Cell. Signal. Vol. 10, No. 9, pp. 619–628, 1998

ISSN 0898-6568/98 $19.00
PII S0898-6568(98)00023-0

TOPICAL REVIEW

Regulation of Proliferation, Differentiation and Survival by the IL-3/IL-5/GM-CSF Receptor Family

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ABSTRACT. The receptors for the Il-3/IL-5/GM-CSF cytokine family are composed of a heterodimeric complex of a cytokine-specific α chain and a common β chain (βc). Binding of IL-3/IL-5/GM-CSF to their respective receptors rapidly induces activation of multiple intracellular signalling pathways, including the Ras-Raf-ERK, the JAK/STAT, the phosphatidylinositol 3-kinase PKB, and the JNK/SAPK and p38 signalling pathways. This review focuses on recent advancements in understanding how these different signalling pathways are activated by IL-3/IL-5/GM-CSF receptors, and how the individual pathways contribute to the pleiotropic effects of IL-3/IL-5/GM-CSF on their target cells, including proliferation, differentiation, survival, and effector functions. CELL SIGNAL 10;9:619–628, 1998. © 1998 Elsevier Science Inc.

KEY WORDS. IL-3/IL-5/GM-CSF, Apoptosis, Signal transduction, Hematopoiesis, JAK/STAT, Kinases

INTRODUCTION

Cytokines of the interleukin-3 (IL-3), IL-5 and granulocyte/macrophage colony-stimulating factor (GM-CSF) family are important regulators of haematopoiesis through the modulation of proliferation, differentiation and survival of various haematopoietic cell lineages and their precursors [1]. Whereas IL-3 and GM-CSF act on various lineages such as granulocytes, macrophages, erythrocytes, megakaryocytes and early haematopoietic progenitors, the action of IL-5 in humans is restricted to the eosinophilic and basophilic lineage. In the past decade, numerous studies have contributed to the understanding of how these cytokines can regulate a large number of distinct biological processes in multiple cell types. In particular, the cloning and mutational analysis of the receptors for IL-3/IL-5/GM-CSF have produced valuable information on the action of these cytokines. In this review, a concise overview will be given of recent advances in the understanding of IL-3/IL-5/GM-CSF receptor signalling.

A COMMON β RECEPTOR CHAIN RESULTS IN REDUNDANCY IN IL-3/IL-5/GM-CSF SIGNALLING

Although IL-3/IL-5/GM-CSF cytokines have distinct effects on different target cells, they elicit similar responses in cells responsive to all three cytokines [1, 2], and they even cross-compete for binding to the same cell [3]. These observations suggested that they might share the same receptor (component). Indeed, the molecular cloning of the IL-3/IL-5/GM-CSF receptors revealed the existence of a shared receptor subunit named the common β chain (βc). The IL-3/IL-5/GM-CSF receptors are composed of a cytokine-specific α chain (IL-3Rα, IL-5Rα and GM-CSFRα) complexed to the βc receptor [2, 4–6]. In mice, there are two β chain genes, one of which (βc) functions similarly to the human βc, whereas the other (βIL-3) dimerises only with the mIL-3Rα, providing the mouse with two different receptors for IL-3 [7]. Both the α and the βc chains are members of the superfamily of cytokine receptors, characterised by conserved structural features such as four conserved cysteine residues in their extracellular domain and a typical WSXWS motif in the juxtamembrane region [2]. The α chains can bind their ligands with low affinity, whereas the βc chain does not bind ligand itself but, when complexed with an α chain, forms a high-affinity signalling-competent receptor [2, 4–6]. Although the βc chain plays a major role in signal transduction through these receptors, the cytoplasmic domains of the IL-3, IL-5 and GM-CSF receptor α chains including conserved proline-rich domains have been implicated in the regulation of proliferation and differentiation by these cytokines [8–15]. The importance of the βc chain for IL-3/IL-5/GM-CSF function was also demonstrated by gene-targeting studies. Eosinophil numbers were reduced in the βc mutant mice, a phenomenon accompanied by the lack of an eosinophilic response to parasites, and IL-5 and GM-CSF failed to stimulate colony formation in clonal cultures of bone marrow cells [16, 17]. In contrast, IL-3 function was

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Received 8 January 1998; and accepted 9 February 1998.

normal, showing that IL-3 can also signal through receptors containing the βIL3 subunit [16, 17], whereas knock-out studies of βIL-3 demonstrate that IL-3 can also signal through the βc-containing receptor [18]. In addition, deletion of βc leads to mice showing lung pathology consisting of lymphocytic infiltration and areas resembling alveolar proteinosis [16, 17].

Recent evidence suggests that a bona-fide receptor is composed of two βc molecules and one α chain [19]. However, others have shown that at least two functional α chains are necessary for a signalling-competent receptor [20]. In addition, the GM-CSF receptor (α + βc) was recently demonstrated to exist in a pre-formed complex in unstimulated cells [21]. These studies clearly show that both α and βc subunits are important for high-affinity ligand binding and receptor function.

THE JAK/STAT PATHWAY

Although the IL-3/IL-5/GM-CSF receptors do not posses any intrinsic kinase activity, tyrosine phosphorylation of cellular substrates is rapidly observed in any cell stimulated with IL-3/IL-5/GM-CSF. A large number of cytoplasmic tyrosine kinases have been implicated in IL-3/IL-5/GM-CSF signalling, including Lyn, Btk, Tec, Fyn, Hck, FAK and Syk [22–32]. However, one of the major discoveries in cytokine signalling of the past 5 years was the elucidation of the JAK/STAT signalling pathway [33–35]. Janus kinases (JAKs) are a family of cytoplasmic tyrosine kinases that are associated with cytokine receptors and play a major role in cytokine signalling. Upon ligand binding, the JAKs are activated by trans-phosphorylation of two receptor-bound JAK molecules and subsequently phosphorylate a number of substrates including the cytokine receptor [33–35]. The phosphorylated receptor then provides docking sites for a variety of Src homology 2 (SH-2) domain-containing proteins, including a novel family of cytoplasmic transcription factors, termed STATs (signal transducers and activators of transcription). STATs are then phosphorylated on a single tyrosine residue by the JAKs, after which the STATs dimerise, migrate into the nucleus and regulate gene transcription (Fig. 1). Although the signalling pathway seems rather simple, the availability of four different JAKs (JAK1, JAK2, JAK3 and Tyk2) and at least eight different STATs (STAT1α, STAT1β, STAT2, STAT3, STAT3β, STAT4, STAT5A, STAT5B and STAT6), all with different DNA binding and trans-activation properties, allows cellular specificity in this signalling pathway.

Larner *et al.* [36] were the first to demonstrate that this pathway is involved in signalling by receptors for IL-3/IL-5/GM-CSF. Since then, many studies have demonstrated the involvement of different STATs and JAKs in IL-3/IL-5/GM-CSF signalling (see Table 1). There is a general consensus that activation of IL-3/IL-5/GM-CSF receptors results in the rapid activation of JAK2, although Tyk2 activation also has been observed (see Table 1). In unstimulated cells, JAK2 is already bound through its N-terminal domain

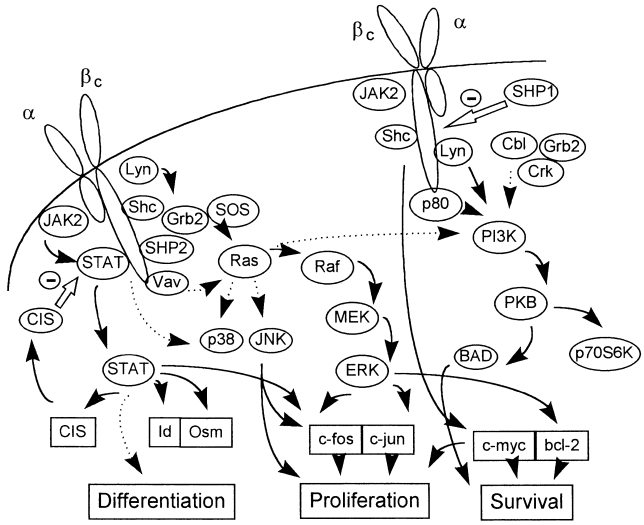

FIGURE 1. Schematic representation of signal transduction pathways activated through IL-3/IL-5/GM-CSF receptors. Summary of studies performed with either IL-3, IL-5 or GM-CSF receptors in different cell types. Receptor activation leads to the activation of multiple cytoplasmic signalling molecules (oval boxes). These pathways eventually lead to altered gene transcription (rectangles) or directly contribute to function, such as differentiation, proliferation or survival. Dashed lines represent connections that remain to be demonstrated. Open arrows indicate repressive signals. See text for details.

to a membrane proximal region of βc containing Box I (Fig. 2) [10, 12, 37–39]. Deletion of this region renders the βc chain unable to activate JAK2 [10]. However, the cytoplasmic region of the IL-3/IL-5/GM-CSF α-chain receptor also appears to have a role in the activation of JAK2 [10, 12, 14, 15, 40]. When JAK2 is activated, it phosphorylates a number of tyrosine residues in the βc chain, including Y577, Y612, Y695 and Y750 [41–44]. These sites then form docking sites for STAT proteins [45, 46]. Although activation of STAT1, STAT3 and STAT6 by IL-3/IL-5/GM-CSF receptors can be observed, depending on the cell type studied, STAT5 (A, B and truncated STAT5 proteins) seems to be the most predominant STAT activated by these receptors (see Table 1).

After βc phosphorylation, STAT5 binds to one or more tyrosine residues through its SH2 domain [45, 46] and is phosphorylated by JAK2, after which it can dimerise, translocate to the nucleus and regulate gene expression. Mutational analysis of the α and βc chains of the IL-3/IL-5/GM-CSF receptors shows that JAK2 is essential for STAT activation [14, 15, 47–49]. Interestingly, a C-terminal deletion mutant of βc (βc 541), which is still able to activate JAK2, fails to activate STAT5, showing that JAK2 activation is necessary but not sufficient for STAT activation [49]. Moreover, mutation of four tyrosine residues, Y577, Y612, Y695 and Y750, of the βc receptor completely blocks IL-5 induced STAT5-mediated transcription, indicating that STAT5 docking to the βc chain is necessary for its activation [43] (Fig. 2). Single mutation of any of these tyrosine residues does not influence STAT activation by IL-3/IL-5/GM-CSF, suggesting a

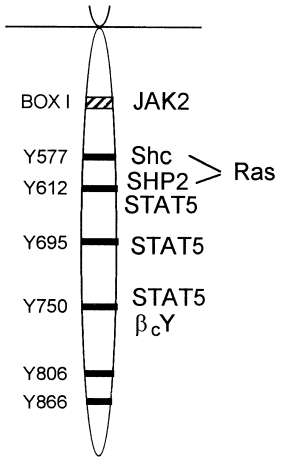

FIGURE 2. Schematic representation of βc residues involved in signalling. Only the cytoplasmic part of βc is shown. Box l mediates JAK2 binding and activation. Y577 is the docking/activation site for Shc. Y612 is the major binding site for SHP2. Both Y577 and Y612 contribute to activation of Ras. Y612, Y695 and Y750 are involved in STAT5 activation. Y750 is important for the tyrosine phosphorylation of βc.

high degree of redundancy in the Y residues of βc [41, 43]. In contrast, Okuda *et al.* [50] recently showed that GM-CSF can activate STAT5 in BaF3 cells expressing a βc chain with all of the cytoplasmic tyrosine residues mutated to phenylalanine. In these cells, STAT activation might well be mediated by endogenous mouse βc, because the GM-CSF receptor has been shown to exist in a pre-formed dimeric complex [21]. Alternatively, STATs may well interact directly with JAK2, as was previously shown for cells over-expressing exogenous STATs [47, 51].

Several studies have suggested that STAT5 might play a role in proliferation induced by the IL-3/IL-5/GM-CSF receptors. Mutations in the α or βc chains that fail to support STAT induction are defective in IL-3/IL-5/GM-CSF-mediated proliferation [9, 10, 12, 15, 52]. Similarly, over-expression of dominant-negative JAK2 strongly inhibits GM-CSF-mediated proliferation and c-*fos* induction in BaF3 cells, although, in these cells also STAT-independent signalling pathways are blocked [39]. Moreover, inhibition of STAT5 activation in BaF3 cells with the use of dominant negative STAT5 significantly repressed IL-3-dependent growth [53], although this effect was not observed in 32D3 cells [54]. This study also identified a number of STAT5 target genes that might be implicated in IL-3 function, including c-*fos*, *pim-1*, *osm* and *cis* (Fig. 1) [53–55]. Finally, macrophages from STAT5A-deficient mice grew more slowly in the presence of GM-CSF compared with wild-type macrophages, and the expression of CIS and A1 was markedly inhibited in the cells derived from the knockout mouse [56]. Interestingly, induction of c-*myc* was not dependent on STAT5 [53] but rather seemed to depend on another JAK2-mediated pathway resulting in the activation of transcription factor E2F [39, 57].

STAT activation by IL-3/IL-5/GM-CSF might also play a role in differentiation processes. STAT5 was implicated in the differentiation of myelomonocytic cells [58, 59] and erythroid cells [60]. Similarly, evidence from studies with other cytokines suggests that STAT3 is likely to play a role in macrophage differentiation [61–63], neutrophil differentiation [64], osteoblast differentiation [65] and even early embryonic development [66]. A role for IL-3/IL-5/GM-CSF-induced STAT5 in myeloid differentiation remains to be demonstrated. However, recent studies have shown that IL-3/IL-5/GM-CSF cytokines induce activation of C-terminally truncated forms of STAT5 (p77, p80) in immature myeloid cell lines, whereas, in mature myeloid cell lines, full-length STAT5 is activated [67–69] (Table 1). These truncated forms are generated by proteolytic cleavage [69]. In contrast, in the differentiation of primary CD34+ progenitor cells to eosinophils or neutrophils, there is a switch from IL-5/GM-CSF-activated wild-type STAT5 in undifferentiated cells to p80 in the differentiated cells (manuscripts submitted). Interestingly, p77 and p80 have distinct DNA-binding properties, whereas synthetic deletions equivalent to p77 and p80 be-

TABLE 1. Summary of JAKs and STATs activated by IL-3/IL-5/GM-CSF

JAK/STAT	Cell type	Reference
JAK2	Eosinophils, PMNs	[22, 70–73]
	Cell lines	[9, 10, 12, 23, 37, 39, 72–74]
Tyk2	BaF3 cell line	[74]
STAT1	Eosinophils, dendritic cells, PMNs	[22, 70, 73, 75]
	Cell lines	[74, 76]
	Transient transfections	[47, 77, 78]
STAT3	PMNs, dendritic cells	[73, 75]
	Cell lines	[47, 74]
	Transient transfections	[47, 77–79]
STAT5A/B	Monocytes, CD34+ cells, dendritic cells	[75, 80][a]
	Cell lines	[14, 48, 54, 68, 74, 81–84][a]
STAT5 p80	Eosinophils, neutrophils, monocytes	[70][a]
	Cell lines	[54, 68, 69]
STAT6	Dendritic cells	[75]
	Cell lines	[83]

[a] Plus unpublished results [manuscript(s) submitted].

have as dominant-negative regulators of STAT5-mediated transcription *in vitro* (manuscript submitted) [53, 68, 85]. It is therefore likely that different target genes are regulated by STAT5/p80 in undifferentiated versus differentiated cells, suggesting a role for STAT5 in myeloid differentiation. However, direct evidence that IL-3/IL-5/GM-CSF-induced STAT activity is causally involved in differentiation processes is currently lacking. Therefore, it would be very interesting to perform a detailed analysis of the differentiation of various haematopoietic lineages in STAT5A or 5B or even 5A/5B knockout mice.

ACTIVATION OF MULTIPLE MAP KINASE SIGNALLING PATHWAYS BY IL-3/IL-5/GM-CSF

The ERK1 and ERK2 members of the MAPK family have been shown to be activated by IL-3/IL-5/GM-CSF in multiple primary cellular lineages and cell lines [22, 86–90]. Analogous to ERK activation by growth factor receptors, ERK activation by IL-3/IL-5/GM-CSF is expected to occur through activation of Ras and c-Raf. Indeed, IL-3/IL-5/GM-CSF cytokines rapidly induce activation of Ras [22, 89–93] and c-Raf [22, 94–96]. Activation of this pathway will eventually result in enhanced transcription of c-*fos* and c-*jun* [52, 91, 97] and might contribute to IL-3/IL-5/GM-CSF-induced proliferation (Fig. 1) [98]. A region between amino acid 544 and amino acid 763 in the cytoplasmic part of the βc receptor takes part in the activation of this pathway [52, 97]. The adaptor protein Shc is likely to have a role in coupling the receptor to the activation of this pathway. Shc is rapidly phosphorylated on tyrosine residues after IL-3/IL-5/GM-CSF stimulation [23, 42, 97, 99]. Interestingly, overexpression of Shc potentiates ERK activation and the proliferative response to GM-CSF [99]. Shc directly binds to the phosphorylated Tyr 577 residue in the βc upon IL-3/IL-5/GM-CSF signalling [100] (Fig. 2). After phosphorylation, Shc interacts with the Grb2 adaptor protein, which, in turn, interacts with mSOS, the nucleotide exchange factor for Ras [101]. Tyrosine 577 of the βc chain is essential for phosphorylation of Shc and the Shc-associated p140, as well as the interaction of Shc with Grb2 and βc [41, 50, 52, 99], whereas tyrosine 750 also seems to be involved in Shc phosphorylation [42]. These studies suggest the following order of events in the activation of ERK2 by IL-3/IL-5/GM-CSF: Y577-Shc-Grb2/mSOS-Ras-Raf-MEK-ERK (Fig. 1). However, alternative mechanisms also are likely to be involved, because mutation of Y577 did not block Raf-ERK activation and proliferation by GM-CSF in BaF3 cells [41]. The phosphatase SH-PTP2 (PTP-1D, SHP2, Syp) interacts with Y612 [44, 102] or both Y577 and Y612 [52] of βc after IL-3/IL-5/GM-CSF stimulation (Fig. 2). SH-PTP2 interacts with Grb2-SOS in eosinophils and is involved in IL-5-induced ERK activation in these cells [44].

ERK1 and ERK2 are not the only MAPKs that are activated by IL-3/IL-5/GM-CSF. Activation of p38, a kinase involved in cellular responses to stress, by GM-CSF was observed in human neutrophils [103] (Fig. 1), although this was not observed in other studies [104]. Similarly, IL-3 can activate p38 in FDC-P2 cells [105]. p38 induction by GM-CSF might be implicated in activation of cytosolic phospholipase A2 [103]. Activation of the third group of MAPKs, the JNK/SAPK kinases, by IL-3/IL-5/GM-CSF in TF1, BaF3, FDCP2 and MC/9 cells also has been reported [106–110] (Fig 1). The mechanism through which this pathway is activated is not completely clear. Y577 of the βc chain and JAK2 are likely to take part [106, 107, 110], as does the cytoplasmic domain of the α chain [106]. However, the role of Ras is unclear, because both *ras*-independent [106] and *ras*-dependent [107] activation of this pathway were observed. SEK-1 might be involved upstream of JNK/SAPK activation, because SEK1 phosphorylation is induced by IL-3 in MC/9 cells [109], whereas dominant-negative SEK1 partly blocks JNK/SAPK activation by IL-5 [106]. However, IL-3 fails to activate SEK-1 in FDC-P2 cells [108]. Downstream targets for JNK/SAPK might be transcription factors involved in c-*jun* and c-*fos* regulation such as TCF, ATF2 and cJun, because dominant-negative SEK partly inhibits TRE- and DSE-dependent transcription induced by IL-5 [106]. Blocking JNK/SAPK activation with a serine/threonine/tyrosine phosphatase suggests that this pathway contributes to IL-3-dependent growth but not survival [111]. However, elucidation of the precise function of this pathway in primary cells awaits the availability of *specific* pharmacological inhibitors of its components.

ACTIVATION OF THE PHOSPHATIDYLINOSITOL 3-KINASE (PI3K) PATHWAY

The lipid kinase PI3K, which generates the signalling molecule phosphatidyl-inositol 3,4,5-triphosphate, is involved in the regulation of multiple cellular processes [112]. IL-3/IL-5/GM-CSF rapidly activates PI3K in multiple cell types, an event dependent on tyrosine phosphorylation (Fig. 1), [23, 89, 90, 97, 113–115]. Kinases that are downstream of PI3K are also activated by IL-3/IL-5/GM-CSF. Recently, PKB was shown to be rapidly activated by IL-3/IL-5/GM-CSF in human granulocytes, a process dependent on PI3K [90, 116]. Similarly, IL-3 activates PKB/Akt activity in multiple cell lines, a process thought to be involved in cellular survival [117, 118]. Similarly, the more downstream p70 S6 kinase also can be activated by IL-3 and GM-CSF [97, 119]. Interestingly, the blocking of IL-3-induced p70S6K in BaF3 cells by rapamycin partly inhibited IL-3-dependent [^{3}H]-thymidine incorporation, suggesting a role for this pathway in cellular proliferation [119].

The initiation of this pathway at the receptor level in not yet completely clear. PI3K and p70 S6K activation in BaF3 cells is dependent on the same region of the βc chain that is involved in Ras activation; it also depends on the cytoplasmic domain of the α chain [97]. PI3K can interact with the βc *in vitro* [37]. This interaction might be mediated by a novel adaptor protein (p80), which interacts with βc and the p85 subunit of PI3K in IL-3/IL-5/GM-CSF stimulated

cells [120]. Interestingly, this complex also contains the Src-family kinases Yes and Lyn, which themselves are activated by IL-3/IL-5/GM-CSF [113, 120, 121]. Recently, it was shown that Lyn is likely to be the kinase responsible for the activation of PI3K in response to GM-CSF [115]. Cloning of p80 and further characterisation of its binding partners will be necessary to elucidate the precise mechanism by which IL-3/IL-5/GM-CSF cytokines activate the PI3K pathway. Another mechanism by which PI3K can be activated includes tyrosine phosphorylation of the p120 Cbl protein, which was recently observed for IL-3 and GM-CSF [122–124]. Interestingly, Cbl forms a stable complex with the adaptor proteins Grb2, Crk and Shc [122–125]. More importantly, PI3K also can be observed in this complex [123, 126]. However, a causal relationship between Cbl phosphorylation and activation of the PI3K pathway by IL-3/IL-5/GM-CSF remains to be demonstrated.

SURVIVAL THROUGH IL-3/IL-5/GM-CSF RECEPTOR SIGNALLING

It is well understood that one of the major functions of IL-3/IL-5/GM-CSF cytokines is the inhibition of apoptosis in their target cells, both in mature blood cells and in early progenitors [127]. However, the signalling pathways utilised by IL-3/IL-5/GM-CSF to overcome death signals have only recently started to become evident. The first molecular approach to tackle this problem was taken by Kinoshita *et al.* [128], who showed that deletion of the cytoplasmic tail of the βc chain down to amino acid 544 completely inhibited cellular survival by GM-CSF in BaF3 cells. This deletion includes the region of the βc chain involved in activation of the Ras-ERK pathway. Moreover, over-expression of an activated Ras Val12 in cells containing the βc 544 mutant could overcome the defect in GM-CSF- or IL-3-induced survival, further stressing the importance of Ras signalling in survival [18, 129], although this effect might also be mediated by PI3K. Similarly, over-expression of oncogenic Raf, a downstream target of Ras, also suppressed apoptosis induced by IL-3 withdrawal in 32D3 and BaF3 cells [130, 131], and inhibition of ERK activation with a dominant-negative MAPKK suppresses IL-3-dependent survival in BaF3 cells [132]. A role for ERK in eosinophil survival was also suggested by studies showing that activation of the tyrosine phosphatase SHPTP2/SHP2 by IL-5 is necessary for both ERK2 activation as well as IL-5-mediated survival [44]. Similarly, activation of the Src-like kinase Lyn, which is thought to act upstream of the Ras-ERK pathway [22], is necessary for survival in IL-5- or GM-CSF-treated eosinophils and neutrophils [31, 32].

The targets important for cellular survival induced by IL-3/IL-5/GM-CSF seem to be proteins of the anti-apoptotic *bcl-2* gene family (Fig. 1). Expression of *bcl-2* and *bcl-x* is rapidly induced by IL-3 or activated Ras in multiple cell types [128, 133–135]. Similarly, GM-CSF induces the expression of *A1*, a novel haemopoietic-specific homologue of Bcl-2 [136]. Jak2 activation by these cytokines seems to be involved in the induction of Bcl2 [137]. Interestingly, over-expression of *bcl-2* or *A1* block apoptosis induced by IL-3 withdrawal in cell lines [138–140]. Importantly, IL-5 treatment of primary eosinophils results in a significant increase in *bcl-2* expression, which is a prerequisite for IL-5-mediated survival, as demonstrated by anti-sense oligonucleotide experiments [141]. In addition, IL-3-induced Bcl-2 phosphorylation on serine 70 contributes to survival, because Bcl-2 (serine 70–alanine) is unable to support IL-3-independent survival [139].

The aforedescribed studies suggest that IL-3-induced Ras-Raf-ERK-Bcl2 activation plays an important role in cellular survival (Fig. 1). However, recent reports have shown that this might be an over-simplification. Gotoh *et al.* [142] showed Ras–ERK-independent pathways also can mediate survival by IL-3 in BaF3 cells. They identified two tyrosine residues on Shc, Y239 and Y240, that mediate induction of the anti-apoptotic c-*myc* gene but not Ras-ERK activation. Moreover, cells expressing Shc Y239/240F, which activates Ras-ERK but not c-*myc*, were sensitive to apoptosis. On the other hand, cells expressing Shc Y317F, which are unable to activate Ras-ERK but still enhance c-*myc* expression, showed resistance to apoptosis. Similarly, mutation of Y750 in the βc chain results in a decrease in Shc phosphorylation accompanied by a decrease in GM-CSF-induced survival [42]. However, Shc phosphorylation is not absolutely required for GM-CSF-mediated survival, because a βc mutant unable to induce Shc phosphorylation is still able to support survival of BaF3 cells [50]. Others have also hinted at the role of ERK-independent pathways in survival. An activated form of the R-Ras protein, Q87L, was shown to suppress cell death in IL-3-starved BaF3 cells in a manner dependent on the presence of serum IGF-I [143]. Interestingly, this effect could be blocked not only with the MEK-MAPK inhibitor PD98059, but also with the PI3K inhibitors LY294002 and wortmannin, suggesting that the PI3K pathway also contributes to survival. Indeed, R-Ras was previously reported to activate PI3K [144]. Similarly, the apoptosis-suppressing effect of another Ras mutant (G12V/V45E) in BaF3 cells could be overcome by PI3K inhibitors or by the inhibition of p70S6K by rapamycin [131]. Blocking the PI3K/p70S6K pathways in other cell types also hints at the involvement of these kinases in survival [145]. Recent advances in the elucidation of this signalling pathway indicated that the PKB/Akt kinase, which lies downstream of PI3K, is involved in cell survival in multiple cellular systems [146]. Indeed, over-expression of active PKB/Akt results in IL-3-independent survival of 32D3 and BaF3 cells and promotes the expression of the anti-apoptotic c-*myc* and *bcl-2* genes [117, 147] (Fig. 1). Moreover, apoptosis after IL-3 withdrawal is accelerated by a kinase-dead mutant of PKB/Akt with dominant-negative properties [117]. A major target for PKB/Akt in survival was shown to be the pro-apoptotic *bcl-2* family member BAD. PKB phosphorylates BAD at the same residues that are phosphorylated in response to IL-3, thereby blocking its pro-apoptotic activity [118, 148] (Fig. 1).

Although the aforedescribed studies clearly show that both the Ras-ERK and the PI3K-PKB pathways can have

roles in cellular survival, depending on the cell type studied, a number of questions remain unanswered. It would be important to test whether Y239/240 of Shc, which can induce c-*myc* and signal to survival, are implicated in the activation of the PI3K-PKB pathway. The observation that PI3K can interact with Shc in chronic myelogeneous leukaemia cells suggests that this might well be the case [149]. Because most of the work was performed in cell lines, it is extremely important to test the relative contribution of the different signalling pathways to cellular survival by using specific pharmacological inhibitors in primary target cells such as eosinophils and neutrophils. However, the up-regulation of anti-apoptotic proteins (Bcl-2 and c-Myc) and the down-regulation of pro-apoptotic proteins (BAD) are clearly involved in survival, disregarding their upstream regulators.

CELLULAR EFFECTOR FUNCTIONS

As heretofore described, most of the work on IL-3/IL-5/GM-CSF signalling has focussed on the regulation of proliferation, differentiation and cellular survival by these cytokines. It is widely acknowledged that IL-3/IL-5/GM-CSF receptors also modify various effector functions in myeloid cells, such as cellular migration and respiratory burst in granulocytes. Pre-incubation of neutrophils or eosinophils with IL-3/IL-5/GM-CSF strongly enhances effector functions regulated by other stimuli, such as FMLP, a phenomenon known as "priming" [150]. However, studies on the molecular mechanisms by which these effector functions are regulated by IL-3/IL-5/GM-CSF receptors are relatively sparse. A role for ERK activation by IL-3/IL-5/GM-CSF in neutrophil effector functions was suggested [151,152]. However, blocking ERK activation with PD098059 showed that ERK does not have a role in GM-CSF- primed neutrophil respiratory burst or migration [89], analogous to the observation that IL-8-induced ERK activation does not play a role in neutrophil migration [153]. In contrast, IL-3/IL-5/GM-CSF-induced Erk activation might play a role in phospholipase A2-mediated release of the lipid mediator PAF [89] and granule secretion [154].

The role of the PI3K pathway in cellular effector functions has been more widely studied. FMLP-induced respiratory burst in neutrophils is dependent on PI3K, as was demonstrated by using the PI3K inhibitors wortmannin and LY294002 [154–156]. Recently, priming and activation of the PI3K pathway by GM-CSF were shown to take part in the activation of the respiratory burst and chemokinesis by GM-CSF in neutrophils [89] and eosinophils [90]. One of the targets of PI3K in the activation of the respiratory burst may be p47phox, which is thought to play a major role in the activation of the NADPH oxidase complex. Interestingly, wortmannin blocks FMLP-induced p47phox phosphorylation [156]. Moreover, over-expression of active PI3K in the monoblastic phagocyte cell line GM-1 caused constitutive phosphorylation of p47phox [157]. However, clarification of the precise mechanism of respiratory burst activation in granulocytes awaits further study. The availability of specific inhibitors of the MAPK and PI3K pathways will undoubtedly be of great help in determining the roles of these pathways in various cellular effector functions regulated by IL-3/IL-5/GM-CSF.

TURNING OFF THE SIGNAL OF PHOSPHATASES AND STAT-INDUCED STAT REPRESSORS

Continuous activation of the aforedescribed signalling pathways would lead to cytokine-independent growth of the target cells [158]. Therefore, feedback mechanisms exist to ensure a transient response to the IL-3/IL-5/GM-CSF cytokines. The tyrosine phosphatase HCP (also called PTP1C, SHPTP1, SHP or SHP1) appears to be a major player in this process. Increased levels of HCP lead to a reduction of the level of βc phosphorylation as well as the suppression of IL-3-dependent cell growth, whereas reduced HCP levels produce the opposite effect [159, 160]. After ligand binding, HCP specifically associates with the βc chain, probably at Y612 [102, 159, 160] (Fig. 1). The role for HCP in the down-regulation of IL-3/IL-5/GM-CSF signalling might explain the dramatic haematopoietic abnormalities observed in HCP mutant (moth-eaten) mice, which die soon after birth owing to over-proliferation and accumulation of macrophages in the lung [161–163].

Other phosphatases also have been implicated in IL-3/IL-5/GM-CSF signalling. SHPTP2 also interacts with Y612 of the activated βc chain, whereas this residue is a good substrate for SHP2 *in vitro* [89]. However, the precise role of SHP2 in IL-3/IL-5/GM-CSF signalling remains to be determined, because it also seems to play a positive role in IL-3/IL-5/GM-CSF signalling, coupling the receptor to Grb2 and PI3K [44, 164]. Recently, a number of different inositol phosphatases, including SHIP and pp135, were implicated in the negative regulation of IL-3/IL-5/GM-CSF signalling [110, 165, 166]. However, the exact contribution of this class of phosphatases to IL-3/IL-5/GM-CSF signalling remains to be determined.

A novel mechanism of terminating IL-3/IL-5/GM-CSF signalling was demonstrated by Yoshimura *et al.* [167]. They cloned the cytokine-inducible gene named *cis* (cytokine inducible SH2-containing protein). CIS is rapidly and transiently induced by various cytokines including IL-3 and GM-CSF. Interestingly, CIS binds stably to the tyrosine phosphorylated βc chain [167]. CIS is thought to be a negative regulator of IL-3/IL-5/GM-CSF signalling, because over-expression of CIS represses IL-3-dependent growth [167], oncostatin M induction and STAT5 activation [168]. CIS is up-regulated by a STAT5-dependent mechanism (Fig. 1), because BaF3 cells over-expressing dominant-negative STAT5 fail to induce CIS in response to IL-3 [53]. Moreover, in bone marrow-derived macrophages from STAT5A-deficient mice, CIS induction by GM-CSF was markedly inhibited [56]. Indeed, the *cis* promoter contains four STAT binding sites that are activated by STAT5 [168]. Recently, it was shown that CIS is a member of a large family. Three CIS-related *SOCS* genes (suppresser of cytokine signalling) that also are

rapidly induced by a variety of cytokines including IL-3 and GM-CSF were cloned [169]. Similarly, JAB (JAK-binding protein), SSI-1 (STAT-induced STAT inhibitor) and CIS2-4 are CIS-related SH2-containing proteins that are rapidly induced by a variety of cytokines [170–172]. Additionally, STAT3 function can be blocked by a novel protein, PIAS3 (protein inhibitor of activated STAT), which associates with STAT3 only in cytokine-stimulated cells [173]. SOCS, SSI-1, JAB and CIS3 also inhibit activation of the JAK – STAT pathway, apparently by directly interacting with the JAKs [170, 171]. The precise mechanism by which this occurs is at present unclear but might include SOCS/JAB/SSI-associated phosphatases that directly dephosphorylate JAK, thereby inhibiting its activity.

FUTURE DIRECTIONS

Since tyrosine phosphorylation of a common set of substrates was understood to play an important role in IL-3/IL-5/GM-CSF signalling [174], much progress has been made in understanding the mechanisms by which IL-3/IL-5/GM-CSF cytokines regulate a wide variety of cellular processes. The cloning of the IL-3/IL-5/GM-CSF receptor components and their mutagenesis have revealed some, but not all, of the mechanisms by which IL-3/IL-5/GM-CSF cytokines induce signalling cascades such as the Ras-ERK, p38/JNK, PI3K and JAK/STAT pathways. Moreover, the availability of specific inhibitors of some of these pathways has opened the way to more extensive studies on the relative contribution of each of these pathways to cellular functions regulated by IL-3/IL-5/GM-CSF. New insights into IL-3/IL-5/GM-CSF signalling are also expected to arise from studies using the yeast two-hybrid system to clone (novel) proteins that interact with the IL-3/IL-5/GM-CSF receptors. In particular, it is important to determine whether the α chains interact with cytoplasmic proteins in a cytokine-specific manner, inasmuch as the ligand-specific effects of IL-3/IL-5/GM-CSF [1, 175] are likely to be mediated through the α chains. Similarly, the availability of techniques to generate lineage-specific knockout mice will also contribute to an understanding of IL-3/IL-5/GM-CSF signalling. Finally, relevant IL-3/IL-5/GM-CSF target genes are now beginning to be cloned with the use of novel techniques such as differential display PCR [176]. It is therefore likely that much progress will be made in the coming years in delineating the mechanistic aspects of IL-3/IL-5/GM-CSF-regulated signal transduction, proliferation, differentiation, survival and cellular effector functions.

References

1. Arai K. I., Lee F., Miyajima A., Miyatake S., Arai N. and Yokota T. (1990) *Annu. Rev. Biochem.* **59**, 783–836.
2. Miyajima A., Hara T. and Kitamura T. (1992) *Trends Biochem. Sci.* **17**, 378–382.
3. Lopez A. F., Vadas M. A., Woodcock J. M., Milton S. E., Lewis A., Elliott M. J., Gillis D., Ireland R., Olwell E. and Park L. S. (1991) *J. Biol. Chem.* **266**, 24741–24747.
4. Mui A., Muto A., Sakamaki K., Sato N., Kinoshita T., Watanabe S., Yokota T., Arai K. and Miyajima A. (1994) *Adv. Exp. Med. Biol.* **365**, 217–223.
5. Hara T. and Miyajima A. (1996) *Stem Cells* **14**, 605–618.
6. Bagley C. J., Woodcock J. M., Stomski F. C. and Lopez A. F. (1997) *Blood* **89**, 1471–1482.
7. Hara T. and Miyajima A. (1992) *EMBO J.* **11**, 1875–1884.
8. Polotskaya A., Zhao Y., Lilly M. L. and Kraft A. S. (1993) *Cell Growth Differ.* **4**, 523–531.
9. Takaki S., Kanazawa H., Shiiba M. and Takatsu K. (1994) *Mol. Cell. Biol.* **14**, 7404–7413.
10. Quelle F. W., Sato N., Witthuhn B. A., Inhorn R. C., Eder M., Miyajima A., Griffin J. D. and Ihle J. N. (1994) *Mol. Cell. Biol.* **14**, 4335–4341.
11. Polotskaya A., Zhao Y., Lilly M. B. and Kraft A. S. (1994) *J. Biol. Chem.* **269**, 14607–14613.
12. Cornelis S., Fache I., Van der Heyden J., Guisez Y., Tavernier J., Devos R., Fiers W. and Plaetinck G. (1995) *Eur. J. Immunol.* **25**, 1857–1864.
13. Ronco L. V., Doyle S. E., Raines M., Park L. S. and Gasson J. C. (1995) *J. Immunol.* **154**, 3444–3453.
14. Kouro T., Kikuchi Y., Kanazawa H., Hirokawa K., Harada N., Shiiba M., Wakao H., Takaki S. and Takatsu K. (1996) *Int. Immunol.* **8**, 237–245.
15. Matsuguchi T., Zhao Y., Lilly M. B. and Kraft A. S. (1997) *J. Biol. Chem.* **272**, 17450–17459.
16. Robb L., Drinkwater C. C., Metcalf D., Li R., Kontgen F., Nicola N. A. and Begley C. G. (1997) *Proc. Natl. Acad. Sci. USA* **92**, 9565–9569.
17. Nishinakamura R., Nakayama N., Hirabayashi Y., Inoue T., Aud D., McNeil T., Azuma S., Yoshida S., Toyoda Y., Arai K. *et al.* (1995) *Immunity* **2**, 211–222.
18. Nicola N. A., Robb L., Metcalf D., Cary D., Drinkwater C. C. and Begley C. G. (1996) *Blood* **87**, 2665–2674.
19. Muto A., Watanabe S., Miyajima A., Yokota T. and Arai K. (1996) *J. Exp. Med.* **183**, 1911–1916.
20. Lia F., Rajotte D., Clark S. C. and Hoang T. (1996) *J. Biol. Chem.* **271**, 28287–28293.
21. Woodcock J. M., McClure B. J., Stomski F. C., Elliott M. J., Bagley C. J. and Lopez A. F. (1997) *Blood* **90**, 3005–3017.
22. Pazdrak K., Schreiber D., Forsythe P., Justement L. and Alam R. (1995) *J. Exp. Med.* **181**, 1827–1834.
23. Sato S., Katagiri T., Takaki S., Kikuchi Y., Hitoshi Y., Yonehara S., Tsukada S., Kitamura D., Watanabe T., Witte O. and Takatsu K. (1994) *J. Exp. Med.* **180**, 2101–2111.
24. Machide M., Mano H. and Todokoro K. (1995) *Oncogene* **11**, 619–625.
25. Appleby M. W., Kerner J. D., Chien S., Maliszewski C. R., Bondada S., Perlmutter R. M. and Bondadaa S. (1995) *J. Exp. Med.* **182**, 811–820.
26. Li Y., Shen B. F., Karanes C., Sensenbrenner L. and Chen B. (1995) *J. Immunol.* **155**, 2165–2174.
27. Anderson S. M. and Jorgensen B. (1995) *J. Immunol.* **155**, 1660–1670.
28. Mano H., Yamashita Y., Sato K., Yazaki Y. and Hirai H. (1995) *Blood* **85**, 343–350.
29. Kume A., Nishiura H., Suda J. and Suda T. (1997) *Blood* **89**, 3434–3442.
30. Burton E. A., Hunter S., Wu S. C. and Anderson S. M. (1997) *J. Biol. Chem.* **272**, 16189–16195.
31. Wei S., Liu J. H., Epling Burnette P. K., Gamero A. M., Ussery D., Pearson E. W., Elkabani M. E., Diaz J. I. and Djeu J. Y. (1996) *J. Immunol.* **157**, 5155–5162.
32. Yousefi S., Hoessli D. C., Blaser K., Mills G. B. and Simon H. U. (1996) *J. Exp. Med.* **183**, 1407–1414.
33. Darnell J. E. (1997) *Science* **277**, 1630–1635.
34. Schindler C. and Darnell J. E. (1995) *Annu. Rev. Biochem.* **64**, 621–651.

35. Watanabe S., Itoh T. and Arai K. (1996) *J. Allergy Clin. Immunol.* **98**, S183–S191.
36. Larner A. C., David M., Feldman G. M., Igarashi K., Hackett R. H., Webb D. S., Sweitzer S. M., Petricoin E. F. and Finbloom D. S. (1993) *Science* **261**, 1730–1733.
37. Rao P. and Mufson R. A. (1995) *J. Biol. Chem.* **270**, 6886–6893.
38. Zhao Y., Wagner F., Frank S. J. and Kraft A. S. (1995) *J. Biol. Chem.* **270**, 13814–13818.
39. Watanabe S., Itoh T. and Arai K. (1996) *J. Biol. Chem.* **271**, 12681–12686.
40. Takaki S. and Takatsu K. (1994) *Int. Arch. Allergy Immunol.* **104**, 36–38.
41. Durstin M., Inhorn R. C. and Griffin J. D. (1996) *J. Immunol.* **157**, 534–540.
42. Inhorn R. C., Carlesso N., Durstin M., Frank D. A. and Griffin J. D. (1995) *Proc. Natl. Acad. Sci. USA* **92**, 8665–8669.
43. van Dijk T. B., Caldenhoven E., Raaijmakers J. A., Lammers J. W., Koenderman L. and de Groot R. P. (1997) *FEBS Lett.* **412**, 161–164.
44. Pazdrak K., Adachi T. and Alam R. (1997) *J. Exp. Med.* **186**, 561–568.
45. Chin H., Nakamura N., Kamiyama R., Miyasaka N., Ihle J. N. and Miura O. (1996) *Blood* **88**, 4415–4425.
46. Woodcock J. M., Bagley C. J., Zacharakis B. and Lopez A. F. (1996) *J. Biol. Chem.* **271**, 25999–26006.
47. Caldenhoven E., van Dijk T., Raaijmakers J. A., Lammers J. W., Koenderman L. and de Groot R. P. (1995) *J. Biol. Chem.* **270**, 25778–25784.
48. Mui A. L., Wakao H., O'Farrell A. M., Harada N. and Miyajima A. (1995) *EMBO J.* **14**, 1166–1175.
49. Smith A., Metcalf D. and Nicola N. A. (1997) *EMBO J.* **16**, 451–464.
50. Okuda K., Smith L., Griffin J. D. and Foster R. (1997) *Blood* **90**, 4759–4766.
51. Fujitani Y., Hibi M., Fukada T., Takahashi Tezuka M., Yoshida H., Yamaguchi T., Sugiyama K., Yamanaka Y., Nakajima K. and Hirano T. (1997) *Oncogene* **14**, 751–761.
52. Itoh T., Muto A., Watanabe S., Miyajima A., Yokota T. and Arai K. (1996) *J. Biol. Chem.* **271**, 7587–7592.
53. Mui A. L. F., Wakao H., Kinoshita T., Kitamura T. and Miyajima A. (1996) *EMBO J.* **15**, 2425–2433.
54. Wang D., Stravopodis D., Teglund S., Kitazawa J. and Ihle J. N. (1996) *Mol. Cell. Biol.* **16**, 6141–6148.
55. Yoshimura A., Ichihara M., Kinjyo I., Moriyama M., Copeland N. G., Gilbert D. J., Jenkins N. A., Hara T. and Miyajima A. (1996) *EMBO J.* **15**, 1055–1063.
56. Feldman G. M., Rosenthal L. A., Liu X., Hayes M. P., Wynshaw-Boris A., Leonard W. J., Hennighausen L. and Finbloom D. S. (1997) *Blood* **90**, 1768–1776.
57. Watanabe S., Ishida S., Koike K. and Arai K. (1995) *Mol. Biol. Cell* **6**, 627–636.
58. Woldman I., Mellitzer G., Kieslinger M., Buchhart D., Meinke A., Beug H. and Decker T. (1997) *J. Immunol.* **159**, 877–886.
59. Barahmand pour F., Meinke A., Kieslinger M., Eilers A. and Decker T. (1996) *Curr. Top. Microbiol. Immunol.* **211**, 121–128.
60. Iwatsuki K., Endo T., Misawa H., Yokouchi M., Matsumoto A., Ohtsubo M., Mori K. J. and Yoshimura A. (1997) *J. Biol. Chem.* **272**, 8149–8152.
61. Nakajima K., Yamanaka Y., Nakae K., Kojima H., Ichiba M., Kiuchi N., Kitaoka T., Fukada T., Hibi M. and Hirano T. (1996) *EMBO J.* **15**, 3651–3658.
62. Minami M., Inoue M., Wei S., Takeda K., Matsumoto M., Kishimoto T. and Akira S. (1996) *Proc. Natl. Acad. Sci. USA* **93**, 3963–3966.
63. Yamanaka Y., Nakajima K., Fukada T., Hibi M. and Hirano T. (1996) *EMBO J.* **15**, 1557–1565.
64. Shimozaki K., Nakajima K., Hirano T. and Nagata S. (1997) *J. Biol. Chem.* **272**, 25184–25189.
65. Bellido T., Borba V. Z., Roberson P. and Manolagas S. C. (1997) *Endocrinology* **138**, 3666–3676.
66. Takeda K., Noguchi K., Shi W., Tanaka T., Matsumoto M., Yoshida N., Kishimoto T. and Akira S. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 3801–3804.
67. Rothman P., Kreider B., Azam M., Levy D., Wegenka U., Eilers A., Decker T., Horn F., Kashleva H., Ihle J. *et al.* (1994) *Immunity.* **1**, 457–468.
68. Azam M., Erdjument Bromage H., Kreider B.L., Xia M., Quelle F., Basu R., Saris C., Tempst P., Ihle J. N. and Schindler C. (1995) *EMBO J.* **14**, 1402–1411.
69. Azam M., Lee C., Strehlow I. and Schindler C. (1997) *Immunity* **6**, 691–701.
70. van der Bruggen T., Caldenhoven E., Kanters D., Coffer P., Raaijmakers J. A. M., Lammers J. W. J. and Koenderman L. (1995) *Blood* **85**, 1442–1448.
71. Bates M. E., Bertics P. J. and Busse W. W. (1996) *J. Immunol.* **156**, 711–718.
72. Brizzi M. F., Zini M. G., Aronica M. G., Blechman J. M., Yarden Y. and Pegoraro L. (1994) *J. Biol. Chem.* **269**, 31680–31684.
73. Brizzi M. F., Aronica M. G., Rosso A., Bagnara G. P., Yarden Y. and Pegoraro L. (1996) *J. Biol. Chem.* **271**, 3562–3567.
74. Nagata Y. and Todokoro K. (1996) *Biochem. Biophys. Res. Commun.* **221**, 785–789.
75. Welte T., Koch F., Schuler G., Lechner J., Doppler W. and Heufler C. (1997) *Eur. J. Immunol.* **27**, 2737–2740.
76. Aronica M. G., Brizzi M. F., Dentelli P., Rosso A., Yarden Y. and Pegoraro L. (1996) *Oncogene* **13**, 1017–1026.
77. Wang Y.-D., Wong K. and Wood W. I. (1995) *J. Biol. Chem.* **270**, 7021–7024.
78. Lai C. F., Ripperger J., Morella K. K., Wang Y., Gearing D. P., Horseman N. D., Campos S. P., Fey G. H. and Baumann H. (1995) *J. Biol. Chem.* **270**, 23254–23257.
79. Caldenhoven E., Vandijk T. B., Solari R., Armstrong J., Raaijmakers J. A. M., Lammers J. W. J., Koenderman L. and de Groot R. P. (1996) *J. Biol. Chem.* **271**, 13221–13227.
80. Rosen R. L., Winestock K. D., Chen G., Liu X., Hennighausen L. and Finbloom D. S. (1996) *Blood* **88**, 1206–1214.
81. Gouilleux F., Moritz D., Humar M., Moriggl R., Berchtold S. and Groner B. (1995) *Endocrinology* **136**, 5700–5708.
82. Mui A. L., Wakao H., Harada N., O'Farrell A. M. and Miyajima A. (1995) *J. Leukocyte Biol.* **57**, 799–803.
83. Quelle F. W., Shimoda K., Thierfelder W., Fischer C., Kim A., Ruben S. M., Cleveland J. L., Pierce J. H., Keegan A. D., Nelms K., Paul W. E. and Ihle J. N. (1995) *Mol. Cell. Biol.* **15**, 3336–3343.
84. Pallard C., Gouilleux F., Charon M., Groner B., Gisselbrecht S. and Dusanter Fourt I. (1995) *J. Biol. Chem.* **270**, 15942–15945.
85. Moriggl R., Gouilleux Gruart V., Jahne R., Berchtold S., Gartmann C., Liu X., Hennighausen L., Sotiropoulos A., Groner B. and Gouilleux F. (1996) *Mol. Cell. Biol.* **16**, 5691–5700.
86. Raines M. A., Golde D. W., Daeipour M. and Nel A. E. (1992) *Blood* **79**, 3350–3354.
87. Okuda K., Sanghera J. S., Pelech S. L., Kanakura Y., Hallek M., Griffin J. D. and Druker B. J. (1992) *Blood* **79**, 2880–2887.
88. Welham M. J., Duronio V., Sanghera J. S., Pelech S. L. and Schrader J. W. (1992) *J. Immunol.* **149**, 1683–1693.
89. Coffer P., Geijsen N., M'Rabet L., Schweizer R. C., Maikoe T., Raaijmakers J. A. M., Lammers J. W. J. and Koenderman L. (1998) *Biochem. J.* **329**, 121–130.
90. Coffer P., Schweizer R. C., Dubois G. R., Maikoe T., Lammers J. W. J. and Koenderman L. (1998) *Blood* **91**, 2547–2557.

91. Satoh T., Nakafuku M., Miyajima A. and Kaziro Y. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 3314–3318.
92. Satoh T., Uehara Y. and Kaziro Y. (1992) *J. Biol. Chem.* **267**, 2537–2541.
93. Duronio V., Welham M. J., Abraham S., Dryden P. and Schrader J. W. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 1587–1591.
94. Carrol M. P., Clark Lewis I., Rapp U. R. and May W. S. (1990) *J. Biol. Chem.* **265**, 19812–19817.
95. Kanakura Y., Druker B., Wood K. W., Mamon H. J., Okuda K., Roberts T. M. and Griffin J. D. (1991) *Blood* **77**, 243–248.
96. Muszynski K. W., Ruscetti F. W., Heidecker G., Rapp U., Troppmair J., Gooya J. M. and Keller J. R. (1995) *J. Exp. Med.* **181**, 2189–2199.
97. Sato N., Sakamaki K., Terada N., Arai K. and Miyajima A. (1993) *EMBO J.* **12**, 4181–4189.
98. Okuda K., Ernst T. J. and Griffin J. D. (1994) *J. Biol. Chem.* **269**, 24602–24607.
99. Lanfrancone L., Pelicci G., Brizzi M. F., Aronica M. G., Casciari C., Giuli S., Pegoraro L., Pawson T., Pelicci P. G. and Arouica M. G. (1995) *Oncogene* **10**, 907–917.
100. Pratt J. C., Weiss M., Sieff C. A., Shoelson S. E., Burakoff S. J. and Ravichandran K. S. (1996) *J. Biol. Chem.* **271**, 12137–12140.
101. Rozakis Adcock M., Fernley R., Wade J., Pawson T. and Bowtell D. (1993) *Nature* **363**, 83–85.
102. Bone H., Dechert U., Jirik F., Schrader J. W. and Welham M. J. (1997) *J. Biol. Chem.* **272**, 14470–14476.
103. Nahas N., Molski T. F., Fernandez G. A. and Sha'afi R. I. (1996) *Biochem. J.* **318**, 247–253.
104. Yuo A., Okuma E., Kitagawa S. and Takaku F. (1997) *Biochem. Biophys. Res. Commun.* **235**, 42–46.
105. Nagata Y., Moriguchi T., Nishida E. and Todokoro K. (1997) *Blood* **90**, 929–934.
106. de Groot R. P., van Dijk T. B., Caldenhoven E., Coffer P. J., Raaijmakers J. A., Lammers J. W. J. and Koenderman L. (1997) *J. Biol. Chem.* **272**, 2319–2325.
107. Terada K., Kaziro Y. and Satoh T. (1997) *J. Biol. Chem.* **272**, 4544–4548.
108. Nagata Y., Nishida E. and Todokoro K. (1997) *Blood* **89**, 2664–2669.
109. Foltz I. N. and Schrader J. W. (1997) *Blood* **89**, 3092–3096.
110. Liu L., Damen J. E., Ware M. D. and Krystal G. (1997) *J. Biol. Chem.* **272**, 10998–11001.
111. Smith A., Ramos-Morales F., Ashworth A. and Collins M. (1997) *Curr. Biol.* **7**, 893–896.
112. Toker A. and Cantley L. C. (1997) *Nature* **387**, 673–676.
113. Corey S., Eguinoa A., Puyana Theall K., Bolen J. B., Cantley L., Mollinedo F., Jackson T. R., Hawkins P. T. and Stephens L. R. (1993) *EMBO J.* **12**, 2681–2690.
114. Gold M. R., Duronio V., Saxena S. P., Schrader J. W. and Aebersold R. (1994) *J. Biol. Chem.* **269**, 5403–5412.
115. al Shami A., Bourgoin S. G. and Naccache P. H. (1997) *Blood* **89**, 1035–1044.
116. Tilton B., Andjelkovic M., Didichenko S. A., Hemmings B. A. and Thelen M. (1997) *J. Biol. Chem.* **272**, 28096–28101.
117. Songyang Z., Baltimore D., Cantley L. C., Kaplan D. R. and Franke T. F. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 11345–11350.
118. del Peso L., Gonzalez-Garcia M., Page C., Herrera R. and Nunez G. (1997) *Science* **278**, 687–689.
119. Calvo V., Wood M., Gjertson C., Vik T. and Bierer B. E. (1994) *Eur. J. Immunol.* **24**, 2664–2671.
120. Jucker M. and Feldman R. A. (1995) *J. Biol. Chem.* **270**, 27817–27822.
121. Torigoe T., O'Connor R., Santoli D. and Reed J. C. (1992) *Blood* **80**, 617–624.
122. Odai H., Sasaki K., Iwamatsu A., Hanazono Y., Tanaka T., Mitani K., Yazaki Y. and Hirai H. (1995) *J. Biol. Chem.* **270**, 10800–10805.
123. Anderson S. M., Burton E. A. and Koch B. L. (1997) *J. Biol. Chem.* **272**, 739–745.
124. Barber D. L., Mason J. M., Fukazawa T., Reedquist K. A., Druker B. J., Band H. and D'Andrea A. D. (1997) *Blood* **89**, 3166–3174.
125. Chin H., Saito T., Arai A., Yamamoto K., Kamiyama R., Miyasaka N. and Miura O. (1997) *Biochem. Biophys. Res. Commun.* **239**, 412–417.
126. Sattler M., Salgia R., Okuda K., Uemura N., Durstin M. A., Pisick E., Xu G., Li J. L., Prasad K. V. and Griffin J. D. (1996) *Oncogene* **12**, 839–846.
127. Brandt J. E., Bhalla K. and Hoffman R. (1994) *Blood* **83**, 1507–1514.
128. Kinoshita T., Yokota T., Arai K. and Miyajima A. (1995) *EMBO J.* **14**, 266–275.
129. Terada K., Kaziro Y. and Satoh T. (1995) *J. Biol. Chem.* **270**, 27880–27886.
130. Cleveland J. L., Troppmair J., Packham G., Askew D. S., Lloyd P., Gonzalez Garcia M., Nunez G., Ihle J. N. and Rapp U. R. (1994) *Oncogene* **9**, 2217–2226.
131. Kinoshita T., Shirouzu M., Kamiya A., Hashimoto K., Yokoyama S. and Miyajima A. (1997) *Oncogene* **15**, 619–627.
132. Perkins G. R., Marshall C. J. and Collins M. K. L. (1996) *Blood* **87**, 3669–3675.
133. Leverrier Y., Thomas J., Perkins G. R., Mangeney M., Collins M. K. and Marvel J. (1997) *Oncogene* **14**, 425–430.
134. Rinaudo M. S., Su K., Falk L. A., Halder S. and Mufson R. A. (1995) *Blood* **86**, 80–88.
135. Kinoshita T., Yokota T., Arai K. and Miyajima A. (1995) *Oncogene* **10**, 2207–2212.
136. Lin E. Y., Orlofsky A., Berger M. S. and Prystowsky M. B. (1993) *J. Immunol.* **151**, 1979–1988.
137. Sakai I. and Kraft A. S. (1997) *J. Biol. Chem.* **272**, 12350–12358.
138. Lin E. Y., Orlofsky A., Wang H. G., Reed J. C. and Prystowsky M. B. (1996) *Blood* **87**, 983–992.
139. Ito T., Deng X., Carr B. and May W. S. (1997) *J. Biol. Chem.* **272**, 11671–11673.
140. Baffy G., Miyashita T., Williamson J. R. and Reed J. C. (1993) *J. Biol. Chem.* **268**, 6511–6519.
141. Ochiai K., Kagami M., Matsumura R. and Tomioka H. (1997) *Clin. Exp. Immunol.* **107**, 198–204.
142. Gotoh N., Tojo A. and Shibuya M. (1996) *EMBO J.* **15**, 6197–6204.
143. Suzuki J., Kaziro Y. and Koide H. (1997) *Oncogene* **15**, 1689–1697.
144. Marte B. M., Rodriguez-Viciana P., Wennstrom S., Warne P. H. and Downward J. (1997) *Curr. Biol.* **7**, 63–70.
145. Scheid M. P., Lauener R. W. and Duronio V. (1995) *Biochem. J.* **312**, 159–162.
146. Marte B. M. and Downward J. (1997) *Trends Biochem. Sci.* **22**, 355–358.
147. Ahmed N. N., Grimes H. L., Bellacosa A., Chan T. O. and Tsichlis P. N. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 3627–3632.
148. Datta S. R., Dudek H., Tao X., Masters S., Fu H., Gotoh Y. and Greenberg M. E. (1997) *Cell* **91**, 231–241.
149. Harrison-Findik D., Susa M. and Varticovski L. (1995) *Oncogene* **10**, 1385–1391.
150. Coffer P. J. and Koenderman L. (1997) *Immunol. Lett.* **57**, 27–31.
151. Gomez-Cambronero J., Huang C. K., Gomez-Cambronero T. M., Waterman W. H., Becker E. L. and Sha'afi R. I. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 7551–7555.

152. Gomez Cambronero J., Colasanto J. M., Huang C. K. and Sha'afi R. I. (1993) *Biochem. J.* **291**, 211–217.
153. Knall C., Worthen G. S. and Johnson G. L. (1997) *Proc. Natl. Acad. Sci. USA* **94**, 3052–3057.
154. Sue-A-Quan A. K., Fialkow L., Vlahos C. J., Schelm J. A., Grinstein S., Butler J. and Downey G. P. (1997) *J. Cell Physiol.* **172**, 94–108.
155. Vlahos C. J., Matter W. F., Brown R. F., Traynor-Kaplan A. E., Heyworth P. G., Prossnitz E. R., Ye R. D., Marder P., Schelm J. A., Rothfuss K. J. *et al.* (1995) *J. Immunol.* **154**, 2413–2422.
156. Ding J., Vlahos C. J., Liu R., Brown R. F. and Badwey J. A. (1995) *J. Biol. Chem.* **270**, 11684–11691.
157. Didichenko S. A., Tilton B., Hemmings B. A., Ballmer-Hofer K. and Thelen M. (1996) *Curr. Biol.* **6**, 1271–1278.
158. Towatari M., Iida H., Tanimoto M., Iwata H., Hamaguchi M. and Saito H. (1997) *Leukemia* **11**, 479–484.
159. Yi T., Mui A. L., Krystal G. and Ihle J. N. (1993) *Mol. Cell. Biol.* **13**, 7577–7586.
160. Yi T. and Ihle J. N. (1993) *Mol. Cell. Biol.* **13**, 3350–3358.
161. Van Zant G. and Shultz L. (1989) *Exp. Hematol.* **17**, 81–87.
162. Shultz L. D., Schweitzer P. A., Rajan T. V., Yi T., Ihle J. N., Matthews R. J., Thomas M. L. and Beier D. R. (1993) *Cell* **73**, 1445–1454.
163. Tsui H. W., Siminovitch K. A., de Souza L. and Tsui F. W. (1993) *Nat. Genet.* **4**, 124–129.
164. Welham M. J., Dechert U., Leslie K. B., Jirik F. and Schrader J. W. (1994) *J. Biol. Chem.* **269**, 23764–23768.
165. Liu L., Jefferson A. B., Zhang X., Norris F. A., Majerus P. W. and Krystal G. (1996) *J. Biol. Chem.* **271**, 29729–29733.
166. Odai H., Sasaki K., Iwamatsu A., Nakamoto T., Ueno H., Yamagata T., Mitani K., Yazaki Y. and Hirai H. (1997) *Blood* **89**, 2745–2756.
167. Yoshimura A., Ohkubo T., Kiguchi T., Jenkins N. A., Gilbert D. J., Copeland N. G., Hara T. and Miyajima A. (1995) *EMBO J.* **14**, 2816–2826.
168. Matsumoto A., Masuhara M., Mitsui K., Yokouchi M., Ohtsubo M., Misawa H., Miyajima A. and Yoshimura A. (1997) *Blood* **89**, 3148–3154.
169. Starr R., Willson T. A., Viney E. M., Murray L. J. L., Rayner J. R. R., Jenkins B. J., Gonda T. J., Alexander W. S., Metcalf D., Nicola N. A. and Hilton D. J. (1997) *Nature* **387**, 917–921.
170. Endo T. A., Masuhara M., Yokouchi M., Suzuki R., Sakamoto H., Mitsui K., Matsumoto A., Tanimura S., Ohtsubo M., Misawa H., Miyazaki T., Leonor N., Taniguchi T., Fujita T., Kanakura Y., Komiya S. and Yoshimura A. (1997) *Nature* **387**, 921–924.
171. Naka T., Narazaki M., Hirata M., Matsumoto T., Minamoto S., Aono A., Nishimoto N., Kajita T., Taga T., Yoshizaki K., Akira S. and Kishimoto T. (1997) *Nature* **387**, 924–928.
172. Masuhara M., Sakamoto H., Matsumoto A., Suzuki R., Yasukawa H., Mitsui K., Wakioka T., Tanimura S., Sasaki A., Misawa H., Yokouchi M., Ohtsubo M. and Yoshimura A. (1997) *Biochem. Biophys. Res. Commun.* **239**, 439–446.
173. Chung C. D., Liao J., Liu B., Rao X., Jay P., Berta P. and Shuai K. (1997) *Science* **278**, 1803–1805.
174. Kanakura Y., Druker B., Cannistra S. A., Furukawa Y., Torimoto Y. and Griffin J. D. (1990) *Blood* **76**, 706–715.
175. Mire Sluis A., Page L. A., Wadhwa M. and Thorpe R. (1995) *Blood* **86**, 2679–2688.
176. Ofir R., Qing W., Krup M. and Weinstein Y. (1997) *J. Interferon Cytokine Res.* **17**, 279–286.