Activating mutations in JAK2, a tyrosine kinase that serves as a critical signaling protein for multiple cytokines, are associated with BCR-ABL1–negative myeloproliferative neoplasms. In this issue of Blood, Zheng and colleagues report that the constitutively activated kinase CK2 binds to JAK2 and is required for JAK-Stat signaling, suggesting that CK2 inhibitors may provide a second, complimentary therapeutic for diseases with elevated JAK2 signaling, such as myeloproliferative neoplasms.\(^1\)

CK2 increases JAK2 activity and signaling. As reported by Zheng and colleagues, the binding of active CK2 to JAK2 appears to be essential for oncostatin M (OSM)–dependent activation of JAK2 and signaling molecules downstream of JAK2, including Stats, ERKs, and expression of target genes including the gene for suppressor of cytokine signaling-3 (SOCS-3).\(^1\) HEL cells and cells from patients with PV express the hyperactive JAK2 V617F. In these cells, the binding of CK2 to JAK2 V617F is essential for maximal JAK2 activity and the expression of the antiapoptotic protein Bcl-xL. Inhibition of CK2 activity results in a substantial decrease in JAK2, Stat3, and ERK activity, decreased Bcl-xL, and increased apoptosis.

JAK family tyrosine kinases (JAK1, JAK2, JAK3, Tyk2) bind to cytokine receptors and are activated on cytokine binding. The activated JAKs phosphorylate tyrosines in themselves and in the associated cytokine receptors. The resulting phosphotyrosines form binding sites for multiple signaling proteins, including the Stat transcription factors that mediate many of the actions of the activating cytokines.\(^2\) JAK2 is activated by more than 15 different cytokines including erythropoietin, oncostatin M (OSM), GM–SCF, thrombopoietin, some interleukins, leukemia inhibitory factor, and IFN-γ as well as by some G protein–coupled receptors.\(^4\) Activating mutations in JAK2 have been found in patients with myeloproliferative neoplasms, with the most common mutation being V617F. This mutation is present in more than 95% of patients with polycythemia vera (PV), 32% to 57% of patients with essential thrombocytemia (ET), and 35% to 50% of patients with primary myelofibrosis (PMF).\(^3\) This has made JAK2 an attractive target for therapeutic intervention, and in fact, multiple JAK2 inhibitors are currently in phase 1, 2, and 3 trials.\(^2\) For these reasons, it is critical to identify the proteins that bind to JAK2 and understand how they regulate and/or mediate JAK2 function. Although quite a few proteins have been identified that are recruited to phosphorylated tyrosines in the cytokine receptors per se, including Stat proteins, very few proteins have been identified that regulate JAK2 signaling as a consequence of binding to JAK2.

Zheng and colleagues provide the first evidence that the serine/threonine kinase CK2 binds to JAK2 and is critical for activation of the JAK2–Stat signaling pathway in response to ligand stimulation and for the constitutive activation of JAK2 in cells expressing JAK2 V617F.\(^1\) CK2 is a ubiquitously expressed kinase that exists primarily as a tetramer composed of 2 catalytic subunits (CK2α) and 2 regulatory subunits (CK2β). CK2 is found in multiple subcellular compartments, and has more than 300 known substrates, many associated with cell viability.\(^6,7\) Zheng et al show that depleting cells of CK2α and/or CK2β using siRNA greatly decreases the ability of the cytokine oncostatin M to stimulate tyrosyl phosphorylation of Stat3 and express SOCS3, a Stat3-dependent gene.\(^4\) Consistent with CK2 being required for ligand activation of JAK–Stat signaling, 2 inhibitors of CK2 (TBB and emodin) block oncostatin M–dependent tyrosyl phosphorylation of Stats 1, 3, and 5, and expression of SOCS3. Depletion of CK2 also decreases IFN-γ–stimulated tyrosyl phosphorylation of Stats1 and 3 and GH-stimulated phosphorylation of Stat5, providing further evidence that the stimulatory effect of CK2 on JAK–Stat signaling may be a direct effect of CK2 on JAK2. The authors report that CK2 binds to JAK2, that binding is independent of phosphotyrosines in JAK2 and increases with oncostatin M, and that CK2 can phosphorylate JAK2. Depletion of CK2β alone or with CK2α has a modest inhibitory effect on JAK2 autophosphorylation while CK2 inhibitors have a more robust inhibitory

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Christin Carter-Su and Lawrence S. Argetsinger

UNIVERSITY OF MICHIGAN MEDICAL SCHOOL


**MYELOID NEOPLASIA**
effect, suggesting that CK2 binding to JAK2 is required for maximal ligand activation of JAK2. However, the degree of JAK2 inhibition is significantly less than the inhibition of Stat signaling, raising the possibility that CK2 may have additional effects on Stat signaling.

Based on these findings, Zheng and colleagues hypothesize that CK2 inhibitors will be potent inhibitors of constitutively activated JAK2 V617F and pathways downstream of JAK2 V617F. They find in cells from PV patients and in cultured human erythroid leukemia (HEL) cells, both of which express endogenous JAK2 V617F, that indeed, JAK2 binds CK2 and that CK2 inhibitors significantly depress the amount of active JAK2, Stat3, Stat5, and ERK. More importantly, CK2 inhibitors decrease proliferation and induce apoptosis in cells expressing JAK2 V617F, assessed by proliferation assays, cell-cycle analysis, increased levels of annexin V and decreased levels of pro-caspase 8, pro-caspase 3, and/or Bcl-xL.

These studies raise a number of interesting questions related to both basic and clinical science realms. How does CK2 bind to JAK2 and is it regulated in any way that could be manipulated? Given the large number of CK2 substrates, manipulation of the JAK2/CK2 interaction would provide a more precise therapeutic intervention than simply inhibiting JAK2 or CK2. What sites in JAK2 does CK2 phosphate and what are the ramifications of those phosphorylations on JAK2 activity and binding partners? Is the effect of CK2 direct on JAK2 or on some other JAK2 binding partner? Interestingly, Zheng et al find CK2 also binds to JAK1, and that CK2 inhibitors decrease oncostatin M-induced JAK1 activation to a much greater extent than oncostatin M-induced JAK2 activation. Is it possible that CK2 acts primarily on JAK1 in the context of oncostatin M signaling? Finally, might CK2 inhibitors provide an additional therapeutic intervention for myeloproliferative neoplasms associated with constitutively activated JAK2 or for the variety of other diseases that are associated with elevated JAK-Stat signaling, such as solid tumors (eg, androgen-independent prostate cancer) and rheumatologic diseases.

Conflict-of-interest disclosure: The authors declare no competing financial interests.

REFERENCES

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Joel Anne Chasis

In this issue of Blood, Satchwell and colleagues provide compelling evidence that during the genesis of the plasma membrane in differentiating erythroblasts, key components of band 3/Rh multiprotein complexes can traffic to the membrane already linked or assemble together for the first time in the membrane.

During erythropoiesis the plasma membrane is in a dynamic state of organization as newly synthesized proteins incorporate into the lipid bilayer. Throughout terminal differentiation the erythroblast membrane facilitates key cellular properties including adhesive interactions with macrophages, active endocytosis of iron, a progressive decrease in cell volume and increased hemoglobin synthesis. Although the timing of expression of individual proteins on the plasma membrane has been described, little is known about the assembly of these individual components into large multiprotein complexes that are known to be present in the mature erythrocyte membrane. Among the important physiologic roles of one of these multiprotein complexes are anion, NH3, and CO2 transport and maintenance of membrane cohesion. Major questions still to be answered include: At what point in erythropoiesis do these complexes assemble? And are these complexes assembled after incorporation of individual components in the membrane or are they assembled intracellularly and then trafficked to the membrane?

The most abundant multiprotein complex is the ankyrin-based band 3 macrocomplex, impressive in size, and containing both a band 3 subcomplex and a Rh subcomplex. The band 3 subcomplex includes the erythrocyte anion transporter band 3, which associates with glycoporphin A and links to the cytoskeleton via protein 4.2 and ankyrin. These interactions are important for membrane cohesion and their absence in hereditary spherocytosis (HS) leads to surface area loss. The NH3 and CO2 transporter RhAG1/2 forms a heterotetramer with RhCe and RhD (termed Rh polypeptides), and this core complex interacts with proteins CD47, LW, and glycoporin B, together forming the Rh subcomplex. These linked proteins are deficient in the Rh null phenotype leading to hemolytic anemia with stomatocytes and spherocytes because of loss of surface area.

To begin to explore the spatio-temporal mechanisms of the band 3 macrocomplex formation during erythropoiesis, Satchwell and colleagues focused their studies on 2 of the crucial linkages within the macrocomplex, band 3/protein 4.2 and RhAG/Rh. Using erythroid cultures from human peripheral blood mononuclear cells, the authors applied confocal immunofluorescent microscopy and biochemical techniques to study the intracellular trafficking of these proteins and the dynamics of their interactions. They observed that the bulk of band 3 and RhAG is delivered to the plasma membrane early during differentiation. Moreover, in basophilic erythroblast plasma membranes, band 3 and protein 4.2 are already present in a linked state. Interestingly, they found band 3 associating with protein 4.2 in the early stages of the secretory
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Christin Carter-Su and Lawrence S. Argetsinger