activation — indicating that clustering modulates signalling.

So it seems that agrin uses a common mechanism in immune cells and neurons — it clusters rafts to concentrate signalling factors. The question now is how agrin causes lipid rafts to cluster.

Alison Schuldt

References and links

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SIGNAL TRANSDUCTION

Mae leads the way

Phosphorylation of transcription factors by the Ras/mitogen-activated protein kinase (MAPK) pathway links signal transduction to gene expression. Reporting in *Nature*, Baker *et al.* have identified an intermediary protein, Mae, that links MAPK to the *Drosophila melanogaster* Ets transcription factor, Yan, indicating that cells might respond differently to MAPK according to their repertoire of adaptor/coupling proteins.

Baker *et al.* cloned Mae, modulator of the activity of Ets, from a yeast two-hybrid screen of a *Drosophila* embryo library using full-length *yan* as bait. This interaction between Yan and Mae requires their Pointed domains.

In mobility-shift assays, Mae interfered with Yan binding to a consensus Ets DNA-binding site. As Yan mediates transcriptional repression, what is the consequence of Mae binding to Yan? Using a reporter assay, the authors showed that Mae is required for the Ras/MAPK-induced inhibition of Yan. Mutants of Yan that cannot bind Mae or be phosphorylated were insensitive to Ras-induced inactivation, indicating that Mae might be needed for MAPK to phosphorylate, and thereby inactivate, Yan.

So, which of the nine potential sites of MAPK phosphorylation in Yan are important? Mutation of Ser127 to alanine has previously been shown to inhibit Ras/MAPK-mediated inactivation of Yan. *In vitro*, activated MAPK could not induce phosphorylation of Ser127 unless Mae was present. This ability of Mae to mediate MAPK-directed phosphorylation of Ser127 requires that Mae binds Yan through Pointed domain interactions, probably causing Yan to change conformation and thereby allowing MAPK access to Ser127. As Mae also similarly regulates Pointed-P2, a transcriptional activator, this might be a common mechanism to selectively couple substrates to MAPK.

Katrin Bussell

References and links

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time of duplication (see figure).

As ZYG-1 is required throughout development, the authors propose that it forms part of the basal centrosome duplication machinery, but what it interacts with remains to be seen. Interestingly, they note that the function of ZYG-1 resembles that of Mps1 during assembly of the spindle pole body (yeast's equivalent of the centrosome), indicating that, despite the differences between the division apparatus in mammals and yeast, the basic molecular mechanisms for nucleating microtubules during division might be conserved.

Alison Schuldt

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CHROMOSOME BIOLOGY

A complex link

Sister-chromatid cohesion is established during S phase, but the mechanisms behind this intimate physical association have remained elusive. One model invokes pairing upon passage of the replication fork, when the sister chromatids are close together. Renewed support for this model comes from a report in *Molecular Cell* by Mayer *et al.*, who describe the identification of a complex that links components of the DNA replication machinery to the establishment of sister-chromatid cohesion.

Mayer and colleagues started with a screen for *Saccharomyces cerevisiae* mutants that mis-segregate a chromosome fragment, and identified a gene called *CTF8*. They found that a *ctf8Δ* strain shows chromosome instability and that it is sensitive to microtubule-depolymerizing drugs, implicating the Ctf8 gene product in chromosome segregation. To test this possibility, the authors used fluorescence-activated cell sorting to check for defects in cell-cycle progression, and saw that homozygous *ctf8Δ* diploids accumulate at the G2/M transition. Then, to pinpoint the aspect of chromosome segregation in which Ctf8 is involved, Mayer *et al.* combined a deletion of *CTF8* with mutations in genes involved in the generation and maintenance of sister-chromatid cohesion. They found these combinations to be synthetically lethal, indicating a role for Ctf8 in this process.

Does Ctf8 act alone in sister-chromatid cohesion?

Immunoprecipitations revealed that it associates with no fewer than six other proteins — Ctf18, Dcc1, and four 'RFC' proteins (Rfc2, Rfc3, Rfc4 and Rfc5), so called because they are part of the replication factor C (RFC) complex. This complex, comprising Rfc1, 2, 3, 4 and 5, loads a processivity factor called proliferating cell nuclear antigen (PCNA) onto DNA during DNA replication. *CTF18* has also been identified previously in a screen for chromosome mis-segregation mutants, and it shows considerable homology to the RFC proteins, especially to Rfc1.

Mayer and colleagues propose that they have identified an 'alternative' RFC complex, which is involved specifically in sister-chromatid cohesion. They suggest that Ctf18 replaces Rfc1 in this RFC complex, and that Ctf18 mediates the association of Rfc2, 3, 4 and 5 with Ctf8, probably through Dcc1. The next step will be to work out how the RFC (Ctf18, Ctf8, Dcc1) complex links replication to sister-chromatid cohesion. One possibility, say the authors, is that this alternative RFC complex may load DNA polymerases selectively onto the sites at which cohesin (the complex that holds the sister chromatids together) is deposited.

Alison Mitchell

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ORIGINAL RESEARCH PAPER Mayer, M. L. *et al.* Identification of RFC(Ctf18p, Ctf8p, Dcc1p): An alternative RFC complex required for sister chromatid cohesion in *S. cerevisiae*. *Mol. Cell* **7**, 959–970 (2001)