CELL ADHESION

Making new contacts

Synapses are sites where nerve impulses are transmitted from the axon of one neuron to the dendrite of an adjacent neuron, and the transformation of an initial axon–dendrite contact into a functional synapse requires the accumulation of the synaptic machinery at the contact site. This accumulation is mediated by intracellular organelles of *trans*-Golgi network (TGN) origin, but how are these organelles 'captured' and stabilized at neurite–neurite contact sites? New insights have now been provided by Schachner and colleagues in *The Journal of Cell Biology*.

In *Drosophila*, fasciclin II is important for synapse development, so the authors looked at the relationship between the distribution of the closest mammalian homologue of fasciclin II — neural cell adhesion molecule (NCAM) — and the localization of intracellular organelles in hippocampal neurons. They found that a subpopulation of organelles, which could be seen moving along neurites, were associated with clusters of cell-surface-localized NCAM.

Using organelle-specific markers, Schachner and co-workers showed that these NCAM clusters colocalize with markers of TGN organelles. In addition, they found that NCAM180 — which binds spectrin — is the NCAM isoform that interacts with TGN organelles. TGN organelles are lined with a spectrin cytoskeleton, and the authors showed that the interaction between cell-surface NCAM180 and TGN organelles is mediated by spectrin.

Cell-surface NCAM180 clusters and intracellular organelles were seen to move bidirectionally along neurites. However, within several minutes of neurite–neurite contact, the authors found that these mobile NCAM180 clusters and the associated organelles became 'trapped' at the contact site. So, is this 'trapping' relevant to synaptic differentiation?

Schachner and colleagues observed 29 cases of synaptic differentiation and found that 26 coincided with NCAM180 clusters. Only one of these 26 nascent synapses was unstable, compared with two of the three

TRANSCRIPTION

Signal to reshape

In eukaryotes, ATP-dependent chromatin-remodelling complexes have an important function in regulating promoter accessibility for transcription. Complexes such as SWI2–SNF2 'remodel' the nucleosome architecture using energy from ATP hydrolysis. Little is known about how chromatin-remodelling activity is regulated. However, two reports in *Science Express* now show a link between the small-molecule inositol polyphosphates and chromatin remodelling.

In a genetic screen for mutants defective in transcription of the phosphate-responsive *PHO5* gene, Erin O'Shea and colleagues identified mutations in *ARG82/IPK2* — a gene encoding a nuclear inositol polyphosphate kinase. The importance of this kinase in *PHO5* induction was confirmed, as the *PHO5* messenger RNA level and chromatin-remodelling activity were reduced in an *arg82/ipk2* mutant strain.

O'Shea and colleagues then showed, by assaying *PHO5* induction in various strains, each containing a different defective chromatin-remodelling component, that the SWI–SNF and INO80 complexes, but not others, are required for efficient remodelling of *PHO5* chromatin. Next, they examined the effects of various mutant enzymes of the inositol polyphosphate pathway on *PHO5* transcription. O'Shea and co-workers found that the transcriptional defect is due to the lack of inositol tetrakisphosphate ($InsP_4$) and inositol pentakisphosphate ($InsP_5$) — rather than the accumulation of inositol triphosphate ($InsP_3$), or the lack of inositol hexakisphosphate ($InsP_6$), the final product in the synthesis pathway.

So, what is the connection between inositol polyphosphate metabolism and chromatin remodelling? A preliminary insight was obtained by carrying out chromatin immunoprecipitation experiments. Deletion of *ARG82/IPK2* decreased the recruitment of INO80 to the phosphate-responsive promoters of *PHO5* and *PHO84*, and SNF2 to *PHO84*, which implies that $InsP_4$/$InsP_5$ metabolism regulates the function of chromatin-remodelling complexes.

Using an *in vitro* approach, Carl Wu and colleagues tested the effects of various inositol polyphosphates on several chromatin-remodelling complexes in a nucleosome mobilization assay. Nucleosome mobilization — as well as ATPase activity — by the NURF, ISW2 and INO80 complexes was inhibited by $InsP_6$. By contrast, $InsP_6$ had no effect on SWI–SNF, but $InsP_4$ and $InsP_5$ stimulated nucleosome mobilization and ATP hydrolysis of SWI–SNF — which is consistent with O'Shea's data.

Through *in vivo* studies, Wu and co-workers showed that the mRNA level of the inositol-1-phosphate synthase (*INO1*) gene was reduced in an *arg82/ipk2* mutant strain and rescued by introduction of wild-type *IPK2*, which, confirms that $InsP_4$ and $InsP_5$ products are required for gene expression.

So, inositol polyphosphates can modulate chromatin-remodelling activity of complexes including INO80, SNF2 and ISW2. Yet the mechanisms by which inositol polyphosphates affect the recruitment of chromatin-remodelling complexes are still unclear. Future studies of inositol polyphosphate metabolism and its regulation by different physiological conditions might further uncover this signalling link to chromatin.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPERS Steger, D. J. *et al.* Regulation of chromatin remodeling by inositol polyphosphates. *Science Express* 2002 November 14 (DOI: 10.1126/science.1078062) | Shen, X. *et al.* Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science Express* 2002 November 14 (DOI: 10.1126/science.1078068)

FURTHER READING Tsukiyama, T. The *in vivo* functions of ATP-dependent chromatin-remodelling factors. *Nature Rev. Mol. Cell Biol.* **3**, 422–429 (2002)

synapses that formed in the absence of NCAM180. These results indicate that NCAM is important for synaptic differentiation and stabilization.

The authors then showed that organelles spend more time at neurite–neurite contact sites in wild-type neurons than in NCAM-deficient neurons, and that organelles leave contacts sites four times more often in NCAM-deficient neurons. NCAM therefore functions to 'anchor' TGN organelles at contact sites.

The work of Schachner and colleagues has highlighted a new role for NCAM in anchoring TGN organelles at initial neurite–neurite contact sites during synapse development, and has shown for the first time that recognition molecules like NCAM can "...provide a direct link between extracellular cues and intracellular organelles to stabilize them at nascent synapses".

Rachel Smallridge

References and links

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CELL CYCLE

Endless cycling

Different types of stem cells share certain properties, such as plasticity and self-renewal, which indicates that they might have common cellular machineries. Tsai and McKay now report in *Genes & Development* a nucleolar mechanism that regulates cell-cycle progression in stem cells and cancer cells.

To investigate the mechanism that underlies the proliferative state of stem cells, Tsai and McKay took advantage of the precise differentiation kinetics of dissociated central nervous system (CNS) stem cells in tissue culture. They constructed a subtractive library from which they identified a novel nucleolar protein — nucleostemin — which was highly enriched in cortical stem cells but absent in serum-differentiated cells. Nucleostemin was also present in embryonic stem cells and several human cancer cell lines.

Tsai and McKay showed that, during CNS development, nucleostemin is expressed before nestin expression peaks — nestin is an intermediate filament protein that is characteristic of neuroepithelial precursors — and is downregulated when the expression of the proliferative marker PCNA and the nucleolar protein B23 is still high. This means that cells continue to proliferate after nucleostemin expression is lost, and that nucleostemin downregulation occurs before the differentiation of neurons and glia. So, nucleostemin expression does not reflect the immediate proliferative state, but is characteristic of an early multipotential state.

To understand the functional role of nucleostemin, Tsai and McKay carried out small inhibitory RNA (siRNA) knockdown experiments in which nucleostemin expression was reduced. Compared with the control cultures, the percentage of non-dividing cells was increased in transfected cortical stem cells and the U2OS cancer cell line, indicating that nucleostemin is required for maintaining the proliferative capacity. Intriguingly, overexpression of nucleostemin also caused cells to exit the cell cycle — which is similar to the loss-of-function phenotype.

Tsai and McKay then set out to further dissect the molecular mechanism of nucleostemin function. Deletion studies showed that the amino-terminal basic region of nucleostemin is important for its nucleolar localization and that its two GTP-binding motifs regulate the nucleolar structural integrity.

Overexpression of mutants lacking the GTP-binding motifs blocked DNA replication, indicating that dysregulation of GTP binding hinders cell-cycle progression in late S phase. Overexpression of these mutants also caused an increase in cell death, compared with wild-type nucleostemin, and were partially rescued by deletion of the amino-terminal basic domain. In addition, when the GTP-binding domain deletion mutants were expressed in p53-null cells, no significant increase in cell death was found.

So how is p53 correlated to nucleostemin? Tsai and McKay showed that nucleostemin can bind p53 in glutathione-*S*-transferase (GST) pulldown and co-immunoprecipitation assays, and that the interacting region maps to the amino-terminal basic domain, which explains the rescue phenotype.

Tsai and McKay hypothesize that nucleostemin forms a complex with other nucleolar proteins when it is in a non-GTP-bound state and becomes dissociated on binding to GTP. The interaction of nucleostemin with p53, which presumably takes place in the nucleoplasm, represents a GTP-regulated and stem-cell/cancer-cell-specific control mechanism of cell-cycle progression.

Arianne Heinrichs

References and links

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