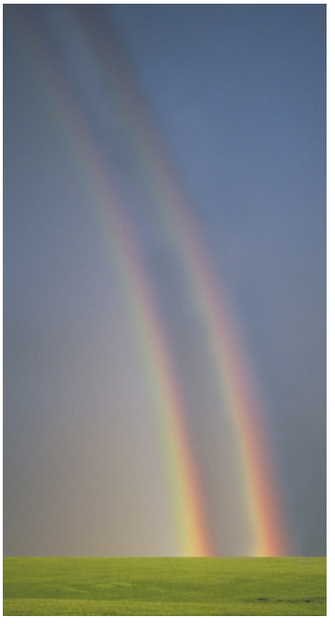
PROTEIN–RNA INTERACTIONS

A repeating rainbow



In *Drosophila* embryos, *hunchback* (*hb*) messenger RNA is tightly regulated by recruitment of a complex that represses its translation at the posterior of the embryo. Pumilio (Pum) binds *hb* mRNA through a defined element in its 3′ untranslated region, and then recruits two other repressive factors, Nanos (Nos) and Brain Tumour (Brat). How is the sequential formation of this complex controlled? The region of Pum that is required for this has been narrowed down to the region that contains Puf repeats, so named after Pum and another protein that contains these repeats, FBF. This critical region is highly conserved in human Pum1, stressing its importance. So how similar are their structures? Very is the answer. The crystal structure of the crucial region of Pum from both is now reported by two groups in *Cell* and *Molecular Cell*; and, in addition to revealing a conserved 'rainbow-like' structure, they reveal some intriguing observations about their similarity to helical-repeat proteins, and the common surface used to mediate interactions with both proteins and RNA.

Both crystal structures show that Puf repeats align to form an extended, curved molecule. Each repeat is related to the next by ~20°, leading to a rainbow-like arc structure. Intriguingly, this structure is similar to repeats found in the family of helical-repeat proteins, including β-catenin and karyopherin-α. Moreover, positive charge is concentrated along the inner concave surface — equivalent to the surface where many helical-repeat proteins show the greatest sequence conservation and interact with their binding partners.

This concentration of charge, together with data from previously characterized mutations in this region, led both groups to propose that the concave surface might provide the binding site for RNA. To investigate this, Edwards and colleagues used mutagenesis to identify the sites important for binding, and found two lines of evidence showing that the concave surface contacts RNA. Does this reflect a general property of the Puf-domain protein family? From an alignment with other members, Wang and colleagues show that the basic nature of the concave surface is conserved, and predict that it probably has the same function in binding RNA.

Next Edwards and colleagues asked which regions bind the two other members of the complex — Nos and Brat. To do this, they tested sets of mutants that bind RNA but cannot interact with one or the other factor. They found that the Pum surface that interacts with Nos includes the eighth repeat and the carboxy-terminal tail region. Focusing on the site that interacts with Brat, they showed that this is limited to repeats seven, eight and nine. The position of this site relative to the Nos-binding region indicates that Nos and Brat might bind cooperatively, and could explain why Brat is recruited only after Nos is present in the complex.

This observation — that Pum binds RNA through its concave surface — indicates that the helical-repeat family, previously characterized by an extended surface that is proposed to mediate protein–protein interactions, is more versatile, and also uses this surface for protein–RNA interactions.

Alison Schuldt

References and links

ORIGINAL RESEARCH PAPERS Edwards, T. A. *et al.* Structure of Pumilio reveals similarity between RNA and peptide binding motifs. *Cell* **105**, 281–289 (2001) | Wang, X. *et al.* Crystal structure of a Pumilio homology domain. *Mol. Cell* **7**, 855–865 (2001)

IN BRIEF

INTRACELLULAR TRANSPORT

Tuberin-dependent membrane localization of polycystin-1: a functional link between polycystic kidney disease and the *TSC2* tumour suppressor gene.

Kleymenova, E. *et al. Mol. Cell* **7**, 823–832 (2001)

Mutation of the *PKD1* gene is responsible for ~85% of cases of autosomal dominant polycystic kidney disease. 63 bp upstream of *PKD1* is the tuberous sclerosis 2 (*TSC2*) tumour-suppressor gene, and a functional link between the *PKD1* gene product — polycystin-1 — and the *TSC2* product, tuberin, has now been made. Kleymenova *et al.* show that, in tuberin-deficient cells, intracellular transport of polycystin-1 is disrupted, implicating tuberin as a determinant in the functional localization of polycystin-1.

EXOCYTOSIS

SNAREs are concentrated in cholesterol-dependent clusters that define docking and fusion sites for exocytosis.

Lang, T. *et al. EMBO J.* **20**, 2202–2213 (2001)

SNARE proteins are highly enriched in lipid rafts in PC12 cells: implications for the spatial control of exocytosis.

Chamberlain, L. H. *et al. Proc. Natl Acad. Sci. USA* **98**, 5619–5624 (2001)

Pairing of cognate SNARE proteins on vesicles and target membranes is believed to be the molecular basis for membrane fusion. It is suspected that several *trans*-SNARE complexes take part in this interaction, but this has not been documented. Two papers now show that SNARE proteins are concentrated in cholesterol-rich microdomains on the plasma membrane, and that their association with these domains is important for exocytosis. This supports the concept that SNARE complexes are organized spatially for efficient membrane fusion.

TECHNOLOGY

Protein quantification from complex protein mixtures using a proteomics methodology with single-cell resolution.

Zhang, H. T. *et al. Proc. Natl Acad. Sci. USA* **98**, 5497–5502 (2001)

Immunodetection amplified by T7 RNA polymerase (IDAT) — a new technique for protein quantification — is at least 10^9 times more sensitive than the most sensitive ELISA technique available today. The technique consists of coupling double-stranded oligonucleotides containing the T7 promoter covalently to antibodies against a given antigen, amplifying RNA from the oligonucleotides with T7 RNA polymerase and quantifying the radioactive reaction product. The method is more sensitive than immuno-PCR, and it is also more accurate owing to the linearity of the amplification reaction by T7 RNA polymerase. The authors suggest that the method has potential as an automated detection system for chip-based proteomics.