mutated. The current evidence for *patched* represents the first strong link between tumour suppressor genes and genes important in embryonic development. In *Drosophila*, the patched protein is a key element in the hedgehog signalling pathway[8]. Hedgehog is responsible for repressing the activity of patched, which normally represses transcription of downstream genes, ultimately resulting in relief of patched inhibition. This pathway is important in many processes including segmentation and the establishment of anterior to posterior polarity in the wing discs of a developing embryo.

In vertebrates, these proteins have been implicated in similar pathways. Theoretically, loss of patched function would result in the expression of downstream genes regardless of other important regulators such as hedgehog. Interestingly, in *Drosophila* patched may act as a regulator of its own expression. In normal human skin and fibroblasts, Gailani *et al.* found virtually no *patched* gene expression[3]. However, sporadic basal cell tumours with *patched* mutations expressed variably high levels of the message consistent with an auto feedback loop in which decreased patched protein would lead to increased transcription of the mutated products. Increased expression of mutated patched may be useful in initial studies of the frequency of genetic alterations in tumour surveys.

Patched is a large protein with a number of membrane spanning domains and two large extra cellular loops. It is interesting to speculate that the *patched* pathway may have similarities with the *APC* system and the development of FAP and sporadic adenomas. APC may also be important in cell adhesion[9] and like patched, leads to the development of relatively benign and indolent lesions. *Patched* and *APC*, unlike other tumour suppressor genes involved in controlling the cell cycle[10], are cytoplasmic proteins regulating a complex signalling cascade. The first effects of not responding appropriately to signals from neighboring cells may be abnormal adhesion and the heaping of cells resulting in small clusters of abnormally proliferating, but not deadly, cells. The initial clusters may provide an increased target population from which other genetic alterations can occur, leading to tumour progression.

Interestingly, other neoplasms also harbour indolent early precursor lesions (such as bladder papillomas) composed of heaped up cells. Despite the ubiquitous nature of p53 mutations[11], most tumour suppressor gene mutations have been quite limited to specific types of tissue. However, there are certainly other sporadic tumours that have displayed limited chromosome 9q losses including bladder cancer, oesophageal cancer and certain types of lung cancer. In addition to validating the role of this gene in the initiation of basal cell carcinoma, it would be of great interest to test for alterations in these other tumour types. Thus, the stage is set for a variety of future genetic and functional studies on the putative gatekeeper of human skin. □

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MYC family ties

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The association of *MYC* and chromosome 8 translocations with Burkitt's lymphoma (BL) remains a landmark in the molecular characterization of cancer. Although many functions have been proposed for *MYC*, the longest chain of evidence suggests a role in activating transcription of growth promoting genes. Unfortunately, the mechanism(s) by which it transactivates and the identities of MYC-regulated genes remain unclear. The paper by Sakamuro *et al.* on page 69 of this issue identifies a new MYC-interacting protein with growth inhibitory properties[1]. This new protein — BIN1 — hints at new connections between MYC and growth control, and may provide a connection between MYC and the transcription apparatus.

Some of the anatomy linking *MYC* to transcription is reasonably clear[2]. MYC–MAX heterodimers transactivate after binding to CACGTG. Protein interactions connecting MYC's transactivation domain to the basal transcription apparatus presumably then increase initiation at TATAA or initiator (INR) elements. Indeed, simple connections between these sites have already been suggested (Fig. 1). For example, the TATA binding protein (TBP) can bind to MYC *in vitro* and this interaction may be antagonized by the retinoblastoma protein (pRb) or the related pocket protein p107[3–8]. Although this model is simple and elegant, details of its *in vivo* function remain controversial. The surfaces available in the TFII-A and TFII-B–TBP–TATAA complexes for additional protein–protein interactions have recently been identified by X-ray crytstallography[9–11]. Mapping MYC binding to specific available sites in those complexes provides a new challenge to those attempting to link MYC directly to the core of the transcription apparatus.

The initiator site provides an alternative to the TATAA site for assembly of the transcription apparatus. Two paths linking MYC to repression at the INR have been suggested. A yeast two-hybrid

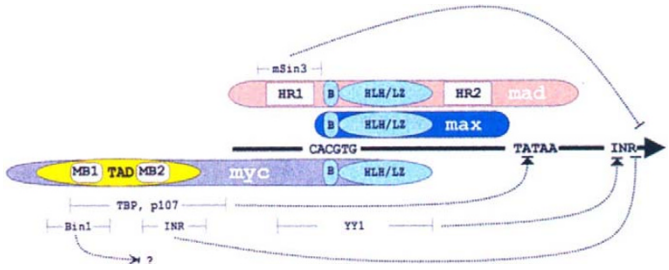

Fig. 1 Proposed protein interactions for MYC. A variety of protein targets for MYC have been identified using various interaction assays. MYC, MAX and MAD are represented schematically with MAD's homology region 1 and 2 (HR1 and HR2) designated. MYC/MAX heterodimers are transcriptionally active and exclusive of MAD. Potential interactions between MYC's transactivation domain and the basal transcription apparatus are illustrated diagramatically. MAX can form homodimers which are either silent or repress transcription. MAD/MAX heterodimers are involved in differentiation, exclude MYC and interact with the transcriptional repressor mSin3. The basic (B), helix-loop-helix (HLH) and leucine zippers (LZ) of each of the three proteins are indicated. The transactivation domain (TAD) of MYC contains conserved sequences termed Myc box 1 (MB1) and 2 (MB2). Regions of interaction with the TATAA-binding protein (TBP), p107, BIN1, the initiator region (INR), Yin Yang 1 (YY1) and mSin3 are indicated by brackets. Potential interactions with the basal transcription apparatus are indicated by dotted lines. The basic domain of MYC, MAX and MAD binds the recognition sequence CACGTG and is thereby thought to activate transcription via the TATAA or initiator (INR) sites in DNA.

screen associated the C terminus of MYC with the transcriptional regulator Yin Yang 1 (YY1)[12]. This connection might explain many of MYC's diverse roles in both activation and repression since YY1 both represses and activates at the INR. An additional intriguing connection to the INR functions either through a factor termed TFII-I or through an unknown mediator. For example, MYC represses INR-driven expression directed by the adenovirus major late promoter both *in vitro* and in transfections[13,14]. Moreover, repression of the cyclin D1 INR can be mapped to a region between amino acids 92 and 106 of MYC[15]. These interactions between MYC and the INR offer a variety of biochemical insights, but they have fallen short of meaningful connections to MYC's biological functions.

Sakamuro *et al.* have continued this search for protein interactions with MYC's transactivation domain using a yeast two-hybrid approach. Non-specific activation of transcription by MYC has generally frustrated this approach in other laboratories. However, by focusing on Myc box 1 (MB1), Sakamura *et al.* eliminated non-specific interactions. As MB1 is a hotspot for mutations in *MYC* in Burkitt's lymphomas[16], this approach was also biologically rational. Human MYC residues 47–62, empirically determined for use as specific bait, identified 19 identical clones corresponding to a new protein. This protein was named BIN1 (Box-dependent Myc-INIteracting protein-1), and it contained extensive similarity to a breast cancer-associated antigen, amphiphysin, and to a negative regulator of the cell cycle in yeast, RVS167.

The similarity of BIN1 to RVS167 implied a functional role in growth suppression. Consequently, *BIN1* expression clones were used in a standard tumorigenicity assay based on *MYC-RAS* cooperation in embryonic fibroblasts. Inhibition of focus formation by BIN1 proved to be dependent on MYC binding and a dominant negative form of BIN1 increased transformation. These data provided strong support for the authors' model. Furthermore, *BIN1* transfected into two cell lines lacking endogenous *BIN1* suppressed G418–resistant colony formation providing additional support for its function in suppressing growth.

As with many interactions identified by the yeast two hybrid approach, coimmunoprecipitation experiments did not identify a cellular complex of BIN1 and MYC *in vivo*. Consequently, an important caveat must be added; the impressive growth suppression seen in their transfection could always represent high levels of expression that are not physiologically relevant. The absence of *BIN1* from breast cancers hints at physiologic relevance but much remains to be accomplished to firmly establish *BIN1* as a tumour suppressor. Its chromosomal locus (2q14) is lost frequently in prostate tumours but cytogenetic support for the proposed linkage to breast cancer is not known.

Although the putative role of BIN1 as a tumour suppressor is of some obvious interest, a chance to further link MYC's transactivation domain to the transcription apparatus may be even more compelling. The expansion of the

Table 1 Candidate* MYC-regulated genes

Gene	Regulated	Screen	Expression	CACGTG	Refs
Adenovirus major late promoter	up and down	Candidate gene	Viral infection	Promoter	14
eIF4E (mRNA cap binding protein)	up	Candidate gene	Ubiquitous	Promoter	18,19
ECA 39	up	Subtraction library	Brain tumour, lymphoma, EC, teratocarcinoma	Exon 1	21
carbamoylphosphate synthase (cad)	up	Candidate gene	Ubiquitous	Exon 1	22
α-prothymosin	up	Subtraction library	MYC-ER, fibroblasts	Intron 1	23, 24
ornithine decarboxylase (ODC)	up	Candidate gene	Ubiquitous	Intron 1	25
CDC-25a (CDK-activating phosphatase)	up	Candidate gene	Ubiquitous	Introns	17

*These candidates were chosen because their regulatory sequences contained the MYC binding site, CACGTG. Strong supporting evidence for their candidacies include the following: Regulation by MYC-oestrogen receptor fusions (eIF4E, MrDb, α-prothymosin, ODC and cdc25A); downregulation by dominant negative MYC (cad and eIF4E); MYC-specific supershifts on EMSA (ECA39); response to specific MYC mutants (ODC, cdc25A); or a role in defining the MYC binding site (adenovirus major late promoter).

network of proteins interacting with MYC through MAX, on to MAD, and further to mSin3 provides an obvious paradigm for further studies of BIN1. The genetics that initiated the authors' approach should compel an additional search for proteins binding to BIN1, perhaps through its SH3 domain. One hopes that at the end of that particular rainbow may lie some portion of the transcription apparatus explaining MYC's ability to activate transcription.

Indeed, the authors hint that they have already looked at ornithine decarboxylase and α-prothymosin as candidate MYC targets and find that BIN1 indeed inhibits MYC-dependent transactivation. Recently, three new MYC targets (CDC25A, a cell cycle regulatory phosphatase; eIF4E, the mRNA cap binding protein; MrDb, a DEAD-box helicase) were shown to be transcriptional targets of MYC[17–20]. The search for MYC targets is rapidly expanding (Table 1). At some point the protein networks interacting with MYC will need to be linked to real activation of a real gene for us to understand MYC's role in growth.

So what is BIN1? Minimally, it is a candidate modifier of transactivation by MYC. Its interaction with MB1 is of particular interest given the frequency of MB1 mutations in Burkitt's lymphoma. It is important to remember, however, that *MYC* is a classical example of an oncogene which can also be activated simply by overexpression. Is *BIN1* a tumour suppressor gene? Certainly loss of expression of some tumour suppressors (p16) is accomplished by transcriptional loss, as suggested in the breast cancers studied by Sakamura and colleagues. However additional cytogenetic, linkage or mutation data would make an even stronger case. □

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New FISH probes — the end in sight

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The various staining techniques introduced during the 1970s enable cytogeneticists to identify all human chromosomes individually by their unique banding patterns. This technical breakthrough permitted clinical cytogenetic laboratories to diagnose numerical and gross structural chromosomal anomalies with confidence and precision. The methodology of prophase chromosome preparation introduced in 1976 by Yunis greatly improved artificial resolution; more recently, fluorescence *in situ* hybridization (FISH) techniques have brought our diagnostic ability to a new level. As new types of FISH probes become available for use in routine clinical diagnostics, such as, potentially, the new set of human telomeric probes described on page 86 of this issue[1], we can now confidently diagnose many previously unresolved and difficult cases, as well as discover new insights into the understanding of human pathology.

Using standard cytogenetic techniques, one can achieve a typical visual resolution of a single band to within 5–10 million base pairs. For cancer cytogenetic diagnosis, the challenges are to identify marker chromosomes and to determine the origin of complex rearranged chromosomes. Further, routine cytogenetic studies are labour-intensive and time-consuming, requiring fresh viable specimens. FISH technology overcomes most of these difficulties: in certain situations, it increases the resolution power of diagnosis for certain disorders, shortens the reporting time and often bypasses the culturing process to accommodate less viable specimens. Commercial availability of many different types of FISH probes has accelerated their rapid acceptance by clinical laboratories as an important adjunct study. Several different FISH probe classes are available (Fig. 1); their strengths and limitations, along with the potential use of the new telomeric probes[1], are discussed below.

Centromeric probes

Centromeric probes contain repetitive DNA sequences found in the centromeric or pericentromeric regions of human chromosomes. Because of these repetitive sequences, these probes require a short hybridization time to generate strong signals and can be utilized for both metaphase