CYTOSKELETON

A joint effort

Proteins of the Dock180 superfamily have recently been identified as novel guanine nucleotide-exchange factors (GEFs) for Rho GTPases in organisms that range from worms to mammals. And ELMO is crucial for the function of Dock180, the prototypical mammalian family member, which functions upstream of Rac in cell migration and phagocytosis. The Dock180–ELMO protein complex has been proposed to function as a bipartite GEF for Rac, but how does it work? In *Nature Structural and Molecular Biology*, Ravichandran and colleagues now show that the GEF activity of Dock180 and the pleckstrin homology (PH) domain of ELMO function in *trans* to regulate Rac activation.

The authors first showed that there was an increased binding of Dock180 to nucleotide-free Rac in the presence of ELMO. In addition, they found that this effect was still observed using Dock180 mutants that do not bind ELMO. It seems that the formation of a nucleotide-free Dock180–Rac complex provides a new binding site for ELMO and that the formation of a trimeric complex further stabilizes the nucleotide-free transition state of Rac.

Next, deletion mutants were used to map the region of ELMO that is involved in forming this trimeric complex, and they found that the PH domain — a domain type that has been implicated in phospholipid binding and membrane localization — was necessary and sufficient for complex formation. This is significant, because all previously identified mammalian GEFs for Rho GTPases contain tandem PH domains, whereas Dock180 does not. Ravichandran and co-workers therefore proposed that the ELMO PH domain and Dock180 function in *trans* to stabilize nucleotide-free Rac.

To test their hypothesis, the authors used sequence alignments of ELMO homologues to identify conserved regions in the PH domains. They selected two regions for further analysis and showed that mutations in these regions disrupted the formation of the trimeric complex: interestingly, these ELMO mutants still bound to Dock180. The authors also showed that such mutations in the PH domain of ELMO blocked its ability to promote Dock180- and Rac-dependent phagocytosis and cell migration, without disrupting the membrane localization of ELMO.

In the final part of this study, the authors showed that a cell-migration defect in worms, which is caused by the loss of the ELMO homologue CED-12, could be rescued by wild-type

TRANSCRIPTION

A non-starter

Most known transcriptional regulators are proteins, and the mechanisms they use to control transcription are well characterized. In recent years, however, a number of RNAs that regulate transcription have been identified, which seem to use different mechanisms. Two reports in *Nature Structural and Molecular Biology* by the Kugel and Goodrich group describe the identification of one such RNA regulator — the mouse B2 RNA — which inhibits transcription by binding to RNA polymerase II (Pol II).

B2 is a small non-coding RNA that is transcribed by RNA polymerase III (Pol III). While studying the transcriptional response to heat shock — which is characterized by the overall repression of Pol-II-mediated transcription and the upregulation of some heat-shock genes such as *Hsp70* — Kugel, Goodrich and co-workers found that the level of nuclear B2 RNA increases.

When they specifically inhibited Pol III, they found that the levels of B2 RNA and Pol-II-mediated transcription remained unchanged after heat shock. This indicates that Pol-III-mediated transcription is somehow involved in blocking Pol-II-mediated transcription. Indeed, B2 RNA inhibited Pol-II-mediated transcription *in vitro* whereas, in mouse cells that were depleted of B2 RNA, mRNA levels remained constant after heat shock.

Next, Kugel, Goodrich and colleagues set out to characterize the mechanism by which B2 RNA inhibits transcription. The addition of B2 RNA to a minimal reconstituted transcription system that contained purified Pol II and three general transcription factors caused a potent and specific inhibition of transcription. An increase in the concentration of Pol II in the system required the addition of a higher concentration of B2 RNA to inhibit transcription, whereas an excess of either of the general transcription factors did not affect the requirement for B2 RNA. Earlier, the authors had shown that B2 RNA co-immunoprecipitated with Pol II in a heat-shock-specific manner. So, together, these data confirm that B2 RNA targets Pol II directly to inhibit transcription. The binding site was found to be a previously characterized RNA-docking site on Pol II.

So, which step in the transcription process does B2 RNA block? To address this question, the authors added B2 RNA to the *in vitro* transcription reaction at different stages, and found that only when it was added before the formation of the transcription pre-initiation complex did B2 RNA block transcription completely. In addition, B2 RNA inhibited the production of very short (up to three-nucleotide) transcripts. So, Kugel, Goodrich and co-workers concluded that inhibition must occur at, or before, transcription initiation. They showed that, in mechanistic terms, this transcriptional inhibition involves the binding of B2 RNA to the pre-initiation complex at the promoter, which renders the complex non-functional.

Understanding how B2-RNA-mediated repression of transcription can be relieved is a challenge for the future — the authors have already shown that the effect of B2 RNA is reversible. Another interesting conundrum is how genes such as *Hsp70* can be transcribed despite the presence of B2 RNA.

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References and links

ORIGINAL RESEARCH PAPERS Allen, T. A. *et al.* The SINE-encoded mouse B2 RNA represses mRNA transcription in response to heat shock. *Nature Struct. Mol. Biol.* **11**, 816–821 (2004) | Espinoza, C. A. *et al.* B2 RNA binds directly to RNA polymerase II to repress transcript synthesis. *Nature Struct. Mol. Biol.* **11**, 822–829 (2004)

FURTHER READING Wassarman, K. M. RNA regulators of transcription. *Nature Struct. Mol. Biol.* **11**, 803–804 (2004)