Author manuscript, published in "Biology of the Cell 100, 11 (2008) 617-31"
DOI : 10.1042/BC20080020

hal-00346793, version 1 - 12 Dec 2008

9654 words

Cytoplasmic signalling by the c-Abl tyrosine kinase in normal and cancer cells.

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Running head: c-Abl cytoplasmic signalling

Key words: tyrosine kinase, c-Abl, growth factor signalling, cancer

ABSTRACT

c-Abl is a non-receptor tyrosine kinase which is localised both in the nucleus and cytoplasm and is involved in the regulation of cell growth, survival and morphogenesis. While c-Abl nuclear function has been extensively studied, recent data point also to an important role in cytoplasmic signalling through mitogenic and adhesive receptors. Here we review the mechanisms by which growth factors promote cytoplasmic c-Abl activation and signalling and its function in the induction of DNA synthesis, changes in cell morphology and receptor endocytosis. The importance of deregulated c-Abl cytoplasmic signalling in solid tumours is also discussed.

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Introduction

The human kinome includes 32 non-receptor tyrosine kinases (Manning et al., 2002) that are thought to be key elements of intracellular signalling induced by extracellular stimuli such as cytokines, growth factors and integrins. Indeed, several of them, including the Src family of kinases (SFK), regulate cell growth and migration and play important roles during embryogenesis (Thomas & Brugge, 1997). c-Abl belongs to this class of tyrosine kinases and is the prototype of a sub-family which includes two members, c-Abl and Arg (Abl-related gene). Both proteins are localised at the cell membrane, actin cytoskeleton and cytosol and c-Abl is present in the nucleus as well (cytoplasmic and nuclear c-Abl in this review). *ABL1* and *ABL2* that encode the human c-Abl and Arg, respectively, are ubiquitously expressed and give rise to the 1a and 1b isoforms. Isoforms 1a originate from an alternative promoter leading to a short amino acid truncation at the N-terminus and omission of the myristoylation site, present in 1b. Abl proteins show a modular organisation similar to that of members of the Src family and are characterised by a unique N-terminus followed by a Src Homology 3 (SH3), a Src Homology 2 (SH2) domain and the catalytic core (Figure 1). However, the short C-terminus tail present in Src is replaced here by a large and unique sequence encoded by the last exon. This C-terminus includes F- (filamentous) and G- (globular) actin binding domains, nuclear export sequences (NES), and Pro-rich sequences with affinity for SH3-contanining proteins. In addition, c-Abl contains nuclear localisation signals (NLS) and DNA binding sequences important for nuclear functions (Pendergast, 2002). Similarly to most tyrosine kinases, the Abl family comprises oncogenic forms that exhibit strict cytoplasmic localisation and a deregulated kinase activity. These include the retroviral oncoprotein v-Abl of the Abelson murine leukaemia virus and the human Bcr-Abl fusion oncoprotein implicated in human chronic myeloid leukaemia and in a subset of lymphocytic leukaemias (Roche & Courtneidge, 1997).

Abl proteins play important roles in cell homeostasis as determined by gene knock-out experiments in mice. *Abl* ablation induces pleiotropic defects which cause postpartum mortality as well as lymphopenia and osteoporosis in the surviving animals, while *Arg* inactivation induces behavioural alterations (Schwartzberg et al., 1991; Tybulewicz et al., 1991). Interestingly, double gene disruption induces embryonic lethality at E9 indicating that,

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like for the Src members, c-Abl and Arg have important redundant functions during embryogenesis, but unique, specific roles later in development (Koleske et al., 1998).

c-Abl shuttles between the nuclear and cytoplasmic compartments. The role of nuclear c-Abl has been largely documented (Van Etten, 1999). Indeed it modulates the cellular response induced by DNA damage, and has been implicated in cell growth inhibition and promotion of apoptosis. In contrast, the role of cytoplasmic c-Abl has not been thoroughly described. Genetic and biochemical analysis point to a central role for cytoplasmic c-Abl in morphogenesis and F-actin dynamics (Woodring et al., 2003). In addition, recent reports suggest a role in signalling induced by extracellular stimuli. The first evidence has been provided by JY Wang et al. who reported activation of a cytoplasmic pool of c-Abl upon integrin engagement (Lewis et al., 1996). Later, Pendergast et al. showed activation of cytoplasmic c-Abl upon growth factors stimulation (Plattner et al., 1999). Since then, several reports have placed c-Abl in different cytoplasmic signalling cascades induced by various extracellular stimuli (Rhee et al., 2002; Woodring et al., 2003; Zipfel et al., 2004; Daniels et al., 2004; Ushio-Fukai et al., 2005; Noren et al., 2006). Here, we will focus on the current understanding of the function of cytoplasmic c-Abl in growth factor receptor signalling and we will discuss its role in solid tumours as well.

Regulation of c-Abl

c-Abl function is tightly controlled in order to prevent oncogenic activity. Similarly to Src (Boggon & Eck, 2004), c-Abl is regulated by intramolecular interactions that keep the kinase domain in a closed and inactive conformation (Figure 2). Strong evidences in favour of this self-inhibitory mechanism have been provided by recent structural and biochemical analysises which are discussed in detail by Hantschel and Superti-Furga (2004). c-Abl closed conformation implicates the interaction between the SH3 domain and the Pro-rich sequence present in SH2-kinase linker (Barila & Superti-Furga, 1998), as previously described for Src (Gonfloni et al., 1997; Williams et al., 1997; Xu et al., 1997). Additionally, c-Abl regulation implicates unique interactions that involve the SH2 domain, the myristoylation site and the first 80 N-terminal amino acids of the 1b protein, that Pluk et al. named the “Cap” (Pluk et al., 2002; Hantschel et al., 2003; Nagar et al., 2003). The SH2 domain interacts with the carboxy-terminal lobe of the kinase domain, while the myristoyl binds to a deep hydrophobic pocket present in the same domain. An alternative mechanism is however suggested for the non-myristoylated 1a isoform. The Cap region interacts with the SH3-SH2 connector and strengthens its clamp function to maintain the kinase domain in an inactive conformation

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(Nagar et al., 2006). All these interactions participate in the kinase auto-inhibition. Opening this conformation by any means is predicted to induce catalytic activation. Accordingly, SH2 and SH3 binders can induce c-Abl activation, and disruption of these interactions by mutation leads to transforming activity, as observed in oncogenic Abl alleles (Figure 1) (Hantschel & Superti-Furga, 2004). Additionally, these data uncovers an unsuspected function for N-myristoylation. This peptidic acylation is thought to be important for protein plasma membrane localisation (Resh, 1999). However, in the present case, it does not modify c-Abl sub-cellular localisation but rather regulates its catalytic activity in vivo (Hantschel et al., 2003). This suggests that myristoyl-transferase/deacylases are potentially important regulators of Abl in vivo.

c-Abl is regulated also by phosphorylation. A phospho-proteomic analysis identified at least 12 phosphorylation sites on Tyr residues, all clustered in the N-terminus (Hantschel & Superti-Furga, 2004). The function of Tyr245 and Tyr412 has been thoroughly analysed and mutagenesis has confirmed that these phosphorylation sites are required for c-Abl activation. Nevertheless, the nature of the involved kinase(s) has not been clearly established. While c-Abl may achieve phosphorylation *in trans* (Brasher & Van Etten, 2000; Dorey et al., 2001), other kinases could also ensure phosphorylation, the best candidates being SFK (Plattner et al., 1999; Dorey et al., 2001; Furstoss et al., 2002 ; Tanis et al., 2003). Moreover, c-Abl is phosphorylated also on Ser and Thr residues that are involved in the catalytic regulation (Hantschel et al., 2003). For example, a structural analysis identified a Ser residue in the Cap region that, upon phosphorylation by a yet unknown kinase, makes contact with the SH3-SH2 connector (Nagar et al., 2006). Similarly, the Serine/Threonine p21-Activated Kinase 2 (PAK2) has been shown to interact with and activate c-Abl via phosphorylation of Ser637 and 638 (Jung et al., 2008). Overall, these data suggest that phosphorylation is an important and complex way for the in vivo regulation of c-Abl.

Activation by growth factors

Cytoplasmic c-Abl is activated by various growth factors. For example, a 2 to 3 fold increase in its activaty has been observed in quiescent fibroblasts stimulated by Platelet-Derived Growth Factor (PDGF) (Plattner et al., 1999). A similar activation has been reported for Arg (Plattner et al., 2004). Mutagenesis analysis has implicated two tyrosines present in the juxta-membrane region of the PDGF receptor (PDGFR), which, when phosphorylated, create a binding site for SFK. A role for these kinases has been confirmed by dominant

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negative and pharmacological approaches (Plattner et al., 1999; Furstoss et al., 2002). Mechanistically, c-Abl forms a complex with activated SFK [(Stanglmaier et al., 2003) and S. Roche, unpublished data] and PDGFR, through its Src binding site (Plattner et al., 2004). Although the role of SFK in c-Abl binding to PDGFR has not been demonstrated, the existence of a ternary tyrosine kinase complex PDGFR/Src/c-Abl has been suggested, enabling Src-induced c-Abl phosphorylation and activation. c-Abl phosphorylation may be, however, reduced by the tyrosine phosphatase and c-Abl interactor PSTPIP1. Accordingly, PDGF-induced c-Abl phosphorylation is increased in cells deficient in PSTPIP1 (Cong et al., 2000) or in cells treated with vanadate, a general tyrosine phosphatase inhibitor (Furstoss et al., 2002).

Unexpectedly, PDGF also uses Phospholipase C γ (PLCγ) to maximise c-Abl activation (Plattner et al., 2003). Remarkably, cellular depletion of the PLC substrate Phosphatidylinositol-4,5-bisphosphate (PIP_2) increases basal c-Abl activity by 20 folds, implicating this phosphoinositide as an important in vivo regulator of c-Abl. Therefore, Plattner et al. (2003) suggested that maximal c-Abl activation is mediated by PLCγ-induced modulation of PIP_2 levels. Moreover, it has been hypothesised that, in vivo, PIP_2 could modulate the F-actin regulation of c-Abl activity. Since F-actin interacts with and inhibits c-Abl kinase activity (Woodring et al., 2001), a decrease in PIP_2 level could alleviate such inhibitory mechanism. We propose, however, an alternative route that implicates the lipid second messenger Sphingosine 1 Phosphate (S1P) (Veracini et al., 2006). By associating with a seven transmembrane receptor encoded by endothelial differentiation genes (EDG), collectively known as the S1P-receptors, S1P may activate a heterotrimeric Gi protein/Src pathway allowing c-Abl activation. Interestingly, PDGF-induced S1P accumulation requires PLCγ (Olivera et al., 1999), which may explain, at least in part, the role of this phospholipase in c-Abl activation. How PDGF uses both Src and a PLCγ for c-Abl activation is not clearly established. One hypothesis involves the existence of two distinct cytoplasmic pools of c-Abl. Accordingly, the use of fluorescent reporters of c-Abl activities has uncovered two distinct populations in PDGF-stimulated cells: the first is associated with cytoplasmic structures and the second is concentrated in membrane ruffles (Ting et al., 2001). Interestingly, a similar situation has been reported for SFK with a pool present in the cholesterol-enriched plasma membrane domains caveolae, which regulates its association with the receptor, and another one associated with F-actin enriched ruffles that is regulated by PLCγ (Veracini et al., 2006). We, thus, propose a model where PDGF activates c-Abl in caveolae by associating with its

receptor and c-Abl in actinic structure through S1P signalling. Such activities may in turn induce distinct signalling cascades and cellular responses (Veracini et al., 2006).

c-Abl is also activated by members of the EGF receptor (EGFR) family (Jones et al., 2006; Plattner et al., 1999). The molecular mechanism underlying this process remains elusive. EGFR does not associate with SFK, whose role in c-Abl activation is questionable. Rather, activation may implicate an AblSH2-pTyr interaction. This hypothesis is supported by the finding of a high affinity binding site for AblSH2 in all members of the EGFR family (ErbB1-4) (Jones et al., 2006). Interestingly, these interactions have been observed in a high throughput protein micro array study, even though they were not predicted by Scansite in silico analysis. Consistent with this notion, c-Abl has been found co-associated with these receptors in various cell lines (Jones et al., 2006; Srinivasan & Plattner, 2006). We thus believe that EGFR activation may create an Abl-SH2 binding site that stabilises the kinase in an open and active conformation, a mechanism previously reported for PDGFR-induced Src activation.

c-Abl is also activated by mitogenic receptors of the non-tyrosine kinase type. These include Transforming Growth Factor beta (TGF-β) (Daniels et al., 2004; Wang et al., 2005) and Angiotensin subtype 1 (AT-1) receptors (Ushio-Fukai et al., 2005). TGF-β receptor is a Serine/Threonine kinase that uses adapters of the Smad family for signalling (Schmierer & Hill, 2007). However, in a subset of mesenchymal cells, it also induces c-Abl activation, via a mechanism independent of the Smad pathway. In these cells, c-Abl activation requires phosphoinositide 3' kinase (PI3K) activity and the Rac effector PAK2 (Wilkes & Leof, 2006). PAK2 probably stimulates c-Abl activity via protein interaction and phosphorylation, but this hypothesis needs to be confirmed in the context of TGF-β stimulation. The AT-1 receptor belongs to the family of G-protein coupled seven transmembrane receptors. By associating with Angiotensin-II, AT-1 activates c-Abl in vascular smooth muscle cells. Src likely mediates AT-1-induced c-Abl phosphorylation and kinase activation. Consistent with this idea, c-Abl forms a ternary complex with AT-1 and Src in caveolae (Ushio-Fukai et al., 2005).

c-Abl cytoplasmic signalling in normal cells

Mitogenesis

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Activation of cytoplasmic c-Abl plays important roles in cellular responses induced by growth factors, including promotion of DNA synthesis, F-actin assembly and receptor trafficking. Previous reports assigned a negative function for c-Abl during cell proliferation (Sawyers et al., 1994; Wen et al., 1996). However, c-Abl appears to have antagonistic function depending on its sub-cellular localisation. Accordingly, nuclear c-Abl can induce a G1 phase block of the cell cycle, while cytoplasmic c-Abl can promote mitogenesis (Vigneri & Wang, 2001). The positive mitogenic role of cytoplasmic c-Abl has been uncovered in *Abl* deficient cells. These cells exhibit a 4-6 hour delay in DNA synthesis following PDGF and serum induction (Plattner et al., 1999; Furstoss et al., 2002). The specific function of cytoplasmic c-Abl has been further confirmed by targeting its cytoplasmic activity without affecting the nuclear function. This has been achieved by microinjection of neutralising antibodies in the cytoplasm or expression of dominant negative, cytoplasmic forms of c-Abl (Furstoss et al., 2002). In both situations, mitogenesis was inhibited. This set of experiments has led to the conclusion that cytoplasmic c-Abl is required during mitogenesis. c-Abl has been then identified as an important effector of Src for mitogenic signalling (Furstoss et al., 2002), which is required for DNA synthesis (Figure 3). Src signalling does not impact on Ras activity, but rather induces a unique cascade that culminates in *c-myc* induction, important for DNA synthesis initiation (Bromann et al., 2004). Hence, growth factors such as PDGF use a tyrosine kinase signalling cascade (i.e., receptor/Src/c-Abl) to promote DNA synthesis.

c-Abl mitogenic substrates have not yet been identified, but they are likely to include regulators of small GTPases of the Rho family, especially Vav and Sos members (Matsuguchi et al., 1995; Chiariello et al., 2001; Sini et al., 2004). This notion is supported by the identification of the small GTPase Rac as an important transducer of Src/c-Abl and v-Abl mitogenic signals (Renshaw et al., 1996; Boureux et al., 2005). Rac further propagates signalling via the induction of NADPH oxidase and Jun N-terminal Kinase (JNK) activities to favour *c-myc* induction and cell cycle progression (Boureux et al., 2005). Finally, c-Abl mitogenic function is not modulated only by receptor tyrosine kinases. Specifically, c-Abl also regulates mitogenesis induced by TGF-β and AT-1 receptors (Daniels et al., 2004; Ushio-Fukai et al., 2005; Wang et al., 2005) through a signalling cascade outside the TGF-β–Smad pathway, but the nature of the downstream effectors is not known. In the case of AT-1, c-Abl participates in trans-phosphorylation of EGFR, implicated in the transmission of the signal.

F-actin assembly

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Abl kinases have important functions in actin cytoskeleton reorganisation. Their role in morphological processes has been illustrated by the significant defect in the actin latticework of *Abl/Arg*$^{-/-}$ mouse embryos (Koleske et al., 1998). In tissue culture models, Abl kinases regulate F-actin organisation needed for lamellipodia, filopodia and neurite extension. These morphological changes mediate cell adhesion, migration and neurogenesis (Woodring et al., 2003). Upon growth factor stimulation, a fraction of active c-Abl is localised at sites of F-actin assembly where lateral ruffles are formed (Ting et al., 2001). It is believed that c-Abl might participate in this process, when promoted by EGF and PDGF, but the associated mechanism has not been further investigated (Sini et al., 2004; Sossey-Alaoui et al., 2007; Stuart et al., 2006). Additionally, c-Abl co-localises with F-actin structures at the dorsal surface of the cell, where circular ruffles are formed. These ruffles are also called waves because of their transient assembly (Buccione et al., 2004). The role of this actinic rearrangement has been puzzling for several decades as it could be specific of the culture conditions. Nonetheless, it has been suggested to be an emanation of a motile state induction, including cytoplasmic remodelling and establishment of cell polarity. Since then, dorsal ruffles have been linked to various cellular processes, including macro-pinocytosis (Dowrick et al., 1993; Dharmawardhane et al., 2000), receptor endocytosis (Orth et al., 2006) and cell invasion (Suetsugu et al., 2003). While EGF and Hepatocyte Growth Factor (HGF) can induce waves in certain conditions, PDGF seems to be the most potent inducer, specifically in fibroblast-like cells. These signalling cascades have been partially elucidated and they implicate c-Abl. Indeed, fibroblasts from *Abl/Arg-/-* mouse embryos show a 8 fold reduction in dorsal ruffles formation (Plattner et al., 1999; Furstoss et al., 2002). Similar results have been obtained also with dominant negative approaches (Veracini et al., 2006). For this cellular response, PDGF may recruit an actinic pool of c-Abl activity which is induced by a S1P/Src pathway (Veracini et al., 2006). Therefore, PDGF uses a spatially regulated tyrosine kinase signalling cascade for F-actin organisation.

How Abl kinases regulate dorsal ruffles has been partially unravelled (Figure 4). c-Abl may function upstream, downstream and in parallel to Rac, a key regulator of F-actin assembly. A role for c-Abl upstream of Rac has been evidenced by the strong reduction of PDGF-induced Rac activation observed in *c-Abl* deficient cells (Sini et al., 2004; Boureux et al., 2005). However, it is yet unknown whether this reduction is linked to the pool of Rac involved in mitogenic signalling and/or in F-actin assembly. c-Abl is thought to regulate Rac activity by phosphorylation and activation of a Rac-specific Guanine Nucleotide Exchange

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Factor (Ras-GEF), a likely candidate being SOS-1. This Ras-GEF shows a specific activity towards Rac when in a complex with the PI3K, Eps8 and Abi-1 adaptors (Innocenti et al., 2002; Innocenti et al., 2003). Moreover, phosphorylation of SOS-1 by c-Abl increases this Rac specific activity (Sini et al., 2004). Evidences for a role of SOS-1 in c-Abl signalling are however lacking. c-Abl may also regulate the sub-cellular localisation of active Rac. For instance, c-Abl can restrain active Rac at dorsal membrane ruffles upon Integrin engagement, thus negatively regulating cell spreading and migration (Jin & Wang, 2007). Dynamin and the c-Abl substrate CrkII have been implicated in this sub-cellular process. This raises the attractive hypothesis that PDGF also recruits c-Abl for targeting active Rac at the dorsal part of the cell for local F-actin assembly. c-Abl may also act downstream and/or in parallel to Rac. It phosphorylates and/or interacts with Rac effectors and members of the Wiskott-Aldrich Syndrome protein-family Verprolin homologous (WAVE) complex including WAVE1-3 (Westphal et al., 2000; Stuart et al., 2006; Sossey-Alaoui et al., 2007) and Abi1/2 (Dai & Pendergast, 1995; Shi et al., 1995). These activated complexes can in turn stimulate Arp2/3 to nucleate actin and induce branching (Stradal & Scita, 2006). Interestingly, WAVE1 and 2 play important roles in dorsal ruffles formation (Suetsugu et al., 2003) by promoting actin polymerisation in this area of the cell. c-Abl may also act in parallel to Rac via phosphorylation and activation of Cortactin. By interacting with Arp2/3, F-actin and Dynamin, Cortactin orchestrates F-actin assembly (Krueger et al., 2003). While c-Abl phosphorylation is required for Cortactin function (Boyle et al., 2007), the underlying mechanism is currently unknown. In summary, these data reveal a complex, but important, role for c-Abl in F-actin assembly leading to dorsal ruffle formation.

Cell migration

c-Abl plays important functions in F-actin dynamics, yet its role in growth factor-induced cell motility is ill defined. An inhibitory role has been originally described during cell adhesion and migration when induced by the extracellular matrix. Accordingly, c-Abl increases F-actin microspikes, but reduces cell spreading, thus revealing a sensory function during cell adhesion (Woodring et al., 2002). A negative role has been suggested in cells stimulated by Collagen (Kain & Klemke, 2001) and HGF (Kain et al., 2003), although in the second case the implication of c-Abl has not yet been firmly established. c-Abl negative effect has been linked to phosphorylation of CrkII leading to p130Cas uncoupling and Rac inhibition. Curiously, the role of c-Abl in cell migration induced by other growth factors has remained elusive. Recently, Plattner et al. (2003) have suggested a positive role for c-Abl in

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the chemotactic response induced by PDGF. Accordingly, c-Abl has been firmly involved in dorsal ruffles induction, a process linked to cell invasion (Suetsugu et al., 2003). Indeed, cell invasion induced by PDGF is reduced in *c-Abl* deficient fibroblasts and restored by re-introduction of endogenous levels of cytoplasmic c-Abl (A. Sirvent and S. Roche, unpublished data). These observations support the view that growth factors use cytoplasmic c-Abl for the promotion of cell migration and invasion in contrast to Integrins.

Receptor endocytosis

Recent data revealed an unsuspected role for c-Abl in receptor endocytosis. This has been first illustrated by the capacity of active c-Abl to reduce EGFR endocytosis. This c-Abl function affects membrane translocation of the Cbl E3 ligase, which is involved in receptor ubiquitination and degradation (Tanos & Pendergast, 2006). The underlying mechanism has not yet been fully established, but recent data suggest a role for the c-Abl substrate Abi in Cbl binding and activity (Tanos & Pendergast, 2007). Additionally, oncogenic c-Abl phosphorylates EGFR at Tyr1193 and this affects receptor endocytosis too (Tanos & Pendergast, 2006). These data raise the interesting idea that deregulated c-Abl could participate in EGFR oncogenic signalling via reduction of receptor endocytosis and degradation, a situation frequently observed in human cancer (Polo et al., 2004). Unfortunately, the role of endogenous c-Abl has not been investigated in this model. Moreover, Nivens et al. identified a novel route for EGFR endocytosis via dorsal ruffles (Orth et al., 2006) that may implicate endogenous c-Abl as a positive regulator of EGFR endocytosis in these cells. Finally, a similar role for endogenous c-Abl has been suggested concerning the AT-1 receptor in caveolae (Ushio-Fukai et al., 2005). Specifically, c-Abl regulates AT-I and EGFR trafficking into and out of caveolae, respectively.

c-Abl cytoplasmic signalling in cancer cells

Deregulation

Cytoplasmic Abl kinases have been implicated in human cancer. They are frequently deregulated in human leukaemias where they drive neoplastic transformation and cancer progression (Krause & Van Etten, 2005). In chronic myeloid leukaemia, deregulation is ensured by translocation of *ABL1* next to the *BCR* gene generating the Bcr-Abl fusion oncoprotein with constitutive cytoplasmic kinase activity. Interestingly, recent observations indicate that c-Abl and Arg are also deregulated in solid tumours. For example, high cytoplasmic kinase activities have been detected in breast carcinomas (Srinivasan & Plattner,

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2006) and non small cell lung cancers (Rikova et al., 2007). An increase in protein levels has been reported in breast carcinomas (Srinivasan & Plattner, 2006) and anaplastic thyroid cancers (Podtcheko et al., 2003); furthermore, Arg over-expression has been correlated with colon carcinoma progression (Chen et al., 1999). Over-expression of c-Abl is however not sufficient and constitutive kinase activity requires protein phosphorylation. For instance, Src-induced c-Abl phosphorylation increases its cytoplasmic kinase activity by 10 folds (Plattner et al., 1999). In breast cancer cells, c-Abl phosphorylation is induced by plasma membrane tyrosine kinases including SFK, EGFR family members and Insulin Growth factor I Receptor (IGFI-R) (Srinivasan & Plattner, 2006; Sirvent et al., 2007; Srinivasan et al., 2007). Additional kinases might be involved in this process, such as the oncogenic receptor tyrosine kinase RET/ Papillary thyroid cancers 3 (PTC3) (Iavarone et al., 2006). While Abl fusion proteins have not been detected in solid cancers, recent data indicate that deregulation could be also caused by a genetic mechanism. This notion is supported by two high through-put genomic analyses of a large set of tumours and cancer cell lines (Greenman et al., 2007; Ruhe et al., 2007). These studies have allowed the identification of somatic mutations and/or sequence deletions in c-Abl transcripts. Interestingly, most of them are clustered in the Cap region, the SH2 and the kinase domains. Due to the important roles of these domains in catalytic auto-inhibition, these alleles might generate oncogenic Abl kinases. These mutations have been detected in skin, ovary, prostate, and colon tumours in addition to lung and breast cancers. It should be stressed that these genetic alterations are rare, an observation which supports the notion that c-Abl deregulation in these cancers primarily involves kinase phosphorylation/activation by upstream oncogenic tyrosine kinases.

Neoplastic transformation

Cytoplasmic c-Abl has been recently implicated in neoplastic transformation (Figure 5). For example, we have demonstrated the requirement of cytoplasmic c-Abl in Src transforming activity. c-Abl function depends on its kinase activity, implying phosphorylation of Tyr245 and Tyr412 (Sirvent et al., 2007). Surprisingly, catalytic activation also requires PI3K and PLC activities, suggesting a role for phosphoinositides in this biochemical process (S. Roche, unpublished data). In addition to Rac/JNK signalling, c-Abl induces an Extracellular Regulated Kinase 5 (ERK5) pathway necessary for maximal transforming activity (Sirvent et al., 2007). c-Abl induces ERK5 by protein stabilisation (Buschbeck et al., 2005) and/or catalytic activation (Sirvent et al., 2007) by a yet unknown mechanism. This pathway impacts on cell cycle progression via *c-fos* and Cyclin D induction (Kato et al., 1997;

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Mulloy et al., 2003) and cytoskeleton rearrangement (Barros & Marshall, 2005), both involved in cell transformation. Interestingly, an additional Abl/ERK8 pathway has been identified upon expression of the oncogenic tyrosine kinase RET/PTC3 (Iavarone et al., 2006), but the biological significance of ERK8 activation in oncogenesis remains elusive. c-Abl also regulates cell transformation induced by Serine/Threonine kinases. For instance, inhibition of Abl kinases abrogates the capacity of TGF-β to induce anchorage-independent growth of human fibroblasts (Wilkes & Leof, 2006). In this context, Integrins and Collagen expression are under the control of c-Abl, which may influence this cellular response. Abl kinases also regulate human cancer cell growth. This notion has been confirmed in cell lines from breast and anaplastic thyroid tumours (Podtcheko et al., 2003). Interestingly, c-Abl requirement has been mostly observed in breast cancer cells whose growth is dependent on SFK activities. Cell growth is under the control of Abl/Rac and Abl/ERK5 signalling pathways (Sirvent et al., 2007). Additionally, a role for c-Abl has been also proposed for the activation of Stat1 and Stat3 transcription factors (Srinivasan et al., 2007), which are important for breast cancer progression. However, the effect of c-Abl on Stat oncogenic activity has not been determined in these cells. Finally, c-Abl seems to be required for DNA synthesis induced by IGF-1 (Srinivasan et al., 2007).

Epithelial to Mesenchymal transition (EMT) and cell invasion

Abl kinases play important roles in cancer cell migration and invasion. Yang et al (2006) have documented an unanticipated role for cytoplasmic c-Abl in EMT. This cellular process includes loss of cell polarity and cell-cell contact followed by induction of a fibroblastoid morphology which is required for cell motility. EMT plays an important role during embryogenesis and possibly during cancer invasion (Thiery & Sleeman, 2006). In colon cancer cells, PDGF-induced EMT requires c-Abl activation. While not tested, Src may probably mediate this activation, since mutation of Src phosphorylation sites prevents EMT. Using a proteomic approach, Yang et al. (2006) have identified the p68 RNA helicase (p68) as an important substrate of c-Abl signalling. β-catenin is a key player in this process (Brembeck et al., 2006). In non stimulated cells, β-catenin is concentrated at adherent junctions by associating with E-cadherin. Scattering factors, such as Wnt, TGF-β, Notch and PDGF, induce β-catenin dissociation and translocation to the nucleus for transcription of EMT genes. When phosphorylated on Tyr593 by c-Abl, p68 displaces β-catenin from the Axin Destruction Complex, thereby promoting its nuclear function (Yang et al., 2006) (Figure

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6). Interestingly, the c-Abl/p68 axis seems to operate upon EGF and TFG-β stimulation and in non transformed cells as well, suggesting that it is a general and conserved pathway for EMT induction (Yang et al., 2006). Additionally, c-Abl can activate β-catenin function by direct phosphorylation, as demonstrated following activation of the Robo receptor (Rhee et al., 2002) and as suggested in anaplastic thyroid cancer cells (Rao et al., 2006). Finally, c-Abl can interact with the cytoplasmic tyrosine kinase Pyk2 to promote breast cancer cell migration (Zrihan-Licht et al., 2004). All this data predict a positive role for cytoplasmic c-Abl in neoplastic cell invasion. Indeed, c-Abl has been shown to regulate the invasive activity of aggressive breast cancers (Srinivasan & Plattner, 2006) and some thyroid cancer cells (Rao et al., 2006). Surprisingly, cytoplasmic c-Abl can also have a negative effect on neoplastic cell invasion. For instance, c-Abl regulates EphB4 tumour suppressive activity in human breast cancer (Noren et al., 2006). EphB4 belongs to the Eph family of tyrosine kinases and is involved in cellular path-finding, when bound to its membrane-associated ligand, Ephrin B2 (Pasquale, 2005). It has been shown that activated EphB4 utilises an Abl-CrkII pathway to inhibit invasion (Noren et al., 2006). Interestingly, EphB4 signalling is inhibited during early carcinogenesis (Noren et al., 2006), hence the idea that Abl kinases may actually promote cancer cell invasion.

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Therapeutic target

c-Abl has become an important therapeutic target in human chronic myeloid leukaemia. Several small inhibitors have been developed that aim at the ATP-binding pocket of this kinase. One of them, Imatinib, shows remarkable activity in vivo and has become the first-line treatment for Bcr-Abl$^+$ leukaemias (Krause & Van Etten, 2005). Although poorly validated in vivo (Podtcheko et al., 2003), the role of cytoplasmic c-Abl in neoplastic cell growth suggests that this class of inhibitors could be useful for the treatment of solid cancers as well. Most carcinoma cells are however rather resistant to Imatinib treatment in vitro, probably because c-Abl phosphorylation by SFK reduces Imatinib potency by 50 fold (Tanis et al., 2003). Hence, inhibitors that target phosphorylated c-Abl might be more promising. Several of them have been now developed, such as the dual Src/Abl inhibitor Dasatinib (Lombardo et al., 2004; Tokarski et al., 2006). In addition to leukaemias, this compound is expected to give a significant therapeutic response in solid tumours, especially those showing oncogenic Src/Abl activities, including lung and breast cancers. Indeed, a large phospho-proteomic survey has identified a subset of non small cell lung cancers with strong Src/Abl activities (Rikova et al., 2007). In the case of breast cancer, two independent studies have

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identified a subset of cancer cells with high sensitivity to Dasatinib (Finn et al., 2007; Huang et al., 2007). Those are mainly cells of basal origin that went through EMT and are triple negative for oestrogen, progesterone and ErbB2 receptors. This type of cancers has a poor prognosis and strong resistance to current therapy. Moreover, genomic analyses have delineated a molecular signature for Dasatinb sensitivity, that, remarkably, has been observed in other breast, lung and ovary primary tumours (Huang et al., 2007), predicting an activity of Dasatinib also in these cancers. While not yet validated in clinical studies, these observations suggest that targeting the Src/Abl oncogenic signalling cascade will be also of therapeutic value in solid cancers.

Concluding Remarks

Although c-Abl was identified more than 30 years ago (Wang et al., 1984), its role in cytoplasmic signalling has emerged only recently. This may be due to the assigned activity of c-Abl in the nucleus, which initially was thought to be incompatible with any cytoplasmic transducer function. However, c-Abl is now becoming a general regulator of signalling processes induced by a growing list of extra-cellular stimuli, viral and bacterial cellular infections being some of the most recent examples (Burton et al., 2003; Zipfel et al., 2004; Reeves et al., 2005; Coyne & Bergelson, 2006). The depicted role of c-Abl in growth factor responses illustrates the complex mechanisms underlying c-Abl cytoplasmic signalling. For instance, the intriguing impact of phosphoinositide on c-Abl activity in vivo (Plattner et al., 2003) has uncovered a novel cross-talk between tyrosine kinases and phosphoinositide signalling. The exact role of these lipids needs however further clarification. c-Abl also integrates a tyrosine kinase signalling cascade (Plattner et al., 1999; Furstoss et al., 2002) that in turn will induce still poorly identified phosphorylation events, which are nevertheless important for cellular responses. Large phospho-proteomic approaches are thus warranted to address the exact nature of the involved substrates and phosphorylation sites. An additional feature of this signalling process lies in the spatio-temporal regulation of c-Abl activity. This has been exemplified by the observation that targeting of active c-Abl in the nucleus leads to apoptosis (Vigneri & Wang, 2001). Moreover, the existence of distinct pools of active cytoplasmic c-Abl (Ting et al., 2001; Veracini et al., 2006) reinforces the notion of signalling specificity ensured by subcellular compartmentalisation. Hence, the mechanism underlying this process may govern signalling specificity and eventually the cellular outcome and this is an important issue to be addressed in the future. Indeed, deregulation of such mechanism is thought to dictate c-Abl function in human solid cancer. For example, inhibition of EphB4

signalling concomitantly with activation of SFK may trigger c-Abl oncogenic activity in breast cancers. This raises the exciting notion of c-Abl as a novel therapeutic target in a subset of solid cancers that need to be precisely identified. Since several c-Abl inhibitors are now available for clinical use, it is becoming timely important to further define c-Abl oncogenic activity together with the associated molecular signatures.

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References

**references of special interest*

Barila D and Superti-Furga G. (1998). An intramolecular SH3-domain interaction regulates c-Abl activity. *Nat Genet*, **18,** 280-2.

Barros JC and Marshall CJ. (2005). Activation of either ERK1/2 or ERK5 MAP kinase pathways can lead to disruption of the actin cytoskeleton. *J Cell Sci*, **118,** 1663-71.

Boggon TJ and Eck MJ. (2004). Structure and regulation of Src family kinases. *Oncogene*, **23,** 7918-27.

Boureux A, Furstoss O, Simon V and Roche S. (2005). c-Abl tyrosine kinase regulates a Rac/JNK and a Rac/Nox pathway for DNA synthesis and c-myc expression induced by growth factors. *J. Cell Science*, **118,** 3717-3726.

Boyle SN, Michaud GA, Schweitzer B, Predki PF and Koleske AJ. (2007). A critical role for cortactin phosphorylation by Abl-family kinases in PDGF-induced dorsal-wave formation. *Curr Biol*, **17,** 445-51.

Brasher BB and Van Etten RA. (2000). c-Abl has high intrinsic tyrosine kinase activity that is stimulated by mutation of the Src homology 3 domain and by autophosphorylation at two distinct regulatory tyrosines. *J Biol Chem*, **275,** 35631-7.

Brembeck FH, Rosario M and Birchmeier W. (2006). Balancing cell adhesion and Wnt signaling, the key role of beta-catenin. *Curr Opin Genet Dev*, **16,** 51-9.

Bromann PA, Korkaya H and Courtneidge SA. (2004). The interplay between Src family kinases and receptor tyrosine kinases. *Oncogene*, **23,** 7957-68.

Buccione R, Orth JD and McNiven MA. (2004). Foot and mouth: podosomes, invadopodia and circular dorsal ruffles. *Nat Rev Mol Cell Biol*, **5,** 647-57.

hal-00346793, version 1 - 12 Dec 2008

Burton EA, Plattner R and Pendergast AM. (2003). Abl tyrosine kinases are required for infection by Shigella flexneri. *EMBO J*, **22,** 5471-9.

Buschbeck M, Hofbauer S, Di Croce L, Keri G and Ullrich A. (2005). Abl-kinase-sensitive levels of ERK5 and its intrinsic basal activity contribute to leukaemia cell survival. *EMBO Rep*, **6,** 63-9.

Chen WS, Kung HJ, Yang WK and Lin W. (1999). Comparative tyrosine-kinase profiles in colorectal cancers: enhanced arg expression in carcinoma as compared with adenoma and normal mucosa. *Int J Cancer*, **83,** 579-84.

Chiariello M, Marinissen MJ and Gutkind JS. (2001). Regulation of c-myc expression by PDGF through Rho GTPases. *Nat Cell Biol*, **3,** 580-6.

Cong F, Spencer S, Cote JF, Wu Y, Tremblay ML, Lasky LA and Goff SP. (2000). Cytoskeletal protein PSTPIP1 directs the PEST-type protein tyrosine phosphatase to the c-Abl kinase to mediate Abl dephosphorylation. *Mol Cell*, **6,** 1413-23.

Coyne CB and Bergelson JM. (2006). Virus-induced Abl and Fyn kinase signals permit coxsackievirus entry through epithelial tight junctions. *Cell*, **124,** 119-31.

Dai Z and Pendergast AM. (1995). Abi-2, a novel SH3-containing protein interacts with the c-Abl tyrosine kinase and modulates c-Abl transforming activity. *Genes Dev*, **9,** 2569-82.

***Daniels CE, Wilkes MC, Edens M, Kottom TJ, Murphy SJ, Limper AH and Leof EB**. (2004). Imatinib mesylate inhibits the profibrogenic activity of TGF-beta and prevents bleomycin-mediated lung fibrosis. *J Clin Invest*, **114,** 1308-16.

Dharmawardhane S, Schurmann A, Sells MA, Chernoff J, Schmid SL and Bokoch GM. (2000). Regulation of macropinocytosis by p21-activated kinase-1. *Mol Biol Cell*, **11,** 3341-52.

hal-00346793, version 1 - 12 Dec 2008

Dorey K, Engen J, Kretzschmar J, Wilm M, Neubard G, Schindler T and Superti-Furga G. (2001). phosphorylation and structure-based functional studies reveal a positive and a negative role for the activation loop of the c-Abl tyrosine kinase. *Oncogene*, **20,** 8075-8084.

Dowrick P, Kenworthy P, McCann B and Warn R. (1993). Circular ruffle formation and closure lead to macropinocytosis in hepatocyte growth factor/scatter factor-treated cells. *Eur J Cell Biol*, **61,** 44-53.

Finn RS, Dering J, Ginther C, Wilson CA, Glaspy P, Tchekmedyian N and Slamon DJ. (2007). Dasatinib, an orally active small molecule inhibitor of both the src and abl kinases, selectively inhibits growth of basal-type/"triple-negative" breast cancer cell lines growing in vitro. *Breast Cancer Res Treat*, **105,** 319-26.

***Furstoss O, Dorey K, Simon V, Barilla D, Superti-Furga G and Roche S.** (2002). c-Abl is an effector of Src for growth factor-induced c-myc expression and DNA synthesis. *EMBO J*, **27,** 514-524.

Gonfloni S, Williams JC, Hattula K, Weijland A, Wierenga RK and Superti-Furga G. (1997). The role of the linker between the SH2 domain and catalytic domain in the regulation and function of Src. *EMBO J*, **16,** 7261-71.

Greenman C, Stephens P, Smith R, Dalgliesh GL, Hunter C, Bignell G, Davies H, Teague J, Butler A, Stevens C, Edkins S, O'Meara S, Vastrik I, Schmidt EE, Avis T, Barthorpe S, Bhamra G, Buck G, Choudhury B, Clements J, Cole J, Dicks E, Forbes S, Gray K, Halliday K, Harrison R, Hills K, Hinton J, Jenkinson A, Jones D, Menzies A, Mironenko T, Perry J, Raine K, Richardson D, Shepherd R, Small A, Tofts C, Varian J, Webb T, West S, Widaa S, Yates A, Cahill DP, Louis DN, Goldstraw P, Nicholson AG, Brasseur F, Looijenga L, Weber BL, Chiew YE, DeFazio A, Greaves MF, Green AR, Campbell P, Birney E, Easton DF, Chenevix-Trench G, Tan MH, Khoo SK, Teh BT, Yuen ST, Leung SY, Wooster R, Futreal PA and Stratton MR. (2007). Patterns of somatic mutation in human cancer genomes. *Nature*, **446,** 153-8.

hal-00346793, version 1 - 12 Dec 2008

***Hantschel O, Nagar B, Guettler S, Kretzschmar J, Dorey K, Kuriyan J and Superti-Furga G**. (2003). A myristoyl/phosphotyrosine switch regulates c-Abl. *Cell*, **112,** 845-57.

Hantschel O and Superti-Furga G. (2004). Regulation of the c-Abl and Bcr-Abl tyrosine kinases. *Nat Rev Mol Cell Biol*, **5,** 33-44.

Huang F, Reeves K, Han X, Fairchild C, Platero S, Wong TW, Lee F, Shaw P and Clark E. (2007). Identification of candidate molecular markers predicting sensitivity in solid tumors to dasatinib: rationale for patient selection. *Cancer Res*, **67,** 2226-38.

Iavarone C, Acunzo M, Carlomagno F, Catania A, Melillo RM, Carlomagno SM, Santoro M and Chiariello M. (2006). Activation of the Erk8 mitogen-activated protein (MAP) kinase by RET/PTC3, a constitutively active form of the RET proto-oncogene. *J Biol Chem*, **281,** 10567-76.

Innocenti M, Frittoli E, Ponzanelli I, Falck JR, Brachmann SM, Di Fiore PP and Scita G. (2003). Phosphoinositide 3-kinase activates Rac by entering in a complex with Eps8, Abi1, and Sos-1. *J Cell Biol*, **160,** 17-23.

Innocenti M, Tenca P, Frittoli E, Faretta M, Tocchetti A, Di Fiore PP and Scita G. (2002). Mechanisms through which Sos-1 coordinates the activation of Ras and Rac. *J Cell Biol*, **156,** 125-36.

Jin H and Wang JY. (2007). Abl tyrosine kinase promotes dorsal ruffles but restrains lamellipodia extension during cell spreading on fibronectin. *Mol Biol Cell*, **18,** 4143-54.

Jones RB, Gordus A, Krall JA and MacBeath G. (2006). A quantitative protein interaction network for the ErbB receptors using protein microarrays. *Nature*, **439,** 168-74.

Jung JH, Pendergast AM, Zipfel PA and Traugh JA. (2008). Phosphorylation of c-Abl by Protein Kinase Pak2 Regulates Differential Binding of ABI2 and CRK. *Biochemistry*, **47,** 1094-1104.

hal-00346793, version 1 - 12 Dec 2008

Kain KH, Gooch S and Klemke RL. (2003). Cytoplasmic c-Abl provides a molecular 'Rheostat' controlling carcinoma cell survival and invasion. *Oncogene*, **22,** 6071-80.

Kain KH and Klemke RL. (2001). Inhibition of cell migration by Abl family tyrosine kinases through uncoupling of Crk-CAS complexes. *J Biol Chem*, **276,** 16185-92.

Kato Y, Kravchenko VV, Tapping RI, Han J, Ulevitch RJ and Lee JD. (1997). BMK1/ERK5 regulates serum-induced early gene expression through transcription factor MEF2C. *Embo J*, **16,** 7054-66.

***Koleske AJ, Gifford AM, Scott ML, Nee M, Bronson RT, Miczek KA and Baltimore D**. (1998). Essential roles for the Abl and Arg tyrosine kinases in neurulation. *Neuron*, **21,** 1259-72.

Krause DS and Van Etten RA. (2005). Tyrosine kinases as targets for cancer therapy. *N Engl J Med*, **353,** 172-87.

Krueger EW, Orth JD, Cao H and McNiven MA. (2003). A dynamin-cortactin-Arp2/3 complex mediates actin reorganization in growth factor-stimulated cells. *Mol Biol Cell*, **14,** 1085-96.

Lewis JM, Baskaran R, Taagepera S, Schwartz MA and Wang JY. (1996). Integrin regulation of c-Abl tyrosine kinase activity and cytoplasmic-nuclear transport. *Proc Natl Acad Sci U S A*, **93,** 15174-9.

Lombardo LJ, Lee FY, Chen P, Norris D, Barrish JC, Behnia K, Castaneda S, Cornelius LA, Das J, Doweyko AM, Fairchild C, Hunt JT, Inigo I, Johnston K, Kamath A, Kan D, Klei H, Marathe P, Pang S, Peterson R, Pitt S, Schieven GL, Schmidt RJ, Tokarski J, Wen ML, Wityak J and Borzilleri RM. (2004). Discovery of N-(2-chloro-6-methyl- phenyl)-2-(6-(4-(2-hydroxyethyl)- piperazin-1-yl)-2-methylpyrimidin-4- ylamino)thiazole-5-carboxamide (BMS-354825), a dual Src/Abl kinase inhibitor with potent antitumor activity in preclinical assays. *J Med Chem*, **47,** 6658-61.

hal-00346793, version 1 - 12 Dec 2008

Manning G, Whyte DB, Martinez R, Hunter T and Sudarsanam S. (2002). The protein kinase complement of the human genome. *Science*, **298,** 1912-34.

Matsuguchi T, Inhorn RC, Carlesso N, Xu G, Druker B and Griffin JD. (1995). Tyrosine phosphorylation of p95Vav in myeloid cells is regulated by GM-CSF, IL-3 and steel factor and is constitutively increased by p210BCR/ABL. *Embo J*, **14,** 257-65.

Mulloy R, Salinas S, Philips A and Hipskind RA. (2003). Activation of cyclin D1 expression by the ERK5 cascade. *Oncogene*, **22,** 5387-98.

Nagar B, Hantschel O, Seeliger M, Davies JM, Weis WI, Superti-Furga G and Kuriyan J. (2006). Organization of the SH3-SH2 unit in active and inactive forms of the c-Abl tyrosine kinase. *Mol Cell*, **21,** 787-98.

Nagar B, Hantschel O, Young MA, Scheffzek K, Veach D, Bornmann W, Clarkson B, Superti-Furga G and Kuriyan J. (2003). Structural basis for the autoinhibition of c-Abl tyrosine kinase. *Cell*, **112,** 859-71.

Noren NK, Foos G, Hauser CA and Pasquale EB. (2006). The EphB4 receptor suppresses breast cancer cell tumorigenicity through an Abl-Crk pathway. *Nat Cell Biol*, **8,** 815-25.

Olivera A, Edsall L, Poulton S, Kazlauskas A and Spiegel S. (1999). Platelet-derived growth factor-induced activation of sphingosine kinase requires phosphorylation of the PDGF receptor tyrosine residue responsible for binding of PLCgamma. *Faseb J*, **13,** 1593-600.

Orth JD, Krueger EW, Weller SG and McNiven MA. (2006). A novel endocytic mechanism of epidermal growth factor receptor sequestration and internalization. *Cancer Res*, **66,** 3603-10.

Pasquale EB. (2005). Eph receptor signalling casts a wide net on cell behaviour. *Nat Rev Mol Cell Biol*, **6,** 462-75.

hal-00346793, version 1 - 12 Dec 2008

Pendergast AM. (2002). The Abl family kinases: mechanisms of regulation and signaling. *Adv Cancer Res.*, **85,** 51-100.

***Plattner R, Irvin BJ, Guo S, Blackburn K, Kazlauskas A, Abraham RT, York JD and Pendergast AM**. (2003). A new link between the c-Abl tyrosine kinase and phosphoinositide signalling through PLC-gamma1. *Nat Cell Biol*, **5,** 309-19.

***Plattner R, Kadlec L, DeMali KA, Kazlauskas A and Pendergast AM**. (1999). c-Abl is activated by growth factors and Src family kinases and has a role in the cellular response to PDGF. *Genes Dev*, **13,** 2400-11.

Plattner R, Koleske AJ, Kazlauskas A and Pendergast AM. (2004). Bidirectional signaling links the Abelson kinases to the platelet-derived growth factor receptor. *Mol Cell Biol*, **24,** 2573-83.

***Pluk H, Dorey K and Superti-Furga G**. (2002). Autoinhibition of c-Abl. *Cell*, **108,** 247-59.

Podtcheko A, Ohtsuru A, Tsuda S, Namba H, Saenko V, Nakashima M, Mitsutake N, Kanda S, Kurebayashi J and Yamashita S. (2003). The selective tyrosine kinase inhibitor, STI571, inhibits growth of anaplastic thyroid cancer cells. *J Clin Endocrinol Metab*, **88,** 1889-96.

Polo S, Pece S and Di Fiore PP. (2004). Endocytosis and cancer. *Curr Opin Cell Biol*, **16,** 156-61.

Rao AS, Kremenevskaja N, von Wasielewski R, Jakubcakova V, Kant S, Resch J and Brabant G. (2006). Wnt/beta-catenin signaling mediates antineoplastic effects of imatinib mesylate (gleevec) in anaplastic thyroid cancer. *J Clin Endocrinol Metab*, **91,** 159-68.

Reeves PM, Bommarius B, Lebeis S, McNulty S, Christensen J, Swimm A, Chahroudi A, Chavan R, Feinberg MB, Veach D, Bornmann W, Sherman M and Kalman D. (2005).

hal-00346793, version 1 - 12 Dec 2008

Disabling poxvirus pathogenesis by inhibition of Abl-family tyrosine kinases. *Nat Med*, **11,** 731-9.

Renshaw MW, Lea-Chou E and Wang JY. (1996). Rac is required for v-Abl tyrosine kinase to activate mitogenesis. *Curr Biol*, **6,** 76-83.

Resh MD. (1999). Fatty acylation of proteins: new insights into membrane targeting of myristoylated and palmitoylated proteins. *Biochim Biophys Acta*, **1451,** 1-16.

Rhee J, Mahfooz NS, Arregui C, Lilien J, Balsamo J and VanBerkum MF. (2002). Activation of the repulsive receptor Roundabout inhibits N-cadherin-mediated cell adhesion. *Nat Cell Biol*, **4,** 798-805.

Rikova K, Guo A, Zeng Q, Possemato A, Yu J, Haack H, Nardone J, Lee K, Reeves C, Li Y, Hu Y, Tan Z, Stokes M, Sullivan L, Mitchell J, Wetzel R, Macneill J, Ren JM, Yuan J, Bakalarski CE, Villen J, Kornhauser JM, Smith B, Li D, Zhou X, Gygi SP, Gu TL, Polakiewicz RD, Rush J and Comb MJ. (2007). Global survey of phosphotyrosine signaling identifies oncogenic kinases in lung cancer. *Cell*, **131,** 1190-203.

Roche S and Courtneidge SA. (1997). Oncogenic cytoplasmic tyrosine kinases. In *Oncogene and Tumour Supressors* (Eds G. Peters and K.H. Vousden) pp87-119. Oxford University Press

Ruhe JE, Streit S, Hart S, Wong CH, Specht K, Knyazev P, Knyazeva T, Tay LS, Loo HL, Foo P, Wong W, Pok S, Lim SJ, Ong H, Luo M, Ho HK, Peng K, Lee TC, Bezler M, Mann C, Gaertner S, Hoefler H, Iacobelli S, Peter S, Tay A, Brenner S, Venkatesh B and Ullrich A. (2007). Genetic alterations in the tyrosine kinase transcriptome of human cancer cell lines. *Cancer Res*, **67,** 11368-76.

Sawyers CL, McLaughlin J, Goga A, Havlik M and Witte O. (1994). The nuclear tyrosine kinase c-Abl negatively regulates cell growth. *Cell*, **77,** 121-31.

hal-00346793, version 1 - 12 Dec 2008

Schmierer B and Hill CS. (2007). TGFbeta-SMAD signal transduction: molecular specificity and functional flexibility. *Nat Rev Mol Cell Biol*, **8,** 970-82.

Schwartzberg PL, Stall AM, Hardin JD, Bowdish KS, Humaran T, Boast S, Harbison ML, Robertson EJ and Goff SP. (1991). Mice homozygous for the ablm1 mutation show poor viability and depletion of selected B and T cell populations. *Cell*, **65,** 1165-75.

Shi Y, Alin K and Goff SP. (1995). Abl-interactor-1, a novel SH3 protein binding to the carboxy-terminal portion of the Abl protein, suppresses v-abl transforming activity. *Genes Dev*, **9,** 2583-97.

Sini P, Cannas A, Koleske AJ, Di Fiore PP and Scita G. (2004). Abl-dependent tyrosine phosphorylation of Sos-1 mediates growth-factor-induced Rac activation. *Nat Cell Biol*, **6,** 268-74 Epub 2004 Feb 22.

***Sirvent A, Boureux A, Simon V, Leroy C and Roche S.** (2007). The tyrosine kinase Abl is required for Src-transforming activity in mouse fibroblasts and human breast cancer cells. *Oncogene*, **26,** 7313-23.

Sossey-Alaoui K, Li X and Cowell JK. (2007). c-Abl-mediated phosphorylation of WAVE3 is required for lamellipodia formation and cell migration. *J Biol Chem*, **282,** 26257-65.

***Srinivasan D and Plattner R.** (2006). Activation of Abl tyrosine kinases promotes invasion of aggressive breast cancer cells. *Cancer Res.*, **66,** 5648-55.

Srinivasan D, Sims JT and Plattner R. (2007). Aggressive breast cancer cells are dependent on activated Abl kinases for proliferation, anchorage-independent growth and survival. *Oncogene* in the press.

Stanglmaier M, Warmuth M, Kleinlein I, Reis S and Hallek M. (2003). The interaction of the Bcr-Abl tyrosine kinase with the Src kinase Hck is mediated by multiple binding domains. *Leukemia*, **17,** 283-9.

hal-00346793, version 1 - 12 Dec 2008

Stradal TE and Scita G. (2006). Protein complexes regulating Arp2/3-mediated actin assembly. *Curr Opin Cell Biol*, **18,** 4-10.

Stuart JR, Gonzalez FH, Kawai H and Yuan ZM. (2006). c-Abl interacts with the WAVE2 signaling complex to induce membrane ruffling and cell spreading. *J Biol Chem*, **281,** 31290-7.

***Suetsugu S, Yamazaki D, Kurisu S and Takenawa T**. (2003). Differential roles of WAVE1 and WAVE2 in dorsal and peripheral ruffle formation for fibroblast cell migration. *Dev Cell*, **5,** 595-609.

Tanis KQ, Veach D, Duewel HS, Bornmann WG and Koleske AJ. (2003). Two distinct phosphorylation pathways have additive effects on Abl family kinase activation. *Mol Cell Biol*, **23,** 3884-96.

Tanos B and Pendergast AM. (2006). Abl tyrosine kinase regulates endocytosis of the epidermal growth factor receptor. *J Biol Chem*, **281,** 32714-23.

Tanos BE and Pendergast AM. (2007). Abi-1 forms an epidermal growth factor-inducible complex with Cbl: role in receptor endocytosis. *Cell Signal*, **19,** 1602-9.

Thiery JP and Sleeman JP. (2006). Complex networks orchestrate epithelial-mesenchymal transitions. *Nat Rev Mol Cell Biol*, **7,** 131-42.

Thomas SM and Brugge JS. (1997). Cellular functions regulated by Src family kinases. *Annu Rev Cell Dev Biol*, **13,** 513-609.

Ting AY, Kain KH, Klemke RL and Tsien RY. (2001). Genetically encoded fluorescent reporters of protein tyrosine kinase activities in living cells. *Proc Natl Acad Sci U S A*, **98,** 15003-8.

hal-00346793, version 1 - 12 Dec 2008

Tokarski JS, Newitt JA, Chang CY, Cheng JD, Wittekind M, Kiefer SE, Kish K, Lee FY, Borzillerri R, Lombardo LJ, Xie D, Zhang Y and Klei HE. (2006). The structure of Dasatinib (BMS-354825) bound to activated ABL kinase domain elucidates its inhibitory activity against imatinib-resistant ABL mutants. *Cancer Res*, **66,** 5790-7.

Tybulewicz VL, Crawford CE, Jackson PK, Bronson RT and Mulligan RC. (1991). Neonatal lethality and lymphopenia in mice with a homozygous disruption of the c-abl proto-oncogene. *Cell*, **65,** 1153-63.

Ushio-Fukai M, Zuo L, Ikeda S, Tojo T, Patrushev NA and Alexander RW. (2005). cAbl tyrosine kinase mediates reactive oxygen species- and caveolin-dependent AT1 receptor signaling in vascular smooth muscle: role in vascular hypertrophy. *Circ Res*, **97,** 829-36.

Van Etten RA. (1999). Cycling, stressed-out and nervous: cellular functions of c-Abl. *Trends Cell Biol*, **9,** 179-86.

Veracini L, Franco M, Boureux A, Simon V, Roche S and Benistant C. (2006). Two distinct pools of Src family tyrosine kinases regulate PDGF-induced DNA synthesis and actin dorsal ruffles. *J Cell Sci*, **20,** 2921-34.

***Vigneri P and Wang JY.** (2001). Induction of apoptosis in chronic myelogenous leukemia cells through nuclear entrapment of BCR-ABL tyrosine kinase. *Nat Med*, **7,** 228-34.

Wang JY, Ledley F, Goff S, Lee R, Groner Y and Baltimore D. (1984). The mouse c-abl locus: molecular cloning and characterization. *Cell*, **36,** 349-56.

***Wang S, Wilkes MC, Leof EB and Hirschberg R.** (2005). Imatinib mesylate blocks a non-Smad TGF-beta pathway and reduces renal fibrogenesis in vivo. *Faseb J*, **19,** 1-11.

Wen ST, Jackson PK and Van Etten RA. (1996). The cytostatic function of c-Abl is controlled by multiple nuclear localisation signals and requires the p53 and Rb tumor suppressor gene products. *EMBO J*, **15,** 1583-95.

hal-00346793, version 1 - 12 Dec 2008

Westphal RS, Soderling SH, Alto NM, Langeberg LK and Scott JD. (2000). Scar/WAVE-1, a Wiskott-Aldrich syndrome protein, assembles an actin-associated multi-kinase scaffold. *EMBO J*, **19,** 4589-600.

Wilkes MC and Leof EB. (2006). Transforming growth factor beta activation of c-Abl is independent of receptor internalization and regulated by phosphatidylinositol 3-kinase and PAK2 in mesenchymal cultures. *J Biol Chem*, **281,** 27846-54.

Williams JC, Weijland A, Gonfloni S, Thompson A, Courtneidge SA, Superti-Furga G and Wierenga RK. (1997). The 2.35 A crystal structure of the inactivated form of chicken Src: a dynamic molecule with multiple regulatory interactions. *J Mol Biol*, **274,** 757-75.

Woodring PJ, Hunter T and Wang JY. (2001). Inhibition of c-Abl tyrosine kinase activity by filamentous actin. *J Biol Chem*, **276,** 27104-10.

Woodring PJ, Hunter T and Wang JY. (2003). Regulation of F-actin-dependent processes by the Abl family of tyrosine kinases. *J Cell Sci*, **116,** 2613-26

***Woodring PJ, Litwack ED, O'Leary DD, Lucero GR, Wang JY and Hunter T.** (2002). Modulation of the F-actin cytoskeleton by c-Abl tyrosine kinase in cell spreading and neurite extension. *J Cell Biol*, **156,** 879-92.

Xu W, Harrison SC and Eck MJ. (1997). Three-dimensional structure of the tyrosine kinase c-Src. *Nature*, **385,** 595-602.

***Yang L, Lin C and Liu ZR.** (2006). P68 RNA helicase mediates PDGF-induced epithelial mesenchymal transition by displacing Axin from beta-catenin. *Cell*, **127,** 139-55.

Zipfel PA, Zhang W, Quiroz M and Pendergast AM. (2004). Requirement for Abl kinases in T cell receptor signaling. *Curr Biol*, **14,** 1222-31.

Zrihan-Licht S, Avraham S, Jiang S, Fu Y and Avraham HK. (2004). Coupling of RAFTK/Pyk2 kinase with c-Abl and their role in the migration of breast cancer cells. *Int J Oncol*, **24,** 153-9.

hal-00346793, version 1 - 12 Dec 2008

hal-00346793, version 1 - 12 Dec 2008

Acknowledgments

This work was supported by the CNRS and the Universities of Montpellier I and II. AS is supported by the SERVIER laboratories and INCa. CB and SR are supported by INSERM.

Abbreviations used in this review

Arg: Abl-related gene/ AT-I: Angiotensin receptor type I / EGF: Epidermal Growth Factor/ EGFR: EGF Receptor/ EMT: Epithelial to Mesenchyme Transition/ ERK: Extracellular-Regulated Kinase/ HGF: Hepatocyte Growth Factor/ HGFR: HGF Receptor/ JNK: Jun N-terminal Kinase/ PAK: serine/threonine kinase p21-activated kinase/ PDGF: Platelet-Derived Growth Factor/ PDGFR: PDGF Receptor/ PIP2: Phosphatidylinositol-4,5-bisphosphate/ PI3K: phosphoinositide 3 kinase/ PLC: Phospholipase C/ S1P: Sphingosine 1 phosphate/ SFK: Src Family Kinase/ SH2: Src Homology region 2/ SH3: Src homology region 3/ TGF-β: Transforming Growth Factor-beta.

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Figure Legends

Figure 1. Modular structure of Abl kinases.

Comparison of the modular structures of Src and Abl kinases. Src and Abl kinases are composed of a variable N-terminus, followed by a SH3 and a SH2 domain, a linker sequence between the SH2 and the kinase domain (linker) and the catalytic core. Additionally, Src and Abl 1b proteins contain a consensus motif for N-terminal myristoylation (/\/\). In contrast to Src, the N-terminus of Abl proteins includes a conserved region called the Cap. Furthermore, the short C-terminus tail in Src is replaced by a large sequence in Abl proteins, which include Pro-rich stretches (PXXP), a DNA binding domain (DNA BD), three nuclear localisation sequences (NLS) in the case of Abl, one nuclear export sequence (NES) and G- (monomeric) and F- (filamentous) actin binding motives (Actin BD). Well-established regulatory phospho-Tyr residues are indicated. The oncogenic forms of v-Abl and Bcr-Abl are also depicted. They all contain modified N-termini that have lost the Cap region and present a viral gag sequence in the case of v-Abl and the N-terminal portion of the breakpoint cluster region protein for Bcr-Abl.

Figure 2. Model of Regulation of Abl kinases.

Inactive c-Abl is in a closed conformation that implicates several intra-molecular interactions: the SH3 domain with the PXXP motif in the linker, the SH2 domain with the C-terminal lobe of the kinase domain, the myristoylation with a hydrophobic pocket present in the C-terminus lobe and the Cap with the SH3-SH2 connector. Opening the conformation by any means, including by interaction with SH3- and/or SH2-binders as shown, induces catalytic activation. Phosphorylation of Tyr245 in the linker and of Tyr412 in the activation loop stabilises Abl in an open and active confirmation. The role of the C-terminus has not been taken into account in this model.

Figure 3. A model of how cytoplasmic c-Abl mediates growth factor mitogenic signalling.

Growth factors (GF) induce SFK activation in caveolae, allowing phosphorylation of c-Abl to increase its catalytic activity. c-Abl then activates a Rac/JNK and Rac/NADPH oxidase (Nox) pathways to induce *c-myc* expression, a transcription factor required for induction of DNA synthesis. JNK and Nox may favour *c-myc* induction through mRNA stabilisation and/or gene transcription. c-Abl substrates for Rac activation are currently unknown. SFK-induced *c-myc* implicates additional substrates (“X”) including Stat3 and Shc. GF also induce additional

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signalling cascades ("others"), such as the "Ras pathway" that may participate in mitogenesis through Myc protein stabilisation and expression of other genes. This set of events promotes G1 phase progression of the cell cycle leading to S phase entry and DNA synthesis.

Figure 4. A model of how cytoplasmic c-Abl mediates growth factor-induced F-actin assembly.

GF receptors recruit Sphingosine 1 Kinase (S1K) to the membrane through a PLCγ-dependent pathway for S1P formation. S1P further activates SFK outside caveolae through the EDG receptor coupled to a heterotrimeric Gi protein allowing cytoplasmic c-Abl activation. Active c-Abl signals upstream Rac for activation and sub-cellular localisation, downstream Rac via phosphorylation and activation of the WAVE complex for Arp2/3 stimulation, and in parallel to Rac via phosphorylation of the actin-binding protein Cortactin. Active WAVE complexes and Cortactin promote F-actin assembly and dorsal ruffle formation. Additional SFK and c-Abl substrates may also participate in this signalling process. Dorsal ruffles may then promote cell invasion. An example of dorsal ruffle is shown in human fibroblasts induced by PDGF and revealed by F-actin immuno-staining.

Figure 5. A model of how cytoplasmic c-Abl mediates oncogenic signalling.

Cytoplasmic c-Abl mediates cell transformation and neoplastic cell growth upon phosphorylation by upstream oncogenic tyrosine kinases, including SFK, and growth factor receptors, such as EGF and IGF-1 receptors. c-Abl can then transmit oncogenic signals by induction of ERK5 and Rac/JNK pathways, all required for cell transformation and neoplastic cell growth (both in anchorage dependent and independent conditions). Additionally, c-Abl may participate in Stat1/3 signalling which is also involved in these cellular processes.

Figure 6. A model of how cytoplasmic c-Abl mediates epithelial to mesenchymal transition induced by growth factors.

Cytoplasmic c-Abl mediates epithelial to mesenchymal transition (EMT) leading to cell migration and invasion upon induction by growth factors. Growth factors induce c-Abl activation through SFK-induced phosphorylation. c-Abl transmits EMT signals by induction of beta-catenin phosphorylation and/or association with phosphorylated p68/RNA helicase. The active and phosphorylated complex is displaced from the Auxin Destruction Complex

and translocates to the nucleus for EMT gene induction eventually leading to cell migration and invasion.

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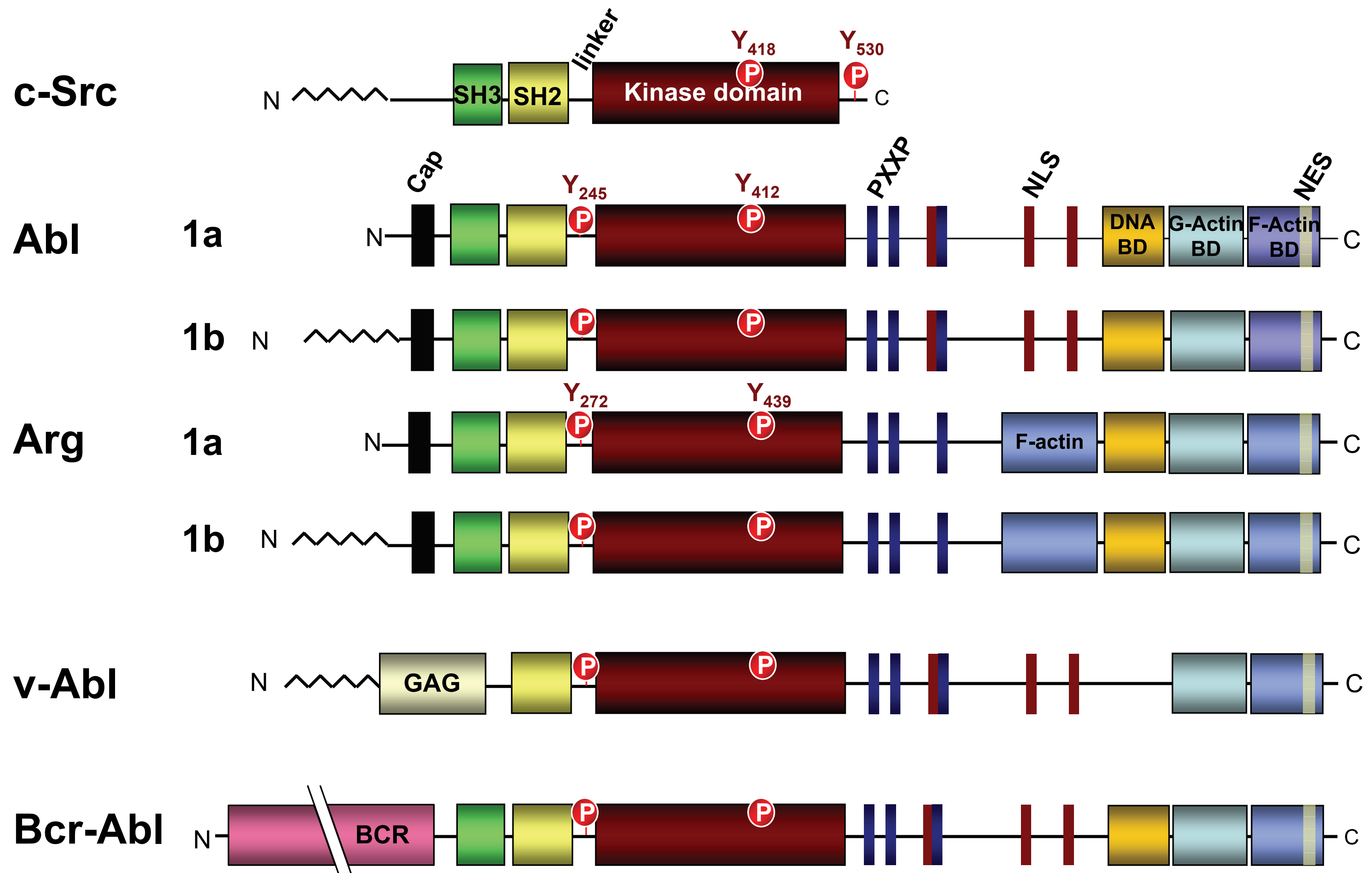

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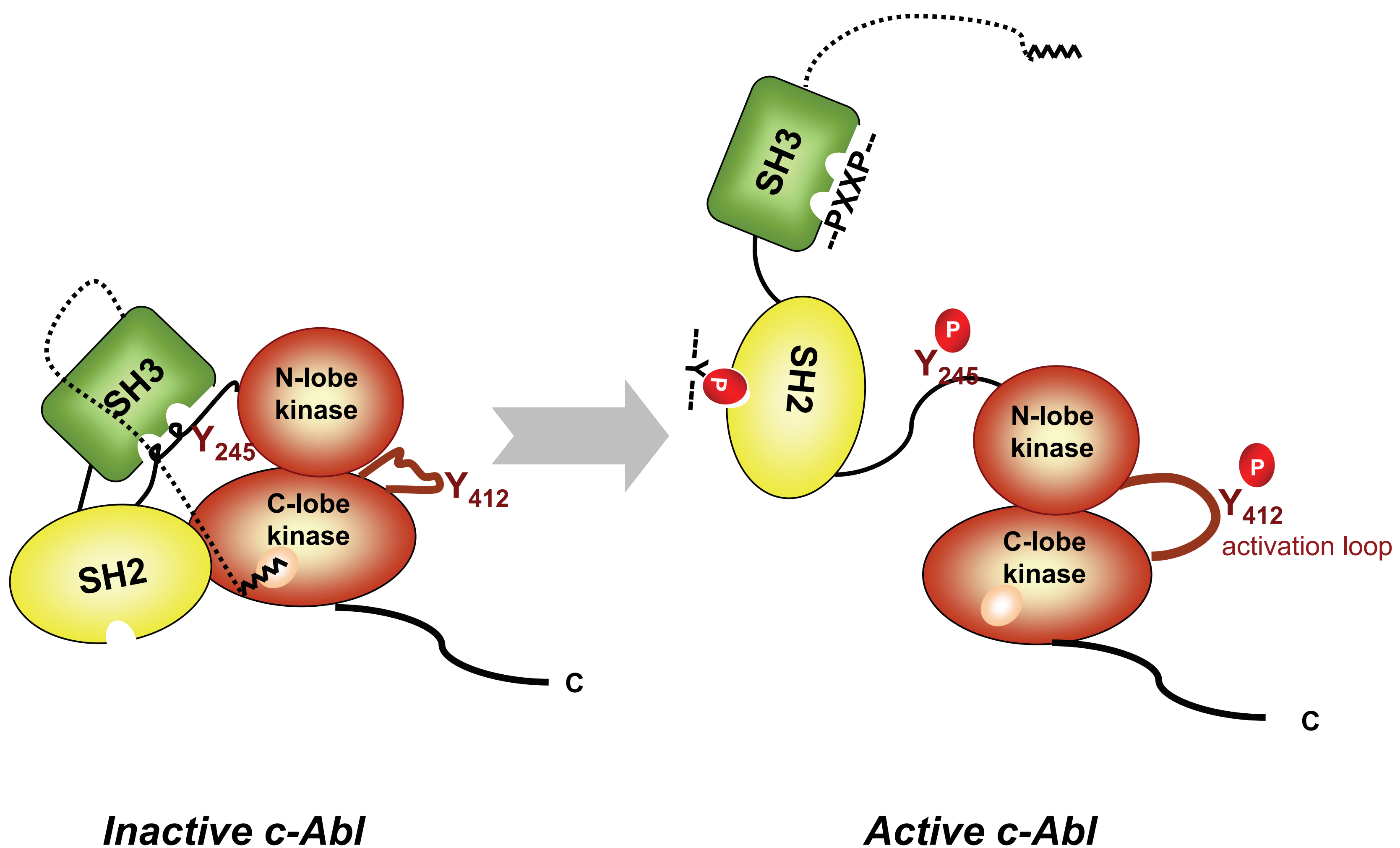

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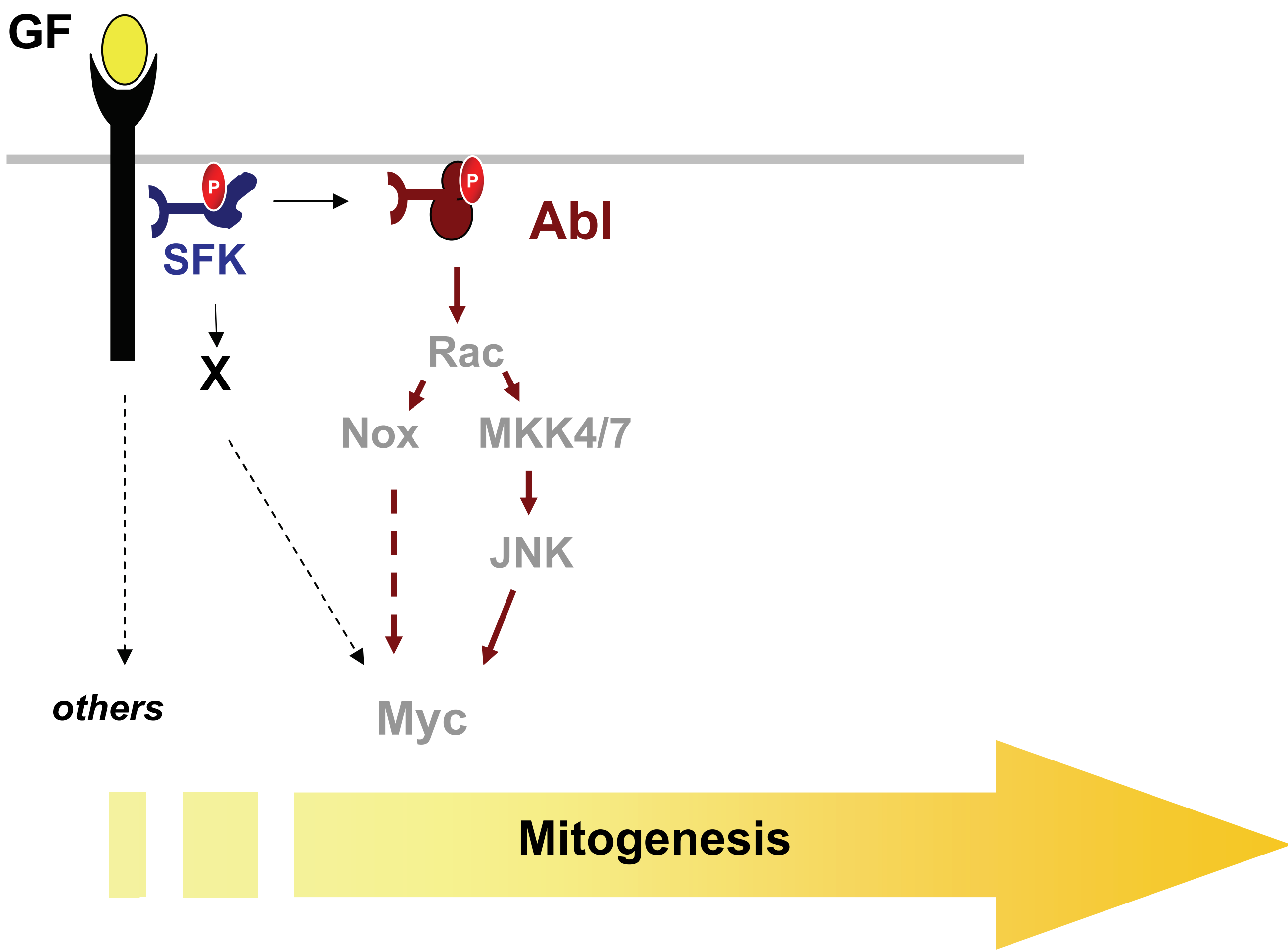

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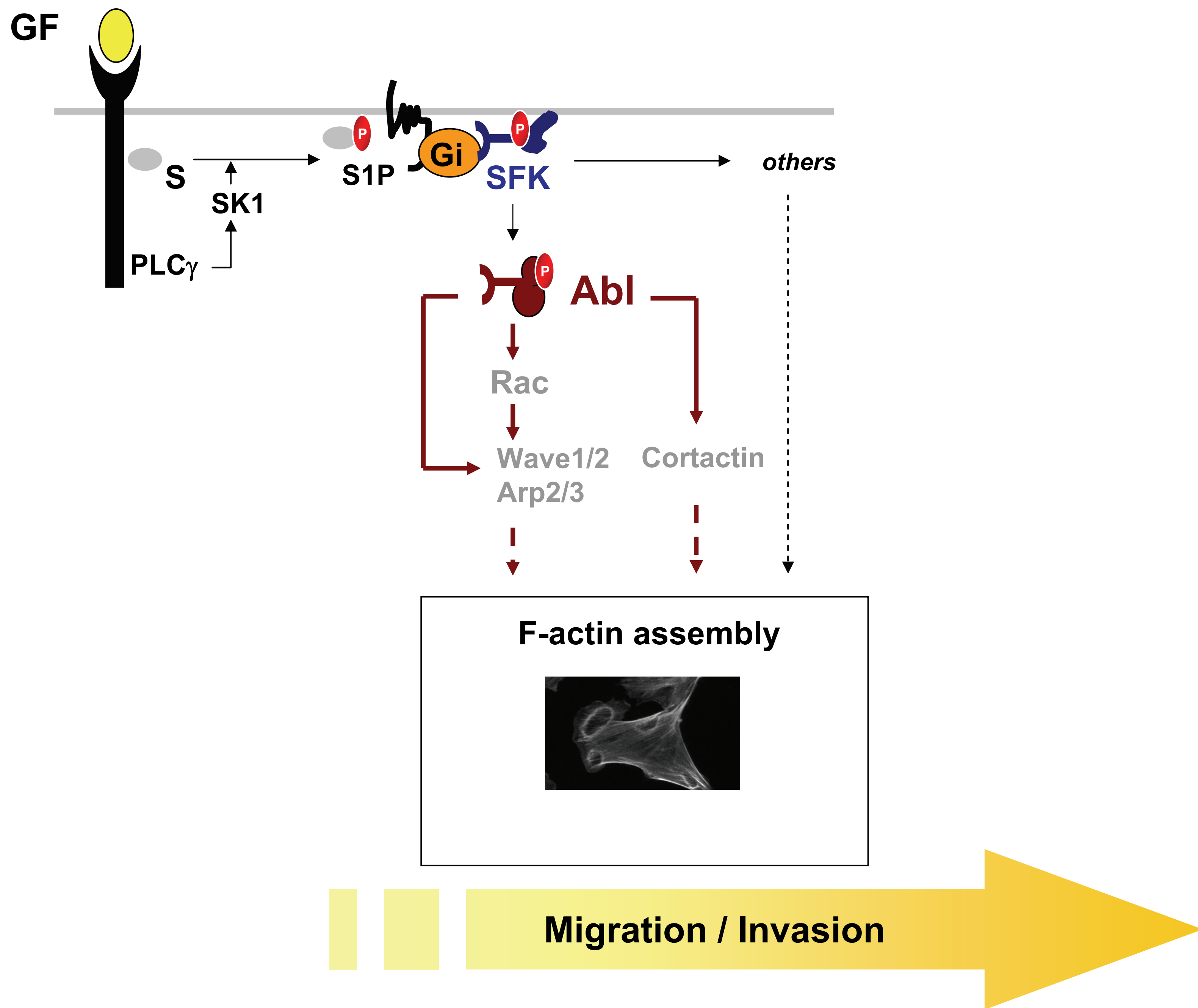
Sirvent et al. Figure 4



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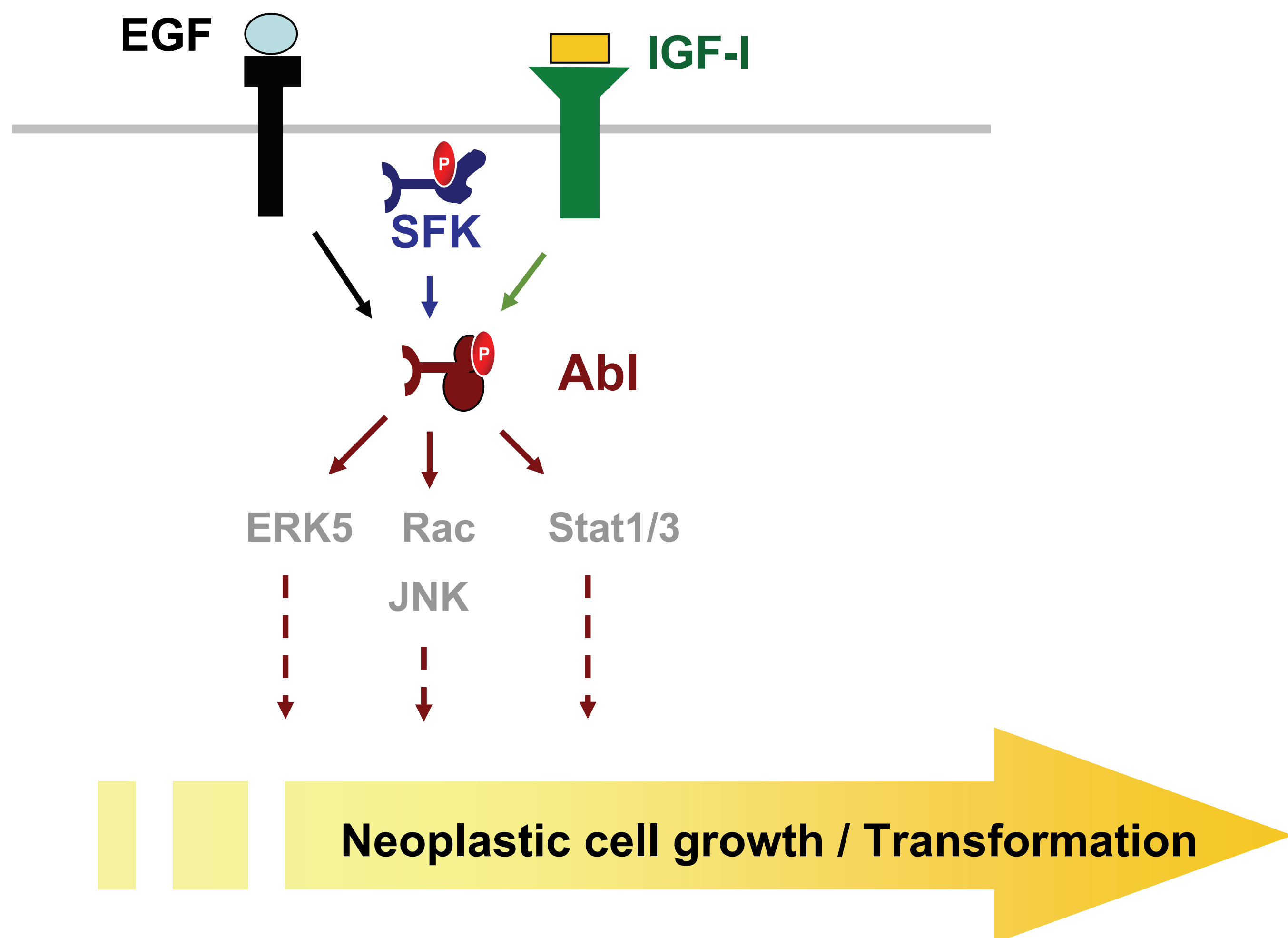
Sirvent et al. Figure 5



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Sirvent et al. Figure 6