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JAKs, STATs and Signal Transduction in Response to the Interferons and other Cytokines [and Discussion]

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JAKs, STATs and signal transduction in response to the interferons and other cytokines

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SUMMARY

The isolation and complementation of mutant human cell lines has established an essential role for the JAK (Janus kinase) family of protein tyrosine kinases and STAT (signal transduction and transcription) factors in the Interferon response pathways. Activation of STATs by JAKs occurs in receptor complexes at the cell membrane. Activated STATs form homo- or heterodimers and, with or without additional factors, migrate to the nucleus to initiate transcription. Different STAT combinations interact differentially with related DNA response elements. Signalling pathways of this novel type are likely utilized by a wide variety of polypeptide ligands. Data from the IL2, IL6 and IFN systems indicate a major role for the tyrosine phosphorylated receptor/JAK complexes (rather than substrate specificity of the JAKs *per se*) in STAT selection. The mutant cell lines lacking individual JAKs and STATs are being used together with kinase-negative JAK mutants which differentially affect the IFN-γ, and IFN-αβ and IL-6 pathways in the further analysis of these and additional systems.

1. INTRODUCTION

Over the last few years work on the interferons (IFNs) has established that they signal though a novel type of signal transduction pathway involving activation of transcription factors at the cell membrane. Activation of these factors now known as STATs (signal transducers and activators of transcription) is by the JAK (Janus kinase) family of protein tyrosine kinases. It is becoming increasingly clear that JAKs, STATs and similar pathways are utilized by a wide variety of cytokines and growth factors (Darnell *et al.* 1994; Ihle & Kerr 1995; Ihle 1995; Larner & Finbloom 1995; Schindler & Darnell 1995). Here recent developments in our knowledge of the IFN response pathways will be briefly reviewed, the use of mutant cell lines defective in the JAKs and STATs in the analysis of these and other pathways will be described as will some contrasting results obtained with kinase-negative mutants of JAK1 and JAK2 in the dissection of the IFN and IL-6 responses. (References to original work are included only for our own data actually presented in this lecture. For general and background information the reader is referred to the many recent reviews on the JAK/STAT pathways a selection of which are also listed.)

The IFNs, first discovered as antiviral agents, can inhibit cell proliferation, promote differentiation and affect cell function particularly in relation to the immune response. There are three major antigenic types of human IFN α, β and γ. The αβ (Type I) IFNs are thought to share the same cell membrane receptor(s) and induce the same or a very similar set of polypeptides whereas IFN-γ (Type II) has a separate receptor and induces a distinct but overlapping set of polypetides. The IFNs induce upwards of 30 polypeptides, the exact number is not known, and down regulate others. Induction is transient and is followed, to a variable degree in different cell types, by a refractory state. Over the years a number of 'classical' second message pathways have been reported to play a role in the IFN responses but no consistent picture has emerged. Their role in modulating or complementing the response through the JAK/STAT pathway remains to be established.

Against this background there have been four major recent developments which have contributed substantially to our understanding of the IFN response. First, the cloning of IFN-inducible genes and the identification in their promoters of DNA response elements both necessary and sufficient to confer a response. Second, the identification and cloning of transcription factors interacting with these elements. Third, the isolation and complementation of mutants in the signal transduction pathways which, in particular, established a role for the JAKs. Fourth, an ongoing process, the identification and cloning of

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receptor subunits. These developments have led to the following models for the IFN response pathways. For IFN-γ two receptor subunits have been cloned, the IFN-binding or α subunit and an accessory factor (AF1) or β subunit. It remains to be established whether more than one accessory factor is involved. Ligand binding triggers receptor dimerization/oligomerization. Tyrosine phosphorylation and activation of JAK1 and JAK2, the receptor and STAT1 all occur in the receptor complex at the cell membrane. Activated STAT1 is released/homodimerizes and, with or without additional factors, migrates to the nucleus to activate transcription through GAS (gamma activation sequence, consensus TTNCNNNAA) and related DNA response elements. The IFN-αβ receptor is less well defined, but again consists of at least two subunits which have been cloned. In this case receptor dimerization/oligomerization leads to the phosphorylation/activation of the receptor, Tyk2 and JAK1 and STATs 1 and 2. STATs 1 and 2 are released/heterodimerize to form ISGF3α which combines with pre-existing ISGF3γ to form ISGF3 which migrates to the nucleus to initiate transcription through ISREs (interferon stimulable response elements – consensus GGAAANNGAAACT).

2. JAKs AND STATs

The JAK family of non-receptor protein tyrosine kinases has four mammalian members JAK1, JAK2, JAK3 and Tyk2 and a Drosophila homologue 'hopscotch'.

JAK1, JAK2 and Tyk2 appear to be universally expressed whereas the expression of JAK3 appears restricted to cells of the immune system. Each is approximately 130 kDa and has a C-terminal protein kinase domain, an adjacent kinase-like domain and five further domains with amino acid similarity within the family extending to the N-terminus. It is reasonable to assume that these additional domains mediate the protein/protein interactions governing the multiple roles of the JAKs in different signal transduction pathways. Seven STATs (1 to 6 including 5A and B) have been cloned. They range from about 80 to 110 kDa. Each has an SH2 and SH3 group. Activation involves phosphorylation of a conserved tyrosine just C-terminal to the SH2 domain towards the C-terminus of the molecule. Homo- or heterodimerization of the STATs, with or without additional polypeptides, yields a spectrum of transcription factors with differing affinities for the family of response elements. Interestingly, although phosphorylation of the above tyrosine is essential for STAT activation phosphorylation on one or more serines appears also to be required for a full transcriptional response.

(*a*) *Mutants in the interferon response pathways*

IFN-inducible promoters driving drug-selectable or cell-surface markers have been used to obtain and complement mutants in the IFN-αβ and -γ response pathways (Pellegrini *et al.* 1989). Recessive mutants in eight complementation groups which affect one or other or both of the major IFN response pathways have been isolated. All have now been complemented. The complementing genes include JAK1, JAK2 and Tyk2, STATs 1 and 2 and ISGF3γ and subunits of the IFN-αβ and -γ receptors (Table 1). In addition to identifying the role of the JAKs, in particular, and the essential nature of all of these components in the IFN response pathways, the mutants are being similarly used in the analysis of the responses to other cytokines and growth factors and are providing a negative background for structure function analysis of the complementing gene products. Some examples are given below.

Table 1. *Interferon response mutants*

	interferon			
	α	β	γ	complemented by
U1	–	P	+	TYK2
U2	–	–	+and–	ISGF3γ (p48)
U3	–	–	–	STAT1 α/β
U4	–	–	–	JAK1
U5	–	–	+	β-subunit IFN-αβ receptor
U6	–	–	+	STAT2 (p113)
γ1	+	+	–	β-subunit IFN-γ receptor JAK2
γ2	+	+	–	JAK2

(*b*) *STAT1 activation in response to Type I IFNs is dependent on STAT2*

STAT 2 is activated in response to IFN-αβ in mutant U3A cells which completely lack STAT1. In contrast, work in the Stark & Darnell labs has shown that STAT 1 is not activated in U6A cells which completely lack STAT2 (Leung *et al.* 1995). The simplest interpretation of the data and current working model is that the recruitment/activation of STAT1 is dependent on the prior recruitment/activation of STAT 2. Additional domain swap experiments have established that in the IFN-γ response the recruitment of STAT1 to the receptor is dependent on the SH2 domain (Heim *et al.* 1995; Qureshi *et al.* 1995).

(*c*) *Kinase-negative JAKs as dominant negative inhibitors*

Kinase-negative mutants of JAK1 and JAK2 can act as dominant negative inhibitors of the IFN-αβ and IL-6 responses (Briscoe *et al.* 1995, Guschin *et al* 1995). Two kinase-negative mutants of JAK1 have been used - in the first (JAK1.KE) a lysine in motif II of the kinase domain was mutated to glutamic acid and in the second (JAK1.SFG) the aspartic acid in the highly conserved DFG motif VII of the kinase domain was changed to a serine. These mutant JAKs were first shown to be without protein tyrosine kinase activity in *in vitro* kinase assays and on over expression in the

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baculovirus system. Consistent with this when introduced into mutant U4A cells, which completely lack JAK1, they failed to restore IFN-αβ or IL-6 responses. Indeed when introduced into wild type cells they can, when overexpressed, function as dominant negative inhibitors of these responses. For comparison the effects of a corresponding mutant of JAK2, JAK2.KE, were also assayed in JAK2 negative γ2A and wild-type cells.

(*d*) *A kinase-negative JAK1 can sustain IFN-γ-inducible gene expression*

In contrast to their dominant negative effects on the IFN-αβ and IL-6 responses the kinase-negative JAK1.KE and JAK1.SFG mutants, when introduced into JAK1-negative U4A cells, can sustain substantial IFN-γ-inducible gene expression. The same is not true for kinase-negative JAK2 when expressed in JAK2-negative γ2A cells. These data provide evidence for a structural as well as an enzymic role for JAK1 in the IFN-γ response. In addition, a more detailed analysis has revealed interesting differences in the ability of the mutant JAK1 and JAK2 to support different steps in the JAK/STAT pathway in response to IFN-γ. For example, with kinase-negative JAK2 there is no detectable activation of JAK1. In contrast, with kinase-negative JAK1, JAK2 activation approaches wild type levels but receptor phosphorylation is greatly reduced, suggesting that this latter function may normally be carried out by JAK1. This and a great deal of additional work from a number of groups has yielded the following working model for the JAK/STAT pathway in response to IFN-γ: On ligand binding and receptor dimerization/oligomerization JAK2 auto-phosphorylates and phosphorylates JAK1. JAK1 phosphorylates tyrosine 440 of the a subunit of the receptor which in turn leads to the recruitment of STAT1 through its SH2 group, its phosphorylation, release and dimerization to form an active transcription factor. The transcriptional activity of this factor is also subject to modulation by the phosphorylation of STAT1 on serine 729 by an as yet unidentified kinase(s) and by interaction with additional factors to modulate interaction with different DNA response elements. These latter points emphasize that the JAK/STAT pathway does not operate in isolation and it is likely that we still have much to learn concerning its modulation by cross talk with different pathways and in different cell types.

(*e*) *The kinase-negative JAK1 mutants do not sustain an antiviral response to IFN-γ*

Despite their ability to sustain substantial IFN-γ-inducible gene expression the kinase-negative JAK1 mutants do not sustain an IFN-γ-mediated antiviral response to encephalomyocarditis or Semliki Forest viruses. In addition, when overexpressed in wild type cells, kinase-negative JAK1 mutants, although without detectable effect on the activation of the JAK/STAT pathway or gene expression in response to IFN-γ, profoundly inhibit the development of an antiviral state. In contrast, kinase-negative JAK2 has a dominant negative inhibitory effect on gene expression but a much less profound effect on the antiviral state. The data from U3A cells, which lack STAT1, established that STAT1 is essential for the antiviral response. The data here indicate that although activation of STAT1 is essential for the antiviral response to IFN-γ it is not sufficient. It appears that a second JAK1 dependent signal is required (Briscoe *et al.* 1995). STATs may not, therefore, be the only targets of JAK activation.

Table 2. *Phosphorylation/activation of JAKs and STATs in response to cytokines and growth factors*

ligand	JAK	STAT
IFN-αβ	JAK1 Tyk2	STAT1 STAT2 STAT3
IFN-γ	JAK1 JAk2	STAT1 STAT3
IL-10	JAK1 TyK2	STAT1 STAT3
IL-6	JAK1 JAK2 Tyk2	STAT1 STAT3
LIF	JAK1 JAK2 Tyk2	STAT1 STAT3
oncostatin M	JAK1 JAK2 Tyk2	STAT1 STAT3
CNTF	JAK1 JAK2 Tyk2	STAT1 STAT3
IL2	JAK1 JAK3	STAT5
IL4	JAK1 JAK3	STAT6
IL7	JAK1 JAK3	STAT5
IL9	JAK1 JAK3 Tyk2	STAT1 STATX
IL13	JAK1 JAK3 Tyk2	
IL15	JAK1 JAK3	
IL3	JAK2	STAT5
IL5	JAK2	STAT5
GMCSF	JAK2	STAT5
IL12	Tyk2	STAT4
erythropoietin	JAK2	STAT5
prolactin	JAK2	STAT5
growth hormone	JAK2	STAT1 STAT3
GCSF	JAK1 JAK2	STAT1 STAT3
thrombopoietin	JAK1 Tyk2	STAT1 STAT3 STAT5
EGF	JAK1	STAT1 STAT3
PDGF	JAK1 JAK2 Tyk2	STAT1 STAT3
CSF1		STAT1 STAT5
angiotensin	JAK2 Tyk2	STAT1 STAT2

(*f*) *Activation of JAKs and STATs in response other ligands*

In addition to their role in the IFN response pathways the JAKs and STATs are phosphorylated/activated in response to a wide variety of other cytokines and growth factors (Table 2). The evidence for involvement in the different response pathways varies from the IFNs, for which the the mutants in the response pathways have established an essential role for the JAKs and STATs in all aspects of the responses assayed to date, through those ligands for which there is evidence (in addition to tyrosine phosphorylation) for association of the JAKs and STATs with the receptor, to those for which the evidence is no more than the demonstration of tyrosine phosphorylation of an immunologically identified product in response to the ligand. In addition, there may well be complexity in the JAK/STAT response. JAKs may target alternative substrates and STATs may be activated by alternative kinases. Our objective has been to use the

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mutant cell lines lacking the individual JAKs and STATs in the further analysis of these systems. Examples include their use in the analysis of the IL-6 and EGF responses.

(*g*) *A major role for JAK1 in the IL-6 response*

A number of groups have reported the activation of JAK1, JAK2, Tyk2 and STATs1 and 3 in response to IL-6. This is also the case in the parental cells with which we are working. In the mutant cells the absence of one kinase does not prevent the activation of the other two: activation does not, therefore, involve a sequential three kinase cascade. In the absence of JAK1, however, the phosphorylation of the gp130 subunit of the IL-6 receptor and the activation of STATs 1 and 3 are greatly reduced. JAK1 is also necessary for the induction of IRF-1 mRNA establishing a requirement for the JAK/STAT pathway in the IL-6 response. JAK2 and Tyk2, although activated, cannot, in the absence of JAK1, efficiently mediate activation of STATs 1 and 3. A major role for JAK1 and the non equivalence of the JAKs in the IL-6 response pathway has, therefore, been clearly established for these cells (Guschin *et al.* 1995).

(*h*) *JAKs and STATs in response to EGF*

Two years ago a number of groups reported the activation of JAK1 and STATs 1 and 3 in response to EGF an PDGF. Subsequent work in the Stark lab has established that activation of STATs 1 and 3 is retained in the mutant cells lacking JAK1 (or JAK2 or Tyk2). It would appear, therefore, that in this system STAT activation occurs independently of the JAKs: whether it is through the EGF receptor kinase a src family or alternative kinase remains to be rigorously established (Leaman *et al.* 1995). This leaves us with the interesting question of the role of the activated JAK1 in this system to which, for the moment, we have no answer. It also raises more general questions concerning what governs STAT activation.

(*i*) *STAT activation*

In general, it appears that STAT activation occurs in receptor complexes at the cell membrane. The basis for specific STAT selection appears, however, to be diverse. It is not readily attributable simply to substrate specificity of the JAKs or, for instance, to phosphotyrosine containing amino acid motifs in the different receptors, although these latter are of undoubted importance in some instances at least. For example, in the IL-2 system there is no activation of STAT1 despite good activation of JAK1, whereas in the IL-6 system STAT1 activation is dependent on JAK1. In response to the IFNs JAK2 or Tyk2 can, under some circumstances at least, activate STAT1. In contrast, with EGF the JAKs do not appear to be required for STAT activation. At the IFN-α receptor STAT1 activation is dependent on STAT2. At the IFN-γ receptor STAT1 is likely recruited through a receptor phosphotyrosine motif whereas with growth hormone, for example, STAT activation has been reported to be independent of receptor tyrosine phosphorylation. Much of this is recent work and not all of it may stand the test of time, but for the moment the most logical conclusion would appear to be that STAT selection/activation is governed in different ways determined by the individual receptor complex involved.

(*j*) *Recent developments*

Recent developments of interest include: The modulation of STAT activity by serine phosphorylation, which emphasizes the likely importance of cross talk between the JAK/STAT and other response pathways. The activation of IRS1 and 2 by a number of cytokines and growth factors. STAT activation in response to angiotensin with the implication of the possible involvement of JAK/STAT pathways downstream of the seven transmembrane receptor family. Finally, the indication (from the requirement for an additional JAK1 dependent signal for the antiviral response to IFN-γ) that the STATs may not be the only targets of JAK activation. Clearly there is much yet to do.

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Discussion

M. Karin (*Department of Pharmacology, Univeristy of California, San Diego, U.S.A.*). Why does Dr Kerr not get STAT1 homodimers in cells treated with IFNα/β, and is there any activtion of the IFNγ-regulated genes?

I. M. Kerr. You do get STAT1 homodimers. Some of IFNγ-regulatable genes are switched on, but the details of their regulation seem to be getting more complex at present, rather than being simplified.

Question. There has been a recent report that angiotensin II activates a JAK/STAT pathway. How does Dr Kerr think that happens?

I. M. Kerr. I don't know, and I think it even remains to be seen whether this is a primary response.

P. J. Parker (*Imperial Cancer Research Fund, London, U.K.*). Does anything control the association of ISGF3γ with the STAT 1 homodimer?

I. M. Kerr. The basis for that interaction is not yet known. As Jim Ihle emphasized, it is proving remarkably difficult to undertake domain structure analyses of the JAKs and STATs, so the definition of functional motifs is still largely in the future.

C. J. Marshall (*Chester Beatty Laboratory, Institute of Cancer Research, London, U.K.*). What does Dr Kerr think is the role of the JAK pathway in controlling cell proliferation? The evidence that JAKs are involved in cytokine stimulation of proliferation seems to come mainly from studies of mutant receptors that do not bind JAKs, and which could simply be disrupting the function of the receptors. Dr Kerr showed studies of cells transfected with dominant negative JAK I which still grew, which seems to suggest that cells do not necessarily require JAKs for proliferation?

J. N. Ihle (*Department of Biochemistry, St Jude Children's Research Hospital, Memphis, U.S.A.*). This is not always the case. For example, over-expression of dominant negative JAK2 can sometimes inhibit proliferative responses to erythropoietin.

I. M. Kerr. We must remember that all of these experiments are being done in transformed cells, in which, for example, we cannot study the growth inhibitory effects of interferons. These cells either grow or die; we cannot get them into G0. So I am not sure that there is a fundamental problem, as the cell systems used so far cannot answer the questions properly.

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