## 1. Phylogeny

ABL1 is a non‐receptor tyrosine‐protein kinase that belongs to the Abl family within the broader group of cytoplasmic protein tyrosine kinases (NRTKs). Phylogenetically, ABL1 is closely related to ABL2 and exhibits significant sequence and functional conservation with other members of the Src and Abl kinase groups. Its kinase domain shares the canonical bilobal fold found in eukaryotic protein kinases, and it is characterized by the presence of regulatory SH2 and SH3 domains that mediate intramolecular interactions governing its activity. Unlike the Src family kinases that contain a C-terminal inhibitory tyrosine, the ABL kinases are regulated by a unique N-terminal myristoylated cap that enforces an autoinhibited conformation. This domain architecture is evolutionarily conserved across metazoans, and orthologs of ABL1 can be identified in a wide range of vertebrate species, reflecting its emergence early in the evolution of multicellular eukaryotes. Comparative studies using yeast as a heterologous expression system have demonstrated conserved kinase‐substrate relationships that underscore the evolutionary retention of substrate recognition motifs in Abl family kinases (corwin2016decipheringhumancytoplasmic pages 13-16, janaki2020unityanddiversity pages 15-18).

## 2. Reaction Catalyzed

ABL1 catalyzes the transfer of the γ‐phosphate from ATP to specific tyrosine residues on substrate proteins. The general reaction can be summarized as: ATP + protein (with an available tyrosine hydroxyl group) → ADP + phospho-protein + H⁺. This phosphorylation event modulates target protein activity, thereby altering downstream signaling pathways that regulate processes such as cytoskeleton remodeling, cell adhesion, and DNA damage response. Although the detailed kinetic mechanism has not been exhaustively dissected in every substrate system, it is generally accepted that the reaction follows a bi-bi sequential mechanism typical of protein kinases, with binding of ATP and the substrate protein followed by phosphoryl transfer that is facilitated by a conserved catalytic base in the active site (fabbro2015tenthingsyou pages 2-4).

## 3. Cofactor Requirements

The catalytic activity of ABL1 is dependent on the presence of divalent metal ions, most notably Mg²⁺. The Mg²⁺ ion is essential for proper positioning and stabilization of the ATP molecule within the active site, thereby facilitating an efficient phosphoryl transfer reaction. In many protein kinases, including ABL1, the presence of Mg²⁺ is an absolute requirement, and in some cellular contexts Mn²⁺ may partially substitute for Mg²⁺ although with altered kinetics. This cofactor dependency is a conserved characteristic among eukaryotic protein kinases and is critical for achieving optimal catalytic turnover (fabbro2015tenthingsyou pages 4-5).

## 4. Substrate Specificity

ABL1 phosphorylates a wide repertoire of substrates involved in diverse cellular processes. Its substrate specificity is defined by linear amino acid sequence motifs surrounding the target tyrosine residues. Experimental evidence derived from yeast-based phosphoproteomics has revealed that Abl family kinases preferentially phosphorylate substrates featuring proline at the +3 position relative to the phosphoacceptor site and aliphatic residues (such as valine, leucine, or isoleucine) at the –1 position. Additional preferences include the presence of negatively charged residues at positions –3, –4, and +1 relative to the tyrosine, thus creating a substrate environment conducive to selective recognition. Though much of the detailed motif data has been derived from studies of the ABL2 kinase, the close evolutionary relationship between ABL1 and ABL2 suggests that similar substrate recognition principles extend to ABL1. Physiologically, ABL1 targets proteins that play key roles in actin dynamics (e.g., WASF3), adaptor protein signaling (e.g., CRK and CRKL), receptor endocytosis (e.g., CBL and RIN1), and DNA repair processes (e.g., RAD51 and TP73). This broad substrate specificity and the conservation of linear motifs across yeast and human orthologs emphasize the evolutionary fine-tuning of Abl kinase substrate modules (corwin2016decipheringhumancytoplasmic pages 126-130, janaki2020unityanddiversity pages 40-54).

## 5. Structure

ABL1 exhibits the classic bi-lobal kinase fold present in eukaryotic protein kinases, comprising a smaller N-terminal lobe predominantly formed by β-sheets and a larger C-terminal lobe consisting mainly of α-helices. The N-terminal region includes a myristoylated cap that is unique to the Abl family, contributing to autoinhibition by docking into a specific pocket in the kinase domain, thereby stabilizing the inactive conformation. In addition to the kinase core, ABL1 contains regulatory modular domains such as an SH3 domain, which typically mediates the binding of proline-rich motifs, and an SH2 domain, which binds phosphotyrosine-containing regions. The juxtaposition of these domains creates an intramolecular network that is critically involved in the regulation of kinase activity. Structural studies using crystal structures and molecular replacement approaches have detailed the active versus inactive conformations of the kinase domain, highlighting conserved elements such as the activation loop (A-loop), the DFG motif, and the hydrophobic spines (R-spine and C-spine) that are essential in dynamic allosteric regulation. Key catalytic residues, including those in the catalytic loop and the αC-helix, are optimally arranged in the active state, and mutations in these regions (e.g., the gatekeeper mutation T315I) have been shown to confer resistance to therapeutic inhibitors. The overall architecture of ABL1 underscores its evolutionary conservation among tyrosine kinases and parallels many of the structural features described in related members of the tyrosine kinase superfamily (kornev2015dynamicsdrivenallosteryin pages 6-7, mcskimming2017classifyingkinaseconformations pages 8-10, tse2015moleculardeterminantsunderlying pages 32-34).

## 6. Regulation

The catalytic activity of ABL1 is tightly regulated by multiple mechanisms that integrate intramolecular interactions with post-translational modifications. Autoinhibition is a hallmark of its regulation, where the N-terminal myristoylated cap, in combination with the SH2 and SH3 domains, maintains the kinase in an inactive conformation under basal conditions. Relief of autoinhibition typically occurs through conformational changes triggered by binding of substrates or regulatory proteins, or by phosphorylation events. ABL1 is known to autophosphorylate on specific tyrosine residues within the activation loop, which helps stabilize the active conformation and promote substrate binding. Moreover, regulatory interactions with inhibitor proteins such as ABI1 modulate ABL1 activity via additional phosphorylation events that can either enhance or suppress kinase activity. In response to cellular stress, such as DNA damage or oxidative stress, ABL1 can translocate to the nucleus where additional regulatory modifications take place to govern roles in DNA repair and apoptosis. Intermolecular phosphorylation by upstream kinases or changes in cellular redox states further contribute to the fine-tuning of its signaling output. This complex regulatory scheme, involving both autophosphorylation and cross-talk with other signaling networks, is an evolutionarily conserved feature that has been elucidated through integrative studies in yeast and higher eukaryotes (corwin2016decipheringhumancytoplasmic pages 152-155, corwin2016decipheringhumancytoplasmic pages 149-152).

## 7. Function

ABL1 serves as a critical signaling node, orchestrating a variety of cellular processes that are central to cell growth, survival, and response to extracellular stimuli. Functionally, ABL1 modulates cytoskeletal dynamics by phosphorylating regulatory proteins such as WASF3, which is essential for lamellipodia formation and cellular migration, as well as other regulators involved in actin cytoskeleton remodeling. Through the phosphorylation of adaptors such as CRK and CRKL, ABL1 integrates signals from multiple receptors to control cell adhesion and motility. Besides its prominent cytoplasmic functions, ABL1 also plays significant roles in receptor endocytosis by phosphorylating components like CBL and RIN1, which are involved in receptor down-regulation and trafficking. Upon DNA damage or in response to oxidative stress, ABL1 translocates to the nucleus where it participates in the DNA damage response by phosphorylating substrates such as RAD51, TP73, and components of the repair machinery, thereby contributing to cell cycle arrest and apoptosis when damage is irreparable. Additionally, ABL1 can influence mitochondrial dynamics and autophagy, further underscoring its diverse roles in maintaining cellular homeostasis. These activities are supported by an extensive network of substrates that not only regulate cellular architecture but also control key transcriptional and apoptotic pathways. The multifunctional nature of ABL1 highlights its importance in both normal physiological contexts and in pathological conditions such as oncogenesis, where aberrant activity driven by gene fusions (e.g., BCR-ABL in chronic myelogenous leukemia) leads to uncontrolled cell proliferation (corwin2016decipheringhumancytoplasmic pages 155-173, corwin2016decipheringhumancytoplasmic pages 97-100).

## 8. Other Comments

Given its central role in cellular signaling, ABL1 is a major target of therapeutic intervention, particularly in the context of leukemias where the BCR-ABL fusion protein is a well-established driver of malignancy. Inhibitors such as imatinib and dasatinib have been developed to target the aberrant kinase activity of BCR-ABL and have achieved notable clinical success. In addition to its roles in cancer, ABL1 is implicated in the regulation of immune cell function and has been linked to pathogen-host interactions whereby certain microbial proteins are phosphorylated by ABL1 to facilitate infection. Mutations and polymorphisms within the ABL1 gene can alter its kinase activity and substrate specificity, thereby impacting disease progression and treatment response. Current research continues to explore the intricate regulation of ABL1, its broad substrate spectrum, and the development of next-generation inhibitors that overcome resistance mechanisms such as the T315I mutation. These advances are supported by structural insights that detail the conformational dynamics of the kinase domain and by proteomic studies that delineate its signaling networks. Resources such as the Chemical Probes portal and the KLIFS database are valuable for comparing the efficacy of ABL1 inhibitors and for revealing new aspects of its regulation and function (OpenTargets Search: -ABL1, janaki2020unityanddiversity pages 15-18, bokhari2022…oflung pages 37-41).

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