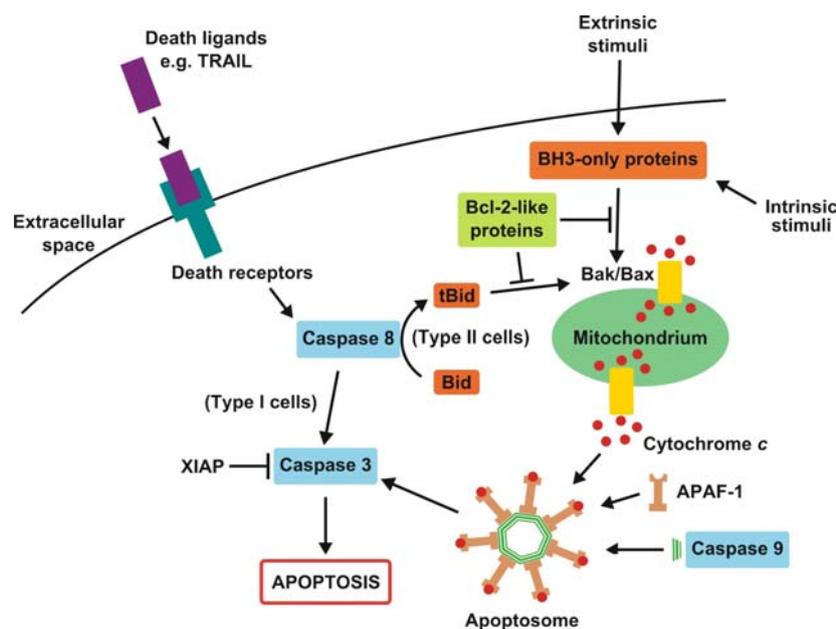


## Molecular mechanisms of apoptosis

Cell death by apoptosis occurs when a specialised intracellular signalling pathway is activated and kills the cell. Apoptosis is the most common way of cells to die *in vivo* but there are other ways (necrosis has been defined as cell death that is not apoptosis; necrosis may in some cases indeed be due to simple physical injuries but there also seems to be at least one signalling pathway that causes necrosis; pyroptosis is when a cell dies as a consequence of the activity of caspase-1, a protease involved in the maturation of cytokines; autophagy has also been linked to cell death. Programmed cell death used to be a term for cell death especially during development, where a cell has the predetermined fate to die. The term is now commonly used to describe any cell death that is the result of intracellular signal transduction (a 'program') and therefore especially encompasses apoptosis).

The pathways to apoptosis are incompletely but still fairly well understood. A simple diagram is here:

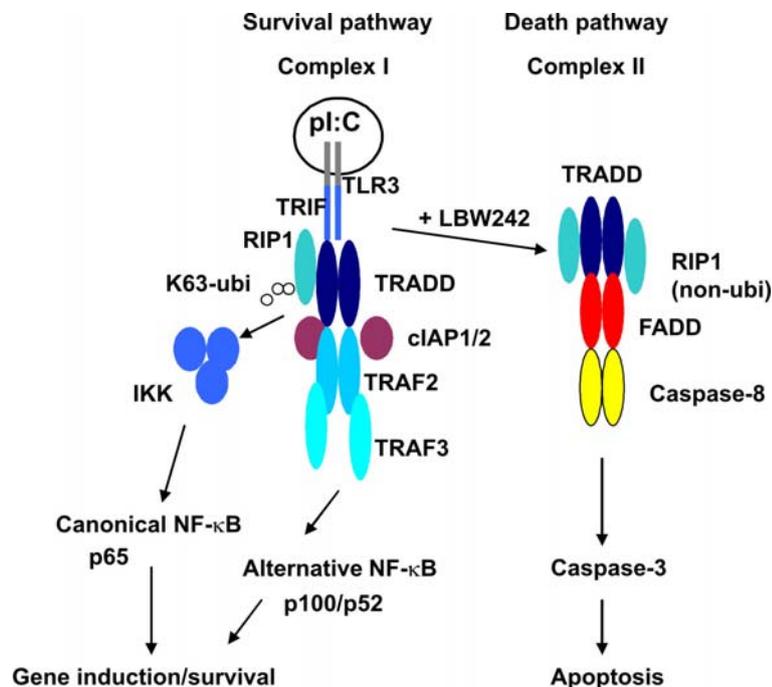


### Caspase-8-activation: death receptors and TRIF

Put simply, apoptosis probably always involves caspase-3, and caspase-3 is activated either by caspase-8 or by caspase-9. The upstream mechanisms therefore activate either caspase-8 or -9. Caspase-8 is typically activated by death receptors (Fas, TRAIL-receptors, TNFR) but it can also be activated by the immune signalling adapter TRIF. In some situations the activation of caspase-8 seems fairly straightforward, where it is recruited by death receptors and activated by reaching a high local concentration. It turns out more and more however that the

signalling downstream of death receptors (in particular the TNF receptor) is amazingly complex, with balancing apoptosis-inducing and non-apoptosis-inducing functions. Similarly, the signalling molecule TRIF appears to be able to activate caspase-8. TRIF is better known for its immune ability of inducing interferons and NF- $\kappa$ B upon recognition of viral RNA but TRIF can also recruit the TNF-receptor signalling machinery, including caspase-8.

One aspect we are looking at in this context is the role of the cellular inhibitor of apoptosis proteins (cIAPs). cIAPs are ubiquitin ligases, and a fair bit about their molecular function is still unclear. One function appears to be the ubiquitylation of RIP1, which at least determines composition of the signalling complexes. One in part speculative model of how signalling downstream of TRIF could work is shown here:



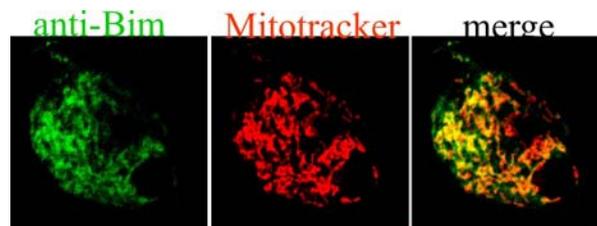
cIAPs appear to govern in part a decision of whether a TRIF-signal leads to activation of caspase-8 or not. This is easily demonstrated by using a synthetic drug that is currently under clinical evaluation (we use LBW242 from Novartis but other companies have similar molecules). These drugs induce the loss of especially cIAP1 and sensitize e.g. tumour cells to TRIF-induced apoptosis. How this works and what the function also in immune cells may be is an intriguing question we are working on.

### Mitochondrial apoptosis

It appears that mitochondrial apoptosis is much more common than caspase-8-dependent apoptosis *in vivo*. Mitochondrial apoptosis is regulated through the Bcl-2-family of proteins:

within this family, the BH3-only proteins ('triggers') often regulate the activation of the effectors Bax and Bak; active Bax and Bak cause the release of cytochrome *c* from the mitochondrial intermembrane space into the cytosol, where it binds to Apaf-1, inducing its oligomerisation and thereby causing the activation of caspase-9.

Cytochrome *c* sits in between the two mitochondrial membranes and is released through permeabilisation of the outer membrane (there may be something specific there as cytochrome *c* is released more easily than other proteins). Permeabilisation is the result of activation (detectable as conformational change) and oligomerisation of the effector proteins Bax or Bak. Activation of Bax/Bak is probably in most situations achieved by BH3-only proteins but this is a contentious area. The anti-apoptotic members of the Bcl-2-family block apoptosis very likely by binding either BH3-only proteins or Bax/Bak. We have been looking a lot at BH3-only proteins, their functions and their physiological role. A number of BH3-only proteins are localised to mitochondria, where they have functions in activating Bax. The insertion of one BH3-only protein (Bim<sub>S</sub>) is shown here (if you look at higher resolution you can see how Bim is inserted into the outer mitochondrial membrane):

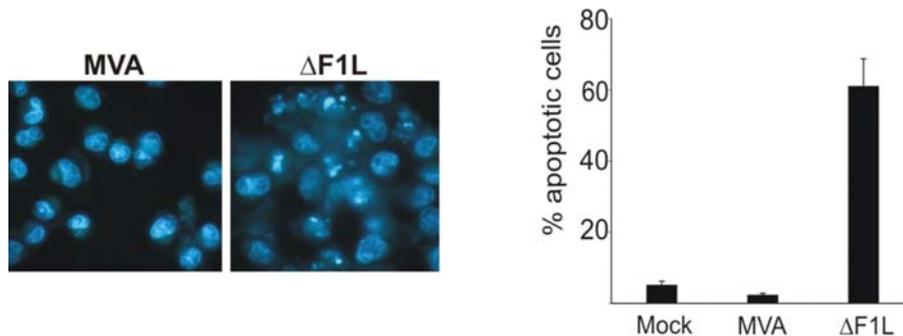


This regulation of apoptosis is something that is still not particularly well understood, and this is an area we are very actively investigating.

### **Apoptosis in host defence**

This is really part of our immunology section. Apoptosis is used in host defence on a number of levels. You'll see in our section *Immunology* how apoptosis regulates the termination of the immune response. What we touch on here is the cell-autonomous defence. This has been best defined in viral infections. Viral infections often kill the cell, and this is because the cell recognises the virus and activates apoptosis. Viruses, on the other hand (in particular the larger DNA viruses such as poxviruses and Herpes viruses) often carry genes whose products inhibit apoptosis (especially Bcl-2-like genes/proteins are common). The understanding of how viruses (and other micro-organisms) are detected (most often by so-called pattern recognition receptors), how this leads to apoptosis, how these recognition pathways are linked

to and distinct from other defence functions such as interferon-induction, and how viruses try to block this is a fascinating area where we are active. The figure shows an instance where a poxvirus called MVA infects a cell (in blue host cell nuclei). Nothing much happens in terms of apoptosis, but if we take away the MVA anti-apoptotic protein F1L (labelled  $\Delta$ F1L), then the virus induces apoptosis (seen by condensation and fragmentation of the nuclei). Analysis of these pathways, in part by rebuilding them synthetically, is one of our research areas.



### Apoptosis in tumours

It is generally assumed that a defect in the ability to undergo apoptosis is one factor that may drive or is otherwise involved in the emergence of tumours. Curiously, a defect in apoptosis is often associated with tumour development but most tumour cells are actually more sensitive than normal cells for apoptosis induced by irradiation or chemotherapy. There are a number of changes known in the apoptosis system that determine this balance; for instance, most tumours depend a lot more than normal cells on the continuous presence of anti-apoptotic Bcl-2 proteins, and these proteins are a very promising target of tumour therapy (a drug furthest in development is the Abbott compound ABT-737, which inhibits some Bcl-2-like proteins very well and can help kill tumour cells). We are applying our knowledge from the above approaches to understand apoptosis deregulation in some tumour cells.

### Publications in this area:

Zall, H., Weber, A., Besch, R., Zantl, N. and Häcker, G. (2010) Chemotherapeutic drugs sensitize human renal cell carcinoma cells to ABT-737 by a mechanism involving the Noxa-dependent inactivation of Mcl-1 or A1. *Molecular Cancer*; 9(1):164

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Weber, A., Kirejczyk, Z., Besch, R., Potthoff, S., Leverkus, M. and Häcker, G. (2010) Pro-apoptotic signalling through Toll-like receptor 3 involves TRIF-dependent activation of caspase-8 and is under the control of inhibitor of apoptosis proteins in melanoma cells. *Cell Death Diff*, 17(6):942-51.

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