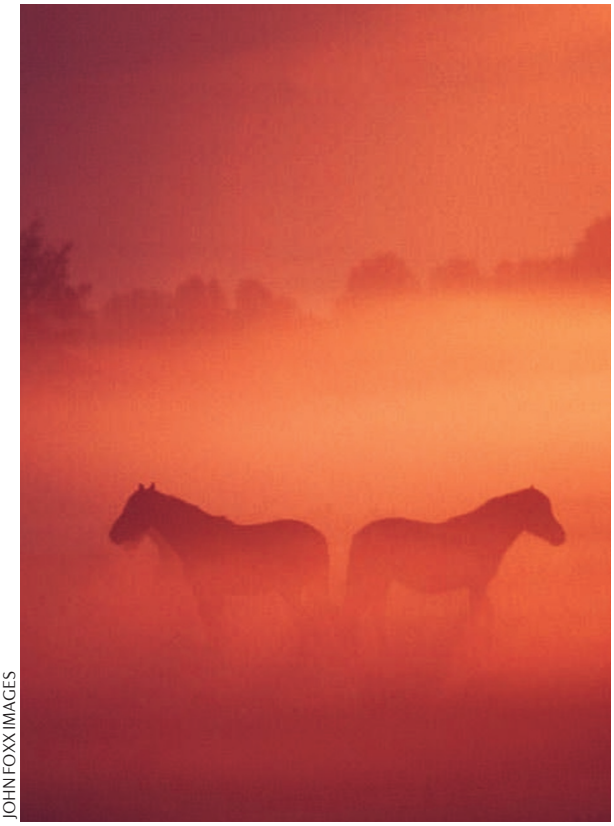
TUMORIGENESIS

MYCN and aurora A: a stable relationship

Deregulation of MYCN expression is implicated in the development of neuroblastoma owing to disruption of the ability of neuroblasts to undergo cell cycle exit and terminal differentiation. Moreover, amplification of *MYCN* is associated with poor prognosis. A recent article in *Cancer Cell* now identifies aurora A as a crucial regulator of the turnover of MYCN in *MYCN*-amplified neuroblastoma.

Otto *et al.* demonstrated that downregulation of aurora A by short hairpin RNA (shRNA) specifically retarded the growth of *MYCN*-amplified neuroblastoma cell lines (including IMR-32), but not of neuroblastoma cells that do not express MYCN. Growth was restored by retroviral expression of MYCN, implying that aurora A stabilizes MYCN. This stabilization is achieved post-translationally, as depletion of aurora A led to a reduction in the steady-state levels of MYCN but not of *MYCN* mRNA.



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So how might aurora A stabilize MYCN? Depletion of FBXW7 in IMR-32 cells led to an accumulation of MYCN, supporting the supposition that, like MYC, MYCN is degraded by the SCF^{FBXW7} ubiquitin ligase when S62 and T58 are phosphorylated. Moreover, expression of FBXW7 in SH-EP cells (a neuroblastoma cell line with a single-copy, silenced *MYCN* gene) decreased the levels of cotransfected MYCN, an effect that was alleviated by coexpression of aurora A. Aurora A had no effect on the levels of a phosphorylation-resistant MYCN mutant (MYCN-*mut*), indicating that aurora A stabilizes MYCN by inhibiting its degradation by SCF^{FBXW7}. Immunoprecipitation experiments in transfected SH-EP cells showed that endogenous MYCN and aurora A interact, but transfection with MYCN-*mut* in place of the wild type caused a reduction in interaction with aurora A that mirrored the reduced interaction with FBXW7, implying that aurora A interacts exclusively with MYCN bound to FBXW7. This effect seems to be specific to MYCN, as aurora A did not affect the degradation of other FBXW7 substrates: cyclin E and MYC.

Exogenous expression of aurora A led to an accumulation of ubiquitylated MYCN, indicating that stabilization is effected at a post-ubiquitylation step. However, transfection of ubiquitin in which all lysine residues except K48 were mutated led to lower levels of MYCN and abolition of the stabilizing effect of aurora A, a result that was partially reversed when K63 or K11 was restored. Thus, the authors concluded that aurora A promotes the accumulation of MYCN ubiquitylated at lysines other than K48, which leads to less efficient degradation by the proteasome and hence stabilization.

The degradation of MYCN requires a priming phosphorylation of S62 by cyclin B–CDK1, followed by phosphorylation of T58 by GSK3, which is inhibited by PI3K–Akt signalling. Aurora A and MYCN levels increased when synchronized IMR-32 cells entered G2 and the proteins colocalized in mitotic cells, which is when cyclin B–CDK1 is most active. MYCN levels were only weakly reduced by activation of GSK3 with the PI3K inhibitor LY294002 in neuroblastoma cells arrested in mitosis, but when aurora A was also depleted MYCN was all but eliminated from the cells. Thus the authors concluded that, in *MYCN*-amplified neuroblastoma, mitosis-specific, PI3K-dependent degradation of MYCN, which in neuronal progenitor cells leads to cell cycle exit and differentiation, is inhibited by aurora A.

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ORIGINAL RESEARCH PAPER Otto, T. *et al.* Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. *Cancer Cell* **15**, 67–78 (2009)

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