VIRAL GENETICS

Interference tactics

The infamous propensity of viruses to mutate their way to evolutionary success makes them a slippery target for antiviral drugs. But a new approach that exploits the effect of dominant–negative viral alleles promises to stem the growth of drug-resistant particles, essentially beating viruses at their own game.

All viruses mutate at high rates, but those with a single-stranded RNA genome, such as poliovirus, top them all and are therefore most adept at evolving resistance to conventional drugs. Scott Crowder and Karla Kirkegaard tried an alternative tactic, which involved identifying mutant lines of poliovirus that could interfere in a dominant–negative way with the growth of wild-type particles. Using reverse engineering, they produced a series of poliovirus genomes and then selected those that generated non-viable (that is, growth-defective) particles. Next, they co-transfected mutant and wild-type viruses into cells and investigated whether the yield of wild-type viruses had been affected.

The dominant mutations, which fell into four classes, were very effective. For example, dominant mutations in several capsid proteins inhibited the growth of wild-type viruses by 93%, on average, in just one round of co-infection. But how successful would such a virus-busting strategy be? One test shows that the predicted benefits translate well into practice: a dominant drug-sensitive virus was able to cut the yield of a co-transfected drug-resistant virus down to 3–7% of its normal titre — presumably because the dominant capsid protein destabilized the multi-subunit coat of the resistant virus.

The genomics screen described in this article has revealed some unexpected details of viral biology. But these results will perhaps be even more attractive to drug developers, whose eye will be on exploiting the dominant proteins as targets for anti-viral compounds.

Tanita Casci

References and links

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WEB SITE

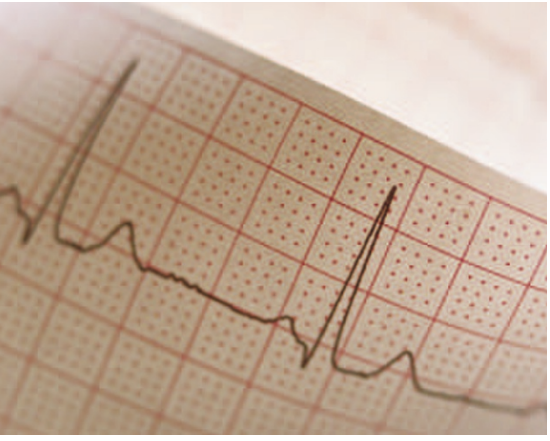
Karla Kirkegaard's laboratory: http://cmgm.stanford.edu/micro/kirkegaard_lab

DEVELOPMENTAL GENETICS

Missing the beat

Recently, phospholipase Cγ1 (PLCG1) was shown to be a key regulator of arterial vasculogenesis in vertebrates. Now, using zebrafish, Wolfgang Rottbauer, Steffen Just and colleagues show that this enzyme also has an earlier and more fundamental role in regulating the contractility of the embryonic heart.

The authors identifed *dead beat* in a forward genetic screen for lethal mutations that perturb cardiac function. As well as showing progressive, ventricle-specific reduction of cardiac contractility, the mutant embryos also have defects in embryonic vasculogenesis, and lack a lumenized dorsal aorta and the posterior cardinal vein.



Rottbauer *et al.* mapped the *dead beat* mutant to the zebrafish *plcg1* gene, and found that it had a premature stop codon induced by a G-to-T transition. Two experiments nailed down *plcg1* as the gene involved in the *dead beat* phenotype. An antisense morpholino to the translational start site or the splice donor site of exon 13, which results in abnormal splicing products, mimicked the effects of the *dead beat* mutation: mutants had lower ventricular contractility and had no blood circulating in lumenized vessels. In addition, injecting wild-type *plcg1* mRNA into *dead beat* mutant embryos completely rescued both the vascular and heart phenotypes in 70% of the cases. The expression pattern of *plcg1* is consistent with its biological function: its RNA is ubiquitously present in zebrafish embryos and is more pronounced in the brain, vasculature and heart.

Plcg1 fuctions downstream of vascular endothelial growth factor (Vegf) signalling in many developmental processes and the authors found that Vegf is essential for zebrafish cardiac contractility. They also found that the effect is mediated by Vegf receptor 1 (Flt1). But does the signalling cascade through VEGF–FLT1–PLCG1 also affect cardiac myocyte contractility in mammals? By using specific pharmacological inhibitors for each of these molecules in rat ventricular cardiomyocytes, they found that PLCG1 signalling controls mammalian cardiomyocyte contractility by modulating calcium cycling.

The authors conclude that heart muscle uses this cascade to control the strength of the heart beat and speculate that the VEGF–PLCG1 pathway might contribute to the normal and pathological regulation of cardiac contractility. Heart failure is a very common disease in humans, and, as no agents are currently available to safely enhance cardiac contractility, it would be of interest to examine whether the VEGF–PLCG1 pathway might offer new opportunities for such treatment.

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References and links

ORIGINAL RESEARCH PAPER Rottbauer, W. *et al.* VEGF–PLCγ1 pathway controls cardiac contractility in the embryonic heart. *Genes Dev.* 1 July 2005 (doi:10.1101/gad.1319405)

WEB SITES

Mark Fishman's web page: http://www.massgeneral.org/cvrc/crvc/fishman.html

Wolfgang Rottbauer's web page: http://www.klinikum.uni-heidelberg.de/index.php?id=4580