CYTOSKELETON

Another two stings in WASP's tail

As direct activators of the Arp2/3 complex, Wiskott–Aldrich syndrome proteins (WASPs) link Cdc42 activation to the formation of actin filaments, and WASP phosphorylation on tyrosine (Y) 291 enhances WASP activity towards the Arp2/3 complex. In *Molecular Cell*, Torres and Rosen further explore Y291 phosphorylation, while Cory *et al.* identify two new WASP phosphorylation sites.

Autoinhibition of WASP — by the carboxy-terminal VCA (verprolin homology, central hydrophobic, and acidic) domain binding to the GTPase-binding domain (GBD) — is relieved by active Cdc42. Torres and Rosen investigated cooperation between Cdc42 and tyrosine kinases in WASP regulation. Y291 is in the GBD, within the fold of the autoinhibited domain, and so is only expected to be phosphorylated when autoinhibition is relieved. Indeed, adding the VCA domain inhibited Y291 phosphorylation; this was relieved by adding a mimic of GTP-bound Cdc42. Similarly, the autoinhibited fold protected phosphoY291 (pY291)-WASP against tyrosine phosphatases. So, WASP Y291 phosphorylation seems to be altered only when activated Cdc42 is present, which, in the context of positive signalling, would allow WASP to remain phosphorylated long after the initial stimulus had subsided.

It has previously been proposed that Y291 phosphorylation might destabilize autoinhibition and constitutively activate WASP. Indeed, the pY291-GBD still bound VCA, but with a lower affinity — its basal activity towards the Arp2/3 complex, though, increased. Another consequence was that Src, through its Src-homology-2 (SH2) domain, could displace pY291-GBD from the VCA, which, again, increases Arp2/3 activity. Src kinase activity might also potentiate WASP activation by phosphorylating Y291.

Cory *et al.* identified two phosphorylation sites — serine (S) 483 and serine 484 — in the VCA domain and showed that these sites in WASP and its relative N-WASP are phosphorylated in various cell types. S483 is within a consensus casein kinase 2 (CK2) phosphorylation

CALCIUM

A local store

Free Ca^{2+} in the nucleus can regulate important functions such as gene transcription. But how is the nuclear level of free Ca^{2+} controlled? In the models that have been proposed so far, Ca^{2+} simply reaches the nuclear interior from the nuclear envelope (NE) by diffusion — a mechanism that would only allow the nucleus to be regulated uniformly by Ca^{2+}. However, this is not what occurs, so could the nucleus have its own local Ca^{2+} store? Nathanson and colleagues now provide the answer to this question in *Nature Cell Biology*.

In SKHep1 epithelial cells, the authors used the endoplasmic reticulum (ER) dye ER-Tracker to detect a nucleoplasmic reticulum — a fine, branching intranuclear network that is continuous with the ER and the NE. They confirmed the presence of this structure by showing that the ER protein calreticulin was distributed in a reticular pattern in the nucleus, as well as in the cytosol, of SKHep1 cells.

Nathanson and co-workers next showed that fluorescent Ca^{2+} dyes also labelled this intranuclear network, and they used fluorescence recovery after photobleaching to confirm that the dyes were membrane-enclosed, rather than membrane-bound. These results therefore show that there is a nuclear Ca^{2+}-storing network that is continuous with the ER and the NE.

Inositol-1,4,5-trisphosphate ($InsP_3$) receptors mediate Ca^{2+} signalling in SKHep1 cells, and the authors found that the type II $InsP_3$ receptor isoform is enriched in the nucleus of these cells. This isoform is expressed, in part, along the nucleoplasmic reticulum, but what do these intranuclear $InsP_3$ receptors do?

The authors developed a new technique to answer this question — a technique that allowed them to photorelease intranuclear nitrophenylethyl ester (NPE)-caged $InsP_3$ in a highly localized fashion in individual cells using two-photon excitation. When they photoreleased $InsP_3$ within 1 μM of the nucleoplasmic reticulum, they detected small increases in Ca^{2+} that began at the nucleoplasmic reticulum and were greatest at the site of $InsP_3$ release. These data allowed them to conclude that "…the nucleoplasmic reticulum is an $InsP_3$-gated calcium store that can give rise to local calcium signals in the nuclear interior".

Finally, Nathanson and colleagues monitored how the distribution of protein kinase C-γ (PKC-γ), which contains a Ca^{2+}-sensitive regulatory domain, is affected by Ca^{2+}. They photoreleased Ca^{2+} in either the nucleus or the cytosol and examined the effect on the distribution of green fluorescent protein (GFP)–PKC-γ. They found that nuclear Ca^{2+} signals altered the distribution of nuclear, not cytosolic, GFP–PKC-γ and vice versa, which indicates that nuclear and cytosolic Ca^{2+} signals can have effects that are independent of one another.

This work has therefore shown that "…the nucleus contains a nucleoplasmic reticulum with the capacity to regulate calcium signals in localized subnuclear regions". This discovery potentially explains how the nucleus can regulate several independent Ca^{2+}-dependent processes simultaneously, and might have revealed a new layer of Ca^{2+} control.

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References and links

ORIGINAL RESEARCH PAPER Echevarria, W. *et al.* Regulation of calcium signals in the nucleus by a nucleoplasmic reticulum. *Nature Cell Biol.* **5**, 440–446 (2003)

WEB SITE

Michael Nathanson's laboratory: http://info.med.yale.edu/intmed/digdis/otherpages/facsum m.html/nathanson.html