Commentary

Putting a bifunctional motor to work: insights into the role of plant KCH kinesins

The plant cytoskeleton consists of actin microfilaments (AFs) and microtubules (MTs), which together provide a structural framework to spatially organize and execute key cellular processes such as cell division, organelle movement and cell expansion (for reviews, see Wasteneys & Galway, 2003; Petrasek & Schwarzerova, 2009). Early electron microscopy studies showed that MTs and AFs coexist in the cell, frequently in close proximity and sometimes appear to be physically connected to each other (Collings, 2008). Colocalization of MTs and AFs occurs in the interphase cortical array, preprophase band and the phragmoplast. In addition, pharmacological experiments and genetic analyses have established that cooperation between the AF and MT cytoskeletal systems is important for the organization and function of these arrays (Collings, 2008; Petrasek & Schwarzerova, 2009). What are the molecular mechanisms that achieve the colocalization of MTs and AFs as well as coordinate the activities of these two cytoskeletal systems? Kinesins containing a calponin homology domain (KCH) are promising candidates for such a function (Fig. 1a). KCHs are a subset of the kinesin-14 family and are unique to plants (Richardson *et al.*, 2006). KCHs can directly bind to both MTs and AFs *in vitro* via the motor domain and calponin homology domain, respectively (Preuss *et al.*, 2004; Xu *et al.*, 2007, 2009; Frey *et al.*, 2009; Umezu *et al.*, 2011). Immunolocalization experiments have also shown that KCHs from both dicots and monocots label MTs and AFs *in vivo* (Preuss *et al.*, 2004; Frey *et al.*, 2009; Xu *et al.*, 2009). Together, the past studies establish KCH to be a bifunctional protein that can interact with both the MT and AF cytoskeleton. But how this ability of KCH relates to its cellular function has been unclear. In this issue of *New Phytologist*, Klotz & Nick (pp. 576–589) used live-cell imaging of a GFP-tagged, full-length KCH from tobacco BY-2 cells (NtKCH) and reported that NtKCH can exist as two subpopulations: one that is associated with the cortical MT cytoskeleton, and is motile; the other associated with the AFs and MTs at the nuclear periphery, and is nonmotile (Fig. 1b). This exciting observation combined with previous genetic analysis of rice KCH (OsKCH1) by the same group (Frey *et al.*, 2010) offers new insights into the role of KCH during different phases of the cell cycle.

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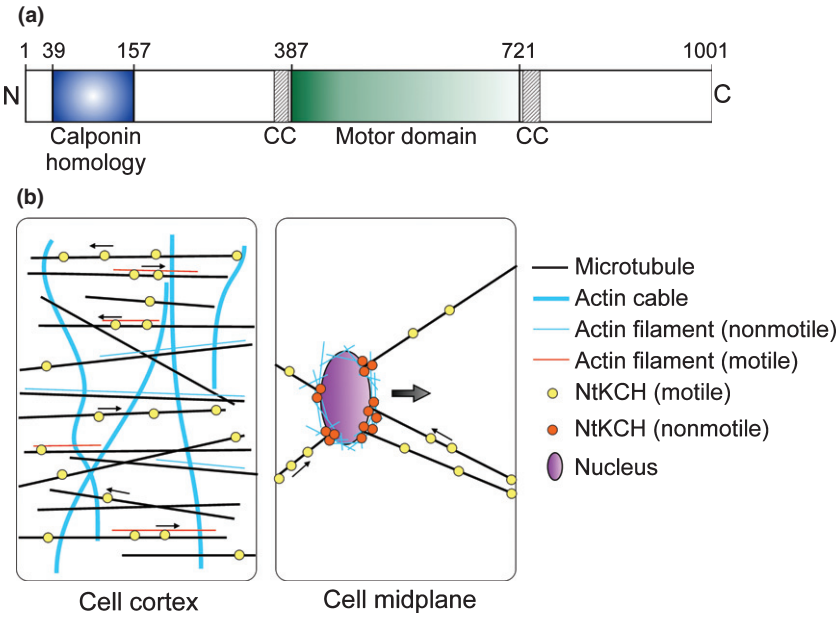

Fig. 1 NtKCH structure and cellular localization. (a) Domain structure of NtKCH. The N-terminal calponin homology domain mediates actin binding while the motor domain contains the microtubule (MT) and ATP binding sites. The motor domain is predicted to be flanked by coiled-coil (CC) domains, which might lead to dimerization. (b) Subcellular localization and proposed functions of NtKCH. During interphase, the cortical NtKCH population is motile (arrows) and might carry cargo such as actin fragments along cortical MT tracks. The perinuclear population of NtKCH is stably bound to actin and is nonmotile. The nonmotile NtKCH population is thought to tether the nuclear actin basket to MTs that extend from the nucleus to the cell cortex. Motile NtKCH molecules exist along these MTs and move inwards towards the nucleus. Cortical NtKCH molecules (or other kinesins) might exert pulling forces on these MTs, leading to movement of the nucleus towards the cell center (large arrow).

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Function of cortical KCH during interphase

The first KCH studied at the cellular level was the cotton KCH, GhKCH1 (Preuss *et al.*, 2004). Immunolocalization of GhKCH1 showed a punctate pattern at the cell cortex that overlapped with the cortical MT array (Preuss *et al.*, 2004). In addition, GhKCH1 also localized to a subset of AFs that were oriented transversely with respect to the elongation axis of the cotton fiber cell. Subsequent studies of other KCHs, including NtKCH, have consistently found a similar localization pattern during interphase: punctate decoration of cortical MTs and decoration of a subset of AFs (Frey *et al.*, 2009, 2010; Xu *et al.*, 2009). The interphase cortical MTs define the axis of cell elongation by influencing the patterning of cellulose microfibrils in the cell wall (Lloyd, 2011). AFs also contribute to this process through an unknown mechanism. So, what does the cortical localization of KCH mean? Preuss *et al.* (2004) found that expression of GhKCH1 peaked during the period of rapid cell elongation and subsequently dropped to lower levels. Similarly, expression of the *OsKCH1* gene from rice was highest in young tissues (Frey *et al.*, 2010), which would be expected to undergo rapid cell elongation. A loss-of-function mutant of *OsKCH1* showed reduced cell length in etiolated coleoptiles (Frey *et al.*, 2010). In addition, overexpression of OsKCH1 in tobacco BY-2 cells led to increased cell length (Frey *et al.*, 2010). Together, these results suggest a role for KCH during cell elongation. How might KCH fulfill this function?

A big step forward in answering this question comes from the observation by Klotz & Nick that the cortical spots of GFP-NtKCH are motile *in vivo*. Klotz & Nick isolated the *NtKCH* gene from a BY-2 cDNA library and generated stably transformed BY-2 cell cultures expressing GFP-tagged NtKCH driven by the 35S constitutive promoter. Using time-lapse imaging, they found that GFP-NtKCH spots moved unidirectionally along individual tracks, consistent with kinesin motor activity. As discrete GFP-NtKCH spots were easily identifiable, they were able to track their movement through time and found the average velocity of these spots to be *c.* 3 μm min^{-1}, about 10-fold lower than the velocity of conventional kinesin (kinesin-1). These data fit very well with the reported ATPase rate of both GhKCH2 (Xu *et al.*, 2007) and rice O12 (Umezu *et al.*, 2011). Specifically, the MT-stimulated ATPase rates of both GhKCH2 and O12 were *c.* 10-fold lower than conventional kinesin. As the ATPase cycle determines the rate at which kinesin motor heads step along the MT lattice, this would be expected to lead to a 10-fold lower velocity for KCH kinesins compared to conventional kinesin. More importantly, the work by Klotz & Nick showed that NtKCH was an active motor protein at the cell cortex. Therefore, NtKCH can now be envisioned to function at the cortex by potentially transporting some sort of cargo along cortical MTs. One potential cargo of NtKCH is AFs, which might bind to the N-terminus of NtKCH where the actin-binding calponin homology domain is located (Fig. 1a). AFs have been observed to be associated with transversely oriented cortical MTs in rapidly elongating cells (Collings, 2008). Indeed, recent live-cell imaging of AFs and MTs in *Arabidopsis* plants has shown that actin fragments move along tracks defined by cortical MTs (Sampathkumar *et al.*, 2011). The velocity of these motile actin fragments was similar to the velocity of NtKCH, supporting the possibility that KCHs might mediate at least some of this movement.

Function of perinuclear KCH during interphase

Besides the cortical localization of GFP-NtKCH during interphase, Klotz & Nick also observed GFP-NtKCH to be localized around the nucleus and in cytoskeletal filaments extending from the nucleus to the cell cortex. They found that the perinuclear localization of GFP-NtKCH was unaffected by treatment with MT depolymerizing drugs but became diffuse and cytoplasmic when cells were treated with actin depolymerizing drugs. Therefore, the perinuclear localization of NtKCH was actin dependent, in contrast to the cortical localization of NtKCH, which was MT dependent. Interestingly, Klotz & Nick found the perinuclear GFP-NtKCH puncta to be nonmotile. As Klotz & Nick pointed out, this observation means that two distinct subpopulations of NtKCH co-exist in the cell: a motile population at the cell cortex and a nonmotile population at the nuclear periphery (Fig. 1b).

One possible explanation for this exciting observation is that binding to actin might cripple NtKCH motor activity. The ATPase activity of rice O12 has been shown to be dramatically reduced in an actin concentration-dependent manner (Umezu *et al.*, 2011). If this is a common feature of KCHs, then binding of NtKCH to actin would predictably impair motor function. *In vitro* biochemical experiments have also shown that KCHs bind to AFs more strongly than to MTs (Xu *et al.*, 2009; Umezu *et al.*, 2011). Given the high density of AFs at the nuclear periphery, this might explain why the perinuclear NtKCH fraction is immotile and requires actin depolymerizing drugs to dislodge it. It is worth noting here that the binding of KCH to MTs and AFs is independent of the integrity of the other cytoskeletal system (Preuss *et al.*, 2004; Xu *et al.*, 2009). In addition, full-length GhKCH2 has been shown to induce bundles containing both MTs and AFs *in vitro* (Xu *et al.*, 2009). Therefore, binding of NtKCH to AFs is not expected to hinder its ability to bind to MTs.

If binding of NtKCH to actin reduces its motor activity, then NtKCH might not be able to transport AFs along cortical MTs as already discussed. Rather, NtKCH might serve to statically cross-link AFs and MTs at the cell cortex. Live-cell imaging in *Arabidopsis* plants has revealed that portions of cortical AFs occasionally interact with cortical MTs and become stably associated with cortical MTs for short periods (Sampathkumar *et al.*, 2011). Such activity might be mediated by KCH proteins. As the cortical AFs are extremely dynamic, their interactions with cortical MTs are likely to be very short-lived compared with the interactions between the more stable perinuclear AFs and MTs. If true, this might explain why most of the cortical NtKCH spots are motile. Alternatively, cortical and perinuclear AFs might have different accessory factors associated with them that differentially regulate NtKCH activity.

Based on the observation of two distinct subpopulations of NtKCH, and their previous finding that nuclear positioning is delayed in BY-2 cells overexpressing OsKCH1, Klotz & Nick proposed a model for nuclear migration. In their model, the static NtKCH fraction at the nuclear periphery serves to anchor the nuclear actin basket to MTs that extend to the cell cortex. These MTs are proposed to interact with motor proteins anchored at the cell cortex, which exert pulling forces on the MTs and thus drive nuclear movement. As noted by the authors, the kinesin performing the pulling function at the cortex may be a fraction of NtKCH that is immobilized or a different kinesin.

Localization and function of KCH during cell division

Besides analyzing the localization and motility of GFP-NtKCH during interphase, Klotz & Nick also used time-lapse imaging to follow the localization of GFP-NtKCH during mitosis and cytokinesis. They found NtKCH localized to the preprophase band and the phragmoplast but not to the spindle, raising the possibility that posttranslational modifications during the course of the cell cycle might regulate NtKCH binding to MTs. Interestingly, NtKCH was motile in both the preprophase band and the phragmoplast. Klotz & Nick took advantage of the uniform polarity of MTs within the phragmoplast to determine the direction of NtKCH movement along MTs. They found that NtKCH spots moved towards the minus-ends of phragmoplast MTs, which agreed with the previous finding that KinG (an *Arabidopsis* KCH) moved towards the minus-end of MTs (Buschmann *et al.*, 2011). Klotz & Nick used semi-quantitative reverse transcription polymerase chain reaction (RT-PCR) to show that NtKCH transcripts were elevated during mitosis. Together, these data suggest that NtKCH plays a role during cell division; however, the precise functions of NtKCH in the preprophase band and phragmoplast are unknown.

Concluding remarks and future directions

The data by Klotz & Nick indicate that actin is an important regulator of KCH motor activity *in vivo*. This finding significantly impacts our understanding of how KCH functions in a cell. For example, actin binding might determine whether KCH serves to transport cargo along MT tracks or whether it acts as a static tether. To directly visualize the impact of actin binding on NtKCH motility *in vivo*, it will be important to generate cell lines that co-express differentially tagged NtKCH, actin and MT markers. Another strategy to dissect how actin binding affects NtKCH motility would be to conduct *in vitro* reconstitution experiments using purified full-length NtKCH in the presence of MTs and AFs.

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