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.

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References and links

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