JAK2 Gets Hist*one* H3 Rolling

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Activation of JAK2 is implicated in normal hematopoiesis as well as oncogenic transformation. A paper in the recent issue of *Nature* demonstrates that phosphorylation of histone H3 by JAK2 releases the transcriptional repressor HP1α from chromatin, resulting in gene transcription.

JAK2 is a nonreceptor tyrosine kinase that forms a complex with the cytoplasmic portions of the receptors for growth hormone, erythropoietin, and related family members. The transcription factor STAT5 (signal transducer and activator of transcription 5) is a major substrate for JAK2, mediating part of its biological response. In neoplasms such as polycythemia vera where JAK2 is constitutively activated because of mutation, STAT5 appears to partially drive transformation. Mice with either JAK2 or STAT5A and STAT5B gene disruption have severely disrupted hematopoiesis (Hennighausen and Robinson, 2008; Ihle and Gilliland, 2007). However, it has been evident for some time that STAT5 is not likely to mediate all of the signaling activities of JAK2.

Dawson et al. (2009) have now reported an important second JAK2-signaling pathway. The authors found that 35% (14) of the top 40 JAK2-regulated genes identified in their study did not contain a putative STAT5 binding site. As one example, *Imo2* was identified as a major JAK2-responsive target gene that is implicated in normal hematopoiesis and leukemic transformation, but did not fit the profile of a STAT5-regulated gene. They found that JAK2 is localized not only to the cytoplasm but also to the nucleus, where it can phosphorylate histone H3 at tyrosine residue 41 (Y41) (Figure 1). In cell lines such as HEL, where JAK2 kinase activity is constitutively activated by a JAK2V617F mutation, phosphorylation of H3Y41 was similarly constitutive, whereas in cell lines with wild-type JAK2, phosphorylation of H3Y41 was induced after activation of JAK2 by an appropriate cytokine. Phosphorylation of the H3Y41 site by JAK2 resulted in release of the transcriptional repressor heterochromatin protein 1α (HP1α) from chromatin, resulting in transcription of genes repressed by HP1α, such as *Imo2*. By contrast, inhibition of JAK2 kinase activity in HEL cells increased recruitment of HP1α to the *Imo2* promoter, but not to the promoter of the nearby β2-macroglobulin gene. They further demonstrate that the phosphorylation of histone H3 on Y41 depends on JAK2 kinase activity via kinase inhibitors and overexpression of JAK2. The levels of H3Y41 were low in the AML cell line HL60 with no detectable levels of active JAK2 as well as in the JAK2-deficient fibrosarcoma cell line γ2A. Ectopical expression of the wild-type JAK2 or the constitutively active JAK2V617F into γ2A cells increased H3Y41 phosphorylation. Conversely, H3Y41 phosphorylation was reduced in HEL cells in response to the JAK2 inhibitors TG101209 and AT9283. It is not clear from these findings whether the nuclear pool of JAK2 is altered upon the stimulation of its kinase activity or how nuclear JAK2 gets activated. A link between JAK2 and HP1α had been previously reported in *Drosophila melanogaster*, although STAT proteins were directly involved in the process in that model system (Shi et al., 2006).

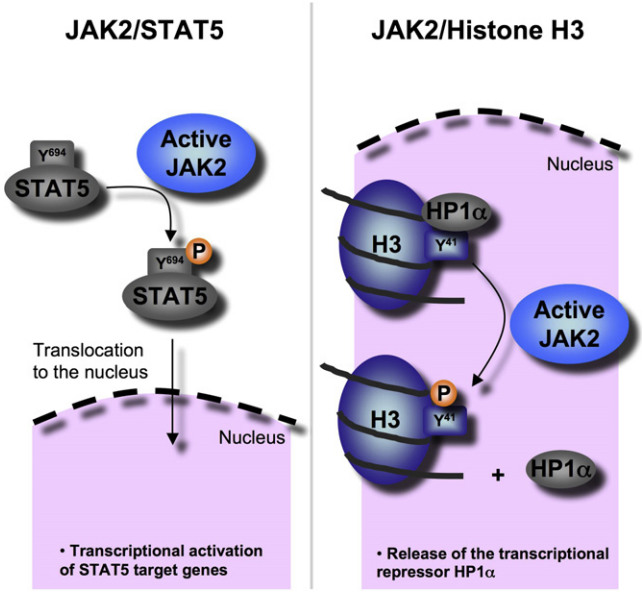

Figure 1. Model for the Regulation of Transcription by Active JAK2
In hematopoietic cells, JAK2 can be transiently activated through cytokine mediated receptor ligation or chronically through multiple mechanisms, including the activating JAK2V617F point mutation. The canonical JAK2 pathway leads to phosphorylation of the STAT5 transcription factor at Y694 within the cytoplasm (Gouilleux et al., 1994). Phosphorylated STAT5 is translocated to the nucleus and drives dependent gene expression as a transcriptional activator. However, through a novel mechanism, active nuclear JAK2 phosphorylates histone H3 at Y41 and thus disrupts the interaction of the HP1α chromo shadow domain with H3 (Dawson et al., 2009). HP1α is thus effectively displaced from H3 and, as a consequence, its chromatin silencing function is disrupted.

Activation and phosphorylation of STAT5 by JAK2 is thought to require binding of JAK2 through its FERM domain to a growth factor receptor (Funakoshi-Tago et al., 2006; Wernig et al., 2008). Thus the results of Dawson et al. (2009) open up the intriguing question whether nuclear activation of JAK2 also requires additional cofactors. It will be interesting to determine the structural

requirements for JAK2 activation in the nucleus and the process guiding plasma membrane-bound active JAK2 to the nucleus. However, basal phosphorylation of histone H3Y41 would hint at some redundancy in this pathway that may not require JAK2.

It has been apparent for some time that STAT5 cannot be the only major transcriptional effector of JAK2 and other substrates of this kinase have been characterized with various impact on gene expression. The identification of histone H3Y41 as a target of JAK2-dependent HP1α regulation adds a new dimension to the field of JAK2 biology. These findings will help to unravel some of the dynamics within the intricate signaling networks required for early hematopoiesis. As correctly pointed out by Dawson et al. (2009), the role of the HP1α binding region in nucleosome mobility and stability as well as that of HP1α itself in mitotic recombination may explain some of the genomic instability associated with malignancies containing active JAK2 (Fernandes et al., 2009; Plo et al., 2008). Nevertheless, it seems likely that other factors might regulate HP1α and the degree of JAK2 requirement is not entirely clear. It will need to be carefully determined whether these novel interactions of JAK2 with histone H3Y41 open up new opportunities for targeted therapeutic approaches that may benefit patients with hematologic neoplasms or other malignancies that involve deregulated JAK2 activity. Finally, it is possible that other members of the Janus kinase family (JAK1, JAK3, and TYK2) may have analogous functions in the nucleus and that should be examined.

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PARsing the Phrase "All in for Axin"—Wnt Pathway Targets in Cancer

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Genetic alternations resulting in constitutive stabilization of β-catenin and altered transcription of β-catenin/TCF-regulated genes are found in many cancers. A recent *Nature* paper offers insights into the role of tankyrase in regulating the Wnt/β-catenin pathway and suggests that compounds targeting tankyrase's poly-ADP-ribosylation (PARsylation) activity may hold promise for cancer therapy.

Defects in conserved signaling pathways are well known to play key roles in the origins and behavior of essentially all cancers. Mutations affecting the Wnt signaling pathway underlie the pathogenesis of cancers, including upwards of 80%–90% of colorectal cancers (CRCs) (MacDonald et al., 2009). The Wnt proteins are a conserved family of secreted molecules with pleiotropic and context-specific effects on cells (MacDonald et al., 2009). In the canonical or β-catenin-dependent Wnt pathway, Wnts regulate the level and subcellular localization of β-catenin. In the absence of an activating Wnt signal, glycogen synthase kinase 3β (GSK3β) collaborates with the AXIN and APC (adenomatous polyposis coli) proteins and other factors to phosphorylate β-catenin at its amino (N)-terminal domain. The phosphorylated β-catenin is recognized and ubiquitinated by a complex containing a β-transducin repeat-containing protein (βTrCP), then degraded by the proteasome. Wnt binding to the Frizzled-low density lipoprotein-related protein (LRP)-5/6 coreceptor complex on the cell surface inhibits the AXIN/GSK3β complex and stabilizes the free pools of β-catenin. In the nucleus, β-catenin can bind to T cell factor (TCF) transcriptional regulators along with other cofactors and modulate transcription of various genes. Mutational mechanisms with major contributing roles in stabilizing β-catenin in human cancer include inactivation of APC or AXIN1 or