability includes transport to the nucleus and protection from degradation by the 26S proteasome. RP stability conditions ribosome abundance and translation efficiency, which certain genes such as ARF2 and ARF3 are sensitive to, as well as cell cycle progression. (Left panel adapted from Xiong et al. [2020], Figure 8.)

To validate this interaction in vivo, Xiong and colleagues (2020) introduced GFP-RPL27a and *KETCH1*-RFP protein fusions driven by the ubiquitous UBQ10 promoter in wild-type and *ketch1-2*+/+ plants. In *ketch1-2* pollen and wild-type roots, the accumulation of GFP-RPL27a signal depended on the coexpression of *KETCH1*-mRFP. GFP-RPL27a also accumulated in response to treatment with the 26S proteasome inhibitor MG132 in roots, suggesting that RPL27a is actively degraded in the absence of *KETCH1* (see figure).
To further understand the consequences of the reduced stability of ribosomal proteins, Xiong and colleagues (2020) generated plants in which KETCH1 was constitutively knocked down by a microRNA driven by the 35S promoter (Pro35S::amiR-KETCH1). In these plants, the accumulation of GFP-RLP27a was compromised in leaf protoplasts and pavement cells. Polysome profiling further revealed that these plants had significantly reduced amounts of 40S and 80S ribosomes, consistent with decreased ribosomal protein stability.

Would this reduction in ribosome affect protein translation efficiency? To test this, Xiong and colleagues (2020) generated GFP fusions with the auxin-responsive factors ARF2 and ARF3, which are sensitive to translation efficiency due to an upstream open reading frame in their 5’ leader sequence. GFP-ARF2 and GFP-ARF3 signals were reduced in Pro35S::amiR-KETCH1 plants compared with the wild type in an upstream open reading frame-dependent manner. Reduced translation efficiency can result in cell cycle progression defects in yeast and mammals (Donati et al., 2012), and loss of KETCH1 resulted in mitotic arrest during gametogenesis. The authors further examined Pro35S::amiR-KETCH1 leaves and found that these had fewer and larger pavement cells compared with the wild type. In addition, DNA content profiling revealed increased frequency of pavement cells with 4C and 8C contents compared with the wild type.

Overall, Xiong and colleagues (2020) demonstrate that KETCH1 functions independently of HYL1 in gametophytes, identified ribosomal proteins as cargos of KETCH1, and uncovered a link between translation efficiency and cell cycle progression. These results provide a framework to further understand transporter-cargo specificity in plants.

Sebastien Andreuzza
Department of Plant Sciences
University of Cambridge
United Kingdom

REFERENCES
Donati, G., Montanaro, L., and Derenzini, M. (2012). Ribosome biogenesis and control of cell proliferation: p53 is not alone. Cancer Res. 72: 1602–1607.
Xiong, F., Duan, C.-Y., Liu, H.-H., Wu, J.-H., Zhang, Z.-H., Li, S., and Zhang, Y. (2020). Arabidopsis KETCH1 is critical for the nuclear accumulation of ribosomal proteins and gametogenesis. Plant Cell 32: 1270–1284.
Zhang, Z., Guo, X., Ge, C., Ma, Z., Jiang, M., Li, T., Koiwa, H., Yang, S.W., and Zhang, X. (2017). KETCH1 imports HYL1 to nucleus for miRNA biogenesis in Arabidopsis. Proc. Natl. Acad. Sci. USA 114: 4011–4016.
Zsögön, A., Szakonyi, D., Shi, X., and Byrne, M.E. (2014). Ribosomal protein RPL27a promotes female gametophyte development in a dose-dependent manner. Plant Physiol. 165: 1133–1143.