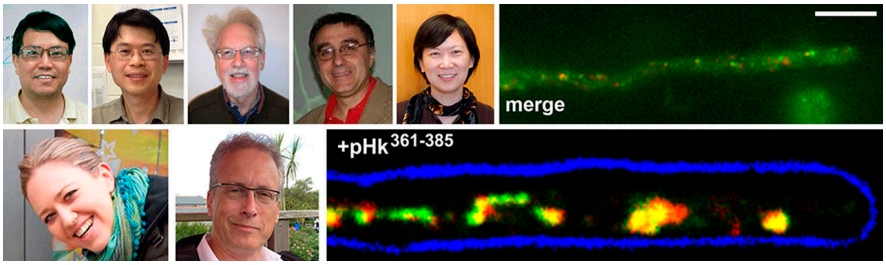
Text by Ben Short
bshort@rockefeller.edu

Motor proteins Hook on to early endosomes

Two studies identify a linker protein that coordinates endosome motility by recruiting microtubule-based motors.

FOCAL POINT



PHOTOS COURTESY OF THE AUTHORS AND GALINA PETUKHOVA (XIANG)

(Top row, left to right) Jun Zhang, Rongde Qiu, Herbert Arst Jr., Miguel Peñalva, and Xin Xiang discover that the cytoplasmic linker protein HookA (green) recruits the motor protein dynein to early endosomes (red) in *Aspergillus nidulans*, promoting the organelles' movement to microtubule minus ends. (Bottom row, left to right) Ewa Bielska, Gero Steinberg, and colleagues (not pictured) find that a related protein, Hok1 (green), plays a similar role in *Ustilago maydis*, and that it also recruits the plus end–directed motor kinesin-3 to regulate the bidirectional transport and distribution of early endosomes (red) throughout the cell. This distribution is disrupted in the presence of a peptide blocking motor recruitment.

Many organelles move through the cytoplasm along microtubules, pulled back and forth by microtubule-based motor proteins. Early endosomes, for example, are transported by the minus end–directed motor dynein and the plus end–directed motor kinesin-3, which are thought to engage in a "tug of war" with each other as they attempt to haul their cargo in opposite directions. Zhang et al. (1) and Bielska et al. (2) describe how these motors are recruited to early endosomes by a member of the Hook family of cytoplasmic linker proteins.

"A big question in the field is how motors are linked to their cargo," explains Xin Xiang from the Uniformed Services University of the Health Sciences, in Bethesda, Maryland. In 2011, Xiang and colleagues, led by research assistant professor Jun Zhang, discovered that the p25 subunit of the dynactin accessory complex was required to recruit dynein to early endosomes in the filamentous fungus *Aspergillus nidulans* (3). "But we didn't know if other proteins were required," says Xiang, "so we performed a genetic screen to look for additional players."

Zhang et al. screened for *Aspergillus* mutants that accumulated early endosomes at their hyphal tips, an indicator that dynein is unable to move these organelles away from the microtubule plus ends concentrated in this part of the cell (1). The researchers focused on mutations that inhibited endosome motility without affecting dynein's function in nuclear distribution. One such mutation was in a gene that the researchers named *hookA* because it encoded a member of the Hook family of proteins thought to connect organelles to the microtubule cytoskeleton. Deleting *hookA* significantly weakened dynein's interaction with early endosomes, preventing their transport away from the hyphal tip.

Hook proteins bind to organelles via their C-terminal domains, and this region of HookA was required for the protein's association with early endosomes. Hook proteins' N termini, on the other hand, contain a microtubule-binding domain, but, in the case of the *C. elegans* Hook protein Zyg-12, this region has also been implicated in binding to dynein (4). Zhang et al. were unable to detect an interaction between HookA and microtubules, but the protein's N terminus was important for HookA's association with dynein, an interaction that relied on components of both dynein and dynactin, including p25. "So it's a very complex interaction," Xiang says. "We still need to figure out how HookA interacts with dynein/dynactin and what other proteins mediate HookA's association with early endosomes."

> "Hok1 controls the number of kinesin-3 molecules on endosomes."

Meanwhile, in the UK, Gero Steinberg, Ewa Bielska, and colleagues at the University of Exeter were also interested in early endosome motility, this time in the plant fungus *Ustilago maydis*, which relies on endosomal transport for its growth and pathogenicity (5). Bielska et al. performed a similar screen to Xiang and colleagues and identified an *Ustilago* Hook homologue, Hok1, that recruited dynein to early endosomes to promote their removal from the hyphal tip (2). Surprisingly, however, a peptide designed to inhibit the Hok1–dynein interaction also impeded the transport of early endosomes to the plus ends of microtubules. "That suggested there was a problem with kinesin-3," Steinberg explains.

Sure enough, the inhibitory peptide reduced the number of kinesin-3 molecules attached to early endosomes, and deleting Hok1 reduced the motor's recruitment still further. Hok1 therefore recruits both dynein and kinesin-3 to early endosomes, a function that required a highly conserved section of the protein's first coiled coil. A chimeric protein containing the equivalent region of human Hook3 was able to rescue Hok1-deficient cells, suggesting that the linker's function is conserved.

Hok1 is thus in an ideal position to referee the tug of war between dynein and kinesin-3, but the motors don't compete for a limited number of attachment sites; endosomes carried five kinesin-3 molecules, regardless of their direction of travel. Instead, Bielska et al. found that early endosomes moving toward the hyphal tip transiently release kinesin-3 and pause, providing passing dynein molecules the opportunity to bind and pull the endosomes in the opposite direction. Kinesin-3 is then re-recruited, probably in an inactive state. "So Hok1 controls the number of kinesin-3 molecules on endosomes and coordinates the activity of kinesin-3 and dynein," Steinberg says. "We're now trying to find out what triggers kinesin-3's release."

1. Zhang, J., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201308009.
2. Bielska, E., et al. 2014. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201309022
3. Zhang, J., et al. 2011. *J. Cell Biol.* 193:1245–1255.
4. Malone, C.J., et al. 2003. *Cell.* 115:825–836.
5. Steinberg, G. 2007. *Nat. Rev. Microbiol.* 5:309–316.