Tips and tricks of the trade

RNAi in *Saccharomyces cerevisiae*

Saccharomyces cerevisiae is an almost perfect model organism. But it lacks one cellular process that is becoming increasingly important: RNAi.

Lab Hint

One could not imagine a better model organism than the yeast *Saccharomyces cerevisiae*: Yeast cells can be grown on defined media, genetic manipulation and transformation of yeast is pretty simple and transformed genes are easily integrated into one of yeast's 16 chromosomes. The yeast genome was the first to be sequenced (in 1996) and researchers know it inside out. A lot of basic cellular functions such as cell devision, DNA replication, stress response and transcription regulation are very similar to higher eukaryotes and disrupted genes may be complimented by introduced homologous genes. And last but not least, there are myriads of yeast strains available, carrying markers or defined mutations.

So what more could you want from a perfect model organism? Probably, RNA-mediated interference (RNAi). Gene silencing via RNAi is indeed one of the few essential cellular processes that is missing in *Saccharomyces cerevisiae*. Yeast cells lack the three proteins Ago2, Dicer and TRBP constituting the pivotal RNA-induced silencing complex (RISC) that cleaves target-mRNAs during RNAi-induced gene silencing. The solution to this problem is pretty obvious: simply introduce the missing genes into yeast cells to reconstitute the desired RNAi system. That's exactly what Frederick Roth's group, then at the Harvard Medical School, has done to reconstitute the human RNAi system in yeast (K. Suk *et al.*, *Nucleic Acids Research*, 2011, Vol.39, e43).

To this end, Suk *et al.* individually cloned human Ago2, Dicer and TRBP behind an inducible GAL1 promoter using the gateway cloning system and introduced the plasmids into a reporter strain expressing the green fluorescence protein (GFP). To check whether the reconstituted RISC system is able to induce the RNAi response and to cleave target mRNAs, Roth's team transformed the Ago2/Dicer/TRBP (ADT)-strain with a plasmid bearing an inducible antisense GFP construct and monitored the generation of GFP-siRNA, using northern blotting. The group detected GFP-siRNA in transformants expressing all three RISC components and also in strains lacking Ago2 or TRBP but not in strains lacking DICER. The latter seems to be essential for siRNA biogenesis in the reconstituted system while Ago2 and TRBP are dispensable.

S. cerevisae is a "model" model organisms. However even yeasts are not perfect models. But with some simple molecular biology they can be brought to (almost) perfection.

However, the reconstituted human RISC complex not only produced GFP-siRNA, it was also capable of silencing the GFP gene expression. After inducing the ADT strain with galactose, Suk *et al.* observed a significant decrease in the GFP fluorescence signal. The group thus came to the conclusion that the human RNAi system may be reconstituted in *Saccharomyces cerevisiae* by simply introducing the human RNAi genes Ago2, Dicer and TRBP into the cells – making yeast an even better model organism than it is already.

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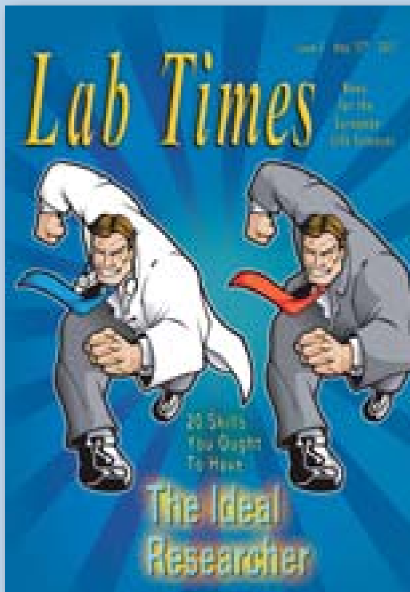

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