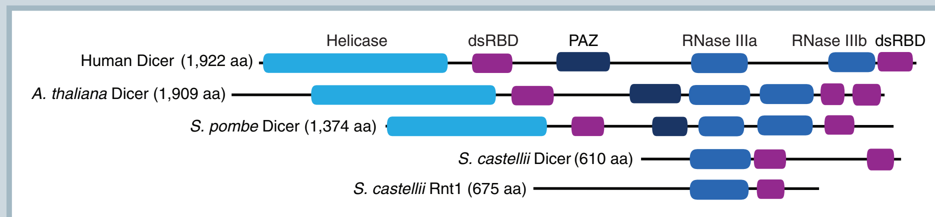
be required for Trigger factor to promiscuously associate with many kinds of nascent chains and full-length substrate proteins. In addition, it could allow different modes of Trigger factor action. Trigger factor may postpone folding of certain nascent chains through strong hydrophobic interactions[19], whereas predominantly hydrophilic interactions could facilitate folding within the proposed Trigger factor cage (see above) and stabilize native-like substrate conformations. It will be challenging to test whether Trigger factor has indeed the versatility to perform different folding tasks in a substrate-specific manner.

ACKNOWLEDGMENTS
We thank E. Martinez-Hackert for providing the structures presented in **Figure 2a,b**, Y. Cully for help in preparation of **Figure 1** and D. Huber and G. Kramer for critical reading of this manuscript.

1. Martinez-Hackert, E. & Hendrickson, W.A. *Cell* **138**, 923–934 (2009).
2. Deuerling, E., Schulze-Specking, A., Tomoyasu, T., Mogk, A. & Bukau, B. *Nature* **400**, 693–696 (1999).
3. Teter, S.A. *et al. Cell* **97**, 755–765 (1999).
4. Kramer, G. *et al. Nature* **419**, 171–174 (2002).
5. Kaiser, C.M. *et al. Nature* **444**, 455–460 (2006).
6. Alix, J.H. & Guerin, M.F. *Proc. Natl. Acad. Sci. USA* **90**, 9725–9729 (1993).
7. Maki, J.A., Schnobrich, D.J. & Culver, G.M. *Mol. Cell* **10**, 129–138 (2002).
8. Genevaux, P. *et al. EMBO Rep.* **5**, 195–200 (2004).
9. Merz, F. *et al. J. Biol. Chem.* **281**, 31963–31971 (2006).
10. Lakshmipathy, S.K. *et al. J. Biol. Chem.* **282**, 12186–12193 (2007).
11. Merz, F. *et al. EMBO J.* **27**, 1622–1632 (2008).
12. Hoffmann, A. *et al. J. Biol. Chem.* **281**, 6539–6545 (2006).
13. Yao, Y., Bhabha, G., Kroon, G., Landes, M. & Dyson, H.J. *J. Biomol. NMR* **40**, 23–30 (2008).
14. Ferbitz, L. *et al. Nature* **431**, 590–596 (2004).
15. Schlünzen, F. *et al. Structure* **13**, 1685–1694 (2005).
16. Baram, D. *et al. Proc. Natl. Acad. Sci. USA* **102**, 12017–12022 (2005).
17. Rutkowska, A. *et al. J. Biol. Chem.* **283**, 4124–4132 (2008).
18. Patzelt, H. *et al. Proc. Natl. Acad. Sci. USA* **98**, 14244–14249 (2001).
19. Agashe, V.R. *et al. Cell* **117**, 199–209 (2004).

Silence of the budding yeast

RNA interference (RNAi) involves the targeting and silencing of cognate transcripts by small RNAs. This pathway has been found to function in gene expression regulation across species and indeed Kingdoms, but there are some key organisms in which RNAi has not yet been found. The budding yeasts, including *Saccharomyces cerevisiae*, have been considered to be a set of species in which this pathway is not present, whereas the fission yeast, *Schizosaccharomyces pombe*, has an active RNAi pathway involved in heterochromatin formation and centromeric silencing. By examining homologs of key RNAi pathway components in species closely related to *S. cerevisiae*, Bartel and colleagues (*Science Express* doi:10.1126/science.1176945, published online 10 September 2009) have now found that some budding yeasts do in fact have this silencing pathway, although it has been lost, as previously suggested, in *S. cerevisiae*.



By high-throughput sequencing of small RNA species, the authors found abundant small RNAs in the 22–23-mer range in three budding yeast species, *Saccharomyces castellii*, *Candida albicans* and *Kluyveromyces polysporus*. These RNAs carry many of the signatures of small interfering RNAs (siRNAs) in other species, such as enrichment for an A or a U at the 5′ end, and map to repetitive elements and transposons. Meanwhile, *S. cerevisiae* RNAs in this size range seem to represent decay intermediates. Further supporting the idea that the small RNAs identified in *S. castellii*, *C. albicans* and *K. polysporus* are RNAi triggers, many map to the genome in a fashion that suggests they originate from a double-stranded RNA (dsRNA) precursor. When paired to each other, the small RNAs tend to have 2-nt overhangs, also a signature of production by Dicer, a key enzyme that cleaves precursor dsRNAs into siRNA duplexes in RNAi-competent organisms. However, these species lack a clear Dicer homolog. The authors therefore relaxed constraints on homology and went in search of other RNase III homologs, focusing on an ortholog of *RNT1*, which encodes an RNase III family enzyme involved in ribosomal RNA (rRNA) and noncoding RNA processing. The species generating putative Dicer products carried the *RNT1* ortholog (named *DCR1* by the authors). This homolog is an RNase III family enzyme that also carries dsRNA-binding domains (see image). Despite lacking other domains that are conserved in canonical Dicers, such as the PAZ domain, proposed to help 'measure' and set the length of Dicer products, deletion of *S. castellii DCR1* resulted in a loss of dicing function in extracts from this yeast. Argonaute is the small RNA–binding effector of RNAi across species. The authors showed that the *S. castellii* Argonaute homolog co-purifies with small RNAs and is needed to maintain levels of small RNAs produced *in vivo*. Deletion of either *S. castellii AGO1* or *DCR1* also resulted in higher levels of the 'Y' element transcript, which seems to be heavily targeted by the budding yeast small RNAs. As a final proof that some budding yeast species are RNAi competent, the authors showed that *S. castellii* could both generate siRNAs from a hairpin transgene targeting a GFP reporter and silence the reporter's expression.

Having provided genetic and biochemical evidence for the functionality of the enzymes needed for RNAi, the authors carried out a sort of 'reverse evolution' experiment and expressed the *S. castellii AGO1* and *DCR1* genes in *S. cerevisiae*. These genes are sufficient to generate an *S. cerevisiae* strain that can now mediate RNAi. Futhermore, in the RNAi-competent *S. cerevisiae* strain, endogenous retrotransposons are silenced.

The study not only indicates the presence of RNAi in some budding yeast species, giving insight into the evolution of this pathway, but also suggests that RNAi has a quite different function in these organisms from its role in *S. pombe*. In addition, the mechanism by which an enzyme lacking a PAZ domain can precisely dice precursors into small RNAs is an interesting avenue for future work and may shed light on regulation by the canonical Dicer orthologs. Another issue up for discussion is why and how *S. cerevisiae* lost the key enzymes needed for RNAi and thus this pathway. Finally, the work has implications for organisms where Dicer, and indeed an RNAi pathway, have yet to be found, given that domains considered to be hallmarks of Dicer can be dispensed with in evolution without compromising the functionality of the pathway.

Sabbi Lall

© 2009 Nature America, Inc. All rights reserved.
npg