

frequencies<sup>11</sup>. Therefore, in the cases reported, lack of wild-type BRCA1 protein expression needs to be confirmed.

Although differential toxicity to cells of the luminal lineage cannot be ruled out in these experiments, the data presented by Wicha and colleagues suggest that BRCA1 loss of function causes a block in progenitor-cell differentiation, which results in the accumulation of early progenitor cells with a basal phenotype. This may explain the basal phenotype of breast cancers arising in carriers of BRCA1-mutations. Nevertheless, although the BRCA1-tumour phenotype is frequently denoted simply as 'basal', this is an oversimplification. In fact, most human BRCA1 'basal' tumours also express some luminal markers, such as cytokeratins 8/18, albeit at lower levels<sup>12</sup>. Similar basal and luminal differentiation has been reported in a mouse model of *Brca1*-associated breast cancer in which a Cre-*loxP* strategy was used to generate *Brca1* null alleles in the basal stem/progenitor cells<sup>13</sup>. Interestingly, generation of *Brca1* null alleles in the luminal layer of the mouse mammary epithelium also resulted in basal phenotype tumours<sup>14</sup>. The existence of both basal and luminal differentiation in human BRCA1 tumours, and in certain mouse models, suggests that BRCA1 loss of function affecting the undifferentiated stem/progenitor-cell

compartment exclusively is uncommon and any differentiation-block probably occurs after the formation of separate progenitors of the basal and luminal lineages. Furthermore, BRCA1 inactivation in cells displaying at least some degree of luminal differentiation causes expression of basal differentiation markers, in conjunction with a partial maintenance of the luminal phenotype (ref. 7, 14; C. James and P. Harkin, personal communication). Hence, the phenotype of BRCA1 tumours could also be interpreted as resulting from either trans-differentiation (switching from a luminal to a basal lineage) or reversion (acquisition of a more primitive basal progenitor-like phenotype). It is possible that the mechanisms driving this involve the roles of BRCA1 in the regulation of transcription and in the maintenance of genomic integrity. Loss of BRCA1 function induces profound transcriptional changes, which may facilitate phenotypic reprogramming, as well as genetic instability, causing genetic alterations that may prevent escape from this phenotypic reprogramming. This may also generate a pool of genetically unstable tumour-initiating cells on which clonal selection could subsequently act.

Knowledge of the cellular origin of different breast cancer subtypes will be important in understanding mechanisms of sensitivity and resistance to therapies<sup>15,16</sup>. Moreover,

the relationship between these subtypes and stem cells will inform strategies to prevent the disease. Perhaps of more immediate relevance, the existence of large patches of breast tissue in carriers of BRCA1 mutations<sup>5</sup>, which may have already undergone the early steps in carcinogenesis, could provide a biomarker, both to assess risk and to monitor the effects of preventative strategies. PARP inhibitors, which are selectively lethal to cells with loss of BRCA1 function<sup>15</sup>, provide one such potential therapeutic strategy.

- 1 Smalley, M. & Ashworth, A. *Nature Rev. Cancer* **3**, 832–844 (2003).
- 2 Turner NC, Tutt A. & Ashworth, A. *Nature Rev. Cancer* **4**, 814–819 (2004).
- 3 Foulkes, W. D. J. *Med. Genet.* **41**, 1–5 (2004).
- 4 Dontu, G., El-Ashry, D. & Wicha, M. S. *Trends Endocrinol. Metab.* **15**, 193–197 (2004).
- 5 Liu, S. *et al. Proc. Natl Acad. Sci. USA* **105**, 1680–1685 (2008).
- 6 Ashworth, A. *Cancer Cell* **8**, 95–97 (2005).
- 7 Hoseney, A. *et al. J. Natl Cancer Inst.* **99**, 1683–1694 (2007).
- 8 Turner, N. C. & Reis-Filho, J. S. *Oncogene* **25**, 5846–5853 (2006).
- 9 Ginestier, C. *et al. Cell Stem Cell* **1**, 555–567 (2007).
- 10 Novelli, M. *et al. Proc. Natl Acad. Sci. USA* **100**, 3311–3314 (2003).
- 11 Clarke, C. *et al. Br. J. Cancer* **95**, 515–519 (2006).
- 12 Palacios, J. *et al. Breast Cancer Res. Treat.* **90**, 5–14 (2005).
- 13 Liu, X. *et al. Proc. Natl Acad. Sci. USA* **104**, 12111–12116 (2007).
- 14 McCarthy, A. *et al. J. Pathol.* **211**, 389–98 (2007).
- 15 Farmer, H. *et al. Nature* **434**, 917–921 (2005).
- 16 Edwards, S. *et al. Nature* **451**, 1111–1115 (2008).

## Dam1 complexes go it alone on disassembling microtubules

Melissa K. Gardner and David J. Odde

**Kinetochores maintain a mechanical grip on disassembling microtubule plus ends, possibly through a 16-member Dam1 ring that acts as a sliding clamp. It turns out, however, that a ring is not required for maintaining grip: individual Dam1 complexes *in vitro* can diffuse on the microtubule lattice and track shortening microtubule tips.**

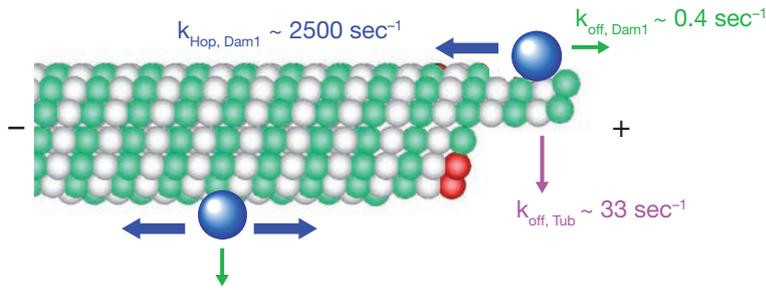
During mitosis, dynamic microtubule ends mediate the faithful segregation of replicated chromosomes into each of two daughter cells.

Melissa K. Gardner and David J. Odde are in the Department of Biomedical Engineering, University of Minnesota, 312 Church Street SE, 7-132 Hasselmo Hall, Minneapolis, Minnesota 55455, USA.  
e-mail: oddex002@umn.edu

Kinetochores provide the essential mechanical link between dynamic microtubule ends and the replicated DNA. A key question in mitosis is how kinetochores remain attached specifically to the dynamic ends of microtubules. This question is particularly challenging given that, in *Saccharomyces cerevisiae* for example, approximately 2000 tubulin subunits are added

and lost per minute at dynamic microtubule tips, whereas kinetochores remain stably associated with microtubule tips during metaphase for 20 min or longer<sup>1,2</sup>.

The Dam1 (DASH) complex is a key kinetochore component in *S cerevisiae* that mechanically links individual kinetochores to microtubule ends<sup>3</sup>. When reconstituted *in vitro*, the Dam1



**Figure 1** A biased-diffusion model for tip-tracking of single Dam1 complexes on depolymerizing microtubule ends. **(a)** The unphosphorylated Dam1 complex (blue sphere) at a microtubule plus end (red, GTP-tubulin subunits, green, GDP-tubulin subunits) has three possible fates. First, the Dam1 complex could dissociate from the microtubule (green arrow), with a calculated off-rate constant ( $k_{\text{off, Dam1}}$ ) of approximately  $0.4 \text{ s}^{-1}$ . Second, the Dam1 complex could diffuse away from the end subunit to the penultimate tubulin subunit (blue arrow), with a calculated hopping rate constant ( $k_{\text{Hop, Dam1}}$ ) of approximately  $2500 \text{ s}^{-1}$ . Third, the Dam1 complex could remain stably associated with the end tubulin subunit during depolymerization, such that the Dam1 complex is lost with the end tubulin subunit (magenta arrow). Tubulin dimers at the ends of depolymerizing microtubules leave the lattice with a rate constant ( $k_{\text{off, Tub}}$ ) of approximately  $33 \text{ s}^{-1}$ . Given these rates, the most likely fate by far is for the Dam1 complex to diffuse away from the tip, a strong diffusional bias that naturally gives rise to the experimentally observed tip-tracking of Dam1 complexes at depolymerizing microtubule ends.

complex oligomerizes to form a ring around the microtubule<sup>4,5</sup>. This ring can diffuse along the microtubule and be driven toward the minus end by a depolymerizing plus end<sup>5</sup>. To explain ring transport, it was hypothesized that microtubule protofilament-curling during depolymerization mechanically pushes the ring towards the minus end of the microtubule, a mechanism referred to in earlier studies as a 'conformational wave'<sup>6</sup>, or more recently as either a 'power-stroke' or a 'forced-walk'<sup>4,7,8</sup>. On pages 407 of this issue, Gestaut *et al.* show that a single Dam1 complex can diffuse along a microtubule and track a depolymerizing end, thus demonstrating that a ring is not necessary for depolymerization-driven movement of the Dam1 complex<sup>9</sup>. Their results suggest that protofilament-curling is not important for kinetochore motility, and instead favour a biased-diffusion mechanism, consistent with a model described earlier<sup>10</sup>.

As with all *in vitro* studies, the connection to an *in vivo* mechanism is potentially problematic. For example, for kinetochores *in vivo*, it is reasonable to posit that molecular motors may be important in maintaining attachment<sup>10,11</sup>. However, recent studies in *Schizosaccharomyces pombe* showed that deletion of every known minus-end-directed molecular motor failed to block chromosome bi-orientation<sup>12</sup>, suggesting that passive kinetochore complexes such as Dam1 directly mediate attachment to, and end-tracking of, microtubules. Thus, the kinetochore *in vivo* seems to track the microtubule end by a process that is intrinsic to the kinetochore complex itself. Reconstituting *in vitro*

versions of the kinetochore should provide insights into tracking mechanisms; the single-complex studies by Gestaut *et al.*<sup>9</sup> represent the most simplified *in vitro* reconstitution to date.

Although previous studies showed Dam1 ring movement along microtubules, Gestaut *et al.* used total internal reflection fluorescence (TIRF) microscopy to allow imaging of the single Dam1 complex. Using picomolar concentrations to prevent oligomerization, they found that single Dam1 complexes bind to and diffuse along microtubules, with a stepping rate of  $2500 \text{ s}^{-1}$  (assuming a distance of 8 nm per step). By comparison, the dissociation rate of a single Dam1 complex was  $0.4 \text{ s}^{-1}$ , so that a Dam1 complex takes an average of approximately 6000 steps along a microtubule lattice before dissociating. As illustrated in Fig. 1, this large disparity in rates implies that a Dam1 complex bound to the most distal tubulin in a protofilament will almost always diffuse back toward the penultimate tubulin (that is, toward the minus end) rather than dissociate. In fact, given the rate of microtubule depolymerization in these studies ( $260 \text{ nm s}^{-1}$ , or 33 tubulin dimers per second per protofilament), it is far more likely that the tubulin itself, rather than the Dam1 complex, will dissociate from the lattice. Even so, tubulin dissociation is slow compared with the stepping rate of diffusion on the lattice. Thus, there is a large free energy keeping the Dam1 complex on the microtubule (estimated to be approximately  $19 k_B T$  by Gestaut *et al.*), compared with the relatively small barrier limiting diffusional hopping to the adjacent site.

It is puzzling that, with such a high frequency of diffusional hops along the microtubule lattice, the single Dam1 complexes track with the depolymerizing ends at all. It seems that they may rapidly diffuse away from the shortening ends and distribute randomly along the lattice. However, although the diffusional hops are frequent, they are directionally unbiased, so the Dam1 complexes are just as likely to diffuse towards the shortening microtubule tip as away from it. Consequently, it is possible that such a system could, in principle, still track a shortening end. In our view, this constitutes a simple diffusion-convection problem, with the concentration of Dam1 complex expected to obey an exponential decay away from the tip, of length  $D/v = 0.3 \mu\text{m}$  (where  $D$  is the diffusion constant,  $0.08 \mu\text{m}^2 \text{ s}^{-1}$  and  $v$  is the depolymerization rate,  $0.26 \mu\text{m s}^{-1}$ ). Such a steep gradient of expected position relative to the end would probably seem to be tip-tracking of the shortening end. Thus, the slow, but biased, microtubule-end depolymerization will tend to keep catching the fast, but unbiased, diffusing Dam1 complexes. Once the Dam1 complexes reach the very end of the microtubule, they would at that moment be very biased in their diffusion back toward the microtubule, as depicted in Fig. 1.

The biased-diffusion model advanced by Gestaut *et al.* is similar to that of Hill<sup>10</sup>, which suggests that the kinetochore could be viewed as a sleeve that hops axially along the microtubule. With greater insertion of the microtubule into the sleeve, there would be a decreasing (favourable) free energy because of the increased number of bonds between the kinetochore and the microtubule. Of course, the Dam1 complexes in the study by Gestaut *et al.* are in a non-ring form and so do not constitute a 'sleeve'. However, the key features of the Hill model are that the linker be able to diffuse, and that there is a favourable free energy between the linker and the microtubule. More generally, this model can be viewed as one in which there is a favourable interfacial surface tension between the kinetochore and microtubule. As the tip-tracking behaviour of rings and single complexes is similar, it seems reasonable that Dam1 ring motion during depolymerization could also be driven by a biased-diffusion mechanism.

An alternative to the biased-diffusion model is a 'conformational wave' model, in which protofilament-peeling drives Dam1 ring/kinetochore motion<sup>6</sup>. However, it is not clear how Dam1 in a non-ring form could be driven by this mechanism. Perhaps most telling is that

growing microtubules, which do not have curling protofilaments, still maintain their mechanical connections to rings *in vitro* and to kinetochores *in vivo*, even when they are under tensile loads similar to those on shortening microtubules<sup>13,14</sup>. The biased-diffusion model provides a consistent explanation for force generation and stable kinetochore attachment to both growing and shortening microtubules.

For chromosomes to segregate properly, kinetochores must maintain their grip on the microtubule. However, if a kinetochore is improperly attached to the spindle then corrective action is needed to loosen the grip. Failure to correct the error will produce aneuploidy, a correlate and possible cause of cancerous transformation<sup>15</sup>. Several studies have established that the Aurora B kinases, including Ipl1p in *S. cerevisiae*, mediate the detachment and correction of misattachments<sup>16</sup>. Gestaut *et al.* compared Dam1 complexes that were phosphorylated at Ser 20 by Ipl1p with unphosphorylated Dam1 complex. They found that the binding affinity of the phosphorylated form for microtubules was two-fold lower, compared with the dephosphorylated form. This is consistent with microtubule-associated proteins (MAPs) generally, which tend to dissociate from microtubules more readily when phosphorylated. In the case

of the Dam1 complex, the lower affinity will make kinetochore detachment from the microtubule more likely, which will presumably aid correction. Although Gestaut *et al.* observed only a modest two-fold effect on the affinity of a single molecule of Dam1 for microtubules, this converts into a 2<sup>16</sup>-fold effect if all members of a ring are phosphorylated, which would reduce the characteristic force required to pull the ring off the microtubule.

What do these results mean for kinetochores *in vivo*? Although the single-molecule analysis helps to elucidate fundamental motility principles, the binding analysis suggests that higher-order oligomers are still functionally important. In particular, the finding by Gestaut *et al.* that Dam1-complex binding to microtubules is cooperative suggests that oligomers, perhaps even rings, will form *in vivo*. As the Dam1 complex interacts with other kinetochore components to transmit forces from the microtubule to the DNA, these components will also presumably affect the thermodynamics of Dam1-complex self-association in the presence of microtubules. So, it may be that small oligomers (for example, 2–4 units) may form functional units that do not coalesce into a complete encircling ring. Given that electron microscopy fails to reveal the existence of rings *in vivo*<sup>17</sup>, it

may well be that an intermediate oligomeric state is the functional unit *in vivo*. The results of Gestaut *et al.* now give us a clear indication that it is physically possible for small, non-encircling couplers to do the important job of tracking disassembling microtubule ends.

1. Maddox, P., Bloom, K. & Salmon, E. D. *Nature Cell Biol.* **2**, 36–41 (2000).
2. Pearson, C. G. *et al. Mol. Biol. Cell* **17**, 4069–4079 (2006).
3. Cheeseman, I. M. *et al. J. Cell Biol.* **155**, 1137–1145 (2001).
4. Miranda, J. J., De Wulf, P., Sorger, P. K. & Harrison, S. C. *Nature Struct. Mol. Biol.* **12**, 138–143 (2005).
5. Westermann, S. *et al. Mol. Cell* **17**, 277–290 (2005).
6. Koshland, D. E., Mitchison, T. J. & Kirschner, M. W. *Nature* **331**, 499–504 (1988).
7. Efremov, A., Grishchuk, E. L., McIntosh, J. R. & Ataullakhanov, F. I. *Proc. Natl Acad. Sci. USA* **104**, 19017–19022 (2007).
8. Westermann, S. *et al. Nature* **440**, 565–569 (2006).
9. Gestaut, D. R. *et al. Nature Cell Biol.* **10**, 407–414 (2008).
10. Hill, T. L. *Proc. Natl Acad. Sci. USA* **82**, 4404–4408 (1985).
11. Inoue, S. & Salmon, E. D. *Mol. Biol. Cell* **6**, 1619–1640 (1995).
12. Grishchuk, E. L., Spiridonov, I. S. & McIntosh, J. R. *Mol. Biol. Cell* **18**, 2216–2225 (2007).
13. Franck, A. D. *et al. Nature Cell Biol.* **9**, 832–837 (2007).
14. Skibbens, R. V., Skeen, V. P. & Salmon, E. D. *J. Cell Biol.* **122**, 859–875 (1993).
15. Weaver, B. A. & Cleveland, D. W. *Cancer Res.* **67**, 10103–10105 (2007).
16. Pinsky, B. A., Kung, C., Shokat, K. M. & Biggins, S. *Nature Cell Biol.* **8**, 78–83 (2006).
17. McIntosh, J. R. *Nature Struct. Mol. Biol.* **12**, 210–212 (2005).

## Securin' M-phase entry

Mark J. Solomon and Janet L. Burton

**The anaphase-promoting complex (APC) mediates the ubiquitination and degradation of key M-phase regulators, including cyclins and the anaphase inhibitor securin. Intriguingly, securin can also inhibit the degradation of cyclin B. This competition between substrates permits the accumulation of enough cyclin to drive entry into M phase.**

The APC is a large ubiquitin ligase that promotes the degradation of several cell-cycle regulators during G1 and M phase. Securin is a key APC substrate and the one whose degradation gave rise to the name APC: securin

binds to and inhibits a protease termed separase, which, when released following securin degradation, cleaves proteins that hold sister chromatids together, thereby initiating anaphase. On page 445 of this issue, Marangos and Carroll<sup>1</sup> show that securin also functions as an APC inhibitor, facilitating the accumulation of another APC substrate, cyclin B, to promote mitosis. Thus, securin joins a growing family of mechanistically related

APC inhibitors that range from pure inhibitors to those that are also APC substrates.

As with other ubiquitin ligases (or 'E3s'), the APC brings substrates into close proximity of a ubiquitin-conjugating enzyme (an 'E2') so that the E2 can transfer ubiquitin to the substrate. Although it contains at least thirteen distinct proteins, the APC still needs help to bind most substrates. The so-called APC activators, Cdc20 and Cdh1, fulfill this role

Mark J. Solomon and Janet L. Burton are at Yale University School of Medicine, Department of Molecular Biophysics and Biochemistry, 333 Cedar Street, New Haven, CT 06520-8024, USA.  
e-mail: Mark.Solomon@yale.edu