EMBO reports

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# The phosphorylation state of Drp1 determines cell fate

Recent research has opened new avenues for the evaluation of mitochondrial function; for example, mitochondria are no longer perceived as thread-like static entities within the cytosol, but instead are viewed as highly dynamic organelles that can change in shape and size, and are transported to strategic locations within the cell. Mitochondrial morphology, size and position within cells are maintained through a balance of fission and fusion events. Perturbation of the steady state between these opposing processes has been directly implicated in several human disorders (Chan, 2006). Although the list of genes for mitochondrial morphogenesis is rapidly increasing, dynamin-related protein 1 (Drp1)—a cytosolic dynamin GTPase—was among the first fission proteins to be discovered; however, the mechanism by which Drp1 function is regulated is poorly understood.

In this issue of *EMBO reports*, Cribbs & Strack identify a new mechanism by which second messengers—cAMP and calcium—modulate mitochondrial shape and function through the regulation of Drp1 phosphorylation. Cyclic-AMP-dependent protein kinase (PKA)-mediated phosphorylation of Drp1 at Ser 656 induces mitochondrial elongation and resistance to apoptotic stimuli, whereas dephosphorylation of Ser 656 by calcineurin promotes mitochondrial fragmentation and increases cell vulnerability to apoptosis. These studies provide a new mechanistic insight into the link between the mitochondrial fission machinery and cell death signalling.

Drp1 is recruited to the mitochondrial surface at potential fission sites (Ingerman *et al*, 2005; Okamoto & Shaw, 2005). The energy generated by GTP hydrolysis is believed to provide the mechanical force required to execute fission (Ingerman *et al*, 2005). Although gain- and loss-of-function studies of Drp1 correlate mitochondrial fission with apoptosis (Frank *et al*, 2001; Germain *et al*, 2005), there is no evidence to show that Drp1 alone, or mitochondrial fission by itself, can induce apoptosis. In addition, although Drp1 GTPase can be regulated by ubiquitination and sumoylation (Nakamura *et al*, 2006; Wasiak *et al*, 2007), there is little insight as to how Drp1 activation might be regulated during apoptosis signalling. In this issue, Cribbs & Strack have addressed some of these important questions and provide a mechanistic link for second messenger regulation of Drp1 GTPase activity and apoptosis signalling.

First, Cribbs & Strack show that PKA-mediated-phosphorylation of Drp1 at Ser 656 attenuates the GTPase activity of Drp1 and promotes cell survival, suggesting that cAMP might mediate survival partly through the inhibition of Drp1 (Fig 1). An independent study (Chang & Blackstone, 2007) corroborates these findings by showing that PKA-dependent phosphorylation of Drp1 within the GED domain at Ser 637 blocks Drp1 GTPase activity. Phosphomimetic substitution at Ser637Asp was also shown to block mitochondrial fission (Chang & Blackstone, 2007). The question of how phosphorylation at Ser 656 and Ser 637 might differ in regulating the GTPase activity remains to be resolved. One possibility is that a spatiotemporal relationship exists whereby phosphorylation at one site regulates modification of the second site. In addition, a third Drp1 phosphorylation site has been reported and is believed to be involved in breaking down the mitochondrial network during mitosis (Taguchi *et al*, 2007). It is likely that Drp1 phosphorylation at different sites might have different physiological consequences. This hypothesis is supported further by the recent finding that phosphorylation modulates substrate processing

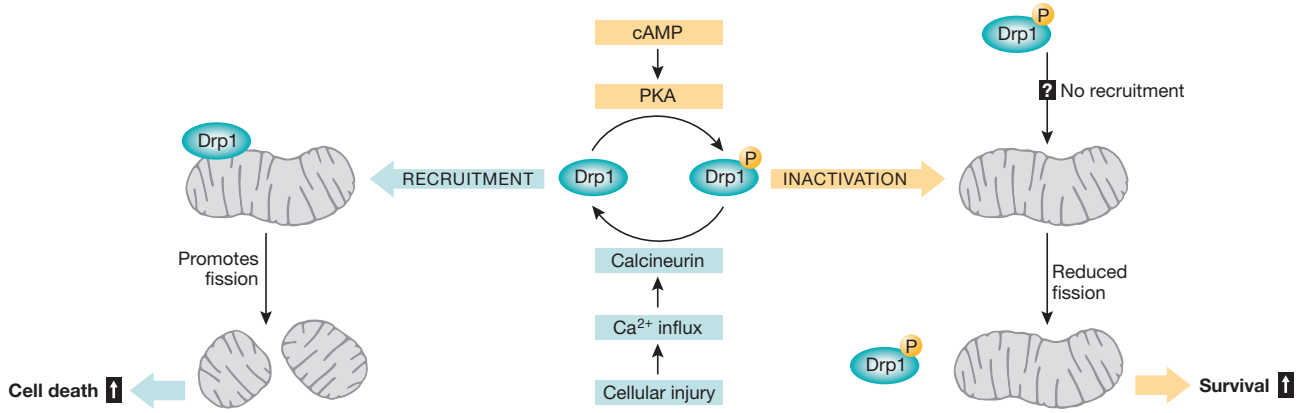


**Fig 1** | Missing links between mitochondrial fission and apoptosis. Cyclic-AMP-dependent protein kinase (PKA) phosphorylates dynamin-related protein 1 (Drp1) and induces mitochondrial elongation and resistance to apoptosis. Calcineurin dephosphorylates Drp1, promotes mitochondrial fragmentation and cell vulnerability to apoptosis. Whether phospho-Drp1 confers protection through inhibiting mitochondrial fission or through other regulatory signalling molecules remains to be discovered.

in a site-specific manner (Jahani-Asl *et al*, 2007a). The complexity imposed by phosphorylation on the three-dimensional structure and oligomerization of Drp1 protein requires further investigation.

A link between mitochondrial fission and death signalling pathways converging on mitochondria has been a hotly debated topic for several reasons. Mitochondrial fission is required under normal physiological conditions to ensure biogenesis and to respond to changes in energy demands (Yaffe, 1999). Although mitochondrial fission has been shown to occur as an early event during cell death (Barsoum *et al*, 2006; Jagasia *et al*, 2005; Jahani-Asl *et al*, 2007b), and Bax/Bak-mediated Drp1-induced mitochondrial fission promotes cell death (Arnoult *et al*, 2005), it has also been shown that inhibiting the fission machinery does not prevent Bax/Bak-dependent apoptosis (Parone *et al*, 2006). In addition, Drp1-mediated fission of mitochondria has been reported to protect against cell death (Szabadkai *et al*, 2004). More importantly, although *in vitro* studies often focus on death pathways evoked by single inducers, the scenario *in vivo* is quite complex (Cheung *et al*, 2007). Previous findings show that recruitment of Drp1 at the scission sites occurs simultaneously with $Ca^{2+}$ uptake by mitochondria (Breckenridge *et al*, 2003). In this issue, Cribbs & Strack show that calcium induces mitochondrial fission through Drp1 dephosphorylation (Fig 1). This finding has broad implications pertaining to several modes of cell death including death induced by staurosporine (a kinase inhibitor), etoposide (topoisomerase inhibitor), calcium ionophore A23187 and oxidative stress. The question of how Drp1 phosphorylation might protect against cell death remains open: one possibility is that phosphorylation might modulate Drp1 interaction with other regulatory proteins that assist in targeting Drp1 to mitochondria. Finally, Cribbs & Strack show that Drp1 phosphorylation protects against apoptotic insult despite the fact that a population of mitochondria exhibit ultrastructure abnormalities. This apparent inconsistency suggests that Drp1 might have other roles in addition to regulating mitochondrial fission.

In summary, these new studies suggest that components of the mitochondrial fission–fusion machinery are linked to cellular signalling pathways and identify a new mechanism by which second messengers might regulate mitochondrial structure and function. Future research towards identifying upstream and downstream regulators of the fission–fusion machineries might identify new approaches to modulate the onset of cell death that becomes deregulated in many human diseases.

ACKNOWLEDGEMENTS

R.S.S. is supported by grants from the Canadian Institute of Health Research (CIHR) and the Heart and Stroke Foundation of Canada (HSFC). A.J.-A. is supported by a CIHR doctorate research award.

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Keywords: Drp1; phosphorylation; calcium; calcineurin; cell death

**Submitted 9 August 2007; accepted 21 August 2007**

EMBO *reports* (2007) **8,** 912–913. doi:10.1038/sj.embor.7401077