PIKK-ing a New Partner: A New Role for PKB in the DNA Damage Response

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The protein kinase PKB/Akt has long been associated with regulating signaling pathways that promote cell survival and cell growth, for example, in response to growth factors. In contrast, the DNA-dependent protein kinase (DNA-PK) is required for the repair of DNA damage and for cell survival after exposure to DNA-damaging agents, such as ionizing radiation. In a recent paper published in *Molecular Cell*, Hemmings and colleagues provide evidence that DNA-PK is required for the activation of PKB in response to exposure to ionizing radiation, suggesting that these two protein kinases may act together to promote survival after DNA damage.

Protein kinase B (PKB) (also called AKT) is a classical serine/threonine protein kinase that plays a critical role in cell signaling in eukaryotes (reviewed in Manning and Cantley, 2007). The classical PKB signaling pathway involves growth factor-dependent activation of class 1A phosphoinositide 3 kinase (PI3K), phosphorylation of plasma membrane-bound phosphatidylinositol 4,5 bis-phosphate (PIP_2) to phosphatidylinositol 3,4,5 trisphosphate (PIP_3), and PIP-dependent activation of phosphoinositide kinase-1 (PDK1), which phosphorylates PKB on threonine 308 in the protein kinase activation loop. Also required for PKB activation is phosphorylation on serine 473 by a kinase or kinases referred to as PDK2. The most well-described PDK2 is mTORC2, a complex composed of the PI3K-like protein kinase (PIKK) mTOR, with Rictor and other proteins, which phosphorylates PKB on serine 473 in response to activation by growth factors (Manning and Cantley, 2007). However, other protein kinases have also been shown to phosphorylate PKB on serine 473, including other PIKK family members, DNA-PKcs (Feng et al., 2004) and ATM (Viniegra et al., 2005).

DNA-PKcs (the catalytic subunit of the DNA-dependent protein kinase) is required for the repair of ionizing radiation (IR)-induced DNA damage via the nonhomologous end-joining pathway (NHEJ). The first step in NHEJ is recognition of IR-induced DNA double-strand breaks (DSBs) by the Ku heterodimer, followed by recruitment of DNA-PKcs, and stimulation of its kinase activity. The protein kinase activity of DNA-PKcs is required for NHEJ, but to date its physiological targets have remained elusive (Weterings and Chen, 2007).

In a recent paper in *Molecular Cell*, Hemmings and colleagues (Bozulic et al., 2008) show that PKB is phosphorylated on both threonine 308 and serine 473 in response to either IR or the DNA-damaging agent doxorubicin. Using selective kinase inhibitors, siRNA targeting, and PIKK-defective cell lines, the authors show that DNA-PKcs is required for the phosphorylation and activation of PKB in response to DNA damage. This newly identified pathway for IR-induced phosphorylation and activation of PKB is distinct from insulin-mediated activation of PKB, since inactivation of DNA-PK only disrupts activation of PKB in response to DNA-damaging agents. In addition, the authors show that PKB interacts with DNA-PKcs in an IR-inducible, phosphorylation-dependent manner. Not only do these studies reveal a new potential role for PKB in the DNA damage response but they also identify PKB as a new target for DNA-PKcs.

Given the traditional view of PKB-mediated signaling that originates from the plasma membrane, a question that immediately comes to mind is where in the cell this pathway occurs. It turns out that PKB as well as its activating kinases (PI3K and PDK1) and their phosphoinositide substrates are known to occur in the nucleus as well as at the plasma membrane (reviewed in Deleris et al., 2006). Here, Hemmings and colleagues demonstrate that DNA-PK-dependent, IR-induced phosphorylation of PKB occurs in the nucleus and that phosphorylated PKB colocalizes (to some extent) with phosphorylated histone-H2AX, which is widely regarded as a marker for IR-induced DSBs (Stucki and Jackson, 2006). Given these findings, it will be interesting to determine whether DNA damage has effects on other components of the nuclear phosphoinositide signaling pathway.

Most cells express multiple isoforms of PKB, termed PKBα/AKT1, PKBβ/AKT2, and PKBγ/AKT3, which have overlapping but distinct phenotypes. Mice lacking one or even two of the PKB isoforms are viable, but disruption of all three genes is lethal (Dummler and Hemmings, 2007). Bozulic et al. suggest that PKBα is likely the major player in IR-induced cell death, as cells from PKBα null animals undergo IR-induced apoptosis and deletion of PKBα reduces the presence of nuclear phospho-PKB foci. It will therefore be interesting to examine more closely the phenotype of PKBα null cells to determine whether they display other defects characteristic of NHEJ-defective cells, and to determine whether absence of PKBα causes defects in the repair of IR-induced DSBs.

The authors also find that PKBα is required for upregulation of the cycle-dependent kinase inhibitor p21 and sug-

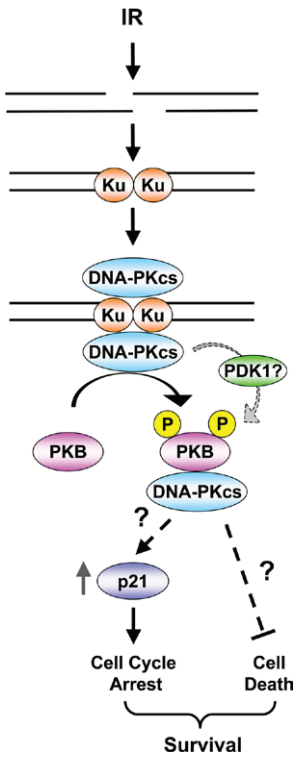

Figure 1. Activation of PKB/Akt in Response to DNA Damage

gest that DNA-PKcs plays a role in this process. This is unexpected, since cell cycle regulation in response to IR has long been considered the function of the related PIKKs, Ataxia-Telangiectasia Mutated (ATM) and ATM-, Rad3-related (ATR) (Lobrich and Jeggo, 2007). More work will be required before we understand precisely how DNA-PKcs and PKB are involved in this process.

In summary, the findings presented by Bozulic et al. suggest the following model (Figure 1). DNA damage induces DSBs (indicated by broken solid lines), which are detected by the Ku70/80 heterodimer. DNA-PKcs is recruited to the DSB via its interaction with Ku, which stimulates the protein kinase activity of DNA-PKcs, promoting the interaction of DNA-PKcs with nuclear PKB. Activation of DNA-PK results in phosphorylation of PKB on threonine 308 and serine 473 (indicated by P in yellow circles) and activation of PKB, which, in turn phosphorylates downstream targets, which results in survival after IR. Whether DNA-PKcs directly phosphorylates PKB or acts through PDK1 is not clear, since the authors also show that PDK1 has a role in the phosphorylation and activation of PKB in response to IR. Also, whether the interaction between DNA-PKcs and PKB is direct or indirect and whether it requires the phosphorylation of DNA-PKcs and/or PKB is not yet known. Once activated, PKB induces the upregulation of the cyclin-dependent kinase inhibitor p21, which induces cell-cycle arrest and promotes cellular survival. Whether this occurs through known PKB/AKT pathways that negatively regulate apoptosis, such as phosphorylation and negative regulation of proapoptotic proteins (such as BAD) or phosphorylation and regulation of the activity of transcription factors (such as FOXO) (see Manning and Cantley, 2007) or by other, yet to be discovered, means remains to be determined.

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