In This Issue

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A cohort study of kinetochore proteins

Samejima et al. use quantitative proteomics to investigate how protein subcomplexes assemble into kinetochores on mitotic chromosomes.

Approximately 100 different proteins assemble on centromeric chromatin to form the kinetochores that attach chromosomes to spindle microtubules during mitosis. To better understand the assembly process, Samejima et al. used quantitative mass spectrometry to compare the composition of kinetochores on chromosomes isolated from cell lines lacking various kinetochore components.

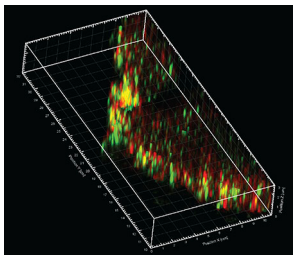
The researchers identified groups of proteins whose incorporation into kinetochores was similarly affected in each of the mutant cell lines. These "cohorts" probably represent subcomplexes within the kinetochore and, while some corresponded to known protein complexes, others suggested new details about the kinetochore's organization in situ. CENP-T, for example, which links the centromere-associated inner kinetochore to the microtubule-binding outer kinetochore, may form a complex with the inner kinetochore proteins CENP-N and CENP-L, because the incorporation of all three proteins into kinetochores was tightly coordinated. Similarly, the outer kinetochore RZZ complex, which is involved in mitotic checkpoint silencing, formed a cohort with three other proteins: Mad1, CENP-E, and Spindly.

Incorporation of the microtubule-binding Ndc80 complex was closely correlated with several different cohorts, suggesting it acts as a "hub" during kinetochore assembly. In fact, Samejima et al.'s data support the idea that there are two pools of Ndc80; one, associated with the microtubule-binding factors Mis12 and Knl1, that would help keep chromosomes attached to the mitotic spindle, and another associated with the putative RZZ/Mad1/CENP-E/Spindly complex, which might coordinate microtubule attachment with checkpoint silencing.

The researchers now want to confirm the physical interactions suggested by their proteomic data and to analyze how the composition of kinetochores is altered by microtubule attachment.

Samejima, I., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201508072

Secretase complexes let it RIP



Superresolution microscopy shows that α-secretase (green) and γ-secretase (red) partially colocalize at the cell periphery.

Chen et al. reveal the existence of large, multiprotease complexes that can process amyloid precursor protein (APP) and other substrates at the plasma membrane.

Like many transmembrane proteins, APP can be sequentially cleaved by different proteases in a process called regulated intramembrane proteolysis (RIP). APP's extracellular domain is first removed by α-secretases such as ADAM10, or β-secretases like BACE1. γ-Secretase then cleaves APP within its transmembrane helix, releasing the protein's intracellular domain and, if the protein was initially processed by β-secretase, the toxic Aβ peptide implicated in Alzheimer's disease. The different proteases have been assumed to remain separate from each other in vivo, but Chen et al. discovered that γ- and α-secretases can associate with each other at the cell surface.

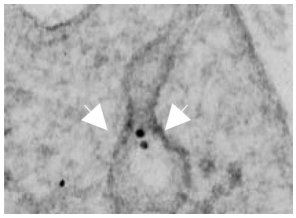
Members of the tetraspanin family of membrane proteins promoted the secretases' interaction, which would likely allow substrates to be processed more efficiently. Indeed, the complexes were able to sequentially cleave APP-based substrates at their α and γ target sites.

Surprisingly, when cells were treated with γ-secretase inhibitors, α-secretase activity was enhanced (and β-secretase activity was reduced), indicating that cells possess feedback mechanisms to regulate the initial steps of RIP. In contrast, a compound that modulates γ-secretase's cleavage site specificity had no effect on α- or β-secretase activity, suggesting that this class of drugs may have fewer side effects in Alzheimer's patients.

Chen et al. also identified a separate interaction between β- and γ-secretase. The researchers now want to investigate whether other proteases involved in RIP form similar complexes.

Chen, A.C., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201502001

B cell, heal thyself



When B cell plasma membranes are wounded, lipid rafts (marked by cholera toxin B, black dots) are internalized into long tubular structures.

Miller et al. reveal that B cells are able to repair damage to their plasma membranes, but this process may inhibit their ability to mount an immune response.

Eukaryotic cells quickly repair plasma membrane wounds through a pathway that culminates in the endocytosis of the damaged membrane through caveolae internalization. B lymphocytes may be particularly susceptible to damage as they squeeze their way through various tissues, sometimes directly encountering the pore-forming toxins secreted by certain pathogens. But B cells don't express caveolins, the main components of caveolae, making it unclear whether they can repair any damage they sustain.

Miller et al. found that B cells initially respond to plasma membrane wounds like other cell types do; calcium influx induced lysosome exocytosis and the release of an enzyme, acid sphingomyelinase, that generates ceramide-rich lipid rafts at the wound site. In most cell types, caveolins are then recruited to these rafts in order to induce their endocytosis. In B cells, however, raft endocytosis and wound repair occurred independently of caveolins.

Lipid rafts are also critical for the activation and endocytosis of B cell receptors (BCRs). Miller et al. found that plasma membrane wounding inhibited BCR signaling and internalization, probably because lipid raft components were diverted towards the process of wound repair. Senior author Wenxia Song says that the competition for lipid rafts might therefore delay the activation of damaged B cells. She now wants to investigate how B cell plasma membrane wounding and delayed activation affect immune responses.

Miller, H., et al. 2015. *J. Cell Biol.* http://dx.doi.org/10.1083/jcb.201505030