FUNGAL PATHOGENICITY

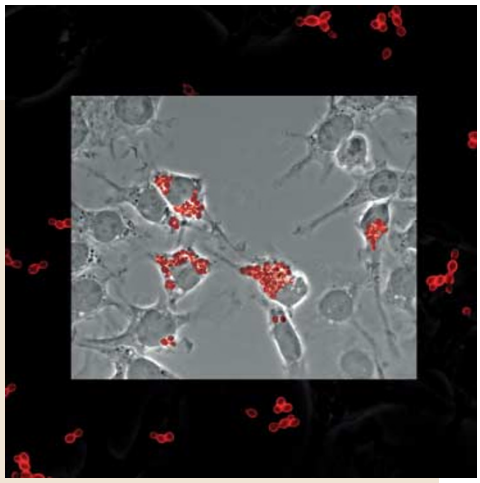
Interference comes good

RNA interference (RNAi) experiments have provided the long-awaited experimental proof that the cell-wall component α-(1,3)-glucan is a virulence factor in the dimorphic fungal pathogen *Histoplasma capsulatum*.

The yeast form of *H. capsulatum* is an intracellular pathogen and is found within macrophages, where it survives and proliferates. Techniques for the direct genetic analysis of *H. capsulatum* have been developed and can be used in conjunction with genome-sequence data to identify virulence factors. However, these techniques are slow, often taking weeks or months to obtain results. Now, Rappleye *et al.* have developed an efficient RNAi system for use in *H. capsulatum*.

The system is plasmid-based and was designed and optimized using an easily quantifiable target — green fluorescent protein (GFP). Test vectors were constructed to determine the most efficient plasmid design for gene silencing in *H. capsulatum*. Transformation of an *H. capsulatum* strain that stably expresses GFP with a construct in which a *gfp* hairpin is formed resulted in an average sixfold reduction in fluorescence. Serial passage of the *H. capsulatum* strain expressing this plasmid showed that the vector and the RNAi effect were stably maintained. Rappleye *et al.* went on to show that the RNAi effect was still observed once the transformed *H. capsulatum* cells had colonized murine macrophages.

α-(1,3)-glucan has long been suspected to be a virulence factor in *H. capsulatum* — spontaneous avirulent mutants can be isolated that are referred to as 'smooth' colony variants as they lack α-(1,3)-glucan. However, until now, there has been no direct experimental evidence of a causal link between α-(1,3)-glucan and virulence. Rappleye *et al.* followed up their proof-of-concept work by targeting the *H. capsulatum* α-(1,3)-glucan synthase gene, *AGS1*, which encodes an essential enzyme for α-(1,3)-glucan biosynthesis. Transformation of *H. capsulatum* with an RNAi vector targeting *AGS1* generated 'smooth' colonies, and immunofluorescence studies confirmed that *AGS1* had been effectively silenced in most transformants. The relative virulence of *H. capsulatum* yeast cells expressing the *AGS1*–RNAi construct was analysed in a macrophage monolayer and in a murine infection model and it was shown that the virulence of these cells was substantially reduced compared with controls, thus providing functional evidence that α-(1,3)-glucan is a virulence factor in *H. capsulatum*.



Histoplasma capsulatum proliferating within macrophages. The red fluorescence corresponds to immunoreactivity to α-(1,3)-glucan. Image kindly provided by Christian Coleman, Chad Rappleye and William Goldman and used with permission of Blackwell Publishing © (2004).

Sheilagh Clarkson

References and links

ORIGINAL RESEARCH PAPER Rappleye, C. A., Engle, J. T. & Goldman, W. E. RNA interference in *Histoplasma capsulatum* demonstrates a role for α-(1,3)-glucan in virulence. *Mol. Microbiol.* (21 May 2004) doi: 10.1111/j.1365-2958.2004.04131.x

CELLULAR MICROBIOLOGY

Polar exploration

Many bacteria show polar characteristics, such as the positioning of a flagellum at one end of the cell. However, the mechanisms involved in establishing this polarity are poorly understood. A recent study from Lucy Shapiro and colleagues has made important progress in understanding these processes, identifying a master regulator of polarity in *Caulobacter crescentus*.

The *C. crescentus* life cycle involves two different cell types, both with specialized structures located at one pole of the cell. Several proteins that are involved in the development of these structures and that have corresponding polar distributions have been identified, providing useful markers of polarity. Shapiro and colleagues made use of these features to investigate whether the actin-like protein MreB is required for polarization in *C. crescentus*.

MreB has a distinctive localization pattern, forming a spiral structure that extends along the length of *C. crescentus* cells. By analogy to eukaryotic actin, MreB molecules might have an intrinsic polarity, so the spirals they form could be used for the asymmetric localization of molecules required for the development of polar structures. To test this, the authors analysed the effect of MreB depletion on the distribution of four signalling proteins — PleC, DivJ, CckA and DivK — that are required for polar development in *C. crescentus*. Depletion of MreB abolished the polar foci that are usually formed by all four proteins at certain points in the cell cycle, consistent with a role for MreB as a global regulator of polarity.

Importantly, MreB seems to be actively required for specifying polarity, rather than having a passive role in protein localization. Unlike CckA and DivK, which form foci at both poles of *C. crescentus* cells, PleC and DivJ are asymmetrically distributed at certain points in the cell cycle, localizing to only one pole. When MreB was depleted and then re-expressed, although polar foci of PleC and DivJ were restored, these were located at the wrong pole in ~50% of cells. This indicates that when MreB is depleted, cells lose all memory of their initial polarity, so that when MreB is re-expressed polarity becomes randomized. MreB must therefore be required for the initial decision that determines which pole of the cell is which.

This study shows intriguing similarities between the establishment of polarity in *C. crescentus* and the corresponding processes in eukaryotic cells, in which actin has a central role. It will be interesting to see whether similar mechanisms operate in other bacteria.

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References and links

ORIGINAL RESEARCH PAPER
Gitai, Z., Dye, N. & Shapiro, L. An actin-like gene can determine cell polarity in bacteria. *Proc. Natl Acad. Sci. USA* **101**, 8643–8648 (2004)

WEB SITE
Lucy Shapiro's laboratory: http://caulo.stanford.edu/shaplab/