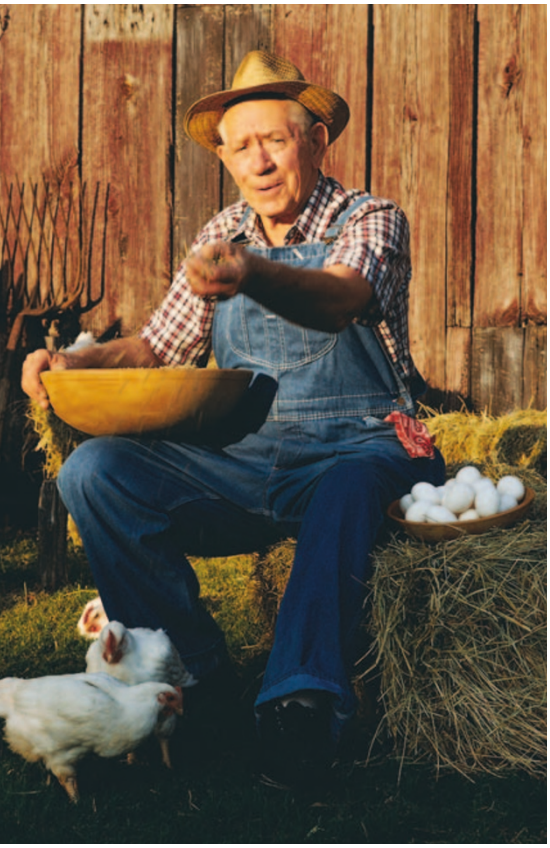

BIOTECHNOLOGY

# Human antibodies from chicken eggs

Growing numbers of monoclonal antibodies (mAbs) are being approved for therapeutic use, and the demand for these looks set to increase. However, building mAb-production facilities based on the established technique of mammalian cell culture is expensive and time-consuming, which has fuelled interest in alternative strategies for large-scale mAb production. Reporting in *Nature Biotechnology*, Zhu *et al.* now describe the development of chimeric chickens that secrete milligram-quantities of fully human mAbs with suitable characteristics for therapeutic use into their eggs.

To drive expression of human mAbs in chicken eggs the authors developed vectors in which the antibody genes are flanked by the regulatory elements responsible for the oviduct specificity and steroid induction of expression of the gene coding for ovalbumin, a protein that is produced exclusively in very high quantities in egg white. The vectors were further designed to allow the rapid insertion of unique variable-region genes for the production of different mAbs. In addition, the successful generation of the chimeric chickens was facilitated by the development of a method for maintaining chicken embryonic stem (cES) cells indefinitely and a system for transfecting cES cells with very large transgenes before injection into chicken embryos.

Although the cES cells failed to contribute to the germline of chimeric hens, a substantial proportion showed contribution of the cES cells to the oviduct. Here, the vectors delivered tissue-restricted, hormonally induced and developmentally regulated gene expression, and fully assembled mAbs with non-antigenic glycosylation patterns were exported from tubular gland cells and deposited in the egg white. For high-grade chimaeras, the concentration of the mAbs in the eggs reached peaks of up to 50–150 μg per ml, and remained relatively stable for several months.

The bioactivity of the egg-derived antibodies proved excellent. Compared with the equivalent antibodies generated in mammalian cell culture they showed similar binding and internalization capacities, despite differences in the glycosylation patterns. Although the *in vivo* half-life of the egg-derived antibodies was shorter, they

ANTIFUNGAL DRUGS

# A helping hand

**Heat-shock protein-90 (HSP90) is well known for its role as a molecular chaperone — a protein that assists the folding of other proteins ('clients'). Now, research published in *Science* reveals that HSP90 might also influence the evolution of new traits, by potentiating the phenotypic effect of genetic variation. Studies of the evolution of drug resistance in several strains of pathogenic fungi demonstrate an essential role for HSP90 and its client protein calcineurin, and implicate HSP90 as a novel antifungal target.**

**As a chaperone for many signal transducers, HSP90 is able to 'buffer' the effects of genetic variation by enabling the cell to tolerate mutations. Although HSP90 is highly inducible following environmental stress, the demands of stress-induced protein misfolding can outpace its induction, enabling previously silent mutations to act combinatorially and generate new phenotypes. It now seems that HSP90 has yet another role to play in the emergence of new traits: by allowing a mutation to have immediate effects rather than buffering against it, HSP90 might actually potentiate the appearance of new phenotypes.**

**Leah Cowen and Susan Lindquist examined the role of HSP90 in the evolution of resistance to antifungal drugs. Using rapid selection of three strains of *Saccharomyces cerevisiae* with varying levels of HSP90, they demonstrated that the development of resistance depended on high-level expression of HSP90. Moreover, HSP90 was required to maintain resistance rather than only to cope with the initial selection stress.**

**The HSP90-dependent effect was specific to mutants generated by rapid selection, which favours mutations that prevent the accumulation of toxic metabolites, rather than gradual selection which involves upregulation of a multidrug transporter. However, all of 11 previously identified *S. cerevisiae* drug-resistant deletion strains were found to be HSP90-dependent, showing that HSP90 can influence the resistance caused by a variety of different genetic lesions.**

**But how does HSP90 achieve this? One possibility is that a common regulator exists to mediate HSP90-dependent effects on different mutations. An obvious candidate was calcineurin, an HSP90 client known to regulate the cell's response to azoles. Accordingly, inhibition of calcineurin strongly reduced fluconazole resistance in all HSP90-dependent resistant strains.**

**These findings reveal an attractive therapeutic strategy against fungal infection, as similar results were seen with several fungal pathogens. It is particularly significant that HSP90 was crucial for the evolution of antifungal resistance in clinical isolates of *Candida albicans* collected from an HIV-infected individual. With continued exposure to fluconazole, the clinical isolates evolved towards HSP90-independent resistance, prompting speculation that HSP90 initially allows the phenotype to be expressed but that environmental stress drives the cell towards stabilizing the resistant phenotype. Inhibiting HSP90 early in infection could therefore render resistant fungal pathogens sensitive to conventional treatment or could prevent the initial development of antifungal drug resistance. Moreover, HSP90 inhibitors are already being evaluated in clinical trials for cancer, and seem to be well tolerated at levels that achieve significant inhibition.**

*Joanna Owens*

**References and links**

**ORIGINAL RESEARCH PAPER** Cowen, L. & Lindquist, S. Hsp90 potentiates the rapid evolution of new traits: drug resistance in diverse fungi. *Science* **309**, 2185–2189 (2005)

**FURTHER READING** Rutherford, S. L. Between genotype and phenotype: protein chaperones and evolvability. *Nature Rev. Genet.* **4**, 263–274 (2003)