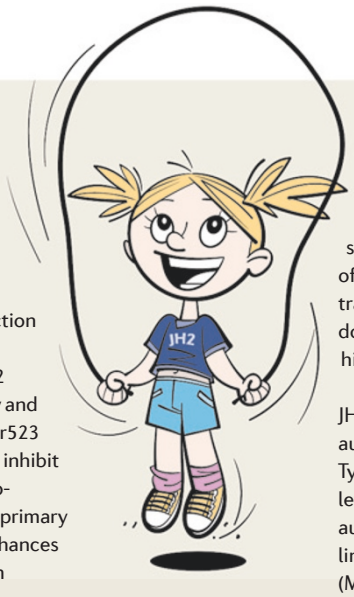
CELL SIGNALLING

JH2 is active!



MACMILLAN MEXICO/Andres Martinez Ricci

Janus kinase 2 (JAK2) is a cytoplasmic Tyr kinase that has a crucial function in mediating the signalling of haematopoietic hormones and cytokines. JAK2 is regulated both positively and negatively by phosphorylation. Its Tyr kinase domain JAK homology 1 (JH1) is regulated both by *trans*-acting proteins and by a regulatory domain, JH2, which inhibits JAK2 activation through an unknown mechanism. Silvennoinen and colleagues now show that JH2 functions by phosphorylating itself on two sites — Ser523 and Tyr570.

JH2 had previously been classified as a pseudokinase domain, as it was predicted to adopt a protein kinase fold but to be catalytically inactive. By obtaining a recombinant JH2 domain *in vitro*, the authors found that JH2 is actually an active protein kinase, although its activity is considerably lower (10%) than that of JH1. Using an *in vitro* kinase assay, they tested the function of wild-type and kinase-inactive JH2 domains. They found that JH2 has autophosphorylation activity and that it phosphorylates itself at Ser523 and Tyr570, which enables JH2 to inhibit JAK2 function. Furthermore, auto-phosphorylation of Ser523 is the primary event in JH2 activation, which enhances subsequent autophosphorylation of Tyr570.

The authors went on to analyse the function of JH2 in mammalian cells by expressing JH2-inactive JAK2 in JAK2-deficient cells. In these cells, autophosphorylation of Ser523 and Tyr570 did not occur and, importantly, this resulted in higher levels of phosphorylation of JH1, an indicator of JAK2 activation. This shows that active JH2 inhibits JAK2 activity through the phosphorylation of Ser523 and Tyr570, which act as two negative regulatory sites. The inhibitory function of the JH2 kinase activity was further confirmed by the finding that, in response to cytokine stimulation, the basal phosphorylation of signal transducer and activator of transcription 1 (STAT1) and STAT5 — downstream targets of JAK2 — was higher if JH2 was mutated.

> “JH2 functions by phosphorylating itself”

Together, these results show that JH2 activity, which is dependent on its autophosphorylation at Ser523 and Tyr570, is required to maintain a low level of JAK2 activity. Importantly, the authors also find that JH2 activity is linked to myeloproliferative neoplasms (MNPs), as known MNP mutations reduce JH2 activity by abrogating Ser523 and Tyr570 phosphorylation. This connection may lead to the development of targeted therapies against JAK-mediated diseases.

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ORIGINAL RESEARCH PAPER Ungureanu, D. *et al.* The pseudokinase domain of JAK2 is a dual-specificity protein kinase that negatively regulates cytokine signaling. *Nature Struct. Mol. Biol.* 14 Aug 2011 (doi:10.1038/nsmb.2099)