PROTEIN DEGRADATION

A dangerous route to take

The prion protein (PrP) is a cell-surface glycoprotein that has been linked to various neurodegenerative diseases. It seems that the cytoplasm provides an environment which promotes the conversion of PrP to PrP^{Sc} — a form of PrP that is associated with transmissible spongiform encephalopathies — but does PrP ever exist in the cytoplasm under normal circumstances? In the *Proceedings of the National Academy of Science*s, Ma and Lindquist now report that PrP can accumulate in the cytoplasm, which has implications for the pathogenesis of prion-related diseases.

It is difficult to detect misfolded or mistargeted proteins in the cytoplasm because they are efficiently degraded by the proteasome, so Ma and Lindquist studied the effect of proteasome inhibitors on the cellular distribution of PrP. In untreated neuroblastoma cells, the authors found that PrP was localized mainly to the cell surface, but treatment with a proteasome inhibitor caused substantial amounts of PrP to accumulate internally.

Ma and Lindquist showed that antibodies against a cytoplasmic form of Hsp70 — Hsc70 — strongly colocalized with intracellular PrP aggregates in inhibitor-treated cells, but not in untreated cells, and concluded that PrP can accumulate in the cytoplasm when proteasome activity is compromised.

The authors found that the internally accumulated PrP was unglycosylated and lacked both the amino- and carboxy-terminal signal sequences, indicating that it had been fully processed in the endoplasmic reticulum and had arrived in the cytoplasm through retrograde transport.

Using various cell lines, protease inhibitors and transfection procedures, Ma and Lindquist showed that cytoplasmic accumulation of PrP is a general consequence of proteasome inhibition rather than the result of using a particular method. They also showed, by overexpressing PrP, that this accumulation is not the result of proteasome inhibition *per se*.

The authors proposed that if cytoplasmic PrP accumulation is involved in disease, it should occur more readily in cells expressing disease-related PrP mutants. They therefore created a point mutation in mouse PrP, which corresponds to one of the most common human PrP mutations associated with transmissible spongiform encephalopathies.

In the absence of proteasome inhibitors, the authors found that a substantial fraction of mutant, but not wild-type, PrP accumulated in the cytoplasm, and that, compared to wild-type PrP, a smaller fraction of the mutant protein had passed through the ER quality-control system. After proteasome inhibition, both wild-type and mutant PrP accumulated in the cytoplasm, although the mutant protein accumulated to higher levels.

PROTEIN PROCESSING

Cutting tool

Activation of the single-pass transmembrane receptor Notch by the ligands Delta or Serrate mediates various cell-fate effects, which arise from signal transduction through a conserved pathway. This begins with ligand-induced cleavage of the extracellular domain (ECD) of Notch, and ends with the regulation of target genes through the association of Notch's intracellular domain (ICD) with a transcription factor. After extracellular cleavage, a γ-secretase complex — containing Presenilin, Nicastrin and other proteins — releases the intracellular domain of Notch from the membrane. Whereas Presenilin is thought to provide the catalytic activity, Nicastrin's involvement has remained elusive. Now, three groups show that Nicastrin also has an obligate role in Notch signalling.

All three groups showed that the phenotype of *nicastrin* (*nct*)-mutant *Drosophila melanogaster* resembles that of *Notch*-mutant embryos. The groups then examined the signalling potential of different forms of Notch in *nct*-mutant cells. Neither wild-type Notch nor a form lacking the ECD could activate Notch-mediated signalling, whereas the ICD could, indicating that Nicastrin is required to release the ICD of Notch. Moreover, as *nct*-null cells produced Delta and therefore induced Notch signalling in surrounding wild-type cells, but couldn't themselves respond to Delta, this role is cell-autonomous.

Chung and Struhl assayed for nuclear import of the Notch ICD to show that Nicastrin is needed for the transmembrane cleavage. They used Notch chimeric receptors of full-length Notch, Notch lacking the ECD or Notch containing only the ICD, fused to a transcriptional activator. Nuclear import — as measured by reporter gene activation — occurred in wild-type embryos using all three chimaeras, but only the ICD chimaeras translocated to the nucleus in *nct*-null embryos.

Consistent with this, López-Schier and St Johnston used antibodies directed against both the ICD and ECD of Notch to show that, in $nct^{-/-}$ cells, the Notch ICD remains at the membrane, whereas the ECD is cleaved normally. Fortini's group also provided direct biochemical evidence that Nicastrin is required for the transmembrane cleavage of Notch. Nicastrin loss-of-function mutants show an inhibition of specific proteolysis of Notch that is identical to that induced by a γ-secretase inhibitor.

While these data provide irrefutable evidence that Nicastrin is required for Presenilin-dependent Notch transmembrane cleavage, we have yet to find its precise role. Clones of follicle cells in late-stage egg chambers of *nct*-mutant embryos had reduced levels of Presenilin protein, but not messenger RNA, as did *Drosophila* S2 cells in which Nicastrin expression was abolished by RNA interference. Furthermore, Presenilin failed to accumulate at the apical plasma membrane of $nct^{-/-}$ wing disc cells, implying that defective Presenilin transport might lead to a decrease in protein stability, or *vice versa*. Perhaps Presenilin can form a stable complex which is capable of moving to the membrane only when Nicastrin is present. As Nicastrin can bind Notch, it is also highly plausible that it functions to recruit the Presenilin-containing complex to Notch.

Katrin Bussell

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These data indicate that PrP does appear in the cytoplasm under normal circumstances, a conclusion that was also reached by Yedidia and colleagues in a recent *EMBO Journal* paper. In unpublished experiments, Ma and Lindquist have shown that cytoplasmic PrP is selectively toxic to neuronal cells, and that a fraction of PrP can convert to PrP^{Sc}, depending on the rate of cytoplasmic accumulation. These results indicate that cytoplasmic accumulation of PrP might contribute to the pathogenesis of prion diseases.

Rachel Smallridge

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WEB SITE

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CHROMOSOME BIOLOGY

Stuck in the middle

Timing is everything in cell division. Take newly replicated sister chromatids, for example, which must stick together until a certain time, after which they separate and migrate to opposite poles of the cell. In many organisms, a region of silent heterochromatin in and around each centromere is known to be important for this process, and results from the Allshire and Javerzat labs now show why.

The authors studied *Schizosaccharomyces pombe*, the centromeres of which contain two distinct silenced regions — the outer-repeat regions, which flank a central domain and are coated with a protein called swi6. As rad21 — a component of the yeast cohesin complex, which maintains cohesion between sister chromatids — has been shown to associate with the outer repeats, Allshire, Javerzat and colleagues tested for a link between this region and cohesion.

To do this, the authors asked what happens if the synthesis of swi6 is turned off in wild-type or *rad21-K1* cells (which are conditionally defective in cohesin function). Reporting in *Science*, they show that although swi6 alone is not essential for viability, its withdrawal from the *rad21-K1* cells leads to a loss of viability, indicating a functional interaction between swi6 and rad21.

To examine this interaction further, the authors carried out fluorescence *in situ* hybridization (FISH) using probes at the centromeres and on the chromosome arms. Cells lacking swi6 or rad21 showed two separate FISH signals at the centromeres, indicating a lack of cohesion. However, cells with no swi6 (*swi6Δ*) showed only a single spot when probed along the chromosome arms, suggesting that a lack of swi6 disrupts cohesion only at the centromere.

Could swi6 therefore be required for the specific association of cohesin with centromeres? Allshire, Javerzat and co-workers used chromatin immunoprecipitation to examine the association of rad21 with centromeres in wild-type and *swi6Δ* cells. They found that recruitment of rad21 to the centromeric outer repeats is lost in the absence of swi6, and subsequent experiments confirmed that swi6 is needed for the association of rad21 with centromeres only, and not with chromosome arms.

Finally, the authors asked whether swi6 needs to be in the context of a functional centromere to recruit rad21. As swi6 contributes to silent heterochromatin at mating-type loci and telomeres too, they examined the association of rad21 with these regions. In both cases, an association was detected in wild-type cells, but not in *swi6Δ* cells, suggesting that silent swi6 chromatin is required to recruit rad21, but that a functional centromere is not.

These results neatly show what centromeric heterochromatin might be needed for — to attract cohesin and allow the sister chromatids to stick together until anaphase. The results are also consistent with the fact that metazoan chromosomes are initially attached along their length, but that chromosome-arm cohesins dissociate during prophase, leaving the centromeres as the only link until anaphase. The authors end by speculating that "if this role for centromeric heterochromatin is conserved… deficiencies in heterochromatin formation will contribute to… driving tumour formation and the production of aneuploid offspring".

Alison Mitchell

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