

STATS: TRANSCRIPTIONAL CONTROL AND BIOLOGICAL IMPACT

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Extracellular proteins bound to cell-surface receptors can change nuclear gene expression patterns in minutes, with far-reaching consequences for development, cell growth and homeostasis. The signal transducer and activator of transcription (STAT) proteins are among the most well studied of the latent cytoplasmic signal-dependent transcription-factor pathways. In addition to several roles in normal cell decisions, dysregulation of STAT function contributes to human disease, making the study of these proteins an important topic of current research.

SIGNALLING

SH2 DOMAIN
(Src-homology-2 domain). A protein motif that recognizes and binds tyrosine-phosphorylated sequences, and thereby has a key role in relaying cascades of signal transduction.

This year marks the tenth anniversary of the recognition of the STAT proteins, named after their dual role as signal transducers and activators of transcription^{1–4}. These transcription factors are latent in the cytoplasm until they are activated by extracellular signalling proteins (mainly cytokines and growth factors, but also some peptides) that bind to specific cell-surface receptors. These extracellular-signalling proteins can activate various tyrosine kinases in the cell that phosphorylate STAT proteins. The activated STAT proteins accumulate in the nucleus to drive transcription. The duration and degree of gene activation are under strict regulation by a series of negatively acting proteins (FIGS 1,2). This review concentrates on recent progress in studying these sets of proteins, highlighting important issues in which recent progress has been made or that still remain unresolved.

Pathways leading to STAT activation

As is the case for **STAT proteins 1 and 2**, which were discovered as targets of interferon activation⁴, all the STAT proteins can be activated after one or more cytokines interact with their cognate receptors (BOX 1)^{5,6}. The basic model for these cytokine pathways depends on a series of three tyrosine phosphorylations that are carried out by Janus kinase (**JAK**) proteins that are non-covalently bound to specific receptors (FIG. 1). Receptor dimerization or oligomerization leads to JAK apposition

and transphosphorylation on tyrosine residues, releasing their intrinsic catalytic activity. Tyrosine phosphorylation by activated JAKs of cytokine-receptor cytoplasmic domains then provides binding sites for the Src-homology-2 (**SH2**) DOMAIN of the STAT proteins. The STAT proteins are then recruited to the JAKs, whereupon they are phosphorylated on a single tyrosine residue (around residue 700 of their 750–850-amino-acid sequence). Although the interactions and consequences of STAT binding to JAKs are the best studied, **STAT1**, **STAT3** and **STAT5** at least can also be activated by other receptor and tyrosine-kinase interactions (BOX 1).

Regardless of how STATs are tyrosine phosphorylated, it is clear that STAT–STAT interaction occurs immediately through reciprocal phosphotyrosine–**SH2** interactions⁷. **STAT1**, **STAT3**, **STAT4**, **STAT5A** and **STAT5B** all form homodimers. **STAT1** and **STAT2**, and **STAT1** and **STAT3** can also form heterodimers, depending on the nature and concentration of the activating ligand. *In vitro* tyrosine phosphorylation is accompanied by quantitative dimer formation⁸, and there are no reports of monomeric tyrosine-phosphorylated STAT proteins. It seems possible, if not probable, that the dimeric (or higher-order) nature of the activating receptor is accompanied by near-simultaneous activation of two STAT molecules — one at each receptor in a complex — followed⁹ by STAT **SH2**–phosphotyrosine-mediated

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doi:10.1038/nrm909

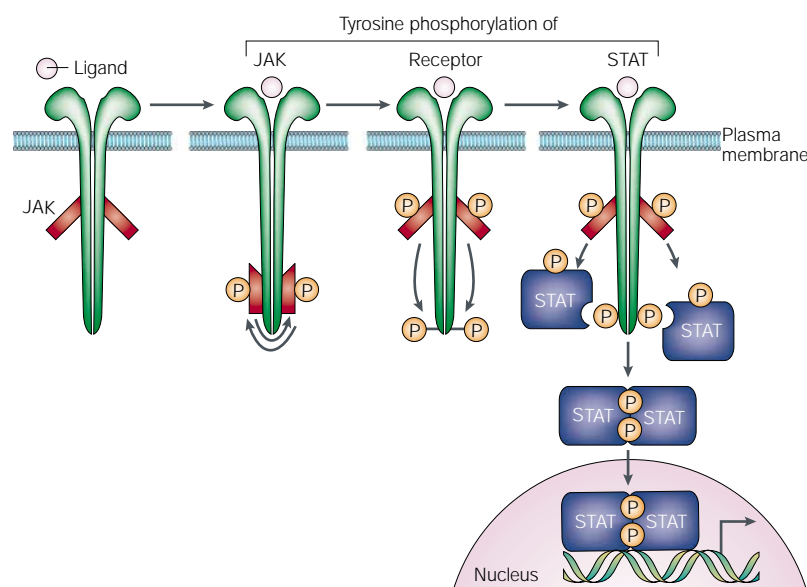


Figure 1 | Canonical JAK–STAT pathway. Sequential tyrosine phosphorylations triggered by cytokine–receptor interaction. Receptor dimerization allows transphosphorylation and activation of Janus kinases (JAKs). This is followed by phosphorylation of receptor tails and the recruitment of the signal transducers and activators of transcription (STAT) proteins through their Src-homology-2 domains. STAT tyrosine phosphorylation then occurs. Dimerization of activated (tyrosine phosphorylated) STAT is followed by nuclear entry.

dimerization as a means of disengagement from the receptor or kinase¹⁰.

JAKs

JAKs are characterized by a carboxy-terminal catalytic domain and a related, but enzymatically inactive, adjacent pseudo-kinase or kinase-like domain^{5,6,11}. They also share five additional blocks of sequence similarity throughout the amino-terminal region (FIG. 2). These sequence features define four members of the JAK family (TABLE 1). The draft sequences of the human and mouse genomes indicate that there are no other JAKs. Gene targeting in mice has shown that each enzyme has mostly non-redundant functions that can be largely — if not completely — explained as features of cytokine signalling (TABLE 1).

No JAK structure is yet available but the existing evidence indicates that this is a fruitful area for further study. Their kinase domains, in addition to an obvious role in catalysis, are also selective targets for inhibition by the suppressor of cytokine signalling (SOCS) proteins (see below). Moreover, the pseudo-kinase domains also seem to regulate catalysis negatively and might have a role in substrate recognition. Mutations in this region, at least in *Drosophila melanogaster*, can lead to **leukaemia**-like cell-proliferation diseases as a result of unopposed kinase activity^{12,13}. Sequence-based structure predictions have suggested that these proteins have an SH2 domain, although the function of this domain remains unclear. In fact, the conserved arginine that is found in all SH2 domains — which hydrogen bonds to phosphotyrosine residues in interacting proteins — is a histidine in tyrosine kinase 2 (TYK2) and has been mutated to an alanine in JAK1 without consequence. This

raises the interesting possibility that this domain is a binding site for something other than phosphotyrosine that might regulate JAK activity.

The more proximal amino-terminal regions of JAK proteins are directly involved in selective interactions with cytokine receptors. In addition, as was first shown for the interferon α (IFN- α) receptor but has now been documented for several others, this interaction contributes a CHAPERONE function that facilitates the expression of the receptor–kinase complex at the cell surface¹⁴. Interactions between some of these amino-terminal domains and the catalytic domain might also function in the regulation of enzymatic activity. Understanding how these long-distance interactions function at a mechanistic level, however, will require a JAK structure to be determined at atomic resolution.

Unphosphorylated cytoplasmic STAT proteins

The molecular state of the STATs in the cytoplasm before activation is not fully understood, and several possibilities have been described that need further exploration. There are clearly proteins that can bind STATs in the cytoplasm, although physiological roles for these interactions have yet to be detailed. STAT-interacting partners that might facilitate recruitment to receptors for enhanced signalling have been identified^{15,16}. One such protein is also a component of a RNA-polymerase-II elongation complex and so might also facilitate STAT-dependent transcriptional activity after nuclear translocation¹⁷. Furthermore, the dimerization of unphosphorylated STATs in the cytoplasm^{5,6}, possibly in association with additional cellular proteins^{18,19}, has also been reported and could be a common state for unphosphorylated STAT1 and STAT3. On the basis of the crystal structure of phosphorylated²⁰ and unphosphorylated STAT1, these complexes might not have the same structure as the dimers that bind DNA (X. Chen *et al.*, unpublished observations). STAT3 in HEPATOMA cells has been reported to exist mainly in association with large protein aggregates²¹. Such complexes could provide a mechanism for sequestering STAT proteins before activation or be a platform that allows efficient access to plasma-membrane receptors.

Effects of crosstalk on STAT activation

The extent of STAT activation can be altered by prior exposure of cells to other stimuli that bind other receptors, commonly known as crosstalk. Because cells in the body often produce more than one cytokine or growth factor, crosstalk probably represents a physiologically important control for STAT activity.

Positive crosstalk. A well-defined example of positive crosstalk is the effect of type-I IFN (α and β) on signalling from the type-II IFN (γ) receptor and vice versa, and on receptors of the interleukin-6/glycoprotein-130 (IL-6/gp130) family. For example, pretreatment of cells with IFN- γ strongly augments a subsequent IFN- α response⁴. This crosstalk can be at least partly explained by the increase in abundance of interferon regulatory factor 9 (IRF9; formerly called p48), the product of an

CHAPERONE

Protein that mediates polypeptide folding or the assembly of another polypeptide-containing structure but does not form part of the completed structure or participate in its biological function.

HEPATOMA

Cancer of the liver.

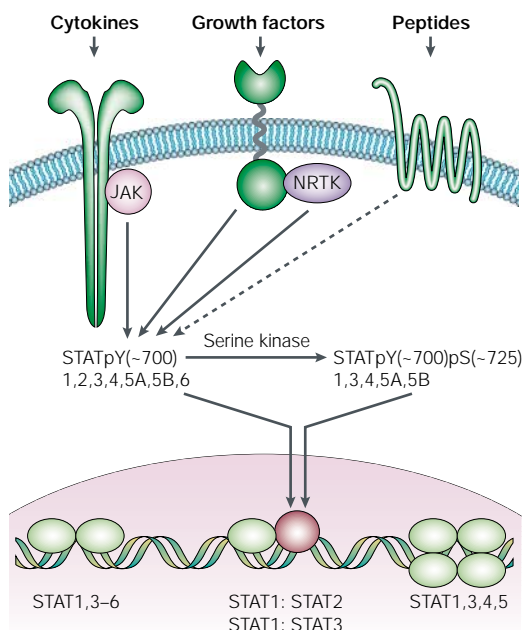
INNATE IMMUNE RESPONSE

This is crucial during the early phase of host defence against infection by pathogens (such as bacteria and viruses), before the antigen-specific, adaptive immune response is induced.

Box 1 | Variations in mechanisms of STAT activation

Tyrosine phosphorylation of signal transducers and activators of transcription (STAT) proteins at or around residue 700 occurs in response to cytokine receptors through Janus kinases (JAKs). However, at least several dozen receptors with intrinsic tyrosine kinase activity (RTKs), such as those for epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), seem to be able to mediate the activation of STAT proteins^{148–151}. Apparently, this activation can be direct (as in the case of STAT1 activation by PDGF receptor) or indirect. The latter case involves the recruitment of complexes of proteins to the phosphorylated RTK. Non-receptor tyrosine kinases (NRTKs), such as Src — the first tyrosine kinase to be discovered — are among the recruited proteins. STAT3 and Src can interact independently and STAT3 probably becomes phosphorylated by Src on the EGF and PDGF receptors. Furthermore, it is clear that seven-transmembrane (7TM) receptors can, after binding their peptide or short polypeptide ligands, also activate STAT proteins^{152–154}. It has been proposed again that the tyrosine kinase involved is Src — or perhaps the JAKs become activated by associating with 7TM receptors^{155–157}. STAT1, STAT3, STAT4, STAT5 and STAT6 homodimerize. STAT1 and STAT2, and STAT1 and STAT3 can form heterodimers, and several STAT proteins can form tetramers (or potentially higher order complexes).

One final comment on STAT activation is needed. Direct recruitment of latent STAT proteins to activated cytokine receptors might be the most common and is certainly conceptually the simplest mechanism of cytokine activation, but pre-association of STAT1 and STAT2 with the interferon- α (IFN- α) receptor before ligand stimulation has also been described. Furthermore, it is known that, after IFN- α treatment, STAT2 must be phosphorylated before STAT1, at least in some cell types^{5,121}. So, in this case, the general model for phosphotyrosine-dependent recruitment might not operate. However, the exact role of pre-associated STAT2 at the IFN- α receptor and that of inducible receptor phosphorylation in the IFN- α pathway remains uncertain¹⁵⁸. STATpY, tyrosine-phosphorylated STAT; pS, serine phosphorylated.



Augmentation at the level of the receptor signalling complex has also been observed, possibly owing to the presence of multiple cytokine receptors within discrete plasma-membrane domains known as LIPID RAFTS^{25,26}. Proximity might lead to signalling reinforcement when ligand-dependent activation of one receptor leads to the activation of a neighbouring receptor, possibly through cross-phosphorylation²⁷ or down-modulation of the non-specific inhibitory effect on signalling by caveolin-1, a main constituent of lipid rafts²⁸.

Negative crosstalk. A well-characterized example of negative crosstalk involves negative feedback by SOCS proteins (see below). These cytokine-inducible kinase inhibitors are fairly promiscuous, both in their effect on the induction of different cytokines and in their ability to inhibit distinct receptor-kinase complexes²⁹. Therefore, induction of SOCS proteins by one cytokine can inhibit other pathways owing to the already-enhanced abundance of an inhibitor. Negative crosstalk from non-STAT signalling pathways also occurs, although the underlying molecular mechanisms are less clear. For instance, stimulation of cyclic AMP, mitogen-activated protein kinases or glucocorticoid-dependent signalling pathways can lead to inhibition of subsequent cytokine-dependent stimulation of STAT phosphorylation^{30–32}. Such crosstalk presumably has a role in normal homeostasis and control of inflammation. Determining the extent and specificity of both positive and negative crosstalk will require further study.

Structure of STAT proteins

The modulatory nature of STAT proteins was first realized by sequence comparisons and mutagenesis studies³³, which showed that the carboxyl terminus was a TRANSACTIVATION DOMAIN (TAD), that an SH2 domain preceded the TAD and that the tyrosine residue that became phosphorylated lay between the two. Furthermore, the DNA-binding domain was in the centre of the molecule (FIG. 3b). These conclusions were confirmed and greatly extended by crystallographic studies of the core amino acids (residues ~130–710) of either dimeric STAT1 or dimeric STAT3 bound to DNA, which have very similar characteristics^{20,34} (FIG. 3a).

Beginning at residue 130, there is a four-stranded helical coiled coil that presents extensive possibilities for protein-protein interaction. Interactions with several proteins have been documented. A DNA-binding fold between residues 320 and 490 contains several β -sheets that are folded similarly to those found in the DNA-binding domains of the transcription factors nuclear-factor κ B (NF- κ B) or p53. Contact with DNA is limited but occurs in both major and minor grooves. For example, in STAT1, N460 and K336 contact the major groove, and E421 contacts the minor groove. (N represents asparagine, K is lysine and E is glutamic acid). A linker domain of highly conserved structure but unknown function follows from residues 490 to 580. Mutations within this domain affect the stability of DNA binding, which leads to a rapid off-rate and an inability to activate genes after IFN- γ induction³⁵. A classic SH2 structure

LIPID RAFTS

Dynamic assemblies of cholesterol and sphingolipids in the plasma membrane.

TRANSACTIVATION DOMAIN

A domain that is present within a transcription factor that enables the transcription factor to interact with proteins that are involved in binding RNA-polymerase to DNA in a sequence-specific manner.

IFN-inducible gene, which is part of the IFN- α -induced transcription complex, IFN-stimulated gene factor 3 (ISGF-3). Similarly, IFN- α treatment increases the cellular abundance of STAT1, making the subsequent response to IFN- γ much stronger^{22,23}.

Post-translational modification of STATs also leads to positive crosstalk. For instance, phosphorylation of STAT1 on S727 greatly augments its transcriptional potency^{5–7}. This phosphorylation occurs in response to bacterial infection independently of tyrosine phosphorylation and is a significant component of pathogen-dependent INNATE IMMUNE RESPONSES, greatly augmenting the transcriptional competence after subsequent exposure to IFN²⁴.

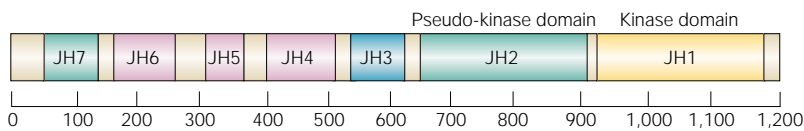


Figure 2 | **Janus kinase domain structure.** The domains of Janus kinases (JAKs) 1–7, based primarily on sequence homology. The domains JH1–7 are regions of sequence similarity in the four known JAKs. Domain JH1 is the kinase domain and domain JH2 is the pseudo-kinase domain. The amino-terminal domains (JH6 and JH7) contain sites that bind JAK to receptors. JH, JAK homology. Scale bar indicates amino acid residues.

extends between residues 580 and 680, followed by the tyrosine residue (~700) that is phosphorylated. The carboxy-terminal TAD (38–200 residues in length in various STAT proteins), the structure of which is unknown and might be evident only after it binds to other proteins, completes the proteins.

The crystal structure of the amino terminus (residues 1–130) of STAT4 was solved independently of the core structure and comprises a series of eight short interactive helices (FIG. 3)³⁶. Other STAT amino termini probably have similar structures, because of the similarity of structurally important amino acid residues in their amino termini, although experiments in which the domains were swapped indicate that they might not be completely equivalent³⁷. The presence in the phosphotyrosine dimer of the amino terminus of the STAT proteins is important in gene activation. There are two STAT-binding sites ~20 base pairs apart in many genes, and these are occupied by tetramers (dimer–dimer pairs). Without the amino terminus, the STAT core can bind single sites but no tetramers are formed and therefore binding to tandem sites is not facilitated^{8,38}. Tetramer formation greatly strengthens STAT–DNA interaction at adjacent sites⁸ and is needed to activate transcription maximally on certain natural promoters^{39,40}. The amino-terminal domain influences transcriptional induction in additional ways, such as influencing receptor recognition and phosphorylation³⁷, nuclear translocation⁴¹, and dephosphorylation^{41,42}.

There is, as yet, no crystal structure of a whole STAT molecule, phosphorylated or unphosphorylated, and no structure of a STAT dimer without DNA, so we do not know how the amino terminus packs with the core structure either before or after DNA binding. Similarly, any structural change that occurs upon dimerization remains to be uncovered.

NF-κB (Nuclear factor of κB). A widely expressed transcription factor that is activated by cellular stress and can induce the expression of numerous anti-apoptotic genes.

COILED-COIL DOMAINS A protein domain that forms a bundle of two or three α-helices. Short coiled-coil domains are involved in protein interactions, whereas long coiled-coil domains forming long rods occur in structural or motor proteins.

Nucleocytoplasmic transport
Importing STAT proteins into the nucleus. Shortly after ligand-dependent tyrosine phosphorylation and dimerization, STATs accumulate in the nucleus³. Such large protein complexes (~180 kDa for the STAT dimer) require facilitated transport into the nucleus⁴³. Binding of STAT1 to **importin-α5**, one of the subunits of the nucleocytoplasmic transport machinery, has been described^{44,45}, and recent mutagenic studies established L407, K410 and K413 (within the DNA-binding domain) in STAT1 as crucial residues in the nuclear import of tyrosine-phosphorylated STAT1 (REFS 45,46). When the importin is bound, STAT dimers cannot bind DNA and, if the importin–STAT complex enters the nucleus, it might require DNA exploration to dislodge the importin^{45,47}. In at least some cells, some (unphosphorylated) STAT1 can enter the nucleus, and this is not affected by mutations of L407 (REF 48). This is especially clear in genetically selected human cancer cells that lack STAT1 completely (U3A cells; BOX 2). Several messenger RNAs (mRNAs) that are required for STAT-directed apoptosis are not formed in these cells⁴⁹ unless STAT1 is expressed. Even when STAT1 is expressed, however, the mRNAs in question are formed in the absence of STAT1 tyrosine phosphorylation; the cells can then undergo induced apoptosis⁴⁹. The unphosphorylated STATs are hypothesized to interact with other transcription factors on DNA and to help stimulate transcription¹⁹. The mechanistic details of how tyrosine phosphorylation and dimerization favour nuclear entry await crystallographic solution of the structures of unphosphorylated STAT proteins, and perhaps even crystallization of full-length unphosphorylated and tyrosine-phosphorylated STATs.

Nuclear export of STAT proteins. The export of STAT1 from the nucleus seems to depend on residues in the COILED-COIL DOMAIN and also in the DNA-binding domain^{50,51}. Whether residues in analogous positions are involved in nucleocytoplasmic transport of other STATs is not yet clear, nor is it known what structural state is assumed by STAT proteins in transit. Dephosphorylation of STATs occurs in the nucleus⁵² and is an important signal for export back to the cytoplasm⁵¹. For STAT1 and STAT3, there is evidence that implicates TC45 (a nuclear tyrosine phosphatase⁵³) as a relevant STAT nuclear phosphatase. Cells that lack this enzyme retain tyrosine-phosphorylated STAT1 for much longer than normal cells⁵⁴, and overexpression of

Table 1 | Role of Janus kinases as revealed by gene-targeting in mice*

Enzyme	Phenotype of null mice
Jak1	Perinatal lethality, probably caused by failure of cytokine signalling in neurogenesis; immunological impairments caused by failure of multiple haematopoietic cytokines
Jak2	Embryonic lethality caused by failure of erythropoiesis; additional immunological impairments caused by impaired cytokine signalling
Jak3	SCID caused by failure of cytokine signalling from γc-containing receptors
Tyk2	Increased pathogen susceptibility caused by impaired responses to interferon and IL-12

*Reviewed in REFS 5 AND 6. IL-12, interleukin-12; Jak, Janus kinase; SCID, severe combined immunodeficiency; Tyk2, tyrosine kinase 2.

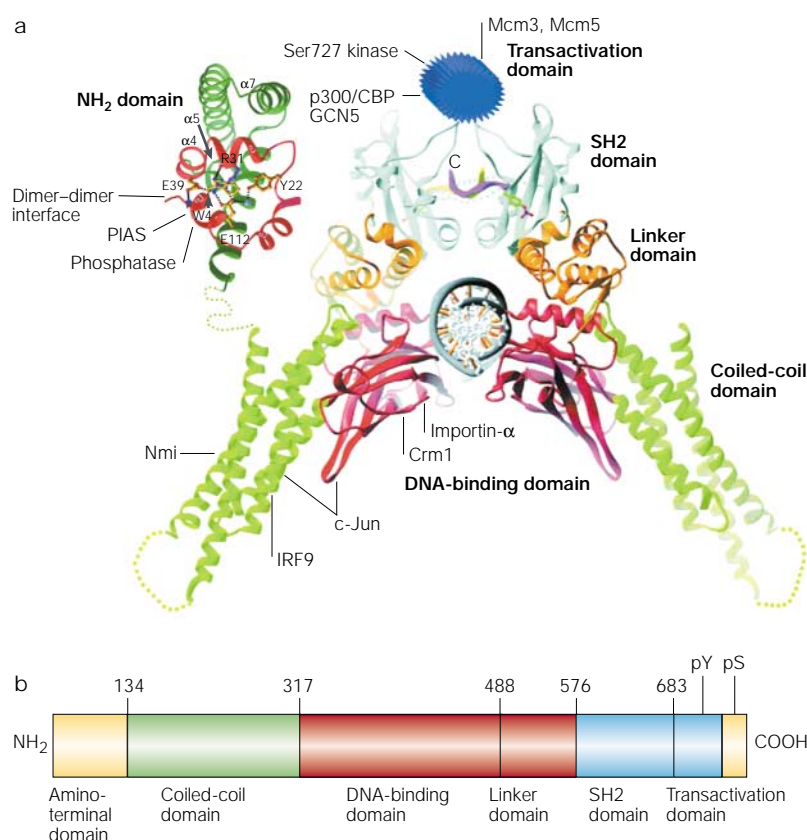


Figure 3 | STAT domain structure and protein binding sites. **a** | The core structure (amino acids ~130–712) shows binding of a STAT1 dimer to DNA and the location of binding sites of various proteins in various domains. The amino-terminal structure, the placement of which in the intact structure is undefined, also interacts with various partners, as does the carboxy-terminal transactivation domain, the structure of which is unknown. Modified with permission from REF. 36 © 1998 American Association for the Advancement of Science, and from REF. 161 © 1998 Elsevier Science Ltd. CBP, CREB binding protein; IRF, interferon regulatory factor; Mcm, minichromosome maintenance; Nmi, N-Myc interactor; PIAS, protein inhibitor of activated STAT. **b** | STAT structure. STAT, signal transducer and activator of transcription. SH2, Src-homology-2 domain.

signal⁴⁵ — the tyrosine-phosphorylated STAT1 dimer remains nuclear until it loses its phosphorylated tyrosine residue, at which point it is returned to the cytoplasm. On the basis of staurosporin inhibition of further tyrosine phosphorylation, the cycle time (activation–inactivation) for an individual STAT1 molecule in EUPLOID fibroblasts is ~20 min (REF. 52). This indicates that several cycles of STAT phosphorylation, nuclear migration, dephosphorylation and export, and re-phosphorylation and re-import occur during a full transcriptional response to cytokine stimulation. The duration of STAT phosphorylation (and therefore of transcriptional activity) is regulated by the balance of receptor-driven JAK catalytic activity and constitutive nuclear dephosphorylation⁶⁰.

Much remains to be done to validate various points of this model, especially extending it to other cell types and to other STATs.

Negative regulators of STAT signalling
As mentioned above, and as will be made clear in the section on cell-biological integration of STAT activity, activation versus inactivation/inhibition of STAT proteins is crucial to their biological actions (FIG. 4).

Cytoplasmic tyrosine phosphatases. There are several types of negative regulator of STAT proteins in the cell cytoplasm. Tyrosine dephosphorylation of receptor or kinase sites by SHP1, SHP2 or PTP1B limits further STAT tyrosine phosphorylation^{57–60}. Humans with mutations in phosphatase recruitment sites on cytokine receptors and transgenic mice with mutations in these phosphatases confirm the importance of regulated phosphorylation^{61,62}. For instance, absence of Ptp1b in mice leads to **leptin** hypersensitivity and enhanced Stat3 tyrosine phosphorylation; this is probably due to impaired **Jak1** dephosphorylation⁶³.

SOCS proteins block continued signalling. An entire subfield of STAT biology was ushered in when SOCS proteins were shown to prevent further receptor signalling by binding to receptor sites and/or JAK catalytic sites in such a way as to block further STAT–protein activation²⁹. These cytokine-induced proteins have been variously termed by the independent discoverers as suppressor of cytokine signalling (SOCS), JAK-binding proteins (JABs), STAT-induced STAT inhibitors (SSIs)^{64–66} or cytokine-induced SH2 (**CIS**) protein⁶⁷.

The SOCS proteins, the genes for which are induced transcriptionally in response to cytokine stimulation, are recruited to active receptor complexes to cause inhibition. SOCS proteins can also cause protein turnover of the receptor through a UBIQUITIN–PROTEASOME-mediated process²⁹. The multiple functions of the large SOCS family provide a rich territory for studying the need for balanced STAT transcriptional output in development and in adult functions (TABLE 2). For example, STAT4 induction by **IL-12** is crucial to differentiation of T_H1 (T_H1) cells, whereas STAT6 induction by IL-4 is necessary for T_H2 (T_H2) cell induction. The levels of STAT activity in the two cell types is

TC45 leads to dephosphorylation of STAT5 (REF. 55). However, TC45 has also been implicated in regulating cytoplasmic dephosphorylation events, such as the dephosphorylation of JAK1 and JAK3 (REF. 56). Other phosphatases, such as SH2-containing phosphatase 1 (SHP1), SHP2 and protein-tyrosine-phosphatase 1B (**PTP1B**) have also been implicated as cytoplasmic regulators of JAK or STAT phosphorylation^{57–59}.

A general scheme, not yet extended to all STAT proteins, emerges for the nucleocytoplasmic transport of STAT1 after IFN treatment of cells: tyrosine phosphorylation and dimerization favour importin binding, perhaps by conferring a change in conformation that exposes the region containing L407. Importin binding and GTP-dependent translocation then occur^{44,48,50}. Either because the nuclear tyrosine-phosphorylated STAT1 now has a conformation that is less favourable to proteins of the export machinery, such as chromosome region maintenance protein (Crm1 — a nuclear export protein)⁵⁰, or because the phosphorylated STAT1 associates reversibly with DNA while exploring for favourable binding sites — which occludes a necessary export

EUPLOID

A term that describes cells whose nuclei have an exact multiple of the haploid set of chromosomes.

UBIQUITIN

A 76-amino-acid protein that can be covalently attached to specific lysine residues in target proteins. This often forms multimeric polyubiquitin chains, which is thought to target the protein for destruction by the 26S proteasome.

PROTEASOME

Protein complex responsible for degrading intracellular proteins that have been tagged for destruction by the addition of ubiquitin.

Box 2 | Transcriptional stimulation without phosphorylation

The first indication that signal transducers and activators of transcription (STAT) proteins might have a role in gene expression as unphosphorylated molecules came from experiments using genetically selected human cancer cells in culture that lacked STAT1 (U3A cells). Such cells did not undergo apoptosis upon challenge until STAT1 expression was restored⁶. The U3A cells lacked a full component of CASPASES, which are present in the U3A cells that express STAT1. However, the apoptotic response did not require wild-type STAT1; a STAT1 Y701F mutant (in which Y is tyrosine and F is phenylalanine), which cannot be phosphorylated on tyrosine, still conferred the ability to respond to apoptotic signals⁴⁹. Subsequently, several other proteins involved in apoptosis were also found to be restored to U3A cells by the STAT1 Y701F mutant^{159,160}.

More recently, gene-array experiments using U3A cells (lacking STAT1) and cells with restored STAT1 were compared without IFN- γ stimulation. A set of genes was found in which the genes were not dependent on IFN and whose mRNAs were present when STAT1 was added back to the U3A cells¹⁶⁰. One of these genes, termed low molecular weight polypeptide 2 (LMP-2), was studied in detail. STAT1 was constitutively bound to the promoter of this gene in cells not stimulated by IFN and in which no tyrosine phosphorylation of STAT1 could be found. The STAT1 was associated with the promoter, presumably because of its association with interferon regulatory factor-1 (IRF1 — a known binding partner of STAT1) that was also present at the LMP-2 promoter, and can bind DNA on its own¹⁹. So, it seems clear that unphosphorylated STAT proteins can have a role in transcription, even though it is evident that the main transcriptional stimulation by STATs follows tyrosine phosphorylation, dimerization and nuclear accumulation.

T HELPER 1/T HELPER 2
(T_H1/T_H2). Subsets of CD4⁺ T cells that are characterized by their cytokine-production profiles. T_H1 cells primarily produce interferon- γ , and generally provide protection against intracellular pathogens, whereas T_H2 cells mainly produce interleukin-4 (IL-4), IL-5 and IL-13, and are important for immunity to helminth parasites.

CASPASES
Cysteine proteases involved in apoptosis that cleave at specific aspartate residues.

HAEMATOPOIESIS
The commitment and differentiation processes that lead from a haematopoietic stem cell to the production of mature cells of all lineages — erythrocytes, myeloid cells (macrophages, mast cells, neutrophils and eosinophils), B and T cells, and natural killer cells.

RNA HELICASE
An ATP-dependent enzyme that catalyses the unwinding of RNA helices.

DOMINANT-NEGATIVE
A defective protein that retains interaction capabilities and so distorts or competes with normal proteins.

apparently mediated by SOCS — SOCS1 is expressed at fivefold greater levels in T_H1 cells than in T_H2 cells, whereas T_H2 cells express SOCS3 at a 23-fold higher concentration than T_H1 cells⁶⁸.

As another example, it is clear from studies of *Socs1*-knockout (*Socs1*^{-/-}) mice — this mutation is perinatal lethal — that unregulated *Stat1* activity is lethal, owing largely to the unopposed action of *Ifn- γ* and *Il-4*. Animals that are null for both *Socs1* and *Stat1* or null for *Ifn- γ* and *Socs1* are resistant to the liver degeneration that is associated with a continuous high level of *Stat1* activity in the *Socs*-knockout animals^{69,70}. Similarly, deletion of the *Socs2* gene leads to dysregulation of the insulin-like-growth-factor-1 (*IGF-1*) pathway, which causes gigantism⁶⁴. Loss of *Socs3* results in embryonic lethality owing to impaired HAEMATOPOIESIS and placental defects^{71,72}, further emphasizing the importance of negative regulation for proper cytokine action. Loss of *Socs6* causes growth retardation owing to a requirement for this protein in the proper regulation downstream of insulin receptor substrate⁷³.

Nuclear regulators. The above-mentioned negative regulator functions operate in the cytoplasm. There are also at least two negative nuclear regulators. As mentioned previously, there is a short (~10–15 min) half-life for nuclear dephosphorylation of activated STAT proteins⁵². Loss of this dephosphorylation would lead to prolonged STAT activation and possible untoward consequences that are mentioned below. Recently, the negative activity on STAT proteins of a group of proteins termed PIAS — proteins that inhibit activated STATs^{74,75} — has been discovered. *PIAS1* and *PIAS3* were first shown in cultured mammalian cells to interact only with tyrosine-phosphorylated STAT1 and STAT3, respectively, and to block

DNA binding *in vitro*. Upon transfection and overexpression of the *PIAS* genes, transcriptional increases that are directed by demonstrably active STAT1 and STAT3 were also blocked.

Genetic interaction between the single *Drosophila* *PIAS* gene (*dPIAS*) and the single *Drosophila* *STAT* gene (*92E*) indicates that PIAS might modulate STAT activity *in vivo*. The JAK-STAT pathway is required for development of the eye in the fly⁷⁶. PIAS overexpression decreases eye size and somatic-cell removal of PIAS prevents differentiation of the lens⁷⁷. Furthermore, a hyperactive JAK allele, *tumorous lethal* (*tuml*), causes fly 'leukaemia', which increases in incidence with reduced PIAS levels and decreases in incidence with PIAS overexpression.

PIAS proteins have also been implicated in various processes that have no apparent connection to STAT proteins, including induction of apoptosis, modulation of ion channels, interaction with androgen receptors and interaction with RNA HELICASE. Recently, some PIAS proteins were shown to have an E3-ligase-like activity for the small ubiquitin-related modifier SUMO (REF. 78). PIAS proteins mediate the conjugation of SUMO to several proteins, including p53 and c-Jun, and this represses their activities. However, the biological role of SUMO modification remains mysterious and so, the relationship between this enzymatic activity and the negative regulation of STATs by PIAS proteins remains to be elucidated.

Naturally occurring truncated STATs. In the first purification of STAT1, a carboxy-terminal truncated molecule (STAT1 β) was found that could not drive IFN- γ -induced and STAT1-homodimer-dependent gene transcription. However, STAT1 β could participate in IFN- α -induced transcription as part of a three-protein interaction of STAT1 β , STAT2 and IRF9. This was the first evidence of carboxy-terminal TADs⁴. Subsequently, STAT3 and STAT5 were also found to have alternative carboxy-terminal truncated forms^{5,6}. These shortened proteins function as DOMINANT-NEGATIVES when overexpressed in cultured cells. In mice with a targeted knockout of *Stat3 β* , which still have full-length *Stat3 α* , the pattern of *Stat3*-induced transcription is distorted, which results in impaired recovery from ENDOTOXIC SHOCK⁷⁹.

A naturally occurring truncated form of the *Drosophila* *Stat92E* has recently been uncovered. This protein lacks the first 130 amino acids and arises from an alternative transcriptional start site. The two different primary transcripts are spliced differentially to yield either a full-length 86-kDa form or a 71-kDa form⁸⁰. The short form acts as a negative regulator of the long form. For example, expression of full-length *Stat92E* is required for *even-skipped* (*eve*) expression in stripes 3 and 7 in the developing *Drosophila* embryo^{81,82}. By contrast, overexpression of the short form of *Stat92E* suppresses *eve* expression in stripes 3 and 7. So, it seems certain that balanced STAT-protein transcriptional activity is required at many points in development and in adults, and this balanced transcription requires the participation of negative regulators.

ENDOTOXIC SHOCK
Also known as septic shock. This is a serious, abnormal condition that occurs when an overwhelming infection leads to low blood pressure and low blood flow. Vital organs such as the brain, heart, kidneys and liver might not function properly or might fail.

DNA MICROARRAY
Array of polymerase chain reaction products (corresponding to either genomic or cDNA sequence) that is deposited onto solid glass slides.

MACROPHAGE
Any cell of the mononuclear phagocyte system that is characterized by its ability to phagocytose foreign particulate and colloidal material.

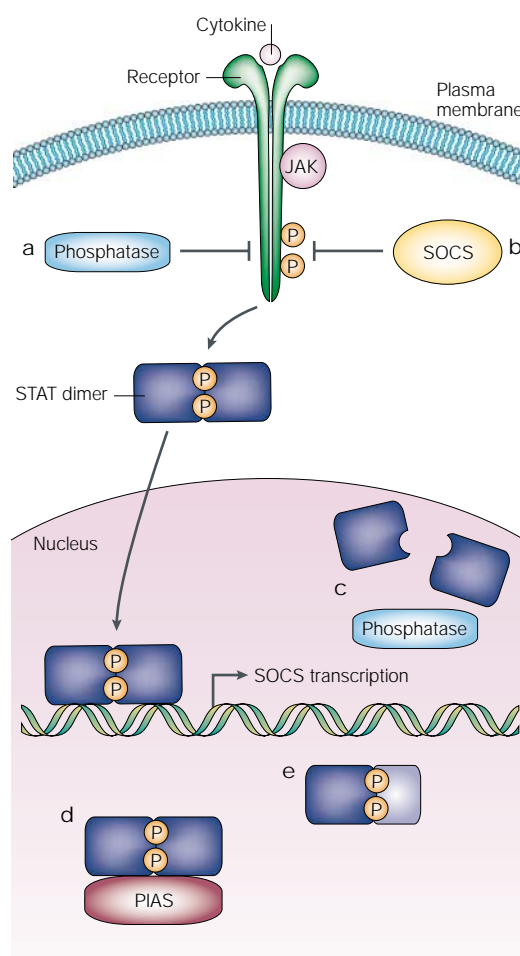


Figure 4 | The negative regulators of STAT proteins. Phosphatases (a) and suppressors of cytokine signalling (SOCS proteins) (b) block further STAT activation in the cell cytoplasm. In the nucleus, nuclear phosphatases (c) can mediate STAT dephosphorylation, and interactions with proteins that inhibit activated STAT proteins (PIAS) (d) can also occur. In addition, naturally occurring short forms of STATs can potentially act as dominant-negative proteins by occupying DNA as non-functional protein or by binding to a wild-type STAT protein (e). JAK, Janus kinase; STAT, signal transducers and activators of transcription.

STAT proteins in transcription
The basic outlines of the biochemistry of STAT activation–inactivation, nuclear import–export and negative regulation have been referred to above. The ultimate biochemical effect of activated STATs, however, lies in

their ability to increase — in a matter of minutes — the transcriptional activity of previously quiescent genes and/or to increase the transcription of less-active genes. Once STATs reach the nucleus, different STAT proteins activate different genes, owing, at least in part, to different binding affinities for natural sites and, in part, to the recruitment of distinct co-activators^{83,84}.

The number of genes activated by particular STAT pathways is a topic of much current research using DNA MICROARRAYS. For example, on the basis of STAT1 and STAT2 activation, IFN- α or IFN- γ increases the concentration of mRNAs from at least several dozen different genes over fourfold, and perhaps doubles the concentration of another 50–100 gene products⁸⁵. These include mRNAs of the ten or so genes that were shown some years ago to be transcribed much more rapidly after IFN treatment. The gene-array experiments have been carried out using cells that lack STAT1 or that have been reconstituted with wild-type or mutant STAT1, which clearly shows an important role for phosphorylated STAT1, but also for unphosphorylated STAT1, in gene expression (BOX 2).

It is known that STAT proteins bind DNA and that they associate with other transcription factors and co-activators (FIG. 3), but details of how transcriptional activation is increased are sparse.

Post-translational modification. Post-translational chemical modifications and tyrosine phosphorylations of STAT proteins have been described that affect STAT-driven transcription. Arginine methylation at the amino-terminal domain⁸⁶ increased transcriptional effectiveness by blocking the association of STATs with PIAS proteins. Acetylation of STATs by associated co-activator proteins might also enhance their transcriptional activity^{87,88}. Finally, serine phosphorylation within the TAD is definitely required for full transcriptional potency of at least STAT1, STAT3 and STAT4 (reviewed in REF. 24). The carboxy-terminal regions of STAT1, STAT3, STAT4 and STAT5 each contain a single serine residue that becomes phosphorylated in a ligand-dependent fashion for full transcriptional activation by the STAT proteins. Because serine phosphorylation can be targeted independently of tyrosine phosphorylation, synergy between two activating signals can be achieved through combined serine and tyrosine phosphorylation. For instance, STAT1 serine phosphorylation is stimulated in MACROPHAGES in response to bacterial products, which renders the subsequent transcriptional response to IFN- γ much more robust²⁴.

Co-activator–STAT interactions. The transcriptional activity of all STATs depends on the carboxy-terminal TAD that binds co-activators. Other regions (such as the amino terminus) might also bind such co-activators, but most studies have concentrated on the TAD^{7,89}. As independent activators in transfection tests, the TADs of STAT2 and STAT6 have at least a tenfold greater stimulatory activity than the TADs of other STAT proteins. All STAT TADs can substitute for STAT1 in IFN-stimulated transcription, which implies that little or no obligate gene specificity is mediated by the carboxy-terminal TAD⁹⁰.

Table 2 | Role of SOCS proteins as shown by mouse genetics

SOCS protein	Phenotype
Cis	No phenotype of null, but enhanced T-cell signalling in mice that overexpress Cis
Socs1	Perinatal lethality owing to unopposed lfn- γ -induced liver degeneration
Socs2	Gigantism owing to unopposed signalling by growth hormone and Igf-1
Socs3	Embryonic lethality owing to multiple placental and haematopoietic defects

*See REFS 68–73. Cis, cytokine-induced SH2 protein; lfn- γ , interferon- γ ; Igf-1, insulin-like growth factor-1; Socs, suppressor of cytokine signalling.

Table 3 | Role of STAT proteins as revealed by gene-targeting in mice*

STAT protein	Phenotype of null mice
Stat1	Impaired responses to interferons; increased susceptibility to tumours; impaired growth control
Stat2	Impaired responses to interferons
Stat3	Embryonic lethality; multiple defects in adult tissues including impaired cell survival (both positive and negative) and impaired response to pathogens
Stat4	Impaired T _H 1 differentiation owing to loss of IL-12 responsiveness
Stat5A	Impaired mammary gland development owing to loss of prolactin responsiveness
Stat5B	Impaired growth owing to loss of growth hormone responsiveness
Stat6	Impaired T _H 1 differentiation owing to loss of IL-4 responsiveness

*Reviewed in REFS 4–7. IL, interleukin; T_H1, T helper 1 cell.

The mechanism underlying the enhanced transcriptional activity of serine-phosphorylated STAT proteins involves the selective recruitment of co-activators. Several proteins, including minichromosome maintenance 5 (MCM5)⁹¹ and MCM3 (J.J. Zhang and J.E.D., unpublished observations), bind more avidly to the TAD of STAT1 when it is phosphorylated. The MCM proteins are known to function in DNA replication as helicases, but how they might participate in RNA synthesis is not known.

STATs and HATs. Like many eukaryotic transcription factors, the carboxy-terminal TADs of STAT1, STAT2, STAT3, STAT5 and STAT6 all interact with the co-activator HISTONE ACETYLTRANSFERASES (HATs), especially p300/CBP (CREB-binding protein)^{7,92}. Whether STAT proteins use unusual transcriptional mechanisms remains unknown. However, at least STAT2 seems to deviate from the normal pathways of transcriptional activation. This protein is activated by IFN- α to mediate the defence against infections. Therefore, STAT2 must be capable of functioning under the stressful conditions of a viral infection. STAT2 recruits the acetyltransferase protein GCN5 (general control non-repressed; a protein originally discovered in yeast)⁹³, which leads to acetylation of HISTONES in the promoters of IFN- α -regulated genes. Surprisingly, STAT2 can stimulate transcription through a complex that lacks some of the general proteins of transcriptional initiation, such as TATA-binding protein, which allows STAT2-mediated transcription to continue under conditions in which general host-cell transcription is inhibited.

One of the most widely used co-activators in eukaryotic (probably including mammalian) cells is the so-called mediator complex⁹⁴, a group of 20 or more proteins that is conserved from yeast to humans. This complex has not yet been reported to interact with STAT proteins but it would be surprising if it did not.

STATs and other DNA-binding proteins. STAT proteins probably seldom act alone in transcriptional activation but, like most other mammalian activators, act in concert with other site-specific DNA-binding proteins (FIG. 3). Interactions necessary for maximal STAT-dependent transcription have been reported between: STAT1, Sp1 and upstream stimulatory factor^{95,96}; STAT5 and glucocorticoid receptor (GR)⁹⁷; STAT6 and CCAAT/enhancer

binding protein (C/EBP)⁹⁸; several STAT proteins with N-Myc interactor (Nmi)⁹⁹; and STAT3 with various other proteins, including c-Jun, GR, androgen receptor and similar to mothers against decapentaplegic (SMAD); SMAD proteins are a group of proteins that respond to transforming growth factor- β ^{89,100–102}. The first instance of this type of interaction to be noted was in the IFN- α -induced STAT1–STAT2 heterodimer with IRF9, which contacts STAT1 (REF. 4). This trimeric complex contacts a composite DNA element consisting of juxtaposed binding sites for both IRF9 and STAT1 (reviewed in REFS 4,5,7).

STAT proteins side-by-side. STAT proteins also interact with each other on tandem DNA sites to achieve maximum transcriptional stimulation^{8,82}. The amino terminus is required for these dimer–dimer interactions, which occur for all STATs except STAT2. No dimer–dimer interaction occurs between different STAT proteins, which is not surprising given that different STATs are activated by different ligands and have different targets.

Although protein segments of interacting partners and even specific amino acids important for interaction have been located, we do not know precisely how such interactions increase initiation rates, but this will be a fertile area for future investigation. It is not clear how STAT proteins are positioned precisely within gene-activating clusters of proteins, but it has been shown that STATs are physically present in chromatin at the time of transcriptional activation. CHROMATIN IMMUNOPRECIPITATION (ChIP) ASSAYS show the IFN-dependent presence of STAT2 on the *ISG54* (interferon-stimulated-gene 54) promoter⁹³, of STAT1 on the *IRF1* (interferon-regulatory-factor 1) promoter (E. Yang and J.E.D., unpublished observations) and the class-II transactivator (CIITA) promoter¹⁰³, and of STAT3 on the α 2-macroglobulin promoter (L. Lerner *et al.*, unpublished observations).

Influence of STATs on biological functions
Transcription factors are, of course, crucial to biological outcomes in whole organisms. Many of the proteins discovered in the well-known Nusslein-Vollhard–Wieschaus screens for early developmental mutants in *Drosophila* proved to be transcription factors¹⁰⁴. So, it is no surprise that STAT proteins are widely involved in developmental decisions.

HISTONE ACETYLTRANSFERASE (HAT). An enzyme that adds acetyl groups to histones. Many HATs function as co-activators.

HISTONE
A family of small, highly conserved basic proteins, found in the chromatin of all eukaryotic cells, that associate with DNA to form a nucleosome.

CHROMATIN IMMUNOPRECIPITATION (ChIP) ASSAYS
ChIP assays can be used to monitor the association of DNA-binding proteins with specific promoters *in vivo*. Briefly, live cells are treated with crosslinking agents to tether the proteins to the DNA. The selected protein is then recovered by immunoprecipitation, the crosslinking is reversed and the co-precipitating DNA is screened for the enrichment of specific promoter fragments using the polymerase chain reaction (PCR).

Table 4 | Tissue-specific roles of Stat3 as revealed by conditional gene targeting in mice*

Target tissue	Phenotype
Skin	Impaired second hair cycle, wound repair and keratinocyte migration
Thymic epithelium	Age-dependent thymic hypoplasia, hypersensitivity to stress
T lymphocytes	Impaired IL-6-dependent survival and IL-2 α expression
Monocytes/neutrophils	Enhanced inflammatory responses and T _H 1 differentiation, chronic colitis
Granulocytes	Enhanced proliferation owing to impaired negative feedback
Mammary epithelium	Defective apoptosis, delayed mammary involution
Liver	Impaired acute phase response
Neurons	Impaired cell survival

*REFS 123–126. IL-2 α , interleukin-2 receptor- α ; T_H1, T helper 1 cell.

HYPOMORPHIC ALLELE

A mutant gene that has a function similar to but weaker than the wild-type gene.

MONOCYTES

Large leukocytes of the mononuclear phagocyte system found in bone marrow and the bloodstream. Monocytes are derived from pluripotent stem cells and become macrophages when they enter the tissues.

ERYTHROPOIETIN

A hormone secreted by certain cells in the kidney, in response to a reduction in the amount of oxygen reaching the tissues, that stimulates red blood cell production.

CRE-*LOXP*

A site-specific recombination system derived from the *Escherichia coli* bacteriophage P1. Two short DNA sequences (*loxP* sites) are engineered to flank the target DNA. Activation of the Cre recombinase enzyme catalyses recombination between the *loxP* sites, which leads to the excision of the intervening sequence.

NATURAL KILLER CELLS

(NK cells). Lymphocytes that confer innate immunity. They were originally defined on the basis of their cytolytic activity against tumour targets, but it is now recognized that they serve a broader role in host defence against invading pathogens.

HAEMATOPOIETIC

PROGENITORS

Cells that have the ability to generate all types of haematopoietic cell (multipotentiality) and to replace themselves (self-renewal) during the whole lifespan of an individual. Multipotentiality can be assessed *in vitro* and *in vivo*, whereas self-renewal can only be determined by the *in vivo* detection of long-term reconstitution activity.

Stat92E in *Drosophila*. *Drosophila* has only one JAK (**hopscotch**) and one STAT (Stat92E)¹³. There is one known ligand, outstretched (**os**) or unpaired (unp), and, recently, receptors that have at least a distant resemblance to cytokine receptors were reported to activate the pathway¹⁰⁵. The discovery of the developmental pathways that are affected by STAT mutations began with experiments showing that a null allele was lethal^{81,82,106}. A role in early embryogenesis was uncovered through localized gene expression — proteins or mRNAs expressed in stripes 3, 5 and 7 were decreased or absent in HYPOMORPHIC ALLELES. Subsequently, the list of tissues (structures) known to be affected extended to — but is not limited to — wing veins, trachea, MONOCYTES, eye disc and eye development, as well as sex determination and germ cells^{13,106,107}.

Of considerable interest are recent publications indicating new roles for Stat92E. First, stem cells in the *Drosophila* testis require JAK–STAT signalling for self-renewal but not for differentiation^{107,108}. In addition, Stat92E activity was required for migration of the border cells in the development of the ovary¹⁰⁹ — overexpression of the ligand unpaired or the *Drosophila* JAK, hopscotch, caused increased migration. In light of the fact that STAT3 and STAT5 are persistently active in many invasive human cancers, these results on cell migration in *Drosophila* are particularly interesting.

Lessons from mice. Primary roles for the seven mammalian STAT proteins were defined originally by tissue distribution, specific cell responses *in vitro* and gene-targeted removal in mice of each of the seven genes. TABLE 3 summarizes the main phenotypes originally discovered by these procedures. However, the original experiments failed to give a complete picture of the tissue/cell-specific roles of STAT proteins in mice. For example, even with complete removal of Stat5A and the consequent failure of breast tissue development^{110,111}, other tissue required a more careful assessment for a Stat5A role to be apparent. Early embryos lacking Stat5A are mildly anaemic¹¹² but, when adults devoid of both Stat 5A and Stat 5B were stressed by oxygen deprivation, the consequent increase in ERYTHROPOIETIN failed to stimulate red blood cell production¹¹³. Stat5A also has a

role in T-cell proliferation, and its absence results in a severe autoimmunity that might also contribute to the observed anaemia¹¹⁴. Stat5A is also required for lymphomyeloid repopulation of suppressed bone marrow¹¹⁵. So, there is a more subtle, but important, role for Stat5 in haematopoiesis and immune regulation compared with that in breast development.

Stat3 is the only family member whose loss results in embryonic lethality¹¹⁶. Recently, investigators have succeeded in removing Stat3 from individual tissues by the CRE-*LOXP* method¹¹⁷, which circumvents the problem of embryonic lethality and has uncovered roles for Stat3 in a wide variety of tissues (TABLE 4). A surprising result from such studies was the multitude of and sometimes contradictory roles for Stat3 in biological processes. The phenotypes resulting from the loss of Stat3 in adult tissues include failure of cell survival, impaired apoptosis, loss of negative feedback regulation and impaired cell migration and wound healing¹¹⁸ (see below). How a single transcription factor contributes to these distinct and opposing processes remains to be determined at a molecular level.

Role in infection. Several STAT proteins in mammals have a crucial role in host defence (TABLE 4). Stat1 and Stat2 are largely restricted to mediating the effects of IFNs; Stat4 and Stat6 mediate the effects of IL-12 and IL-4, respectively; and Stat3 mediates the effects of IL-6 and other gp130 ligands. Animals that lack either Stat1 or Stat2 are exquisitely sensitive to microbial infections^{119–121}, and subtle mutations of STAT1 in humans lead to decreased resistance to mycobacterial infection¹²². The absence of STAT6 blocks the differentiation of T_H2 cells, and lack of STAT4 impairs IFN- γ production by T cells and development of NATURAL KILLER CELLS during bacterial and viral infections (reviewed in REF 6).

As mentioned above, study of the absence of STAT3 must be done by Cre-*loxP*-mediated removal in specific tissue. The absence of STAT3 causes several biological effects. For example: in adult liver, STAT3 absence leads to significantly impaired responses to acute phase activators commonly associated with bacterial infection¹²³; in the thymus, it impairs T-lymphocyte survival¹²⁴; in macrophages, it disrupts resistance to intestinal microbes¹²⁵ and, in HAEMATOPOIETIC PROGENITORS, it leads to increased accumulation of granulocytes¹²⁶.

PICORNAVIRUS

Any of a group of RNA animal viruses that consist of naked, icosahedral 27-nm capsids with single-stranded infectious RNA (plus-strand) of 2.7 MDa.

MYELOMA

A malignant tumour of the bone marrow.

Further evidence of the importance of STAT-mediated signals for resistance to infection can be seen from the multitude and variety of pathogen-encoded mechanisms that decrease STAT function and the cellular attempts to avoid these detrimental effects¹²⁷. Several viruses target STAT or JAK proteins for degradation^{128–131} or inhibit their activation in other ways¹³². Vesicular stomatitis virus inhibits the nuclear translocation of proteins^{133–135}, but STAT1-mediated induction of the nuclear pore protein Nup98 overcomes this block. PICORNAVIRUSES inhibit cellular transcription by targeting transcription factors for degradation¹³⁶, but STAT2-dependent transcription somehow remains resistant to this effect⁹³.

Role in growth control. Signalling pathways that originate at the cell surface and send active transcriptional proteins to the nucleus are frequently dysfunctional in cancer cells¹³⁷. The STAT proteins are certainly no exception. Mice that lack Stat1 are much more susceptible to chemically induced primary tumours and to tumours that can be readily transplanted^{138–140}, and human cancer cells have often lost STAT responses to IFN, which normally imposes growth restraint¹⁴¹.

Of great current interest is persistently active STAT3, which is known to occur in a wide variety of human tumours¹⁴². Furthermore, STAT3 can, by experimental mutation, be converted into an oncogene¹⁴³. The persistently active protein is required because introduction of a dominant-negative form of STAT3 into **head and neck cancer** cells or into multiple MYELOMA cells causes apoptosis of recipient cancer cells (reviewed in REF. 144). Persistent activation of STAT3 in head and neck cancer is associated with mutations in the epidermal growth factor (EGF) receptor or mutations that result in the production of excess ligand or normal receptor¹⁴⁵. In some multiple myelomas, excess production of IL-6 might be the underlying defect. Two recent reports of persistently active STAT3 highlight the importance of negative factors in STAT control. First, in hepatocellular

carcinoma, silencing of the *SOCS3* gene locus by methylation was associated with persistent STAT3 activation¹⁴⁶. Second, loss of *PIAS3* was found in a leukaemia in which STAT3 was persistently activated¹⁴⁷. So, mutations that cause continued signalling or ineffective negative upstream regulation of STAT3 seem to be important in promoting cancer. It is very likely that mutations in *STAT3* itself are not the reason for its persistent activity in cancer.

Conclusions and perspectives

The enormous variety of experiments on the STAT genes and the proteins they encode in all animals reflect the widespread importance of these transcription factors and of the delicate balance normally exercised on the extent and time of their activation. Biochemistry of the mechanistic role of STAT proteins in transcription initiation has lagged behind the exploration of the biological decisions in which the STAT proteins participate. However, studies of the association of STAT proteins with various nuclear proteins have begun. With the reagents developed in the many studies on transcription both *in vivo* and *in vitro*, passage of another ten years should see answers to presently unanswered biochemical questions and show how integration into biological decisions is achieved by this important group of transcription factors.

Increasing our knowledge of how STAT proteins affect transcriptional regulation is important as basic information for understanding coordinate control in mammalian cells. Furthermore, as a practical consideration — if pharmacological intervention of specific transcriptional activity is ever to be achieved — it is necessary to know the most common interacting partners of specific activators. Therefore, many experiments have been — and are being — done using mutagenized STAT proteins that fail to function at various steps in transcriptional activation, and experiments on STAT–STAT and STAT–protein interactions will very probably provide targets for drug development.

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Acknowledgements
Work in the authors' laboratories was supported by grants from the NIH.

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