ELMO, but not by ELMO mutants in which the conserved PH-domain residues were mutated. Similar CED-12 mutants were also unable to rescue this defect. These results indicate that the roles of the PH domain and of the conserved residues within this domain have been conserved through evolution and are required for CED-12 function in the context of the whole organism.

So, this work has provided mechanistic insights into how Dock180 and ELMO function as a bipartite GEF for Rac. In addition, it has indicated that PH-domain-mediated regulation might be a general way to control Dock180 family members, with the PH domain being provided by an independent protein. Finally, this work has highlighted the fact that PH domains should not be thought of only as phospholipid-binding domains — it might be that the PH domains of other proteins can contact small GTPases, which would have significant functional and regulatory implications.

Rachel Smallridge

References and links

ORIGINAL RESEARCH PAPER Lu, M. *et al.* PH domain of ELMO functions *in trans* to regulate Rac activation via Dock180. *Nature Struct. Mol. Biol.* **11**, 756–762 (2004)

NUCLEAR TRANSPORT

New role for an old favourite

The calmodulin-like centrin proteins have a well-characterized role in the duplication of microtubule organizing centres (MTOCs). Intriguingly, though, in *Nature Cell Biology*, Fischer *et al.* have now shown that the yeast centrin Cdc31 seems to be multi-talented, as it has an unexpected role in the nuclear mRNA-export machinery.

The recently identified components of the yeast mRNA-export machinery — Sac3, Thp1 and Sus1 — form a complex that could connect the processes of transcription and mRNA export. Sac3 interacts with components of the nuclear pore, and Thp1 and Sus1 are both also involved in transcription.

The carboxy-terminal 'C domain' of Sac3 is toxic when overexpressed and causes strong mRNA-export defects. Fischer *et al.* analysed deletion mutants of this region and showed that a region comprising residues 733–851 was important for both toxicity and localization to the nuclear periphery. However, overexpression of Sac3^{733–860} alone, although still toxic, didn't affect mRNA export.

The toxicity of the C-domain fragments might be caused by the fact that they bind, and thereby sequester, an essential protein. So, the fragments were tagged and affinity purified to identify potential binding partners. Two proteins, Cdc31 and Sus1, co-purified with the toxic C-domain fragments. And, in a Sus1-negative yeast strain, the Sac3^{733–860} fragment remained toxic, and was still able to recruit Cdc31. This 130-residue segment was therefore called the Cdc31-interacting domain (CID).

Overexpression of Sac3^{CID} in synchronized cells caused cell-cycle arrest and produced large budded cells with a single spindle pole body (SPB; the yeast equivalent of the MTOC). This effect could be significantly reduced by the

co-expression of Cdc31. So, Sac3^{CID} interferes with SPB duplication by titrating Cdc31.

Next, the authors used several biochemical approaches to confirm that Cdc31 is a genuine component of the Sac3–Thp1–Sus1 complex. These included the 'split double tag method', which used a yeast strain with chromosomally integrated versions of Sus1 and Thp1 that each had a different molecular tag. The eluate from an affinity-purification step for tagged Sus1 was subsequently affinity purified for the alternatively tagged Thp1 protein. This purification scheme efficiently isolated the components of the Sac3–Thp1–Sus1 complex, as well as Cdc31. Furthermore, tagged Cdc31 correspondingly co-purified the other members of this complex as well as the SPB component Sfi1.

The authors then sought further confirmation that Cdc31 was implicated in both SPBs and the nuclear envelope, and, indeed, fluorescently tagged Cdc31 labelled both structures. Also, of the various temperature-sensitive alleles of Cdc31 that were studied, several caused defects in SPB function and one caused the accumulation of poly(A) RNA inside the nucleus.

Finally, deletion of the CID motif caused defects in mRNA export and a failure of Sac3ΔCID, Sus1 and Thp1 to associate with the nuclear envelope. Nor was Sac3ΔCID able to recruit Cdc31 or Sus1. Therefore, the CID motif is needed to recruit both Cdc31 and Sus1 to a Sac3–Thp1 heterodimer, and the Sac3–Thp1–Sus1 complex to the nuclear periphery.

This new role for Cdc31 in transcription-coupled mRNA export is consistent with the increasing evidence of additional roles for other centrans. And, the authors point out that it is "...not uncommon that proteins with an important role in distinct cellular pathways are physically linked to the nuclear pore complex", as this might provide a way for major cellular pathways to influence each other.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPER Fischer, T. *et al.* Yeast centrin Cdc31 is linked to the nuclear mRNA export machinery. *Nature Cell Biol.* **6**, 840–848 (2004)