some movements in oocyte spindles. They speculate that these attachments could drive meiotic chromosome segregation because Klp-19, the plus-end motor driving congression in oocyte spindles, is removed from chromosomes at anaphase[15], potentially resulting in a net increase in minus-end-directed lateral chromosome movement towards spindle poles. To prevent chromosome segregation errors, however, chromosomes would have to bi-orient, with homologues (meiosis I) or sister chromatids (meiosis II) establishing lateral interactions with microtubules that extend from opposite spindle poles, before anaphase. It is possible that the site-specific and overlapping localizations of Klp-19 and the chromosomal passenger complex (Aurora kinase), which is known to regulate chromosome–microtubule attachments[16], could contribute to establishing bi-orientation in meiosis in *C. elegans*. Additional studies should shed light on how the restricted localization of factors that mediate bi-orientation ensures accurate chromosome spindle attachments.

The powerful assays developed by Sakuno *et al.*, which allow different sister chromosome geometries to be engineered through artificial tethers, may need to be combined with electron microscopy analysis and, in the future, extended to other systems, to fully explore how chromosome geometry influences different attachment modes and how inter-conversions between these attachment states are possible. Tracking the different routes chromosomes take to the spindle equator during cell division will no doubt continue to intrigue and surprise us.

1. Maiato, H., DeLuca, J., Salmon, E. D. & Earnshaw, W. C. *J. Cell Sci.* **117,** 5461–5477 (2004).
2. Wadsworth, P. & Khodjakov, A. *Trends Cell Biol.* **14,** 413–419 (2004).
3. Karsenti, E. & Vernos, I. *Science* **294,** 543–547 (2001).
4. Khodjakov, A., Copenagle, L., Gordon, M. B., Compton, D. A. & Kapoor, T. M. *J. Cell Biol.* **160,** 671–683 (2003).
5. Cai, S., O'Connell C. B., Kohdjakov, A. & Walczak, C. E. *Nature Cell Biology* **11,** 832–838 (2009).
6. Wignall, S. M. & Villeneuve, A. M. *Nature Cell Biology* **11,** 839–844 (2009).
7. Sakuno, T., Tada, K. & Watanabe, Y. *Nature* **458,** 852–858 (2009).
8. Kapoor, T. M. *et al. Science* **311,** 388–391 (2006).
9. Wood, K. W., Sakowicz, R., Goldstein, L. S. & Cleveland, D. W. *Cell* **91,** 357–366 (1997).
10. Manning, A. L. & Compton D. A. *Curr. Biol.* **17,** 260–265 (2007).
11. Skold, H. N., Komma, D. J. & Endow, S. A. *J. Cell Sci.* **118,** 1745–1755 (2005).
12. Brunet, S. *et al. J. Cell Biol.* **146,** 1–12 (1999).
13. Hauf, S. & Watanabe, Y. *Cell* **119,** 317–327 (2004).
14. Hoffman, D. B., Pearson, C. G., Yen, T. J., Howell, B. J. & Salmon, E. D. *Mol. Biol. Cell* **12,** 1995–2009 (2001).
15. Powers, J. *et al. J. Cell Biol.* **166,** 991–1001 (2004).
16. Kelly, A. E. & Funabiki, H. *Curr. Opin. Cell Biol.* **21,** 51–58 (2009).

Cavin fever: regulating caveolae

Ivan R. Nabi

SDPR is a new regulator of caveolae biogenesis. SDPR overexpression results in increased caveolae size and leads to the formation of caveolae-derived tubules containing Shiga toxin. SDPR may therefore be a membrane curvature-inducing component of caveolae.

Caveolae are morphologically defined plasma membrane invaginations of a relatively constant size (50–100 nm) that function as endocytic carrier vesicles. Formation of caveolae is dependent on expression of the caveolar coat protein, caveolin-1 (Cav1) or, in muscle, Cav3 (ref. 1). Recently, PTRF (polymerase I and transcript release factor, also known as cavin-1), was found to be required for caveolae formation[2,3]. However, regulators of caveolae dynamics remain poorly characterized. On page 807 of this issue, Hansen *et al.*, show that a PTRF-related protein, SDPR (serum deprivation protein response; also known as cavin-2), is required for caveolae formation and that it regulates caveolae morphology and dynamics by inducing membrane tubules, similar to those induced by the glycosphingolipid-binding Shiga toxin[4].

As reported previously for PTRF[2,3], Hansen *et al.* found that depletion of SDPR, which is known to associate with caveolae[5], reduces Cav1 expression levels and limits caveolae formation[4]. Furthermore, knockdown of *SDPR* reduces PTRF levels and vice versa. SDPR, PTRF and Cav1 were found to co-immunoprecipitate, suggesting that they form a protein complex in which expression of one member critically regulates expression of the other complex members. The dependence of Cav1 expression on these two cytoplasmic proteins could explain the requirement of these adaptor proteins for caveolae formation. However, in cells overexpressing both Cav1 and PTRF, and concomitantly presenting increased caveolae, SDPR depletion still reduced caveolae formation. More importantly, whereas overexpression of PTRF did not affect caveolae morphology, overexpression of SDPR resulted in the formation of larger caveolae and also induced caveolae-associated tubule formation. These findings not only define a clear role for SDPR in caveolae biogenesis that is distinct from that of PTRF, but they also demonstrate a role for this protein in caveolae morphology[4].

Another PTRF homologue, SRBC (sdr-related gene product that binds to c-kinase; also known as cavin-3), has recently been shown to regulate caveolae endocytosis[6]. A fourth member of this family, MURC (muscle restricted coiled-coiled protein; also known as cavin-4), is muscle specific, but whether it interacts with Cav3 and regulates caveolae function in muscle remains to be determined[7,8]. Knockdown of *SRBC* does not affect Cav1 levels, but limits budding and intracellular trafficking of Cav1-positive vesicles from the plasma membrane[6]. Importantly, Hansen

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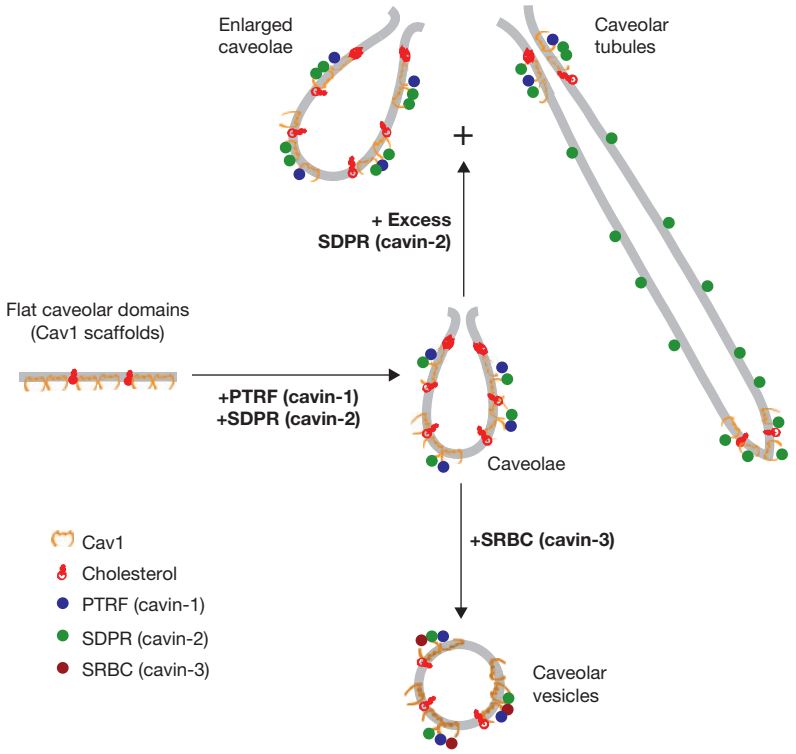

Figure 1 Regulation of caveolae morphology and dynamics by cavin family members. Cav1 is associated with both flat caveolar domains and Cav1 scaffolds[11] as well as invaginated smooth plasmalemmal vesicles or caveolae. PTRF (cavin-1) and SDPR (cavin-2) are required for caveolae invagination and SRBC (cavin-3) for caveolae budding to form caveolar vesicles. Overexpression of SDPR induces enlarged caveolae and caveolar tubules, defining critical roles for cavin family members in caveolae formation, morphology and dynamics.

et al.[4] show that all four cavins, PTRF, SDPR, SRBC and MURC, are recruited to caveolae. Overexpression of individual complex members alters caveolae biogenesis, suggesting that altered stoichiometry of these members may affect caveolae formation, morphology, dynamics and function (Fig. 1).

A requirement for PTRF and SDPR in caveolae formation is consistent with functional roles for Cav1 that are independent of caveolae[9]. In cells that do not express PTRF or SDPR, Cav1, even at reduced expression levels, will necessarily adopt caveolae-independent functions. Indeed, in cells expressing reduced levels of Cav1, oligomerized Cav1 domains or scaffolds regulate EGFR (epidermal growth factor receptor) signalling and dynamics independently of caveolae[10,11]. In addition, PC3 prostate cancer cells lack PTRF and caveolae, yet express elevated Cav1 levels as well as tyrosine phosphorylated Cav1 that regulates focal adhesion turnover and cell migration[2,12]. Indeed, Cav1 is a multifunctional protein that has specific functions in different tissues and can function as both tumour suppressor and promotor, in cancer[1,13]. By affecting the ability of Cav1 to form caveolae, as well as the nature and stability of the caveolae formed, tissue-specific expression of regulatory cavin proteins may selectively modulate Cav1 function in different cell types.

Caveolae function as endocytic carriers for various ligands, including the SV40 virus. In *Cav1*$^{-/-}$ fibroblasts, SV40 and the cholera toxin-b subunit are internalized through cholesterol-dependent, lipid raft-associated tubular endocytic carriers that necessarily form independently of caveolae[14,15]. Hansen *et al.* demonstrate that SDPR induces tubules that are similar to Shiga toxin-induced membrane tubules[16] and can be labelled with Cav1 (ref. 4). SDPR may recruit the Shiga toxin receptor Gb3 to caveolar domains and therefore to SDPR-induced tubules. The requirement of SDPR, PTRF and Cav1 for Shiga toxin recruitment to tubules suggests that Shiga toxin is internalized through a caveolar domain[4]. Internalization of Shiga toxin through caveolae-derived tubules in SDPR-overexpressing cells therefore expands the repertory of raft-derived endocytic intermediates[17], suggesting that vesicular and tubular endocytic intermediates can derive from both Cav1-positive and Cav1-negative raft domains. Together with the ability of SRBC to regulate caveolae-derived vesicle formation[6], these data indicate that cavins may prove to be critical regulators of the endocytic potential of caveolae.

The ability of SDPR overexpression to increase caveolae size as well as to induce elongated Cav1-containing tubules suggests that caveolae morphology is not immutable[4]. How the oligomeric caveolar coat can change size and give rise to dynamic tubules remains unclear. Cav1 forms stable oligomers, is immobile at the plasma membrane and has been proposed to form a stable coat of ~150 monomers on individual caveolae[1]. Indeed, if Cav1 oligomers form a stable vesicular coat, SDPR must in some way be altering Cav1 oligomeric structure, perhaps by disrupting larger caveolae-associated Cav1 oligomers, causing them to form smaller, less stable oligomers or monomers that permit tubule formation. The recent observations from electron microscopy tomography of a spiked caveolar coat[18] may be suggestive of a more flexible coat structure conducive to changes in caveolar size, shape and tubulation.

It remains to be determined whether tubules derive directly from caveolae, flat caveolar domains or disrupt Cav1 oligomeric structure. Using immunofluorescence, Hansen *et al.* found that overexpressed SDPR was distributed along elongated membrane tubules that were enriched for Cav1 at either the plasma membrane-associated origin or at the distal end of a long tube. Electron microscopy showed that these tubules were labelled for Cav1 throughout their length. However, the discrete distribution of SDPR and Cav1 along the tubules indicates that these proteins are not forming stochiometric complexes along the length of the tubule. The region of SDPR responsible for membrane tubulation is a coiled-coiled domain followed by a conserved basic domain that is distinct from its PTRF-binding site. Hansen *et al.* speculate that the coiled-coiled domain is responsible for homo- and/or hetero-oligomerization, whereas the basic domain promotes interaction with the inner leaflet of the plasma membrane, perhaps through binding to phosphatidyl serine[4]. The lack of homology between these domains and other curvature-inducing proteins suggests that SDPR induces membrane curvature through a new mechanism. Furthermore,

how the formation of Shiga toxin-containing tubules, which are apparently derived from and are dependent on caveolae and Cav1, in intact cells can be reconciled with the ability of Shiga toxin to induce tubules in cell-free liposomes[16] is unclear. It does, however, suggest that complex mechanisms underlie membrane curvature in biological membranes, including endocytic events associated with lipid raft domains. Whereas the specific impact of SDPR on caveolae function remains to be determined, its ability to regulate caveolae expression, morphology and endocytosis implicates it, and other cavin family members, as critical regulators of both caveolae and Cav1 function.

1. Parton, R. G. & Simons, K. *Nature Rev. Mol. Cell Biol.* **8,** 185–194 (2007).
2. Hill, M. M. *et al. Cell* **132,** 113–124 (2008).
3. Liu, L. *et al. Cell Metabolism* **8,** 310–317 (2008).
4. Hansen, C., Bright, N., Howard, G. & Nichols, B. *Nature Cell Biol.* **11,** 807–814 (2009).
5. Mineo, C., Ying, Y. S., Chapline, C., Jaken, S. & Anderson, R. G. *J. Cell Biol.* **141,** 601–610 (1998).
6. McMahon, K.-A. *et al. EMBO J.* **28,** 1001–1015 (2009).
7. Ogata, T. *et al. Mol. Cell. Biol.* **28,** 3424–3436 (2008).
8. Tagawa, M. *et al. Am. J. Physiol. Cell Physiol.* **295**, C490–498 (2008).
9. Head, B. P. & Insel, P. A. *Trends Cell Biol.* **17,** 51–57 (2007).
10. Lajoie, P. *et al. J. Cell Biol.* **179,** 341–356 (2007).
11. Lajoie, P., Goetz, J. G., Dennis, J. W. & Nabi, I. R. *J. Cell Biol.* **185,** 381–385 (2009).
12. Joshi, B. *et al. Cancer Res.* **68,** 8210–8220 (2008).
13. Goetz, J. G., Lajoie, P., Wiseman, S. M. & Nabi, I. R. *Cancer Metastasis Rev.* **27,** 715–735 (2008).
14. Kirkham, M. *et al. J. Cell Biol.* **168,** 465–476 (2005).
15. Damm, E. M. *et al. J. Cell Biol.* **168,** 477–488 (2005).
16. Romer, W. *et al. Nature* **450,** 670–675 (2007).
17. Lajoie, P. & Nabi, I. R. *J. Cell. Mol. Med.* **11,** 644–653 (2007).
18. Richter, T. *et al. Traffic* **9,** 893–909 (2008).

Delta traffic takes a sh-Arp turn

Eyal D. Schejter

In the Notch pathway, the transmembrane ligand Delta is internalized and then re-established on the surface of signal-sending cells to allow the productive binding and activation of the Notch receptor on neighbouring cells. Arp2/3-dependent actin polymerization directs Delta trafficking through this circuit.

The Notch signalling pathway uses a broad array of basic cellular machineries to convey instructions between cells throughout metazoan development[1,2]. At first glance, the molecular mechanisms for signal transduction involving this pathway appear relatively simple. The Notch receptor and its transmembrane ligands (DSL-family, Delta, Serrate and Lag-2) are expressed on the surface of neighbouring signal-receiving and signal-sending cells, and associate through their extracellular domains. This interaction results in proteolytic release of the intracellular portion of Notch, which then enters the nucleus where it functions as a transcriptional co-activator of target genes.

Closer examination, however, has revealed a far more complex picture. The Notch receptor undergoes extensive post-translational modification and several cleavage events; Notch receptor trafficking through endocytosis also has a critical role in modulation of signalling levels[3–5]. Regulation of the activity of the Notch ligands seems just as complex[6]. In particular, there is an intriguing requirement for ligand endocytosis into the signal-sending cell, which has been the focus of intensive recent research[7,8]. On page 815 of this issue, Bellen and colleagues[9] describe an essential involvement of the WASp–Arp2/3 (Wiskott-Aldrich syndrome protein and actin-related protein 2/3, respectively) actin polymerization machinery in DSL-ligand trafficking and signalling.

The authors studied the assignment of distinct fates to the four cell types that constitute the numerous sensory organs located along the cuticle of the adult *Drosophila melanogaster* fly, and which are characterized by their external mechanosensory bristle. This process relies on asymmetric cell divisions, mediated by the Notch signalling pathway[10,11]. Single sensory organ precursors (SOPs) are selected within a neurogenic field[12] and enter a program of three rounds of cell division[13,14]. Each division generates a pair of daughter cells that interact in a stereotypic fashion: one of the daughters assumes the signal-sending role and uses DSL-type ligands[15] to activate Notch in its sibling, the signal-receiving cell. Preferential activation of a Notch-induced genetic program in the signal-receiving cell results in the acquisition of cell features and functions that are distinct from those shown by the signal-sending cell. Disruption of Notch-based signalling in this context leads the siblings to assume an identical 'default' cell fate, normally only shown by the signal-sending cell.

The evolutionarily conserved Arp2/3 system is a primary mediator of the dynamic organization of the actin cytoskeleton. When stimulated by actin nucleation-promoting factors such as WASp, the seven-subunit Arp2/3 complex induces the formation of extensive, branched arrays of microfilaments, which can be used for cell motility, intracellular movement of membrane-bound particles and endocytosis[16]. The single *Drosophila* WASp homologue has been implicated previously in Notch-mediated sensory organ development[17,18]. However, the nature of the branched microfilament structures involved, as well as the mechanistic basis for their function, remained unknown.

In their recent study, Bellen and colleagues concentrated on the regulation of the DSL- ligand Delta in the pair of cells generated by division of the SOP cell itself, pIIb (signal-sending) and pIIa (signal-receiving; Fig. 1). They took advantage of a newly-identified mutant allele of *Arp3*, which encodes one of the subunits of the Arp2/3 complex, as well as existing mutant alleles of a second Arp2/3 subunit and WASp. The authors show that regions of the adult fly cuticle lacking Arp2/3 function show sensory organ cell-fate transformations that are highly similar to those observed following disruption of Notch signalling. They obtained genetic evidence that Arp2/3 is required upstream of Notch, within the signal-sending cell. The authors rule out two possible explanations for the observed phenotypes; they show that cell-fate determinants are

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