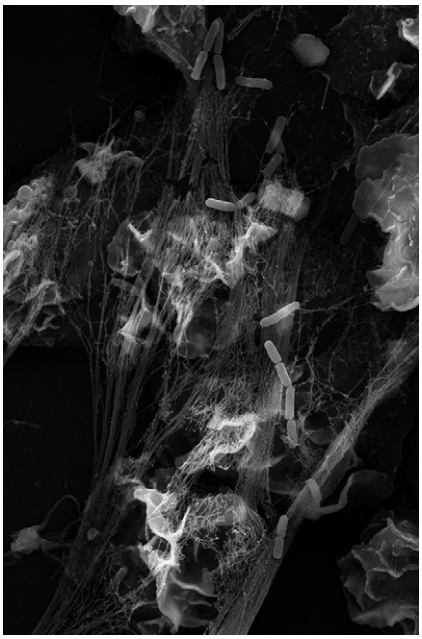
Cell Death and Destruction

Programmed cell death and autophagy, a limited form of cellular destruction, are implicated in an ever-expanding range of biological processes. In this issue's Select, we highlight recent insights into how these events impinge on cancer progression, Crohn's disease, innate immune responses, and development.



Web-like neutrophil extracellular traps (NETs) catch and neutralize the dysentery-inducing rod-shaped bacteria, Shigella flexneri. Image courtesy of V. Brinkmann.

Neutrophils Make the Ultimate Sacrifice

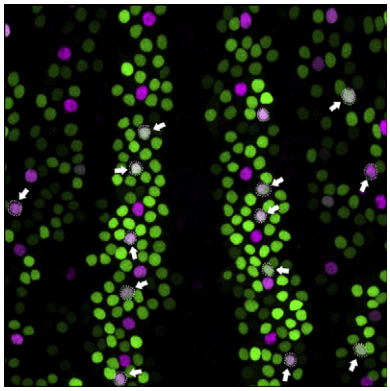
Upon contact with their bacterial targets, neutrophils fire a barrage of antimicrobial peptides, sometimes releasing structures called neutrophil-associated extracellular traps, or NETs. In releasing their NETs, they commit suicide—unleashing their entire antibacterial arsenal via a specialized form of programmed cell death called netosis. Hakkim et al. (2011) now provide new insight into the molecular triggers for netosis. The authors first employ a high-throughput approach to rapidly and quantitatively examine the cellular changes that accompany NET formation in isolated human neutrophils. They then screen for small molecules that inhibit or delay netosis, isolating two classes of compounds that block NET formation. One includes chemicals that block the production of reactive oxygen species (ROS), which have been implicated in netosis, and one contains molecules that are known to inhibit transduction through the Raf-MEK-ERK signaling cascade. The authors go on to dissect how this pathway influences NET formation by challenging neutrophils with either proinflammatory lipids or *Helicobacter pylori*, the stomach bacterium that causes ulcers. Despite some differences, all stimuli cause netosis by elevating production of ROS by stimulation of NADPH oxidase via the Raf-MEK-ERK pathway. Though NET formation can be beneficial, excessive netosis is associated with a variety of autoimmune diseases, and this new understanding of the molecular player that is responsible for netosis could provide a foothold for an assault on excessive innate immune responses.

Hakkim, A., et al. (2011). Nat. Chem. Biol. 7, 75–77.

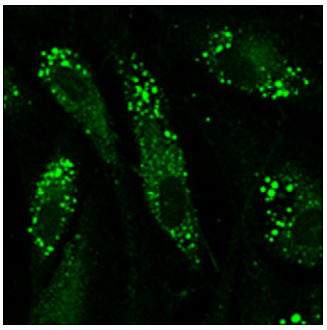
Death Silences Developmental Noise

Apoptosis is well known for its role in removing excess cells during normal developmental processes, including the destruction of the skin cells that form webs between our fingers and toes. Recent findings by Koto et al. (2011) show that apoptosis is also deployed to remove mis-specified cells that result from inborn errors of development. The authors make movies of developing sensory organs in living fly pupae and then ask a few simple questions. When, where, and how frequently are aberrant sensory cells produced? Though much is known about how Notch-Delta signaling regulates the patterns that arise during maturation of sensory organ precursors (SOPs), this study provides new insight into how fluctuations in Notch signaling synergize with neural specification factors to promote either cell differentiation or cell death. The authors suggest that transcriptional noise during cell fate determination causes nearly 20% of epithelial cells to initiate neuronal differentiation in the wrong place at the wrong time. These mis-specified SOP-like cells appear confused, following neither the neuronal nor epithelial differentiation program. Further examination of the SOP-like cells indicates that a caspase-dependent mechanism kills them, ensuring that sensory bristles on the fly notum occur at regularly spaced intervals. The data provide several hints that activation of Notch, in the presence of neural determinants, favors apoptosis. Though this study focuses on the development of sensory organs on a specific patch of the fly, it is tempting to speculate that similar failsafe systems operate in many places and in many organisms, guaranteeing robust control over the number, type, and patterns of cells during development.

Koto, A., et al. (2011). Curr. Biol. 21, 1–10.



Aberrant SOP-like cells (white arrows) express both neurogenic genes (magenta) and high Notch activity (green) before they die. Image courtesy of A. Koto and M. Miura.



High levels of Ras-induced autophagy trigger the death of human ovarian epithelial cells. Image courtesy of S. Martin.

One Way or Another, Ras Is Gonna Getcha

Although activating mutations in oncogenic proteins, such as Ras^{v12}, promote hyperproliferation in a wide variety of cancers, including squamous cell carcinomas and neuroblastomas, these same mutated proteins can also trigger senescence and cell death. How an activated oncoprotein promotes programmed cell death has been controversial. Elgendy et al. (2011) now supply evidence that oncogenic Ras signals through the kinase ERK, stimulating excessive autophagy that culminates in caspase-independent cell death. The authors conditionally induce Ras^{v12} in a variety of epithelial cell lines and then study how these cells die. They show that induction of the constitutively active Ras^{v12}, but not the dominant-negative Ras^{N17}, promotes proliferative arrest followed by cell death. Prior to death, these cells upregulate the BH3-only protein Noxa, which has been linked to initiation of apoptosis. Surprisingly, the dying cells display none of the typical hallmarks of apoptosis, instead exhibiting excessive autophagy. Generally, BH3-only proteins promote apoptosis by binding to antiapoptotic members of the Bcl-2 family. Recently, several Bcl-2-related proteins have been found to bind and inhibit a proautophagy protein, Beclin-1. The authors tested whether Ras^{v12}-induced upregulation of Noxa promoted autophagy by antagonizing the inhibition of Beclin by Bcl-2. Consistent with this notion, Elgendy and colleagues find that Noxa can bind Bcl-2 family members and sequester them from Beclin-1. In addition, they show that downregulation of Beclin-1 or the autophagosome protein Atg5 prevents Ras^{v12}-induced cell death. The authors speculate that this same mechanism of induced autophagic cell death may be responsible for the spontaneous regression of some human neuroblastoma tumors, raising the possibility that understanding the innate potential of oncogenic cells to switch from unrestrained proliferation to cell death may provide a new avenue for cancer therapies.

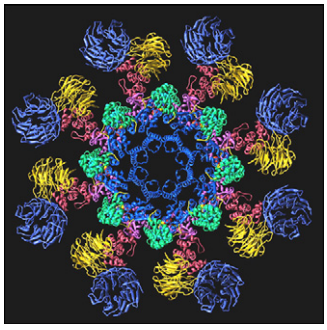
M. Elgendy et al. (2011). Mol. Cell, in press. Published online February 24, 2011. 10.1016/j.molcel.2011.02.009.

miRs Link Autophagy and Crohn's Disease

Increased risk of Crohn's disease has been linked to more than 70 genetic polymorphisms in loci-encoding proteins that are required for myriad cellular processes, including cell fate determination, inflammation, and autophagy. However, the pathobiology of this chronic disorder remains poorly defined. In a recent study, Brest et al. (2011) connect deregulation of a specialized form of autophagy that degrades phagocytosed bacteria, xenophagy, with the pathology of Crohn's disease. The authors find that a synonymous single-nucleotide polymorphism (SNP) in the coding region of *IRGM*, a gene that is required for xenophagy, has profound implications for the regulation of IRGM expression. They show that, rather than changing protein function, this nucleotide change makes IRGM transcripts less susceptible to regulation by the mircoRNA, miR-196. Because the disease-linked SNP is located within the miRNA seed region of *IRGM*, it alters the binding affinity between the mRNA-miRNA duplex and the RNA-induced silencing complex, RISC. Brest and coworkers find that miR-196 binds more tightly to transcripts containing the protective (nondisease associated) allele and suggest that altered control of IRGM levels by miR-196 explains, in part, the risk of developing Crohn's disease. They go on to investigate how deregulation of IRGM could explain the massive inflammation observed in the intestinal mucosa of Crohn's patients. Under normal circumstances, gut inflammation in response to *E. coli* infection dampens autophagic flux and eventually downregulates the immune response. However, in the inflamed intestinal epithelium of Crohn's patients, levels of autophagy and xenophagy remain high, prolonging the inflammatory immune response that leads to bowel dysfunction.

Brest, P., et al. (2011). Nat. Genet., in press. Published online January 30, 2011. 10.1038/ng.762.

The Shape of Death's Dark Shadow



The eight subunits of the Dark apoptosome form a ring-like platform with a central hub, topped by an octagonal crown of CARDs (shown in green). Image courtesy of C. Akey.

The multimeric ring-shaped apoptosome binds specific procaspases, initiating the caspase cascade that is responsible for the intrinsic programmed cell death pathway. With their most recent study, Yuan et al. (2011) offer new insights into apoptosome assembly, function, and evolution. The authors use cryo-electron microscopy and homology-based modeling to determine the structure of the biologically active apoptosome formed by the *Drosophila* Apaf-1-related killer protein, Dark. They then compare their latest structure with those from other species. In all organisms, apoptosomes are homo-oligomers, comprised of a variety of domains that bind nucleotides, facilitate inter- and intramolecular interactions, and recruit procaspases. The authors highlight differences in caspase recruitment domain (CARD) organization in different organisms' apoptosomes, suggesting that the complex evolutionary history of the apoptotic pathways is reflected in these variations. Additional structural insights from this work include variability in the necessity of cytochrome *c* and ATP for apoptosome formation, as well as the presence of distinct nucleotide binding motifs. Despite many differences among these divergent procaspase activating platforms, including distinct cylindrical symmetries, the authors find that the pattern of connections between apoptosome subunits in flies, worms, and humans is remarkably similar.

Yuan, S., et al. (2011). Structure 19, 128–140.

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