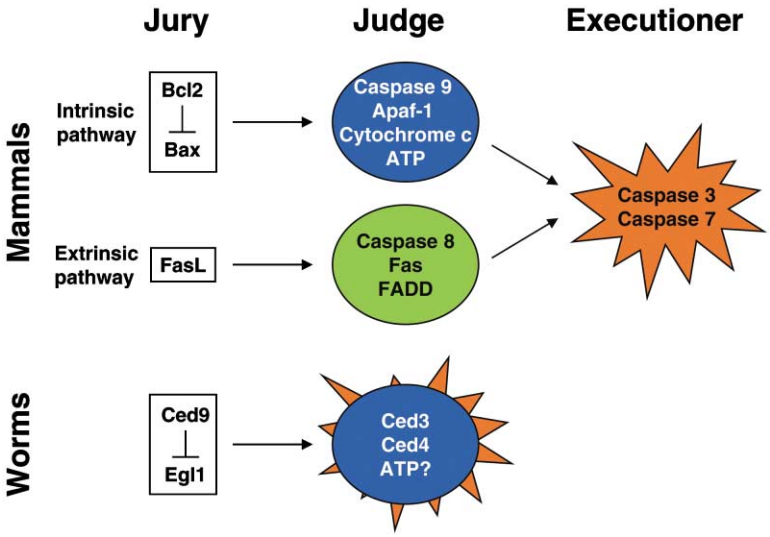

Phases in the Arbitration of Cell Fate Decisions

Protein interactions dominate the trial phase of apoptosis, serving as control points to mediate the integration of disparate signals. The fatal judgement is pronounced by molecular assemblies, which in mammals pass the job on to the executioner caspases. In worms, the pathway is streamlined, as the same molecular assembly serves the judge and executioner functions, since there appears to be no caspases downstream of CED3.

most exciting news from the apoptosome is that it provides just enough of a look at the machine to predict ways to turn it off and on artificially, thereby providing major therapeutic advantages.

Assuming there is enough ATP in vivo to allow oligomerization, displacing the head of Apaf-1 from between its feet may be sufficient to activate the molecule, or activation may require the gap to be filled by something the size of cytochrome c. If the former, then one could imagine a simple, small molecule that would interfere with the CARD-WD40 interface and allow the activation of caspase-9. If the latter, then it would be more challenging to design molecules which can intervene and activate caspase-9. If the polymerization interface is dominated by the CED4 domain, then again, one could imagine a small drug that would block it, and render the molecule incompetent to activate the caspase. However, to get to this point scientists are going to need atomic resolution structures of at least the CED4 domain, the WD40-CARD, and the WD40-cytochrome c complexes, and these structures are going to be just as exciting as this first look at the death machine.

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Fusion of Vacuoles—Where Are the Membranes, and Where Are the Holes?

In the February 8th issue of *Cell*, Wang et al. report the surprising finding that vacuolar fusion occurs at the periphery of the contact area of the vacuoles and not by the expansion of a central fusion pore. During fusion, a disk of boundary membrane is excised and left behind within the fused vacuoles.

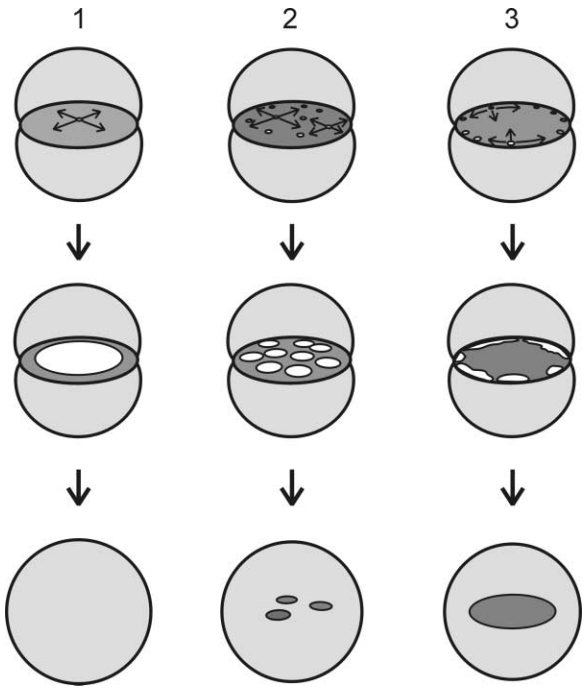
Eukaryotic cells continuously generate transport vesicles, move them around within the cytoplasm, and fuse

them with other vesicles or with the plasma membrane. Before a vesicle fuses, it needs to bind to the target membrane, a process referred to as tethering or docking. Binding involves many different proteins that not only proofread the membrane's identity but also bring them close together. Fusion proteins then initiate the opening of an aqueous fusion pore that subsequently expands.

Fortunately for scientists in the field, vesicle fusion does not require intact cells. Indeed, transport vesicles fuse with surprising efficiency in cell-free extracts. Such assays have been instrumental in assigning proteins to specific jobs in the reaction cascade, and they offer unsurpassed flexibility if the vesicles are derived from an organism with easy genetics such as baker's yeast. One of the best-studied fusion reactions is the homotypic fusion of yeast vacuoles (Wickner and Haas, 2000). Like lysosomes, vacuoles serve as the cell's recycling station in which macromolecules are degraded. Several vesicular trafficking pathways end at the vacuole including endocytosis of extracellular material, delivery of new enzymes from the Golgi apparatus, and delivery of captured cytoplasm by autophagocytotic vacuoles during starvation (Bryant and Stevens, 1998). Spanning several micrometers in diameter, vacuoles are large and occupy most of the cytoplasm of the small yeast cells. Each cell usually contains only a single or a few vacuoles. During cell division, the vacuole vesiculates and ships a string of small vesicles into the growing bud. After cytokinesis, these smaller vacuoles fuse to regenerate a large vacuole.

To measure fusion, Haas et al. (1994) designed an assay in which vacuoles from two different strains were used, one containing a normal set of vacuolar proteases but lacking the inactive proform of a vacuolar enzyme, the second containing the proenzyme but lacking the activating protease. Upon fusion, the protease activates the proenzyme, whose activity can easily be measured photometrically. In recent years, this assay has been instrumental in differentiating priming, docking, and fusion. A multitude of proteins have been assigned to these steps (Wickner and Haas, 2000), many of which were previously identified in genetic screens (Bryant and Stevens, 1998; Klionsky and Emr, 2000). Since several of the key proteins belong to protein families operating in other fusion reactions, vacuolar fusion was regarded—perhaps a bit prematurely—as a prototype for eukaryotic fusion events.

As attractive as they are, the readout from such assays is very indirect, and nobody knows what exactly is happening in those test tubes. Wang et al. have now labeled various vacuolar membrane proteins with the cell biologist's lantern, GFP, and watched vacuolar fusion under the microscope (Wang et al., 2002). Big surprises awaited these scientists, showing again that it is dangerous to believe too much in the cartoons we all like to publish at the end of our papers. Before fusion, clusters form in which large areas of adjacent vacuoles are sandwiched against each other like soap bubbles. Surprisingly, several of the proteins needed for docking and fusion, including the GTPase Ypt7, the syntaxin-related SNARE Vam3p, and components of the so-called HOPS complex, are not evenly distributed over the connected membrane area but accumulate at the edges (Wang et al., 2002). Accumulation depends on activation steps preceding fusion, since it does not occur when vacuoles are clustered mechanically by cosedimentation. Apparently, whole sets of membrane-associated proteins move laterally in the plane of the membrane, over large distances, before fusion occurs. Even more surprisingly, fusion itself did not proceed as would be expected, that is, by the radial expansion of a central fusion pore. Rather, an elongated slit forms along the rim of the connecting membranes. The slit appears to expand until it encircles the central membrane sandwich, which then drops into the interior of the fused vacuoles and is degraded (see Figure).



Possible Scenarios during Fusion of Two Big Vesicles with a Large Contact Area (Shaded)

1. Radial expansion of a single central fusion pore.
2. Radial expansion of multiple, randomly scattered fusion pores. If all pores expand radially, small pieces of the connecting double-membrane layer are expected to be left in the lumen.
3. Expansion of fusion pores aligned along the rim of the connected area. A central membrane disk is excised.

In hindsight, it is perhaps not too surprising that membrane rearrangements during fusion of large organelles are far more complex than previously envisioned. At the onset of fusion, the contact area between yeast vacuoles is in the range of 1–2 μm^2. This is about 10,000 times larger than the contact area of a neuronal synaptic vesicle, which is not much larger than a single SNARE protein complex. During exocytosis of synaptic vesicles it is likely that a single fusion pore forms—there is simply no space for a second. In contrast, in vacuolar fusion it is

more than likely that multiple pores are involved. Indeed, considering the vast area of the connected membranes, many dozen pores could form even if the density of pore-forming proteins (SNAREs or others) is 100-fold lower than at the synapse. Multiple fusion pores add a whole new layer of complexity to the fusion reaction. The pattern of holes and the direction of phospholipid flow in the plane of the connected membranes depend upon the spatial arrangements of the fusion pores and the timing of their opening (see Figure). If fusion pores are not randomly distributed but rather concentrate at the rim as suggested by the observed accumulation of fusion-relevant proteins, a central membrane disk would be left behind when the pores connect upon radial expansion, exactly what has been found in vacuolar fusion.

Is the excision of a membrane disk during fusion a freakish feature of yeast vacuoles or is it more widespread? Neuroscientists will probably question your sanity if you propose that a membrane fragment is released into the synaptic cleft each time a synaptic vesicle exocytoses. However, excision may represent a special adaptation for membrane degradation in the endosomal-lysosomal pathway. In fact, if the excised membrane disk vesiculates it would appear as an internal vesicle, thus complementing other membrane degradation pathways.

There is another, possibly more important lesson to be learned from this study. Despite all of the focus on conserved mechanisms, there are vast differences between intracellular fusion events with respect to dimensions and timing. A synaptic vesicle is not much larger than some protein complexes whose composition is already known, and the millisecond speed of its exocytosis does not leave time for multistep reaction sequences. In contrast, fusion that occurs between membrane surfaces whose molecular dimensions are orders of magnitude larger than a small vesicle will need additional steps such as lateral organization of fusion pores and bulk movement of macromolecules over large distances in the plane of the membrane. Many more proteins are likely to be involved, and their identification and assignment will be a major task for the future.

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