simulate diastole. The authors showed that JTV519 prevents the depletion of calstabin-2 that is observed in mice during exercise and significantly reduced P_O in calstabin-$2^{+/-}$ mice but not in calstabin-2-null mice. However, JTV519 had no direct effect on this phosphorylation, indicating that it increases the affinity of calstabin-2 for RyR2 even when the channel is phosphorylated by PKA. Indeed, further experiments showed that the affinity of calstabin-2 for both wild-type and a mutant, constitutively PKA-phosphorylated RyR2 was increased in the presence of JTV519, regardless of phosphorylation status of the channel.

The authors speculate that calcium leakage resulting from calstabin-2-deficient RyR2 alone might not be sufficient to cause SCD, but that when combined with exercise, the resulting phosphorylation of RyR2 and subsequent dissociation of already depleted levels of calstabin-2 is enough to cause a fatal arrhythmia. JTV519 has therefore provided both an insight into this mechanism and a potential therapeutic strategy to prevent SCD.

Joanna Owens

References and links

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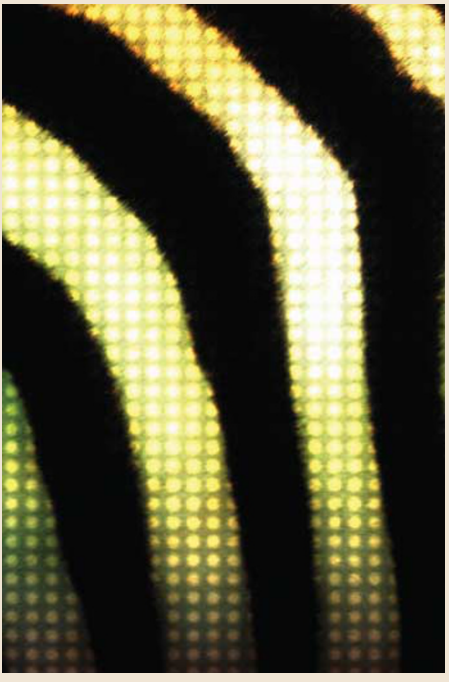

CHEMICAL GENETICS

New use for the cellular dustbin

Protein degradation is essential in cellular homeostasis, and the proteasome — a large protease complex in the cell that destroys appropriately tagged proteins — has a key role in this process. Now, as reported in the *Journal of the American Chemical Society*, Craig Crews and colleagues have harnessed the proteasome by using small molecules to direct the selective degradation of particular proteins in whole cells, thereby providing a novel general method to study the effects of the removal of selected proteins.

Proteins are normally tagged for destruction by the proteasome with a polymer chain formed from a small protein called ubiquitin, which is attached in a three-part process. First, the carboxyl terminus of ubiquitin is activated by an enzyme known as E1. The activated ubiquitin is then transferred to a ubiquitin-conjugating enzyme (E2). Finally, ubiquitin is transferred to a lysine of the protein substrate that is specifically bound by a ubiquitin ligase (E3). Successive attachment of ubiquitin to internal lysines in previously added ubiquitins results in the formation of the polyubiquitin destruction tags.

Crews and colleagues have exploited this process by developing bifunctional molecules — dubbed PROTACs — that comprise a ligand for the target protein, a linker and a ligand for an E3 ligase. By bridging the target protein and an E3 ligase, the PROTAC molecule initiates the ubiquitination of the target protein, leading to its proteasome-mediated degradation.

The authors had previously provided proof of principle that this strategy worked *in vitro* on one stable protein, and so set out to investigate whether it would be capable of significantly reducing the level of proteins in whole cells simply by adding PROTACs to the cells. They designed a PROTAC molecule that consisted of a heptapeptide that binds to an E3 ligase, a linker, a known ligand for a mutant form of FK506-binding protein (FKBP12) and a poly-D-arginine tag, as such tags are known to facilitate translocation of peptides into cells.

To assess the impact of this molecule on protein levels after addition to cells, they monitored the fluorescence from a fusion protein of FKBP12 and enhanced green fluorescent protein. Fluorescence was lost in cells treated with the PROTAC, but not with either of the two protein ligands separately, confirming that the PROTAC worked as expected. Further analogous experiments — this time targeting the androgen receptor by using a second PROTAC incorporating dihydrotestosterone as the protein ligand — confirmed the robustness of the approach for inducing intracellular protein degradation.

The strategy described should in principle be applicable to 'knocking out' any protein for which an appropriate ligand is available, potentially providing a convenient and rapid complementary approach to conventional genetic knockouts for studying protein function. Indeed, libraries of PROTACs might prove useful in chemical-genetic screens for phenotypic effects. This approach might also allow more temporal or dosing control than gene inactivation at the RNA level with RNA interference (RNAi), a feature that could be important if molecules based on the PROTAC principle were developed as potential therapeutics for diseases that depend on the overexpression or presence of a particular protein.

Peter Kirkpatrick

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