**Metabolism**

**Major finding:** AKT-mediated phosphorylation of NAD kinase induces synthesis of NADP⁺ and NADPH.

**Mechanism:** Phosphorylation of the amino-terminal region of NADK by AKT relieves its autoinhibitory function.

**Impact:** Stimulation of NADP⁺ production by growth factor signaling serves to increase cellular reducing power.

**NAD KINASE IS AN AKT SUBSTRATE**

The PI3K–AKT–mTORC1 signaling axis promotes a number of anabolic processes by directly phosphorylating metabolic enzymes and indirectly regulating downstream transcription factors. Several of these processes require significant reducing power in the form of NADPH, which is oxidized to NADP⁺ during biosynthesis. Hoxhaj and colleagues demonstrate that AKT activity regulates the availability of NADPH and NADP⁺ via phosphorylation of NAD kinase (NADK), the sole cytosolic enzyme that produces NADP⁺ from a larger pool of NAD⁺. Stimulation of cells with insulin-like growth factor 1 (IGF1) or insulin resulted in AKT-dependent phosphorylation of NADK and increases in NADP⁺ and NADPH independent of glucose metabolism. Expression of constitutively active AKT increased phosphorylation of NADK on three amino-terminal serine residues: S44, S46, and S48. A phospho-NADK antibody confirmed that growth factor stimulation results in AKT-dependent phosphorylation of NADK, primarily at S44 and S46, and these sites are constitutively phosphorylated in cancer cells with aberrant activation of AKT. Expression of NADK harboring mutated phosphorylation sites impaired NADP⁺ synthesis in response to growth factor stimulation or as a result of constitutively active AKT. Although wild-type and phospho-mutant NADK exhibited similar basal enzymatic activity, in vitro phosphorylation by AKT increased the catalytic activity of wild-type but not mutant NADK. An NADK mutant lacking the N-terminal region where the AKT phosphorylation sites reside exhibited increased basal NADP⁺ synthesis, which was not increased further by AKT. Collectively, these findings indicate that the N-terminal domain exerts an autoinhibitory effect on NADK activity and that phosphorylation by AKT relieves this inhibition to stimulate synthesis of NADP⁺. This signaling axis provides a means to acutely regulate the availability of reducing power in the cell in response to growth factor stimulation, a regulatory pathway predicted to be chronically activated in cells with oncogenic activation of PI3K–AKT signaling.


**Transcription**

**Major finding:** MYCN recruits BRCA1 to activated gene promoters to suppres accumulation of stalled RNAPII.

**Mechanism:** Stabilization of MYCN and BRCA1 by USP11 allows efficient clearance of RNAPII from active promoters.

**Impact:** MYCN-driven tumors utilize a distinct method of coping with aberrant RNAPII function.

**MYCN RECRUITS BRCA1 TO LIMIT ACCUMULATION OF STALLED RNA POLYMERASE**

MYC is an oncogenic transcription factor that can promote transcriptional elongation by RNA polymerase II (RNAPII). Herold, Kalb, Büchel, and colleagues show that the neuronal and neuroendocrine lineage-specific paralog MYCN can similarly mediate escape of RNAPII from promoters and recruits BRCA1 to prevent RNAPII stalling at promoter-proximal regions. In neuroblastoma cells, activation of a MYCN–ER fusion protein via 4-hydroxytamoxifen treatment generated a MYCN-specific expression profile, with elongating RNAPII increased in the body of activated genes. A shRNA screen revealed that MYCN–ER activation led to increased dependence on BRCA1, suggesting that BRCA1 might control the transcriptional functions of MYCN. Genome-wide methylation data showed that the BRCA1 promoter was significantly hypomethylated in high-risk and MYCN-amplified neuroblastoma, and MYCN activation resulted in recruitment of BRCA1 to promoter-proximal regions of activated genes, overlapping closely with MYCN binding sites; the extent of BRCA1 recruitment correlated with expression of the downstream gene. MYCN activation increased the association of BRCA1 with promoter-proximal RNAPII, and blocking RNAPII elongation further increased this association. Depletion of BRCA1 resulted in accumulation of RNAPII at promoter-proximal regions following MYCN activation. Activation of MYCN also suppressed R-loop formation in a BRCA1-dependent manner, and BRCA1 depletion resulted in complete loss of the mRNA decapping enzyme DCP1A from promoter-proximal regions, indicating that recruitment of BRCA1 by MYCN enables mRNA decapping when RNAPII stalls. MYCN and BRCA1 did not directly interact, but formed a stable complex with the ubiquitin hydrolase USP11, which stabilized both MYCN and BRCA1 protein levels to maintain their recruitment to promoter-proximal regions. MYCN, but not MYC, promotes association of USP11 with BRCA1, and MYCN recruited BRCA1 to chromatin much more efficiently than MYC. Taken together, these results suggest that recruitment of BRCA1 by MYCN comprises a cell lineage–specific transcriptional stress response that enables MYCN-driven tumors to mitigate dysregulated RNAPII function.

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Access the most recent version of this article at: doi:10.1158/2159-8290.CD-RW2019-038

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