The initial flurry (1976–1985) in the discovery of onco-
genes — mutated genes that allow unregulated cell
growth — yielded a large number of proteins that are
involved in cell signalling1,2. Signalling pathways begin
with extracellular proteins — ligands — which bind to
specific cell-surface receptors that dimerize or oligomer-
ize 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 cells2. 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 intra-
cellular molecules that are connected to ligand-acti-
vated 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 cate-
gory. It is mutated in ~15% of all human cancers3. 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 oncogenes2.
Such proteins will be the main focus of this article.

In addition to oncogenes, tumour-suppressor genes
were discovered more recently4. 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 apoptosis5 — an event in carcinogenesis that is of
equal importance to growth dysregulation.

Appropriately enough, inhibition of oncogenic pro-
teins 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 success6. Although some clever
strategies have been devised to attempt to correct defects
that are caused by the deletion or mutation of tumour
suppressors5,6, it has remained difficult to reinstate nor-
mal cell regulation by returning missing tumour-sup-
pressor 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 goal6.

Choosing an antitumour target
Given that small-molecule inhibitors of an overactive
process are thought to be the most useful mechanism of
tumour inhibition6, what are the most logical targets?
First consideration would seem to indicate a protein or
One or more latent cytoplasmic transcription factors (such as STATs, NF-kB, 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.

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 are affected in cancer.
- 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, 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. 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.

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. In 1987, JUN was shown to be an oncogene — first, the v-jun of an oncogene, 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
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 and participate in activating transcription. Activation is brief because dephosphorylation occurs in the nucleus within 20 minutes for any given molecule. 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.

**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). 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. 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 and participate in activating transcription. Activation is brief because dephosphorylation occurs in the nucleus within 20 minutes for any given molecule. 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.**

**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, be the ‘missing protein’ in these gene-activating complexes. For example, STAT5 interacts with the glucocorticoid receptor (GR) and STAT1 interacts with GR and the transcription factors SP1 and PU.1 (Ref. 21, 22). STAT3 also activates transcription in association with other proteins. STAT3 can bind c-JUN 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 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. 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.

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. 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 Ca2+ 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. 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. 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, 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.

**SRC-LIKE**

The generic name for proteins that are similar to v-src, the oncogene of Rous sarcoma virus.
dephosphorylate nuclear STATs to allow the protein to return to the cytoplasm. 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. 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 STAT 3 is involved in transformation is persuasive. First, all SRC-transformed cell lines have persistently activated STAT 3, and dominant-negative STAT 3 blocks this transformation. Second, STAT 3 — C is 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. STAT 3 functions in normal lymphocyte development to combat apoptosis and the same is true in STAT 3-C transformed cells. So, one contribution of persistently active STAT 3 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 STAT 5 is associated with several types of leukaemia and lymphomas. One type of lymphoma depends on STAT 5 that is persistently activated because of a chromosomal translocation. When a dominant-negative STAT 5 is introduced into these tumour cells, they undergo apoptosis.

STAT 3 is persistently activated in almost all head and neck cancers due to the dysregulation of the EGF pathway. All human multiple myelomas that were tested had persistently activated STAT 3, which is caused in some, but not all, cases by the overproduction of IL-6. Introducing dominant-negative STAT 3 into cell lines derived from either of these tumours resulted in apoptosis. Hepatocellular carcinoma has been reported to have persistently active STAT 3 in association with hypermethylation, and hence suppression, of SOCS1 (one of the negative regulators of STAT activity). Finally, persistently active STAT 3 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. At present, there is no reported mutation in STAT 3 that results in persistent activation. So, the widespread occurrence of active STAT 3 and STAT 5 in cancers is probably due to the dysregulation of signalling molecules or mutations and deletions in the proteins that negatively regulate STAT 3. Logic dictates, given these conditions, that inhibition of STAT 3 as a transcriptional activator is a prime anticancer target.

Molecular targets for inhibition of persistently active STAT 3 include homodimer–homodimer interaction (tetramer formation) on DNA (Table 1), and interactions between STAT 3 and c-Jun, CBP/p300 or GR. All of these interactions are required for maximal

Table 1 | Target sites for inhibition of oncogenic transcription factors

<table>
<thead>
<tr>
<th>Transcription factors</th>
<th>Ligand-receptor</th>
<th>Receptor</th>
<th>Kinase effector</th>
<th>Protease effectors</th>
<th>Importin interaction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cytoplasm</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>STAT3,5</td>
<td>+</td>
<td>SH2–pTyr</td>
<td>JAKs RTKs</td>
<td>+++(?)</td>
<td></td>
</tr>
<tr>
<td>NF-xB</td>
<td>+</td>
<td>kB</td>
<td>IkB, p100</td>
<td>+++(?)</td>
<td></td>
</tr>
<tr>
<td>β-catenin</td>
<td>+</td>
<td></td>
<td></td>
<td>+++(?)</td>
<td></td>
</tr>
<tr>
<td>Notch (NICO)</td>
<td>+</td>
<td>Protease</td>
<td></td>
<td>+++(?)</td>
<td></td>
</tr>
<tr>
<td>GLI</td>
<td>+</td>
<td></td>
<td></td>
<td>++++</td>
<td></td>
</tr>
<tr>
<td>c-JUN</td>
<td>+</td>
<td>Enhancosomes (other transcription factors)</td>
<td>HATs Cofactors Serine kinases DNA binding</td>
<td>+++(?)</td>
<td>++++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nucleus</td>
<td>STAT-STAT, c-JUN</td>
<td>GR, ICF</td>
<td>++</td>
<td>++(?)</td>
<td>+++(?)</td>
</tr>
<tr>
<td>NF-xB</td>
<td>c-JUN</td>
<td>++</td>
<td>++(?)</td>
<td>++(?)</td>
<td>++(?)</td>
</tr>
<tr>
<td>β-catenin</td>
<td>TCF/LEF</td>
<td>+++</td>
<td>++(?)</td>
<td>++(?)</td>
<td>++(?)</td>
</tr>
<tr>
<td>Notch (NICO)</td>
<td>HLH proteins</td>
<td>+++</td>
<td>++(?)</td>
<td>++(?)</td>
<td>++(?)</td>
</tr>
<tr>
<td>GLI</td>
<td>++(?)</td>
<td>++</td>
<td>++(?)</td>
<td>++(?)</td>
<td>++(?)</td>
</tr>
<tr>
<td>c-JUN</td>
<td>++(?)</td>
<td>++</td>
<td>++(?)</td>
<td>++(?)</td>
<td>++(?)</td>
</tr>
</tbody>
</table>

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; ICF, interferon regulatory factor; NICO, notch intracellular domain; RTK, receptor tyrosine kinase; ICF/LEF, T-cell factor/lymphocyte-enhancer factor.
**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.

![Diagram of NF-κB activation](image)

**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.

**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. 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. 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 proteosome; 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. 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. 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.

The interaction between NF-κB and other proteins in the β-interferon (IFN) enhanceosome 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 HM G1(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. Active NF-κB is found in the nucleus of many different cancer cells (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. 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 processes that activate NF-κB is appealing (Table 1). The major targets that activate NF-κB are appealing (Table 1). The major targets,
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 (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 — with many more reports on β6,13 — 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)6,13. 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 factor or lymphocyte-enhancer factors) that lack a transcription-activation domain (TAD)15. β-catenin either provides a TAD16 or associates with additional nuclear factors that, together, activate transcription17–19. This latter suggestion is prompted by recent results from Drosophila showing that the wingless pathway uses two proteins (LGS/BCL9 and PYGO6,17) 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 signalling8. 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 colon10,18. 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 adenomas. A recent report indicates that a particular form of TCF1 is most often the β-catenin partner in human cancer19. Further mutagenesis in this growing cell population leads to cancer.

Overexpression and/or mutation of β-catenin was also reported in a recent study of hepatocellular carcinomas20, 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 desmoids21.

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 H edgehog 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 Drosophila60,61. 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 DNA21. The NICD has a transcriptional-activation domain and activates specific genes according to which HLH it binds22,23. The precise mechanism of transcriptional activation is complicated. One role of the NICD is to attract the HATs, CBP/p300, and possibly also

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

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

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**REL PROTEINS**

Family name for a group of proteins that have sequence similarity to the oncogene in the chicken virus (v-rel) that causes reticuloendothelial 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.
75-kDa transcriptional repressor, as a result of serine phosphorylation by protein kinase A and, subsequently, casein kinase. So, HH activation of smoo 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. GLI1 was originally discovered as an overproduced product in a glioma, and is thought to serve as a transcriptional activator. The details of how the GLI proteins activate transcription are not completely clear, although cleavage of GLI3 produces a repressor protein. 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 and of rhabdomyosarcomas, 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. 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. Many oncogenic cytoplasmic proteins feed into serine kinase cascades that end in the phosphorylation of one or more of the resident nuclear transcription factors2,3,15,23,80,81,83,84. Just as the family size of DNA-binding proteins with DNA is difficult to determine82 but requires specific phosphorylation on serine or 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 genes2,31,32,83,85. 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 proteins39,40 or by mutation41. 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 JUN3. Many dozens of overactive receptor tyrosine kinases (RTKs), ligands, non-receptor tyrosine kinases (NRTKs) and down-stream 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 oncoactive fusion proteins. Perhaps most prominent among these is MYC, which is pervasively increased in cancer37,38. MYC does not regulate transcription on its own, but dimerizes with another protein, MAX49. 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 to the retinoblastoma protein (RB, the first identified tumour-suppressor gene) 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 cells1. 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 entry86. 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 E2F38.

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 proliferation of literature of experiments that estimate mRNA profiles in cells by gene-array hybridization analysis80–84. 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 difficult87,88. 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 disruption of interaction of these specific factors with importins. There are at least six importins49 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, 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 interactions that are specific enough not to block all transcription is problematic, 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 elucidated102–104. 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 literature102,103, 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 cells223.

With the availability of robotic screening procedures, huge chemical libraries (~10^10 compounds) need to be screened in assays that might uncover small molecules that target any of the 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?

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