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<i>H. sapiens</i>	611-PIMPAS SPQK GHAVNLL-DVPVPV-- ARKLS AREQRD	
<i>R. norvegicus</i>	630-PIMPAS SPQK GHAVNLL-DVPVPV-- ARKLS AREQRD	
<i>C. elegans</i>	587---SKT SPQEK QSANFLPEVPETQ-LGR KL TSREQRD	
<i>D. melanogaster</i>	611--NNIV SP --VKPVNLLDPVPANH-NP RRL TDKEQKD	
<i>X. laevis</i>	573-PAFPAS PLR GHAVNLL-DVPVPV-- ARKLS AREQRD	

Fig 1 | Sequence alignment of the Drp1 sequence surrounding the PKA phosphorylation site in the indicated species. The position of the PKA phosphorylation site in Drp1 identified by Chang & Blackstone (2007) and Cribbs & Strack (2007) is indicated by an asterisk, and the consensus sequence is shaded in yellow. The position of the Cdk1/cyclin B phosphorylation site (Taguchi *et al*, 2007) is indicated by an arrowhead, and the consensus sequence is shown in orange. Both the PKA and Cdk1/cyclin B phosphorylation sites are conserved in all species shown. Boundary amino-acid residues are indicated to the left. Numbering for the rat sequence is derived from Cribbs & Strack (2007). Cdk1, cyclin-dependent kinase 1; Drp1, dynamin-related protein 1; PKA, cAMP-dependent protein kinase.

emphasize the important roles of protein phosphorylation by cAMP-dependent protein kinase (PKA) in the regulation of the dynamin-related protein 1 (Drp1) GTPase and mitochondrial fission. However, the Literature Report (Jahani-Asl & Slack, 2007) that accompanied the Cribbs & Strack article led to incorrect interpretations, which we would like to clarify here.

Foremost among these, the Literature Report discusses cAMP-dependent phosphorylation at Ser637 in human Drp1 splice variant 1 (Chang & Blackstone, 2007) and Ser656 in rat Drp1 splice variant 1 (Cribbs & Strack, 2007) as if they are distinct sites. However, both our study and that of Cribbs & Strack present sequence alignments that clearly indicate that these sites are the same; thus there is only one PKA phosphorylation site in Drp1 (Fig 1). In this regard, it is important to emphasize that there are several splice variants in the Drp1 protein and that protein size varies among species, making protein sequence alignments important when comparing results of studies investigating different species or variants. In addition, the Literature Report states that the Cribbs & Strack study showed that phosphorylation at this site attenuates GTPase activity. In fact, these authors reported no effect using a phosphomimetic substitution (Supplementary Figure 1C in Cribbs & Strack, 2007), although our study did report attenuation of GTPase activity in response to both direct phosphorylation by cAMP-dependent protein kinase, as well as with the same phosphomimetic mutant (Figure 3 in Chang & Blackstone, 2007). The reason for this discrepancy, despite using similar *in vitro* approaches and the same phosphomimetic mutation,

is unclear; however, the attenuation of Drp1 GTPase activity that we observed would provide a mechanistic explanation for the findings in both papers that mitochondrial fission is impaired in cells, as well as for the resistance to pro-apoptotic stimuli reported by Cribbs & Strack.

Our study also showed that the intramolecular association of Drp1 is altered by the phosphomimetic substitution (Figure 2 in Chang & Blackstone, 2007); other mutations in the GTPase effector domain that alter intramolecular interactions also attenuate GTPase activity (Zhu *et al*, 2004). Even so, we cannot eliminate the possibility that conformational changes owing to phosphorylation at this site also affect interactions with other proteins involved in mitochondrial fission. Importantly, the two-dimensional phosphopeptide mapping experiments in our study showed that there is no basal phosphorylation at the PKA phosphorylation site in HeLa cells (Figure 1 in Chang & Blackstone, 2007), indicating that any regulation of Drp1 function by dephosphorylation at this site in these widely studied cells would need to occur in conjunction with activation of cAMP-PKA signalling. Conversely, we visualized other basal sites of Drp1 phosphorylation, one of which might correspond to a site of mitotic phosphorylation previously identified by Taguchi *et al* (2007).

The functional regulation of Drp1 by post-translational modifications such as protein phosphorylation, ubiquitination and sumoylation clearly provide cells with an impressive array of regulatory mechanisms to modulate mitochondrial morphology within cells. Further studies promise to clarify the role of these mechanisms in the dynamic regulation of mitochondrial morphology and distribution within cells.

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Response by Arezu Jahani-Asl & Ruth S. Slack

A recent study by Cribbs & Strack (2007) identified a new mechanism for the integration of the second messengers Ca^{2+} and cAMP in the regulation of mitochondria form and function. This study was the first to provide a mechanistic link between phosphorylation of dynamin-related protein 1 (Drp1)—a component of mitochondrial fission machinery—and the regulation of apoptosis. Our report also referred extensively to two previous studies that investigated the role of DRP1 phosphorylation in mitochondrial fission: one study published by Chang & Blackstone (2007a) also identified a cAMP-dependent protein kinase (PKA) phosphorylation site on human DRP1, and a report by Taguchi *et al* (2007) showed that DRP1 is phosphorylated by Cdk1/cyclin B (Cdk1 for cyclin-dependent kinase 1) in a cell-cycle-dependent manner.

Together, these three studies provide evidence that the post-translational modification of DRP1 has a crucial role in the regulation of mitochondrial dynamics and cellular function. Chang & Blackstone (2007b) have responded to our report to clarify two points. First, they have provided a sequence alignment between the rat and human DRP1 sequence and have indicated that the PKA phosphorylation of DRP1 at Ser 656 in rat (reported by Cribbs & Strack, 2007) and Ser637 in human (reported by Chang & Blackstone, 2007a) represent the same serine residue. Second, we had referred to the loss of GTPase activity in the Cribbs & Strack (2007) study where we should have described it as a loss of Drp1 fission activity. Therefore, although Drp1 activity and GTPase activity were interchangeably used, it is important to emphasize that in the Cribbs & Strack study no reduction in GTPase activity was shown, whereas the Chang & Blackstone (2007a) study did show a reduction in both fission and GTPase activity. Although we appreciate this clarification, we would like to re-emphasize that the crucial point of our Literature Report (Jahani-Asl & Slack, 2007) was to highlight the physiological relevance of PKA-mediated Drp1 phosphorylation that had not been previously shown. The findings of the Cribb & Strack (2007) paper bring the regulation of Drp1 phosphorylation

into a broader context by showing its physiological importance for cell death. Understanding the physiological outcomes of post-translational modifications such as phosphorylation in the context of cellular function—including apoptosis and cell cycle regulation—is the ultimate challenge for cell biologists.

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Response by J. Thomas Cribbs & Stefan Strack

We agree with the letter by Chang & Blackstone (2007a) in its entirety. Their report (Chang & Blackstone, 2007b), which appeared online while our manuscript was under review, independently identified the main cAMP-dependent protein kinase (PKA) phosphorylation site in dynamin-related protein 1 (Drp1) (Ser637 in human, Ser656 in rat splice variant 1). Although some discrepancies between our reports in regard to the biochemical characterization of the phospho-site mutants need to be resolved with future experiments, the combined data provide unequivocal evidence for inhibition of Drp1-mediated mitochondrial scission through PKA phosphorylation.

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