Cell biology. Apoptosis--the calcium connection

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cisional lesions that slice the exposed amnioserosa from canthus to canthus, and then measuring the consequential gaping of the leading-edge epithelium. This epithelium twangs back at a rate about 80 times the rate of its previous forward motion, until it reaches a new equilibrium where the applied tissue forces are again in balance. The quantitative data gathered from these "mechanical-jump experiments" revealed that the amnioserosa and the force-generating mechanisms in the adjacent epithelium make comparable contributions to the advancement of the epithelial leading edge.

These data raise several fascinating issues. For example, it is remarkable that the zipper rate increases in the unperturbed canthus when the opposite canthus is laser-ablated. It is tantalizing to imagine how cells in the embryo might regulate such a response. Presumably, cells at the healthy canthus detect some change in the global stress pattern that directs their compensatory response. Indeed, this sort of mechanism illustrates how the cues "read" by cells during development may often be mechanical, with cells sensing tension, stretch, and so forth, as opposed to diffusible growth factor signals. Indeed, what more ideal signal to announce that one embryonic tissue movement is complete, and the next can commence, than some mechanical cue arising from the completion of the preceding morphogenetic episode.

Important for any mathematical modeling of a developmental process is that it should feed back to the experimental scientist with some predictions about the episode being modeled. As their test case, Hutson and colleagues selected the *myospheroid* fly mutant, which has a defective β_{PS} integrin and fails in dorsal closure. Their model predicts that the failure of dorsal closure in this mutant results from defective zippering as opposed to aberrant epithelial sweeping forces, suggesting unexpectedly that integrins are involved in the zippering process. Time, and some tough future experiments, should tell us whether this is indeed where integrins fit into the dorsal closure story.

As the authors point out, many other fly mutants could do with a similar experimental treatment. A good example might be the *Rho1* mutant, in which the small guanosine triphosphatase Rho is nonfunctional, resulting in failure of the normal assembly of the leading-edge actin cable (*7, 8, 14*). Surprisingly, these embryos can generally close the dorsal hole, albeit in a rather haphazard fashion. Information about the rejigged balance of tissue tensions within the leading-edge epithelium and the adjacent amnioserosa would be invaluable in the interpretation of how the embryo compensates for the loss of Rho and the actin cable. This knowledge would in turn help us to determine more precisely the normal function of Rho and actin cables during embryonic development in vivo.

Can this kind of mathematical modeling be done for other morphogenetic events, and will it be as revealing? Dorsal closure is the morphogenetic episode about which we have the most genetic clues, partly because it takes place late, after the maternal pools of mRNA and protein are diminished. Also, because it occurs superficially (unlike the epithelial invagination events of fly gastrulation) and essentially in one plane, it is particularly amenable to imaging and to experimental manipulations such as laser ablation. But, with some effort, similar approaches will be possible for gastrulation and other tissue movements in the developing fly embryo, such as the amnioserosa-driven germ band retraction episode that precedes dorsal closure (*15*). These tissue movements are very different from dorsal closure and will reveal other components of the machinery that the embryo uses to shape itself.

The Hutson *et al.* study investigating the forces directing dorsal closure is a good first step toward dissecting the forces governing tissue movements in embryos. There are almost certainly morphogenetic episodes in human embryogenesis that mirror the events of dorsal closure, for example, closure of the eyelids during midgestation. And, as Hutson and colleagues point out, what is true for morphogenetic hole closure may also serve as a good model for damage-triggered hole closure during wound healing (*10, 16, 17*).

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CELL BIOLOGY

Apoptosis—the Calcium Connection

Nicolas Demaurex and Clark Distelhorst

The cells of our body are able to quickly commit suicide in response to genetic or environmental cues, a process termed apoptosis. This process is essential for development, tissue homeostasis, and defense against pathogens. Organized life requires cell death, and execution of cell death relies on the very machinery of life. Mitochondria, the organelles that produce energy through cellular respiration, integrate death signals mediated by proteins belonging to the Bcl-2/Bax family, and kill cells by releasing critical factors such as cytochrome c that activate executioner caspase proteases (*1*, *2*). Calcium ions (Ca^{2+}), the cellular messengers that control every aspect of cell and tissue physiology, can be turned into death signals when delivered at the wrong time and place (*3*, *4*). Mitochondria eventually decide whether Ca^{2+} signals are decoded as life or death signals (*5*), but it is not clear whether Ca^{2+} is an additional stress factor that "tips the balance" or is an obligatory signal for death. On page 135 of this issue, Scorrano *et al*. (*6*) demonstrate that the transfer of Ca^{2+} from the endoplasmic reticulum (ER) to the mitochondria is required for initiation of programmed cell death by some, but not all, apoptotic signals (see the figure). Their elegant approach of genetically inactivating crucial proteins and reconstituting them in specific organelles reveals that the Ca^{2+} content of the ER determines the cell's ability to commit suicide, defining the ER as a new gateway to apoptosis.

By using Ca^{2+} as an intracellular messenger, cells walk a tightrope between life and death. Because of the toxicity of Ca^{2+} ions, a low Ca^{2+} concentration must be maintained in the cytoplasm, and most of the cellular

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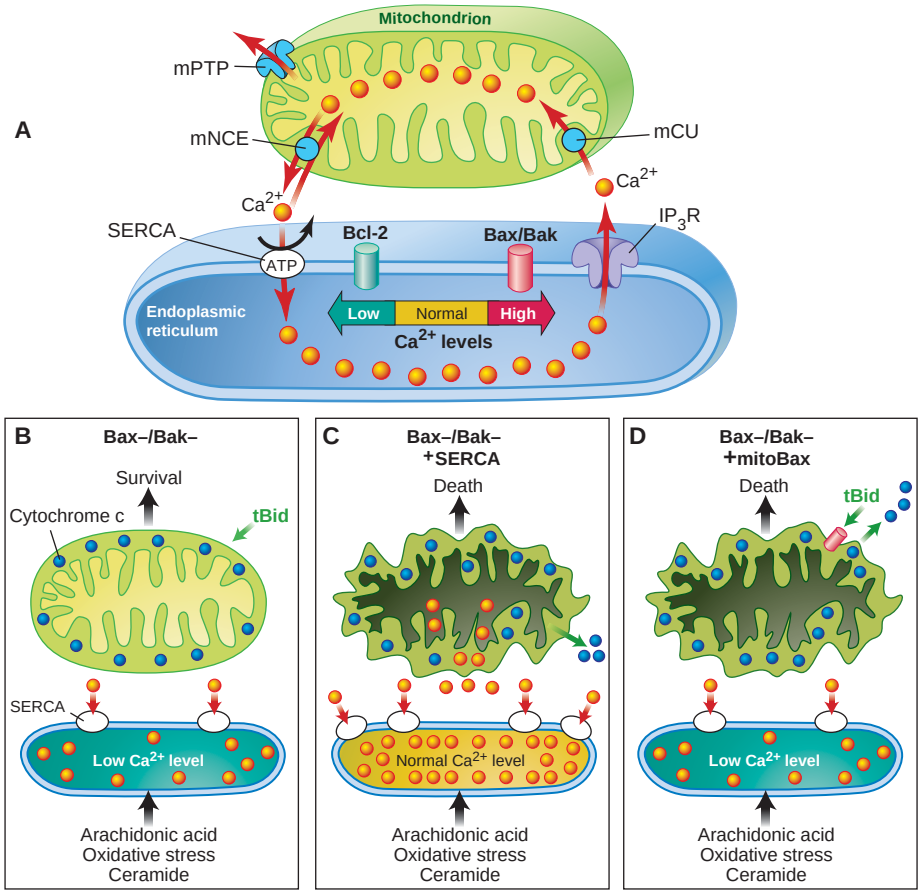

The ER Ca^{2+} apoptotic gateway. (**A**) Under normal conditions, Ca^{2+} continuously cycles between the ER and mitochondria. Ca^{2+} is pumped into the ER by Ca^{2+} ATPases (SERCA), and released by IP_3–gated channels (IP_3R). Ca^{2+} enters mitochondria by a Ca^{2+} uniporter (mCU) and is released by a Na^+/Ca^{2+} exchanger (mNCE). The ER Ca^{2+} load reflects the balance between Bcl-2 and Bax/Bak proteins. (**B**) Ablation of Bax/Bak decreases the ER Ca^{2+} load and protects cells from apoptosis (*6*). (**C**) Correction of the ER Ca^{2+} load by overexpressing SERCA proteins or selective expression of Bax in mitochondria (**D**) defines three classes of apoptotic stimuli: (i) stimuli that engage the ER Ca^{2+} gateway and do not require Bax/Bak in mitochondria, such as arachidonic acid, ceramide, and oxidative stress; (ii) stimuli that do not engage the ER Ca^{2+} gateway but require mitochondrial Bax/Bak, such as the "BH3-only" protein tBid; and (iii) stimuli that engage both pathways, such as T cell receptor activation, staurosporine, etoposide, and brefeldin A.

Ca^{2+} is stored in the ER. Ca^{2+} is pumped into the ER by SERCA ATPases (adenosine triphosphatases) and is released only transiently during bouts of signaling by the opening of inositol 1,4,5-trisphosphate (IP_3) or ryanodine receptor (RyR) Ca^{2+}-release channels (*3*). A significant fraction of the released Ca^{2+} is captured by mitochondria, which are strategically located near Ca^{2+}-release channels (*7*). This close connection allows mitochondria to modulate, propagate, and synchronize Ca^{2+} signals (*8*) and to prevent ER depletion by recycling Ca^{2+} to the ER (*9*) (see the figure). This ER-mitochondria connection enables Ca^{2+} signals not only to fine-tune cellular metabolism (*10*) but also to modulate the ability of mitochondria to undergo apoptosis (*5*, *11*). The switch from a life to a death signal involves the coincidental detection of Ca^{2+} and proapoptotic stimuli, and depends on the amplitude of the mitochondrial Ca^{2+} signal (*12*). The magnitude of mitochondrial Ca^{2+} signals, in turn, depends largely on the Ca^{2+} content of the ER, which is maintained by the balance between active Ca^{2+} pumping by SERCA and passive leakage through Ca^{2+}-release channels (see the figure).

Several studies indicate that the Ca^{2+} content of the ER determines the cell's sensitivity to apoptotic stress. Procedures that decrease the Ca^{2+} concentration in the ER, such as genetic ablation of the ER Ca^{2+}-buffering protein calreticulin or overexpression of plasma membrane Ca^{2+} ATPases, protect cells from apoptosis (*13*, *14*). Conversely, procedures that increase the ER Ca^{2+} load, such as overexpression of SERCA or calreticulin, sensitize cells to apoptotic stress (*14*, *15*). Sensitivity to apoptosis correlates with the total ER Ca^{2+} load, rather than with the free ER Ca^{2+} concentration, and depends on the ability of cells to transfer Ca^{2+} from the ER to the mitochondria. Accordingly, procedures that enhance the transfer of Ca^{2+} from the ER to mitochondria augment ceramide-induced cell death (*16*).

Further studies suggest that the balance between pro- and antiapoptotic Bcl-2/Bax family members regulates the ER Ca^{2+} content. Overexpression of the antiapoptotic protein Bcl-2 decreases the ER Ca^{2+} load and protects cells from death (*17*, *18*). Apoptosis is restored by correcting ER Ca^{2+} levels with SERCA and correlates with an increase in mitochondrial Ca^{2+} (*14*). Conversely, Bax/Bak overexpression favors the transfer of Ca^{2+} from ER to mitochondria and induces cell death (*19*, *20*). How Bax, Bak, and Bcl-2 interfere with the ER Ca^{2+} load is uncertain, but clearly this interference depends on the ER-mitochondria connection. Bcl-2 protects from death only when targeted to the ER, but kills when directed at mitochondria (*21*). ER-targeted Bcl-2 protects from death induced by mitochondria-targeted Bax (*22*), suggesting that the ER exerts a dominant role in its coupling to mitochondria.

All of these gain-of-function studies point to the central part played by Bcl-2/Bax in regulating ER Ca^{2+} and suggest that the transfer of Ca^{2+} from the ER to mitochondria induces apoptosis. But do specific apoptotic signals really require this Ca^{2+} connection to kill? Scorrano *et al.* use a unique model to answer this question. They discovered that mice deficient in both Bax and Bak, so-called double knockouts, are resistant to a wide range of apoptotic stimuli (*23*) and show a decrease in ER Ca^{2+} load. Double knockout cells from these mice exhibit no intrinsic defects in the handling of mitochondrial Ca^{2+} but cannot deliver enough Ca^{2+} to the mitochondria because their ER Ca^{2+} content is too low. Genetic correction of the ER Ca^{2+} load by overexpression of SERCA restored the killing ability of Ca^{2+}-mobilizing apoptotic agents. This demonstrates that Ca^{2+} acts on mitochondria independently of Bax or Bak. Conversely, expression of mitochondria-targeted Bax restored death selectively to the "BH3-only" protein tBid. This indicates that tBid requires mitochondrial Bax but not Ca^{2+} transfer from the ER to mitochondria to induce cell death. The ability to rescue apoptosis by expressing organelle-targeted proteins in cells deficient in both Bax and Bak neatly defines which apoptosis signals are controlled by ER Ca^{2+} and which by mitochondrial Bax. From a practical standpoint, this provides a useful classification of apoptotic stimuli into three categories (see the figure).

The first category comprises apoptotic signals—such as arachidonic acid, ceramide, and oxidative stress—that engage the ER Ca^{2+} gateway but do not require

Bax/Bak action in mitochondria. These agents release Ca^{2+} themselves and kill more efficiently when Ca^{2+} is further increased by physiological or pathological stimuli, accounting for the "Ca^{2+}-preconditioning" observed in previous studies (*5*). Killing absolutely requires an increase in mitochondrial Ca^{2+}, and thus strictly depends on ER Ca^{2+} levels. In the second category are agents, such as tBid, that require the presence of Bax or Bak in the mitochondria but do not engage the ER Ca^{2+} gateway. These agents do not require mitochondrial Ca^{2+} and kill efficiently at all ER Ca^{2+} loads. The third category is constituted of agents—such as etoposide, staurosporine, brefeldin A, and T cell receptor activation—that engage both pathways. These agents require both Ca^{2+} and the presence of Bax or Bak in mitochondria, and both ER Ca^{2+} and Bax/Bak levels modulate their killing potency.

The Bax/Bak-deficient mouse cells of Scorrano *et al*. are the first loss-of-function model in which an alteration in Ca^{2+} handling is causally linked to cell killing, but the mechanism leading to decreased ER Ca^{2+} is not established. The presence of normal amounts of Ca^{2+} signaling proteins in Bax/Bak-deficient cells suggests that the defect is either directly caused by the Bax/Bak proteins themselves or is mediated by a change in activity, rather than content, of a Ca^{2+} handling protein. A possible candidate for such modulation is the IP_3 receptor, the principal Ca^{2+}-release channel of the ER, whose activity undergoes complex regulation by Ca^{2+}, ATP, and phosphorylation. SERCA proteins are also subject to modulation, and it will be interesting to see whether Bax/Bak inactivation is associated with changes in activity of the IP_3 channel or of SERCA. Another likely partner is the antiapoptotic protein Bcl-2. The effects of Bak/Bax inactivation mimic those of Bcl-2 overexpression, suggesting that the balance between Bax/Bak and Bcl-2, rather than the amounts of the individual proteins, determines ER Ca^{2+} load. Manipulation of Bcl-2 expression in Bax/Bak-ablated cells will allow researchers to test directly this "rheostat" model, and to confirm whether Bcl-2 and Bax/Bak indeed coregulate ER Ca^{2+}.

The Scorrano *et al*. study defines a new role for the ER-mitochondria Ca^{2+} connection. The ER is now envisioned as a gun pointed at the mitochondria, which can be loaded and unloaded with Ca^{2+} by Bax and Bcl-2 proteins. Some, but not all, apoptotic signals are able to pull the ER Ca^{2+} trigger, and hence to kill cells in a strictly Ca^{2+}-dependent manner. Future studies will determine whether this mechanism also occurs when Bcl-2 family members are expressed at physiological levels in vivo, and whether physiological death signals are able to pull the Ca^{2+} trigger.

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CLIMATE CHANGE

Will Ocean Fertilization Work?

Ken O. Buesseler and Philip W. Boyd

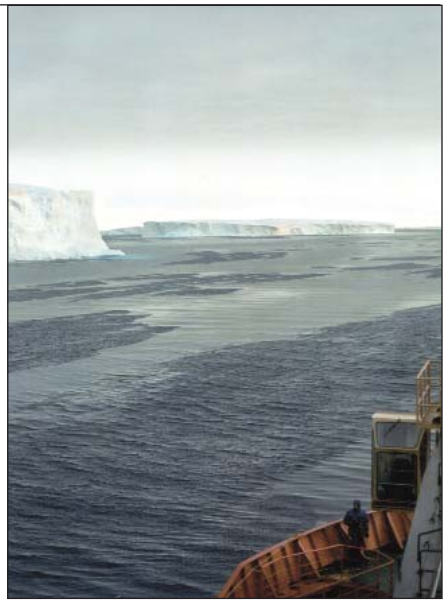
Iron fertilization of the ocean—a potential strategy to remove CO_2 from the atmosphere—has generated much debate among ocean and climate scientists (*1*–*4*). It is viewed as particularly attractive by geoengineers because the addition of relatively small amounts of iron to certain ocean regions may lead to a large increase in carbon sequestration at a relatively low financial cost.

To assess whether iron fertilization has potential as an effective sequestration strategy, we need to measure the ratio of iron added (Fe_{add}) to the amount of carbon sequestered (C_{seq}) (in the form of sinking particulate organic carbon, POC) to the deep ocean in field studies. We must then apply appropriate scaling factors to determine whether globally significant quantities of CO_2 can be removed from the atmosphere to the deep ocean in this way.

The Southern Ocean (see the figure) is the most important region for possible climate regulation by iron fertilization. In this high-nitrate low-chlorophyll (HNLC) region, large quantities of surface macronutrients return to the deep ocean via the flow of intermediate and deep waters. According to the "iron hypothesis" (*5*), adding iron to these nutrient-rich surface waters will increase phytoplankton biomass, resulting in increased uptake of CO_2 by the phytoplankton living in the surface ocean.

In the Southern Ocean, there have been three open-ocean iron-enrichment experiments: SOIREE (Southern Ocean Iron Enrichment Experiment) (*6*), EisenEx-1 [Eisen(=Iron) Experiment] (*7*), and SOFeX (Southern Ocean Iron Experiment) (*8*). All three produced notable increases in biomass and associated decreases in dissolved inorganic carbon and macronutrients. However, evidence of sinking particles carrying POC to the deep ocean was limited.

SOIREE (a 13-day experiment) and EisenEx-1 (21 days) showed no difference between particle fluxes in the fertilized and nonfertilized waters (*7*, *9*–*10*). During SOFeX (28 days), we observed in the fer-



Exploring the Southern Ocean. The research and supply vessel *Aurora Australis* heads into an iceberg field off Antarctica.

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