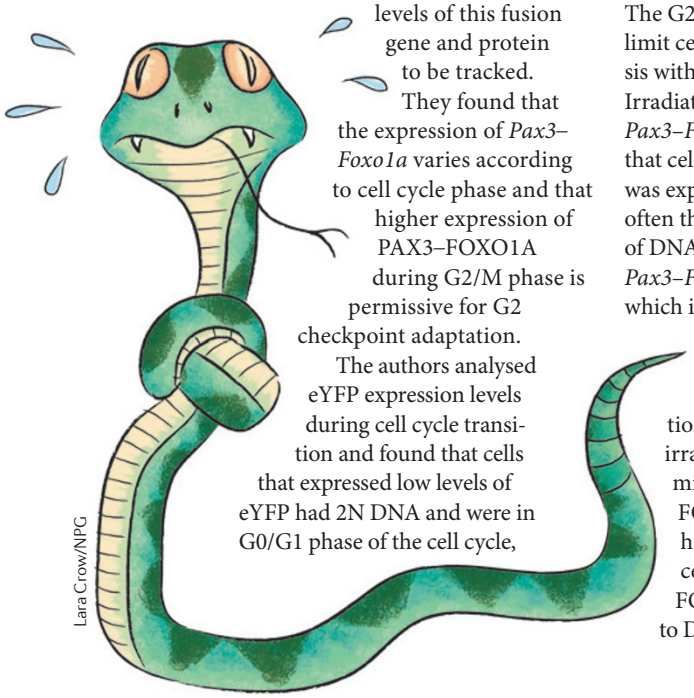
RHABDOMYOSARCOMA

Flexibility could be important

> higher expression of PAX3–FOXO1A during G2/M phase is permissive for G2 checkpoint adaptation.

Rhabdomyosarcoma (RMS) is a rare childhood tumour of muscle cells in which alveolar RMS (aRMS) has a poorer outcome than embryonal RMS. aRMS is characterized by gene fusion-positive and gene fusion-negative subtypes, of which fusion-positive disease is more prone to relapse with a substantial difference in overall survival. To better understand this more aggressive disease subtype, Charles Keller, Ken Kikuchi and colleagues generated an aRMS knock-in mouse model that expresses the common fusion gene paired box 3 (*Pax3*)–forkhead box O1A (*Foxo1a*) under the regulation of the normal *Pax3* promoter. The authors also expressed an enhanced yellow fluorescent protein (eYFP) as a second cistron in the *Pax3–Foxo1a* mRNA, which allowed the expression levels of this fusion gene and protein to be tracked. They found that the expression of *Pax3–Foxo1a* varies according to cell cycle phase and that higher expression of PAX3–FOXO1A during G2/M phase is permissive for G2 checkpoint adaptation.



Lara Crow/NPG

The authors analysed eYFP expression levels during cell cycle transition and found that cells that expressed low levels of eYFP had 2N DNA and were in G0/G1 phase of the cell cycle, and those with high levels were in G2/M phase (4N) or were hyperdiploid (>4N) cells. Further analyses in human and mouse RMS cells with PAX3–FOXO1A expression and with markers specific for either G2 or M phases indicated that PAX3–FOXO1A expression is high in G2 phase but not in M phase. Moreover, analysis of primary myoblasts indicated that PAX3 expression varies in a similar but less accentuated manner during the cell cycle, whereas FOXO1A expression remains consistent.

What are the biological consequences of these findings? Gene expression analyses indicated that in G2/M (4N) cells in which *Pax3–Foxo1a* expression was knocked down, the expression of genes associated with G2 and M checkpoint adaptation was reduced. The G2/M checkpoints function to limit cells progressing through mitosis with unrepaired DNA damage. Irradiation of cells with and without *Pax3–Foxo1a* knock down showed that cells in which *Pax3–Foxo1a* was expressed progressed more often through mitosis with evidence of DNA damage compared with *Pax3–Foxo1a* knockdown cells, which indicates that PAX3–FOXO1A facilitates transition in the presence of DNA damage through checkpoint adaptation. Transplantation of these irradiated cells into syngeneic mice showed that PAX3–FOXO1A-expressing cells had a growth advantage, and cells that expressed PAX3–FOXO1A were more resistant to DNA damage-inducing chemotherapy and to targeted therapies, such as imatinib. The authors conclude that understanding the natural expression patterns of oncogenes and fusion genes in RMS and other cancers could be important for understanding their function in tumorigenesis.

PAX3–FOXO1A is not the only gene known to contribute to RMS development. A comprehensive genome analysis of 147 RMS samples and matched normal samples by Javed Khan and colleagues indicated that, in RMS samples with fusion genes that involve either *PAX3* or *PAX7*, the level of additional somatic mutations is low. In samples that lack fusion genes, the authors found mutations in genes that were previously known to be involved in RMS, such as the RAS genes and fibroblast growth factor receptor 4 (*FGFR4*), as well as mutations in F-box and WD repeat domain containing 7 (*FBXW7*) and BCL6 co-repressor (*BCOR*), which are two genes that were not previously known to be mutated in RMS. Interestingly, these authors also found that 93% of RMS samples have altered receptor tyrosine kinase–RAS–PI3K signalling, which suggests that agents that target this pathway should be investigated as potential therapies for patients with RMS.

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ORIGINAL RESEARCH PAPERS Kikuchi, K. *et al.* Cell-cycle dependent expression of a translocation-mediated fusion oncogene mediates checkpoint adaptation in rhabdomyosarcoma. *PLoS Genet.* **10**, e1004107 (2014) | Shern, J. F. *et al.* Comprehensive genomic analysis of rhabdomyosarcoma reveals a landscape of alterations affecting a common genetic axis in fusion-positive and fusion-negative tumors. *Cancer Discov.* **4**, 216–231 (2014)