Perspective

On the Regulation and Activation of JAK2: A Novel Hypothetical Model

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Abstract

Janus kinase 2 (JAK2) is a protein tyrosine kinase central to a multitude of cellular processes. Here, a novel model of JAK2 regulation and activation is proposed. In the JAK2 dimer, instead of being auto-inhibited by its own JH2 domain, inhibition comes from the JH2 domain of the partnering JAK2 monomer. Upon ligand binding, the receptor undergoes a conformational rotation that is passed to its dimeric partner. The activation is achieved by the rotation of two JAK2 molecules, which relieves the JH1/JH2 inhibitory interface and brings two JH1 domains in proximity for the subsequent trans-phosphorylation event. This hypothetical model is consistent with most of the currently available experimental evidence and warrants further tests. Based on the proposed model, it is possible to rationalize the differential responses of JAK2 signaling involving various receptors and ligands.

Implications: The proposed model of JAK2 regulation and activation is poised to suggest potential alternative drug-discovery strategies that could impact a number of relevant diseases.

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Introduction

Janus Kinase 2 (JAK2) is a protein tyrosine kinase that transduces cellular signals through the JAK–STAT pathways (1). Deregulation of JAK2 is thought to be associated with hematopoietic disorders and oncogenesis (2). However, the regulation mechanism of JAK2 remains elusive, and surprisingly little is known about the detailed molecular interactions involved in JAK2 activation processes (3).

It has been well accepted that the kinase domain of JAK2, JH1, is likely negatively regulated by its adjacent pseudokinase domain, JH2 (4, 5), but the remaining question is how this “autoinhibition” and JAK2 activation are achieved. Recent research advances of JAK2 warrant more testable hypotheses of the JAK2 regulation and activation mechanism. Following key evidences should be considered in a plausible hypothesis:

The JH1/JH2 interfaces are critical for the negative regulation

Currently, there is no experimental structure of the whole JAK2 assembly, and a theoretical structure model has been built using homology modeling methods and coevolution data (6). In this model, the JH1 and JH2 domains face each other in an asymmetrical way, that is, the JH1 N-lobe contacts the JH2 C-lobe, whereas the JH1 C-lobe contacts the JH2 N-lobe, with a helix–helix contact between the JH1 C-helix and the JH2 C-helix.

It has been suggested that the above theoretical model may be able to explain the mutational effect of JAK2 V617F at the atomic level (7). We reported molecular dynamics simulations of the wild-type and V617F-mutant JAK2 protein, and the results strongly support the notion that the JH1 is inhibited by JH2 (8). The simulation results also predicted that F595 is a key residue interacting with F617 in the V617F mutant, which has been experimentally verified by other groups (9, 10). JH2 has been suggested to act as a dual-specificity protein kinase by Ungureanu and colleagues (11). These roles do not exclude the possible inhibitory JH1/JH2 interface. In fact, Ungureanu and colleagues suggested that JH2 could transphosphorylate Y570, which may imply that two JAK2 monomers are brought near to become autoinhibited.

Receptor binding and dimerization are necessary but not sufficient for activation

Various experimental evidences suggest that the binding to the receptor and dimerization of JAK2 are critical in the regulation mechanism. The activation mechanism likely has at least 2 steps: JH1 of each monomer must be released from their corresponding JH2, then two JH1s need to be aligned properly into close proximity to be activated (12, 13). The binding to the receptors, however, is necessary but not sufficient for the activation (14). The V617F mutant can only be activated when it is bound to the receptor and dimerized (15, 16).
Monomer–monomer interactions may be important

Furthermore, the interaction between 2 monomers could play important roles as well. It has been shown that the homozygous Jak2 V617F mutants, the heterozygous Jak2 V617F mutant, and wild-type Jak2 manifest different phenotypes (17). JAKs can form heterodimers in various signaling pathways and have mutual effects on their partner JAKs, for example, JAK1 and JAK3 (18).

The signaling mechanism likely is directionally (orientationally) driven

It has been suggested that the active and inactive states of erythropoietin receptor differ in the relative orientation between monomers (19), and the juxtamembrane domains of receptors must be in precise orientations to properly function (14, 20). Other evidence strongly supports that the Jak2 is activated through the steric information transduced through the transmembrane region into the cell. (3, 21).

The hypothesis

Trying to consolidate all available evidence, we propose a novel hypothesis of the Jak2 activation as follows. The receptor dimer recruits 2 Jak2s to form a Jak2 dimer, possibly the only way that Jak2 dimerizes in the physiologic conditions, because only in vivo dimerized Jak2 has been suggested. In the dimer form, the Jak2s JH1 domain is inhibited by the JH2 domain of the other monomer, rather than its own adjacent JH2. The activation is achieved by the rotation of two Jak2 molecules, which separates the JH1/JH2 interface and brings two JH1 together to be ready for transphosphorylation.

The first scenario

Shown in Fig. 1, the JH1 N-lobe is located on top of its C-lobe, whereas the JH2 C-lobe is on top of JH2 N-lobe. In the inactive state, the JH1 faces and is inhibited by the JH2 of the other monomer. The ligand binding of the receptors causes the rotation of the Jak2 molecules and leads to the active state where JH2s are not interacting with JH1s anymore, and now two JH1s are facing each other.

The second scenario

Shown in Fig. 2, the JH1 N-lobe and C-lobe are arranged side-by-side. JH1 is facing JH2 similar to the first scenario and the ligand binding of the receptors causes the rotation of the Jak2 molecules and leads to the active state.

The difference between these two scenarios is that in the first scenario, two JH1s will face each other symmetrically in the active state, that is, the N-lobe will face the other N-lobe, whereas in the second scenario, they will face each other antisymmetrically, that is, the N-lobe will face the C-lobe of the other JH1.

Explanation of known mutational effects

It is well known that Jak2V617F constitutive activation requires the dimerization of both receptors and Jak2 (15), similar to the wild-type, but may have different activation mechanisms upon ligand binding (22, 23). Suggested by our previous work (8), the Jak2V617F mutation breaks the JH1/JH2 inhibitory interface and separates JH1 and JH2 domains, and, in the proposed model, two Jak2 monomers will not be held by each other and their JH1 domains will be actively accessible to substrates. Compared with ligand-induced activation in wild-type where the JH1/JH2 interface is broken due to the relative rotation between Jak2 dimers, the Jak2V617F directly breaks the interface through weakening the molecular interactions in the interface. Recent simulation work (24) also suggests that several clinically observed mutations within exon 12 to 15 have similar mutational effects on weakening the JH1/JH2 interface, consistent with clinical and experimental evidence.

Possible tests and impacts

Computational models can be built on the basis of this hypothetical model, and subsequent molecular simulations would be able to fine tune the resulting models and provide possible detailed interaction network of relevant residues. Such work can be collaboratively done with site-directed mutagenesis experiments. The binding modes of receptor–ligand complexes can be experimentally determined and compared with the phosphorylation sites of Jak2, either

Figure 1. The proposed Jak2 activation mechanism. The JH1 N-lobe is located on top of its C-lobe, whereas the JH2 C-lobe is on top of JH2 N-lobe. In the inactive state (left), the JH1 faces and is inhibited by the JH2 of the other monomer. The ligand binding of the receptors causes the rotation of the Jak2 molecules and leads to the active state (right) where JH2s are not interacting with JH1s anymore and now two JH1s are facing each other.
known or to be determined. On the basis of the proposed model, it would be possible to rationalize the differential responses of JAK2 signaling transductions with various receptors and ligands (25, 26): the magnitude of the relative rotation between two JAKs apparently will decide the regions of JH1 domains facing each other upon activation, hence the residues to be likely phosphorylated.

Another experimental verification approach can be taken: using the receptors with heterodimers, such as interleukin-6 or gp130, with hetero JAK dimers consisting of mutated JAK and explore the possible cross-mutational effects between different JAK monomers.

If this model is true, it can explain why different extracellular signals can be transduced through only JAK2 but produce different cascade events: the signals differ in the magnitudes of the resulting rotations, which may cause phosphorylation on other sites besides Y1007/Y1008, or may change the extent of JAK2 activation loop phosphorylation, and induces different cascade events. Furthermore, instead of JAK2 ATP site inhibitors, alternative drug discovery strategies (27) may be taken for relevant diseases, based on the proposed receptor--JAK2 complex models. It is our hope that the hypothetical model presented here will stimulate further relevant research and eventually lead to our ultimate understanding of the JAK2 activation mechanism.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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