tightly correlated with its luminosity, and the distance to a Cepheid of known period can thus be readily determined from its apparent brightness (*3*). The Cepheid period-luminosity relation was first discovered in surveys of the Magellanic Clouds, which contain over a thousand known Cepheids (*4*). Using the Cepheids of the LMC, an accurate relative distance scale was quickly established (*5*). The Key Project has extended the Cepheid-based relative distance scale out to the nearest large clusters of galaxies, in Virgo and Fornax, 300 to 400 times as distant as the LMC. However, the absolute calibration of the Cepheid-based distance scale requires independent distance estimates for a large sample of local Cepheids in order to set the zero point of the period-luminosity relation. Unfortunately, Cepheids are intrinsically rare in the solar neighborhood, and none exist within 100 parsecs of the sun. The distance scale zero point therefore has to be estimated indirectly, contributing to the controversy over d_{lmc}.

The European Space Agency's Hipparcos satellite vastly extended the sample of stars for which accurate distances are known beyond ground-based studies. Hipparcos provided high-quality parallaxes for more than two dozen Cepheid variables (*6*). However, the discrepancy between the long and short d_{lmc} was not mitigated. This is because the number of Cepheids with high-quality parallaxes is still so small that statistical biases in sample selection play a dominant role in the determination of the distance scale zero point.

A promising method for the direct determination of d_{lmc} uses eclipsing binary stars. These stellar systems comprise two stars in mutual orbit, whose relative positions yield a regular pattern of variability as each star occults the other in turn. The method is conceptually simple but has only recently become feasible because it requires ultraviolet spectrophotometry and accurate theoretical models of stellar atmospheres. Observations at multiple orbital phases yield an accurate determination of the absolute luminosity of the binary and hence a distance that is independent of local calibrators (see the figure). Early results, based on just one binary, favor a distance of 46 to 48 kpc for the LMC, near the mean of other recent determinations and ≈6% shorter than the value adopted by the HST Key Project (*7*).

During the next decade, a new generation of proposed satellite missions promise to greatly reduce the errors in d_{lmc}. Two planned NASA missions are the Full-Sky Astrometric Mapping Explorer (FAME, launch date 2004) and the Space Interferometry Mission (SIM, launch date 2006) (*8*). FAME will be a survey mission that will extend the work of Hipparcos by increasing the number of reliable Cepheid parallax measurements by an order of magnitude. SIM will be the first astronomical satellite to use optical interferometry. This technological innovation is expected to enable parallax measurements of Cepheids in the LMC for the first time. By eliminating all intermediate steps, it may finally be possible to directly determine the distance to the LMC and, by extension, to the rest of the galaxies in the universe.

References

1. B. F. Madore *et al.*, *Astrophys. J.* **515**, 29 (1999).
2. M. Feast, in *New Views of the Magellanic Clouds*, Y.-H. Chu, Ed. (Astronomical Society of the Pacific, San Francisco, CA, 1999), pp. 542–548.
3. A. Sandage, *Astrophys. J.* **127**, 513 (1958).
4. H. Leavitt, *Harvard Coll. Obs. Circ.* **173** (1912).
5. E. P. Hubble, *The Realm of the Nebulae* (Yale Univ. Press, New Haven, CT, 1936).
6. M. A. C. Perryman *et al.*, *Astron. Astrophys.* **323**, 49 (1997); I. N. Reid, *Annu. Rev. Astron. Astrophys.* **37**, 191 (1999).
7. E. F. Guinan *et al.*, *Astrophys. J. Lett.* **509**, L21 (1998); C. A. Nelson, K. H. Cook, P. Popowski, D. R. Alves, *Astron. J.* **119**, 1205 (2000).
8. See aa.usno.navy.mil/FAME/, sim.jpl.nasa.gov, and astro.estec.esa.nl/GAIA.

PERSPECTIVES: APOPTOSIS

Mitochondria—the Death Signal Integrators

Catherine Brenner and Guido Kroemer

The mitochondrion, the cell's Pandora's box, contains potentially harmful proteins that it keeps hidden away. Activation of these harmful proteins sets in motion programmed cell death (apoptosis) pathways that result in the demise of the cell. In many of these pathways, permeabilization of mitochondrial membranes is a critical event that results in release (from the mitochondrial intermembrane space) of various molecules that are crucial for apoptosis. Such molecules include enzymes called procaspases, cytochrome c (a caspase activator), Smac/Diablo (a caspase coactivator) (*1*), and an apoptosis-inducing factor, which activates the nucleases that chop up DNA into small fragments. Now, on page 1159 of this issue, Li and colleagues (*2*) report the tantalizing discovery that a potential proapoptotic transcription factor, TR3 (also called Nur77 or NGFIB), normally present in the nucleus, can move to mitochondria where it triggers membrane permeabilization and apoptotic cell death.

Like other proteins in the steroid/thyroid receptor superfamily, TR3 is a transcription factor with a central zinc finger DNA binding domain flanked by transactivation domains. In contrast to other steroid receptors, however, the endogenous ligand of TR3 (which is predicted to interact with the carboxyl-terminal half of the receptor) has not yet been identified, making TR3 an "orphan" receptor. TR3 forms homodimers with itself and heterodimers with other proteins from the same family, in particular with the 9-*cis*-retinoic acid receptor (RXR). The TR3 DNA binding domain interacts with a specific DNA octamer sequence, the Nur77/NGFIB-binding response element (NBRE), and, when in a heterodimer with RXR, also with the retinoic acid response element. Usually, TR3 (and RXR) are imported into the nucleus after their synthesis in the cytoplasm. This implies that most if not all of TR3's activity is in the nucleus. Under specific circumstances, however, TR3 can be exported back to the cytoplasm. This halts its transcriptional activity (*2*) and may also suppress that of RXR, which accompanies TR3 back to the cytoplasm (*3*).

Surprisingly, as Li *et al.* show, TR3 may also induce mitochondrial membrane permeabilization. Indeed, in cells undergoing apoptosis, TR3 (fused to a green fluorescent protein marker) specifically translocates to mitochondrial membranes, as revealed by its punctate staining pattern in the cytoplasm. Recombinant TR3 induces the release of cytochrome c when added to purified mitochondria in vitro (*2*), suggesting that TR3 permeabilizes mitochondrial membranes on its own, without the participation of additional proteins.

TR3 is overexpressed in response to certain apoptotic stimuli. For instance, TR3 is up-regulated by external stressors such as seizures or brain ischemia. Upon ligation of the T cell receptor by ligand, T cells switch on expression of the TR3

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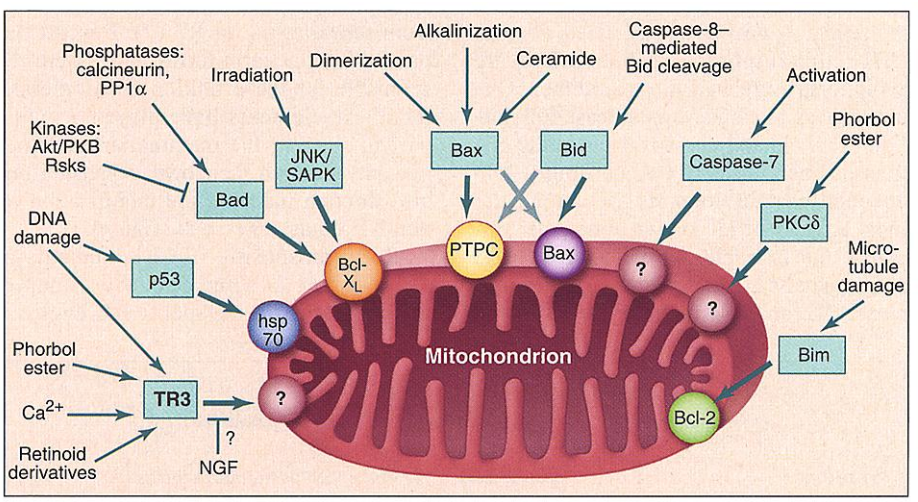
gene (presumably through the Ca^{2+}-induced dissociation of cabin-1 from myocyte enhancer factor 2, a transcription factor that controls TR3 expression) (*4*). Overexpression of dominant-negative mutants of TR3 in transgenic mice prevents the negative selection of autoreactive thymocytes (developing T cells) through apoptosis. In contrast, genetic inactivation of TR3 does not interfere with activation-induced thymocyte apoptosis, indicating that TR3 can be replaced by related orphan receptors, including Nurr1 and Nor-1 (*5*). Indeed, Nurr1 and Nor-1 can activate the expression of target genes through the same DNA element as TR3, and their transcriptional activities are blocked by a TR3 dominant-negative protein (*6*). TR3 is also induced by the retinoid derivative CD437 in lung cancer cell lines, and dominant-negative TR3 inhibits CD437-induced cell death. Moreover, TR3 expression is induced by CD437 analogs, phorbol ester, a Ca^{2+} ionophore, and etoposide. These stimuli also induce the translocation of TR3 from the nucleus to mitochondria, which can be blocked with a dominant-negative TR3 mutant lacking 151 amino acids at the amino terminus (*2*).

That a transcription factor interacts with and acts on mitochondrial membranes is counterintuitive, at least at first glance. However, this observation appears far less bizarre in the context of an ever-growing list of apoptosis-promoting proteins that move to mitochondria to carry out their tasks (see the figure). Translocation to mitochondria has been reported for proapoptotic proteins from the Bcl-2/Bax/Bid family. These proteins permeabilize the outer mitochondrial membrane upon interaction with the permeability transition pore complex (for example, Bax) or, alternatively, independently of such an interaction (for example, Bid) (*7*). Many proteins that are localized in mitochondria can covalently modify members of the Bcl-2/Bax/Bid family, thereby affecting their local ability to regulate apoptosis and/or their subcellular localization. For example, c-Jun NH_2-terminal kinase (JNK, also called stress-activated protein kinase, SAPK) phosphorylates and inactivates the antiapoptotic protein Bcl-X_L (*8*, *9*). Another potential proapoptotic kinase that moves to the mitochondria is protein kinase Cδ (*10*). Intriguingly, it was recently found that p53—a transcription factor thought to induce apoptosis through the induction of proapoptotic genes (*11*)—moves from the nucleus to the mitochondria (*12*) where it interacts with hsp70, a heat shock protein. The impact of the interaction between p53 and hsp70 on mitochondrial membrane integrity has not yet been elucidated. As is true for TR3 (*2*), specific targeting of p53 to mitochondria is sufficient to cause cell death (*12*). The antiapoptotic protein Bcl-2 prevents mitochondrial membrane permeabilization by most if not all of these proteins, including TR3 (*2*).

In a way, TR3 represents just another example of a factor that exerts its proapoptotic function through local effects on mitochondria. One plausible scenario predicts that mitochondria behave as general integrators of proapoptotic signals, and that TR3 constitutes one of the terminal effectors linking lethal signals to these organelles (see the figure). Numerous questions remain unanswered. Is TR3 the only protein of the steroid receptor superfamily to move to mitochondria, or is the phenomenon shared by its close relatives (Nurr1 and Nor-1) and perhaps by more distantly related family members? What is the mitochondrial receptor for TR3? Previous reports indicate that cyclosporin A, which inhibits the calcineurin signaling pathway as well as the permeability transition pore complex, can prevent TR3-mediated apoptosis (*13*). Whether local inhibitory effects of cyclosporin A on mitochondria account for this observation remains unclear.

Finally, it will be important to learn how binding of the hypothetical TR3 ligand and signaling influence the subcellular localization of TR3. Nerve growth factor (NGF) induces the nuclear export of TR3 by stimulating TR3 phosphorylation through the Trk1/Ras/mitogen-activated protein kinase pathway (*3*). However, NGF stimulates TR3 to become reorganized in a diffuse cytoplasmic (rather than a punctate mitochondrial) pattern (*3*). This suggests that movement of TR3 to a mitochondrial rather than a nonmitochondrial (cytosolic?) location may be differentially regulated. If true, this would resolve the apparent paradox that NGF, which blocks cell death signals, stimulates the nuclear export of TR3. Whatever the answers to these questions, it appears that nuclear transcription factors are no longer strangers to mitochondria, at least when it comes to the lethal signaling molecules of apoptosis. Subcellular relocalization of transcription factors, including TR3 and p53, adds a new level of complexity to the regulation of intricate pathways in the cell.



Dicing with death. Proteins that move to and affect mitochondrial membranes. Terminal effectors of apoptosis (shown in boxes) are activated by different proapoptotic pathways. Upon activation, these proteins move from the cytoplasm or nucleus to the mitochondrial membranes, where they interact with known (circles) or unknown (question marks) receptors. When translocated to mitochondrial membranes, these proteins promote permeabilization of the membranes, with the consequent release of caspases or nuclease activators from the mitochondrial intermembrane space. Proapoptotic secondary messenger molecules produced by mitochondria and/or that have effects on these organelles include Ca^{2+}, ceramide, ganglioside GD3, sphingosine, palmitate, reactive oxygen species, and nitric oxide. (PTPC, permeability transition pore complex.)

References

1. C. Du *et al.*, *Cell* **102**, 33 (2000); A. M. Verhagen *et al.*, *Cell* **102**, 43 (2000).
2. H. Li *et al.*, *Science* **289**, 1159 (2000).
3. Y. Katagiri *et al.*, *Nature Cell Biol.* **2**, 435 (2000).
4. H.-D. Youn *et al.*, *Science* **286**, 790 (1999).
5. B. Osborne, *Curr. Opin. Immunol.* **8**, 245 (1996).
6. L. E. Cheng *et al.*, *EMBO J.* **16**, 1865 (1997).
7. G. Kroemer and J. C. Reed, *Nature Med.* **6**, 513 (2000).
8. C. Tournier *et al.*, *Science* **288**, 870 (2000).
9. S. Kharbanda *et al.*, *J. Biol. Chem.* **275**, 322 (2000).
10. P. K. Majumder *et al.*, *J. Biol. Chem.* **275**, 21793 (2000).
11. E. Oda *et al.*, *Science* **288**, 1053 (2000).
12. N. D. Marchenko *et al.*, *J. Biol. Chem.* **275**, 16202 (2000).
13. K. Yazdanbakhsh *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **92**, 437 (1995).