CELL BIOLOGY

Reversible Polo

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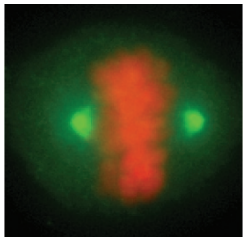

Centrioles are microtubule-based organelles that generate centrosomes, the main microtubule-organizing centers of animal cells. During mitosis, centrosomes form the opposing ends of the bipolar mitotic spindle. Surprisingly, more than 100 years after their discovery, it is still not clear whether centrosomes are needed for cellular proliferation. Previous attempts to remove centrosomes either surgically or through laser ablation resulted in transient centrosome loss with little to no disruption of cell division. An approach that would 'permanently' remove centrosomes would be to target Plk4, a serine/threonine kinase that regulates centriole assembly. Although a number of small-molecule inhibitors of Plk4 have been described, none deplete centrioles and centrosomes from cells. Wong *et al.* have synthesized two selective Plk4 inhibitors, centrinone and centrinone B, based on the pan–Aurora kinase inhibitor VX680. The centrinones are >1,000-fold more selective for Plk4 than Aurora A or B because they target an uncommon hinge region methionine of Plk4. Treating cancer cells with the centrinones resulted in the progressive loss of centriolar and pericentriolar markers and in continuous proliferation, albeit with increased mitotic errors. Interestingly, centrinone inhibition was reversible, with centrioles re-forming within one day of compound removal. In contrast to its effects on cancer cells, the authors found that centrinone-mediated loss of centrosomes in normal cells was associated with a G_1 cell-cycle arrest. This arrest was attributed to increased p53 levels, as knockdown of p53 allowed cells to proliferate in the absence of centrosomes. This p53-mediated cell-cycle arrest was not due to changes in DNA damage or stress signaling, suggesting a potentially new mechanism. Numerous cancer cells that proliferate in the presence of centrinone contained p53-pathway mutations. Overall, the use of centrinone has addressed a longstanding question about the functional requirement of centrosomes and offers a potential route for investigating additional cell biological questions. *GM*

ANTIBIOTIC RESISTANCE MECHANISMS

WTAs get tailored

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The cell wall of the bacterial pathogen *Listeria monocytogenes* (*Lm*) contains a peptidoglycan layer that serves as a scaffold for proteins, glycopolymers and teichoic acids (called wall teichoic acids, WTAs). Among their various functions, WTAs protect bacteria from the antibiotic effects of antimicrobial peptides (AMPs). Modification of WTAs by D-alanylation is a known mechanism by which some Gram-positive pathogens can evade killing by AMPs. Because *Lm* WTAs are decorated strictly with monosaccharides, Carvalho *et al.* explored whether glycosylation could contribute to AMP resistance by *Lm*. They focused on a previously identified gene cluster they named *rmlABCD*, whose homologs in other bacteria are responsible for L-rhamnose biosynthesis, after they found that transcription of this cluster is increased throughout infection. As well, a significant number of bacteria that harbor a *rmlABCD* gene cluster are pathogenic. The authors found that *Lm rmlABCD* deletion strains completely lacked L-rhamnose and that their WTA composition was perturbed. They found a similar result—that WTAs contained no L-rhamnose—upon deletion of an upstream gene, *rmlT*, which they had considered to be important because of its homology to glycosyltransferases. Both *rmlABCD* and *rmlT* mutants were more susceptible to killing by AMPs, which the authors determined was due not to a change in the cell surface charge (a known defense mechanism against AMPs) or to an improved binding efficiency of the AMPs, but rather to an increased ability of the AMPs to cross the cell wall. Accordingly, *Lm* plasma membrane integrity—the efficacy target of AMPs—was more easily compromised in the mutant than in the wild-type strains. Lastly, the authors also found that the two mutant strains were less virulent in an animal model of *Lm* infection. These results suggest that L-rhamnosylation of *Lm* WTAs has a protective effect against penetration by AMPs and is critical for *Lm* pathogenesis. *MB*

POLYMER BIOSYNTHESIS

Rubber ramps up

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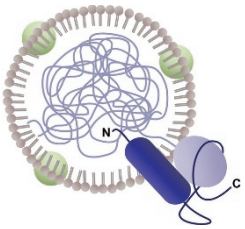
NATURE PLANTS



Natural rubber is a polymer of isoprene made by rubber *cis*-prenyltransferase (CPT) complexes. Failed attempts to reconstitute rubber biosynthesis via heterologous expression of CPTs have suggested that crucial elements of the complex remain unknown; the localization of CPTs at the surface of rubber particles suggests that this missing component includes a membrane-anchoring domain. In eukaryotes, CPTs also synthesize dolichol chains, shorter polymers necessary for glycoprotein synthesis; in this system, the integral membrane protein Nogo-B receptor protein (NgBR), that displays some homology to CPTs but is not an active enzyme, has recently been shown to interact with and stabilize CPTs. Epping *et al.* and Qu *et al.* suspected that an NgBR-like protein might also have a role in long-chain polymer synthesis. To test this hypothesis, Epping *et al.* first examined digested rubber particles from the dandelion *Taraxacum brevicorniculatum*, finding four peptides from an expressed sequence tag homologous to NgBR-like proteins that they named rubber transferase activator (RTA). *TbRTA* mRNA was most abundant in latex, and two tagged constructs further localized the protein in the rubber particle phase of separated latex and at least transiently on the ER membrane. TbRTA interacts with the CPT enzymes, as shown *in planta* by fluorescence analysis and co-immunoprecipitation experiments and in yeast by FRET analysis. Knockdown of *TbRTA* yielded plants that lacked CPT proteins, even though the corresponding mRNAs were present, and had empty rubber particle–like structures that did not produce rubber, though the cells continued to produce dolichol. Qu *et al.* performed similar experiments in the lettuce *Lactuca sativa*, finding that CPTL2, an NgBR-like sequence that is inactive but required for rubber biosynthesis, is localized on the ER and forms a stabilizing complex with the newly discovered enzyme CPT3. These combined results lead to a model in which NgBR-like proteins both stabilize CPTs and anchor them to the rubber particle to facilitate rubber synthesis. *CG*