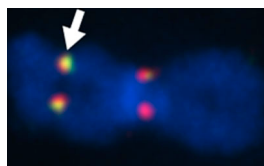


CAL1 builds centromeres on the fly



Mis-targeting CAL1 (green) to a noncentromeric site (arrow) induces the ectopic incorporation of CENP-A (red).

Chen et al. reveal how *Drosophila* cells assemble the specialized histone CENP-A on centromeric DNA.

Eukaryotic centromeres are defined by the histone H3 variant CENP-A, which is specifically incorporated into the nucleosomes that package centromeric chromatin. In mammalian cells, CENP-A is deposited at centromeres by a chaperone protein called HJURP. This protein is conserved in budding yeast but is missing from several eukaryotic lineages, including insects, nematodes, and fish. One candidate to fulfill HJURP's function in flies is a protein called CAL1, whose depletion results in the loss of CENP-A from *Drosophila* centromeres.

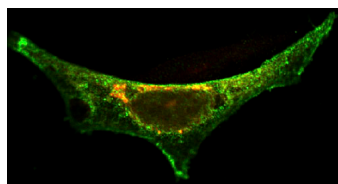
Chen et al. found that mis-targeting CAL1 to noncentromeric

DNA induced the incorporation of CENP-A and the assembly of ectopic centromeres and kinetochores. CAL1's N terminus—which binds to CENP-A—was sufficient to assemble CENP-A into chromatin, whereas CAL1's C terminus localized the protein to centromeres by binding to a centromeric protein called CENP-C.

CAL1 could also incorporate CENP-A, but not histone H3, into nucleosomes in vitro. Nucleosomes containing *Drosophila* CENP-A have been reported to be tetrameric, but CAL1 assembled CENP-A into octameric nucleosomes that wrapped DNA into a negative supercoil, similar to regular, H3-containing nucleosomes and the centromeric, CENP-A-containing nucleosomes of other organisms.

Though apparently unrelated to HJURP, CAL1 is therefore the *Drosophila* CENP-A assembly factor. Senior author Barbara Mellone now wants to investigate why flies evolved to use a different CENP-A chaperone than the one used by yeast and mammals. Chen, C.-C., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201305036>.

A mutual interest in prostaglandin signaling



L-PGDS (red) promotes the delivery of DP1 (green) to the plasma membrane.

Binda et al. describe how the localization and activity of the prostaglandin receptor DP1 is regulated by its association with the enzyme that synthesizes its ligand.

DP1 is a G protein-coupled receptor activated

by the prostaglandin PGD₂. Much of the receptor is retained inside of the cell instead of being exported to the plasma membrane. Binda et al. discovered that the enzyme that synthesizes PGD₂, L-PGDS, binds to DP1 and colocalizes with the receptor in the endoplasmic reticulum and Golgi apparatus.

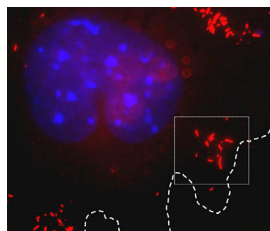
L-PGDS enhances DP1's transport to the plasma membrane, the researchers found. Overexpressing the enzyme boosted DP1's expression on the cell surface, whereas knocking down L-PGDS

reduced the receptor's export. L-PGDS promoted DP1 export by recruiting the chaperone Hsp90 into a complex containing both the receptor and the prostaglandin synthase. Abolishing the interaction between Hsp90 and L-PGDS, or inhibiting the chaperone with geldanamycin, prevented L-PGDS from stimulating DP1 export. The chaperone might help DP1 to fold correctly or regulate the vesicle transport machinery that delivers the receptor to the cell surface.

L-PGDS enhanced prostaglandin signaling by promoting DP1's transport to the plasma membrane. But the receptor-enzyme interaction might also increase DP1 signaling inside the cell; DP1 stimulated L-PGDS' enzymatic activity, generating PGD₂ that could potentially activate the receptor intracellularly. Indeed, L-PGDS and DP1 formed a complex with activated ERK kinases—downstream effectors of DP1—in the perinuclear region of cells. Senior author Jean-Luc Parent now wants to distinguish the effects of intracellular and cell surface DP1 signaling pathways.

Binda, C., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201304015>.

Prion infections come with strings attached



PrP^{Sc} (red) forms multiple strings on the surface of an infected cell.

Misfolded prion proteins form extended amyloid strings on the surface of infected cells, Rouvinski et al. reveal.

The endogenous prion protein, PrP^C, is attached to lipid rafts on the plasma membrane by a C-terminal glycosylphosphatidylinositol (GPI) anchor. Infectious prion particles convert PrP^C to a misfolded conformation, PrP^{Sc}, that is subsequently trimmed down to a protease-resistant C-terminal fragment. PrP^{Sc} forms amyloid fibers when detergent-extracted from infected cells, but whether it aggregates on membranes in vivo and where it localizes in the cell are still uncertain, in part because antibodies to the protein's C-terminal region fail to recognize PrP^{Sc} under physiological conditions.

Rouvinski et al. found, however, that antibodies against PrP^{Sc}'s N terminus could recognize the full-length prion in its native state in infected cells. Using these antibodies, the researchers discovered that PrP^{Sc} forms "strings," up to 8 μm in length, on the surface of cells in culture and in the brains of infected mice. The strings fluoresced with thioflavin T, suggesting an amyloid structure, and contained both the full-length and cleaved forms of PrP^{Sc}, but they didn't contain PrP^C at a detectable level. Live imaging revealed that the strings were largely immobile and remained on the cell surface for several hours. The slow rate of internalization might limit PrP^{Sc}'s degradation by lysosomal proteases and increase the prion's ability to convert PrP^C to the misfolded conformation.

Senior author Albert Taraboulos now wants to investigate PrP^{Sc} string structure and determine how they affect the physiology of infected cells. The strings localize to enlarged lipid raft-like domains, which could have a major impact on cell signaling pathways.

Rouvinski, A., et al. 2014. *J. Cell Biol.* <http://dx.doi.org/10.1083/jcb.201308028>.