WEB WATCH

Protein united

- http://www.ebi.uniprot.org
- http://expasy.uniprot.org
- http://www.pir.uniprot.org

Three world-class protein databases — Swiss-Prot, TrEMBL and the Protein Information Resource-Protein Sequence Database (PIR-PSD) — have united to create the UniProt database. This database is set to become a primary resource for scientific researchers by providing free access to comprehensive and up-to-date information on protein sequence and function.

UniProt is a user-friendly database, which is divided into three sections — the UniProt Archive (UniParc), the UniProt Knowledgebase (UniProt) and UniProt non-redundant reference (UniRef) databases. UniParc is the foundation of UniProt and contains all known, non-redundant protein sequences (including those from PIR-PSD), which are updated daily.

The Knowledgebase is a central database that contains everything there is to know about a specific protein. It comprises two familiar resources — Swiss-Prot, which contains detailed, manually annotated information about protein function, and TrEMBL, which provides computer-generated information on protein function that is obtained by comparison with other proteins. UniRef is the final level that is designed to automatically combine closely related sequences into a single record.

UniProt is available at three mirror sites and each section of the database is easily accessible using the drop-down 'Databases' menu. Information can be retrieved by text and BLAST similarity searches, and downloaded by FTP. Submission to the Knowledgebase involves a new, web-based tool known as SPIN, which allows researchers to include information about specific protein features in addition to the primary sequence.

Emma Croager

RNA INTERFERENCE

RISC assessment

The RNase III Dicer enzyme processes RNAs into small interfering (si)RNAs and micro (mi)RNAs. These small RNAs then function as guides for RISC (RNA-induced silencing complex) to cleave messenger RNA (siRNA) or block its translation (miRNA). New insights into RISC formation, and the role of Dicer in this process, now come from two studies published in *Cell*.

In the first paper, the groups of Sontheimer and Carthew used a sophisticated genetic screen to identify *Drosophila melanogaster* eye mutants with either enhanced or reduced gene silencing — or RNA interference (RNAi) — activity. And among the mutated loci were *dicer-1* (*dcr-1*) and *dicer-2* (*dcr-2*), which encode the two Dicer enzymes present in *D. melanogaster*.

dcr-2 mutants had reduced siRNA levels and a complete RNAi defect in the eye and in the female germline, which indicates that Dcr-2 is important for dsRNA processing into siRNAs.

When the authors injected *D. melanogaster* eggs with a pre-processed siRNA that corresponded to the *bicoid* gene, the level of *bicoid* transcript was reduced in wild-type eggs, but did not change significantly in mutant *dcr-2* eggs due to a defective RNAi response. This means that Dcr-2 must also function downstream of siRNA production in the RNAi pathway.

The *dcr-1* RNAi phenotype was less severe than that of *dcr-2* and siRNA levels were normal, which is consistent with Dcr-2 being the main dsRNA-processing enzyme. So how can the partial RNAi defect be explained? *dcr-1*-mutant eggs injected with pre-processed siRNA complementary to *bicoid* transcripts contained sixfold more *bicoid* mRNA compared with wild-type eggs. This shows that Dcr-1 also has a downstream function in siRNA-dependent RNAi.

dcr-1 mutants also showed some developmental defects, which hints at a role for Dcr-1 in the miRNA-mediated silencing pathway. Dcr-1 is indeed essential for miRNA production, as miRNA levels in *dcr-1*-mutant eggs were undetectable. And, using a genetic assay for miRNA-mediated gene silencing, the authors showed that functional *dcr-1*, but not *dcr-2*, is necessary for this process. So Dcr-1 functions in both siRNA- and miRNA-mediated gene silencing.

Reporting in their second paper, the groups identified an assembly process that incorporates siRNAs into active RISC. This assembly involved formation of three distinct complexes — R1, R2 and R3. These complexes assemble in the presence of siRNAs in extracts derived from wild-type flies, but not from *dcr-2*-null mutants.

Partially purified fractions that could form the R1 complex have dsRNA-processing, but not mRNA-cleavage, activity. In a pulse-chase experiment, R1 complexes were identified in R2 and R3 complexes, which suggests that R1 might be an initiating complex in the RISC-assembly pathway. And, as shown in

UV-crosslinking studies, Dcr-2 associates directly with siRNAs in R1, R2 and R3.

The R2 complex remains to be characterized but is thought to be an intermediate complex that then forms the R3 complex. mRNA-cleavage activity coincided precisely with R3, which suggests that this is a functional RISC. Indeed, in the presence of siRNA, R3 binds specifically to complementary target mRNAs but not to unrelated mRNAs. Other known RISC-associated factors were also present in the R3 fractions, and co-purified as a partially preformed 'holo-RISC' in the absence of siRNAs.

Given that Dcr-2 was present in the active R3 complex, and both studies showed that siRNAs were unable to associate with RISC in the presence of *dcr-2*-mutant-embryo lysate, this indicates that Dcr-2 is necessary for the siRNA-specific assembly of functional RISC. In the presence of *dcr-1*-mutant lysate, R1 was formed, but R2 and R3 could not be detected — so Dcr-1 might be necessary for later steps in the assembly pathway.

Dcr-1 and Dcr-2 have distinct but overlapping roles in the siRNA and miRNA silencing pathways. Dicer enzymes are required for functional RISC assembly and, in *D. melanogaster*, Dcr-1 and Dcr-2 might direct the assembly of functionally different RISCs.

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References and links

ORIGINAL RESEARCH PAPERS Lee, Y. S *et al*. Distinct roles for *Drosophila* Dicer-1 and Dicer-2 in the siRNA/miRNA silencing pathways. *Cell* **117**, 69–81 (2004) | Pham, J. W. *et al*. A Dicer-2-dependent 80S complex cleaves targeted mRNAs during RNAi in *Drosophila*. *Cell* **117**, 83–94 (2004)