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Negative Regulation of Haematopoiesis: Role of Inhibitory Adaptors

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1. Introduction

Cytokine signalling is initiated through ligand interaction with specific members of the cytokine receptor superfamily. The subsequent receptor oligomerization and conformational change result in activation of either an intrinsic kinase domain or receptor associated kinases, notably the Janus (JAK) family of cytoplasmic tyrosine kinases. The activated JAKs phosphorylate tyrosine residues in the receptor and subsequently downstream substrates, such as the signal transducers and activators of transcription (STAT) proteins. Once recruited to the receptor complex, STAT proteins are themselves phosphorylated on tyrosine, dimerize and translocate into the nucleus, where they activate the transcription of genes mediating cytokine-induced responses (Ortmann et al., 2000). Cytokines also activate other signaling cascades, such as the Ras/Mitogen-Activated Protein Kinase (MAPK) and the Phosphoinositide 3-kinase (PI3K)/Akt pathways. These cascades have been implicated in the proliferation, survival, and differentiation of several cell types in the haematopoietic system (Geest and Coffer, 2009; Leevers et al., 1999. However, all these signalling pathways require precise cellular control and their deregulation has been implicated in haematopoietic disorders, autoimmune and chronic inflammatory diseases and cancer, making it important to understand the mechanisms by which these cytokine-mediated signalling pathways are controlled (Schade et al., 2006; Khwaja, 2006).

It is therefore not surprising that multiple levels of control have evolved to finely modulate the threshold, magnitude and specific responses elicited by cytokine stimulation. This regulation is achieved through both positive and negative mechanisms. The aim of the present chapter is to review the current advances in the regulation of haematopoiesis, with special interest on inhibitory pathways. Understanding how haematopoiesis is modulated is essential to provide useful information on its physiological functioning, the pathological origin of many related haematological disorders and to yield potential therapeutic targets.

2. Regulation of cytokine signalling pathways: Role of adaptor proteins

Cytokine binding to their receptors results in tyrosine autophosphorylation of the associated tyrosine kinase and of the receptor cytoplasmic domain at sites where specific signalling molecules can bind. In this way, the cytoplasmic domain of these cytokine receptors serves

to initially localize the signalling response to the plasma membrane. It is the combination of the signalling proteins that are recruited to the receptor that then determines the quality of the response that is generated. Indeed, the location of the proteins inside the cell and the kinetics of their activation are important features of signal-transduction pathways. How the signalling molecules are localized in the cell and how the strength and quality of the signal is regulated is an area of intense research, and increasing attention has focused on the so-called adaptor proteins as key molecules controlling these more complex aspects of signal transduction.

Adaptor proteins lack enzymatic activity or other direct effector function. Adaptors can be transmembrane proteins, reside in the cytoplasm under resting conditions and be recruited to the membrane upon activation, or be localized by specific interactions in intracellular compartments. Regardless of their cellular localization, they possess an array of binding sites and modules that allow them to mediate specific protein-protein and protein-lipid interactions. Examples of binding domains in adaptors include Src-homology 2 (SH2) and Phosphotyrosine-binding (PTB) domains that bind to phosphotyrosine motifs, SH3 domains that bind to proline-rich sequences and Pleckstrin homology (PH) domain that recognizes phospholipids (Pawson and Scott, 1996). With an assemblage of modules and binding sequences, a single adaptor can serve as a scaffold protein for the binding of multiple proteins into complexes, bringing in this way effectors into close proximity to their targets. However, the general ability of adaptor proteins to amplify or inhibit signalling, highly depends on their cell-specific expression and level, as well as on that of their binding partners, their location in the cell, the stability of the interactions between the adaptor and its targets and in certain conditions, on the basal kinase/phosphatase activity in the cell.

Lastly, it should be noted that the domains and motifs found in adaptors are also frequently present in enzymatically active molecules, such as tyrosine phosphatases of the SH2-containing phosphatase (SHP) family and the ubiquitin ligases Casitas B-cell lineage lymphoma (c-Cbl) proteins, where they can mediate true adaptor-like functions and also orchestrate signalling complex formation.

3. Inhibitory adaptors in cytokine signalling regulation

New insights into the biology of adaptor protein function have been possible with the use of a variety of biochemical, cellular and imaging techniques, as well as *in vivo* genetic approaches. All these techniques have helped establish that adaptor proteins can affect the thresholds and the dynamics of signalling reactions by coordinating positive and negative feedback signals. Over the years, the majority of investigations on cytokine signalling pathways have mainly focused on the mechanisms of cytokine-receptor activation, whereas our knowledge of negative regulation has been less explored. However, the most recent research has placed increasing emphasis on the mechanisms by which cytokine signals are attenuated or terminated. Indeed, stringent mechanisms of signal attenuation are essential for ensuring an appropriate, controlled cellular response following cytokine stimulation. One could imagine how the aberrant assembly of macromolecular active signalling complexes could lead to disease: excess positive signalling or insufficient negative signalling may lead to autoimmunity, chronic inflammation or malignant transformation, while excess negative signalling or insufficient positive signalling may lead to immunodeficiency or certain haematological disorders.

A number of mechanisms have been proposed to regulate the initiation, duration, magnitude and specificity of cytokine signalling at multiple levels: 1) receptor internalization and inhibition mediated by soluble receptor antagonists and/or specific inhibitors (such as the Lnk and Dok adaptor proteins); 2) tyrosine dephosphorylation of the receptor and signalling intermediates mediated by tyrosine phosphatases; 3) proteosomal degradation of signalling molecules mediated by the suppressors of cytokine signalling (SOCS) proteins and the Cbl E3 ubiquitin ligases proteins; and lastly 4) transcriptional suppression mediated by specific inhibitors such as the protein inhibitors of activated STATs (PIAS) proteins. In this section, we review what is currently known about the function and regulation of four families of inhibitory adaptor proteins that are key players in some of the regulatory mechanisms mentioned above. These families are the DOK, the Lnk/SH2B3, the Cbl and the SOCS proteins. We first discuss the adaptors without catalytic activity (DOK and Lnk), followed by those possessing an enzymatic function (Cbl and SOCS). The latter ones are also known as scaffold proteins; however we will refer to them as adaptors in hopes that this distinction provides a clear picture on the different properties of each.

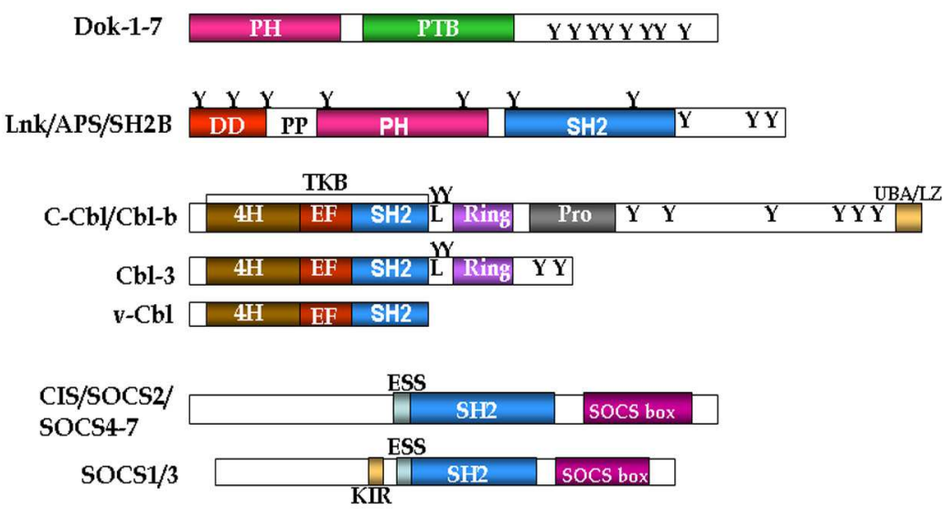

Fig. 1. Schematic representation of the domain structure of inhibitory adaptors

3.1 The DOK family

3.1.1 Structure and cell expression

The Dok (Downstream Of Tyrosine Kinases) family of adaptor proteins consists of seven members, Dok 1 to Dok-7, that differ in the length of their C-terminal region. They are all characterized by an N-terminal PH domain, a central PTB domain and multiple SH2 and SH3 binding motifs in the C-terminal region (Figure 1). Dok-1 ($p62^{dok}$), Dok-2 ($p56^{dok-2}$, also called Dok-R, or FRIP) and Dok-3 (also called DokL) are preferentially expressed in the haematopoietic compartment, as well as co-expressed in haematopoietic progenitors (Carpino et al., 1997; Yamanashi et al., 1997; Di Cristofano et al., 1998; Lemay et al., 2000). All three Dok proteins are expressed in myeloid cells, but differ in their lymphoid lineage expression. While Dok-1 and Dok-2 are highly expressed in T cells, Dok-3 is little or not detected at all. In contrast, Dok-1 and Dok-3 are expressed in B cells, while Dok-2 is not

normally detected in these cells. Moreover, several studies have demonstrated that Dok-1 and Dok-2 expression was up or down-regulated, respectively, by different signalling pathways in immune cells. Dok-1 expression was upregulated in response to the glucocorticoid dexamethasone in RBL-2H3 mast cells (Hiragun et al., 2005). By contrast, its expression was downregulated in bone marrow-derived macrophages in response to lipopolysaccharide (LPS) [Shinohara et al., 2005]. As for Dok-2, its expression increased in response to cytokines such as M-CSF, granulocyte–macrophage colony stimulating factor (GM-CSF), and interleukin-3 (IL-3) in NFS-60 myeloid leukemia cells, suggesting its implication in a negative feedback loop for the regulation of these cytokine pathways (Suzu et al., 2000). The other Dok proteins, Dok-4 (IRS5), Dok-5 (IRS6), Dok-6 and Dok-7 are mainly expressed in non-haematopoietic cells, notably in neural cells. However, Dok-4 was reported to function as negative regulator in human T cells (Favre et al., 2003).

Dok protein	Binding domain	Binding partner	Signalling Receptor
Dok-1	PTB	Abl	BCR
		SHIP-1	FcγRIIB
		Dok-1, Dok-2	CD2
		TCRε	idem
	pY	CD3ε	idem
		p120RasGap	BCR, CD2+CD28, FcεRI
		Abl, Lyn	BCR
		Nck	FcεRI
		SHIP-1	FcγRIIB, FcεRI
Dok-2	PTB	SHIP-1	FcγRIIB
		Dok-1, Dok-2	CD2
		TCRε, CD3ε	idem
	pY	p120RasGAP,Abl	BCR
		Nck	TCR
Dok-3	PTB	SHIP-1, Abl	FcγRIIB
		Dok-3	BCR
	pY	Grb2	BCR
		SHIP-1	FcγRIIB

Table 1. Signalling partners bound to the different domains and motifs of Dok proteins

3.1.2 Signalling partners

The biological functions of the Dok proteins have been defined with the identification and functional analysis of their binding partners, as well as of their subcellular localization. Dok-1 was the first member of this family identified as a tyrosine-phosphorylated 62 kDa substrate of both v-ABL and BCR-ABL and associated with p120RasGap, a negative regulator of Ras. Several studies have later shown that Dok-1/2/3 can be tyrosine phosphorylated by a variety of growth factors, cytokines and immuno receptors, providing multiple docking sites for SH2 and PTB-containing proteins such as Nck, SHP-1, SHIP-1 and p120RasGap (Table 1). The interaction between p120RasGap and Dok-1/2 has been the most extensively studied and the one likely responsible for the negative regulation of the Ras/Erk

pathway mediated by the Dok adaptors. It involves the SH2 domain of p120RasGap and its binding motifs present in the C-terminal moiety of Dok-1/2 (Songyang et al., 2001). In contrast, Dok-3 protein has no YxxP motifs and therefore is unable to associate with p120 RasGap. However, it can negatively regulate Erk activation through its binding with Grb2 (Honma et al., 2006). Fewer signalling molecules have been reported to associate with the PTB domain of Dok-1/2/3 (Table 1). Interestingly, these Dok proteins show homotypic and heterotypic (for Dok-1/2) oligomerization that is dependent on their tyrosine phosphorylation and PTB domains. Moreover, this oligomerization appears crucial to their function, at least for Dok-1/2 (Boulay et al., 2005). Instead, the functional relevance of Dok-3 oligomerization is not yet clear (Stork et al., 2007). The presence of a PH domain in the structure of Dok proteins suggests an important role for this domain in the localization or translocation of the Dok adaptors to cellular membranes. Indeed, it seems that Dok-1 and PI3K activity are required for the recruitment of the adaptor to the membrane and its negative effect on PDGF-mediated ERK activation. Furthermore, Dok-1/2 PH domain can bind tightly to PI(5)P and modulate the negative function and tyrosine phosphorylation of the adaptors in T-cells (Guittard et al., 2009). Conversely, the PH domain of Dok-3 is important for its localization to the membrane in B cells (Stork et al., 2007).

3.1.3 Signalling pathways in immune cells

Studies with Dok-1/2/3 deficient mice and/or cells have helped demonstrate the physiological importance of these inhibitory adaptors to the function and development of immune cells. Using *Dok-1*-deficient splenic B cells, Yamanashi et al. demonstrated a negative role of Dok-1 in antigen receptor-mediated signalling through suppression of MAPK activity and cell proliferation (Yamanashi et al., 2000). Moreover, co-cross-linking of the B-cell receptor (BCR) and FcγRIIB receptor induces the tyrosine phosphorylation of Dok-1 and its subsequent association with RasGap (Vuica et al., 1997). In FcγRIIB signalling, Dok-1 is recruited to the receptor complex at the membrane via SHIP, and in this way, contributes to the negative regulation of the Erk pathway [Figure 2.] (Tamir et al., 2000). On the other hand, Dok-3 is also expressed in B cells and therefore, one can expect a functional redundancy between Dok-1/3 in these cells. Indeed, it was reported that Dok-3 can function as a negative regulator of BCR-mediated responses (Ng et al., 2007). Furthermore, both Dok-1 and Dok-3 were shown to be phosphorylated by Lyn kinase after stimulation of the BCR, suggesting that Lyn can activate these Dok proteins to then function as negative regulators in B cells (Yamanashi et al., 2000; Stork et al., 2007). However, B cells from *Dok-3*-deficient mice exhibited augmented proliferation and $Ca2^+$ influx upon BCR stimulation (Ng et al., 2007), while these responses are not observed in the absence of Dok-1 (Yamanashi et al., 2000). These phenotypic differences could be attributed to Dok-1, but not Dok-3, recruiting p120RasGAP, which can inhibit Ras/Erk signalling; by contrast, Dok-3, but not Dok-1, can recruit Grb2, which can then inhibit $Ca2^+$ signalling in B cells.

Dok-1 and Dok-2 adaptor proteins have been also shown to play a role in the maintenance of T-cell homeostasis. In some cell line systems, Dok-1 is phosphorylated by Lck kinase and associates with RasGap upon CD2 and CD28 stimulation, but not CD3-TCR engagement, indicating a possible role of Dok-1 in T cell signalling (Nemorin & Duplay, 2000). Furthermore, overexpression of Dok-2 results in a dramatic reduction in both thymocytes and splenic T-cell numbers, suggesting a negative role of Dok-2 in T-cell development

(Gugasyan et al., 2002). Although the molecular mechanisms underlying the Dok-mediated inhibition are unclear, Dok proteins can bind to the ITAM motifs of TCRζ and CD3ε through their PTB domain. As these ITAMs are essential for the binding and activation of ZAP-70, interference between the Dok proteins and ZAP-70 might occur through their binding to the ITAMs (Figure 2). Recently, Nunès and colleagues reported that the PH domain of Dok-1 and Dok-2 is necessary for the tyrosine phosphorylation of these Dok proteins and their negative functions in T cells (Guittard et al., 2009). These results demonstrate the functional relevance of the membrane localization of the Dok adaptors.

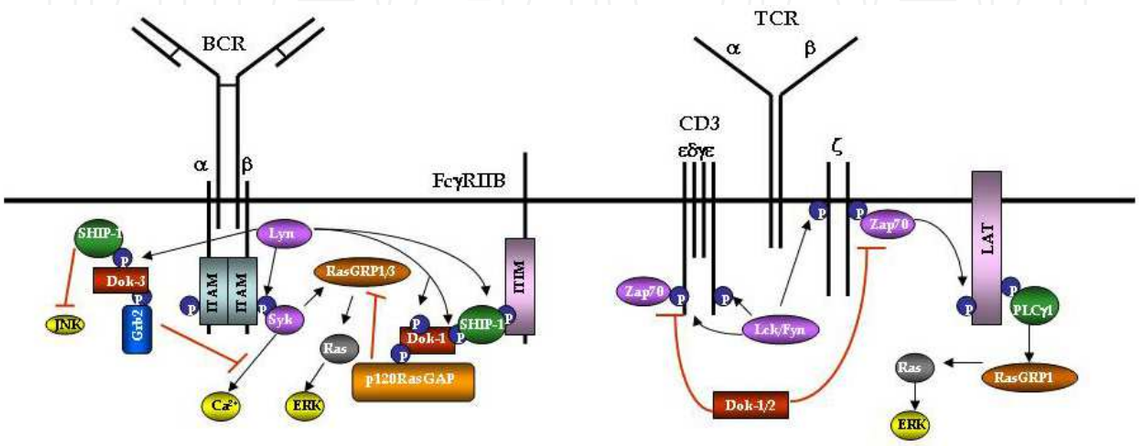

Fig. 2. Dok-mediated signalling pathways in immune cells

Unlike lymphoid cells, myeloid cells express all the immune cell Dok proteins. However, the loss of Dok-1 and Dok-2 causes mainly neoplastic abnormalities in myeloid cells, suggesting an important role in immune and cytokine receptor signalling in these cells. Analysis of *Dok-1* and *Dok-2* deficient myeloid cells showed enhanced proliferation and survival in response to Stem Cell Factor (SCF), IL-3, macrophage-colony stimulating factor (M-CSF), and granulocyte-monocyte-colony stimulating factor (GM-CSF), which are cytokines crucial for myelopoiesis. These findings indicate that Dok-1 and Dok-2 act as key negative regulators of signalling downstream of these cytokine receptors. Indeed, the activation of Erk and Akt in macrophages deficient for *Dok-1* and *Dok-2* was strongly augmented compared with that in wild type controls upon M-CSF receptor stimulation, confirming the role of Dok adaptors as negative regulators for these pathways. On the other hand, the role of Dok-1 and Dok-2 was examined in innate immune signalling in macrophages. Stimulation of macrophages by LPS induces rapid tyrosine phosphorylation of Dok-1 and Dok-2, suggesting the involvement of these adaptors in TLR4 signalling (Shinohara et al., 2005). In addition, the stimulation of *Dok-1* or *Dok-2*-deficient macrophages promoted the activation of Erk and hyperproduction of TNF-α and nitric oxide, two major signalling mediators of innate immunity, indicating that the Dok proteins are key negative regulators of TLR4 signalling in macrophages. The Dok adaptors are also expressed in mast cells where they have been shown to interact exclusively with negative regulators of FcεRI signalling. FcεRI stimulation leads to the tyrosine phosphorylation of only Dok-1 and Dok-2. Nevertheless, Dok-3 associates with tyrosine-phosphorylated proteins upon FcεRI stimulation, implicating a yet undefined function for this adaptor protein downstream of the receptor (Abramson et al., 2003). A complex of Dok-1, RasGAP, and SHIP-1, similar to the one described in B cells after co-

aggregation of the BCR with FcγRIIB, was also described in mast cells after FcεRI and FcγRIIB co-aggregation (Ott et al., 2002). Moreover, Dok-1 has also been involved downstream of activating receptors, like FcεRI, by associating with and negatively regulating signals without the involvement of inhibitory receptors (Ott et al., 2002; Abramson et al., 2003). However, *Dok-1*-deficiency did not affect mast cell activation, suggesting a possible functional redundancy among the different isoforms expressed in these cells.

Recently, two groups have reported the expression and function of the Dok proteins in human platelets. Using a proteomic approach in these cells, it was shown that Dok proteins are tyrosine phosphorylated downstream of main platelet activation receptors (Garcia et al., 2004; Hughan et al., 2007; Senis et al., 2009). Tyrosine phosphorylation of Dok-1 and Dok-3 was primarily Src kinase-independent downstream of the integrin pathway, whereas it was Src-dependent downstream of glycoprotein VI (GPVI) pathway. Both proteins interact in an inducible-fashion with Grb-2 and SHIP-1 in fibrinogen-spread platelets, suggesting that the formation of a multi-molecular negative signalling complex may be a mechanism of down-regulating αIIbβ3 outside-in signalling.

3.2 The Lnk/SH2B family

3.2.1 Structure, origin and cell expression

The Lnk (or SH2B) family of adaptor proteins is composed of 3 members, SH2-B [also known as PSM (proline-rich, PH and SH2 domain-containing signalling mediator) or SH2B1], APS (for Adaptor protein with PH and SH2 domain, also known as SH2B2) and Lnk (SH2B3). They all possess a dimerization (DD) domain and proline-rich motifs at the N-terminus, followed by a PH and SH2 domains, and several potential tyrosine phosphorylation sites, notably a conserved tyrosine residue at the C-terminus [Figure 1](Rudd, 2001). The *SH2B1* gene encodes four isoforms (α, β, γ, δ) resulting from alternative mRNA splicing at their 3' terminus giving rise to proteins differing at their C-terminus (Nelms et al., 1999). SH2-Bα and β isoforms were originally cloned from yeast tribrid and two-hybrid systems screening, respectively, using different proteins as baits (Osborne et al., 1995; Riedel et al, 1997; Rui et al., 1997). Despite its initial identification in immune cells, SH2-B isoforms are mainly expressed and functional, as shown by gene inactivation in mice, in non-haematopoietic tissues. The *APS/SH2B2* gene encodes for two isoforms, SH2B2α and recently identified SH2B2β (Li et al., 2007). The APS protein was also identified in a two-hybrid system screening of human B cells or adipocytes (Yokouchi et al., 1997; Moodie et al., 1999). Like SH2-B, APS adaptor protein is also highly expressed in non-haematopoietic tissues. However, it is also expressed in haematopoietic cells, notably in mature B and mast cells. As for Lnk, it has only one form in mammalians and one invertebrate orthologue in *Drosophila melanogaster* (D-Lnk) to date (Werz et al., 2009). The Lnk adaptor protein was the first member of this family identified (Huang et al., 1995; Takaki et al., 1997). However, it was later found that the Lnk protein was much larger than initially reported (Li et al., 2000; Takaki et al., 2000; Velazquez et al., 2002). In contrast to SH2-B and APS, Lnk is mainly expressed in haematopoietic cells, notably in haematopoietic stem cells (HSC), and haematopoietic (lymphoid and myeloid) progenitors. Moreover, Lnk expression is up-regulated by certain cytokines important for the development and function of these haematopoietic cells, such as SCF, thrombopoietin [TPO], and erythropoietin [EPO] (Kent et

al., 2008; Buza-Vidas et al., 2006; Gerry et al., 2009a, 2009b; Baran-Marszak et al., 2010). Interestingly, Lnk is also highly expressed in endothelial cells and its expression is also induced by Tumor Necrosis Factor (TNF)-α (Fitau et al., 2006; Kwon et al., 2009). These findings suggest the implication of Lnk adaptor in a negative feedback loop for the regulation of these cytokine pathways.

3.2.2 Signalling partners

Over the last years, much effort has gone into understanding the role of the Lnk family as signalling regulators through the identification of the molecules binding to their different functional domains and motifs, as well as their signalling pathways (Table. 2). The SH2 domain of the Lnk adaptor proteins is implicated in most of the key molecular interactions between the adaptors and their partners/effectors and their biological functions. The fist identified binding partner of the Lnk protein was the SCF receptor, Kit. The primary Kit-binding site for Lnk SH2 domain has been identified as phosphotyrosine 567 (pTyr567), which resides in the juxtamembrane region of the receptor (Simon et al., 2008; Gueller et al., 2008). Similarly, the SH2 domain of APS was reported to bind to Y568 and Y936 in the human c-Kit receptor (Wollberg et al., 2003). Interestingly, this region of Kit contains critical tyrosine (Y) residues (Y567/69) for the recruitment of different regulatory signalling molecules (Chan et al., 2003). In this system, a proposed mode of action of Lnk is that once bound to the juxtamembrane region of Kit, it will then block the association of activators with the receptor, resulting in down-regulation of SCF-mediated pathways. Indeed, expression of an SH2-inactive Lnk protein abolishes Lnk-mediated negative regulation of SCF-induced cell proliferation and migration (Simon et al., 2008). Lnk has been also reported to bind through its SH2 domain to other tyrosine kinase receptors, such as the PDGFR and the M-CSF receptor (c-Fms); however the physiological implication of these associations is not yet clear (Gueller et al., 2010, 2011).

The JAK2 tyrosine kinase was the first characterized binding partner of SH2-B and APS, and subsequently of Lnk. This association results in activation of the kinase in the case of SH2-B and APS or in its inhibition when bound to Lnk. Different biochemical studies have shown that the interaction of the SH2 domains of SH2-B and APS occurs preferentially with kinase-active, tyrosyl phosphorylated JAK2 (Rui et al., 1997). The primary JAK2-binding site for the SH2 domain of the Lnk family has been identified as pTyr813, which resides within the regulatory JH2 pseudokinase domain of JAK2 (Kurzer et al., 2004, 2006). Crystallographic studies have demonstrated that the SH2 domain of APS dimerizes when binding to the insulin receptor, whereas the SH2 domain of SH2-B, binds JAK2 as a monomer (Hu et al., 2003; Hu & Hubbard, 2006). Less is known on how the Lnk SH2 domain binds JAK2. However, it has been shown that Lnk is capable of binding JAK2 wild-type form, as well as the constitutive active JAK2-V617F form present in myeloproliferative neoplasms (Bersenev et al., 2008; Gery et al., 2009; Baran-Marszak et al., 2010). In addition to the SH2-dependent interaction of the Lnk adaptor family with pTyr813 in JAK2, there appears to be one low-affinity interaction involving amino acids outside the SH2 domain in the adaptors and inactive JAK2 that might prevent abnormal activation of the kinase. (Rui et al., 2000; Kurzer et al., 2006; Baran-Marzak et al., 2010).

The N-terminal region of the Lnk adaptor family contains a dimerization domain whose crystal structure has revealed a "phenylalanine zipper" motif. This domain mediates SH2-B

Lnk proteins	Binding domain	Binding partner	Cells System
Lnk	N-term	Lnk	COS
	Inter PH-SH2	ABP-280	COS, T cells (TCR)
	SH2	Kit	Mast (SCF)
		JAK2	Myeloid (EPO, TPO)
		c-Fms	Myeloid (M-CSF)
		PDGFR	COS (PDGF)
APS	N-term	APS, SH2B	HEK293, CHO (In)
	PH	Vav3	NIH3T3
	SH2	Kit	Mast (SCF)
		JAK2	Myeloid (GH)
		IR	Adipocyte (In)
	pY618	Cbl	Adipocyte (In)
SH2B	N-term	SH2B, APS	CHO (In), COS
		Rac	CHO, COS (GH)
	SH2	JAK2	Myeloid (GH)
		GHR, IR	Adipocyte (GH, In)

GHR, growth hormone receptor; IR, insulin receptor; In, insulin

Table 2. Signalling partners bound to the different domains and motifs of Lnk proteins.

and APS homo and heterodimerization that appears crucial to their function (Dhe-Paganon et al., 2004). Instead, Lnk homodimerization has only been shown in an over-expressed system (Takizawa et al., 2006) and therefore, its functional relevance is not yet clear.

The presence of a PH domain in the structure of Lnk proteins suggests an important role for this domain in the localization or translocation of these adaptor proteins to cellular membranes. Indeed, previous reports showed that Lnk PH mutants (W191A or W270A) proteins moderately affected Lnk modulation of TPO-, EPO- or SCF-dependent biological responses (Tong & Lodish, 2004; Tong et al., 2005; Simon et al., 2008). Moreover, the Lnk PH domain seemed to display moderate affinity and little specificity to phosphoinositides *in vitro*. It is therefore possible that the Lnk PH domain may down-regulate membrane targeting of Lnk in the absence of docking site for the SH2 domain and increase binding stability to membrane receptors when the SH2 domain is engaged.

Association of Lnk, APS and SH2-B with growth factor, cytokine receptors or the JAK2 kinase allows phosphorylation of these adaptors and their proper localization at the signalling complex. The conserved C-terminal tyrosine residue present in all members of this family has been shown to be a main site for phosphorylation upon growth factor or cytokine stimulation. In Lnk, this residue, Y536, was suggested to be phosphorylated upon SCF stimulation in a mast cell line (Takaki et al., 2002). However, an Lnk form mutated at this tyrosine still gets phosphorylated upon Kit activation in primary mast cells (Simon et al., 2008). This result suggested that Lnk could be phosphorylated at sites other than Y536. Indeed, a similar result was reported with human Lnk mutated at this residue (Li et al., 2000). On the other hand, the biological relevance of Lnk Y536 seems to depend on the signalling pathway analyzed. Lnk Y536 is dispensable for lymphoid development, TPO- or SCF-dependent signalling pathways, but it might play a regulatory role in IL3- and EPO-mediated proliferation (Takaki et al., 2003; Tong & Lodish, 2004; Simon et al., 2008).

However, no Lnk binding partners for this site has so far been identified. In contrast, APS C-terminal tyrosine, Y618, has been shown to get phosphorylated by activated growth factor (IR), cytokine (EPO) and immune (BCR) receptors and then serve as binding site for the Cbl protein (Moodie et al., 1999; Yokouchi et al., 1997; Wakioka et al., 1999). The APS/Cbl association plays an important role in down-regulation of IR signalling (Kishi et al., 2007). Other binding proteins have been identified that associate with other regions of Lnk, APS and SH2-B that are involved in actin regulation. In particular, an amino acid sequence in the N-terminal region of SH2-B has been shown to bind to Rac, a mayor actin regulating protein, while a similar sequence in APS can associate with Vav3, a guanine nucleotide exchange factor for Rac (Diakonova et al., 2002; Yabana and Shibuya, 2002). Lnk was demonstrated to associate with the actin binding protein ABP-280 via a sequence between the PH and SH2 domains of human Lnk in Jurkat T cells (He et al., 2000). These findings suggest a role for the Lnk family members in the regulation of actin cytoskeleton and cell motility.

3.2.3 Signalling pathways in haematopoietic cells

The initial *in vitro* biochemical analysis was done on SH2-B and APS and showed that these adaptors were phosphorylated and became positive mediators of receptor and protein tyrosine kinases cascades. However, APS can also function as negative regulator in the BCR and JAK2 signalling pathways (Yokouchi et al., 1997; Wakioka et al., 1999). Conversely, Lnk is considered as a negative regulator of growth factor and cytokine receptor-induced proliferation and migration (Takaki et al., 2000, 2002; Velazquez et al., 2002; Tong & Lodish, 2004; Tong et al., 2005; Fitau et al., 2006; Simon et al., 2008; Gueller et al., 2010, 2011). Nonetheless, Lnk seems to play a positive role in mouse platelets for the stabilization of thrombus through the integrin αIIbβ3 outside-in signalling and in human vascular endothelial cells via the PI3K/Akt pathway activated by TNF-α (Takizawa et al., 2010; Fitau et al., 2006). Together with data on *in vivo* ablation of these adaptors, these findings demonstrate that these adaptor proteins can function as positive and/or negative regulators depending on their cell expression and on the growth factor or cytokine receptor–mediated pathway.

The generation of mice and cell lines deficient for members of this family has confirmed Lnk and APS, but not SH2-B, specific function in the haematopoietic system, while establishing SH2-B implication in other tissues. As stated before, Lnk is highly expressed in HSC, so as expected, *Lnk*$^{-/-}$-derived HSC show an increased capacity to proliferate and to self-renew together with an increase in the quiescent fraction. These effects on HSC homeostasis are due to abnormal TPO signalling in these cells, that results from an enhanced TPO hypersensibility, increased TPO-dependent activation of Akt, STAT5 and down-regulation of p38MAPK (Ema et al., 2005; Buza-Vidas et al., 2006; Seita et al., 2007; Bersenev et al., 2008). These findings therefore confirm that Lnk controls TPO-induced self-renewal, quiescence and proliferation of HSC. Moreover, *Lnk* deficiency enhances the ability of HSC and haematopoietic progenitors to reconstitute the haematopoietic system in irradiated hosts. Indeed, transient inhibition of endogenous Lnk significantly increased the repopulating capacity of the transduced cells and thereby, engraftment (Takizawa et al., 2006). Moreover, analysis of *Lnk*$^{-/-}$-derived haematopoietic progenitors show an hypersensibility to several cytokines resulting in sustained MAPK, JAK/STAT activation and cell proliferation (Takaki et al., 2000; Velazquez et al., 2002; Tong, 2005; Takizawa et al., 2008).

Lnk-deficient mice have also revealed an essential role for Lnk in B cell lymphopoiesis with the selective expansion of pro-/pre-B and immature B cells in bone marrow and spleen. This abnormal proliferation is partly due to hypersensitivity to SCF and IL-7 (Takaki et al., 2000; Velazquez et al., 2002). Alternatively, Lnk over-expression in transgenic mice show impaired B production in an Lnk dose-dependent manner confirming the negative control mediated by this adaptor in B-lineage cell production (Takaki et al., 2003). However, no evident effect on mature B cells was observed in the absence of Lnk, suggesting either a lack of role for Lnk in this population or a functional compensation by APS in these cells. APS has also been shown to play a role in B cell development and function. Ablation of *APS* in mice caused an increase in B-1 cell number and an enhanced humoral immune response against a thymus-independent type 2 antigen, suggesting a role for APS in mature B cell proliferation (Iseki et al., 2004). Accordingly, APS transgenic mice showed reduced numbers of peritoneal B-1 and splenic B cells and impaired BCR-induced proliferation of mature B cells (Iseki et al., 2005). In these cells, APS co-localized with pre-activated capped BCR complexes and filamentous actin, indicating a negative regulatory role for APS in BCR signalling and actin reorganization.

Lnk has been also shown to control erythropoiesis and megakaryopoiesis. Studies on primary *Lnk*$^{-/-}$ erythrocytes and megakaryocytes indicate an abnormal proliferation due to the absence of negative regulation of EPO and TPO signalling pathways (Figure 3). Indeed, Lnk, through its SH2 domain, negatively modulates MPL, and EPO receptor (EPOR) signalling by attenuating three major signalling pathways: JAK2/STAT, MAPK and Akt (Tong et al., 2005; Tong & Lodish, 2004). Moreover, Lnk is capable of binding and regulating MPL-W515L and JAK2-V617F, the mutated forms expressed in Myeloproliferative Neoplasms [MPN] (Gery et al., 2007, 2009; Bersenev et al., 2008; Baran-Marszak et al., 2010). In addition, Lnk also regulates thrombopoiesis through control of crosstalk between integrin- and TPO-mediated pathways implicated in the megakaryocyte maturation and platelet release process (Takizawa et al., 2008). Furthermore, Lnk plays an important role in stabilizing thrombus formation through positive regulation of integrin signalling pathways. In this way, it contributes to platelet cytoskeleton rearrangement and spreading (Takizawa et al., 2010).

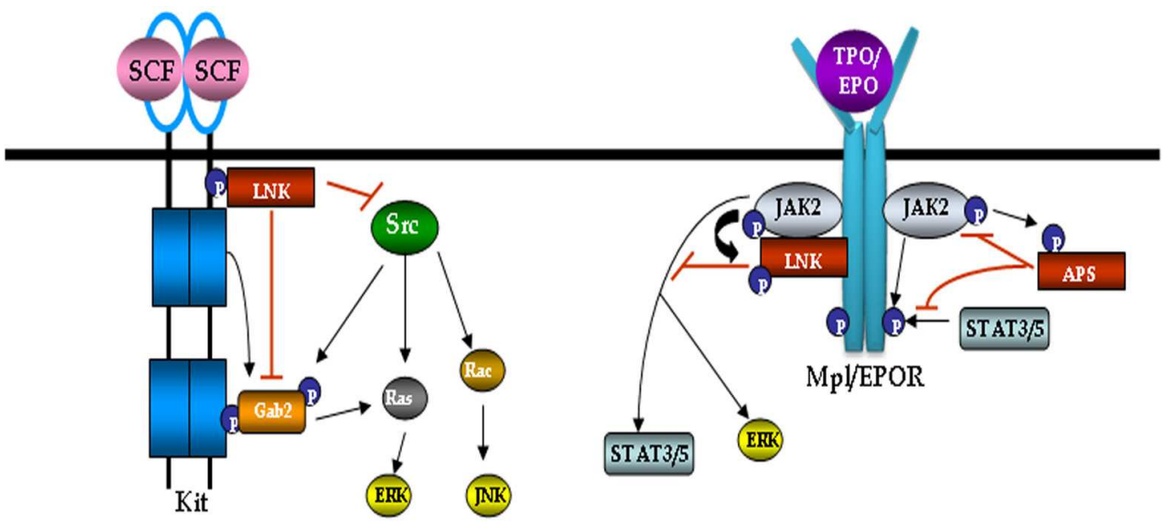

Fig. 3. Lnk family signalling pathways in haematopoietic cells

Studies on *Lnk*$^{-/-}$ and *APS*$^{-/-}$ mast cells demonstrated their physiological implication in mast-cell functions. Lnk regulates SCF-mediated signalling pathways controlling proliferation (MAPK and JNK) and migration (Rac and p38MAPK) in these cells (Takizawa et al., 2006; Simon et al., 2008). These functions are mainly mediated by binding of its SH2 domain to specific effectors involved in actin rearrangement. On the other hand, APS controls actin cytoskeleton and magnitude of degranulation induced by FcεRI receptor cross-linking (Kubo-Akashi et al., 2004). Besides its specific expression in the haematopoietic system, Lnk is also highly expressed in endothelial-like cells in the aorta-gonad-mesonephros (AGM) region and in endothelial progenitor (EPC) and mature cells [EC](Nobuhisa et al., 2003; Fitau et al., 2006; Kwon et al., 2009). Fitau *et al.* have shown that the pro-inflammatory cytokine TNF-α rapidly evokes Lnk phosphorylation together with down-regulation of vascular cell adhesion molecule 1 (VCAM-1) expression in activated vascular ECs. These results implicate Lnk as an important negative regulator of TNF-α signalling pathway (Fitau et al., 2006).

3.3 The Cbl family

3.3.1 Origin, cell expression and structure

The Cbl (for Casitas B-lineage Lymphoma) family comprises multidomain regulators with dual function, as E3 ubiquitin ligases and adaptor proteins. It consists of three mammalian homologues, c-Cbl, Cbl-b and Cbl-c/Cbl-3 (Blake et al., 1991; Keane et al., 1995, 1999), as well as invertebrate orthologues (Thien & Langdon, 2001). The first isoform of this family identified was the oncogenic protein v-Cbl, a Gag-fusion transforming protein of Cas NS-1 retrovirus, which induces pre- and pro-B lymphomas and the transformation of rodent fibroblasts (Langdon et al., 1989). The cellular form, c-Cbl was subsequently cloned and revealed that v-Cbl was a result of a large truncation of its C-terminal portion and that overexpression of c-Cbl did not promote tumorigenesis. The 120 kDa c-Cbl protein is ubiquitously expressed, primarily cytoplasmic, with highest expression levels in haematopoietic organs (thymus) and testis. In contrast, Cbl-3 is expressed mainly in epithelial cells of the gastrointestinal system.

All Cbl proteins share highly conserved N-terminal regions, but their C-terminal sequence differs and is less well-conserved (Figure 1). The N-terminal half encompasses two important domains: a tyrosine kinase-binding (TKB) and a C3HC4 zinc-binding RING finger domains, both separated by a small linker sequence. The TKB domain contains three distinct subdomains comprising a four-helix bundle (4H), a calcium-binding EF hand and a modified SH2 domain, all necessary for its function as phosphotyrosine-binding (PTB) module. The second conserved domain in the N-terminal region is a zinc-binding RING finger domain responsible for the E3 ubiquitin ligase activity of c-Cbl (Joazeiro et al., 1999). The C-terminal sequences are less homologous among Cbl proteins; however, they all have proline-rich regions that are involved in numerous SH3-domain interactions (Keane et al., 1995) and the major sites of tyrosine phosphorylation, which enable interactions of Cbl with SH2 domain containing proteins. The C-terminus of c-Cbl, Cbl-b contains a sequence homologous to both the leucine zipper (LZ) and the ubiquitin associated (UBA) domain. The LZ domain has been shown to mediate Cbl dimerization (Bartkiewicz et al., 1999; Liu et al., 2003), while only Cbl-b and not c-Cbl can bind to ubiquitin through its UBA domain (Davies et al., 2004).

3.3.2 Signalling binding partners

The TKB domain is unique to Cbl proteins and its feature role is to determine Cbl substrate specificity by engaging specific phosphorylated tyrosine residues on proteins that are to be ubiquitylated by Cbl. Some of Cbl TKB domain targets include: receptor tyrosine kinases (RTKs), non-receptor protein tyrosine kinases (PTKs) of the Syk family, several adaptors and regulatory proteins (Table 3). In contrast to SH2 binding motif, the TKB phosphotyrosine recognition consensus sequence displays an specificity conferred by amino acid residues C-terminal to the tyrosine (Lupher et al., 1997). These findings argue that interaction of the TKB domain with RTKs may then primarily be to ensure the appropriate orientation of the receptor such that Cbl's E3 ligase activity can promote the transfer of ubiquitin. TKB domain interactions may therefore determine the number of ubiquitin molecules transferred to the substrate and thus regulate the extent to which activated RTKs are ubiquitylated. Thus the TKB domain appears to have at least two important roles in regulating E3 ligase activity, and, as such, it is functionally more complex than classical SH2 or PTB domains.

Binding domain	Binding partner	Signalling receptor
TKB	Syk, Zap-70	AgR
	c-Src	AgR, GFR, CyR
	APS	HR
	EGFR, PDGFR	GFR
RING	E2s(Ub-conjugated enzyme)	GFR, AgR, CyR
Pro-rich	Grb2	GFR
	Nck	GFR, AgR
	Src kinases	GFR, AgR, CyR
pY	Vav, CrkL, Src kinases	AgR
	p85 (only with c-Cbl)	AgR, GFR
UBA	Ub (only with Cbl-b)	GFR
LZ	c-Cbl, Cbl-b	GFR, HR

AgR, antigen receptor; GFR, growth factor receptor; CyR, cytokine receptor; HR, hormone receptor.

Table 3. Signalling proteins bound to the different domains and motifs of c-Cbl/Cbl-b proteins.

Separating the TKB and the RING domains, a short linker sequence extends which has been shown crucial for Cbl ubiquitin ligase activity (Thien et al., 2001). The TKB domain makes intramolecular contacts with the linker α-helix and these contacts are centred on conserved residues Y368 and Y371 in human c-Cbl (Zheng et al., 2000). Interestingly, deletion of these tyrosines causes c-Cbl to become oncogenic (Thien et al., 2001). Molecular modelling data predicted that this structural alteration, in addition to loss of E3 activity, is required to activate fully the oncogenic potential of Cbl proteins. The second highly conserved domain among all Cbl proteins is the RING finger. *In vitro* ubiquitylation assays proved that the highly conserved Cbl RING finger has intrinsic E3 ligase activity and can independently recruit E2s or ubiquitin-conjugating (Ubc) enzymes for the transfer of ubiquitin to substrates (Joazeiro et al., 1999). The structural integrity of the RING finger domain is an absolute requirement for Cbl proteins to function as E3 ligases. Moreover, the RING finger domain acts in concert with the TKB domain to facilitate ubiquitylation and degradation of activated

PTKs, with the TKB domain conferring substrate specificity and the RING finger bringing in an E2 ubiquitin-conjugating enzyme. The carboxy-terminal half of c-Cbl is rich in proline residues, which contributes at least 15 and 17 potential SH3-domain-binding sites in c-Cbl and Cbl-b respectively, while Cbl-3 encodes five potential SH3-binding sites. Moreover, the proline-rich region in c-Cbl is also required for the ubiquitylation and proteasomal degradation of activated forms of Src (Yokouchi et al., 2001). In this case, the Cbl substrate is targeted by proline sequence interactions, rather than the TKB domain.

c-Cbl and Cbl-b are prominent substrates of RTKs and PTKs following stimulation of diverse cell surface receptors, as they possess major sites of tyrosine phosphorylation at their C-terminal part that enable them to interact with different SH2 domain containing proteins. Indeed, several studies have demonstrated the important role of tyrosine phosphorylation of Cbl proteins for their adaptor function, as well as for their E3 activity. The best-characterized phosphorylation sites are Y700, Y731 and Y774 in human c-Cbl and Y709 and Y665 in Cbl-b (Tsygankov et al., 1996; Keane et al., 1995). These residues are efficiently phosphorylated by Syk and the Src-family kinases Fyn, Yes and Lyn, but not by Lck or ZAP-70. An important difference between c-Cbl and Cbl-b is the unique presence of Y731 in c-Cbl which binds the SH2 domain of the p85 regulatory subunit of PI3K. Surprisingly, this association enables c-Cbl to function as a positive regulator by recruiting PI3K to the cell membrane (Hunter et al., 1999).

3.3.3 E3 ligase activity and adaptor function

The multi-domain nature of Cbl allows it to interact, directly or indirectly, with a wide range of signalling molecules. In this way, activated Cbl proteins act essentially as attenuators of cellular signals by exerting their function as E3 ubiquitin ligases or as adaptors/inhibitors proteins towards PTK pathways.

Cbl E3 ligase activity. Extensive biochemical studies have demonstrated Cbl-mediated ubiquitylation of its substrates (Figure 4). It is clear that ubiquitylation of a targeted receptor occurs in parallel to the onset of receptor internalization and continues to occur throughout the endosomal pathway. One of the best-studied examples of how Cbl-mediated ubiquitylation affects receptor trafficking, and helps terminate the signal from the activated receptor complex, is the EGF receptor (EGFR). This multistep process was initially described in *C. elegans*, where SLI-1 (the Cbl orthologue) was shown to negatively regulate signalling downstream of the LET-23 receptor [the EGFR orthologue] (reviewed in Thien and Langdon, 2001). This mechanism has become a model for the regulation of other RTKs by Cbl.

Cbl as an adaptor/inhibitor protein. An alternative way for Cbl to ubiquitylate EGFR is indirect and utilises its adaptor/inhibitor function by binding to the adaptor protein growth factor- receptor bound-2 (Grb2). One of the first proteins to be recruited into the complex is Grb2, which can recruit Cbl proteins from the cytoplasm to the plasma membrane through interactions between the proline-rich region of Cbl proteins and the N-terminal SH3 domain of Grb2. In this way, Cbl competes with the guanine-nucleotide-exchange factor son-of-sevenless (SOS) to bind Grb2, thereby blocking signalling through the Ras–mitogen-activated protein kinase (MAPK) pathway and inhibiting proliferation.

3.3.4 Signalling pathways in the haematopoietic and immune systems

Cbl gene deletions primarily affected the haematopoietic, immune and metabolic systems. A recent study on *c-Cbl*$^{-/-}$ HSC showed that the number and ability to reconstitute the haematopoietic system was increased in these cells compared to wild-type HSC. These results suggested that Cbl ubiquitin-mediated protein degradation is important for HSC homeostasis (Rathinam et al., 2008). Furthermore, it was shown that c-Cbl is capable of controlling HSC development and function through negative regulation of TPO-dependent STAT5 activation, an important pathway for HSC maintenance. Indeed, *c-Cbl*-deficient HSC displayed TPO hypersensibility, as well as increased levels of STAT5 and its activated form, phospho-STAT5. Thus, these findings underline the role of c-Cbl as important modulator of the TPO/Mpl/JAK/STAT5 signalling pathway in HSCs.

The importance of c-Cbl and Cbl-b in immunity and immune receptor signalling pathways has been demonstrated clearly by the phenotypes of their respective gene knockout mice. Both *c-Cbl*$^{-/-}$ and *Cbl-b*$^{-/-}$ mice display hyperactive signalling downstream of the TCR. Loss of either Cbl protein results in lower activation threshold for signalling through the TCR, hypersensitivity to low affinity/avidity engagement of the receptor, and activation of downstream signalling pathways without the normal requirement for co-receptor stimulation (Figure 4) (Murphy et al., 1998; Naramura et al., 1998; Bachmaier et al., 2000; Chiang et al., 2007). Interestingly, these c-Cbl and Cbl-b phenotypes are restricted to thymocytes and T-cells, respectively, reflecting a difference in tissue distribution with c-Cbl more prominent in the thymus and Cbl-b highly expressed in peripheral T-cells. Loss of Cbl-b dramatically increases T-cell activation threshold and uncouples T-cell activation from the requirement for CD28 co-stimulation, thus leading to spontaneous autoimmunity (Gronski et al., 2004).

Cbl proteins have been also shown to differentially modulate BCR-dependent signalling. Loss of c-Cbl in primary B cells showed a significant inhibition of BCR-mediated signalling mainly caused, not by down-regulation of Syk, but instead by up-regulation of Lyn kinase (Shao et al., 2004). In contrast, Cbl-b negatively regulates BCR-mediated signalling, this time, by down-regulating Syk in primary B cells (Sohn et al., 2003). Furthermore, the activity of Cbl proteins as adaptors was also implicated in the effect of Cbl proteins on B-cell activation. In this case, it has been shown that c-Cbl negatively regulates the phospholipase C-γ2 (PLC-γ2) pathway in B cells, while Cbl-b was shown to positively regulate this same pathway (Yasuda et al., 2002).

Studies carried out with cell lines have demonstrated that both c-Cbl and Cbl-b negatively regulated FcεRI-mediated mast cell activation (Figure 4). However, experiments on primary mast cells derived from *c-Cbl* and *Cbl-b*-deficient mice revealed a more profound effect with the lack of Cbl-b than of c-Cbl (Zhang et al., 2004). Cbl-dependent FcεRI down-regulation occurs mainly via Cbl E3 ubiquitin ligase activity that promotes receptor β and γ multiubiquitination, providing signals for receptor internalization and sorting into lysosomal compartments for degradation. Interestingly, Syk activity is required for c-Cbl-dependent ubiquitylation of FcεRI receptor (Paolini et al., 2002). On the other hand, Cbl proteins can also down-modulate engaged FcεRI through its adaptor function by interacting with molecules involved in clathrin-mediated endocytosis (Molfetta et al., 2005). Remarkably, Cbl proteins also negatively regulate mast cell activation by selectively ubiquitinating and degrading the activated kinase form of Lyn and Syk proteins (Paolini et al., 2002; Kyo et al., 2003; Qu et al., 2004).

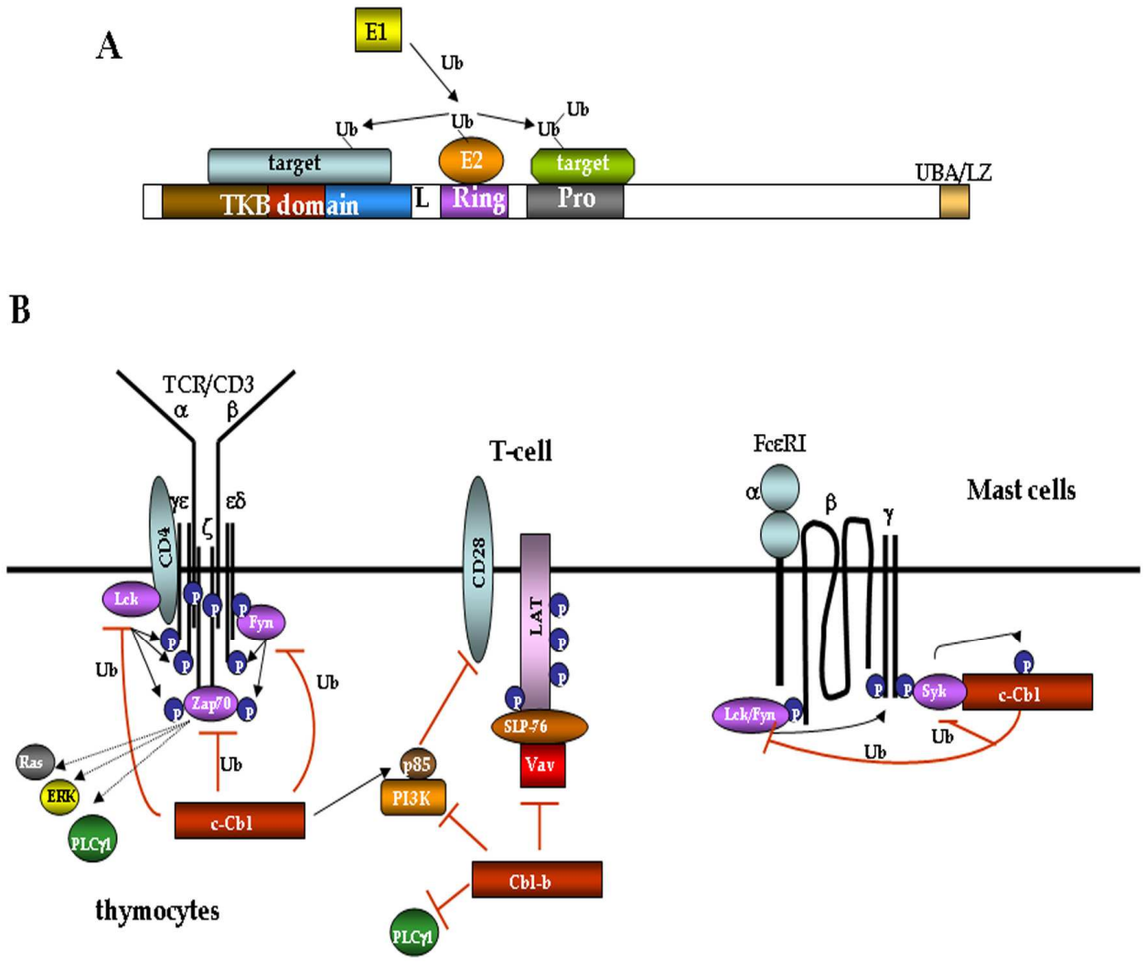

Fig. 4. A) Model of Cbl ligase activity. B) Schematic model of Cbl signalling pathways in T and mast cells

c-Cbl also participates in the modulation of monocyte/macrophage signalling mediated by Fcγ and Colony Stimulating Factor (CSF)-1 receptors through its adaptor functions. c-Cbl is capable of attenuating CSF-1-mediated signalling by binding to a phosphotyrosine residue of this receptor and then ubiquitylating and targeting it for degradation. Lastly, c-Cbl also appears to play a negative regulatory role in platelets as well. This is not so surprising considering that Syk kinase has a biological function in these cells, a known substrate of c-Cbl. The contribution of c-Cbl-dependent ubiquitylation of Syk to the negative effect of c-Cbl on platelet functions is not yet understood, however, it is possible that the biological role of c-Cbl in platelets consists in preventing unwanted platelet activation *in vivo* by increasing the threshold of platelet activation.

3.3.5 Regulation of Cbl function

Cbl proteins are potent regulators of cell function and development through their adaptor function and ligase activity. It is therefore necessary that the Cbl proteins are at their turn, subject to complex and sophisticated regulatory mechanisms that fine-tune the effects that

these proteins have on signalling (Ryan et al., 2006). These include: *cis*-acting structural elements that prevent inappropriate E3 activity until the Cbl proteins interact with their substrate, degradation of the Cbl proteins, inhibition of Cbl protein function mediated by protein interactions, deubiquitination of the Cbl substrates, and negative regulation of trafficking of the ubiquitinated Cbl substrates. Therefore, abnormal Cbl regulation can lead to pathological conditions such as immunological and malignant diseases, thus underscoring the essential role of Cbl in normal homeostasis.

3.4 The SOCS family

3.4.1 Origin, structure and cellular expression

The SOCS (for Suppressors Of Cytokine Signalling) proteins are a family of intracellular molecules that negatively regulate the strength and duration of cytokine receptor signalling cascades, notably the JAK/STAT pathway. This family consists of eight members, CIS and SOCS1-7 that share structural and functional homology. The first family member identified was CIS (for Cytokine-Inducible SH2-containing protein) cloned as an early gene differentially induced following IL-3 and EPO exposure (Yoshimura et al., 1995). The second member identified, SOCS1 (also known as JAB for JAK-Binding protein or SSI for STAT-induced STAT Inhibitor) was identified simultaneously by three separate groups (Starr et al., 1997; Endo et al., 1997; Naka et al., 1997). SOCS-2 and SOCS-3 were cloned using distantly related expressed sequence tags [ESTs] and the rest of the members (SOCS4-7) were identified on the basis of a conserved C-terminal amino acid sequence using various DNA databases (Starr et al. 1997; Minamoto et al., 1997; Hilton et al., 1998). All members of the SOCS family have a similar tripartite domain organization composed of a variable N-terminal region, followed by a central SH2 domain and a conserved C-terminal SOCS box domain (Fig. 1). SOCS1 and SOCS3 differ from other family members in that they possess an extended SH2 domain at the N-terminus (for SOCS1 and SOCS3) or the C-terminus (for SOCS3) of this domain. Additionally, they also present at their N-terminal region and adjacent to their SH2 domain, a Kinase-Inhibitory Region (KIR) required for inhibition of JAK kinase activity (Yasukawa et al., 1999; Sasaki et al., 1999). Another exception is SOCS7 which is unique in its possession of a proline-rich N-terminus and a nuclear localisation signal.

SOCS molecules expression is controlled at the transcriptional, translational and post-translational levels. Many *SOCS* genes contain STAT-binding sequences in their promoter region and their STAT-dependent transcription can be differentially regulated in a factor- and tissue specific manner. SOCS proteins are often low or undetectable at the basal level in unstimulated cells and their expression is rapidly induced to a variable extent in different cell types and tissues by immunoregulatory cytokines, colony stimulating factors, growth factors and hormones that signal via JAK-STAT pathway or via RTK [Table 4] (Starr et al., 1997). Certain cytokines that do not signal via JAK kinases or RTKs, and a number of non-cytokine ligands can also induce *SOCS* gene expression, such as TNF signalling and bacterial products such as LPS and CpG DNA which signal via Toll-like receptors (TLR). Because *SOCS* genes are induced by cytokines and the corresponding proteins inhibit further cytokine-induced signalling, SOCS proteins are believed to form part of a classical negative feedback loop mechanism. Northern blot analysis of murine tissues has shown that *CIS* and *SOCS2* mRNA was ubiquitously expressed, with expression being particularly

strong (CIS) or weak (SOCS2) in kidney, lung, liver, heart, testis and male spleen (Yoshimura et al., 1995; Starr et al., 1997). *SOCS1* and *SOCS3* mRNA was detected at different levels in adult haematopoietic organs such as the thymus and spleen and to some extent in other organs like lung, spleen and testis (Starr et al., 1997). However, *SOCS3* show high expression in fetal liver (Marine et al., 1999). Although some of the SOCS members appear to be co-expressed in only a few organs, the *in vivo* expression of *SOCS* genes may be more pronounced than is detectable by Northern hybridisation, since most cell types seem to depend on cytokine stimuli for SOCS induction.

3.4.2 Signalling targets

The different domains in the SOCS proteins allow them to bind to the cytokine receptors, associated JAKs or other signalling molecules and to attenuate signal transduction directly or indirectly by targeting the receptor complex for ubiquitin-mediated degradation in proteosomes. The N-terminal region of the SOCS proteins is variable in length and sequence. In SOCS1 and SOCS3, there is a 12 amino acid sequence adjacent to the SH2 domain that is essential for the inhibition of JAK2 kinase called KIR. This sequence is supposed to function via a conserved tyrosine residue as a pseudo-substrate, lodging in the catalytic cleft to block further JAK kinase activity. Removal of this tyrosine does not affect binding of the SOCS proteins to the kinase, but does abrogate its inhibition (Sasaki et al., 1999). Inded, a SOCS1-KIR mimetic peptide is sufficient to inhibit IFNγ-mediated JAK2 activity in primary cells (Waiboci et al., 2007). The role of the remaining N-terminal region among the SOCS family members has yet to be elucidated.

The central SH2 domain determines the target protein for degradation of each SOCS protein. It binds to distinct phosphorylated tyrosine motifs on SOCS target proteins (Table 4). Once the SH2 domain binds to its specific target, it brings other domains in proximity to the target protein, directing degradation of the appropriate protein. Mutagenesis studies allowed the identification of small regions at the N-termini of SOCS1 and SOCS3, and at the C-terminus of SOCS3 SH2 domains, critical for phosphotyrosine binding. These regions have been defined as an N- and C-extended SH2 domain (N-ESS and C-ESS, respectively). The solved structure of SOCS3 SH2 domain had shown that the N-ESS sequence directly contacts the phosphotyrosine-binding loop of the kinase and determines its orientation (Babon et al., 2006). The C-ESS of SOCS3 forms part of the SH2 domain that is spatially displaced by a 35 amino acid PEST [for Proline, Glutamic acid (E), Serine and Threonine rich sequence] insertion. This sequence is thought to signal for rapid proteolytic degradation. It is therefore not surprising that removal of this sequence lowers SOCS3 turnover without affecting the SH2 domain folding and function (Babon et al., 2006). Since other SOCS members contain putative PEST sequences, this may prove to be a common mechanism for regulation of SOCS protein levels.

The SOCS proteins are substrate recognition factors for an E3 ligase that targets their specific cargo for ubiquitin-mediated degradation. They serve this function by binding to the E3 complex via the highly conserved SOCS box domain at their C-terminus. The SOCS box is a 40 amino acid motif found not only in the SOCS family members, but also in a vast number of proteins. The SOCS box consists of a three-α-helical structure bound to an E3 ubiquitin ligase complex that in turn covalently binds ubiqutin to lysines in the target protein. The N-terminus of the SOCS box mediates interaction with Elongin C/B, while the C-terminus of

SOCS proteins	SH2 partner	Inducer system	Signalling system inhibited
CIS	EPOR, PRLR, Leptin R	EPO, IL-2/3/6, IFNα, PRL, GH, Leptin	EPO, PRL, IL-2/3, GH
SOCS1	JAK2, IFNGRI	EPO, TPO, GM-CSF, G-CSF,M-CSF,IL-2/4/6/7/9/10/13/15, PRL, LPS, TNFα, GH, In, CpG DNA	IL-2/4/6/7/12/15, IFNα/β/γ, LIF, TNFα, EPO, TPO, GH, PRL, In, Leptin
SOCS2	GHR, Leptin R	GH, IL-6, IFNα/γ, LIF	IL-6, GH, IGF-1
SOCS3	EPOR, gp130, G-CSFR, IL-12Rβ2, Leptin R	IL-1, TGF-β, IL-2/6/9/10/13, GH, LPS, EGF, IFNα/γ, LIF, EPO, GM-CSF, PRL, In, Leptin	IL-2/4/6/9/11, IFNα/β/γ, LIF, EPO, GH, PRL, In, Leptin
SOCS4, SOCS5	EGFR	EGF, IL-6	EGF, IL-4/6
SOCS6, SOCS7	IRS2/4, IR	In, IGF	In, IGF

In, insulin; PRL, prolacftin.

Table 4. Signalling partners, transduction pathways and factors regulating SOCS protein expression and function.

the SOCS box directs the SOCS/Elongin C/B association with Cullin 5 (Cul5) scaffold protein (Zhang et al., 1999). The latter one recruits the stabilizing RING finger protein Rbx and allows Cul5 to bind to an E2 ubiquitin-conjugating enzyme. The resulting complex SOCS/Elongin B/C/Cul5/Rbx/E2 forms a functional E3 ubiquitin ligase (Figure 5a). The SOCS1-SOCS box has been shown to be capable of driving the ubiquitination of specific proteins such as TEL-JAK2 fusion and Vav1, and only in very few cases the receptor complex for subsequent degradation through the proteosome (Frantsve et al., 2001; De Sepulveda et al., 2000; Irandoust et al., 2007; Verdier et al., 1998). The importance of the SOCS box has been further shown by *in vivo* targeted deletion of SOCS1 and SOCS3 in mice that resulted in partial loss of SOCS function with enhanced IFNγ and G-CSF signalling, respectively (Zhang et al., 2001).

3.4.3 Regulation of cytokine receptor signalling pathways

The structural analysis of the SOCS molecules revealed they can control cytokine receptor signalling by several mechanisms (Figure 5):

(1) Direct inhibition of intrinsic kinase activity by binding to JAKs. Immunoprecipitation assays revealed that both SOCS1 and SOCS3 were able to co-precipitate with JAK kinases upon cytokine stimulation, and that this association blocked JAK kinase activity, although with a different affinity and kinetics (Endo et al., 1997; Sasaki et al., 1999). Structure-function studies using truncated versions of SOCS1 identified the regions essential for SOCS-JAK association and for inhibition of JAK activity. SOCS1 inhibits tyrosine kinase activity upon interaction with phosphorylated tyrosine Y1007 located in the activation loop of JAK.

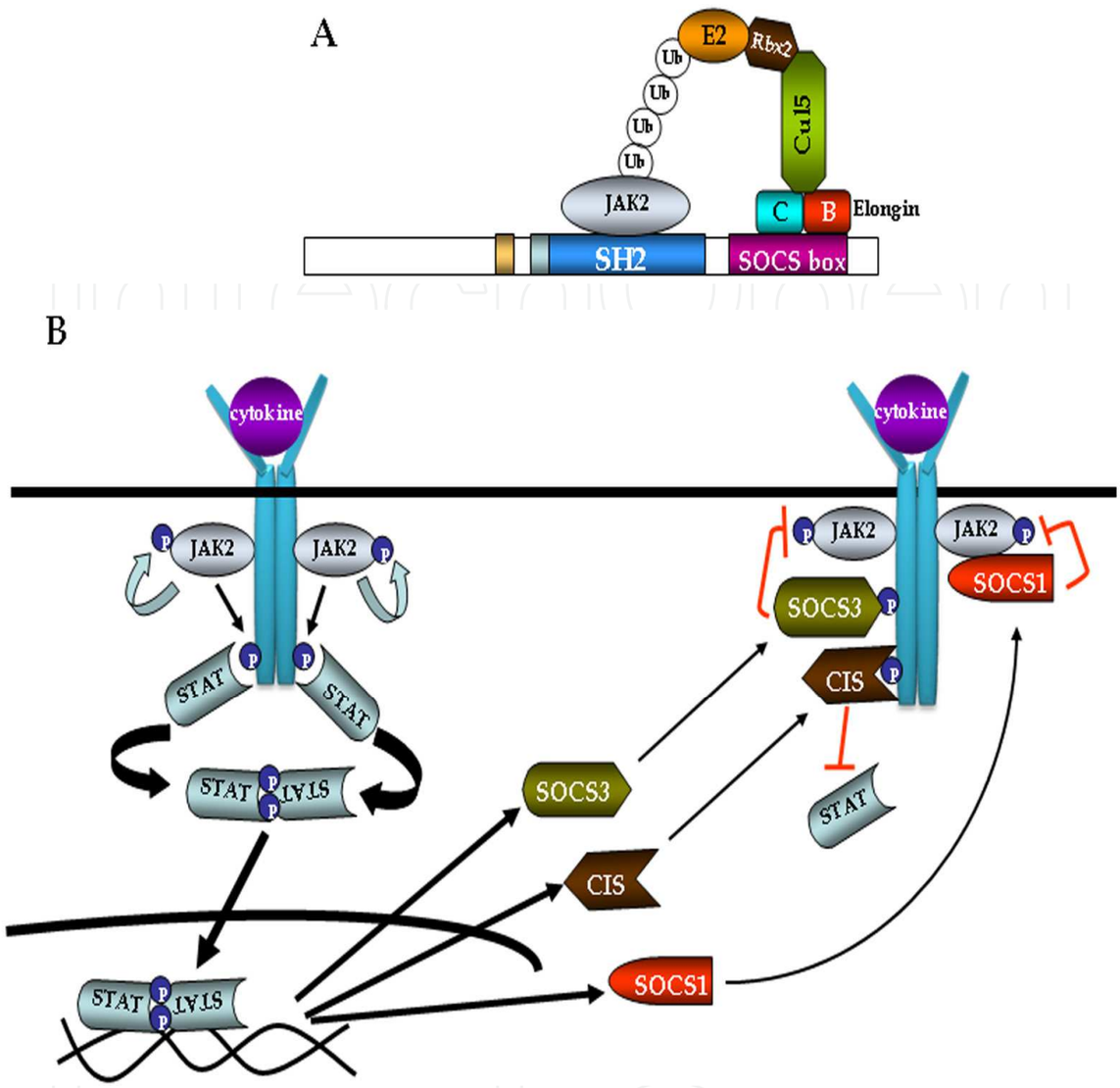

Fig. 5. A) Schematic representation of the SOCS box function, B) Mechanisms of suppression by the SOCS proteins.

However, complete inhibition of the kinase activity requires not only the SH2 domain but also the KIR region (Narazaki et al., 1998; Nicholson et al., 1999). The critical amino acids for the KIR region's function are conserved between SOCS1 and SOCS3, suggesting a common inhibitory mechanism for these two family members.

(2) Indirect inhibition of JAKs by binding to the receptor. As with SOCS1, the SOCS3 SH2 domain was initially shown to interact with Y1007 in JAK2, albeit with lower affinity than SOCS1. However, subsequent studies demonstrated that SOCS3 SH2 domain exhibited a higher affinity for phosphotyrosine residues located within the receptor subunits than for JAK2 (Sasaki et al., 1999). Accordingly, it was found that SOCS3 associated with higher affinity with phosphorylated residues in the IL-6 receptor subunit, gp130, notably Y759, than with the activation loop of JAKs (Nicholson et al., 2000). SOCS3, therefore, in contrast

to SOCS1, has to be recruited to the receptor complex in order to inhibit IL-6 signal transduction (Schmitz et al., 2000). Subsequently, SOCS3 might inhibit the kinase activity of JAKs through the pseudo-substrate, KIR, in the same way as SOCS1, but only after recruitment and binding to critical phosphotyrosine residues in the cytokine receptor. Given that SOCS1 and SOCS3 can interact with both receptor and JAK, a two-step interaction model has been proposed, whereby the SOCS1/3 SH2 domains are first recruited to the receptor and subsequent bi-modal binding to nearby JAK through the SH2 domain and KIR region results in a high affinity interaction, inhibition of JAK kinase activity and potential proteosomal degradation.

(3) Blocking binding of downstream signalling molecules to the receptor. This mechanism has been shown for CIS and SOCS2 for EPO and GH signalling inhibition, respectively. Upon stimulation of the receptors, the SOCS proteins bind to tyrosine residues at the membrane distal region of receptor chains that are docking sites for downstream signalling molecules, such as STAT5 or SHP-2. By masking these phosphorylated residues, CIS/SOCS2 competes with STAT thereby down-modulating the signalling (Yoshimura et al., 1995; Ram & Waxman, 1999). On the other hand, SOCS3-mediated inhibition of signalling via EpoR, gp130 and LeptinR could partially result from competitive inhibition of SHP-2 binding to gp130, LeptinR, and EpoR, resulting in the blockade of SHP-2-mediated ERK activation (Schmitz et al., 2000; Bjorbaek et al., 2001; Sasaki et al., 2000).

(4) Ubiquitination and subsequent proteasomal degradation of JAKs and receptor. Studies on EPO receptor using proteasome inhibitors showed that these compounds protected the EpoR and STAT5 from the normal reduction in phosphorylation upon CIS expression, indicating proteasome involvement of both EPO-receptor and STAT5 inactivation (Verdier et al., 1998). These results allow proposing a model where the phosphorylated target molecules may become a substrate of the proteolytic machinery by binding to SOCS. In this situation, the SOCS box acts as an adaptor molecule, bringing into this complex the Elongin B/C/E3 ligase for ubiquitination of the target protein. Subsequently, the substrate and the associated SOCS proteins may be destroyed, and the cell is ready to respond again to stimulation. Therefore, targeting the signalling proteins for degradation by the SOCS box seems to be another mechanism by which cytokine signalling might be inhibited under physiological levels of SOCS proteins.

3.4.4 Regulation of SOCS proteins

Besides elaborate transcriptional regulation, another control point of the expression of SOCS molecules is their protein stability. SOCS proteins exhibit a rapid turnover rate, and the half lives of SOCS1, SOCS2 and SOCS3 have been estimated to be less than 2 h (Siewert et al., 1999). Several mechanisms have been proposed to regulate SOCS expression. The presence of a PEST sequence in SOCS3 appears to mediate non-proteosomal degradation, while SOCS box-dependent ubiquitination of SOCS3 on lysine 6, at least *in vitro*, contributes to proteosomal degradation of the SOCS3 protein (Sasaki et al., 2003). Furthermore, SOCS3 is uniquely phosphorylated within the SOCS box on Y204 and Y221 and this appears to have two consequences, interaction with the Elongin B/C complex is lost, destabilizing the SOCS3 protein, and signalling through the Ras/MAPK pathway can be potentiated (Haan et al., 2003; Cacalano et al., 2001). Nevertheless, the full implication of SOCS3 phosphorylation on its regulation remains to be explored.

4. Animal models of inhibitory adaptors: Definition of their physiological significance

Central to understanding the physiological role of families of inhibitory adaptors in the haematopoietic and immune systems, has been the generation of mice deficient for these proteins when possible. This has also helped dissect regulatory and/or compensatory mechanisms through the functional, complete or partial, reconstitution in these mice, in particular for members of multi-gene families. These approaches have allowed 1) analyse changes in the expression level of the adaptor that can also affect its signalling pathway; 2) establish how deficiency of an adaptor can have dramatically different effects in different cell lineages; 3) understand the functional synergy between members of the same family of adaptors; 4) identify null mutations leading to the complete absence of some cell types, while leaving others with no discernable defect and 5) define how some deficiencies are selective within a cell type, disrupting only particular pathways while leaving others intact. Mice deficient for the different families of inhibitory adaptors discussed in this chapter have certainly provided important new insights into the biology and function of these adaptors. However, in some cases, these animal models have also raised important questions regarding the mechanisms of action of these regulators and their potential therapeutic application.

4.1 *Dok* deficient mice

Although Dok-1, Dok-2, and Dok-3 have been shown to act as negative regulators downstream of a wide range of immunoreceptors, cytokine, and LPS receptors mediated signalling, insights into their physiological importance in immune cells have and will continue to come from studies with mice or cells lacking individually or in combination Dok-1, Dok-2, and Dok-3. *Dok-1* or *Dok-2*-deficient mice displayed normal steady-state haematopoiesis. By contrast, mice lacking both *Dok-1* and *Dok-2* succumbed to a myeloproliferative disease resembling human chronic myelogenous leukaemia (CML) and chronic myelomonocytic leukaemia (CMMoL) at around one year of age (Yasuda et al., 2004; Niki et al., 2004) [Table 5]. These animals displayed medullary and extramedullary hyperplasia of granulocyte/macrophage progenitors with leukemic potential, and their myeloid cells showed hyperproliferation and hypo-apoptosis upon treatment and deprivation of cytokines, respectively. Consistently, the mutant myeloid cells showed aberrant Ras/MAP kinase and Akt activation upon cytokine stimulation. Strikingly, ablation of *Dok-1* and *Dok-2* markedly accelerated leukaemia and blastic crisis onset in *bcr-abl* transgenic mice known to develop a CML-like disease. These results demonstrate the critical role of Dok-1 and Dok-2 in myeloid homeostasis and suppression of leukaemia. Interestingly, half of the double-deficient mice also developed histiocytic sarcoma (HS) of macrophage origin. These results suggest an involvement of additional genetic changes.

Similar to *Dok-1* and *Dok-2* deficiency, *Dok-3* inactivation did not result in development of aggressive tumors in haematopoietic cells (Ng et al., 2007). However, ablation of all three Dok proteins (Dok-1/2/3) in mice has recently shown drastic phenotypic consequences. These mice showed earlier mortality due to development of aggressive HS with multiple organ invasions, but no incidence of other types of tumors (Mashima et al., 2010). This disease is a haematological malignancy characterized by cells displaying a tissue macrophage-like (histiocytic) morphology (Grogan et al., 2008). Indeed, loss of *Dok-1/2/3*

causes aberrant proliferation of macrophages in the lung, already detectable before the onset of morphologically recognizable HS. These cells displayed an exaggerated proliferative response to M-CSF or GM-CSF compared to wild type littermates. These results suggest that Dok proteins can mutually compensate and inhibit macrophage proliferation and therefore suppress the aggressive transformation of HS.

4.2 *Lnk/APS/SH2-B* deficient mice

Mice deficient for members of this family have demonstrated the positive (SH2-B) and negative (Lnk and APS) physiological role of these adaptors in growth factor, cytokine, and immune receptors signalling (Table 5). Deletion of the *SH2B1* gene resulted in severe obesity, hyperphagia and both leptin and insulin resistance as well as infertility, which might be a consequence of resistance to IGF-1 (Ohtsuka et al., 2002; Ren et al., 2005). Thus, *SH2-B*-deficient mice support a role for this adaptor as a positive regulator of JAK2 signalling pathways initiated by leptin, insulin and potentially, by IGF-1.

Interestingly, *APS*-deficient mice also displayed an insulin-related phenotype. They showed a hypersensibility to insulin and enhanced glucose tolerance, a finding that is consistent with APS playing a negative role in insulin signalling (Minami et al., 2003). Moreover, these mice present also a haematopoietic phenotype with defects in degranulation of mast cells and cytoskeleton rearrangement in both mast and B-1 cells (Kubo-Akashi et al, 2004; Iseki et

Gene	Approach	System	Mouse phenotype/Human disease
Dok-1 Dok-2	KO	mu	Impaired immunoreceptor signaling in lymphocytes, allergic responses in mast cells, enhanced ERK signaling, hyperresponsiveness to LPS
Dok-1/2	KO	mu	MPN, Lupus Renal disease/CML, CMMoL
Dok-1/2/3	KO	mu	HS
Lnk	KO	mu	MPN-like (ET/PMF), CML (aged mice)
	Tg	mu	Impaired lymphopoiesis
	Mu (SNP)	hu	MPN, autoimmune, cardiovascular and inflammatory diseases
APS	KO	mu	Insulin hypersensitivity, enhanced glucose tolerance, enhanced B1 cells proliferation
	Tg	mu	Reduced B1 and B2 cell number, impaired B-cell development and BCR-dependent proliferation, reduced mast cell degranulation and actin assembly
SH2B	KO	mu	Severe obesity, hyperphagia, increased leptin and insulin resistance, infertility
c-Cbl	KO	mu	Enlarged thymus, splenomegaly, extramedullar haematopoiesis, enhanced thymocyte function/ myeloid malignancies
	Mu	mu	Oncogenesis, mastocytosis, myeloid leukemia, tumourigenesis

Gene	Approach	System	Mouse phenotype/Human disease
c-Cbl	Mu	hu	AML, MPD/MPN, aCML, JMML
Cbl-b	KO	mu	Impaired immunological tolerance, autoimmune diseases with inflammatory organ and tissue damage, enhanced peripheral T-cell function, rejection of certain tumours
c-Cbl/ Cbl-b	KO	mu	Embryonic lethal
	T-cell KO	mu	Autoimmune-like vasculitis, SLE-like autoimmune disease
CIS	KO	mu	No specific phenotype
	Tg	mu	Fewer T-cells, NK, and NK-T cells, lactation deficiency, similar phenotype to STAT5 KO
SOCS1	KO	mu	perinatal lethality, enhanced IFNγ production and responsiveness, lymphopenia and inflammation with multi-organ infiltration
	Tg(Tcell)	mu	Inhibited T-cell response to IFNγ, IL-6 and IL-7, increased CD4+T-cells, reduced peripheral T-cell activation
		hu	Increased expression in Th-driven inflammatory diseases (RA, UC, Crohn, dermatitis), decreased expression and/or hyper-methylation in certain cancers
SOCS2	KO	mu	Gigantism, deregulated GH signalling
	Tg	mu	Gigantism
SOCS3	KO	mu	Embryonic lethality due to placental deficiency, erythrocytosis, deregulated LIF response
	Tg	mu	Embryonic lethality due to anemia
	Tg(Tcell)	mu	High TGFβ1 and IL-10 production
	Mu (SNP)	hu	Allergic diseases
		hu	Increased expression in Th2-driven asthma patients and inflammatory diseases, hyper-phosphorylation in MPN
SOCS5	KO	mu	No specific phenotype
	Tg	mu	Reduced IL-4-mediated STAT6 activation, reduced Th2 development and cytokine production
SOCS6	KO	mu	Mild growth retardation
SOCS7	KO	mu	Growth retardation (strain-dependent), hypoglucemia, hydrocephalus, altered glucose homeostasis, enlarged pancreatic islets

KO, knock out; Tg, transgenic; Mu, mutations; hu, human; mu, murine, RA, theumatoid arthritis; UC, ulcer colitis.

Table 5. Mouse phenotype and human diseases related to deficiencies in inhibitory adaptors.

al., 2004). These results suggest a negative role for APS in controlling actin dynamics in these cells. Furthermore, *APS*-deficient mice display an increase in B-1 cells in the peritoneal cavity and humoral responses to type-2 antigen, indicating a negative regulatory role for APS in BCR-mediated cell proliferation and cytoskeletal regulation.

By contrast, mice deficient for *Lnk* display a profound perturbation in haematopoiesis that confirmed its role as a key negative regulator of cytokine signalling in the haematopoietic system. Indeed, these mice exhibit splenomegaly together with fibrosis, expansion of HSC, B lymphoid and myeloid progenitors that confer an enhanced repopulating ability. *Lnk*-deficient mice have also revealed an important role for Lnk in B-cell lymphopoiesis, megakaryopoiesis and erythropoiesis, as a result of the absence of negative regulation of SCF, TPO and EPO signalling pathways. One important feature of the *Lnk*$^{-/-}$ mice phenotype is its resemblance to human myeloproliferative neoplasms (MPN): hypersensitivity to cytokines, increased number of haematopoietic progenitors, high platelet counts, splenomegaly together with fibrosis and extramedullary hematopoiesis (Velazquez et al., 2002; Campbell & Green, 2008). This has suggested an important role for Lnk in the development of these diseases. Indeed, loss of Lnk cooperates with oncogenes, such as JAK2 and BCR-ABL, to induce MPN in mice. These animals exhibit a disproportionate expansion of myeloid progenitors and immature precursors *in vitro* and *in vivo* (Bersenev et al., 2010). Moreover, aged *Lnk*$^{-/-}$ mice seem to spontaneously develop a Chronic Myeloid Leukemia (CML)-like MPN, suggesting a role for Lnk in myeloid expansion *in vivo*. However, this myeloid cell hyperproliferation fails to trigger blast crises, reflecting the need of *Lnk*-deficiency for additional oncogenic events to promote blast transformation. *Lnk*-deficient mice also exhibited an increase in endothelial progenitor cells (EPC) numbers that display an enhance capacity for colony formation. Different molecular, physiological and morphological approaches have shown that *Lnk* deficiency promotes vasculogenesis/angiogenesis and osteogenesis through the mobilization and recruitment of HSCs/EPCs via activation of the SCF/Kit signalling pathway in the ischemic and perifracture zone, respectively, thereby establishing an optimal environment for neovascularisation, bone healing and remodelling (Kwon et al., 2009, Matsumoto, et al., 2010). Taken together, these findings strongly suggest that Lnk regulates bone marrow EPC kinetics during vascular and bone regeneration.

4.3 *Cbl*-deficient mice

Mice deficient for *c-Cbl* and *Cbl-b* have been invaluable in demonstrating the important roles played by these proteins in fine-tuning signalling thresholds in immune cells. Despite close structural similarities, loss of c-Cbl and Cbl-b proteins evokes prominent phenotypic differences (Table 5). *c-Cbl*-null mice have an enlarged thymus, expanded hematopoietic progenitor pools with increased repopulating capacity, splenomegaly with extramedullary hematopoiesis, as well as changes in energy metabolism, and reduced fertility of male mice (Murphy et al., 1998; Naramura et al., 1998; Molero et al., 2004; El Chami et al., 2005, Rathinam et al., 2008). However, the most marked alteration in *c-Cbl* and *Cbl-b*-deficient animals is being associated with thymocyte and peripheral T-cell activation, respectively. *c-Cbl*$^{-/-}$ mice exhibit strong effects on thymocytes, with increase cell numbers in the thymus of the young adult and enhanced signal strength following TCR engagement. Moreover, perturbed thymocyte signalling does not depend on the TKB domain of c-Cbl, as a TKB knock-in did not rescue the phenotype (Thien et al., 2003). In contrast, *Cbl-b* ablation results in an impaired immunological

tolerance induction and animals succumb to spontaneous and/or induced autoimmune diseases with widespread inflammatory organ (pancreas, lung) and tissue (adipose) damage (Bachmaier et al., 2000, 2007; Hirasaka et al., 2007). Importantly, *Cbl-b*-null mice are able to reject multiple types of tumours spontaneously (Chiang et al., 2007; Loeser et al., 2007).

The redundant and overlapping functions of Cbl family proteins are more evident from the striking phenotypes of *Cbl, Cbl-b* double-deficient mice. Deletion of both proteins in the germline leads to early embryonic lethality (Naramura et al., 2002). In contrast, T-cell specific double knock-out mice are viable, however, their T-cells develop independent of MHC-restricted thymic selection and these mice eventually succumb to autoimmune-like vasculitis early in adult life (Huang et al., 2006; Naramura et al., 2002). Similarly, B-cell specific *Cbl, Cbl-b* double deficiency leads to a failure to acquire tolerance to self antigens and the animals developed Systemic Lupus Erythematosus (SLE)-like autoimmune diseases (Kitaura et al., 2007). At the molecular level, cells from these double knock-out mice display delayed down-modulation of cell surface antigen receptors and prolonged activation of downstream signalling pathways.

Loss of *c-Cbl*, but not of *Cbl-b*, led to a significant expansion of HSC and haematopoietic progenitors. Strikingly, ablation of both proteins enhanced this phenotype and eventually all mice succumbed to aggressive myeloproliferative disease-like leukemia with peripheral organ involvement within two to three months after birth (Naramura et al., 2010). Moreover, blastic transformation of chronic myelogeneous leukemia in a *bcr/abl*-transgenic model is accelerated in the *c-Cbl* null background (Sanada et al., 2009). Combined, these observations support that c-Cbl can act as a tumor suppressor and that complete loss of Cbl functions is required to promote myeloid malignancy. In contrast to the tumor suppressor function of the wild-type c-Cbl, c-Cbl mutants isolated from human and murine neoplasms, as well as v-Cbl, show clear transforming capacity in terms of anchorage-independent growth in soft agar *in vitro* and tumour generation in nude mice *in vivo* (Sanada et al., 2009; Thien et al., 2001). Bone marrow cells transduced with c-Cbl mutants in the linker (70Z) or in the RING finger domain (R420Q) generate generalized mastocytosis, a myeloproliferative disease, and myeloid leukemia in lethally irradiated mice with long latency but high penetrance (Bandi et al., 2009). The transforming activity of these mutant forms of c-Cbl seems to be mediated by alteration of the E3 ubiquitin ligase activity. Most c-Cbl mutations in myeloid neoplasms are missense changes at highly conserved amino acid positions within the linker and RING finger domains, or involve splice-site sequences, leading to amino acid deletions within them. Supporting these findings, the generation of knock-in mutants carrying single point mutations in the TKB, linker or RING domains of c-Cbl protein has further validated the importance of these domains in Cbl-mediated tumorigenesis. Interestingly, mice with an equivalent RING finger domain mutation in *Cbl-b* do not show comparable changes in the haematopoietic compartment, indicating that Cbl-b is not capable of inhibiting c-Cbl functions (Rathinam et al., 2010). Taken together, mouse models with Cbl family ablation or point mutations have convincingly established the role of these proteins as important E3 ubiquitin ligases for the homeostasis of the haematopoietic and immune systems and as tumour suppressors.

4.4 *SOCS*-deficient mice

Since the discovery of SOCS proteins, much attention has been drawn to their physiological roles and their involvement in human diseases. Many of their common inhibitory activities

on cytokine signalling demonstrated *in vitro*, do not seem to be essential *in vivo* as genetic ablation of CIS, SOCS1, SOCS2 and SOCS3 genes has demonstrated remarkable cytokine specificity for different SOCS molecules (Table 5).

SOCS1-deficient mice are growth retarded and died within 3 weeks of birth with a syndrome characterized by severe lymphopenia, activation of peripheral T cells, fatty degeneration and necrosis of the liver and multi-organ failure with rampant inflammation due to macrophage infiltration of major organs (Alexander et al., 1999; Marine et al., 1999a, Starr et al., 1998). The neonatal phenotypes appear primarily as the result of deregulated IFNγ signalling, because *SOCS1*$^{-/-}$ mice that are also deficient for IFNγ, do not die neonatally. Moreover, constitutive STAT1 activation and IFNγ-inducible genes were observed in *SOCS1* deficient mice, indicating that IFNγ signalling is regulated by SOCS1 and that its deregulation contributes to the lethal phenotype. However, the *SOCS1, IFNγ* double deficient mice ultimately died 6 months after birth with inflammation and polycystic kidneys, which suggests that SOCS1 regulation is not exclusive to IFNγ (Metcalf et al., 2002). The lethality in *SOCS1*$^{-/-}$ mice is also significantly delayed in the *RAG2*$^{-/-}$, *STAT1*$^{-/-}$, *STAT6*$^{-/-}$ and *STAT4*$^{-/-}$ backgrounds, thus implicating SOCS1 as a critical regulator of IFNγ, IL-4 and IL-12 signalling pathways (Alexander and Hilton, 2004).

In recent years, it has become clear that some of the SOCS members play critical roles in regulating T-cell differentiation, maturation and function by controlling different signalling events, as shown by the phenotypes displayed by *SOCS1* and *SOCS3* deficient mice. In this sense, T-cell specific conditional deletion of *SOCS1* showed that it is not sufficient to induce the lethal multi-organ disease; however it does cause T-cell specific abnormalities that include increased numbers of $CD8^+$ T cells and increased sensitivity to cytokines with common γ-chain receptors. SOCS1 also plays an important role in the regulation of Tregs. Higher number of Tregs is observed in the thymus and spleen of T-cell-specific *SOCS1*-deficient mice (Horino et al., 2008). This is probably due to higher IL-2 responses, because IL-2 enhances Tregs proliferation. Moreover, Lu and colleagues have recently showed that SOCS1-specific ablation in Tregs induced the development of spontaneous dermatitis, splenomegaly and lymphoadenopathy in these mice (Lu et al., 2009). These results point out SOCS1 as an important controller of Tregs.

Analysis of mice bearing the deletion of the SOCS box of *SOCS1* demonstrated the *in vivo* importance of the SOCS box for inhibition of IFNγ signalling by SOCS1. SOCS box deleted-deficient mice also die prematurely and suffer from reduced body weight. Inflammatory lesions are observed in skeletal and heart muscle, cornea, pancreas and dermis.

SOCS2 is thought to play a major role in the regulation of GH signalling. Indeed, mice deficient for *SOCS2* develop gigantism with enlargement of visceral organs and 3 months after birth weight 30-40% more than control mice, elevated mRNA levels of insulin-like growth factor (IGF-1) and enhanced responses to exogenous GH (Metcalf et al., 2000; Greenhalgh et al., 2005). Interestingly, over-expression of SOCS2 increases GH signalling and *SOCS2* transgenic mice develop mild gigantism (Favre et al., 1999). These results suggest a more complex role of SOCS2 in regulating GH signalling. To date, there are no evidences on the role of SOCS2 in the regulation of immune responses.

Deletion of *SOCS3* leads to embryonic lethality with embryos dying between 12 and 16 days of gestation (Marine et al., 1999b). Lethality was initially thought to result from excessive

erythropoiesis due to enhanced EPO signalling. However, further studies showed that lethality was in fact due to placental insufficiency with poor development of embryonic vessels, spongiotrophoblasts, as well as increase in trophoblast giant cell differentiation (Roberts et al., 2001). Tetraploid aggregation assays resulting in *SOCS3*-deficient foetal components with SOCS3 sufficient placental tissues, generated live birth *SOCS3*$^{-/-}$ mice. These mice were smaller than littermates, exhibited cardiac hyperthrophy and finally succumbed by 25 days. Importantly, SOCS3 lethality could be rescued if mice were also deficient for Leukemia-Inhibitory factor (LIF) or its receptor (LIFR), indicating that SOCS3 is required for modulating LIF signalling in giant trophoblast cell differentiation (Takahashi et al., 2003, Robb et al., 2005).

SOCS3 has also been shown to play an important role in Th1 and Th2 cell differentiation. Indeed, blocking SOCS3 either by a dominant-negative mutant or in heterozygous *SOCS3* mice diminishes the differentiation of Th2 cells, resulting in the skewing of T-cells towards the Th1 phenotype and reduced allergic responses (Seki et al., 2003; Kubo et al., 2006). Furthermore, over-expression of SOCS3 in T-cells provokes exacerbating Th2 cell-mediated eye-allergy, with inhibition of SOCS3 ameliorating the severity of the disease.

The receptors to which SOCS3 binds mostly activate STAT3, therefore, SOCS3 has been considered as a negative regulator of inflammation and an inhibitor relatively specific to STAT3. Indeed, mice with a conditional deletion of SOCS3 in haematopoietic and endothelial cells die as young adults due to severe inflammatory lesions in the peritoneal and pleural cavities (Croker et al., 2004, Robb et al., 2005). If G-CSF is administered to these mice, mimicking emergency granulopoiesis during infection, they exhibit massive neutrophil infiltration and destruction of liver, lung, muscle and spinal tissue, resulting from increased intensity and duration of G-CSF-induced STAT3 activation. These results thus indicate SOCS3 as a negative regulator of G-CSF and STAT3 in myeloid cells. Mice with *SOCS3*-deficient haematopoiesis display also high susceptibility to inflammatory joint disease in an IL-1-induced inflammatory model (Wong et al., 2006). Adenoviral gene transfer of SOCS3 or dominant negative STAT3 indeed reduced the proliferation of RA synovial fibroblasts and the severity of the disease in a mouse model that is also dependent on Th17 cells (Shouda et al., 2001). The generation of these same cells is enhanced in T-cell specific *SOCS3*-deficient mice and abrogation of SOCS3 binding site in gp130 knock-in mutant mice results in Th17-like arthritis (Taleb et al., 2009; Ogura et al., 2008). Together, these results show that the absence of SOCS3 has dramatic pro-inflammatory effects by promoting Th17 development and Th17-mediated disease.

Mice lacking *CIS* did not display any specific phenotype (Marine et al., 1999a). However, CIS-over-expressing transgenic mice recreate a phenotype quite similar to *STAT5*-deficient mice with defects in growth and lactation, GH and prolactin signalling, as well as in natural killer, natural killer T-cell and T-cell development (Matsumoto et al., 1999). These results support its role in JAK/STAT5 pathway.

The role of SOCS4 *in vivo* or in immune responses is to date unknown. However, *in vivo* studies on SOCS5 reveal its possible role in adaptive immunity, notably in Th1 and Th2 cell differentiation. *SOCS5* transgenic mice show attenuation of IL-4-mediated STAT6 signalling, as well as reduced Th2 cell development and production of Th2-type-cytokines (Seki et al., 2002). Interestingly, SOCS5 over-expression augmented eosinophilic airway inflammation

and septic peritonitis in mice (Ohshima et al., 2007; Watanabe et al., 2006). In contrast, *SOCS5*-deficent mice appear to have normal T-cell development and differentiation to both Th1 and Th2 cells (Brender et al., 2004). These contradictory phenotypes might be explained with the high homology between SOCS4 and SOCS5 denoting a redundant role of these proteins *in vivo*.

SOCS6-deficient mice do not display overt abnormalities, but just mild growth retardation, suggesting its role in cell growth (Krebs et al., 2002). However, SOCS6 over-expression results in inhibited insulin signalling and improvement in glucose tolerance, similarly to *p85*-deficient mice (Li et al., 2004). *SOCS7* ablation *in vivo* has mainly delineated its essential role in insulin signalling. *SOCS7*-deficient mice shows multiple defects at an early age with half of the homozygous progeny displaying severe hydrocephalus and growth retardation concomitant with hypoglycaemia and enhanced glucose metabolism that resulted in perinatal lethality (Banks et al., 2005; Krebs et al., 2004). On the other hand, SOCS7 seems to have a role in the regulation of allergic inflammatory disease. *SOCS7*-deficient mice have a propensity toward spontaneous development of cutaneous disease with infiltration of degranulated mast cells (Knisz et al., 2009). Thus, these studies suggest a role for SOCS7 in modulating the development of allergic diseases.

5. Inhibitory adaptors in human haematological diseases

As described in the previous section, animal models for the Dok, Lnk, Cbl and SOCS families have been invaluable in demonstrating the important roles played by these adaptor proteins in fine-tuning signalling thresholds in haematopoietic and immune cells. Some of these *in vivo* models recapitulate exactly or almost, essential features of different haematological and immune diseases, allowing us to identify new molecular players and mechanisms implicated in the initiation and progression of these malignancies.

Dok proteins

Studies on *Dok-1* and *Dok-2*-deficient mice demonstrated that these adaptors are essential to suppress the blastic transformation of the Bcr-Abl–induced CML-like disease. However, it is of note that in case of patients with CML, blast crisis mostly results in myeloid or B cell leukemia/lymphoma, usually not in the T cell variety. That Bcr-Abl mice carrying a p53 mutation also developed T cell lymphoma suggests involvement of genetic background (Honda et al., 2000). Moreover, as double *Dok-1/2* knockout mice developed myeloproliferative disease in the absence of Bcr-Abl, Dok-1 and Dok-2 may oppose a wide range of myeloid leukemogenesis in humans. Consistently, undetectable or marginal levels of their expression was observed in about half of the leukemic cell lines established from patients with myeloid leukaemia, regardless of whether it is CML or not (Yasuda et al., 2004). Further investigation of the tumour suppressive function of Dok-1 and Dok-2 in human malignancies, especially myeloid leukaemia including CML and CMMoL, might lead to an understanding of the molecular mechanisms of such diseases and contribute to designing effective therapies against them (Table 5).

Histiocytic sarcoma (HS) is a malignant proliferation of cells showing morphological and immunophenotypic features of mature histiocytes, which represent tissue-resident macrophages. Until recently, HS, which was also known as malignant histiocytosis, was often confused with anaplastic large B-cell lymphoma or with other malignant lymphomas

(Weiss et al., 2009). As the molecular etiology of this disease is unknown, there remains a need for realistic animal models. Mouse models that have been reported for HS frequently show multiple lesions including lymphomas and severe renal failure. The syndrome elicited in mice lacking all the three proteins, Dok-1, Dok-2, and Dok-3, more specifically resembles the disease found in humans and hence may serve as a useful model for the study of HS. Although elucidation of the mechanisms by which the ablation of Dok proteins specifically causes HS and how the tumour gains its aggressive phenotype awaits further studies, such studies will help unveil the hidden etiology of this rare aggressive human malignancy.

Lnk proteins

Lnk-deficient mice display a phenotype reminiscent of BCR-ABL negative (Ph^-) MPNs, notably Essential Thrombocythemia (ET) and Primary Myelofibrosis (PMF) that has suggested a role for Lnk in the development of these diseases (Table 5). All MPNs are clonal disorders of HSC characterized by excessive proliferation of haematopoietic cells due to hypersensibility to normal cytokine regulation and absence of negative feed back regulation. The recent discovery of the Val617Phe acquired mutation of the *JAK2* gene (*JAK2*-V617F) represents the first reliable molecular marker of Ph^- MPN (Campbell & Green, 2008). The pathogenic role of JAK2-V617F constitutive active kinase and more recently of mutated Mpl (*MPL*W-515L) most likely will go through abnormal activation of signalling molecules, among which Lnk likely plays an important negative regulatory role through its binding to JAK2-V617F and MPL-W515L forms. Indeed, recent work has demonstrated a modulation of Lnk level in megakaryocyte/platelets and $CD34^+$ cells from MPNs patients (Baran-Marszak et al., 2010). Recently, the first *LNK* mutations in JAK2-V617F-negative MPN patients (ET and PMF) were identified (Oh et al., 2010). Both mutations are on exon 2, one (E208Q) is a missense mutation in the PH domain (ET patient), while the second mutation lead to a premature stop codon resulting in the absence of the protein (PMF patient). The prevalence of such mutations is rare (5% or less). However, other *LNK* mutations have been identified in leukemic transformation of MNP at a higher frequency [13%] (Pardani et al., 2010). Moreover, *LNK* exon 2 mutations were also found in pure erythrocytosis (Lasho et al., 2010). In this case, one mutation (A215V) was previously described in PMF blast crisis and another (E208X) results in absence of the protein. This finding suggests that the *LNK* mutations induce an MPN phenotype that may depend on different parameters, such as the presence of other mutations (Lasho et al., 2011).

Genome-wide association studies (GWAS) have recently revealed that different diseases share susceptibility variants. The *LNK/SH2B3* gene maps on chromosome 12q24 and a non-synonymous single nucleotide polymorphisms (SNP) in this gene has been reported in exon 2 resulting in a missense mutation at position 262 leading to a R262W amino acid substitution in the PH domain. Surprisingly, this nsSNP has recently been associated with inflammatory disorders, such as celiac disease (Hunt et al., 2008; Zhernakova et al., 2010), type 1 diabetes (Lavrikova et al., 2010), asthma (Gudbjartsson et al., 2009) multiple sclerosis (Alcina et al., 2010), and also to eight clinically relevant haematological parameters (Soranzo et al., 2009; Ganesh et al., 2009) in different populations. Furthermore, the *LNK* R262W variant has also been associated to cardiovascular diseases such as myocardial infarction, coronary heart disease and hypertension (Gudbjartsson et al., 2009; Ikram et al., 2010). All these data suggests that Lnk nsSNP could be a risk variant for these diseases contributing to their pathogenesis, and in consequence, providing a useful diagnostic marker.

Cbl proteins

Animal models have demonstrated the enhanced biological responses in the haematopoietic and immune system of Cbl family members when they are either genetically ablated or point mutated. It is in this context that the recent identification of mutations in CBL in patients with myeloid malignancies provides an important milestone (Table 5). Two groups simultaneously identified CBL mutations in Acute Myeloid Leukaemia (AML) patient samples (Sargin et al., 2007; Caligiuri et al., 2007). CBL mutations are most frequently observed in a distinct group of myeloid disorders named myelodysplastic syndromes/myeloproliferative neoplasms (MDS/MPN) that include: the Chronic Myelomonocytic Leukaemia (CMML), atypical Chronic Myeloid Leukaemia (aCML) and Juvenile Myelomonocytic Leukaemia (JMML). They originate from immature haematopoietic progenitors and are characterized by the production of dysplastic blood cells and myeloproliferative features. In most adult cases, mutations seem to be somatic, but germline mutations were reported in some JMML cases in children (Loh et al., 2009; Niemeyer et al., 2010; Martinelli et al., 2010). Genetic alterations in these haematological malignancies are strongly associated with hyperactivation of the Ras/MAPK signalling pathway due to activating mutations in signalling molecules involved in this pathway accounting for approximately 75%cases of JMML (Schubbert, et al., 2007). Strikingly, *CBL* mutations are found in 5% of aCML and up to 15% of JMML and CMML (Grand et al., 2009; Loh et al., 2009; Muramatsu et al., 2010; Shiba et al., 2010; Dunbar et al., 2008; Sanada et al., 2009). Most *CBL* mutations associated with myeloid malignancies are clustered around the linker and the RING finger domains and *in vitro* studies with these mutants have shown their lack of E3 ubiquitin ligase activity (Sargin et al., 2007; Grand et al., 2009; Sanada et al., 2009). Complete loss of *CBL*, deletions or mutations outside the linker/RING finger domains are rare, as well as mutations in *CBLB*. These findings suggest that expression of mutant Cbl proteins act as dominant negative inhibitor of wild-type Cbl or even of lost Cbl expression. A remarkable genetic feature of *c-CBL* mutations in these myeloid neoplasms is that mutations are homozygous in most cases, as a result of duplication of the mutated parental copy of 11q (where the *CBL* gene resides) and loss of the remaining wild-type allele, a genetic process called "acquired uniparental disomy" (aUPD) (Grand et al., 2009). This feature underlies the gain-of-function nature, rather than a loss-of-function, of the CBL mutations and may represent a defining oncogenic event. Indeed, mutations involving *RUNX1*, *JAK2*, *FLT3* and *TP53* have been shown to coexist with CBL mutations in myeloid neoplasms (Sanada et al., 2009; Perez et al., 2010; Tefferi, 2010; Makishima et al., 2011) suggesting that additional oncogenic events may contribute to the mutant Cbl-driven leukemogenic process.

SOCS proteins

There is now emerging evidence that SOCS expression is differentially regulated during Th cell differentiation and in Th-driven inflammatory diseases (Table 5). In Th1 inflammatory diseases (rheumatoid arthritis, ulcerative colitis, Crohn disease, contact and atopic dermatitis), SOCS1 expression is observed in lymphocytes and macrophages, as well as keratinocytes and stromal cells that are capable of antigen presentation, but granulocytes are negative (Egan et al., 2003; Federici et al., 2002). Importantly, SOCS3 expression levels seem to correlate with the severity of this type of inflammatory diseases (Seki et al., 2003; Shouda et al., 2001; Suzuki et al., 2001). The same is true for Th2-driven asthma that promotes

lymphocyte, basophile and mast cell infiltration, where SOCS3 expression in Th2 lymphocytes is elevated. Furthermore, the association of the function of SOCS molecules in the allergic response has been supported by human studies analyzing the association of polymorphisms in *SOCS* genes with allergic disease in people. Interestingly, an association of a promoter polymorphism leads to a promoter with modified activity *in vitro*, suggesting that changes in *SOCS1* expression can have considerable effects on disease manifestations in patients (Harada et al., 2007). SOCS1 expression has been found decreased in some human cancers including hepatocellular carcinoma and myeloproliferative neoplasms and this is frequently associated with hyper-methylation of one or more *SOCS* genes (Yoshikawa et al., 2001; Watanabe et al., 2004; Quentmeier et al., 2008; Chaligné et al., 2009). In some cases, this methylation has been correlated to the degree of malignancy (Okochi et al., 2003; Yoshida et al., 2004). These observations strongly suggest that SOCS proteins may be tumour suppressors. Loss of SOCS expression may then contribute or favour tumour progression in synergy with other oncogenes. SOCS expression has also been implicated in the resistance to interferon in haematopoietic malignancies, such as leukaemia, lymphomas and multiple myeloma (Sakamoto et al., 2000, Sakai et al., 2002). Persistent expression of SOCS1 and/or SOCS3 has been detected in cutaneous T-cell lymphoma (CTCL), chronic myeloid leukaemia (CML), and some acute leukaemia (Brender et al., 2001b; Cho-Vega et al., 2004; Roman-Gomez et al., 2004). In these circumstances, elevated expression of SOCS coincides with constitutive activation of JAK/STAT pathways. Moreover, in certain myeloproliferative neoplasm, SOCS3 has been found hyper-phosphorylated, which enhances the half-life of the protein but interferes with its regulatory function (Hookham et al., 2007; Elliott et al., 2009; Suessmuth et al., 2009). These data suggests that perturbed SOCS expression may contribute to the malignant phenotype and favour disease progression, rather than being an early event in the oncogenic process.

6. Therapeutic application of inhibitory adaptors

Given the central role played by inhibitory adaptors in the regulation of different aspects of haematopoietic and immune cell function, they are predicted to serve as excellent new targets in the development of anti-oncogenic, anti-inflammatory and immunosuppressor reagents. The advantage of these adaptors as targets is their restricted expression in cells of the haematopoietic and immune system, at least of some of the members in the different families discussed here. The disadvantage for some of them (Dok, Lnk) is their lack of enzymatic activity for drug targeting. However, this can be surpassed by utilizing strategies based in the direct inhibition or blockade of their specific protein-protein interactions for targeting a particular signalling pathway. This approach relies on the current information available on the molecular structure of the adaptor functional domains and the identification of specific sequences or residues involved in the adaptor/partner interaction. In some pathological contexts, the association of the inhibitory adaptor with mutated or oncogenic forms of its targets is modified from that engaged with its normal counterpart (Baran-Marszak et al., 2010). These findings open the possibility to use the binding sequence in the adaptor to exclusively inhibit the oncogenic protein and signalling pathway, while sparing the normal cell signalling cascades. Indeed, successful development of small molecule inhibitors of protein-protein interaction has begun to emerge, validating it as a practical approach (Azmi & Mohammad, 2009). The use of dominant negative forms of these inhibitory proteins has also proved to be advantageous, notably for adaptors that have

shown dual functions, as positive and negative regulators, as they allow modulate specifically their function depending on the cell type and biological response to be addressed. In the case of Lnk, its loss or inhibition causes the abnormal expansion and enhanced ability for engraftment of HSC (Takizawa et al., 2006); this feature can be used for bone marrow transplantation where the scarce number of these cells is always the limiting factor for the use of this therapy.

On the other hand, adaptors with catalytic activity, like the Cbl and SOCS proteins, have the double advantage of being used as adaptors and as E3 ligase. In the case of Cbl, it is clear that its ligase activity is central to the regulation of many oncogenic proteins, so drugs that could enhance this activity may provide new therapeutic strategies for limiting their constitutive signalling. A potential strategy is the screening for molecules that could mimic the activation of its E3 activity while retaining its targets binding. This can be use in basophile and mast cells of allergic patients as a therapy to treat allergy diseases. In the case of SOCS, a strategy based in the delivery of the SOCS3 protein using a recombinant cell penetrating moiety has proved to increase the concentration and activity of SOCS3 in the cells and suppressed the effects of acute inflammation (Jo et al., 2005). Moreover, therapeutic trials using SOCS antisense oligonucleotide, small hairpin RNA and peptide mimetics are currently investigated in animal models (Yoshimura et al., 2007). Importantly, a better understanding of the spectrum of signalling alterations provoked by mutant forms of the inhibitory adaptors identified in human pathologies is likely to reveal therapeutic strategies for patients with these mutations. In this context, the association of single nucleotide polymorphisms (SNP) in the genes of some of these adaptors (*LNK*, *SOCS*) with different inflammatory, myeloproliferative and vascular diseases suggests the implication of these molecules as risk factor and their potential use as biomarkers in these diseases. Lastly, future challenges in the study of inhibitory adaptors lie also in the development of performing techniques that will allow accurate monitoring of their signalling complexes at the molecular level. Indeed, precise regulation of protein interactions is of medical relevance, as modifications in the composition or localization of crucial components of these signalling networks can lead to the development of human diseases.

7. Conclusions

Over the past decade, it has become clear the pivotal role that cytokines play in the development and pathology of human diseases, including those of the haematopoietic and immune system. They perform their actions by regulating essential biological functions, such as cell proliferation, differentiation, cell morphology and migration. It is therefore not surprising that cytokine signal transduction pathways are tightly regulated. To achieve this, they have set up a variety of mechanisms and the rate at which the signal is turned off will be due to the net effect of all of these regulatory pathways. Although initially identified and best understood as mediators of positive signalling, adaptors have also shown an equally critical role in the negative control of signalling events. Inhibitory adaptors have been demonstrated important for maintaining homeostasis by preventing inappropriate cellular activation (Lnk, Cbl), by localizing enzymatically active regulatory molecules to specific subcellular compartments (Dok), and/or by terminating signalling cascades once they are initiated through targeting activated mediators to degradative pathways (Cbl, SOCS). In doing so, these molecules act upon three key signalling intermediates (the receptor,

JAK/other kinases, and STATs/downstream effectors) to completely switch off the signal. In contrast to SOCS and Lnk, which are induced in response to cytokines, Cbl and Dok (with the exception of Dok-2 in some cases) are constitutively present in the cell and may therefore function as more acute, early response regulators. The timing and specificity of each of these mechanisms, as well as how the inhibitors interact and cooperate with each other, is still an area for future investigation. Furthermore, the fact that the expression of these adaptors is itself regulated, points out further the complexity of the regulatory system. While gene-targeting studies have highlighted critical roles for the inhibitory adaptors in immune function, haematological malignancy and inflammation, the complexity of the mouse models, particularly with regard to multi-gene families, suggests that these studies should be carefully interpreted, and certainly more work is required before we can predict the consequences of using these molecules or their agonists/antagonists in a clinical setting. Thus, one of the challenges for the future is sorting out the roles of negative regulators of cytokine signalling in all the existing pathways activated in response to cytokines. This knowledge is likely to yield both new and confirmatory findings, with the anticipation of a better understanding of how these adaptors orchestrate the functional activity and fate of many partners to produce the desired intensity of a signalling response. Although a great deal of research remains to be done to clarify the roles of these inhibitors and their mutant forms in human diseases such as cancer and inflammation, it can be foreseen that it will lead to the development of strategies based on the up- or down-regulation of their properties for therapeutic purposes.

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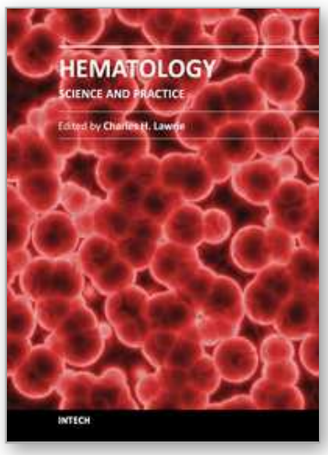

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Hematology encompasses the physiology and pathology of blood and of the blood-forming organs. In common with other areas of medicine, the pace of change in hematology has been breathtaking over recent years. There are now many treatment options available to the modern hematologist and, happily, a greatly improved outlook for the vast majority of patients with blood disorders and malignancies. Improvements in the clinic reflect, and in many respects are driven by, advances in our scientific understanding of hematological processes under both normal and disease conditions. Hematology - Science and Practice consists of a selection of essays which aim to inform both specialist and non-specialist readers about some of the latest advances in hematology, in both laboratory and clinic.

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