When death starts with a caspase

When a cytotoxic lymphocyte delivers granzyme B to a target cell, current models emphasize that the enzyme induces apoptosis through a mitochondria-centered pathway, cleaving the proapoptotic Bcl-2 family member Bid to cause mitochondrial permeabilization. On page 875, Metkar et al. overturn this view, showing that in the dominant pathway granzyme B begins by processing procaspase-3, whereas mitochondria are secondary components that amplify the signal once caspase-3 has been activated. The work adds to recent evidence that caspases can initiate apoptosis, whereas mitochondria serve as signal amplifiers.

The authors used a variety of strategies to avoid common pitfalls during granzyme-mediated apoptosis assays. In past assays, uncomplexed granzyme B was added to cell extracts. Granzyme B in the new assays was delivered into cells using adenovirus particles, and was presented in a complex with the proteoglycan serglycin to mimic the enzyme’s natural state. Doing the apoptosis assays with whole cells minimized the risk of proteolysis occurring at sites not normally frequented by granzyme B.

In this system, Bid cleavage was not detected, and cells deficient in procaspase-3 failed to undergo mitochondrial depolarization or DNA fragmentation, indicating that granzyme B acts first through procaspase-3 rather than the mitochondria. Caspase-3–induced permeabilization of the mitochondria amplifies the death signal but, based on assays with cells harboring a Bax/Bak deletion, this amplification is not essential for apoptosis.

Fusion failure for flies

Liu et al. (page 899) genetically disrupted the gene for paramyosin, a major component of invertebrate muscle thick filaments, in Drosophila melanogaster. In addition to the expected finding, that the disruption damages myofibril structure, the work identifies a surprising requirement for paramyosin in myoblast fusion.

Mutant flies in which a P-element has been excised from the paramyosin promoter region die during embryonic development. In addition to defects in myofibril assembly, the embryos exhibit sporadic failures in myoblast fusion, leading to the absence of some muscle fibers. Antibody localization experiments show paramyosin in discrete foci at sites near the junctions of fusing myoblasts, and nonmuscle myosin appears to colocalize to the same sites.

The authors propose that, in the fly, paramyosin functions as a cytoskeletal protein during early development. In this model, paramyosin and nonmuscle myosin form minifilaments that interact with the actin cytoskeleton and promote myoblast fusion. After myoblast fusion, nonmuscle myosin is then replaced by muscle myosin to produce thick filament precursors, ultimately leading to the construction of normal thick filaments. With or without its myosin partners, paramyosin could function as intermediate filament proteins do in vertebrates, attaching to membrane bound proteins to aid in fusion. Since Drosophila lacks cytoskeletal intermediate filaments, paramyosin may have been borrowed for this function during evolution.

When the channels leak, the muscle is weak

Patients with heart failure often have reduced exercise tolerance that cannot be explained solely by their impaired blood flow. On page 919, Reiken et al. identify the molecular defect that may underlie this phenomenon, revealing promising therapeutic targets and supporting a new model for the pathogenesis of this widespread disease.

Coupling of excitation and contraction in skeletal muscle requires the type 1 ryanodine receptor (RyR1), a calcium channel that forms a complex with several regulatory proteins. The authors identified a unique site on RyR1 that is phosphorylated by protein kinase A; they then generated mutant channels that either cannot be phosphorylated or that mimic constitutively phosphorylated RyR1. Phosphorylation of the channel causes it to dissociate from the regulatory protein FKBP12, allowing the channel to open more frequently. Compared with RyR1 in normal skeletal muscle, RyR1 from animal models of heart failure is hyperphosphorylated, and the rate of calcium release and decay of calcium transients are both slowed.

Reiken et al. propose that the hyperadrenergic state of heart failure causes hyperphosphorylation of ryanodine receptors in both cardiac and skeletal muscle, allowing calcium leakage through the channels. The muscles’ effort to compensate for the leakage of calcium stores may increase energy consumption, thus decreasing exercise tolerance.