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Janus Kinases in Cytokine Signalling [and Discussion]

James N. Ihle, P. T. Hawkins and P. J. Parker

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Janus kinases in cytokine signalling

JAMES N. IHLE

Department of Biochemistry, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, Tennessee 38101, U.S.A.

SUMMARY

Hematopoiesis is largely regulated by the binding of cytokines to receptors of the cytokine receptor superfamily. Although lacking catalytic domains, members of the cytokine receptor superfamily mediate ligand dependent activation of tyrosine phosphorylation which is critical for all receptor functions. Recent studies have demonstrated that this is mediated through the association and activation of members of the Janus kinase (Jak) family of protein tyrosine kinases. The activated Jaks phosphorylate the receptors, creating docking sites for SH2 containing signalling proteins which are tyrosine phosphorylated following their association with the receptor complex. Among the substrates of tyrosine phosphorylation are members of the signal transducers and activators of transcription family of proteins (Stats). Various cytokines induce the tyrosine phosphorylation and activation of one or more of the six family members. The pattern of Stat activation provides a level of cytokine individuality that is not observed in the activation of other signalling pathways. Although not required for mitogenic responses, it is speculated that the Stats may mediate many of the cytokine specific functional responses of hematopoietic cells.

1. INTRODUCTION

Hematopoiesis is physiologically regulated through the availability of a variety of cytokines which are variably produced by cells of hematopoietic and non-hematopoietic origins. Functionally, many cytokines mediate comparable effects on cells indicating considerable redundancy which is speculated to allows multiple opportunities for overall regulation. Cytokines are also typically quite pleiotropic and induce quite different responses in cells of different lineages or at different stages of development. This observation is interpreted to indicate that the cellular environment is critical to defining the type of response. To approach the mechanisms accounting for both the redundancy and pleiotropy, it is necessary to understand receptor structures and signalling pathways. Over the past several years the structures for almost all of the cytokine receptors have been elucidated and within the last few years the signalling pathways used have been extensively characterized.

Most of the cytokines that affect hematopoiesis use receptors of the cytokine receptor superfamily. These receptors have conserved extracellular motifs involved in ligand binding (Bazan 1990). The similar structures suggest a common evolution from the structurally related fibronectin type III modules. Depending on the cytokine, the receptors are composed of one to three chains which contribute to ligand binding and/or signal transduction. The cytoplasmic domains have limited similarity in the membrane proximal region of one or more chains.

The first critical ligand induced event is the dimerization–oligomerization of the receptor components. In the case of the single chain receptor for growth hormone this occurs through the ability of the single receptor chain to bind two sites on the ligand (De Vos *et al.* 1992). In cases such as the Epo receptor this may be mediated through ligand dimers. The importance of dimerization is illustrated by the constitutive activation of the Epo receptor by an Arg→Cys mutation which results in constitutive receptor aggregation through the formation of disulphide-linked homodimers (Youssoufian *et al.* 1993). In the case of the IL-6 receptor, the primary function of the ligand binding component is to associate with, and cause the aggregation of, gp130 (Murakami *et al.* 1993) as exemplified by the ability of the soluble complex to activate signal transduction. The importance of dimerization–oligomerization of the cytoplasmic domains of the cytokine receptors has been particularly evident from a variety of studies which have used chimaeric receptors. In these studies various extracellular domains have been used to allow antibody or ligand dependent aggregation of cytoplasmic domains of cytokine receptors.

Although lacking kinase domains, or other catalytic motifs, a variety of studies had indicated that members of the cytokine receptor superfamily couple ligand binding to the induction of protein tyrosine phosphorylation. The importance of tyrosine phosphorylation was initially indicated by the observation that mutations of the receptors, which eliminated functional activity, similarly eliminated the ability to couple ligand binding to induction of the tyrosine phosphorylation. Substrates of ligand-induced tyrosine phosphorylation include one or more of the receptor chains, suggesting the close association of a kinase. Based on these observations it was hypothesized that the cytokine receptor superfamily members as-

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sociate with cytoplasmic kinases which are activated following ligand binding.

2. JANUS KINASES IN CYTOKINE SIGNALLING

The most consistently implicated family of protein tyrosine kinases in cytokine signalling has been the *Janus* protein tyrosine kinase (Jaks). The Jaks were identified during a period when numerous new protein tyrosine protein kinases were being cloned by homology approaches (Ziemiecki *et al.* 1994; Ihle *et al.* 1994; Ihle *et al.* 1995). Their structure is unique in containing a carboxyl-kinase domain and, immediately amino terminal, a psuedokinase domain. The Jaks contain no src homology (SH) domains nor any other previously identified protein motifs. Among the family members there are regions of homology which have been referred to as Jak homology (JH) domains. The mammalian family currently consists of four members, Jak1, Jak2, Jak3 and tyrosine kinase 2 (Tyk2) which vary in size from 120 kDa to 140 kDa. Jak1, Jak2 and Tyk2 are widely expressed whereas Jak3 is primarily expressed in hematopoietic lineages where its levels are affected by T-cell activation (Kawamura *et al.* 1994), macrophage activation (Kawamura *et al.* 1994) and terminal differentiation of granulocytes (Rane & Reddy 1994).

The murine Jaks have been genetically mapped by interspecific hybrids (O. Silvennoinen, N, Jenkins, N. Copleland and J. N. Ihle, unpublished data). *Jak1* gene is very tightly linked to *Pgm2* on chromosome 4, which would correspond to human chromosome band 1p22.1 and be consistent with the human mapping data (Pritchard *et al.* 1992). The *Jak2* gene is genetically linked to *Fas* on chromosome 19 which would correspond to human 10q23-q24.1, although the human gene had been previously mapped to 9p24 (Pritchard *et al.* 1992). The basis for this discrepancy is not known, but may have arisen from using the murine cDNA to localize the human gene in the initial studies. Finally, the *Jak3* gene is located distal of *JunD* in the middle of chromosome 8, a region that has homology with human chromosome 19p13. Interestingly, this would place *JAK3* very near the human *TYK2* gene at 19p13.2 (Firmbach-Kraft *et al.* 1990).

The involvement of Jaks in cytokine signalling has been primarily established by examining the ability of cytokines to induce the tyrosine phosphorylation of specific Jaks and to activate their catalytic activity. Phosphorylation occurs at multiple sites, but includes the tryptic peptide containing the sequence KDYY (Jak3) or KEYY (Jak1,Jak2,Tyk2) which is in the catalytic core and is often associated with increasing catalytic activity (Hanks *et al.* 1988). Consistent with a critical role for tyrosine phosphorylation of one or both Ys, mutation of this sequence to KEFF eliminates catalytic activity (B. Witthuhn and J. N. Ihle, unpublished data).

The abilities of a variety of cytokines to phosphorylate and activate Jaks have been examined. Until now, the known cytokines that use receptors of the cytokine receptor superfamily have been shown to phosphorylate and activate one or more Jaks (it should be noted that only Jak2 is activated as a single Jak). The basis for this is not known, although the possibility exists that Jak2 is uniquely able to 'autophosphorylate' and activate kinase activity. The other Jaks may rely on cross phosphorylation for activation. For example, the activation of Jak1 catalytic activity may rely upon the phosphorylation of the activation site by Jak2 or Tyk2. Evidence to support this possibility is considered below.

In a number of systems, the ability of Jaks to associate with one or more of the receptor chains has also been demonstrated. As seen during examinations, the association specifically occurs with the critical membrane proximal region of the cytoplasmic domain containing the box1/box2 motifs. This was initially demonstrated with the Epo receptor (Witthuhn *et al.* 1993) through the use of receptor truncations and internal deletions. Furthermore, point mutations in the membrane proximal region which eliminate receptor function also eliminate the ability of Jak2 to associate with the receptor (Miura *et al.* 1994*c*). Using GST fusion proteins, Jak2 associates with the receptor independent of activation. However, under appropriate conditions it can be demonstrated that the ability of Jak2 to associate with the receptor increases following ligand binding *in vivo*. The results are consistent with the concept that there is a significant affinity for Jak2/EpoR association, but that this affinity is increased, or the association stabilized, by the receptor aggregation that occurs following ligand binding.

Although the receptor membrane proximal domain is essential for Jak association, the domains of the Jaks are not known. Indeed, experiments with various Jak mutants have failed to define a simple domain that associates with the receptor (Tanner *et al.* 1995) suggesting that multiple, perhaps non-linear, regions are involved. The possibility also exists that other receptor components are required for assembly of a functional receptor complex.

Based on the above observations, the general model proposed for EpoR hypothesizes that the first critical event is receptor aggregation induced by ligand binding. As a consequence of receptor aggregation the associated Jak2 molecules are brought into sufficiently close proximity to cross phosphorylate and activate kinase activity. The activated Jak2 molecules then go on to phosphorylate the receptor as well as substrates that are recruited to the receptor complex by either the phosphorylated receptor or Jak2. Consistent with this hypothesis, mutations that cause the constitutive, ligand independent, aggregation of the Epo receptor also cause constitutive Jak2 activation.

Similar to the Epo receptor, Jak2 associates with the membrane proximal region of the cytoplasmic domain of the prolactin (DaSilva *et al.* 1994) and growth hormone (Goujon *et al.* 1994; VanderKuur *et al.* 1994; Tanner *et al.* 1995) receptors as well as with a comparable region in the G-CSF receptor (K. Shimoda & J. Feng, unpublished data) and gp130 (Tanner *et al.* 1995). In the IL3/GM-CSF/IL5 receptors, association of Jak1 and Jak2 occurs with the membrane proximal region of the shared β_c chain

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(Quelle *et al.* 1994). Importantly, the use of chimaeric receptors has demonstrated that ligand induced aggregation of the β_c cytoplasmic domains is sufficient for a mitogenic response (Eder *et al.* 1994). In contrast, aggregation of the GM-CSF α chain cytoplasmic domain is not capable of initiating a mitogenic response. Therefore it is hypothesized that the α chain may primarily function to increase the ligand binding affinity of the α chain and to facilitate ligand dependent aggregation of β chains.

The family of cytokines including IL6, LIF, OSM, CNTF and IL11 all use gp130 or the related LIFRβ chain as a signal transducing receptor subunit. This is best illustrated in the case of IL6 for which a ligand binding ας chain exist, but which can function without a cytoplasmic domain as a soluble, extracellular ligand binding protein. Thus, as above, the primary function of the IL6/IL6 receptor α complex is to associate with and cause the aggregation of gp130 (Murakami *et al.* 1993). Recent studies have demonstrated that gp130 associates with Jak1, Jak2 and Tyk2 (Narazaki *et al.* 1994; Lutticken *et al.* 1994; Stahl *et al.* 1994). Thus it is hypothesized that gp130 aggregation can bring together and cause the activation of multiple Jaks. Using a series of cell lines, described below, that lack Jak1, Jak2 or Tyk2, the absence of one Jak does not affect the ability of IL6 to induce the tyrosine phosphorylation and activation of the remaining Jaks (Guschin *et al.* 1995). However, downstream signalling only occurs when Jak1 is present; indicating that the activated Jaks are not functionally equivalent. Furthermore, a dominant negative of Jak2 can suppress the activation of both Jak1 and Tyk2. The results are interpreted to indicate that either Jak2 or Tyk2 is required in the receptor complex to activate Jak1 which then is uniquely capable of initiating the down stream signalling events.

Perhaps the most intriguing example of a role for Jak family members in cytokine signalling is in the responses to IFN-α/β and IFN-γ (Velazquez *et al.* 1992; Watling *et al.* 1993; Muller *et al.* 1993). The involvement of Jak family members was established through the use of a series of mutants that were selected for their inability to respond to IFN-α/β or IFN-γ (McKendry *et al.* 1991; John *et al.* 1991; Pellegrini *et al.* 1989). The U1 mutant fails to respond to IFN-α/β while retaining the ability to respond to IFN-γ. Using expression cloning, a gene was identified which restored the IFN response which, when sequenced, was found to be Tyk2 (Velazquez *et al.* 1992). The γ mutants lack IFN-γ responsiveness but retain the ability to respond to IFN-α/β. The IFN-γ responsiveness can be restored by transfecting the cells with Jak2, but not Jak1 or Tyk2, expression constructs (Watling *et al.* 1993). Thus the initial genetic evidence demonstrated that Jak2 is required for an IFN-γ response and Tyk2 is required for an IFN-α/β response. Consistent with this, tyrosine phosphorylation and activation of Jak2 has been observed in the response to IFN-γ (Silvennoinen *et al.* 1993; Shuai *et al.* 1993).

The complexity of the system became more evident from studies with another mutant, U4 (Muller *et al.* 1993). This mutant is unable to respond to either IFN-α/β or IFN-γ, has a truncated Jak1 transcript and lacks serologically detectable Jak1. Responsiveness to either IFN is restored by introducing Jak1, thus demonstrating that both Jak1 and Tyk2 are essential for an IFN-α/β response whereas Jak1 and Jak2 are essential for an IFN-γ response. The requirement for two kinases suggested the existence of a kinase cascade. This possibility is excluded by the observations that whereas IFN-γ stimulation of parental cells induces the tyrosine phosphorylation of both Jak1 and Jak2, no tyrosine phosphorylation of Jak1 is seen in the γ-1 mutant. Conversely, in the U4 mutant, lacking functional Jak1, no Jak2 tyrosine phosphorylation occurs. Reconstitution of either mutant results in the tyrosine phosphorylation of both kinases in response to IFN-γ. Thus both must be functionally present for activation to occur. The situation is identical in the case of IFN-α/β; both Jak1 and Tyk2 must be present to see tyrosine phosphorylation of either. The basis for the interdependence of two Jaks is not known; however, the current data suggest that the ligand induced receptor complex may require Jak heterodimers. In particular, the receptors for IFN-γ and IFN- consist of at least two chains (Soh *et al.* 1994; Novick *et al.* 1994). Thus it can be proposed that individual receptor chains bind Jak1, Jak2 or Tyk2. Consistent with this, the newly cloned chain of the IFN-α/β receptor binds Jak1 (Novick *et al.* 1994). In the case of IFN-γ, co-immunoprecipitation studies indicated that Jak1 is associated with the first chain to be cloned and that following ligand binding, Jak2 became associated with the complex (Igarashi *et al.* 1994). Irrespective, the current hypothesis suggests that ligand binding causes receptor aggregation and may bring Jaks into a heterodimeric complex. Whether the absolute requirement for two Jaks is unique to the IFN receptor systems or extends to other receptor systems in which two Jaks are activated is not currently known.

In the case of the IL2 receptor the heterodimerization of the cytoplasmic domains of both the β and the α chains is required for signalling (Nelson *et al.* 1994; Nakamura *et al.* 1994). The importance of the γ chain is particularly evident from the observation that X-linked severe combined immunodeficiency (X-SCID) is associated with truncations or mutations of this subunit (Noguchi *et al.* 1993). The β chain contains a membrane proximal region that has a typical box1/box2 motif whereas the γ chain does not, but has been suggested to have a sequence that resembles part of an SH2 domain. Using co-expression approaches it was shown that Jak1 specifically associates with the membrane proximal region of the β chain whereas Jak3 specifically associates with the γ chain (Russell *et al.* 1994; Miyazaki *et al.* 1994). Furthermore, point mutations in the γ chain that are associated with X-SCID, also disrupt the ability of the γ chain to associate with Jak3.

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2. ACTIVATION OF MULTIPLE SIGNALLING PATHWAYS BY CYTOKINES

A number of cytokines activate the ras pathway. In the responses to IL-3 and Epo, these include the tyrosine phosphorylation of SHC; its association with GRB2 and SOS; increases in ras bound GTP; activation of raf-1; tyrosine phosphorylation of mitogen activated protein (MAP) kinases; and induction of immediate early genes such as c-fos (Carroll *et al.* 1991; Satoh *et al.* 1991; Sakamaki *et al.* 1992; Sato *et al.* 1993; Cutler *et al.* 1994; Damen *et al.* 1993; Carroll *et al.* 1990). The abilities of various receptor mutants to couple ligand binding to activation of Jaks, activation of elements of the ras pathway and mitogenesis, have been examined. In the response to IL-3, carboxyl truncations of the IL-3 receptor β chain could be identified in which the ability to activate the ras pathway was lost although the ability to activate Jak2 and to induce a mitogenic response were retained (Quelle *et al.* 1994). Similarly, carboxyl-truncations of the Epo receptor uncoupled activation of the ras pathway from activation of Jak2 and mitogenesis (Miura *et al.* 1994). Because the truncations eliminate receptor tyrosine phosphorylation, it can be hypothesized that the inability to activate the ras pathway is associated with the loss of a docking site for the SH2 domain of SHC.

Although most cytokines activate the ras pathway, IL-4 is a notable exception (Satoh *et al.* 1991). However, the IL-4 response is associated with the phosphorylation of a protein that is related to the main substrate of the insulin receptor (IRS-1) (Wang *et al.* 1993*a*). Myeloid cells lacking this protein fail to respond to IL-4 and the ability to respond can be conferred on the cells by introduction of IRS-1 (Wang *et al.* 1993*b*). Recent studies have suggested that, among a variety of IL-4 receptor mutants, there is a correlation between the ability to phosphorylate IRS-1 and to support mitogenesis (Keegan *et al.* 1994). The region required for IRS-1 phosphorylation is membrane distal relative to the box1/box2 motifs and contains a potential tyrosine phosphorylation site with sequence similarity to a site in the insulin and insulin-like growth factor 1 receptors.

Many cytokines induce increases in PI 3-kinase activity (Gold *et al.* 1994; Damen *et al.* 1995; Miura *et al.* 1994*b*; Merida *et al.* 1991; Corey *et al.* 1993; He *et al.* 1993). The mechanism of activation is proposed to involve phosphorylation of a p85 subunit which contains two SH2 domains and functions as an adaptor molecule that targets the catalytic 110 kDa subunit to the activated receptor complex (Otsu *et al.* 1991; Escobedo *et al.* 1991; Hiles *et al.* 1992). Where examined, the phosphorylation of p85 requires the membrane distal region of cytokine receptors. Consistent with this, mutation of the most carboxyl-terminal tyrosine (Y^{505}) of the Epo receptor eliminates the p85 phosphorylation (Damen *et al.* 1995). Therefore it is hypothesized that, in general, receptor phosphorylations create docking sites for p85 and recruit it to the receptor complex. As with SHC it has not been determined whether Jaks or other receptor associated kinases mediate p85 phosphorylation. Importantly, mutation of the carboxyl-terminal tyrosine, while eliminating PI 3-kinase activation, has no detectable effect on proliferation or tyrosine phosphorylation events.

The *VAV* gene encodes a 95 kDa protein that contains an SH2 domain, two SH3 domains as well as other structural motifs including a helix-loop-helix and leucine zipper-like domains, a zinc finger-like domain and regions with homology to GDP-GTP exchange factors. Vav is transiently tyrosine phosphorylated during activation of the T-cell receptor complex (Margolis *et al.* 1992; Bustelo *et al.* 1992), by IL-3 in mast cells (Margolis *et al.* 1992), in B cells by engagement of the immunoglobulin receptors (Bustelo & Barbacid 1992), by IFN α (Platanias & Sweet 1994) and by stem cell factor (Alai *et al.* 1992). Recent studies (Miura *et al.* 1994*a*) have also demonstrated the tyrosine phosphorylation of Vav by Epo and the receptor membrane proximal region is necessary and sufficient for this function (see figure 3). Recent studies (Matsuguchi *et al.* 1995) have further indicated that Vav may directly associate with the phosphorylated Jaks, rather than being recruited into the receptor complex through a physical association with the receptor chains.

3. CYTOKINE ACTIVATION OF SIGNAL TRANSDUCERS AND ACTIVATORS OF TRANSCRIPTION (STATS)

Cytokines also activate members of the signal transducers and activators of transcription (Stat) family, transcription factors initially identified in studies of IFN regulated gene expression (Darnell, Jr *et al.* 1994). Stat1 and Stat2 are inducibly tyrosine phosphorylated in response to IFNα/β. Phosphorylation triggers formation of a complex with a p48 DNA binding protein which translocates to the nucleus and binds the interferon response element (ISRE). In response to IFNγ, Stat1 phosphorylation results in its dimerization (Shuai *et al.* 1994), translocation to the nucleus and binding to a gamma activated sequence (GAS) in IFNγ responsive genes. The utilization of cytokine receptors and Jaks by the IFNs suggested other cytokines might also use Stats. This hypothesis was soon supported by the cloning of the genes for Stat3 (Zhong *et al.* 1994*a*; Akira *et al.* 1994), Stat4 (Yamamoto *et al.* 1994; Zhong *et al.* 1994*b*), Stat5 (Wakao *et al.* 1994) and Stat6 (Hou *et al.* 1994; Quelle *et al.* 1995). Indeed, to date, virtually all cytokines induce the activation of one or more Stat proteins.

The Stats contain a carboxyl SH2 domain, an SH3-like domain and blocks of homology among themselves throughout the amino terminal region. The DNA binding domain has recently been localized to a conserved region in the middle of the protein (Horvath *et al.* 1995). Tyrosine phosphorylation of a carboxyl site mediates homo- or heterodimerization through the SH2 domains which is necessary for nuclear translocation and DNA binding activity. More recently, serine–threonine phosphorylation has also been impli-

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cated in activation which may be mediated by the MAP kinases (Zhang *et al.* 1995; Lutticken *et al.* 1995) thereby linking Stat activation to the activation of the ras pathway.

Cytokine induced Stat tyrosine phosphorylation requires Jak activation. Mutations that eliminate Jak receptor associations eliminate Stat phosphorylation in all cases examined. Furthermore, Jaks can directly phosphorylate purified Stats *in vivo* at the single tyrosine required for activation of DNA binding activity (F. W. Quelle & J. N. Ihle, unpublished results). In the *in vitro* assays, individual Jaks exhibit no detectable specificity for specific Stats indicating that remarkable *in vivo* specificity described below is determined by other factors.

Cytokines typically induce both overlapping and unique biological responses. With the identification of the signalling pathways used, explanations for both redundancy and pleiotropy have become obvious. The diversity in cytokine signalling is illustrated by some of the cytokines that regulate T cells. IL-2, IL-4 and IL-9, all of which use the IL-2 receptor γ chain, activate Jak1 and Jak3, whereas IL-10 and IL-12 activate Jak2 and Tyk2. The diversity is particularly evident in the Stats, with each cytokine activating its own distinguishing pattern of Stats. It can be anticipated that some of the biological differences which these cytokines have on T-cells (Paul & Seder 1994) will be attributable to the constellation of signalling pathways activated. Alternatively, a number of cytokines often have remarkably similar biological activities. Part of this redundancy is associated with the use of common signalling chains. However, some of the similarities can be accounted for by the Stats. For example, G-CSF, a cytokine that promotes granulocyte differentiation, shares a number of properties with IL-6 when examined on the same cells. Much of this redundancy can be ascribed to their common activation of Stat3.

The specificity in Stat activation, relies on recruitment of a specific Stat to the receptor complex. In several cases recruitment is dependent upon Stat SH2 domain recognition of receptor tyrosine phosphorylation docking sites (Stahl *et al.* 1995; Quelle *et al.* 1995). In the above examples, docking sites exist on the IL-2 receptor β chain (Fujii *et al.* 1995), the IL-4 α chain (Quelle *et al.* 1995) and the IL-10 α chain (R. D. Schrieber, unpublished results) for Stat5, Stat6 and Stat3 respectively, Indeed, addition of relatively short peptide sequences, containing a docking site, to a receptor allows Stat recruitment and activation (Stahl *et al.* 1995). Alternatively, switching the SH2 domain of Stats can change their ability to be recruited to specific receptor complexes (Heim *et al.* 1995).

In some cases receptor sites of tyrosine phosphorylation are not required for Stat recruitment and activation such as in the case of growth hormone (Wang & Wood 1995) and erythropoietin (F. W. Quelle and J. N. Ihle, unpublished results). The potential complexity of Stat recruitment is illustrated (Leung *et al.* 1995) by the requirement for Stat2 to recruit and phosphorylate Stat1 in the response to IFNα/β. In this case it is hypothesized that Stat2 is first recruited to the receptor complex and phosphorylated. It then provides the docking sites for recruitment of Stat1. Such a model is particularly interesting because it would favour the formation a Stat1/Stat2 heterodimer.

A variety of genes are known to be regulated by Stats. Numerous genes have been identified in IFN responses (Darnell, Jr *et al.* 1994). Similarly, Stat3 is required for the expression of a subset of the genes, the acute phase response genes, induced by IL-6 (Kishimoto *et al.* 1994). Stat5 mediates prolactin induced transcription of a number of mammary gland proteins secreted in milk (Wakao *et al.* 1994). Lastly, Stat6 binds to an element in the immunoglobulin locus that is required for IL-4 induced class switching (Coffman *et al.* 1993; Rothman *et al.* 1991) and mediates IL-4 upregulation of the major histocompatibility locus class II antigen, various immunoglobin receptors and other cell surface proteins.

A common property of the Stat regulated genes is their involvement in functional rather than mitogenic responses. The lack of involvement of the Stats in mitogenic responses is further suggested by other observations. First, IFNs do not generally induce proliferation and, indeed, are more characterized by their antiproliferative properties (Pestka *et al.* 1987). More directly, there are receptor mutations that uncouple activation of Stat3, Stat5, and Stat6 from IL-6 (Stahl *et al.* 1995), IL-2 (Fujii *et al.* 1995) and IL-4 (Quelle *et al.* 1995) induced cell proliferation, respectively.

4. CONCLUSIONS AND PERSPECTIVES

Considerable data now leads to the conclusion that the Jaks play a central role in cytokine signalling. For this reason it will be particularly interesting to observe the phenotypes of mice in which individual Jaks are disrupted by homologous recombination. It can be anticipated that this information will be available in the very near future. Although the data suggest a critical role, relatively little is known regarding the functional domains of the Jaks. Based on research with other kinases, it can be anticipated that knowledge of the multiple phosphorylation sites will be important. The role of the pseudokinase domain is of particular interest and little is known about the Jak domains involved in binding to cytokine receptors. Finally, it can be hypothesized that the Jaks, perhaps through unique domains or sites of tyrosine phosphorylation, activate novel signalling pathways.

Receptor mutants have been particularly important for assessing the importance of activation of various signalling pathways in the functional responses to cytokines. Somewhat surprisingly, they raise a question about the role of activation of the ras pathway in the response to cytokines. It can be anticipated that, as more functional responses are defined and examined, many of these will require ras activation. Similarly, the functional consequences of p85, PLC-γ1 and Vav need to be further explored.

The Stats provide an important addition to the pathways activated. The results clearly demonstrate the absence of a role for Stat activation in mitogenesis;

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suggesting a role in functional responses. As with the Jaks, it can be anticipated that the phenotype of mice in which individual Stats are disrupted will begin to help to define their individual roles in various cell lineages. As with the Jaks, it can be anticipated that such mice will be available in the very near future. Like the Jaks however, much remains to be determined regarding Stat structure and function. The remarkable effects that the tyrosine phosphorylation of a single site can have on cellular localization and DNA binding activity are poorly understood. Also, relatively little is known regarding the basis for DNA binding. Irrespective of this, the successes of the last couple of years have provided the well from which it can be anticipated that a number of new and exciting concepts will be drawn.

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Discussion

P. T. Hawkins (*The Babraham Institute, Cambridge, U.K.*). How does Professor Ihle envisage that the activated STATs get into the nucleus?

J. N. Ihle. This is one of the most interesting unanswered questions. There is obviously a need for studies to determine whether there are particular domains in the STATs necessary for nuclear translocation, but that evidence is not yet available. Evidence from Chris Shendler shows that STAT 1 is diffusely distributed in the cytoplasm before IFN stimulation, but that after stimulation STAT 1 quickly localizes in the nucleus.

P. J. Parker (*Imperial Cancer Research Fund, London, U.K.*). When deletions are made that block STAT signalling, does this happen because the STAT is incapable of being activated or because it is not properly recruited by the receptors? If domains are switched between STATs, can receptor specificity be switched?

J. N. Ihle. This has been done very nicely by George Yancopolous. If the STAT3 phosphotyrosine recognition site of gp130 is incorporated into the erythropoietin receptor (which does not normally activate STAT3), then the erythropoietin receptor has an ability to activate STAT3.

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