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Tubulin Folding Cofactors: Half a Dozen for a Dimer

Dispatch

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Tubulin folding cofactors control the availability of tubulin subunits and microtubule stability in eukaryotic cells. Recent work on *Arabidopsis* mutants has provided a new experimental system for understanding the cellular functions of tubulin folding cofactors.

All living cells use protein polymers to move, measure and position different objects. For example, in bacterial cells, polymers of the FtsZ protein are required to measure the midpoint of the cell and to execute cytokinesis. Eukaryotic cells use microtubules, polymers of FtsZ-related α and β tubulin, to locate and pull duplicated chromosomes, position nuclei, move organelles and define sites of cytokinesis. A dimer of an α and β tubulin subunit is the basic microtubule building block, and the asymmetry of the tubulin dimer is reflected in the structure and polarity of the microtubule (reviewed in [1]). A microtubule is an intrinsically dynamic structure, which has a fast growing 'plus' end that can undergo transition from a rapidly growing state to one in which the microtubule undergoes complete depolymerization. The switch from microtubule polymerization to depolymerization is sensitive to the intrinsic rate of hydrolysis of the GTP molecule bound to the β tubulin subunit. The 'minus' end of a microtubule has a slower rate of dimer addition and loss compared to the plus end, and is often capped *in vivo*.

In cells, the dynamic properties of microtubules are controlled by diverse microtubule-binding proteins which nucleate, stabilize, depolymerize and cluster microtubules. The amount and isotype composition of available tubulin dimers also dramatically affect microtubule function. Six tubulin folding cofactors — TFC-A, TFC-B, TFC-C, TFC-D, TFC-E and ARL2 — regulate tubulin subunit synthesis, dimer formation, and microtubule stability (reviewed in [2]). Three groups [3–5] have now used genetic analysis of morphogenesis in *Arabidopsis* to demonstrate the importance of tubulin folding cofactors during plant development.

Tubulin dimers are incredibly stable structures, but isolated α and β tubulin subunits are difficult to purify. Recent measurements of a dimer dissociation rate constant of 9.6 hours and a dissociation binding constant of 10^{-11} M explain the scarcity of isolated subunits in cell extracts [6]. *In vitro* translation of soluble α and β tubulin is also not trivial: newly synthesized α and β tubulin polypeptides bind tightly to the cytosolic chaperonin complex on their journey to the native state, and are released from the complex only after binding to TFC-B and TFC-A, respectively [7,8]. But binary complexes between tubulin and tubulin folding cofactors do not yield polymerization-competent subunits.

Biochemical analyses of the tubulin folding pathway identified three additional proteins that are required for the synthesis of polymerization-competent tubulin subunits. TFC-D binds tightly to β tubulin and displaces TFC-A. The subsequent association of the β tubulin–TFC-D complex with TFC-C/TFC-E leads to the release of polymerization competent β tubulin [7]. Subsequently Cowan's group [8] showed that α tubulin is recruited to the tubulin folding cofactor complex via sequential interactions with TFC-B and TFC-E. A model was derived in which α and β tubulin folding pathways converge with the formation of a multimeric complex consisting of β tubulin–TFC-D, α tubulin–TFC-E and TFC-C, through which subunit association and dimer release occur (Figure 1). Tian *et al.* [8] showed that TFC-D has potent dimer-splitting activity and the proposed folding reactions are reversible, but the *in vivo* importance of the tubulin folding cofactor-mediated back-reaction is not known.

Yeast tubulin folding cofactor genes were identified in mutant screens for cells that were compromised in microtubule-dependent functions. In the budding yeast, *Saccharomyces cerevisiae*, tubulin folding cofactor genes are not essential, but their mutations confer an increased frequency of chromosome loss and hypersensitivity to microtubule-disrupting drugs, and show genetic interactions with tubulin mutations [9,10]. Consistent with the notion that tubulin folding cofactors function in a common pathway, double and triple combinations of mutation affecting tubulin folding cofactors did not cause more severe phenotypes [10]. The fission yeast *Schizosaccharomyces pombe* has more constrained requirements for microtubules during development; this may explain why tubulin folding cofactor mutations in this species are lethal and cause severe cell shape and polarity defects. Although tubulin folding cofactor mutants in both yeast species display an altered microtubule cytoskeleton, the defects are not clearly attributable to decreases in the steady state levels of α or β tubulin [11,12].

The genetic analyses in yeast identified a clear functional hierarchy among tubulin folding cofactors. Overexpression studies revealed clear differences between the various tubulin folding cofactors in their ability to bypass the requirement for each other. In fission yeast, for example, overproduction of either TFC-D or TFC-E can bypass the requirement for TFC-B, but while overproduction of TFC-D can functionally substitute for TFC-E, the converse is not true [13]. Similar results were obtained with budding yeast [9]. Mutations in the budding yeast gene *CIN1*, which encodes a TFC-D like protein, are epistatic to mutations in two other genes for tubulin folding cofactor-like proteins, *CIN2* and *CIN4* [14]. It may be that the

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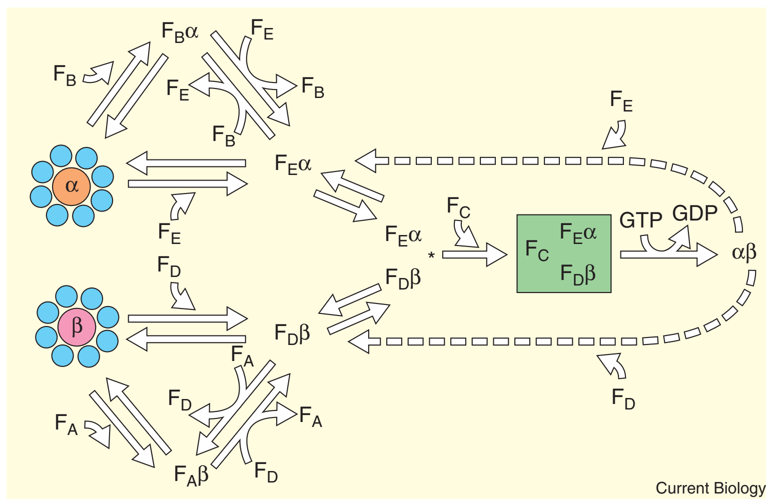

Figure 1. A model of the tubulin folding pathway based on biochemical data.

Newly translated α and β tubulin subunits sequentially associate with the chaperonin complex (blue circles), then interact with tubulin folding cofactors TFC-B (F_B) and TFC-A (F_A), respectively. The pathways converge following transfer of the subunits to TFC-E (F_E) and TFC-D (F_D), respectively, and formation of the F_D–β/F_E–α complex (green). F_C promotes GTP hydrolysis and dimer release from the complex. Broken arrows indicate the back reaction between the dimer and cofactors F_D and F_E. (Adapted from [8].)

yeast tubulin folding cofactors function sequentially and activate downstream folding cofactors. It is also possible that the tubulin folding cofactors function as a complex, but there are differences among cofactors in the extent to which their activities can be substituted. In either case, TFC-D appears to have the most specialized function — it is the only cofactor shown to co-localize with microtubules, and is negatively regulated by ARL2, itself a tubulin folding cofactor with GTPase activity [9,15,16].

Although tubulin folding cofactor activity was originally discovered with vertebrate proteins, the extent to which tubulin folding cofactors are important during multicellular development has not been clear. But now two different morphology-based mutant screens have identified tubulin folding cofactors as essential players in plant morphogenesis. In the first of these screens [5], map-based cloning of several genes required for early embryo development identified *Arabidopsis* homologues of TFC-A, TFC-C, TFC-D, TFC-E and ARL2.

First some background on the cell biology of early plant development: in the developing *Arabidopsis* zygote, a transient preprophase band of cortical microtubules marks future sites of cell division. The preprophase band microtubules are recycled into the mitotic spindle, then to the phragmoplast microtubule array that directs vesicles containing new wall material to the expanding cell plate. In the endosperm, several rounds of nuclear division occur in the absence of cytokinesis and pre-prophase band formation. Prior to endosperm cellularization, microtubules emanate from nuclear membrane and appear to position new cell plate synthesis at points where microtubules from the two adjacent nuclei are stabilized in an anti-parallel orientation (reviewed in [17]).

In their screen, Steinborn *et al.* [5] identified what they dubbed 'Pilz' group embryo-lethal mutants, which display a range of zygotic defects: abortive embryos with a severely reduced cell number, defective cell plates and enlarged nuclei (Figure 2). With the exception of *KIESEL*^{A} (*KIS*), which encodes a TFC-A-like protein, the Pilz group tubulin folding cofactors were also found to be required for normal endosperm development. *KIS*^{A} gene activity may be more important during pre-prophase band formation. Surprisingly, the haploid phase of the plant was not affected by the tubulin folding cofactor mutations, as the mutant allele was efficiently transmitted through both male and female gametophytes.

Functional homologues of TFC-C have not been studied in yeast, although database searches using the sequence of the *Arabidopis PORCINO*^{C} (*POR*^{C}) gene identified a possible fission yeast homologue. Developing *por*^{C} embryos display a dramatic reduction in α tubulin; as a result, localization of the cell plate specific syntaxin KNOLLE and cell-plate formation is defective in these mutant embryos. The effects seem to be specific to the microtubule cytoskeleton, as the organization of the actin cytoskeleton, and the localization of the actin-dependent plasma membrane cell polarity marker PIN1, were found not to be dramatically altered. Although lacking TFC-A-like activity, *kis*^{A} null embryos display an extensive cortical microtubule signal similar to the wild type. The less severe *kis*^{A} phenotype is consistent with the TFC-A-like gene being the only non-essential cofactor in fission yeast. POR^C co-purifies with α tubulin, and is partitioned between a monomeric form and two discrete high molecular weight complexes of approximately 400 kDa and 600 kDa. With an anti-POR^C antibody, the signal was distributed throughout the cytoplasm and did not co-localize with microtubules. These results are consistent with the notion that *POR*^{C} and the tubulin folding cofactor complex is required for the polymerization and stability of microtubules during cell division.

Two other recent studies [3,4] indicate that tubulin folding cofactors are also important in plants during growth of non-dividing cells, such as the leaf hair cells known as trichomes. *Arabidopsis* trichomes are multibranched, unicellular structures that have distinct requirements for microtubules and actin filaments during their morphogenesis (reviewed in [18]). Weak alleles of *KIS*^{A} and *POR*^{C} were identified by a reduction in trichome branching, a clear indicator of a microtubule-based defect. The *kis*^{A} and *por*^{C} mutant plants also displayed dwarfism, sporophytic sterility and

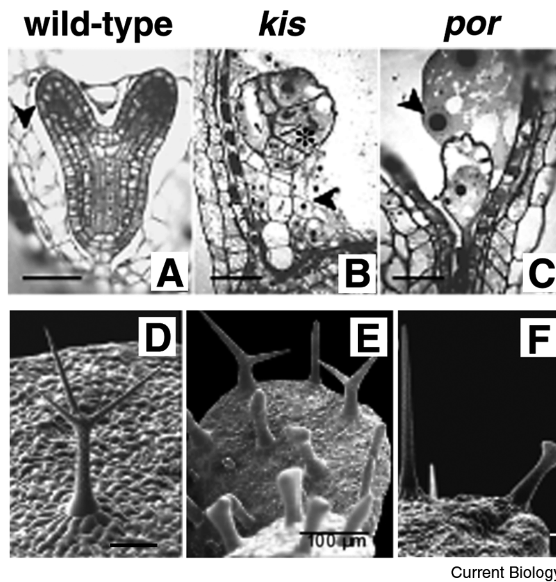

Figure 2. Phenotypes of *kis*[A] and *por*[C] mutant *Arabidopsis* embryos and trichomes.

(A–C) Thick sections through developing (A) wild-type, (B) *kis*[A] and (C) *por*[C] heart stage embryos. The two elongated structures at the top of the wild-type embryo are developing cotyledons. The root axis is clearly defined at this stage. Mutant embryos contain defective cell walls (asterisk) and endosperm that cellularizes (arrowheads) in the wild-type and *kis*[A], but not in *por*[C]. (D–F) Scanning electron micrographs of (D) wild-type, (E) *kis*[A], and (F) *por*[C] trichomes. Wild-type trichomes usually contain three branches. *kis*[A] and *por*[C] trichomes display reduced branching and blunt branch tips. Similar phenotypes are obtained by treating trichomes with microtubule, but not actin filament-disrupting drugs. Scale bars: A–C,D,F 50 μm; E, 100 μm. (Reproduced with permission from [3–5].)

hypocotyl cell shape defects in elongating dark-grown plants. The loss of *POR*[C] and *KIS*[A] function could be rescued by overexpression of either the endogenous gene or a vertebrate homologue.

If the function of TFC-A factors is to promote β tubulin synthesis, it is surprising that overproduction of α tubulin, but not β tubulin, is sufficient to bypass the requirement for *KIS*[A] [4]. Perhaps the *kis*[A] phenotype is due to the presence of toxic levels of free β tubulin [19], and excess α tubulin relieves the toxicity via dimer formation. In wild-type trichomes, the microtubule cytoskeleton reorients from a transverse to parallel orientation with respect to the elongating branches. In *por*[C] and *kis*[A] plants trichome branches display an extensive microtubule cytoskeleton, but the orientation remains fixed in the transverse direction.

It is not clear exactly how defects in tubulin folding cofactors affect microtubule function. Plant microtubules display a higher degree of dynamic instability than animal microtubules [20]. The extent of instability may depend on tubulin isotype composition: *Arabidopsis* has six α and nine β tubulin genes. Do tubulin folding cofactors regulate the supply and dimerization specificity, as well as the turnover, of existing dimers and microtubules? Microtubule dynamics is dramatically affected by the concentration of free tubulin dimers. A spatially regulated supply of dimers would be a very efficient method for regulating microtubule polymerization and stabilization in large eukaryotic cells. Perhaps the location and number of active tubulin folding cofactor complexes control the availability of polymerization-competent tubulin dimers. In *Arabidopsis*, the tools are accessible to address the relationship between microtubule dynamics, tubulin folding cofactor function, and morphogenesis in living cells. It will be interesting to see how this tubulin story unfolds.

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