IN THE NEWS

To be hairless or wise?
Hairless — a protein that is essential for hair-follicle regeneration — has been in the news following a report that it can help a bald mouse sprout a coat of fur. Research published in the *Proceedings of the National Academy of Sciences* shows that the interaction between hairless and another protein, wise, is responsible for the "furry flurry" (*Scientific American*, 27 September 2005).

Hair is maintained through the periodic regeneration of hair follicles. Mutations in the hairless gene cause a specific defect in this process in both humans and mice: initial hair growth is normal, but once a strand is shed it cannot grow back. But how hairless regulates hair regeneration has been unclear.

Catherine Thompson and colleagues from the Kennedy Krieger Research Institute, Maryland, USA now show that hairless represses the expression of wise, a modulator of Wnt signalling. Without hairless, wise accumulates in the follicles and prevents the hair cycle from entering the regrowth phase. So, hairless regulates regeneration by controlling the timing of this signalling pathway. "It is important to understand the hair cycle because the timing of the signaling required [for] regenerating a hair follicle is critical," Thompson said (*National Geographic News*, 27 September 2005).

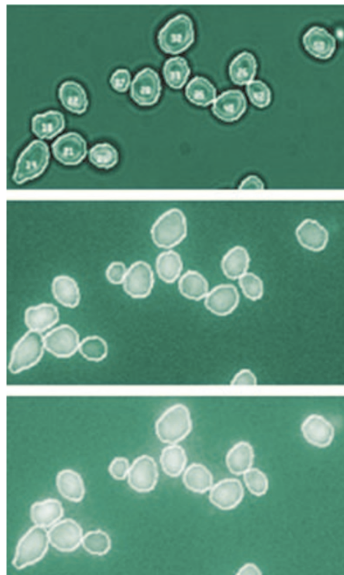
"There are certainly parts of the puzzle that remain to be solved. But this work has given us a better understanding of the molecular mechanisms, or step-by-step process, by which hair growth is regulated," Thompson added (*National Geographic News*, 27 September 2005).

This study sheds light on the mechanisms that control hair regrowth and could help in the development of treatments for baldness. But whether you want to be bald or wise is entirely your choice.

Ekat Kritikou

SYSTEMS BIOLOGY

The variable cell



Cells expressing either yellow or cyan fluorescent protein in response to pheromone treatment (a bright-field image is also shown). Images courtesy of Alejandro Colman-Lerner, The Molecular Sciences Institute, Berkeley, California, USA.

The activation of signalling cascades in genetically identical cells often leads to variable responses, raising the question about the origin of that variation. Colman-Lerner, Gordon, Brent and colleagues now report their studies of cell-to-cell variation in the output of a cell-fate decision system — the pheromone-response pathway in budding yeast.

The pheromone-response pathway, which includes a mitogen-activated protein kinase (MAPK) cascade, is induced by the α-factor pheromone. Pheromone induction triggers a cell-fate decision by switching normal, vegetative growth to the initiation of mating events, including the induction of gene expression and cell-cycle arrest. Colman-Lerner *et al.* measured the pheromone-induced expression of fluorescent-protein reporter genes as the readout of this cell-fate decision system — the output combines differences in the signal-transduction pathway and in gene expression from the reporters. To distinguish between these contributions, the authors generated a series of yeast strains containing the genes for yellow and cyan fluorescent protein (YFP and CFP, respectively). The results of experiments in which YFP and CFP were controlled by identical α-factor-responsive promoters were compared to those of experiments in which YFP was driven by an α-factor-responsive promoter and CFP by an α-factor-independent promoter.

This experimental set-up was accompanied by an analytical framework in which the system could be divided into two subsystems, 'pathway' and 'expression', and in each subsystem the authors distinguished between differences in 'capacity' and stochastic fluctuations in the workings of each subsystem ('noise'). This approach enabled the authors to discriminate among the contributions of four sources of variation — differences in pathway power or the ability to transmit a signal; differences in expression capacity or the ability to express proteins from genes; and noise in the two subsystems.

PROTEIN DEGRADATION

Yet another job for a gas

How does the cell know when it's time to degrade a protein? According to a study by Alexander Varshavsky and colleagues in *Nature*, for proteins with an N-terminal Cys residue, the ubiquitin-dependent N-end-rule pathway of protein degradation relies on sensing the cellular levels of nitric oxide (NO) and, possibly, oxygen (O_2).

The N-end-rule pathway controls various cellular processes by manipulating the levels of important regulatory proteins. It selects proteins for ubiquitylation and degradation on the basis of the identity of their N-terminal residue. In yeast, the arginylation branch of the N-end-rule pathway requires that the N-terminal residues Asp and Glu are conjugated to Arg by the Arg-tRNA-protein transferase Ate1 before a substrate can be recognized by specific ubiquitin ligases.

Colman-Lerner *et al.* showed that most of the cell-to-cell variation in the activation of gene expression in response to pheromone is caused by pre-existing differences among the cells and not noise, and that a main source of variation arises from differences in expression capacity. The cells also differed in the strength with which they carry the signal from the plasma membrane into the nucleus: at high pheromone concentrations most of the variation was due to cell-to-cell differences in their expression capacity; at low pheromone concentrations, the relative contribution of pathway strength was much higher — indicating that high levels of input might conceal pre-existing differences in pathway capacity.

These findings posed new questions about the regulation of cellular functions by signal-transduction cascades. For example, how can pheromone-induced gene expression be accurately regulated if cells vary so widely in their expression capacity? The authors show evidence suggesting that yeast cells adjust the strength of the mating cascade to compensate for the variability in expression capacity, allowing the amount of protein made to reflect the activity of the pathway more accurately.

Furthermore, Colman-Lerner *et al.* showed that the two MAPKs that transduce the signal, Fus3 and Kss1, function to regulate cell-to-cell variation in pathway capacity. Fus3 suppressed variation when cells are stimulated with high pheromone levels, whereas Kss1 enhanced variation at low pheromone levels.

In conclusion, the identification of the mechanisms that control the quantitative behaviour of a cell-fate decision system, and the first attempts to characterize the functional components that regulate cell-to-cell variation in its performance, form a crucial first step to deepen the understanding of biological systems.

Arianne Heinrichs

References and links

ORIGINAL RESEARCH PAPER Colman-Lerner, A. *et al*. Regulated cell-to-cell variation in a cell-fate decision system. *Nature* **437**, 699–706 (2005)

The same is true in mammals, but there, Cys can also become arginylated, whereas it is a stabilizing residue in yeast.

Varshavsky and colleagues found that an N-terminal Cys needs to be oxidated to $CysO_3H$ before it can be arginylated. Levels of the G-protein-coupled-receptor regulatory proteins RGS4, RGS5 and RGS16 were increased in arginylation-deficient *Ate1*$^{-/-}$ mouse embryos, but not through increased transcription. Moreover, expression of a dominant-negative inhibitor of the N-end-rule pathway also increased the level of RGS16. These RGS proteins were therefore identified as the first physiological substrates of the N-end-rule pathway in mammals.

But how do N-terminal Cys residues of N-end-rule substrates become oxidized *in vivo*? Studies showed that treating mouse cells with either an NO synthase (NOS) inhibitor or an NO scavenger increased the levels of RGS4, as did knocking out *NOS1*. RGS4 levels were also much higher in cells grown in low-O_2 conditions. Furthermore, using an *in vitro* arginylation system, the authors showed that prior exposure of a Cys-RGS4 protein to an NO donor was essential for its arginylation. Together, these results revealed that NO, and probably also O_2, are required for Cys arginylation. It also explains why Cys is a stabilizing residue in yeast — yeast do not have NOS enzymes.

The arginylation branch of the N-end-rule pathway has therefore been discovered to function as an NO sensor, which allows the levels of NO to affect cellular processes that are controlled by regulatory proteins bearing an N-terminal Cys. The many implications of this link between NO signalling and a protein-degradation pathway open up new possibilities for understanding both processes.

Lesley Cunliffe

References and links

ORIGINAL RESEARCH PAPER Hu, R.-G. *et al*. The N-end rule pathway as a nitric oxide sensor controlling the levels of multiple regulators. *Nature* 13 Oct 2005 (doi:101038/nature04027)

WEB SITE

Alexander Varshavsky's lab: http://biology.caltech.edu/Members/Varshavsky

STRUCTURE WATCH

Spikes point the way

New possibilities for the design of coronavirus vaccines have been opened up with the elucidation of the structure of the receptor-binding domain (RBD) of severe acute respiratory syndrome (SARS) spike protein. The RBD of the SARS spike protein is responsible for virus attachment to its receptor, angiotensin-converting enzyme-2 (ACE2), on human cells. The RBD of the human epidemic strain differs from that of the animal virus by just two amino acids. Harrison and colleagues determined the complex of the interacting segments of these proteins, which consists of residues 19–615 of the peptidase domain of human ACE2 and residues 323–502 of the RBD, to 2.9-Å resolution.

The ACE2 peptidase domain comprises two lobes that close during substrate binding, and the spike RBD contacts the tip of one lobe when ACE2 is either open or closed. The RBD has a five-stranded, antiparallel β-sheet core, and an extended-loop subdomain that lies along one edge of the core. A two-stranded β-sheet in this loop forms a concave surface that cradles the N-terminal helix of ACE2, and ridges along either side of the loop interact with structural features of ACE2. This loop (residues 424–494) was named the receptor-binding motif (RBM) and makes the only contacts with ACE2, producing a 1,700 $Å^2$ interface that is consistent with the high binding affinity of these proteins (K_d ~10^{-8}). The detailed analysis of the contacts between these two proteins provides useful information for rational vaccine design.

REFERENCE Li, F. *et al*. Structure of SARS coronavirus spike receptor-binding domain complexed with receptor. *Science* **309**, 1864–1868 (2005)

Modelling splicing regulation

The solution structures of the four RNA-binding domains (RBDs) of polypyrimidine-tract-binding protein-1 (PTB1) in complex with RNA have now been solved by Oberstrass *et al.*, leading to new models for the function of PTB1 as a repressor of alternative splicing. As an abundant eukaryotic RNA-binding protein, PTB1 is implicated in various aspects of RNA metabolism, but how it functions in these processes is not well understood. Oberstrass *et al.* used NMR to look at the structure of RBD1–4 in complex with a 5′-CUCUCU-3′ oligonucleotide, which is a common feature of intronic regulatory sequences.

Each RBD binds independently to one RNA molecule and recognizes a different consensus sequence within the oligonucleotide. The nucleotides interact with the flat β-sheet surface of each RBD but, unlike other RBD–RNA structures, the third β-strand is only weakly involved in RNA binding. Whereas most RBDs have four β-strands, RBD2 and RBD3 have a fifth β-strand, which allows these domains to interact with a larger nucleotide sequence.

Significantly, RBD3 and RBD4 interact extensively and have a fixed orientation such that their bound RNAs are antiparallel to each other and must be separated by a linker sequence. A single PTB1 molecule can therefore bring two distant pyrimidine tracts into close proximity and induce RNA looping — a feature that has led to the proposal of various models for the function of this protein in alternative splicing.

REFERENCE Oberstrass, F. C. *et al*. Structure of PTB bound to RNA: specific binding and implications for splicing regulation. *Science* **309**, 2054–2057 (2005)