BIOTECHNOLOGY

Putting a finger on gene expression

Methods to control endogenous gene expression are important both for functional genomics and for potential therapeutic applications. One possible strategy is to develop artificial transcription factors, and much progress has been made in recent years with the design of transcription factors based on a DNA-binding domain known as a zinc finger, which have been shown to be capable of regulating the expression of selected genes in tissue culture. Writing in *Nature Medicine*, Rebar, Huang and colleagues now describe the first application of a designed zinc finger protein (ZFP) transcription factor in a whole organism — up-regulation of an endogenous gene encoding a protein that stimulates the growth of blood vessels.

Natural transcription factors usually contain two domains: an effector domain, which interacts with other components of the transcriptional machinery to up- or down-regulate gene expression, and a DNA-binding domain, which targets the transcription factor to specific sites on chromosomal DNA. Zinc-finger domains are commonly used for DNA binding by natural transcription factors, and greater understanding of how these domains recognize specific DNA sequences now allows the design of ZFP-based transcription factors that will bind specifically to selected target sequences.

Rebar *et al.* designed ZFP-based transcription factors — which contained a zinc-finger domain fused to an activating effector domain — to target DNA sites around the mouse gene encoding vascular endothelial growth factor A (Vegfa) that were predicted to lead to a strong influence on gene transcription. Viral vectors were then used to express the ZFP-based transcription factors in the ears of mice, which resulted in new blood vessel growth due to an increase in expression of *Vegfa*. Further experiments showed that such ZFP-based transcription factors could accelerate wound healing in a mouse model.

Using ZPF-based transcription factors for gene regulation could have advantages over alternative strategies, especially in the regulation of complex processes, such as blood vessel growth, which involve more than one variant of a gene, or multiple genes. In

MUSCULAR DEGENERATION

Body building best without myostatin

Blocking the activity of the protein myostatin could be an attractive therapeutic strategy for the treatment of muscle degeneration diseases, indicates research in the 28 November issue of *Nature*. Khurana and colleagues found that blocking myostatin activity in the *mdx* mouse model of Duchenne muscular dystrophy causes a significant increase in muscle size and improves muscle function. Such blockade could be beneficial for a variety of primary and secondary myopathies, including the muscular dystrophies (MD), and muscle loss for other reasons.

Duchenne MD is an X-linked muscle disease caused by an absence of the protein dystrophin. Dystrophin and a similar protein, utrophin, are structural proteins in skeletal and cardiac muscle cells. Affected boys show signs of disease early in life, stop walking at the beginning of the second decade, and usually die by 20 years of age. Mutations in genes encoding members of the dystrophin-associated glycoprotein complex lead to different MDs. There is no cure for these diseases, but corticosteroids, including prednisone and a related compound, deflazacort, can delay the loss of muscle strength and function in Duchenne MD patients. Experimental approaches include gene therapy, cell transplantation and upregulation of alternative therapeutic proteins.

Myostatin is a negative regulator of muscle mass — a mutation in the myostatin gene is responsible for the enormous musculature in 'double-muscled' Belgian blue cattle. After injecting month-old *mdx* mice with myostatin-blocking antibodies for three months, the authors found that the levels of utrophin in the muscle were not elevated, suggesting that blockade ameliorated the dystrophic phenoptype via a utrophin-independent mechanism. Elevated serum creatine kinase (CK) concentration is a sign of dystrophin deficiency in *mdx* mice; however, after three months of myostatin blockade the serum CK concentration was almost normal. Not all dystrophic changes in *mdx* mice were reversed by blocking myostatin; the mice were still susceptible to muscle-fibre damage by lengthening contractions. However, the authors suggest that this might not be the case if the treatment was started at birth, rather than a month later.

The pharmacological intervention of injecting antibodies to myostatin would obviate a number of practical difficulties associated with other experimental approaches to MDs. Interestingly, myostatin has also been implicated in the development of obesity and insulin resistance. This protein could represent a target for the design of small-molecule inhibitors.

Melanie Brazil

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WEB SITES Khurana's lab: http://www.uphs.upenn.edu/~pmi/members/khurana/

contrast to conventional gene therapy, in which just one variant of the desired gene is introduced directly into cells, the expression of all the variants of the endogenous gene can be regulated using ZPF-based transcription factors, which could have important implications for the success of the approach as the authors show. The *Vegfa* gene codes for three major variants of Vegfa, which are not functionally equivalent or redundant, and the blood vessels produced using ZFP-based transcription factors seem to be more mature than those produced by expression of just the main variant, $Vegfa_{164}$. And taking things one step further, the ZFP-based approach should, in principle, be applicable to the up- or down-regulation of multiple genes, which would be considerably more challenging using conventional gene therapy.

Peter Kirkpatrick

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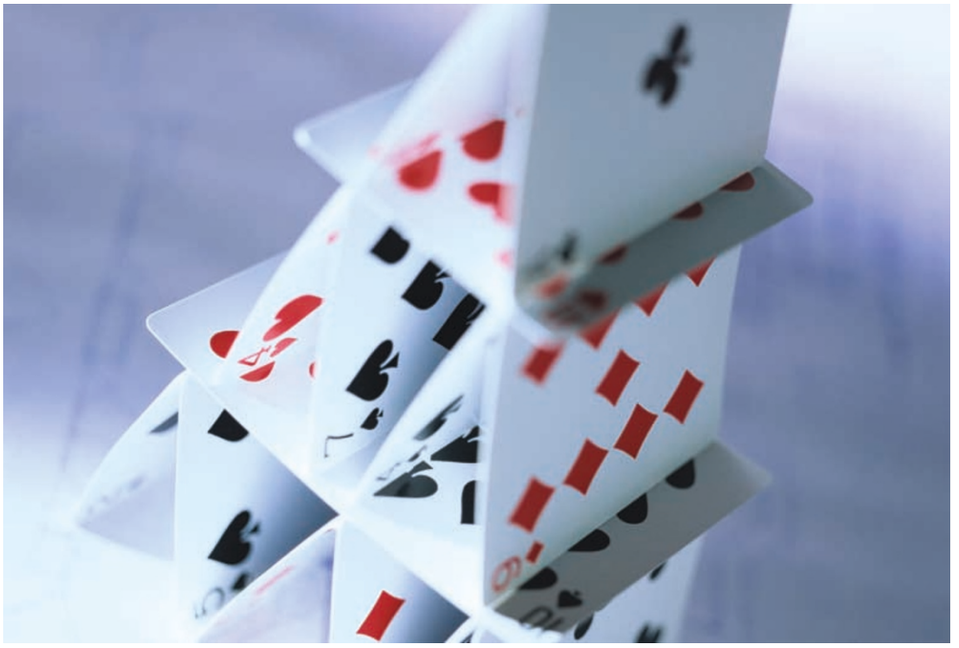

AMYLOID DISEASES

Assembly instructions

Whether the formation of insoluble amyloid-fibril deposits is the causative agent or merely symptomatic in amyloid diseases such as Alzheimer's disease and Creutzfeldt–Jakob disease is a point of great debate at present. But what is certain is that understanding the driving forces behind what causes proteins to misfold, aggregate and form amyloid fibrils is crucial to designing therapeutic strategies for these diseases. Now, reporting in *Proceedings of the National Academy of Sciences*, Luis Serrano and colleagues show how regions of proteins as small as six amino acids in length are all that is needed to turn a normal protein into an amyloid fibril.

The use of model proteins or fragments of proteins in *in vitro* models, which remove many of the complications that exist in the crowded environment of the cell, is revealing an emerging theme in amyloid-disease research. Despite the differences in amino-acid sequences and three-dimensional structures of the proteins involved, there seem to be common mechanistic rules for fibril formation.

To find these characteristic features, Serrano and colleagues have created an even more simplified model for fibril formation. They used a computer-designed algorithm to search for small peptide sequences of six amino acids in length that have a high propensity to form fibrils. The small size of this model system made it easy to study sequence preferences for amyloid-fibril formation as it allowed systematic substitutions of each of the six positions with any amino acid.

What they found was that fibril formation is due to a delicate balance between specific side-chain and charge–charge interactions within the sequences. Point mutations that changed the amino acid (but did not affect the overall structure) inhibited fibril formation, and peptides with charged residues only formed amyloid fibrils when the net charge was ±1 — a net charge of zero led to amorphous aggregates and a net charge of ±2 led to no aggregates being formed. This presumably occurs as a result of how fibrils form — the peptides form flat secondary protein structures, called β-sheets, which stack on top of each other to form the ordered structure of the fibril. If the net charge is zero, there are neither attractive or repulsive forces between the β-sheets and therefore non-ordered, amorphous aggregates are formed. A small net positive or negative charge favours the organization of ordered aggregates as the distances between charges of the same sign are maximized. However, too great a net charge leads to energetically unfavourable conditions for aggregation to occur, so the peptides remain in solution.

The findings show that small regions, and not the whole protein, might be involved in fibril formation. How this translates to the more complicated situation found in larger proteins, in which the number of charged residues may be high and their distribution more complex, will need to be assessed. But this does provide the fascinating possibility that fibril-forming regions of proteins can be predicted from sequence alone, from which molecules can be designed to disrupt this process.

Simon Frantz

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