TRANSCRIPTION FACTORS AS TARGETS FOR CANCER THERAPY

James E. Darnell, Jr

A limited list of transcription factors are overactive in most human cancer cells, which makes them targets for the development of anticancer drugs. That they are the most direct and hopeful targets for treating cancer is proposed, and this is supported by the fact that there are many more human oncogenes in signalling pathways than there are oncogenic transcription factors. But how could specific transcription-factor activity be inhibited?

Laboratory of Molecular Cell Biology, The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA.
e-mail: darnell@rockefeller.edu
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The initial flurry (1976–1985) in the discovery of oncogenes — mutated genes that allow unregulated cell growth — yielded a large number of proteins that are involved in cell signalling[1,2]. Signalling pathways begin with extracellular proteins — ligands — which bind to specific cell-surface receptors that dimerize or oligomerize at the cell surface to begin the intracellular phase of signalling (FIG. 1). Activation of the now dimeric or oligomeric receptor most often depends on serine or tyrosine kinases that are either intrinsic to the receptor or bound to the internal domain of the receptor. Cytoplasmic proteins — either activated transcription factors or proteins that activate transcription factors — then relay the signal into the nucleus and change the transcription pattern of the cell.

Proteins in these pathways can be made oncogenic by mutation or overexpression. For example, epidermal growth-factor receptor (*EGFR*) and platelet-derived growth-factor receptor (*PDGFR*) — both of which contain an intrinsic tyrosine kinase — can be either overexpressed or mutated in cancer cells[2]. Some of the earliest discovered oncogenes were *SRC*, *ABL* and *RAS*. SRC and ABL are among the intracellular tyrosine kinases that are mutated in cancer, and their persistent activation is fairly common in cancer cells. Other intracellular molecules that are connected to ligand-activated receptors can also be activated by mutation, and RAS — a small GTPase that connects the cell surface to, and activates, a kinase cascade — falls into this category. It is mutated in ~15% of all human cancers[3]. The overactivity of transcription factors that are activated directly by specific phosphorylation or that become activated following phosphorylation of other cellular proteins were later discovered to act as oncogenes[2]. Such proteins will be the main focus of this article.

In addition to oncogenes, tumour-suppressor genes were discovered more recently[4]. The normal function of proteins that are encoded by tumour-suppressor genes is to provide growth restraint. Both activation of oncogenes and loss of tumour-suppressor genes allow cancer cells to avoid apoptosis[5] — an event in carcinogenesis that is of equal importance to growth dysregulation.

Appropriately enough, inhibition of oncogenic proteins or reactivation of tumour suppressors has become the goal for those developing anticancer drugs. For example, antibodies that block cell-surface receptors (preventing them from binding their ligand), kinase inhibitors and RAS inhibitors have all been described and are being tested clinically or are approved for use with varying degrees of success[6]. Although some clever strategies have been devised to attempt to correct defects that are caused by the deletion or mutation of tumour suppressors[7,8], it has remained difficult to reinstate normal cell regulation by returning missing tumour-suppressor proteins in enough tumour cells to eradicate cancer. Inhibition of proteins by small molecules — not restitution of missing proteins — is an eminently more tractable pharmacological goal[6].

Choosing an antitumour target

Given that small-molecule inhibitors of an overactive process are thought to be the most useful mechanism of tumour inhibition[6], what are the most logical targets? First consideration would seem to indicate a protein or

Summary

- Signalling proteins, which are often mutated in cancer, change transcription patterns.
- Many more signalling proteins are affected in cancer than transcription factors, electing transcription factors as cogent targets.
- One or more latent cytoplasmic transcription factors (such as STATs, NF-κB, β-catenin and Notch intracellular domain (NICD)) have increased activity in most human cancers, and in many cases prevent apoptosis of cancer cells.
- Necessary physical interaction among transcription factors and cofactors in the nucleus affords selective sites of potential drug action.
- Should pharmacology of transcription-factor inhibition be the wave of the future? It might be difficult, but it should not be impossible.

protein complex that is most frequently overactive in the full range of human tumours. It has been argued that the fundamental molecular requirements for human cancer might be the same in all tissues[9], and these include loss of growth inhibition by ligands such as transforming growth factor-β (TGF-β), dispensing with the need for growth stimulation (for example, by epidermal growth factor (EGF)), limitless replication potential (including telomere maintenance) and avoidance of apoptosis. (The final development of clinical cancer also includes the capacity to initiate angiogenesis and metastasis.) All of these properties are hypothesized to depend on dysregulated transcription. The number of oncogenes that could go awry and that underlie this dysregulated transcription makes it likely that no one protein, or even a few proteins, would always be overactive in a given human cancer. However, a specific group of transcription-factors that are overactive in a large percentage of cancers have much to recommend them as the most appropriate targets. There are many more potentially oncogenic proteins upstream of these same transcription factors than there are oncogenic transcription factors themselves, so one effective anti-transcription-factor drug might combat various upstream oncogenes. Although the inhibition of specific transcription factors or their interactions with accessory proteins might be a formidable task, it is not an impossible one.

Finally, it should be noted that what is being proposed is selective inhibition of transcription — not general inhibition, which would be expected to be too toxic; however, general inhibitors in the anthracycline family have been used clinically[10]. Of course, even well-chosen targets might carry the risk of some toxicity, but the benefit of stopping overactive, cancer-specific transcription should outweigh the risk.

Transcription factors in cancer

Three main groups of transcription factors are known to be important in human cancer. The first to be recognized were the steroid receptors — for example, oestrogen receptors in breast cancer and androgen receptors in prostate cancer. Anti-oestrogen and anti-androgen compounds such as tamoxifen and bicalutamide have been in clinical use for many years, and because of the ability to induce apoptosis — at least in lymphoid cells — active glucocorticoids have also been widely used[6,11,12].

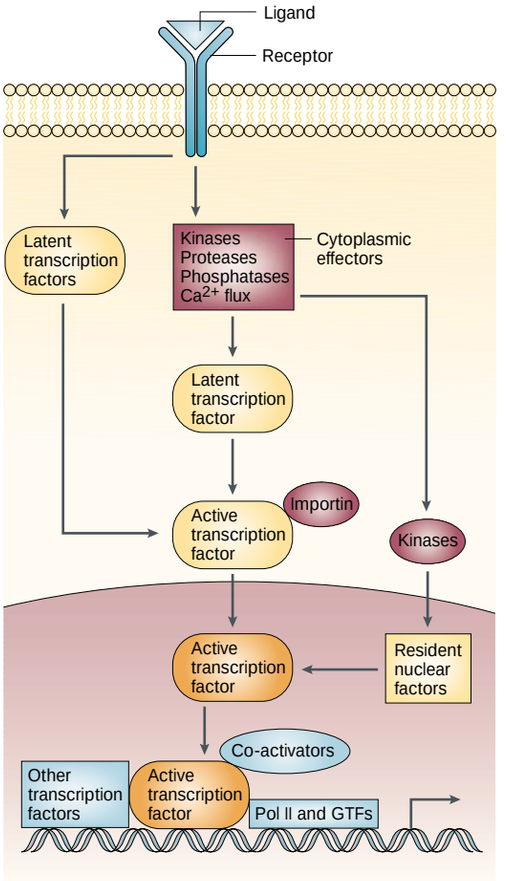
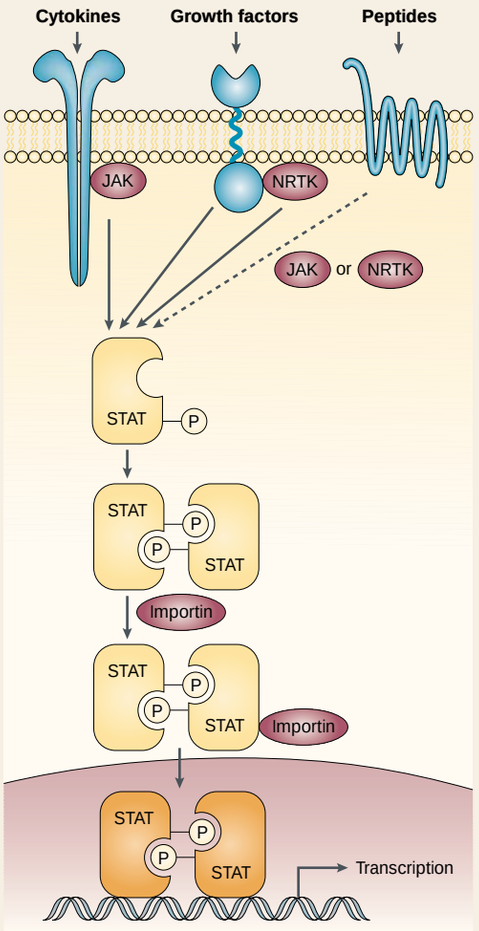


Figure 1 | **Generalized signalling pathway.** The human genome contains information for several thousand cell-surface protein receptors and ligands for these receptors (in general, small secreted proteins). The biochemistry of intracellular signalling has been very widely studied. The diagram summarizes key reactions. Latent transcription factors might be activated at the cell membrane by either tyrosine (STATs) or serine (SMADs) phosphorylation. They then bind to the transport proteins called importins and enter the nucleus to participate in regulated gene transcription. Other pathways of activation for latent cytoplasmic factors are less direct, involving phosphorylation-stimulated or -inhibited events that most often then block or trigger proteases with a final result of importin-mediated delivery of 'active factor' (orange) to the nucleus. In some pathways, the kinase enters the nucleus and effects an activation of resident nuclear-transcription factors. The active factors (in combination with DNA) bind other transcription factors (forming an enhanceosome) that attracts co-activators (this includes several dozen possible proteins), leading to the final step of attracting the actual RNA synthesis machinery that includes RNA polymerase II (Pol II) and general transcription factors (GTFs).

The second group of transcription factors that were recognized to have a role in cancer are resident nuclear proteins, which are activated by serine kinase cascades[13]. In 1987, JUN was shown to be an oncogene — first, the v-jun of a retrovirus, then the normal cellular form, c-JUN — and, soon thereafter, JUN was found to be a transcription factor. This was initially based on its similarity in a DNA-binding domain to an

Box 1 | STAT activation pathways

In the canonical pathway (below), JAK tyrosine kinases associate with cytokine receptors, and in a three-step tyrosine phosphorylation cascade — JAK(pTyr)–receptor(pTyr)–STAT(pTyr) — they activate the STATs (signal transducers and activators of transcription). Growth-factor receptors, such as epidermal growth factor (EGF) and platelet-derived growth factor (PDGF), which have intrinsic tyrosine kinases (RTKs, receptor tyrosine kinases) activate STATs either directly or by recruiting other non-receptor tyrosine kinases (NRTKs) (centre). In addition (not shown), the STATs can also bind to SRC-LIKE cytoplasmic kinases and might become directly phosphorylated. Finally, ligand-activated GPCRs (G-protein-coupled receptors) can activate STATs, perhaps by activating either SRC-like proteins or JAKs (right). The STATs have SH2 domains and, after tyrosine phosphorylation, dimerize by reciprocal phosphotyrosine SH2 interactions. After binding to importins, the STAT phosphoproteins accumulate in the nucleus[105] and participate in activating transcription. Activation is brief because dephosphorylation occurs in the nucleus within 20 minutes for any given molecule[106]. There are seven mammalian STATs; mouse knockouts show each to have distinctly different roles. STAT1 restricts growth and is often absent or inactive in human cancers, whereas STATs 3 and 5 have been implicated in human cancer[18].



SRC-LIKE
The generic name for proteins that are similar to v-src, the oncogene of Rous sarcoma virus.

already identified yeast transcription factor, GCN4 (REFS 3,14). This highly significant discovery provided the first concrete example, other than steroid receptors, that linked cancer to transcriptional control. Subsequent work showed that c-JUN was phosphorylated on serine 63 and serine 73 following activation of signalling cascades, which made c-JUN maximally effective in stimulating transcription[3,15]. c-JUN is just one of hundreds of nuclear proteins that are targets of serine kinase cascades that are initiated in the cytoplasm, and there are more than 500 serine kinases[2] (FIG. 1).

The third group of transcription factors that have oncogenic potential, and the most recently recognized, are latent cytoplasmic factors, activation of which is normally triggered by receptor–ligand interaction at the cell surface[13]. The latent cytoplasmic proteins can be activated directly by tyrosine or serine kinases at the cell surface, or by various different cytoplasmic biochemical events that also feature kinases (some regulated by Ca^{2+} flux) or specifically regulated proteolysis. Despite their dissimilarity in detail, these pathways are all similar in that a protein–protein interaction at the cell surface triggers cytoplasmic events, which result in delivery to the nucleus of a protein that increases transcription through interaction with one or more of the many proteins that affect the initiation of transcription (FIG. 1).

An oversupply or overactivity of one or more transcription factors from these three classes might well be required for the survival, unrestrained growth and metastatic behaviour of all human cancers[3,13,16]. Inhibition of excess transcription-factor activity therefore seems to offer a direct and promising target to develop effective anticancer therapy. Therapeutics have already been developed to inhibit the steroid receptors[11,12], but not the other transcription-factor classes. Increased knowledge of the salient features of their activation and function, particularly involving interaction with other nuclear proteins, should reveal therapeutic targets. We now turn to the details of transcription-factor activation and the interactions of a set of such factors with other proteins to illustrate potential pharmacological targets.

Latent transcription factors

STATs. STATs (signal transducers and activators of transcription) are transcription factors that are latent in the cytoplasm until activated by any of a large variety of receptors (for reviews, see REFS 17,18) (BOX 1).

They often, if not always, increase transcription by interacting with other transcription factors on chromatin and might, until delivered to the nucleus[19], be the 'missing protein' in these gene-activating complexes. For example, STAT5 interacts with the glucocorticoid receptor (GR)[20] and STAT1 interacts with GR and the transcription factors SP1 and PU.1 (REFS 21,22). STAT3 also activates transcription in association with other proteins. STAT3 can bind c-JUN[23] and GR (L. Lerner *et al.* manuscript in preparation), and these interactions are required for maximal interleukin (IL)-6 induction of the *A2M* (α2-macroglobulin) gene: c-JUN and GR can

Table 1 | Target sites for inhibition of oncogenic transcription factors

Transcription factors	Ligand–receptor	Receptor	Kinase effectors	Protease effectors	Importin interaction
Cytoplasm					
STAT3, 5	+	SH2–pTyr ++	JAKs RTKs ++		+++(?) ++++
NF-κB	+		IκB ++	IκB, p100 ++++	+++(?)
β-catenin	+		++++	++++	+++(?)
Notch (NICD)	+	Protease(s) +			+++(?)
GLI	+		GSK +++		+++(?)
c-JUN	+				+++(?)

Transcription factors	Enhanceosomes (other transcription factors)	HATs	Cofactors	Serine kinases	DNA binding
Nucleus					
STAT3,5	STAT–STAT, c-JUN, GR,IRF ++++	+++(?)	+++(?)		+++(?)
NF-κB	c-JUN ++++	+++(?)	+++(?)		+++(?)
β-catenin	TCF/LEF ++++	+++(?)	+++(?)		
Notch (NICD)	HLH proteins +++	+++(?)	+++(?)		
GLI		+++(?)			+++(?)
c-JUN				++++	+++(?)

Possible pharmacological targets in transcription-factor activation and activity. The most economical point of inhibition for any pathway ending in transcription-factor activation is to block nuclear activity of the factor. All transcription-factor activity involves nuclear entry, association with cofactors, including histone acetyltransferases (HATs), and, of course, DNA binding. As discussed in the main text, these are all possible points of quite specific inhibition (+++(?)). A particularly vulnerable and specific locus in this chain of events is the cooperative interaction between an oncogenic transcription factor and its nuclear partners. ++++, most specific; +++(?), possible high specificity; ++, partial specificity; +, least specific. GR, glucocorticoid receptor; GSK, glycogen synthetase kinase; HLH, helix–loop–helix; IRF, interferon regulatory factor; NICD, notch intracellular domain; RTK, receptor tyrosine kinase; ICF/LEF, T-cell factor/lymphocyte-enhancer factor.

be present on the chromosome without gene activation, but it is only after STAT3 binds that transcription is activated (L. Lerner *et al.*, manuscript in preparation).

STATs 1, 3, 4 and 5 also function through homodimer–homodimer interaction, thereby creating homotetramers on tandem STAT DNA-binding sites[13,17,18]. The tetramer formation depends on the amino terminus of the protein and is specific between STATs — that is, homotetramers form, not heterotetramers. For STATs to activate the transcription of chromosomal genes, they must interact with CREB binding protein (CBP)/p300 or other histone acetyltransferases (HATs) through the carboxy-terminal STAT domain[18]. All of these interactions, which are required for STAT activation, are potential targets for drug intervention in STAT activity.

There are also numerous negative regulators for STATs, including cytoplasmic tyrosine phosphatases and cytokine-induced proteins called SOCS (suppressors of cytokine signalling) that prevent further STAT activation[24]. Nuclear tyrosine phosphatases dephosphorylate nuclear STATs to allow the protein to return to the cytoplasm[25]. At least two members of the PIAS protein family — a newly recognized group of proteins — have the capacity to decrease STAT dimer-dependent transcription *in vivo* and to block DNA binding *in vitro*[26]. As a group, the negative regulators of the STATs determine the transcriptional impact of the STAT-induced signalling events that emanate from the cell-surface-receptor–ligand interaction.

In cell lines, the evidence that STAT3 is involved in transformation is persuasive. First, all SRC-transformed cell lines have persistently activated STAT3, and dominant-negative STAT3 blocks this transformation[27,28]. Second, STAT3-C — a constitutively active mutant that is dimerized by cysteine–cysteine bridges instead of the pTyr–SH2 interaction — can transform cultured cells that form tumours when injected into mice[29]. STAT3 functions in normal lymphocyte development to combat apoptosis[30,31] and the same is true in STAT3-C transformed cells[32]. So, one contribution of persistently active STAT3 to oncogenesis is probably to protect cancer cells from apoptosis.

Persistently activated STATs — that is, tyrosine phosphorylated, DNA-binding STATs — have been reported in a wide variety of human tumours without evidence of mutation in the STAT genes themselves. The persistent activity of STAT5 is associated with several types of leukaemia and lymphomas[28]. One type of lymphoma depends on STAT5 that is persistently activated because of a chromosomal translocation. When a dominant-negative STAT5 is introduced into these tumour cells, they undergo apoptosis[33,34].

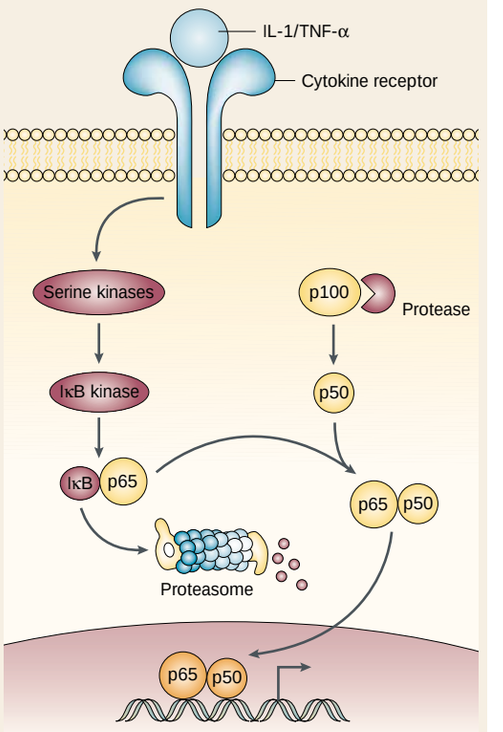
STAT3 is persistently activated in almost all head and neck cancers due to the dysregulation of the EGF pathway[35]. All human multiple myelomas that were tested had persistently activated STAT3, which is caused in some, but not all, cases by the overproduction of IL-6 (REFS 28,36). Introducing dominant-negative STAT3 into cell lines derived from either of these tumours resulted in apoptosis[36]. Hepatocellular carcinoma has been reported to have persistently active STAT3 in association with hypermethylation, and hence suppression, of SOCS1 (one of the negative regulators of STAT activity)[37]. Finally, persistently active STAT3 is present in lymphomas and leukaemias, and, in at least one leukaemia, a deletion of *PIAS3* — another of the negative regulators of STAT activity — might be the cause of this[38]. At present, there is no reported mutation in *STAT3* that results in persistent activation. So, the widespread occurrence of active STAT3 and STAT5 in cancers is probably due to the dysregulation of signalling molecules or mutations and deletions in the proteins that negatively regulate STAT3. Logic dictates, given these conditions, that inhibition of STAT3 as a transcriptional activator is a prime anticancer target.

Molecular targets for inhibition of persistently active STAT3 include homodimer–homodimer interaction (tetramer formation) on DNA (TABLE 1), and interactions between STAT3 and c-JUN, CBP/p300 or GR. All of these interactions are required for maximal

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.

Box 2 | NF-κB activation

The cytoplasmic activation of nuclear factor of κB (NF-κB) can occur through a number of different pathways that activate serine kinases. The most direct from the cell surface is activation by binding of interleukin (IL)-1 or tumour necrosis factor-α (TNF-α)-like protein ligands to their receptors. This triggers serine kinases that phosphorylate inhibitor of κB (IκB) kinase with subsequent IκB phosphorylation and proteolytic destruction. Serine kinases are activated through other pathways that phosphorylate the IκB kinase with the same effect — p65 is released, joins p50 (a proteolytic cleavage product of p100) and enters the nucleus to participate in transcriptional activation.



transcriptional activation by STAT3 (REF. 18). Most of these interactions have been mapped to small protein domains and some to specific amino acids.

NF-κB. The nuclear factor of κB (NF-κB) family of proteins in mammals is crucial in the inflammatory-response incident to cellular injury, and they are also important in the immune response[39,40]. They are cytoplasmic until cells encounter bacterial toxins, common virus infections or the extracellular signalling proteins IL-1 or tumour necrosis factor-α (TNF-α) (BOX 2). The classic NF-κB transcriptional activator is a heterodimer of p65–p50 and was the first latent cytoplasmic transcription factor to be discovered[41]. Three additional family members have been identified, but are not as well studied as p65–p50. p65 is bound in the cytoplasm by an inhibitor termed inhibitor of κB (IκB). Several different serine kinases can phosphorylate a 700-kDa kinase complex that then phosphorylates IκB, marking it for ubiquitylation and destruction by the proteasome; p65 is released during this proteolysis. The smaller NF-κB subunit, p50, is derived by proteolytic cleavage of a p100 primary translation product, which itself is retained in the cytoplasm by ANKYRIN REPEATS. After proteolytic release, the p50 subunit binds p65 — probably in the cytoplasm — to form an active transcription factor[42]. p65 contains nuclear-localization sequences that are exposed following destruction of IκB; it then binds to an IMPORTIN that translocates the complex into the nucleus.

The duration of the active NF-κB cycle might be determined by the synthesis of IκB, which is itself transcriptionally activated by NF-κB[42]. The newly synthesized inhibitor is hypophosphorylated, which allows it to enter the nucleus, where it binds NF-κB; NF-κB is then returned to the cytoplasm, where IκB is hyperphosphorylated: this strengthens the bond with p65 (R. Sen, personal communication). In addition to the importance of IκB proteins in nuclear–cytoplasmic partitioning of NF-κB, a protein called A20 that blocks any further NF-κB activation by binding to the IL-1 or TNF-α receptor is induced by NF-κB[43].

The interaction between NF-κB and other proteins in the β-interferon (IFN) ENHANCEOSOME[44] was among the first and most extensively studied case in mammalian cells of cooperative transcription-factor interactions. In addition to NF-κB, interferon regulatory factor 3 (IRF3), AP2/c-JUN and HMG1(Y) all bind to an ~60-bp DNA fragment. NF-κB also interacts with c-JUN and probably with other proteins to stimulate the transcription of other genes[39,45]. Active NF-κB is found in the nucleus of many different cancer cells[45-49] (see TABLE 2), including solid tumours (such as breast cancer), some multiple myelomas and the REED–STERNBERG CELLS of Hodgkin's disease.

When the p65 and p100 genes that encode NF-κB were first sequenced, a close homology was noted with v-rel, an oncogenic retrovirus[39]. That NF-κB proteins are PROTO-ONCOGENES is consistent, therefore, with the ability of v-rel of a retrovirus to induce tumours. The importance of NF-κB in cell transformation is emphasized by induction of apoptosis in multiple myeloma and Hodgkin's disease cells when dominant-negative NF-κB subunits were introduced into such cells.

In considering the development of antitumour agents, the possibility of inhibiting cytoplasmic proteases that activate NF-κB is appealing (TABLE 1). Protease inhibitors might block the destruction of IκB that releases p65 or the cleavage of p100 that produces p50. Second, if a limited number of serine kinases activate the destruction of IκB, or if the interaction site of several kinases with IκB is similar enough, this limited number of kinases might be reasonable targets. However, these upstream targets would not necessarily be as effective as drugs that were targeted directly to nuclear NF-κB protein interactions. For example, NF-κB almost always drives transcription by association

ANKYRIN REPEATS
Short amino-acid repeats that were first identified in the protein ankyrin, to which a number of cytoplasmic proteins bind.

IMPORTIN(S)
A family of proteins (also called karyopherins) that combine with 'cargo' proteins in the cytoplasm and engage the nuclear import machinery to bring proteins into the nucleus.

ENHANCEOSOME
A group of transcription factors that are bound to regulatory DNA elements that act in concert to activate gene transcription.

REED–STERNBERG CELLS
Characteristic large stellate lymph-node cells that are associated with Hodgkin's disease.

PROTO-ONCOGENES
Normal cellular genes that, when mutant or overactive, contribute to cancerous transformation in cells.

Table 2 | Tumours with persistently high levels of NF-κB

Gene	Haematopoietic tumours	Solid tumours
c-REL	Diffuse large-cell lymphoma; primary mediastinal B-cell lymphoma; follicular large-cell lymphoma; follicular lymphoma; diffuse large-cell lymphoma	Non-small-cell lung carcinoma
RELA	B-cell non-Hodgkin's lymphoma; multiple myeloma; diffuse large-cell lymphoma	Squamous head and neck carcinoma; breast adenocarcinoma; stomach adenocarcinoma; thyroid carcinoma cell lines; non-small-cell lung carcinoma
NFKB1	Acute lymphoblastic leukaemia	Non-small-cell lung carcinoma; colon cancer cell lines; prostate cancer cell lines; breast cancer cell lines; bone cancer cell lines; brain cancer cell lines
NFKB2	Cutaneous T-cell lymphoma; B-cell non-Hodgkin's lymphoma; B-cell chronic lymphocytic leukaemia; multiple myeloma	Breast carcinoma; colon carcinoma
BCL2	B-cell non-Hodgkin's lymphoma; B-cell chronic lymphocytic leukaemia	

with other transcription factors. NF-κB can interact with IRFs, AP1, steroid receptors and cofactor proteins (see below). These sites offer great potential for interruption that should be highly specific. (NF-κB is used generically here; different REL PROTEINS might require different inhibitors, whether the targets were cytoplasmic or nuclear.)

The WNT–β-catenin signalling pathway. WNT proteins — of which there are more than 20 in mammals — are small secreted proteins that signal most effectively when prebound in the extracellular matrix[50,51] (FIG. 2). WNT receptors — Frizzled proteins — are members of the low-density lipoprotein (LDL) receptor family, termed LRP in mice. These receptors transmit signals to a family of intracellular proteins; Dishevelled was the first recognized family member in *Drosophila*. Activation of this signalling pathway leads to inhibition of a serine kinase, termed GSK3β for its original discovery as a glycogen synthetase kinase. Among the targets of GSK3β are catenins — β and γ[52], with many more reports on β[50,53]— which were originally discovered as cytoplasmic structural proteins bound to the cytoplasmic domain of E-cadherins (transmembrane proteins that help govern cell–cell adhesion). Excess β-catenin that is not occupied in a structural role is bound in a large protein complex that includes axin and adenomatous polyposis coli (APC; a tumour suppressor that is often deficient in colon cancer cells)[50,53]. Normally, GSK3β phosphorylates both APC and any excess β-catenin, targeting both proteins for proteolytic destruction. After binding of WNT to Frizzled, GSK3β is inhibited, phosphorylation of β-catenin is decreased and the molecules of β-catenin that are spared from destruction enter the nucleus. β-catenin does not bind DNA but rather binds to DNA-binding proteins called TCFs/LEFs (for T-cell factors or lymphocyte-enhancer factors) that lack a transcription-activation domain (TAD)[51]. β-catenin either provides a TAD[54] or associates with additional nuclear factors that, together, activate transcription[55]. This latter suggestion is prompted by recent results from *Drosophila* showing that the WINGLESS pathway uses two proteins (LGS/BCL9 and PYGO[56,57]) that are downstream of the *Drosophila* β-catenin product, Armadillo, to increase transcription.

There are at least four LEF/TCF proteins in mammals — the mRNAs for some of which are differentially spliced — and there are homologues of each of the *Drosophila* proteins that are involved in wingless signalling[58]. The most prevalent derangement that leads to β-catenin overactivity in human cancer is caused by *APC* mutations or deletions in the epithelial cells of the colon[50,58]. An interesting model (reviewed in REF. 50) for the development of colon cancer is that of growth-favouring mutations that occur in one of the limited number of stem-cell progenitors. These cells are found at the base of epithelial crypts and divide to give rise to new epithelial cells as older cells are shed. It is supposed that clones of cells bearing the *APC* mutations gradually take over in a site of the colonic epithelium. The β-catenin that is released as a consequence of this, in combination with TCF4 (at least in a mouse model of colon cancer), causes a premalignant continuously replicative adenomatous growth. A recent report indicates that a particular form of TCF1 is most often the β-catenin partner in human cancer[59]. Further mutagenesis in this growing cell population leads to cancer.

Overexpression and/or mutation in β-catenin were also reported in a recent study of hepatocellular carcinomas[53], and deranged β-catenin metabolism has been reported in a rare tumour that is termed hepatoblastoma, a non-malignant brain tumour and skin growths called desmoids[58].

Inhibition of β-catenin to treat cancer might be most effective by preventing interaction with TCF/LEF factors, a family that is limited in number (TABLE 1).

Notch and Hedgehog as targets. Notch proteins are an evolutionarily conserved family that often has a role in determining cell fate. In mammals, there are four family members — Notch1–4, in contrast to the single founding member in *Drosophila*[60,61].

Notch proteins are translated as an ~300-kDa precursor to a transmembrane receptor protein. An initial proteolytic cleavage leaves a large extracellular fragment still associated with a transmembrane fragment. After binding to its ligand (Delta/Serrate in *Drosophila*; Delta-like/Jagged in mammals) — presented as another transmembrane protein on a neighbouring cell or as an extracellular-matrix-bound protein — two additional proteolytic cleavages within Notch release the Notch intracellular domain (NICD). This fragment translocates to the nucleus where it can interact with negative-acting helix–loop–helix (HLH) proteins that are bound to DNA[61]. The NICD has a transcriptional-activation domain and activates specific genes according to which HLH it binds[62,63]. The precise mechanism of transcriptional activation is complicated. One role of the NICD is to attract the HATs, CBP/p300, and possibly also

REL PROTEINS
Family name for a group of proteins that have sequence similarity to the oncogene in the chicken virus (v-rel) that causes reticulo-endothelial tumours.

WINGLESS
(Wg). The gene discovered early in *Drosophila* genetics that encodes a protein that is very similar to a DNA integrase named Int that is encoded by a retrovirus. The original term Int was melded with Wg to produce the current term WNT.

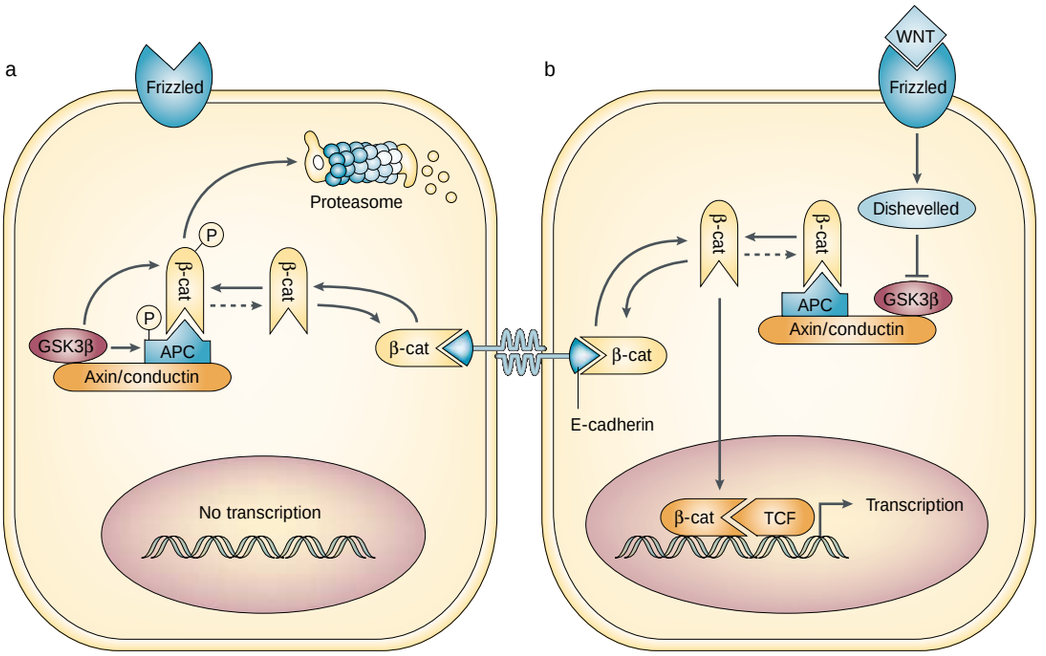


Figure 2 | **WNT signals through β-catenin. a** | Cells without WNT bound to its receptor Frizzled destroy excess β-catenin (β-cat) because it is phosphorylated by GSK3β (glycogen synthetase kinase-3β) and binds strongly to phosphorylated APC (adenomatous polyposis coli). This complex is destroyed by the proteasome. **b** | When WNT binds to Frizzled, GSK is inhibited and excess β-catenin enters the nucleus to furnish a transcriptional-activation domain to the site-specific DNA-binding protein TCF (T-cell factor), also called LEF (lymphocyte-enhancer factor).

pCAF/GCN5 co-activating complexes[64]. In this role, there seems to be a competition with p53 so that the apoptotic role of the tumour suppressor p53 is diminished, thereby making activated Notch an anti-apoptotic transcription factor.

Notch was originally identified as an oncogene due to a chromosomal translocation in T-cell leukaemia[65]. This event resulted in the fusion of a Notch fragment, equivalent to the NICD, to a fragment of the T-cell β-receptor, which is normally expressed only in T cells. Such NICD fragments expressed in breast also transform breast epithelial cells (*in vivo* and *in vitro*)[66,67]. So, there seems little doubt now that the Notch internal domains are, in fact, transcription factors[61,64], and that their overactivity can contribute to oncogenesis[65–69].

The Hedgehog (HH) pathway was discovered initially in *Drosophila* because of its crucial role in development[70]. HH is a ligand that is processed from a larger transmembrane precursor protein, leaving a cholesterol-attached extracellular signalling fragment[50,71]. Several mammalian homologues to proteins in the HH pathway are known[50], but the molecular pathway is best understood in *Drosophila*. Two complex transmembrane proteins — patched (*ptc*) and smoothened (*smo*) — act as an inhibited cell-surface complex; release of *smo* signalling activity is brought about by the HH ligand that interacts with *ptc*. As a result of *smo* activity, a transcriptional activator — CI155 (Cubitus interruptus 155 kDa) — is released from a cytoplasmic cluster of proteins and enters the nucleus. In the absence of HH signalling, the CI155 is cleaved to a 75-kDa transcriptional repressor, as a result of serine phosphorylation by protein kinase A and, subsequently, casein kinase[72]. So, HH activation of *smo* somehow blocks this proteolysis, perhaps by inducing a phosphatase.

In mammals, there are three proteins — GLI1, 2 and 3 — that have strong sequence homology with the CI protein[73]. GLI1 was originally discovered as an overproduced product in a glioma, and is thought to serve as a transcriptional activator[74]. The details of how the GLI proteins activate transcription are not completely clear, although cleavage of GLI3 produces a repressor protein[75]. There is a DNA-binding zinc-finger region of the protein, but association with other nuclear proteins is not yet well studied. What is clear is that overactivity of the GLI proteins is associated with a number of proliferative diseases. Basal-cell carcinoma of the skin — the most common carcinoma of humans — is routinely accompanied by excess GLI1 (REF. 76). In addition, cells of a rare childhood cancer, medulloblastoma[77], and of rhabdomyosarcomas[78], overexpress GLI1. Because of the scant knowledge about GLI function in transcription, it is difficult to suggest how the GLI proteins might be targeted for drug inhibition, but the stage is set for a thorough study of these transcription factors and information on how they might be inhibited should come soon.

Resident nuclear proteins in cancer

Many transcription factors enter the nucleus automatically after synthesis[13]. Some of the largest groups of structurally related proteins fall into this category; for

example, the bZip proteins — including c-JUN, JUNB, JUND, c-FOS, FRA, the ATFs and the CREB-CREM family, the cEBP family, the ETS proteins and the MAD-box family. These groups include at least several hundred individual proteins in mammals, only a few of which have been extensively studied[2,14,79–81]. Many oncogenic cytoplasmic proteins feed into serine kinase cascades that end in the phosphorylation of one or more of the resident nuclear-transcription factors[1,2,13] (FIG. 1). To take one well-studied example, *RAS* mutations lead to persistent activation of mitogen-activated protein kinase pathways that end in the phosphorylation of one or more of the transcription factors mentioned above, and it is presumably the consequent change in transcriptional profile that changes the cell phenotype[1–3].

A general picture has emerged of the events that control maximal transcriptional activity of the resident nuclear-transcription factors. They might be constitutively bound to DNA[82] but require specific phosphorylation on serine/threonine residues to be fully active in stimulating transcriptional initiation. These proteins frequently stimulate transcription by associating with other transcription factors while bound to chromosomal DNA, or by interacting with proteins that act as co-activators of genes[2,3,15,23,80,81,83,84]. Just as the family size of these resident nuclear proteins is very large, so are the number of genes, the transcription of which is affected by these proteins[85]. Indeed, it is rare for regulated genes not to be partially dependent on one or more of these resident nuclear factors.

Perhaps the two groups of resident nuclear transcription factors that are most often considered to have a role in human cancers are the ETS proteins and proteins such as c-JUN that form the AP1 transcriptional complexes. The ETS family of transcription factors can be oncogenic because of overexpression (for example, in ovarian cancer), by contributing a DNA-binding domain through fusion with other proteins[83,86] or by mutation[16]. Although no mutations of *JUN* have been found in human cancers, a great many of the mutations that have been identified in human cancers are upstream activators of JUN[3]. Many dozens of overactive receptor tyrosine kinases (RTKs), ligands, non-receptor tyrosine kinases (NRTKs) and downstream serine kinases lead to serine phosphorylation and activation of c-JUN. So, an inhibitor of c-JUN interaction(s) with nuclear proteins or chromatin should be more beneficial than blocking individual upstream targets, such as the EGF or PDGF receptors, or even any individual single kinase that phosphorylates serines on c-JUN.

In addition to serine-phosphorylated nuclear proto-oncogenes, there are a number of resident nuclear-transcription factors that are simply overexpressed in human cancers or expressed as overactive fusion proteins. Perhaps most prominent among these is MYC, which is pervasively increased in cancer[87,88]. MYC does not regulate transcription on its own, but dimerizes with another protein, MAX[89]. Inhibitors of this pairing might be useful in cancer therapy, although MYC is so widely used, such inhibition could be toxic.

E2F is a member of a transcription-factor family that is bound in the nucleus to the retinoblastoma protein (RB, the first identified tumour-suppressor gene[4]) until cells enter S phase — at which point, the E2F proteins are liberated and increase specific gene transcription. Because of the almost universal loss of RB in human cancer, E2F proteins are free in cancer cells[4]. Cultured cell studies show that E2F1–3 are important in normal cell-cycle progression, although deletion of E2F3 has the most marked effect on preventing S-phase entry[90]. Although most cancers do not have abnormal levels of activity of E2F proteins, E2F derangement in cancer has been reported. Some 90% of cases of small-cell lung cancer, but not squamous-cell or adenocarcinoma of the lung, have elevated amounts of E2Fs[91].

Although future investigations might pinpoint a high-frequency involvement of specific resident nuclear-transcription factors in specific common cancers, at the moment this is not the case. However, even in the absence of mutations or elevated concentrations, resident nuclear-transcription factors might still be required in cancer cells and their inhibition could prove beneficial.

Future prospects

It is now commonplace to assume that cancer cells have a different and pathological transcriptional pattern compared with the normal cells from which they originate — witness the proliferating literature of experiments that estimate mRNA profiles in cells by gene-array hybridization analysis[92–95]. Many of these studies are undertaken with the notion that 'key target genes' will be transcriptionally activated, and that inhibition of their gene products will be beneficial. The facts reviewed here indicate that a limited number of transcription factors are indeed overactive in many human cancers and that these overactive transcription factors themselves are the appropriate targets. They are appropriate both because they are less numerous than upstream activators and are at a focal point in the deregulated pathway. The question naturally follows, how can a transcription factor best be inhibited?

It has been stated by investigators, both industrial and academic, that specific inhibition of the interaction of DNA-binding proteins with DNA is difficult[6,96]. But in the face of overwhelming evidence that increased activity of a limited set of transcription factors have crucial and frequent roles in cancer, more intense scrutiny of compounds that might specifically inhibit the binding of these particular factors seems warranted (TABLE 1).

Even if the specific interruption of DNA binding of a particular transcription factor cannot be achieved, interrupting the function of this limited group of transcription factors in other ways still offers ample opportunity for extensive pharmacological searches. One of the generic sites for interruption of STATs, NF-κB and β-catenin, NICD or c-JUN, which might or might not yield fruit, is interaction of these specific factors with importins. There are at least six importins[97] and only in a few cases is it clear which importins are chiefly responsible for translocating which transcription factor. In *Drosophila*, it has been recently shown that different

nuclear proteins use different importins (C.S. Parker, personal communication). It is certainly conceivable that drugs that inhibit nuclear transport might be found that have enough specificity to inhibit the nuclear arrival of particular overactive transcription factor(s) in a particular cancer (TABLE 1).

Protein–protein interactions of the overactive factors within the nucleus of the cancer cell certainly offer a great list of possibilities for pharmacological interference. These include interactions between the factors themselves, and also with the multitude of co-activators and proteins of the transcription machinery. For example, all of the oncogenic transcription factors interact with one or another site in the large p300/CBP complex, the HATs that seem to be most commonly used to acetylate histones. In addition, as more is learned of the mediator complex[98], the more likely it seems that different transcription factors will bind to different proteins in this huge ~20-protein complex. Whether inhibitors of CBP/p300 or mediator interactions might be found that are specific enough not to block all transcription is problematical, but without seeking such inhibitors for specific transcription factors, no test of this possibility is forthcoming. Finally, and potentially most importantly, there are already known contacts between STAT3, NF-κB, β-catenin and the NICD of Notch, and other transcription factors, that could be directly targeted. These have been summarized in the previous sections on each of these factors (TABLE 1).

The real stumbling block to the successful inhibition of these transcription factors is that the principles of successful inhibition of protein–protein interaction have yet to be fully elucidated[99–102]. The standard and logical explanation for difficulty in this regard is that compared with enzyme–substrate interactions, relatively larger interactive surfaces are involved and small-molecule interruption of large surface interactions is difficult or impossible to 'design.' However, optimistic outlooks are described in the literature[102,103], including a recent success in which the MYC–MAX interaction was blocked by derivatives of a combinatorial chemical library, on the basis of synthetic peptidomimetic compounds. Furthermore, the candidate compounds inhibited transformation of cultured cells[104].

With the availability of robotic screening procedures, huge chemical libraries (~10^6 compounds) need to be screened in assays that might uncover small molecules that target any of the specific interactions of transcription factors that are suggested here. In addition, adequately broad, cell-based assays that have embedded within them the possibility of finding such agents should be carried out. Given the potential use of such inhibitory compounds, the risk of sufficiently comprehensive 'smart' screens seems slight. Finally, a query might be offered: what is the benefit to medicine in all the twenty-first century promise of proteomics if we cannot selectively inhibit protein–protein interactions?

1. Varmus, H. E. Oncogenes and transcriptional control. *Science* **238**, 1337–1339 (1987).
2. Blume-Jensen, P. & Hunter, T. Oncogenic kinase signalling. *Nature* **411**, 355–365 (2001).
3. Vogt, P. K. Jun, the oncoprotein. *Oncogene* **20**, 2365–2377 (2001).
   The discoverer of JUN presents an excellent summary of the first nuclear oncogene.
4. Futreal, P. A. *et al.* Cancer and genomics. *Nature* **409**, 850–852 (2001).
5. Green, D. R. & Evan, G. I. A matter of life and death. *Cancer Cell* **1**, 19–30 (2002).
   Authoritative account of the importance of apoptosis in cancer.
6. Gibbs, J. B. Mechanism-based target identification and drug discovery in cancer. *Science* **287**, 1969–1973 (2000).
7. Biederer, C., Ries, S., Bandts, C. H. & McCormick, F. Replication-selective viruses for cancer therapy. *J. Mol. Med.* **80**, 163–175 (2002).
8. McCormick, F. Cancer gene therapy: fringe or cutting edge? *Nature Rev. Cancer* **1**, 130–141 (2001).
9. Hanahan, D. & Weinberg, R. A. The hallmarks of cancer. *Cell* **100**, 57–70 (2000).
   Widely quoted summary of the requirements to make a human cancer cell.
10. Bachur, N. R. in *Encyclopedia of Cancer* 2nd edn Vol. 1 (ed. Bertino, J. R.) 57–61 (Academic Press, 2002).
11. Tilley, W. D., Clarke, C. L., Birrell, S. N. & Bruchovsky, N. Hormones and cancer: new insights, new challenges. *Trends Endocrinol. Metab.* **12**, 186–188 (2001).
12. Barnes, P. J. Anti-inflammatory actions of glucocorticoids: molecular mechanisms. *Clin. Sci.* **94**, 557–572 (1998).
13. Brivanlou, A. H. & Darnell, J. E., Jr. Signal transduction and the control of gene expression. *Science* **295**, 813–818 (2002).
14. Vogt, P. K., Bos, T. J. & Doolittle, R. F. Homology between the DNA-binding domain of the GCN4 regulatory protein of yeast and the carboxyl-terminal region of a protein coded for by the oncogene jun. *Proc. Natl Acad. Sci. USA* **94**, 3316–3319 (1987).
15. Derijard, B. *et al.* JNK1: a protein kinase stimulated by UV light and Ha-Ras that binds and phosphorylates the c-Jun activation domain. *Cell* **76**, 1025–1037 (1994).
16. Denhardt, D. T. Oncogene-initiated aberrant signaling engenders the metastatic phenotype: synergistic transcription factor interactions are targets for cancer therapy. *Crit. Rev. Oncog.* **7**, 261–291 (1996).
17. Stark, G. R., Kerrr, I. M., Williams, B. R., Silverman, R. H. & Schreiber, R. D. How cells respond to interferons. *Annu. Rev. Biochem.* **67**, 227–264 (1998).
18. Levy, D. & Darnell, J. E., Jr. STATs: transcriptional control and biologic impact. *Nature Rev. Mol. Cell Biol.* **3**, 651–662 (2002).
    Most up-to-date review of the STAT protein family, which is important in cancer.
19. Darnell, J. E., Jr. STATs and gene regulation. *Science* **277**, 1630–1635 (1997).
20. Groner, B. *et al.* Regulation of the trans-activation potential of STAT5 through its DNA-binding activity and interactions with heterologous transcription factors. *Growth Horm. IGF Res.* **10** (Suppl. B), S15–S20 (2000).
21. Aittomaki, S. *et al.* Cooperation among Stat1, glucocorticoid receptor, and PU.1 in transcriptional activation of the high-affinity Fc gamma receptor I in monocytes. *J. Immunol.* **164**, 5689–5697 (2000).
22. Look, D. C., Pelletier, M. R., Tidwell, R. M., Roswit, W. T. & Holtzman, M. J. Stat1 depends on transcriptional synergy with Sp1. *J. Biol. Chem.* **270**, 30264–30267 (1995).
23. Zhang, X., Wrzeszczynaska, M. H., Horvath, C. M. & Darnell, J. E., Jr. Interacting regions in Stat3 and c-Jun that participate in cooperative transcriptional activation. *Mol. Cell. Biol.* **19**, 7138–7146 (1999).
24. Starr, R. & Hilton, D. J. Negative regulation of the JAK/STAT pathway. *Bioassays* **21**, 47–52 (1999).
    Authoritative summary of how cells switch off this important signalling pathway.
25. Aoki, N. & Matsuda, T. A nuclear protein tyrosine phosphatase TC-PTP is a potential negative regulator of the PRL-mediated signaling pathway: dephosphorylation and deactivation of signal transducer and activator of transcription 5a and 5b by TC-PTP in nucleus. *Mol. Endocrinol.* **16**, 58–69 (2002).
26. Shuai, K. Modulation of STAT signaling by STAT-interacting proteins. *Oncogene* **19**, 2638–2644 (2000).
27. Yu, C. L. *et al.* Enhanced DNA-binding of a Stat3-related protein in cells transformed by the Src oncoprotein. *Science* **269**, 81–83 (1995).
28. Bowman, T., Garcia, R., Turkson, J. & Jove, R. STATs in oncogenesis. *Oncogene* **19**, 2474–2488 (2000).
29. Bromberg, J. F. *et al.* Stat3 as an oncogene. *Cell* **98**, 295–303 (1999).
30. Takeda, K. *et al.* Stat3 activation is responsible for IL-6-dependent T cell proliferation through preventing apoptosis: generation and characterization of T cell-specific Stat3-deficient mice. *J. Immunol.* **161**, 4652–4660 (1998).
31. Takeda, K. *et al.* Enhanced Th1 activity and development of chronic enterocolitis in mice devoid of Stat3 in macrophages and neutrophils. *Immunity* **10**, 39–49 (1999).
32. Shen, Y., Devgan, G., Darnell, J. E., Jr & Bromberg, J. F. Constitutively activated Stat3 protects fibroblasts from serum withdrawal and UV-induced apoptosis and antagonizes the proapoptotic effects of activated Stat1. *Proc. Natl Acad. Sci. USA* **98**, 1543–1548 (2001).
33. Lacaronique, V. *et al.* A TEL–JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* **278**, 1309–1312 (1997).
34. Lacronique, V. *et al.* Transforming properties of chimeric TEL–JAK proteins in Ba/F3 cells. *Blood* **95**, 2076–2083 (200).
35. Song, J. I. & Grandis, J. R. STAT signaling in head and neck cancer. *Oncogene* **19**, 2489–2495 (2000).
    Importance of persistently active STAT3 in human cancer.
36. Catlett-Falcone, R. *et al.* Constitutive activation of Stat3 signaling confers resistance to apoptosis in human U266 myeloma cells. *Immunity* **10**, 105–115 (1999).
37. Yoshikawa, H. *et al.* SOCS-1, a negative regulator of the JAK/STAT pathway, is silenced by methylation in human hepatocellular carcinoma and shows growth-suppression activity. *Nature Genet.* **28**, 29–35 (2001).
38. Zhang, Q. *et al.* Multilevel dysregulation of STAT3 activation in anaplastic lymphoma kinase-positive T/null-cell lymphoma. *J. Immun.* **168**, 466–474 (2002).
    References 36–38 provide evidence of the importance of STAT3 in clinical cancer.

39. Perkins, N. D. The Rel/NF-κB family: friend and foe. *Trends Biochem. Sci.* **25**, 434–440 (2000).
40. Israel, A. IκB kinase all zipped up. *Nature* **388**, 519–521 (1997).
41. Sen, R. & Baltimore, D. Inducibility of kappa immunoglobulin enhancer-binding protein NF-κB by a posttranslational mechanism. *Cell* **47**, 921–928 (1986).
42. Tam, W. F., Wang, W. & Sen, R. Cell-specific association and shuttling of IκBα provides a mechanism for nuclear NF-κB in B lymphocytes. *Mol. Cell. Biol.* **21**, 4837–4846 (2001).
43. Song, H. Y., Rothe, M. & Goeddel, D. V. The tumor necrosis factor-inducible zinc finger protein A20 interacts with TRAF1/TRAF2 and inhibits NF-κB activation. *Proc. Natl Acad. Sci. USA* **93**, 6721–6725 (1996).
44. Thanos, D. & Maniatis, T. Virus induction of human IFN beta gene expression requires the assembly of an enhanceosome. *Cell* **83**, 1091–1101 (1995).
Early description of the protein complex that functions as the enhanceosome.
45. Rayet, B. & Gelinas, C. Aberrant REL/NF-κB genes and activity in human cancer. *Oncogene* **18**, 6938–6947 (1999).
46. Ni, H. *et al.* Analysis of expression of nuclear factor B (NF-κB) in multiple myeloma: downregulation of NF-κB induces apoptosis. *Br. J. Haematol.* **115**, 279–286 (2001).
47. Izban, K. F. *et al.* Characterization of NF-κB expression in Hodgkin's disease: inhibition of constitutively expressed NF-κB results in spontaneous caspase-independent apoptosis in Hodgkin and Reed–Sternberg cells. *Mod. Pathol.* **14**, 297–310 (2001).
48. Kim, D. W. *et al.* Activation of NF-κB/REL occurs early during neoplastic transformation of mammary cells. *Carcinogenesis* **21**, 871–879 (2000).
49. Tai, D. I. *et al.* Constitutive activation of nuclear factor kappaB in hepatocellular carcinoma. *Cancer* **89**, 2274–2281 (2000).
50. Taipale, J. & Beachy, P. A. The Hedgehog and Wnt signalling pathways in cancer. *Nature* **411**, 349–354 (2001).
Excellent review of two signalling pathways and how they might connect to human cancer.
51. Barish, G. D. & Williams, B. O. in *Signaling Networks and Cell Cycle Control* (ed. Gutkind, J. S) 53–82 (2000).
52. Kolligs, F. T. *et al.* Gamma-catenin is regulated by the APC tumor suppressor and its oncogenic activity is distinct from that of beta-catenin. *Genes Dev.* **14**, 1319–1331 (2000).
53. Wong, C. M., Fan, S. T. & Ng, I. O. L. Beta-catenin mutation and overexpression in hepatocellular carcinoma: clinicopathologic and prognostic significance. *Cancer* **92**, 136–145 (2001).
54. van de Wetering, M. *et al.* Armadillo coactivates transcription driven by the product of the *Drosophila* segment polarity gene dTCF. *Cell* **88**, 789–799 (1997).
55. Kramps, T. *et al.* Wnt/wingless signaling requires BCL9/legless-mediated recruitment of pygopus to the nuclear beta-catenin-TCF complex. *Cell* **109**, 47–60 (2002).
56. Parker, D. S., Jemison, J. & Cadigan, K. M. Nucleotide Pygopus, a nuclear PHD-finger protein required for Wingless signaling in *Drosophila*. *Development* **129**, 2565–2576 (2002).
57. Thompson, B., Townsley, F., Rosin-Arbesfeld, R., Musisi, H. & Bienz, M. A new nuclear component of the Wnt signalling pathway. *Nature Cell Biol.* **4**, 367–373 (2002).
58. Barker, N. & Clevers, H. Catenins, Wnt signaling and cancer. *Bioessays* **22**, 961–965 (2000).
59. Hovanes, K. *et al.* Beta-catenin-sensitive isoforms of lymphoid enhancer factor-1 are selectively expressed in colon cancer. *Nature Genet.* **28**, 53–57 (2001).
60. Artavanis-Tsakonas, S., Rand, M. D. & Lake, R. J. Notch signaling: cell fate control and signal integration in development. *Science* **284**, 770–776 (1999).
61. Weinmaster, G. Notch signal transduction: a real Rip and more. *Curr. Opin. Genet. Dev.* **10**, (2000).
Authoritative summary of Notch signalling.
62. Davis, R. L. & Turner, D. L. Vertebrate Hairy and Enhancer of split related proteins: transcriptional repressors regulating cellular differentiation and embryonic patterning. *Oncogene* **20**, 8342–9357 (2001).
63. Jarriault, S. *et al.* Signalling downstream of activated mammalian Notch. *Nature* **377**, 355–358 (1995).
64. Oswald, F. *et al.* p300 acts as a transcriptional coactivator for mammalian Notch-1. *Mol. Cell. Biol.* **21**, 7761–7774 (2001).
65. Ellisen, L. W. *et al.* TAN-1, the human homolog of the *Drosophila* Notch gene, is broken by chromosomal translocations in T lymphoblastic neoplasms. *Cell* **66**, 649–661 (1991).
Connects the *Notch* pathway to human cancer.
66. Callahan, D. & Callahan, R. The mouse mammary tumor associated gene INT3 is a unique member of the NOTCH gene family (NOTCH4). *Oncogene* **14**, 1883–1890 (1997).
67. Capobianco, A. J., Zagouras, P., Blaumueller, C. M., Artavanis-Tsakonas, S. & Bishop, J. M. Neoplastic transformation by truncated alleles of human NOTCH1/TAN1 and NOTCH2. *Mol. Cell. Biol.* **17**, 6265–6273 (1997).
68. Jang, M. S., Zlobin, A., Kast, W. M. & Miele, L. Notch signaling as a target in multimodality cancer therapy. *Curr. Opin. Mol. Ther.* **2**, 55–65 (2000).
69. Fitzgerald, K., Harrington, A. & Leder, P. Ras pathway signals are required for notch-mediated oncogenesis. *Oncogene* **19**, 4191–4198 (2000).
70. Ingham, P. W. Transducing Hedgehog: the story so far. *EMBO J.* **17**, 3505–3511 (1998).
71. Kalderon, D. Transducing the Hedgehog signal. *Cell* **103**, 371–374 (2000).
72. Price, M. A. & Kalderon, D. Proteolysis of the Hedgehog signaling effector cubitus interruptus requires phosphorylation by glycogen synthase kinase 3 and casein kinase 1. *Cell* **108**, 823–835 (2002).
73. Ruiz i Altaba, A. Gli proteins and Hedgehog in signalling. *Trends Genet.* **15**, 418–425 (1999).
Connects human cancer with the *Hedgehog* pathway.
74. Cheng, S. Y. & Bishop, J. M. Suppressor of Fused represses Gli-mediated transcription by recruiting the SAP18–mSin3 corepressor complex. *Proc. Natl Acad. Sci. USA* **99**, 5442–5447 (2002).
75. Wang, B., Fallon, J. F. & Beachy, P. A. Hedgehog-regulated processing of Gli3 produces an anterior/posterior repressor gradient in the developing vertebrate limb. *Cell* **100**, 423–434 (2000).
76. Riefenberger, J. *et al.* Missense mutations in SMO in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. *Cancer Res.* **58**, 1798–1803 (1998).
77. Goodrich, L. V., Milenkovic, L., Higgins, K. M. & Scott, M. P. Altered neural cell fates and medulloblastoma in mouse patched mutants. *Science* **277**, 1109–1113 (1997).
78. Hahn, H. *et al.* Rhabdomyosarcomas and radiation hypersensitivity in a mouse model of Gorlin syndrome. *Nature Med.* **4**, 619–622 (1998).
79. van Dam, H. & Castelazzi, M. Distinct roles of Jun: Fos and Jun: ATF dimers in oncogenesis. *Oncogene* **20**, 2453–2464 (2001).
80. Hartl, M. & Vogt, P. K. A rearranged junD transforms chicken embryo fibroblasts. *Cell Growth Differ.* **3**, 909–918 (1992).
81. Vandel, L. *et al.* Stepwise transformation of rat embryo fibroblasts: c-Jun, JunB, or JunD can cooperate with Ras for focus formation, but a c-Jun-containing heterodimer is required for immortalization. *Mol. Cell. Biol.* **16**, 1881–1888 (1996).
Emphasizes the importance of multiple events in tumorigenesis.
82. Gille, H., Strahl, T. & Shaw, P. E. Activation of ternary complex factor Elk-1 by stress-activated protein kinases. *Curr. Biol.* **5**, 1191–1200 (1995).
83. Davidson, B. *et al.* Ets-1 messenger RNA expression is a novel marker of poor survival in ovarian carcinoma. *Clin. Cancer Res.* **7**, 551–557 (2001).
84. Shaywitz, A. J. & Greenberg, M. E. CREB: a stimulus-induced transcription factor activated by a diverse array of extracellular signals. *Annu. Rev. Biochem.* **68**, 821–861 (1999).
Excellent summary of CREB — one of the most well-studied transcription factors.
85. Fambrough, D., McClure, K., Kazlauskas, A. & Lander, E. S. Diverse signaling pathways activated by growth factor receptors induce broadly overlapping, rather than independent, sets of genes. *Cell* **97**, 727–741 (1999).
86. Gilliland, D. G. The diverse role of the ETS family of transcription factors in cancer. *Clin. Cancer Res.* **7**, 451–453 (2001).
87. Nesbit, C. E., Tersak, J. M. & Prochownik, E. V. MYC oncogenes and human neoplastic disease. *Oncogene* **18**, 3004–3016 (1999).
88. Grandori, C., Cowley, S. M., James, L. P. & Eisenman, R. N. The Myc/Max/Mad network and the transcriptional control of cell behavior. *Annu. Rev. Cell Dev. Biol.* **16**, 653–699 (2000).
Discoverer of MYC transcriptional activity provides up-to-date summary.
89. Eisenman, R. N. Deconstructing Myc. *Genes Dev.* **15**, 2023–2030 (2001).
90. Wu, L. *et al.* The E2F1-3transcription factors are essential for cellular proliferation. *Nature* **414**, 457–462 (2001).
91. Eymin, B., Gazzeri, S., Brambilla, C. & Brambilla, E. Distinct pattern of E2F1 expression in human lung tumours: E2F1 is upregulated in small-cell-lung carcinoma. *Oncogene* **20**, 1678–1687 (2001).
92. Chen, X. *et al.* Gene expression patterns in human liver cancers. *Mol. Biol. Cell.* **13**, 1929–1939 (2002).
93. Perou, C. M. *et al.* Molecular portraits of human breast tumours. *Nature* **406**, 747–752 (2000).
Among one of the first comprehensive gene-array reports: strengths and weaknesses discussed.
94. Rosenwald, A. *et al.* The use of molecular profiling to predict survival after chemotherapy for diffuse large-B-cell lymphoma. *N. Engl. J. Med.* **346**, 1937–1947 (2002).
95. Davis, R. E. & Staudt, L. M. Molecular diagnosis of lymphoid malignancies by gene expression profiling. *Curr. Opin. Hematol.* **9**, 333–338 (2002).
96. Abbott, A. News Feature: On the offensive. *Nature* **416**, 470–474 (2002).
Summary of where cancer pharmacology stands now.
97. Malik, H. S., Eickbush, T. H. & Goldfarb, D. S. Evolutionary specialization of the nuclear targeting apparatus. *Proc. Natl Acad. Sci. USA* **94**, 13738–13742 (1997).
98. Malik, S. & Roeder, R. G. Transcriptional regulation through mediator-like coactivators in yeast and metazoan cells. *Trends Biochem. Sci.* **25**, 277–283 (2000).
99. Park, H. S., Lin, Q. & Hamilton, A. D. Supramolecular chemistry and self-assembly special feature: modulation of protein–protein interactions by synthetic receptors. Design of molecules that disrupt serine protease-proteinaceous inhibitor interaction. *Proc. Natl Acad. Sci. USA* **99**, 5105–5109 (2002).
100. Ohkanda, J., Knowles, D. B., Blaskovich, M. A., Sebti, S. M. & Hamilton, A. D. Inhibitors of protein farnesyltransferase as novel anticancer agents. *Curr. Top. Med. Chem.* **2**, 303–323 (2002).
101. Peczuh, M. W. & Hamilton, A. D. Peptide and protein recognition by designed molecules. *Chem. Rev.* **100**, 2479–2494 (2000).
102. Cochran, A. G. Antagonists of protein–protein interactions. *Chem. Biol.* **7**, R85–R94 (2000).
103. Eckert, D. M., Malashkevich, V. N., Hong, L. H., Carr, P. A. & Kim, P. S. Inhibiting HIV-1 entry: discovery of D-peptide inhibitors that target the gp41 coiled-coil pocket. *Cell* **99**, 103–115 (1999).
104. Berg, T. *et al.* Small-molecule antagonists of Myc/Max dimerization inhibit Myc-induced transformation of chicken embryo fibroblasts. *Proc. Natl Acad. Sci. USA* **99**, 3830–3835 (2002).
First description of a small molecule that inhibits transcription-factor dimerization.
105. McBride, K. M., Banninger, G., McDonald, C. & Reich, N. C. Regulated nuclear import of the STAT1 transcription factor by direct binding of importin-α. *EMBO J.* **21**, 1754–1763 (2002).
106. Haspel, R. L. & Darnell, J. E. Jr. A nuclear protein tyrosine phosphatase is required for the inactivation of Stat1. *Proc. Natl Acad. Sci. USA* **96**, 10188–10193 (1999).

Online links

DATABASES

The following terms in this article are linked online to:

Cancer.gov: http://www.cancer.gov/cancer_information/
brain cancer | breast cancer | colon cancer | head and neck cancer | hepatocellular carcinoma | Hodgkin's disease | leukaemia | lymphomas | medulloblastoma | multiple myeloma | prostate cancer | rhabdomyosarcomas | skin cancer | small-cell lung cancer

FlyBase: http://flybase.bio.indiana.edu/
Armadillo | Delta | Dishevelled | Hedgehog | *ptc* | Serrate | *smo*

LocusLink: http://www.ncbi.nlm.nih.gov/LocusLink/
A2M | *ABL* | APC | β-catenin | γ-catenin | CBP | CREB | CREM | Delta-like | E2F1 | E2F2 | E2F3 | E-cadherin | EGF | *EGFR* | FOS | Frizzled | GLI1 | GLI2 | GLI3 | GR | GSK3β | HMG1 | BCL9 | IκB | IL-1 | IL-6 | IRF3 | Jagged | JAK | JUN | JUNB | JUND | MAX | MYC | NF-κB | Notch1 | Notch2 | Notch3 | Notch4 | p53 | p300 | PDGFR | *PIAS3* | PU.1 | *RAS* | RB | SP1 | *SRC* | STAT1 | STAT3 | STAT4 | STAT5 | TCF4 | TGF-β | TNF-α | WNT

Saccharomyces Genome Database: http://genome-www.stanford.edu/Saccharomyces/
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