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**SIGNALLING AND FUNCTIONAL DIVERSITY WITHIN THE AXL SUBFAMILY OF
RECEPTOR TYROSINE KINASES**

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ABSTRACT

The related Axl, Sky and Mer receptor tyrosine kinases (RTKs) are increasingly being implicated in a host of discrete cellular responses including cell survival, proliferation, migration and phagocytosis.

Furthermore, their ligands Gas6 and protein S are characteristically dependent on vitamin K for expression of their functions. The Gas6/Axl system is implicated in several types of human cancer as well as inflammatory, autoimmune, vascular and kidney diseases. Each member of the Axl RTK subfamily possesses distinct expression profiles as well as discrete functions. In this article, we review the knowledge so far on the intracellular signalling interactions and pathways concerning each of the Axl RTKs. In this way, we hope to gain a greater understanding of the mechanisms that set each of them apart, and that relay their associated functions.

KEYWORDS: Axl; Gas6; receptor tyrosine kinase; vitamin K; cell survival.

1. Introduction

Growth factors are major effectors of cell differentiation, growth and migration and act via stimulating specific cell surface receptors known receptor tyrosine kinases (RTKs) [1]. The RTKs are a large family of transmembrane proteins exhibiting great diversity in their extracellular regions, however sharing in common a highly conserved intracellular tyrosine kinase domain [2]. Intracellular signalling is triggered through activation of the tyrosine kinase domain and its subsequent phosphorylation of multiple substrates [1].

Subfamilies of RTKs are categorised according to their amino acid sequence identities and extracellular structural similarities; thus members of a subfamily often bind common or similar ligands [2, 3].

The Axl subfamily of mammalian RTKs is composed of Axl, Sky and Mer, which participate in a signalling axis we shall refer to as the Gas6/Axl system. Axl RTK subfamily members each possess a combination of two N terminal immunoglobulin (Ig)-like domains and two fibronectin type III (FNIII) repeats in their

extracellular regions (Fig. 1). The Axl RTK subfamily has long been in the shadow of “classical” growth factor receptors due to the rather enigmatic yet diverse array of biological effects their members have been shown to exert. This article is aimed at providing a comprehensive outline of the multiple intracellular signalling interactions that have been reported for each one of the Axl subfamily of RTKs, which may explain the intriguing diversity of functions with which they are associated.

(FIG. 1)

1.1. Axl RTK – a major effector of cell survival, proliferation and migration

Axl was originally isolated from patients with chronic myelogenous leukaemia [4] and a chronic myeloproliferative disorder [5]. Overexpressing the cDNA for this novel RTK yielded a tyrosine phosphorylated 140 kDa protein with transforming ability. Axl is ubiquitously expressed, having been detected in a wide variety of organs and cells, including cell lines of epithelial, mesenchymal, and hematopoietic origins, as well as non-transformed cells [6]. The Axl ectodomain resembles that of adhesion molecules such as NCAM and L1 [7]. Indeed, an adhesive phenotype is a hallmark of Axl overexpression, accompanied by constitutive receptor activation [8, 9]. However, the actual adhesiveness of the Axl ectodomain is independent of intracellular kinase activity, since cells expressing a receptor lacking entirely the intracellular domain are still able to aggregate [8]. However, the actual oncogenic potential of Axl lies within the tyrosine kinase domain, which can be constitutively activated without extracellular stimulation. For example, a fusion of a viral gag gene to a partial Axl construct beginning 33 amino acids downstream of the transmembrane region was sufficient to induce tumours in nude mice [10]. Subsequent to its original identification in myeloid leukaemias, Axl overexpression has also been reported in several types of human cancers, including colon [11], oesophageal [12], thyroid [13], breast [14] and lung carcinomas [15].

1.2. Sky (Tyro3) RTK – a neurotrophic factor receptor?

After cloning of partial sequences from rat (*tyro-3*; [16]) and human (*TYRO3*; [17]), separate groups in 1994 independently identified the human and murine sequences for a novel Axl-homologous RTK. The novel protein was separately termed Sky [18], Brt [19], Rse [20], DTK [21], Tif [22] and Tyro 3 [23]. The genomic structure of human Sky is identical to that of human Axl, demonstrating close conservation within the Axl subfamily [24]. Sky expression appears to be predominant in the CNS and brain [18, 25], also showing the widest distribution in the CNS out of the three receptors [26]. In addition to suggesting a role for Sky in neurotrophism, Sky is also expressed in osteoclasts in bone, indicating a role in bone resorption [27]. Although able to induce cell transformation experimentally [28], information on the status of Sky expression in human cancer is scant. Sky has been detected in several human leukaemic cell lines and blasts of acute myeloid leukaemia patients [29], and overexpressed in myeloma cells compared to autologous B-lymphoblastoid cell lines [30].

1.3. Mer - a mediator of phagocytosis

Mer RTK was first identified in the form of the chicken protooncogene *c-eyk*, which was derived from the avian retrovirus RPL30 [31]. Human Mer, or c-mer, is so named after its original reported expression pattern (monocytes and epithelial and reproductive tissues) [32, 33]. Northern analysis in the mouse showed almost exclusive expression in the monocytic cell lineage [32]. As with Sky (but in contrast to Axl), very little has been reported to date as to the expression/activity of Mer in cancer. Experimentally, both the transforming and antiapoptotic abilities of Mer have been observed through at least its intracellular region [34, 35]. One microarray study has shown Mer to be overexpressed in ACTH-secreting adenomas compared with normal pituitary [36]. However, in the same study, Mer was intriguingly underexpressed in PRL-

secreting adenomas. Also, since its original detection in neoplastic B- and T-cell lines [32], Mer upregulation has also been observed in mantle cell lymphomas as compared to normal B cells [37]. An novel aspect to Mer function that distinguishes it from its sister RTKs has come to light in recent years. In addition to its transforming ability, several recent studies have brought to light a fascinating alternative function for Mer that seems to set it apart from its sister RTKs. Mer appears to be required for ingestion of apoptotic cells by professional phagocytes such as monocytes/macrophages, retinal pigment epithelial cells and dendritic cells. In 2000, a deletion in the *MERTK* gene was identified as the underlying cause for a classic rat model of retinal dystrophy [38]. This model features an impairment in the ingestion of shed photoreceptor cell fragments by retinal pigment epithelial cells. Thus, Mer appears to be able to induce the cytoskeletal remodelling that is required for engulfment during phagocytosis, which also extends beyond the eye [39, 40]. For example, a general increase in numbers of apoptotic cells has been detected in Axl/Sky/Mer triple knockout mice which are also blind [41]. These triple knockouts also show raised serum levels of autoantibodies, reflecting an inability to clear “junk” and thus develop an autoimmune response [42]. Indeed, single Mer knockout in mice is sufficient to induce such an autoimmune phenotype [43], highlighting the individual importance of Mer in immune homeostasis.

2. Gas6 and protein S, vitamin K-dependent ligands for Axl RTKs

The biological ligands for Axl RTKs are two highly similar vitamin K-dependent proteins, Gas6 (‘product of *growth arrest-specific gene 6*’) [44] and protein S, a negative regulator of blood coagulation [45] (43% amino acid identity) (Fig. 1). Both proteins are composed an N-terminal region containing multiple post-translationally modified γ -carboxyglutamic acid residues (Gla). The Gla region possesses the ability to interact in a conformationally specific manner with negatively charged membrane phospholipids [46], which is thought to mediate the binding of both Gas6 and PS to apoptotic cells. In this way, they are thought to act as recognition bridges between apoptotic cells and the phagocyte cell that ingest them. After the Gla region, there is a loop region followed by four epidermal growth factor (EGF)-like repeats. This loop region is

sensitive to thrombin cleavage only in PS, which is relevant to its role in coagulation, a role which Gas6 lacks [45]. The C termini of Gas6 and PS house a globular sex hormone binding globulin (SHBG)-like region, comprising a pair of laminin G-like (LG) domains [47].

The SHBG domain is believed to bind directly to and activate Axl [48, 49], whilst a supporting role has been demonstrated for a properly γ -carboxylated Gla region in the functional effects of Gas6 [50]. It is noteworthy that inter-species variations in ligand receptor-affinity have been reported by many groups; for example human Sky prefers human Gas6 and bovine PS to human PS, while mouse Sky prefers human PS over human Gas6 [51-53]. This issue of intra species ligand-receptor affinities is an important one that still requires further study, in order to be certain of the roles of each ligand and receptor with respect to each other within a certain tissue and/or physiological condition. It would also address the possibility for functional redundancy within the Gas6/Axl system.

Ample studies using cultured cells have demonstrated an Axl-mediated effect of exogenous Gas6 on cell survival [54-56], proliferation [50, 57, 58], migration [59, 60] and adhesion [61]. In contrast, only a handful of studies point to a direct receptor-mediated effect of PS on cells, independent of its role in the coagulation cascade. PS was shown to promote bone resorbing activity via Sky in osteoclasts [27]. Also, a mouse study of stroke revealed PS administration to protect ischaemic neurons both in vivo and in vitro [62]. Also, both PS and Gas6 have been shown to mediate recognition of dying cells and their subsequent phagocytosis by Mer [63, 64]. This recognition is thought to be mediated by the interaction of the Gas6 and PS Gla regions with phosphatidylserine exposed on apoptotic cell membranes [65].

3. Signal generation and transduction via the Axl RTKs

In general, binding of ligand to an RTK monomer causes a conformational alteration that drives receptor dimerisation [1], and this has been shown for the Gas6-Axl pairing [49]. Moreover, the potential for ligand-independent dimerisation and activation of Axl RTKs also exists [49, 66]. Interactions between the extracellular portions has been described above; however there is also evidence for constitutive dimerisation

of the intracellular regions of these molecules. For example, using the human Axl intracellular domain (ICD) as bait in a library-scale yeast two-hybrid screen, we identified the Axl ICD itself as a common binding partner [67]. Moreover, Axl ICD has been detected in the nucleus [68]. Whether ligand-driven or not, dimerisation appears to stabilise interactions between cytoplasmic domains and leads to activation of the intrinsic kinase [1]. Usually, the activated ICD of one receptor monomer transphosphorylates multiple tyrosine residues on the neighbouring monomer. This autophosphorylation serves to increase the catalytic efficiency of the RTK by phosphorylating a conserved tyrosine residue inside the kinase domain as well as creating docking sites for signal transduction second messengers. Studies over the past ten years have shed more light on the intracellular signalling cascades emanating from activation of the Axl RTKs.

3.1. Axl signalling

Starting from the receptor itself, an early study that screened an expression library revealed the p85 α and p85 β subunits of phosphatidylinositol 3-kinase (PI3K), and phospholipase C (PLC)- γ as binding partners for Axl ICD [69]. Furthermore, individual docking sites for these proteins were located; tyrosine 821 (Y821 of shorter isoform) is an apparent multi-substrate docking site, interacting with the p85 subunits of PI3K, PLC- γ , Grb2, c-Src and Lck. Y779 also exhibited binding affinity for the p85 proteins, while Y866 was an additional docking site for PLC- γ (Fig 2a).

(FIG. 2)

We utilised a yeast two-hybrid system to screen a human heart cDNA library for putative Axl-binding partners [67]. With this approach, we were able to confirm the interaction of Grb2 and PI3K with Axl, and uncovered p55 γ as an additional PI3K regulatory subunit that bound. Furthermore, we identified a number of novel Axl binding partners in SOCS-1, Nck2, RanBPM, and C1-TEN (see below). All of the above binding partners, with the exception of RanBPM, possessed phosphotyrosine-binding Src homology 2 (SH2)

domains, indicating that these are the interfaces for Axl interaction. In addition, Axl ICD itself was amongst the most common Axl-interacting proteins (Fig. 2b), suggesting a constitutive dimerisation process that may be biologically relevant and important for signalling.

It is also clear from these findings that activation of PI3K is an important event in Axl signalling, implicating cell survival, proliferation and migration. Moreover, the interactions with alternate isoforms of PI3K may serve to link Axl to discrete signalling pathways further downstream (Fig. 2a). Further support of previous findings comes from identification of Grb2 adapter protein as an Axl interactor, which suggests a link with the Ras-extracellular regulated kinase (ERK) pathway. In addition, SOCS-1 was originally identified as a negative regulator of cytokine signalling [70], and therefore could serve a similar role in Axl signalling. Furthermore, Nck2 is an adapter protein composed of three tandem SH3 domains and an SH2 domain [71]. These tandem domains may serve to tether Axl to other signalling complexes in the same way that Grb2 does. For example, the Axl-Nck2 interaction may connect Axl to a ternary complex consisting of the PINCH protein and integrin-linked kinase (ILK), which is a signalling platform at focal adhesions that regulates cytoskeletal dynamics and downstream signalling pathways [72].

We cloned and carried out an initial characterisation of the most novel Axl interactor, which we named C1-TEN (C1 domain-containing phosphatase and TENsin homologue) [67]. C1-TEN houses an N terminal region with predicted structural similarity to PTEN, a phosphatidylinositol lipid phosphatase that negatively regulates PI3K/Akt signalling [73]. We further characterised C1-TEN and observed that its overexpression in cells exerted similar negative regulatory effects as PTEN, namely an inhibition of cell survival, proliferation, migration and of specifically the PI3K/Akt signalling pathway [74]. Thus, it appears that C1-TEN is a novel PTEN-like phosphatidylinositol lipid phosphatase with an ability to bind to RTKs such as Axl. We also studied further the interaction between Axl ICD and RanBPM, which is probably part of a protein scaffolding complex that houses multiple signalling proteins [75]. Moreover, RanBPM also bound to Sky ICD, suggesting a common binding motif for Axl and Sky, since the two receptors share 63% sequence identity in their ICDs.

Recently, an unexpected aspect to Axl signalling was uncovered by Budagian and colleagues, who in mouse cells demonstrated a constitutive interaction between Axl and the IL-15 receptor α subunit [76] (Fig. 2b). They showed that IL-15 could transactivate Axl and its associated signalling pathway, with concomitant phosphorylation of the IL-15 receptor. This example of a novel cross-talk mechanism therefore broadens the repertoire of potential regulators of Gas6/Axl signalling, as well as implicating Axl RTKs in the signalling of other receptors. Evidence for the latter phenomenon was provided by Gallicchio and colleagues, who showed that Gas6 stimulation of Axl actually inhibited activation of vascular endothelial growth factor receptor 2 and consequent endothelial cell morphogenesis [77]. This inhibition appeared to be via Gas6/Axl-mediated activation of the SH2 domain-containing tyrosine phosphatase 2 (SHP-2). Further support for this hypothesis came from a large cDNA microarray study by van Ginkel and coworkers, who showed that, while Axl increases the survival of uveal melanoma cells, Gas6 stimulation of Axl caused a down-regulation of Cyr61, a member of the CCN protein family involved in tumor progression [78]. It remains to be seen whether Sky or Mer could also be involved in such heterotypic interactions.

Recently, the role of intracellular oxidation has come to the fore in terms of switching on or off the activation state of many kinases and phosphatases. Indeed, addition of hydrogen peroxide to cells, which generates intracellular reactive oxygen species (ROS), has been shown to activate Axl within a rapid time frame [79, 80]. This could be due to ROS-induced inhibition of a phosphatase that normally maintains Axl in an inactivated state. In terms of signalling further downstream, Fridell *et al.* observed that stimulation with EGF of an EGFR-Axl (extracellular-intracellular) chimera caused activation of the Ras/ERK pathway and cell proliferation; however in cells bearing full length Axl and stimulated with Gas6, PI3K was activated with no proliferation or ERK activation occurring [81]. This showed that the type of signalling and its functional outcome depends on the nature of the stimulus and not solely on activation of the kinase *per se*. Additional studies pointed more towards a survival effect of Gas6 rather than proliferation. In cells that respond to Gas6 with increased survival, such as NIH 3T3 fibroblasts, components of the PI3K pathway, incorporating the serine/threonine kinase Akt/PKB and the rapamycin-sensitive S6K, and Src appear to be

required [55]. Via Akt, Gas6 also causes an increase in the antiapoptotic protein Bcl-2 [82], as well as phosphorylation of the Bcl-2 family member, Bad [83, 84]. A transient ERK, JNK/SAPK and p38 MAPK activation also occurs [56, 83], although blocking ERK did not influence Gas6-induced survival [83]. PI3K also mediates Gas6-supported survival of human oligodendrocytes [85], endothelial cells [82], VSMC [86] and lens epithelial cells [87]. Although more unusual, ERK has in addition to PI3K been implicated in gonadotropin-releasing hormone neuronal survival [88].

Additional signalling entities that have been implicated in Gas6/Axl survival signalling include the NF-kappa B transcription factor system [82, 89]. In serum-starved NIH 3T3 cells, Gas6 stimulated nuclear NF-kappa B binding activity and subsequent transcriptional activation from NF-kappa B-responsive promoters and an increase in Bcl-x(L) protein level [89]. The NF-kappa B activation appeared to be a downstream consequence of PI3K and Akt signalling, with the Akt substrate glycogen synthase kinase 3 (GSK3) implicated as a link. In addition, a reduction in caspase 3 activity has also been reported for Gas6 in endothelial cells [82].

In cells in which Gas6 is a clear mitogen, however, activation of the ERK pathway seems to be paramount. For example, we have shown Gas6-induced activation of ERK in cardiac fibroblasts, which respond by proliferation [50]. Furthermore, the growth effect of Gas6 was additive to that of EGF, suggesting that Gas6 can act as a growth factor through signalling pathways distinct from those utilised by EGF. ERK also mediates Gas6-induced human prostate cancer cell proliferation [58]. In C57MG mouse mammary cells, a requirement for ERK, as well as PI3K/Akt and S6K was demonstrated [90] and Gas6 also induced up-regulation in the cytosol of the proto-oncogene beta-catenin. This stabilisation of beta-catenin was correlated with activation of T-cell factor-driven transcription. Gas6 is also a mitogen for cultured mesangial cells, and activates the STAT3 (signal transducers and activators of transcription) transcription factor pathway [91]. In response to Gas6, STAT3 is phosphorylated and translocates to the nucleus and induces STAT3-dependent transcriptional activation.

In addition to survival and mitogenesis, gonadotropin-releasing hormone neuronal cells respond to Gas6/Axl by migration, and the signalling events associated with this phenotype included activation of the Rho family GTPase Rac and actin cytoskeletal reorganisation, downstream activation of p38 MAPK and MAPK-activated protein kinase 2 and phosphorylation of HSP25, a regulator of actin remodelling [60]. Gas6 has also been shown to induced upregulation of the class A scavenger receptor in humans VSMC, an atherogenic process in which cells ingest lipids to become foam cells [92]. This upregulation was attributed to the PI3K/Akt signalling pathway. Furthermore, Gas6 induces the phosphorylation and ubiquitination of Axl and its interaction with the ubiquitin ligase c-Cbl, which may be a mechanism for downregulation of Axl after activation [80].

3.2. *Sky signalling*

Sky RTK was early on found to be autophosphorylated upon overexpression in a ligand-independent manner, and an association with Src kinase was detected [93, 94] (Fig. 3). Furthermore, a truncated cytoplasmic variant of Sky has been reported, that is capable of functioning as a dimeric tyrosine kinase, even in the absence of an extracellular input [94]. This interesting property of constitutive dimerisation of cytoplasmic domains appears to also exist for Axl [67]. Like Axl, Sky also responds to ligand stimulation as a true receptor and is activated by Gas6 in a matter of minutes [27]. The p85 subunit of PI3K has also been detected in a yeast two-hybrid screen as a binding partner for Sky, and stimulation with human protein S activates rat Sky to induce PI3K and Akt activation [28]. In bone, Gas6 has been shown to stimulate mouse osteoclastic bone resorption via Sky, involving activation of ERK [95]. We have shown Sky ICD to also interact with RanBPM, as did Axl, further highlighting similarities in their signalling interfaces [75].

(FIG. 3)

3.3. *Mer signalling*

A distinction in signalling within the Axl subfamily has been observed when comparing Mer with Axl in 32D cells. In these cells, Axl was shown to cause differentiation of 32D cells whereas Mer produced cytoskeletal alterations and blocked apoptosis, without stimulating growth [96]. Prior to the ligand(s) being known, Lin and colleagues conducted an early analysis of Mer signalling by constructing a chimeric receptor (Fms-Nyk) composed of the extracellular domain of the human colony-stimulating factor 1 receptor (Fms) and the transmembrane and cytoplasmic domains of human Mer [34]. Stimulation of this chimeric receptor in transfected NIH 3T3 fibroblasts led to a transformed phenotype accompanied by proliferation. PLC- γ , PI3K, Shc, Grb2, Raf-1, and ERK were phosphorylated downstream (Fig. 4). They later identified tyrosine residues Y749, Y753 and Y754 as autophosphorylation sites in the activation loop of the Mer kinase domain, and established that full Mer kinase activity required phosphorylation of all three tyrosines [97]. Significantly, the tyrosine trio of Y749, Y753 and Y754 in Mer are all present in the same sequence context in the ICDs of both Axl and Sky, suggesting a conserved set of key residues that are important for kinase activity. In addition, Mer possesses an unusual kinase signature sequence KWIAIES that is also characteristic of the Axl RTK subfamily [32]. Todt and colleagues have also shown PLC- γ to be associated with Mer, and to be at least partly responsible for the phagocytosis of apoptotic thymocytes by Mer-expressing macrophages [39]. Georgescu and co-workers used a chimera of mouse Mer ICD that was constitutively active and activated ERK and proliferation in Ba/F3 pro-B-lymphocyte cells [35]. The Y867 residue in Mer ICD (equivalent to Y872 in human Mer) was demonstrated to bind Grb2, recruit and activate PI3K as well as transcriptionally activate NF-kappaB. Thus, this residue was implicated in a survival signalling pathway, and did not influence the ERK-related proliferative phenotype of these transfected cells. Y872 in Mer is part of a Grb2 consensus binding site, YXNX [98], and its equivalent in Axl receptor is Y821 (YVNM), which is a multi-substrate docking site, potentially interacting with PI3K, PLC- γ , Grb2, c-Src and Lck [69]. In Mer, however, evidence indicated that the Y872 site may instead indirectly bind PI3K p85 subunit via Grb2 [35].

(FIG. 4)

Mer has also been shown to activate the STAT transcription factor pathway, which was associated with Mer-induced transformation [99]. Human Mer activates mainly Stat1, although mutation of the sequence Y933VPL to Y933VPQ (present in the viral Mer homologue) led to increased activation of Stat3 and increased transformation efficiency. Another study utilising microarray analysis has shown Mer to be a strong inducer of chemokines such as interleukin (IL)-8 in human prostate cancer cells, in which Mer causes differentiation [100]. The Mer-induced up-regulation of IL-8 in DU145 depended on the ERK pathway, but not PI3K/NF-kappaB.

Novel aspects to Mer signalling have been observed that could allude to its distinct function as a cell ingestion-inducing molecule. For example, in 32D cells, which normally grow in suspension, expression of an EGFR ECD-Mer ICD chimera caused an altered morphology featuring adherence and cell flattening [96]. Thus, the cytoskeletal alterations induced by Mer appear to set it apart from Axl and Sky, and the mechanisms for these effects appear to involve a link between Mer and small G protein regulators of cytoskeletal dynamics. Mahajan and Earp identified by yeast two hybrid screening the guanine nucleotide-exchange factor Vav1 as a Mer-binding partner [101]. Vav1 binds to Mer ICD in a constitutive and phosphotyrosine-independent manner, and Mer activation results in tyrosine phosphorylation of Vav1 and release from Mer. The released Vav1 subsequently stimulates GDP-GTP exchange on, and activation of, the RhoA family of small GTPases including Rac1, Cdc42 and RhoA. These Rho GTPases are well known effectors of cytoskeletal remodelling that affects morphogenesis, polarity, movement and cell division [102]. Thus, the Rho GTPases may be major effectors of the ingestion-inducing phenotype that Mer activation causes in phagocytic cells such as macrophages and ocular RPE cells. Furthermore, there is evidence that in platelets, Mer (rather than Axl or Sky) may transduce a signal that is relayed into 'inside-out' signalling, where integrin ligation to extracellular matrix components is activated to promote platelet aggregation [103]. Evidence that Mer is directionally and functionally linked to integrin signalling was provided by Wu and

colleagues who found that Mer activation, either with or without Gas6, induced Src-mediated tyrosine phosphorylation of focal adhesion kinase (FAK) and its recruitment to the α v β 5 integrin [64]. This recruitment causes formation of the p130(CAS)/CrkII/Dock180 integrin signalling complex, which activates Rac1. Alternatively, it has also been suggested that Mer signalling occurs downstream of α v β 5 integrin signalling in the RPE phagocytic process [104]. Furthermore, the importance of actin cytoskeletal and integrin interactions for Mer in its signalling was shown through absence of Mer function in genetically α v β 5 integrin-deficient cells, whose phenotype is also impaired retinal phagocytosis [64, 105].

The physiological importance of Mer signalling has also been shown in vivo in mice genetically lacking the entire Mer ICD [106]. LPS challenge caused a dramatic increase in deaths from endotoxic shock in Mer kinase-deficient mice versus wild-type animals. The endotoxic shock response was associated with overproduction of TNF- α by macrophages, apparently as result of LPS-induced activation of NF-kappa B and its nuclear translocation [106]. Thus, Mer signalling appears to actively inhibit proinflammatory signalling, which tantalisingly agrees with the pro-phagocytic property of Mer, that also acts to dampen down an inflammatory response.

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FIGURE LEGENDS

Fig. 1. The Gas6/Axl system. Gas6 and protein S are vitamin K-dependent ligands for the Axl, Sky and Mer RTKs. Both ligands are composed of a Gla region, four EGF repeats and a receptor-binding SHBG domain. Gas6 is known to bind to all three receptors whilst protein S has only been observed to bind to Sky and Mer. The receptors are each composed of two Ig repeats and two FNIII repeats in their extracellular regions, and a tyrosine kinase domain in their intracellular regions. Each RTK appears to signal for distinct functional outcomes, either in a ligand-dependent or independent manner.

Fig. 2. Signalling interactions with the intracellular domain (ICD) of Axl RTK. (A) Protein-protein interactions demonstrated for Axl and their intracellular consequences. Also shown are individual tyrosine phosphorylation sites that have been shown to recruit certain signalling proteins. (B) Activating interactions between Axl ICD and: (i) another Axl ICD (ligand-independent) and (ii) IL-15 receptor alpha ICD (induced by IL-15 stimulation).

Fig. 3. Protein-protein interactions demonstrated for Sky and their intracellular consequences, including interaction with another Sky ICD (ligand-independent).

Fig. 4. Protein-protein interactions demonstrated for Mer and their intracellular consequences. Also shown are individual tyrosine phosphorylation sites that have been shown to recruit certain signalling proteins.

Fig. 1

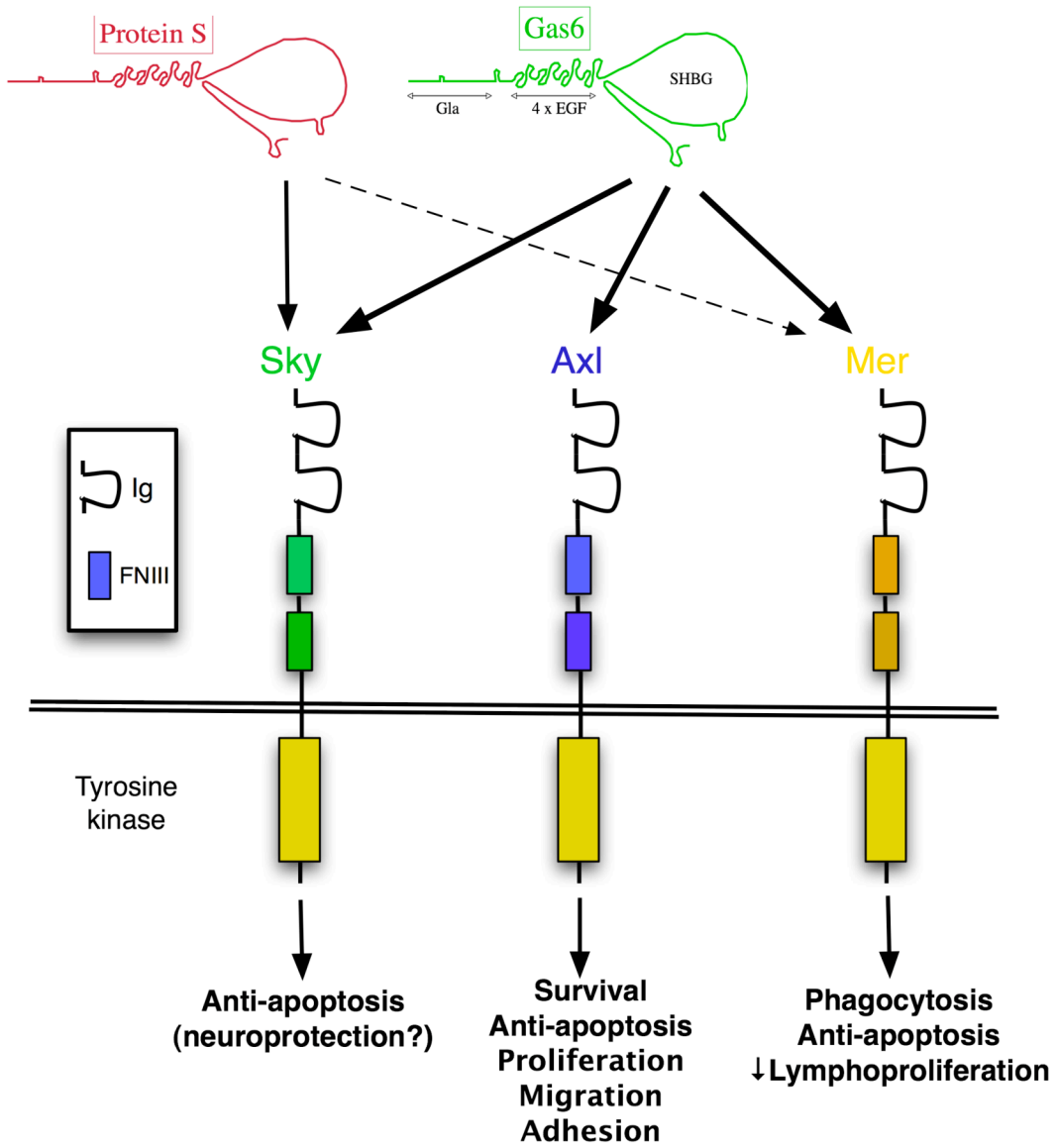


Fig. 2a

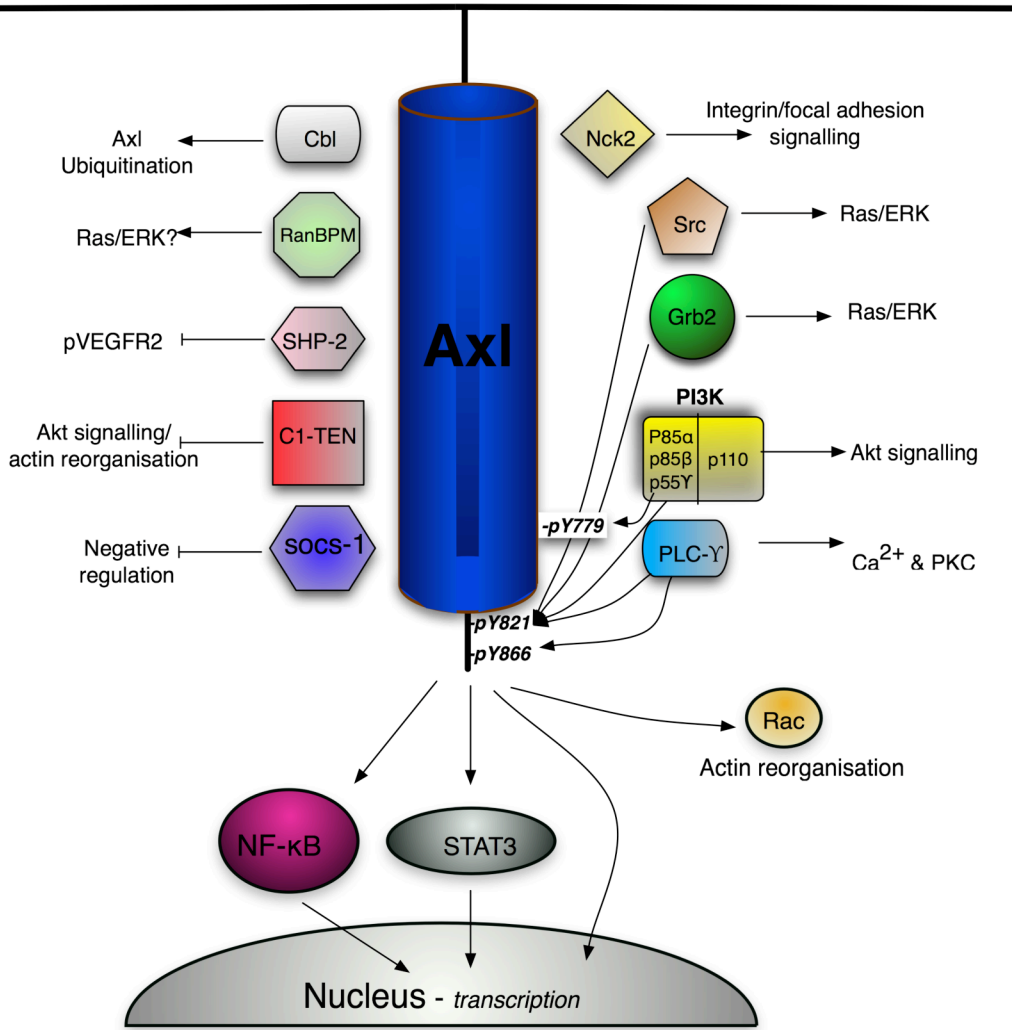


Fig. 2b

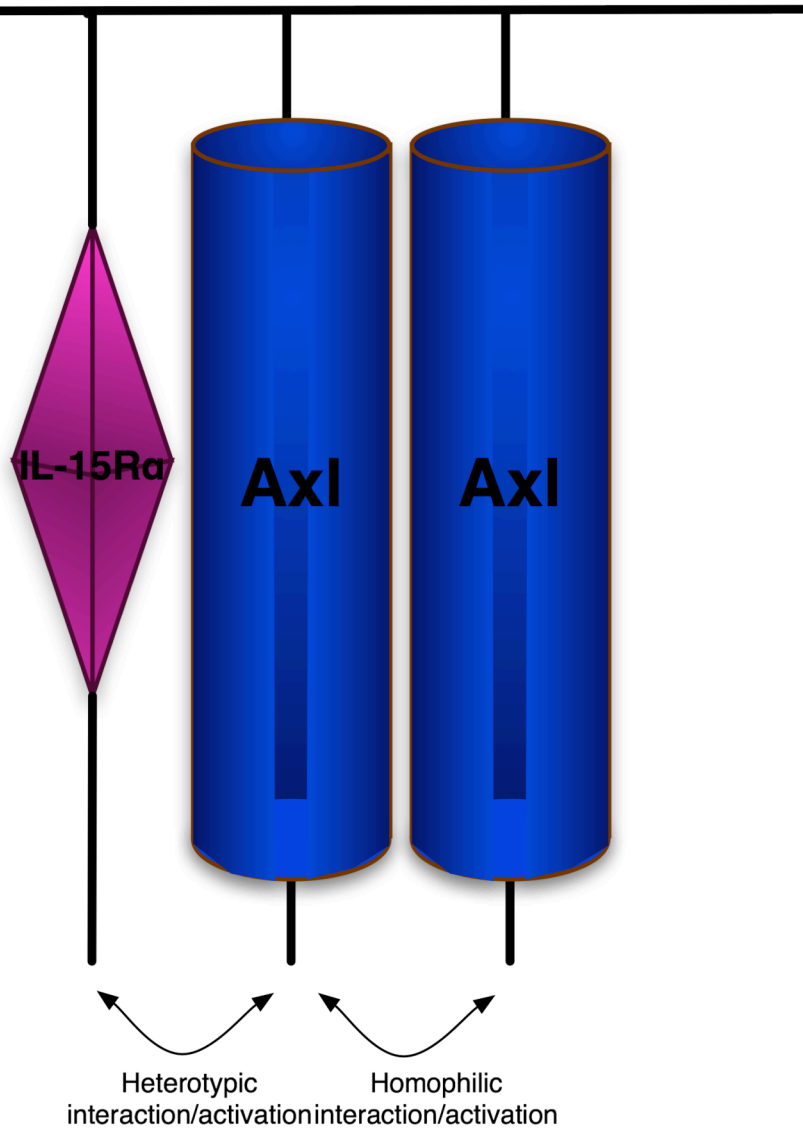


Fig. 3

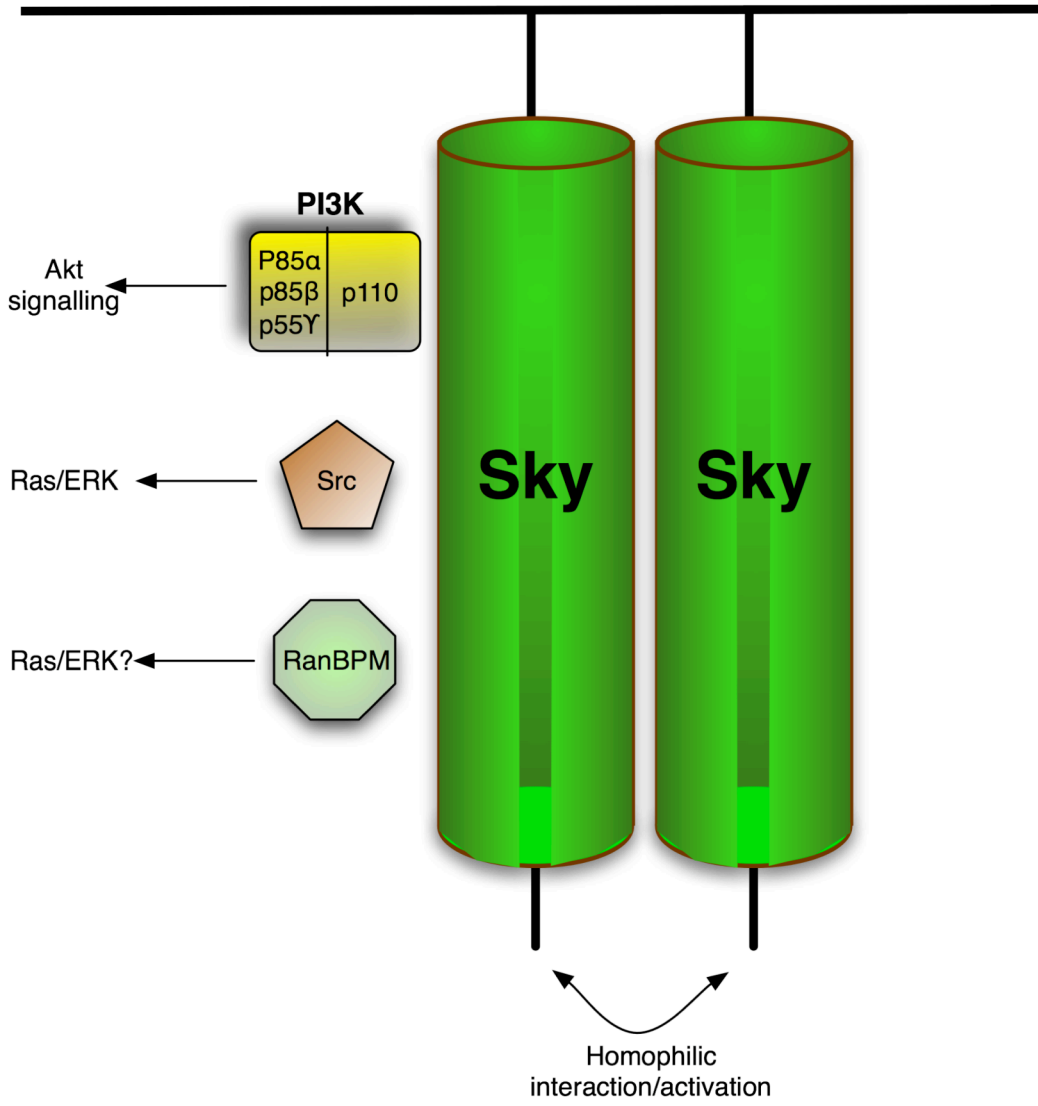


Fig. 4

