Cell. Mol. Life Sci. 62 (2005) 2438–2459
1420-682X/05/212438-22
DOI 10.1007/s00018-005-5199-8

Cellular and Molecular Life Sciences

Review

Functional and physical communication between oncoproteins and tumor suppressors

E. V. Prochownik

Section of Hematology/Oncology, Children's Hospital of Pittsburgh, Rangos Research Center, Room 2100, 3460 Fifth Ave., Pittsburgh, Pennsylvania 15213 (USA), Fax: + 1 412 692 5228, e-mail: procev@chp.edu

Received 10 May 2005; received after revision 3 July 2005; accepted 19 July 2005
Online First 7 September 2005

Abstract. The discovery of oncogenes (c-onc's) and tumor suppressors (TS's) has led to the concept that cancer arises from defects in each of these classes of genes or their products. More recently, it has been appreciated that c-onc and TS proteins often affect one another's functions. Within this context, I review the two classical TS's, p53 and the retinoblastoma protein, and the consequences of their inactivation. The various forms of genomic instability (GI) that underly the high mutation rates of transformed cells are then discussed. Particular emphasis is placed upon the concept that GI is not only an integral part of the transformed state but is a prerequisite. Increased oxidative DNA damage, and/or an inabiliy to repair it, can lead to GI. The review then discusses recent observations showing that loss of the TS protein peroxiredoxin 1 (prdx1) and increased expression of the c-onc protein c-Myc, each leads to increased oxidative DNA damage. The critical nature of the c-onc-TS interaction is underscored by that occurring between prdx1 and c-Myc, with the former protein regulating the production of DNA-damaging reactive oxygen species by the latter. The intimate association between these proteins and others serves as a paradigm for the exquisite balancing act that c-onc's and TS's must maintain in order to properly control normal DNA replication and cellular proliferation while simultaneously minimizing the acquisition of potentially neoplastic mutations.

Key words. CIN; cyclins; E2F; Fanconi's anemia; genomic instability; MIN; Myc; peroxiredoxin; p53, Rb.

Introduction

The modern era of molecular oncology arguably began with the seminal work of Stehelin, Varmus, Bishop and Vogt [1] who demonstrated that the transforming oncogenes (v-onc genes) of replication-defective avian retroviruses were derived from highly conserved cellular sequences, now commonly referred to as c-onc genes. These initial observations were subsequently generalized to the transforming retroviruses of many other species and their distinct v-onc sequences [2.] Later work by Hayward et al. [3] demonstrated that even replication-competent retroviruses, totally devoid of v-onc sequences, induced lymphoid malignancies by recurrently integrating near and de-regulating previously recognized or novel c-onc genes. These observations unified the seemingly disparate mechanisms of retroviral transformation and provided the framework within which to explore the causes of non-retroviral-associated human cancers. The eventual outcome was the realization that many of the c-onc genes previously identified in the retroviral context were also involved in the etiologies of these non-viral associated cancers. It was shown, for example, that DNAs from certain sporadic human cancers harbored point mutations in the same *K-RAS* or *H-RAS* genes first described in retroviruses [4]. Subsequent work showed that not only the same genes but, indeed, the same point mutations were associated with chemical carcinogen-induced murine mammary tumors [5]. Certain sporadic human malignancies, such as Burkitt's lymphoma and chronic

myelogenous leukemia, were also shown to be invariably associated with the deregulated expression of c-onc genes previously identified in retroviruses, namely *C-MYC* and *C-ABL*, respectively. However, the mechanisms leading to their activation differed in that they involved distinct chromosomal translocations. Amplification provided a third mechanism by which c-onc genes could be deregulated. For example amplification of *N-MYC* was demonstrated in approximately 25% of pediatric neuroblastomas, with its level of expression being positively correlated with advanced-stage disease and inversely correlated with survival [6]. Finally, the majority of colon cancers were shown to express abnormally high levels of c-Myc as a consequence of mutations in certain members of the Wnt-APC-β-catenin-Tcf-4 pathway, which controls *C-MYC* gene transcription [6]. Thus, within the short span of only about 20 years, a wide assortment of experimental and naturally occurring malignancies were shown to bear common molecular signatures.

Growth regulation, however, is much too fundamental and complex a process to be subject only to the positive control of c-onc genes. Indeed the existence of negative growth controls (anti-oncogenes or tumor suppressors [TS's]) had been postulated well before the characterization of the first v-onc or c-onc genes. Evidence supporting the existence of TS's was at first indirect in that it demonstrated the transformed phenotype to be often recessive and suppressible by fusion with a normal cell [7]. This fit well with the pioneering work of Knudson [8], who attempted to explain the causes of sporadic and familial forms of retinoblastoma (Rb) based on a single, unifying etiology. He postulated that, in the sporadic form of Rb, loss or inactivation of both alleles of a recessive suppressor gene were necessary for the initiation of tumorigenesis. Hence the rarity of the disease, its unifocal nature and its relatively late age of onset. In contrast, familial Rb would result from the germ-line inheritance of an already inactived allele. Only a single additional mutation involving the remaining normal allele would thus be necessary for the disease to develop. Such tumors would arise with high penetrance, would often be multifocal and would occur early. This two-hit model was also potentially applicable to other constitutional cancer syndromes such as familial breast cancer, hereditary nonpolyposis colo-rectal cancer (HNPCC) and Li-Fraumeni syndrome. Implicit in this model was the belief that inactivation of distinct TS's might underly these various cancers. An even more radical notion was that TS's might play a role in the sporadic cancers that constitute the vast majority of human malignancies.

The 3 decades that have elapsed since the seeds of the TS hypothesis were sown have witnessed an explosion of research in this area and affirmation of these ideas. Direct evidence for TS's was first provided with the molecular cloning of complementary DNAs for p53 and Rb [9, 10]. Ironically, p53 was initially believed to be a c-onc gene by virtue of its ability to cooperate with mutant ras genes in transforming primary rodent fibroblasts [10, 11]. It was eventually appreciated, however, that the cDNA harbored a point mutation, and subsequent studies showed unequivocally that the wild-type version of the molecule did in fact function as a TS (reviewed in [12]). Following these landmark discoveries, a large number of additional TS's, now rivaling in number the known c-onc genes, have been described.

The realization that two quite distinct classes of genes were involved in tumorigenesis raised a number of fundamental questions. Did all tumors demonstrate both c-onc activation and TS inactivation? If so how many such genes were involved? What functions were controlled by each class of proteins? Did they interact with or otherwise communicate with one another, and if so, how, and to what end? Why were these genes recurrently altered in some tumor types but not in others? Lastly, by what mechanisms did such alterations occur? Although answers to these questions remain incomplete, many of the gaps have now been filled.

Both the c-onc and TS fields are large, dynamic and constantly evolving. As such, it is virtually impossible to provide a comprehensive survey of either area in a limited space. Rather, in this review, I concentrate on examining several representative examples of how these two fields are becoming increasingly convergent. Whereas in the not too distant past c-onc's and TS's were thought of as controlling parallel pathways toward tumor development, we now understand that their relationships are often much more intimate and that they often serve to regulate one another's functions via their direct interaction. c-onc and TS proteins must now be viewed as part of a complex molecular circuitry which ensures the integrity of critical cellular functions that often go awry in cancer. This review therefore focuses on examples where these interactions are now well established, or are less clear but nonetheless intriguing. I begin with a discussion of two TS proteins, which not only have significant historical importance but illustrate the relationships with c-onc proteins referred to above.

p53 and Rb

Many excellent recent reviews have comprehensively discussed the history and current understanding of the p53 and Rb TS proteins [13–17]. I therefore provide here only the briefest of summaries to familiarize the reader with their basic molecular biology and to illustrate how these two important TS's cross-talk with one another and with other TS and c-onc genes and proteins.

Rb, the first TS gene to be cloned [9], is actualy a member of a family that contains two relatives, p107 and

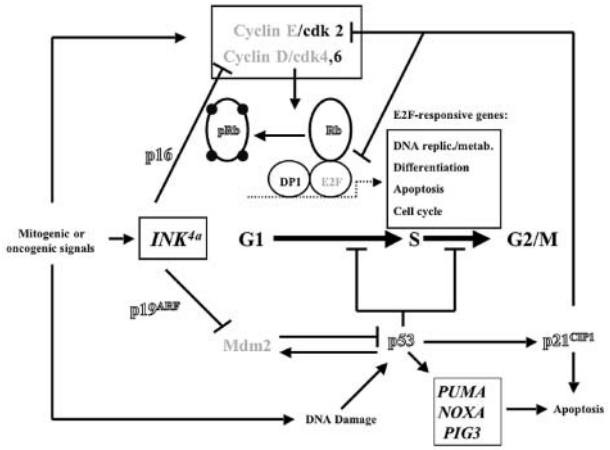

Figure 1. The Rb and p53 pathways: a paradigm for communication among c-onc and TS proteins. Mitogenic or oncogenic signals activate cyclin-dependent kinases such as cyclin D and cyclin E as well as the *INK4a* locus, which encodes p16 and $p19^{ARF}$. The final balance between these is probably determined by a combination of factors, including the nature of the stimulus and its relative strength. Cyclin activation leads to Rb phosphorylation (dark circles), resulting in the reversal of the transcriptional repression it normally exerts on E2F family members. These in turn regulate the large number of E2F-responsive genes that permit S-phase progression. The activity of cyclin D/CDK4,6 is inhibited by the p16 CDKI. Cells can progress to a transformed state as a result of cyclin D or cyclin E overexpression or loss/mutation of p16 or Rb. Oncogenic signals indirectly upregulate p53 via the induction of a second *INK4a*-encoded protein, $p19^{ARF}$. This inhibits the E3-ubiquitin ligase Mdm2, which in turns inhibits p53. DNA damage also upregulates p53, largely via $p19^{ARF}$-independent pathways. p53 in turn regulates a large number of genes whose products then collectively promote cell cycle arrest in G1 or G2/M, or apoptosis. Note that the activity of p53 is also regulated by a positive feedback loop involving the induction of Mdm2. In certain cancers, this pathway can be inactivated as a result of loss/mutation of p53, overexpression of Mdm2, or loss of $p19^{ARF}$. Note that in addition to communicating via *INK4a*-encoded proteins, the Rb and p53 pathways also communicate via $p21^{CIP1/WAF1}$, a direct positive target of p53, which is one of the mediators of p53-induced apoptosis. However, it also suppresses the transcriptional regulatory potential of E2F members and inhibits cyclin E/CDK2 activity. Proteins with proven or putative tumor suppressor function are denoted by outlined lettering, whereas those which function as oncoproteins are depicted in gray. Other p53 target genes that promote apoptosis include *PUMA, NOXA* and *PIG3.*

p130, whose functions overlap those of Rb but which are rarely mutated in human tumors. In Go and early G1 phase cells, Rb typically exists in a hypophosphorylated form in association with one of five of the seven known members of the E2F transcription factor family [16] (fig. 1). These in turn are associated with one of two related partner proteins, DP1 and DP2. These complexes regulate a large number of genes involved in cell cycle progression, DNA replication, differentiation and apoptosis [18, 19]. However, in its hypophosphorylated form, Rb suppresses the transcriptional activity of E2Fs. This occurs via several mechanisms, including the direct inhibition of the E2F transcriptional regulatory domains, the blocking of pre-initiation complex formation, the modification of chromatin structure via the recruitment of histone-modifying enzymes such as deacetylases and methyltransferases, and the alteration of E2F member half-lives [16, 20]. Not all E2Fs are equivalent, however; for example, E2Fs 1–3 have transcriptional activator activity, while E2Fs 4–6 are repressive. The affinities of E2Fs for Rb, p107 and p130 also vary. However, these distinctions are not absolute, and in many cases are based on inferences derived from overexpression studies. Nonethless, they suggest the tremendous potential of the Rb and E2F families for providing context-specific and multi-level control of cell cycle progression.

Early mitogenic signaling is accompanied by the induction of G1 and S-phase cyclins and their association with and activation of cyclin-dependent kinases (CDKs) [21]. Specifically, cyclin D1,2-CDK4/CDK6 and cyclin E-CDK2 inactivate Rb by direct phosphorylation, leading to its release from E2F-DP dimers. Their transcriptional regulatory activity now restored, E2Fs act upon the genes leading to cell cycle progression. The role of Rb in the global control of E2F activities, together with the importance of many E2F target genes in subsequent cell cycle events, may explain why Rb inactivation is much more frequently associated with naturally occurring tumors than is the activation of any single E2F member.

While mutational inactivation of Rb is common in human tumors, indirect ways to bypass this important TS also exist. These include overexpression of either of the cyclins-mentioned above, particularly cyclin D1 [22], the amplification of CDKs such as CDK4 [23] and inactivation of the cyclin-dependent kinase inhibitor (CDKI) p16, whose primary targets are cyclin D/CDK4,6 complexes [24]. Interestingly, although E2F1 itself is oncogenic in vitro [25], it has not been shown to be overexpressed in human cancers. This may be because one of the protein's direct targets is $p14^{ARF}$, the human ortholog of $p19^{ARF}$, which promotes a prompt and robust apoptosis [26].

p53, perhaps the most frequently mutated gene in human cancer, is a transcription factor dubbed the guardian of the genome by virtue of its ability to sense and respond to genotoxic and oncogenic insults and, ultimately, to prevent the perpetuation of damaged DNA [27]. The induction of p53 by these signals results in cell cycle arrest in both G1 and, to a lesser extent, in S and G2/M. Depending upon the degree of DNA damage, cells may either initiate its repair or undergo apoptosis. How this choice is made is still incompletely understoood, but is at least in part governed by the level to which p53 and its downstream target genes are induced, by specific post-translational modifications of p53 that occur in response to DNA damage and by cellular context [28].

Realization that p53 and Rb functions are inextricably intertwined derives from several lines of evidence. Among the first was the observation that the *INK4a* locus, which encodes the p16 CDKI, also encodes a second protein,

$p19^{ARF}$ (or the human ortholog $p14^{ARF}$). The latter arises via the translation of an alternate reading frame of *INK4a* that substantially overlaps with that of p16 [29]. However, unlike p16, which regulates Rb activity by inhibiting cyclin D/CDK4,6 complexes, $p19^{ARF}$ regulates p53 by inhibiting Mdm2 (or the human ortholog Hdm2), a RING-finger E3 ubiquitin ligase, which targets p53 for proteolysis (fig. 1).

Additional evidence linking the p53 and Rb pathways has come from studies on $p21^{CIP1/WAF1}$, whose gene was the first identified p53 target [30]. As a consequence of its interaction with proliferating cell nuclear antigen (PCNA), $p21^{WAF1/CIP1}$ inhibits DNA replication and cell cycle progression. This function is thus in accord with the known cell cycle effects of p53. However, it was subsequently shown that $p21^{WAF1/CIP1}$ also acts as a CDKI and specifically targets cyclinE/CDK2 complexes [31]. This activity results in the accumulation of hyposphorylated Rb and in a further block to cell cycle progression that likely cooperates with the p16-mediated block of cyclin D/CDK4,6 [32]. Finally, to ensure that the block to cell cycle progression is complete, $p21^{CIP1/WAF}$ also interacts with and directly inhibits transcriptional regulation by E2F proteins [32].

In a manner that evokes the loss of Rb control resulting from cyclin D1 overexpression or p16 loss, some tumors inactivate p53 indirectly rather than through direct mutation. One such example is seen in soft tissue sarcomas, which often expresss high levels of Mdm2, thus effectively ablating the p53 response [23]. Similarly, in some experimental tumors, loss of 19^{ARF} is oncogenic at least in part because it leads to an inability to quench Mdm2 [12, 33]. Although inactivation of $p14^{ARF}$ in human tumors has been reported infrequently, recent evidence indicates that the c-onc protein Pokemon, which is frequently overexpressed in human tumors, specifically interacts with and downregulates the $p14^{ARF}$ promoter [34].

Both cyclin D and cyclin E can be classified as c-onc genes by virtue of their ability to promote transformation in vitro and by the fact that they are often overexpressed and/or otherwise deregulated in naturally occurring cancers [35, 36]. However, a rather novel way of utilizing one of these to sabotage both the Rb and p53 pathways in some cases of breast cancer has recently come to light. Wingate et al. [37] have described novel forms of cyclin E, which have been proteolytically clipped of their N-terminal 40–45 amino acids. As a result of this truncation, cyclinE-CDK2 complexes are highly resistant to inhibition by p21 as well as by $p27^{KIP1}$, an alternate cyclin E-CDK2 CDKI. In fact, the affinity of this latter CDKI for the truncated cyclin E is actually higher than for the full-length molecule. This suggests that $p27^{KIP1}$ sequestration may contribute independently to the activation of cyclin E.

The above-described multi-faceted control of the cell cycle by Rb and p53 underscores the fine tuning and coordination of the complex machinery that is mandatory for the orderly and error-free initiation and progression of DNA replication and mitosis. Yet despite the specificity and redundancy with which these networks operate, and despite the presumed fail-safe mechanisms embedded within them, there remain chinks in this molecular armor that cancer cells have exploited. As illustrated above, the study of cancer-associated mutations has provided insights into the most critical nodal points in these pathways. They have also enhanced our appreciation of the relationships these pathways share with some of the c-onc proteins that are deregulated in cancer cells.

So many mutations, so little time

One of the central tenets of molecular oncology is that most tumors arise as a consequence of their having sustained multiple genetic mutations. Some of the first experimental evidence in support of this concept came from the work of Weinberg and his colleagues who first demonstrated that in vitro transformation of primary rodent fibroblasts required the cooperation of at least two oncogenes (for example c-Myc and ras) [11]. In vivo confirmation of these findings was soon provided by Leder's group, which showed that female transgenic mice, with mammary gland-directed expression of c-Myc or v-Ha-ras, developed monoclonal tumors only after long latency periods, whereas interbreeding of the two transgenic strains resulted in the accelerated appearance of multiple tumors [38, 39]. It is important to appreciate that, even in the latter case, only rare mammary cells actually became transformed and that the latency period, while shortened, still remained. Both studies suggested that additional, stochastically acquired events were necessary to generate a tumor, even when two c-onc genes were co-expressed.

A second line of evidence to support the multi-hit hypothesis of cancer derived from the molecular evaluation of human colo-rectal carcinoma surgical specimens. Pioneered primarily by Vogelstein and Kinzler and their colleagues [40], these studies have provided evidence that as many as seven distinct and sequential mutations, accumulating over a span of years to decades, are necessary for the development of a malignant tumor whereas fewer are necessary for the development of precursor polyps [41].

The above conclusions are consistent with more recent studies that have attempted to define the minimal number of genetic elements necessary to transform primary human cells in vitro. For reasons that remain unclear, human cells are considerably more resistant to in vitro transformation than rodent cells and may require as many

as four to six individual events. These include the overexpression of oncogenes such as ras, the inactivation of tumor suppressors such as Rb and p53, an immortalizing signal such as the activation of telomerase and other as yet poorly defined events [42].

Taken at face value, all of the above studies are in direct conflict with a large body of work showing that the mutation rate of normal human cells is much too low to account for the accumulation of this number of genetic alterations over a single individual's lifetime. Thus, it has been proposed that one of the earliest events in the generation of a transformed cell is an enhanced genomic plasticity or instability that greatly accelerates the mutation rate [43]. Many rare familial cancer syndromes, such as Fanconi's anemia, Bloom's syndrome, ataxia-telangiectasia and Nijmegen breakage syndrome have now been shown to be associated with high mutation rates and defects in specific DNA repair pathways, thus providing strong evidence for the central role of genomic instabiity in some cancers [12, 44].

The so-called mutator hypothesis, which explains how cells develop a hypermutable genome, and thus a predisposition to transformation, was originally applied to genes whose products control the fidelity of DNA replication, such as polymerases [43]. More recently, the concept has expanded to embrace genes whose products regulate levels of intracellular mutagens and recognize and repair DNA and chromosomal damage [45]. Cells with a mutator phenotype need not be initially transformed. Nor, theoretically, is transformation even an inevitable consequence of the phenotype. Rather, the mutator phenotype provides for a rate of mutational acquisition that vastly increases the probability that the correct number and mix of mutated c-onc's and TS's, necessary for transformation, will accumulate within the host's lifetime. The mutator hypothesis also explains many additional aspects of tumor cell behavior such as the ability to adapt to the hostile hypoxic and acidotic tumor micro-environment, the ability to develop resistance to chemotherapeutic drugs, and the ability to invade and metastasize. Finally, the mutator hypothesis implies that, at the cellular level, tumors are molecularly heterogeneous, with new mutations arising constantly, randomly and independently of those accumulating in neighboring tumor cells. The vast majority of such mutations would be of no consequence or even detrimental. Those mutations which did confer a survival or proliferative advantage might eventually come to constitute a majority of the tumor cell population, but only under the proper selective circumstances. For example, cells with enhanced ability to catabolize or expel cytotoxic drugs or to survive a hypoxic environment would only be selected for if chemotherapy were administered or if the tumor began to outgrow its blood supply. What is now appreciated is that genes that control genomic stability are in fact TS's, but of a type that is distinct from classical TS's such as Rb and p53. The latter have been termed gatekeepers to denote their function in limiting DNA replication or mitosis until genomic damage can be repaired. In contrast, the former genes have been dubbed caretakers by virtue of their function to maintain genomic integrity [46].

In the following sections, I discuss several forms of genomic instability, some of which have only just recently been appreciated. In keeping with the overall theme of this review, emphasis is placed upon how c-onc and TS proteins communicate with one another and contribute to these properties.

Forms of genomic instability

As mentioned previously, the mutator hypothesis was developed as a way around the fact that, when combined with its intrinsic proofreading activity, the heritable mutation rate of DNA polymerase-δ, the major eukaryotic DNA polymerase, is extraordinarily low (on the order of 10^{-9}–10^{-10} per generation) [43, 45]. Although transgenic mice expressing a proofreading-defective mutant of the enzyme develop lymphomas and epithelial cancers [47], human DNA polymerase-δ mutations have not been described. Thus, other mechanisms must account for the high mutation rates seen in cancer.

In fact, two general types of genomic instability do occur commonly in human cancers, particularly those of epithelial origin. The first, termed microsatellite instability (MIN), involves defects in the repair of short mismatches resulting from slippage of DNA polymerase as it negotiates segments of short repeats, termed microsatellites. These generally occur as single nucleotide repeats (…AAAAAA…) or as di-tri nucleotide repeats (…ATATATAT… or …ATGATGATG…) (fig. 2). The second type of instability, chromosomal instability (CIN), occurs at the whole chromosome level and results from failure of the mitotic spindle checkpoint apparatus to ensure that the proper alignment of chromosomes has occurrred prior to telophase. CIN may also result from the presence of supernumerary centrosomes [48]. The consequences of CIN are chromosomal mis-segregation with resultant daughter cell aneuploidy and allelic imbalance. Interestingly in the case of colon cancers, MIN, which occurs with a frequency of about 15%, rarely coexist with CIN, which occurs in about 85% of cases. This suggests either that these two forms of genomic instability are mutually incompatible or that each on its own is sufficient for the requisite genomic instability leading to cancer. Although translocations, segmental duplications and deletions, and gene amplification can also be considered a form of CIN, the mechanisms giving rise to these are distinct.

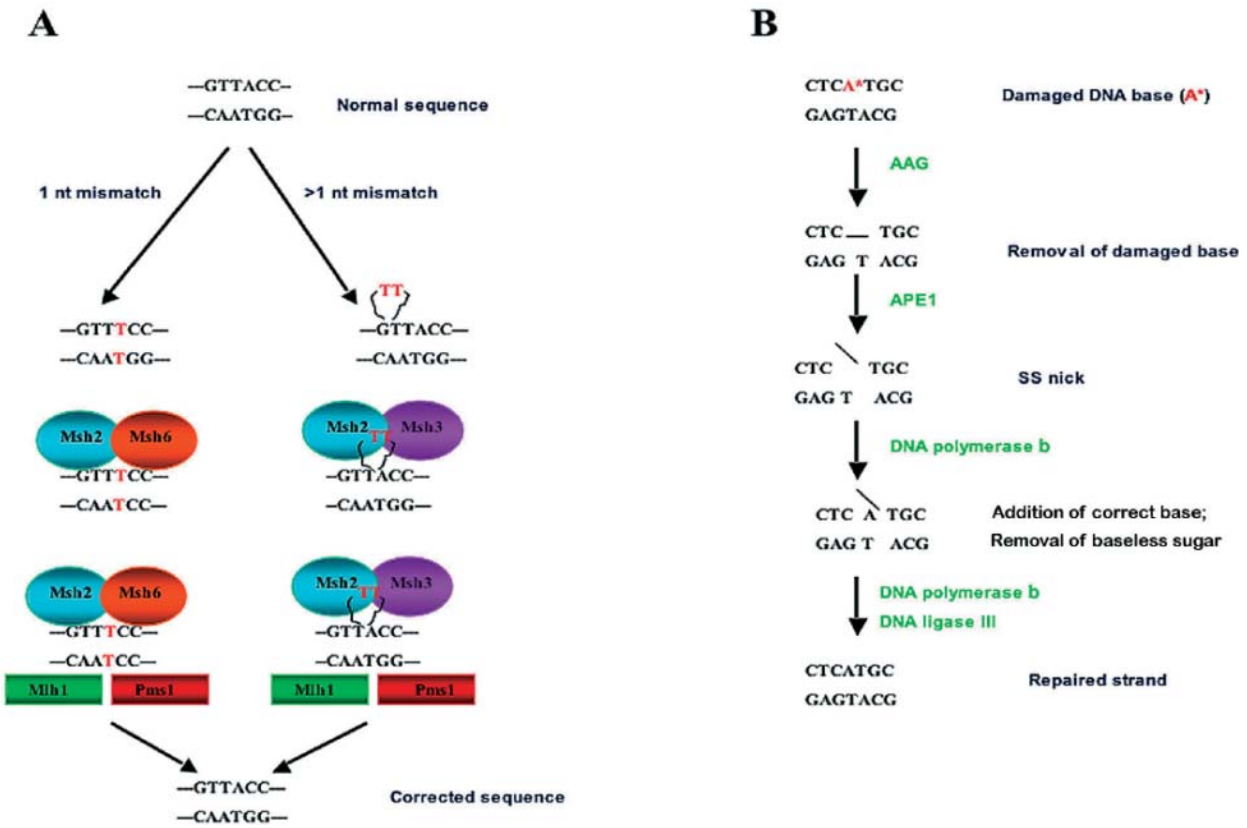

Figure 2. DNA mismatch and base excision repair systems. (*A*) DNA mismatch repair. Misincorporation of DNA bases due either to errors in DNA polmerase or to DNA slippage in microsatellite sequences are generally 1 or >1 nt in length. In either case Msh2 (turquoise) surveys and marks these mismatched regions (red). It then dimerizes with Msh6 (orange) if the mismatch involves a single base, or with Msh3 (magenta) if the mismatch is >1 nt. Mlh1-Pms1 heterodimers are then recruited to catalyze the excision process. (*B*) Base excision repair. Damage of single damaged DNA bases is recognized by AAG (3-methyladenine DNA glycosylase), which excises the base in a single step while preserving the sugar-phosphate backbone. Ape1 (apurinic/apyrimidinic endonuclease) then creates a 5' single-stranded nick, thus allowing DNA pol β to add a new, undamaged base and to remove the baseless sugar via its lyase activity. The 5' nick is then repaired by DNA ligase III.

An early start on the road to MIN

In humans, several independent lines of evidence suggest that MIN arises in pre-neoplastic tissues under certain circumstances. Brentnall et al. [49] examined cells obtained by endoscopy from the pancreatic juice of a small number of patients with pancreatitis and no evidence of pancreatic cancer. MIN was detected at two or more loci in 100% of these individuals, suggesting that it may be the response to a chronically stressed intracellular environment. Similarly, MIN was detected in the inflamed mucosa of 50% of patients with ulcerative colits (UC), which is associated with a high lifetime risk of colon cancer [50]. The incidence was nearly identical in dysplatic versus non-dysplastic tissues. In esophageal dysplasia, a precursor lesion to esophageal squamous cell carcinoma, MIN was detected in 22% of individuals with low-grade dysplasia, 33% of those with high-grade dysplasia and 59% of those with frank carcinoma [51]. The frequency with which MIN is detected in these early pre-neoplastic lesions is striking and consistent with its playing an early and important role in actual cancer pathogenesis.

The DNA mismatch repair machinery involved in MIN consists of three proteins of the mutS family (MSH2, MSH3 and MSH6) and four proteins of the mutL family (MLH1, MLH3, PMS1 and PMS2) (fig. 2). MutS members are involved in nucleotide mis-match recognition, whereas MutL proteins are involved in its repair. Single nucleotide mis-matches are recognized by complexes of MSH2 and MSH6 which then recruit the repair enzymes MLH1 and PMS1. Di- and tri-nucleotide nucleotide mis-matches are recognized by a complex of MSH2 and MSH3, which then recruits the same repair enzymes. HNPCC, which accounts for about 2% of all colo-rectal cancers, is associated with a high frequency of mutations in either MSH2 (approx. 35%) or MLH1 (approx. 60%), each of which participates in both types of repair [52]. Individuals with such germ-line mutations have an approximately 80% lifetime risk of developing colo-rectal cancer as well as a higher than average incidence of

endometrial, gastric and ovarian cancers. 10–15% of non-familial, sporadic colo-rectal cancers show loss of expression of these genes, although this usually results from epigenetic promoter hypermethylation [52].

As noted above, the vast majority of the random mutations resulting from MIN should not confer any growth advantage and indeed may even be deleterious. Tumors which do arise should thus be expected to originate from a subset of damaged cells with mutations in critical genes that regulate key tumor phenotypes. Therefore, one prediction of the mutator hypothesis is that, compared with their non-cancerous precursors, MIN tumors will more frequently contain mutations that activate c-onc genes or inactivate TS's. In fact, several of the latter have now been described, including those which inactivate the transforming-growth factor (TGF-β) receptor, the insulin-like growth factor II (IGF-II) receptor, BAX, and the *MSH3* and *MSH6* genes themselves [52].

Identifying the genes responsible for generating MIN in human cancers has facilitated the development of in vivo models to investigate their causal role directly. Biallelic knockouts of MSH2 or MLH1 in mice do in fact lead to MIN and are associated with an increased incidence of thymic lymphomas and intestinal tumors, respectively [53–55]. MSH2 –/– cells are also more sensitive to the mutagenic effects of methylating agents, most likely due to a defect in p53-dependent apoptosis [55]. Thus, at least in this experimental context, both MSH2 and MLH1 can be considered as true TS's since they meet one of the central requirements for such genes; namely, they protect against the de novo development of tumors in vivo.

MIN in chronic inflammatory diseases may evolve along somewhat different pathways involving the base excision repair (BER) system, which recognizes and replaces DNA bases that have been damaged by oxidation or alkylation. Hofseth et al. [56] showed that abnormally high levels of the enzymes AAG (3-methyladenine DNA glycosylase) and APE1 (apurinic/apyrimidinic endonuclease) could be detected in the inflammed mucosa of UC colonic epithelium. AAG normally removes single damaged DNA bases such as N^6-etheneoadenine and hypoxanthine without cleaving the sugar-phosphate backbone of the DNA strand. APE1 then creates a single-stranded nick immediately 5' to the site of the excised base. Following this, DNA polymerase-β catalyzes the replacement of the damaged base and also removes the baseless sugar that remains from the previous action of AAG. DNA ligase III then repairs the nick. Although the entire process is MutS/MutL-independent, misincorporation in microsatellite sequences can lead to MIN. While it is unclear how this occurs, it has been suggested that higher levels of these enzymes may lead to relative imbalances, thus disrupting the normal efficiency of BER [56]. These findings make some sense given that BER is involved in reversing the DNA base damage that arises from high levels of reactive oxygene species (ROS), which invariably accompany inflammatory states. They imply that MIN, arising in the earliest, pre-cancerous UC lesions, may have a different origin than the MIN of cancers originating from non-inflammed colonic mucosa. They also raise several intriguing points. First, they demonstrate how a TS could, when overexpressed, actually become a c-onc protein. Second, they show that TS dysfunction need not always involve its loss, mutation or inactivation. Finally, they suggest that variation of TS or c-onc protein levels, due for example to genetic polymorphisms, might underly individual, racial or ethnic variations in cancer incidence.

CIN

CIN occurs in the majority of human cancers, and in certain types, such as colon cancer, high-grade aneuploidy is especially common. In yeast, over 100 genes have been identified whose deregulation results in CIN [57]. Many of these have mammalian orthologs and 15% act dominantly, suggesting that they are potential c-onc genes. Others, as discussed below, are previously identified TS genes.

The obvious consequence of CIN is allelic imbalance (AI), in which the inheritance of an extra chromosome by one daughter cell is accompanied by chromosomal loss in its sister cell. The case depicted in figure 3 illustrates how this single step can give rise to daughter cells with unique molecular signatures, and distinct subsequent evolutionary pathways. For example, daughter cell A has received three copies each of a c-onc and TS gene. It would therefore be highly unlikely to evolve further by inactivating all three copies of the latter gene. However, it may already possess a growth advantage by virtue of an increased dosage of the c-onc gene. In contrast, daughter cell B is much more likely to evolve by losing or inactivating its sole remaining copy of the TS gene. However, it may be at a growth disadvantage by virtue of a reduced expression of the c-onc gene. Assuming the survival of both daughter cells, such different baseline states could dictate which events occur next and, ultimately, how the two tumor cell populations that subsequently evolve are hard-wired. Some of these events might involve common genes of high importance such as p53, whereas others might be favored as a result of the different starting molecular environments of the cells. Ultimately, the progeny of each daughter might possess quite different properties, athough all would be transformed. The degree to which each contributes to the final tumor mass would be based upon their relative rates of proliferation and upon their intrinsic abilities to survive and/or adapt to subsequent environmental challenges. This type of mechanism could explain why seemingly identical tumors from different

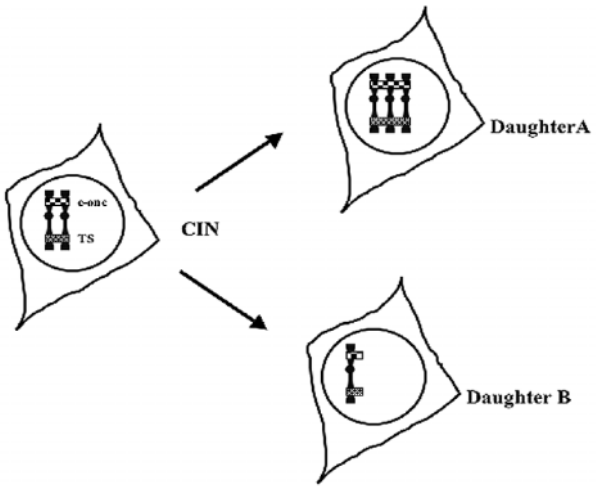

Figure 3. Theoretical tumor genotypes and phenotypes arising as a result of CIN. A normal cell, which has just acquired a mutation leading to CIN, is shown at the left of the diagram. Maternal and paternal copies of a single chromosome are shown. Each contains a hypothetical c-onc gene (c-onc: checkered box) and tumor suppressor gene (TS: stippled box). As a result of CIN, duplicated chromosomes are distributed unequally between daughter cells. In order for daughter cell A to evolve into a malignant clone, it would need to lose or inactivate one copy of the TS and to amplify or otherwise upregulate its c-onc gene. In contrast, in order for daughter cell B to evolve into a malignant clone, it might not need to further amplify c-onc-A gene but would need to sustain three independent inactivating events of TS, which is highly unlikely. A much more likely scenario for cell B is to inactivate both alleles of another TS located on a different chromosome. Thus, the subsequent events needed to become a fully malignant clone would be different and may occur at different rates in the two cell types. Depending upon the similarities of the ultimate genotypes, the actual tumor cells might differ in appearance, behavior or gene expression profiles.

individuals possess different biological properties, why a single tumor can show histologic variability, and why seemingly identical tumors and even individual cells from the same tumor can possess different molecular signatures.

The road to CIN: an equally early start?

How early does CIN arise, what are the mechanisms generating it, and what is the evidence that it is causally related to cancer? In answer to the first question, it appears that, at least in some cases, CIN, like MIN, can arise extremely early. In one study from the Vogelstein laboratory [58], the authors examined 32 small (1–3 mm) colonic adenomas. AI at the adenomatous polyposis coli (APC) locus on chromosome 5q was detected in 55% of tumors. At four other loci tested, the incidence of AI ranged from 10 to 28%. At least one locus other than 5q showed AI in 67% of tumors. Similarly, in a study of uterine cervical dysplasia, Luft et al. [59] demonstrated LOH at multiple chromosomal loci in 50% of high-grade dysplasias. All affected cases were also positive for human papilloma virus, suggesting a possible role for this agent in the initiation of CIN. Perhaps the well-known inactivation of p53, mediated by the virally encoded E6 protein, creates a permissive environment for CIN by preventing apoptosis of chromosomally imbalanced cells. This combination could prove particularly deleterious as it would involve defects in both the gatekeeper and caretaker functions referred to earlier in this review. Finally, Rubin et al. [60] demonstrated the presence of aneuploidy in a significant fraction of mucosal biopsies obtained from individuals with UC and correlated this with the eventual development of dysplasia or frank carcinoma. Although the studies to date remain few and the total cases studied small, they nonetheless emphasize that, like MIN, CIN may occur exceedingly early in tumor cell evolution. Clearly, it will be important not only to corroborate these findings but to establish their generality.

Although the molecular defects leading to CIN are still emerging, it is now generally appreciated that numerical or structural chromosomal defects arise from failure of the mitotic spindle checkpoint (MSC). This surveillance mechanism oversees the proper alignment of chromosomes on the mitotic plate and their attachment to the centromere via microtubles. Its ultimate purpose is to ensure the proper and orderly partitioning of the full chromosomal complement to each daughter cell at the completion of mitosis. Any chromosomal misalignment or asymmetry during this critical process triggers an assortment of molecular alarms designed to freeze mitosis until any lag or malpositioning has been corrected. Failure to do so results in apoptosis, which is largely, although not exclusively, p53-dependent.

Much of the work on CIN has focused on the proteins that bind to the centromere and form what has been termed the kinetochore. One of the major kinetochore components is the anaphase-promoting complex/cyclosome (APC/C), which should not be confused with the adenomatous polyposis coli (APC) protein discussed earlier. APC/C is composed of over a dozen subunits, including a novel ubiquitin ligase and a co-activator, cdc20 [61]. In metaphase cells whose sister chromatids are properly aligned and attached to the mitotic spindle, the major role of APC/C is to initiate a cascade of events culminating in the orderly and equal partitioning of the duplicated chromosomes to each daughter cell. APC/C also binds to the kinetochores of misaligned or improperly attached chromosomes with much different consequences [61–63]. In this case, the complex acts as a recipient of signals from the MSC, which conveys the message that mitosis must halt and not resume until proper chromatid positioning and attachment to the mitotic spindle has been completed [63]. Under these conditions, APC/C initiates the signal for this stop program, the components of which have been characterized over the past several years. As a result

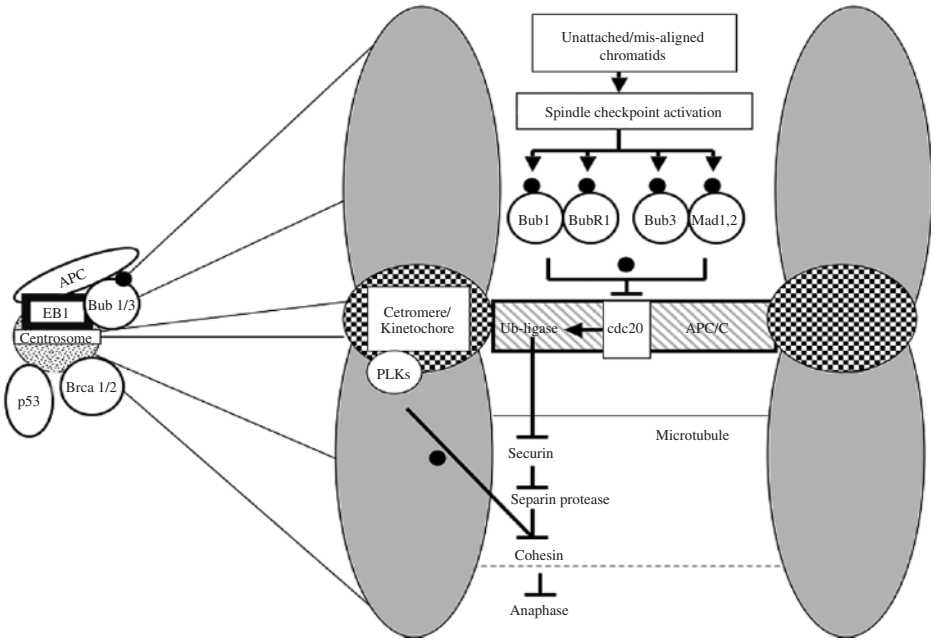

Figure 4. The mitotic spindle checkpoint. The cartoon depicts a pair of metaphase chromosomes attached by the microtubule network (thin solid lines) to one of a pair of centrosomes. As depicted at the top of the figure, misaligned or unattached sister chromatids activate the spindle checkpoint, resulting in the phosphorylation-dependent activation (dark circles) of various members of the Bub and Mad family of kinases. These are then recruited to the kinetochore where, through a series of additional phosphorylation reactions, they inhibit cdc20, a co-activator of the kinetochore-associated anaphase promoting complex/cyclosome (APC/C) and its associated ubiquitin-ligase activity. Failure to ubiquitinate securin blocks separase and its proteolytic degradation of cohesin (dashed line), a molecular glue that prevents chromatid separation and anaphase progression. Polo kinases (PLKs) associated with the kinetochore independently phosphorylate cohesin, making it more sensitive to separase and providing a means by which its rate of degradation can be controlled. Proper positioning of chromatids along the spindle and the exertion of appropriate tension along microtubule network inactivates the checkpoint, thus ultimately allowing for anaphase to proceed. Congenital or acquired mutations in members of the Bub and Mad family result in a CIN phenotype and predisposition to cancer development, presumably due to the unequal partitioning of sister chromatids to daughter cells. Proteins associated with the centrosome include the tumor suppressors p53, Brca1, Brca2 and APC. Loss of any of the first three has been associated with centrosome amplification and aneuploidy.

of this work, new roles for previously known TS's have been appreciated and potentially novel TS's have been identified (fig. 4).

One role for the MSC is to monitor the composition and integrity of kinetochores and the degree to which tension is exerted on sister chromatids by mitotic spindle microtubules. Imperfections in any of these or other defects in spindle dynamics result in activation of the checkpoint, the immediate result of which is the phosphorylation-dependent activation of several protein kinases, including Bub1, BubR1, Bub3, Mad1 and Mad2 [64–66]. These are then recruited to the unattached kinetochore where they form a complex with, phosphorylate and inactivate cdc20, although not necessarily with identical kinetics or stoichiometries. In the absence of the co-activator function of cdc20, the ubiquitin ligase activity of APC/C is greatly suppressed. This leads to the stabilization of securin, an inhibitor of the caspase-like separin protease (separase), whose major target is cohesin, a multi-component chromatid glue that blocks anaphase [67]. In yeast, and possibly in mammalian cells as well, degradation of cohesin at the metaphase-anaphase boundary is further enhanced by its phosphorylation by polo kinase, which also resides within the kinetochore [68, 69].

Perhaps not surprisingly, defects in certain of the above proteins can lead to CIN, both in experimental animal models and in naturally occurring human cancers. For example, haploinsufficiency of either BubR1 or Mad2 in mice leads to CIN and a tumor-prone phenotype [70, 71], and a small number of human colo-rectal cancers harbor heterozygous mutations in the *BUB1* gene [72]. The mutant proteins behave dominantly, as they promote CIN when introduced into diploid cells with wild-type *BUB1* alleles. In other human cancers with CIN, reduced expression of *MAD2* has been described and is due to promoter hypermethylation [73]. Human cells with an engineered knockout of securin developed CIN due to an inability to properly segregate sister chromatids. These cells also show defects in separase activation [67]. Interestingly, overexpression of securin, which is seen in some human tumors, is also transforming in vitro [74]. These studies imply that, at least for some components of the mitotic machinery, the distinction between c-onc and TS proteins is not always a sharp one, even for the same protein.

One of the strongest pieces of evidence for a causative role for MSC dysfunction in cancer has come from the study of individuals with mosaic variegated aneuploidy, a rare constitutional condition characterized by microcephaly, growth retardation, a predisposition to a variety of human cancers and, at the cellular level, mosaicism for chromomal gains and losses. In five such families, biallelic germ-line mutations in BubR1 have been identified [75].

The APC gene is mutated in the germ-line of individuals with familial adenomatous polyposis and Gardner's syndrome as well as in a substantial fraction of sporadic colo-rectal cancers with CIN [40, 76]. Classically, APC has been viewed as a key regulatory component of the Wnt1-β-catenin-Tcf4 pathway, which controls the transcription of numerous genes involved in cell cycle progression, most notably c-Myc. APC or β-catenin mutations can thus contribute to the pathogenesis of colorectal cancers via the upregulation of these target genes [77]. c-Myc deregulation, particularly when combined with p53 inactivation, can lead to tetraploidy, aneuploidy, and various other structural and numerical chromosomal abnormalities [78, 79].

Recently, however, two alternate ways by which APC mutations might promote CIN have been described. The first involves the finding that APC is also a component of the centrosome, which serves as a microtubule organizing center during mitosis (fig. 4). Here, APC associates with Bub1 and BubR1 and is probably an in vivo substrate of at least the former; it also interacts with another centrosome component, EB1, that is closely associated with microtubules [80–82].

Like chromosomes, the centrosome is normally replicated once per cell division. These daughter centrosomes then migrate to opposite sides of the cell where they form the spindle poles typically seen during cytokinesis. However, centrosome amplification and abnormally sized centrosomes have been described in many types of human cancers, particularly those with p53 mutations [83]. Supernumerary centrosomes, in association with abnormal chromosome segregation, are also observed in cells with APC mutations [80,81]. APC appears necessary to stabilize the insertion of microtubules into the kinetochore. Mutant APC proteins promote abnormal chromosomal segregation, probably as a consequence of unstable microtubule-kinetochore connections [80].

APC is illustrative of the dual role that certain TS's may play as both caretakers and gatekeepers. On the one hand, APC may suppress CIN via its role in centrosome and/or microtubule function, thus fulfilling its caretaker function. On the other hand, the ability to regulate cell cycle progression-related genes such as c-Myc and cyclin D1 also places APC squarely within the gatekeeper camp. Perhaps it is this dual functionality that underlies APC's frequent inactivation in colo-rectal tumors.

Because p53 loss creates a cellular environment that is more forgiving of DNA or chromosomal damage, it is not surprising that p53 defects and CIN often go hand in hand. However, a more direct role for p53 in centrosome maintenance and CIN may also exist. Fukasawa et al. [84] have shown that cells from various organs of p53–/– mice often bear multiple centrosomes and show abnormal chromosome segregation. The mechanisms by which p53 controls centrosome duplication involves $p21^{WAF1/CIP1}$ [85]. p53 localizes to the centrosome of several established and primary cell lines, although its function at this location is unclear [86]. Consistent with this finding is the fact that short-term exposure of p53–/– cells to sublethal doses of cytotoxic drugs results in CIN [87]. Ironically, this might represent a means by which the pharmacologic agents long used in cancer therapy might actually accelerate tumor evolution. The high degree of aneuploidy and centrosome amplification that commonly accompanies type 16 papillomavirus infection is also due, at least in part, to the inactivation of p53 [88]. Interestingly, the virally encoded E6 and E7 proteins also de-regulate Polo kinase, which, as discussed above, might potentially contribute to CIN via an independent mechanism involving cohesin destabilization (fig. 4). Taken together, these and previous studies suggest that, with regard to CIN, p53 affects multiple pathways controlling the cell cycle, apoptosis and centrosome function.

Some of the Brca1 familial breast cancer susceptibility protein localizes to the centrosome, along microtubules during chromatid segregation, and at the sites of microtubule insertion on chromatids [89]. These findings suggest an intimate role for Brca1 is controlling centrosome duplication and/or chromosome segregation and are consistent with the finding that Brca1-deficient tumors and primary cells often display centrosome amplification and CIN [90]. However, this remains guilt by association only, as it is not yet clear what Brca1's function is at these sites.

Human T lymphotropic virus type I (HTLV-I) is the causative agent of adult T cell leukemia and lymphoma. HTLV-I-associated malignancies display a considerable degree of aneuploidy and other complex chromosomal aberrancies, both of which are unusual for lymphoid neoplasms. Unlike the malignancies induced by other replication-competent retroviruses, which are associated with their recurrent insertion near specific c-onc genes, those caused by HTLV-I are poorly understood at the molecular level. However, the viral protein Tax appears to play an important role in this process and thus appears to be a novel c-onc gene [91, 92]. Among its other activities, Tax directly interacts with cdc20 and promotes the premature activation of the APC/C complex [93]. This is associated with the early polyubiquitination and degradation of securin and other mitotic regulators such as cyclins A and B1, both of which are components of the APC/C complex [92]. Tax-expressing T cells demonstrate delayed passage through S/G2/M and eventually become aneuploid and bi- or multi-nucleated.

The participation of the APC/C component cyclin B1 in numerical CIN was originally suggested by work demonstrating that c-Myc overexpression promotes aneuploidy in cells treated with mitotic spindle poisons, or spontaneously, and in the absence of such poisons, if p53 is concurrently inactivated. Analysis of various

cell cycle proteins demonstrated that cyclin B1 was overexpressed and its cdc2-associated kinase activity significantly elevated. The cyclin B1 promoter was subsequently shown to be a direct transcriptional target for c-Myc and a direct negative target for p53 [94]. It was subsequently established that cyclin B1 overexpression alone is sufficient for the induction of aneuploidy so long as cells are prevented from undergoing p53-dependent arrest or apoptosis, either by overexpressing dominant-negative forms of p53 or inactivating it with papilloma virus E6 protein [94]. These findings are consistent with observations that many aneuploid tumors overexpress cyclin B1 [95] and with more recent findings that overexpression of human cyclin B1 in yeast disrupts the MSC, promotes unscheduled DNA replication and ultimately leads to CIN [96]. However, the precise mechanism by which this occurs remains to be determined.

Which type of genomic instability?

What determines whether a tumor will display MIN or CIN, and how, if at all, do these two forms of genomic instability differentially contribute to tumor behavior or clinical outcome? It is known that HNPCC and sporadic colon cancers with MIN tend to arise on the right side and are generally associated with a more favorable prognosis than those with CIN, which tend to be left-sided [97–100]. One interpretation of these data is that the epithelia on the two sides of the colon are exposed to different environmental carcinogens, thus leading to distinct forms of genomic instability. Another, non-mutually exclusive possibility is that the mucosa from the two sides are somehow differentially prone to mutation of genes controlling MIN and CIN. Support for this latter interpretation has been provided by microarray analysis of MIN and CIN tumor cell lines, which has shown that they differentially express over 70 genes [101]. Whether these represent actual cell type differences or simply responses and/or adaptations to MIN or CIN remains to be determined.

Genomic instability and oxidative DNA damage

As discussed previously (fig. 2), BER provides the primary means of correcting damaged DNA bases, most of which are generated by ROS. Recent evidence supports the view that a mutator phenotype arises if cells are unable to dispose properly of these highly mutagenic species. In such cases, BER and/or other repair pathways that commonly respond to oxidative DNA damage may simply be overwhelmed. Because of the now appreciated association between chronic inflammation, ROS and preneoplastic genomic instability, it is tempting to speculate that ROS might be a trigger for MIN and CIN.

ROS are produced constantly by normal metabolic processes, acute and chronic inflammation, and exposure to exogenous toxins and radiation [102]. ROS include hydrogen peroxide (H2O2), superoxide ($O2^{-}$), and hydroxyl ($^{\cdot}OH$) radicals. These short-lived but highly reactive agents can mediate the oxidation of proteins, lipids and DNA, particularly in association with certain metal ions such as Fe^{++} [102]. The consequences of DNA damage include the chemical modification of bases with resultant mispairing [103].

Because of the potentially deleterious consequences of maintaining an oxidative environment, a number of defenses have evolved to inactive ROS. These include superoxide dismutase, which converts superoxide to H2O2; catalase and glutatione peroxidase, which convert H2O2 to water; and peroxiredoxins, which utilize thioredoxin as an electron donor and also scavenge peroxide [102]. Non-enzymatic ROS scavengers include ascorbate (vitamin C), tocopherols (vitamin E and its derivatives), glutathione, selenium and carotenoids. The number and functional redundancy of these molecules underscores the importance of ROS inactivation and the need to regulate tightly the cellular re-dox state [102, 104].

To investigate the relationship between ROS scavengers and cancer, several groups have developed knockout mouse strains for various anti-oxidant proteins. Henderson et al. [105] have shown that deficiency of the π-class glutathione-S-transferase (GST) increased the susceptibility of mice to skin tumorigenesis in response to the polycyclic aromatic hydrocarbon 7,12-dimethylbenzanthacene and the tumor promoter phorbol ester 12-O-tetradecanoylphorbol-13-acetate. Using a somewhat different approach that targeted the Nrf2 transcription factor, which is necessary for the induction of genes containing anti-oxidant response elements, Ramos-Gomez et al. [106] achieved a 50–80% knockdown of GSTs and NAD(P)H-: quinone oxidoreductase. These mice were more susceptible to the development of gastric neoplasms following treatment with benzo[a]pyrene. However, given the wide spectrum of the anti-oxidant enzyme knockdown, and their continued partial expression, it was not possible to determine which, if any, single one was responsible for the enhanced cancer susceptibility.

The above knockout models suggested that ROS scavengers can serve as tumor suppressors, albeit under quite restricted and highly contrived circumstances. More recently, however, the knockout of peroxiredoxin I (Prdx1) (formerly known as proliferation-associated gene or pag) has supported the idea that ROS may be involved in spontaneously arising cancers. *prdx1–/–* animals are indeed tumor prone [107], and their tissues contain elevated levels of 8-oxo-deoxyguanosine (8-oxo-dG) and several other damaged DNA bases such as

8-hydroxy-2'-deoxyadenosine, (5'R)-cyclo-2'-deoxyadenosine and (5'S)-cyclo-2'-deoxyadenosine [108]. The absolute and relative levels of these, however, are highly tissue-specific and do not appear to correlate in any simple manner with the cancers that develop [108]. An unexpected finding was that, with the exception of erythrocytes, the tissues of prdx1–/– animals do not actually contain elevated levels of ROS. Rather, the increased DNA damage appears to result from a re-distribution of cellular ROS such that a much higher fraction localizes to nuclei [108]. Although these studies indicate that Prdx1 is a tumor suppressor, it remains to be seen whether it, and for that matter any of the other ROS scavengers discussed above, are inactivated in human cancers. Reports of both over- and underexpression of Prdx1 in human tumors have appeared [109, 110], and the *PRDX1* gene, located on chromosome 1p34, is a candidate TS in meningioma [111]. However, to date there have been no reports of mutational or functional analyses in any of these tumors

Although ongoing ROS-mediated DNA damage on its own might be sufficient to lead to the eventual development of cancer, the role of Prdx1 may be more complex and sinister. Prdx1 associates with at least two c-onc proteins, namely, c-abl and c-Myc [112, 113]. In the former case, Prdx1 interacts with the SH3 domain of c-abl and negatively regulates the protein's tyrosine kinase activity [112]. In MEFs, c-abl also negatively regulates the *prdx1* gene, which contains an oxidative response element in its promoter [114]. In the second case, Prdx1 interacts with the MBII domain of c-Myc, which is necessary for transformation and for the regulation of some c-Myc target genes [113, 115]. Prdx1 overexpression markedly reduces in vitro clonogenicity and in vivo tumorigenicity of c-Myc-transformed fibroblasts and alters the expression of some, but not all, c-Myc target genes [108]. *prdx1–/–* MEFs are also susceptible to transformation by a ras oncogene alone, unlike wild-type rodent fibroblasts, which require the combination of c-Myc and ras [11, 108]. This suggests that the loss of Prdx1's interaction with MBII leads to c-Myc's transcriptional activation. Interestingly, de-regulation of c-Myc has been shown to generate ROS, to promote DNA damage at the chromosomal level and to interfere with double-stranded DNA break repair [79, 116]. Perhaps the interaction with Prdx1 is a means by which ROS can be locally controlled during c-Myc-regulated transcription, when a more open DNA conformation increases its susceptibility to oxidative attack. Taken together, these findings suggest that, in addition to scavenging ROS, Prdx1 may regulate the activities of two important components of intracellular signaling and oxidative stress response (e. g. c-abl and c-Myc).

One of the most abundant, stable and mutagenic products of oxidative DNA damage is 8-oxo-deoxyguanosine (8-oxo-dG). Its incorporation into DNA can lead either to correct base pairing with deoxycytosine or to incorrect pairing with deoxyadenine leading to G:C→T:A transversions following replication [44, 103]. Three separate enzymatic pathways exist to prevent or correct such mistakes. The first, MTH, removes the damaged base from the intracellular nucleotide pool before it can be incorporated into DNA. The second, 8-oxo-dG glycosyase (OGG) utilizes BER to repair 8-oxo-dG:dC pairs. The third, MYH, which is also a glycosylase, replaces the incorrect dA opposite 8-oxo-dG:dA with deoxycytidine [56]. Recently, Al-Tassan et al. [117] have described a defect in BER in a single familial colo-rectal cancer cohort without inherited APC mutations but with a variety of somatically acquired tumor mutations involving G:C→T:A transversions. These individuals were found to be compound heterozygotes for missense mutations in MYH, both of which greatly reduced the adenine glycosylase activity of the enzyme. That MYH also interacts physically with the MSH2 and MSH6 subunits of the MMR machinery (fig. 2A) further suggests that the BER and MMR systems are intertwined [118]. These findings provide reason to suspect that defects in ROS generation and scavenging, and the subsequent repair of the mutagenic lesions they engender, may underly certain naturally occurring human malignancies, particularly those that do not display evidence of MIN or CIN.

c-Myc: another paradigm for c-onc and TS cross-talk

The previous discussion has provided a number of examples of close physical and functional c-onc-TS relationships. In retrospect, such links seem obvious and sensible. The potentially high cost of ceding control over functions as basic as proliferation, survival and genomic integrity would seem to demand that c-onc and TS proteins communicate with one another in order to ensure the monitoring and integrity of these processes.

I have already touched upon the relationship between c-Myc and certain TS proteins such as Prdx1. In fact, the centrality of c-Myc in controlling a significant fraction of the cell's transcriptional repertoire [119, 120] would seem to make it a logical point for TS's to intercede. c-Myc is already widely implicated in the causation of a variety of human tumors, generally as a result of gene amplification or chromosomal translocation [6], and its requirement for normal cell survival and ontogeny beyond the early embryonic stage is well known [121]. Recent work has begun to reveal exciting hints as to how c-Myc may cross-talk with other TS pathways.

c-Myc and its cousins, N-Myc and L-Myc, are basic-helix-loop-helix-leucine zipper (bHLH-ZIP) transcription factors. In addition to the ca. 80–90 amino acid bHLH-ZIP dimerization domain, localized at their extreme

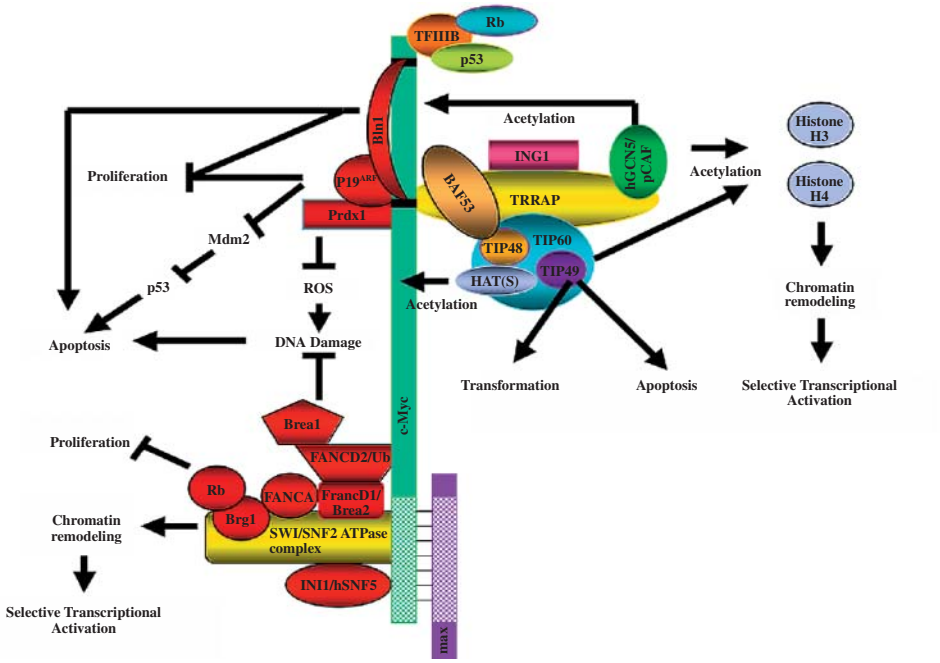

Figure 5. The interaction between c-Myc and other c-onc and TS proteins. The figure shows a schematic diagram of the c-Myc protein in association with its obligate heterodimeric partner, Max, via their HLH-ZIP dimerization domains. The C-termini of both proteins are at the the bottom of the figure. c-Myc is 439 amino acids in length and Max, which exists in two forms as a result of alternate splicing [123], is either 151 or 160 amino acids in length. The Myc Box I (MBI) and Myc Box II (MBII) domains of c-Myc, which are located within the transcriptional regulatory domain (TRD), and which are each about 20–25 amino acids in length, are depicted as black boxes near the protein's N-terminus. Several proteins have been identified which bind to MBII exclusively (e. g. Prdx1), or to MBII and other regions of the TRD (e. g. Bin1, TRRAP). Although all of these proteins are depicted as binding to MBII, it is unknown how each influences the binding of, or is dependent upon, the others. Furthermore, although depicted here as interacting with MBII, p19^{ARF}'s actual binding site in the TRD remains to be established. The largest MBII binding protein, TRRAP, serves as a nucleation center for a large number of proteins, which coordinately regulate chromatin remodeling, DNA unwinding, protein acetylation and transcriptional regulation. Although many of these proteins co-immunoprecipitate with TRRAP or c-Myc, it is not clear in all instances whether their associations with these proteins are direct (as depicted in the diagram for some) or indirect via interactions with other proteins. Putative or established TSs are depicted in red.

C-termini, all contain a ca. 150-amino acid N-terminal segment, termed the transcriptional regulatory domain (TRD), which is required for both transcriptional activation and repression. MBII, referred to in a previous section of this review, resides within the TRD between amino acids ca. 130–150. Another highly conserved region of the TRD, MBI is located between amino acids ca. 42–66 [122].

All of c-Myc's biological properties, including transformation, are dependent upon its interaction with its obligate bHLH-ZIP heterodimerization partner Max [123, 124]. Structural studies have determined that the c-Myc-Max quasi-symmetric heterodimer forms via head-to-head interactions involving multiple hydrophobic and polar contacts involving the H1 and H2 regions of the HLH domain and the entire ZIP domain [125]. Interaction of the basic domain with its cognate DNA recognition element (the E-box: CAC/TGTG) occurs via insertion into the major groove, with each monomer interacting with half of the element [125]. In addition, the DNA bound c-Myc-Max dimer can form a bivalent head-to-tail heterotetramer, resulting in the formation of an anti-parallel four-helix bundle. This may allow for the approximation of otherwise linearly distant E-boxes and the construction of a DNA-c-Myc-Max scaffold that serves as a nucleation site for various transcriptional co-activators and repressors.

Transcriptional activation by DNA-bound c-Myc-Max heterodimers involves the recruitment of a number of proteins by the c-Myc TRD. Central to this process is the interaction of MBII with the extremely large (434 kDa) ATM/PI3 kinase-related protein TRRAP [126] (fig. 5). The importance of TRRAP for transformation is emphasized by the finding that blocking it with anti-sense RNA severely impairs c-Myc's transforming ability , although not its ability to accelerate apoptosis or to restore the growth defect of c-Myc-deficient fibroblasts (126, 127). Preventing the interaction between TRRAP and MBII also impairs the transcriptional activation of some, but not all, c-Myc target genes [128].

A number of additional proteins are recruited to the c-Myc-Max-TRRAP complex, presumably through their direct interaction with the latter protein. Among these are Tip48 and Tip49, both of which possess helicase and ATPase activities and are part of the TIP60 complex [129, 130]. Several histone acetylases, among them hGCN5, H3 acetylase and H4 acetylase are also recruited by the c-Myc-TRRAP complex [131, 132]. Although the precise relationships among these proteins remains to be fully elucidated, it is currently believed that the complex

is involved in gene activation through a process involving chromatin acetylation and DNA unwinding, which presumably culminates in the enhanced access of other non-c-Myc-associated transcriptional activators and co-activators [133]. Recently, the c-Myc protein itself was shown to be a direct acetylation substrate for the Tip60 complex and hGCN5 [134]. This post-translational modification greatly stabilizes the protein and thus serves in a positive feedback capacity to ensure the persistence of gene activation. Together, enhanced chromatin relaxation and increased c-Myc longevity might be expected to augment gene expression more robustly than either one alone.

INI1/hSNF5 is a TS that is commonly mutated in atypical teratoid and rhabdoid tumors of the central nervous system and kidney and in some meningiomas [135–137]. The protein is believed to be involved in chromatin remodeling through an as yet incompletely understood ATP-dependent mechanism [138]. INI1/hSNF5 is a component of the SWI/SNF2-like ATPase chromatin remodeling complex whose human orthologs are BRG1 and BRM [139]. Using a yeast two-hybrid-based screen, Cheng et al. [140] identified c-Myc as an INI1/hSNF5-interacting protein. Interestingly, unlike the other proteins described above, INI1/hSNF5 appears to associate with c-Myc via the latter protein's bHLH-ZIP domain. Whether it also interacts with any of the other above-described TRD-interacting proteins has not been determined. The TS nature of INI1 has been confirmed using a murine conditional knockout model in which such animals are highly susceptible to the development of T cell lymphomas and, less commonly, malignant rhabdoid tumors [141].

The SWI/SNF2 complex, which contains at least seven subunits, appears to play a central role in the coordination between the functions of c-Myc and various TS's. The BRG1 component associates with hypophosphorylated Rb via a binding domain that is shared with viral oncoproteins [142]. In this capacity, it may cooperate with Rb to provide additional transcriptional repression of Rb target genes. BRG1 is mutated in some tumor cell lines [140], although it has not been determined whether these show deficiencies in Rb function as well. SWI/SNF2 also associates with the FANCA gene product [143], mutation of which underlies the most common cause of Fanconi's anemia, a hereditary disorder characterized by bone marrow failure, hypersensitivity to radiation and DNA cross-linking agents, and high cancer susceptibility. This suggests a link between BRG1, DNA damage and its repair, cell cycle arrest, and cancer. A wider implication of these findings, which remains to be tested, is that associations exist between c-Myc and other FANC members, including Brca2, recently identified as the FANCD1 protein [144, 145]. Evidence that the FANC complex may play an additional role in the repair of oxidative DNA damage [146] suggests the possibility that, like Prdx1, FANC proteins might be intimately involved in modulating c-onc proteins such as c-Myc, which generate ROS as a consequence of their deregulation [116].

$p19^{ARF}$ was recently demonstrated to associate with the c-Myc TRD [147]. Under conditions of $p19^{ARF}$ excess, c-Myc relocalizes from its normal nuclear location into the nucleolus where $p19^{ARF}$ typically resides. Conversely, when c-Myc is in excess, $p19^{ARF}$ relocates to the nucleus. The association interferes with c-Myc's ability to transcriptionally activate its target genes. Although some of the effects on gene expression might be attributable to the above-described redistribution of c-Myc, chromatin immunoprecipitation studies have indicated that at least some c-Myc-$p19^{ARF}$ remains bound to its target genes. This suggests that gene silencing by $p19^{ARF}$ may be a direct consequence of its association with the TRD and its resultant dampening of the transcriptional activation function of this domain. These effects are selective, however, as $p19^{ARF}$ does not affect c-Myc's ability to downregulate certain target genes. Indeed, the association appears to enhance c-Myc-mediated apoptosis. These results may help to explain why $p19^{ARF}$ loss commonly accompanies experimental lymphomas induced by transgenice Eμ-c-Myc overexpression [148] and why Bmi-1, which transcriptionally downregulates the $p19^{ARF}$ promoter, also cooperates with c-Myc in lymphomagenesis [149]. The co-dominant behavior of c-Myc and $p19^{ARF}$ is reminiscent of that previously described for c-Myc, Max and other Myc family members, and highlights the dynamic nature of the various c-onc-TS protein interactions that appear necessary to meet various cellular demands [150, 151].

The $p19^{ARF}$-c-Myc interaction complements the $p19^{ARF}$--| Mdm2--| p53 pathway, in which here, too, a known c-onc protein, Mdm2, physically associates with both upstream and downstream TS's. Whereas the $p19^{ARF}$-c-Myc association affects proliferation but not apoptosis [147], activation of the p19--|Mdm2--|p53 pathway ensures a potent pro-apoptotic response to c-Myc deregulation [152]. Perhaps these interactions are necessary to allow c-Myc to tread the fine line between normal levels of expression leading to cell cycle progression and the higher levels which lead to transformation or apoptosis.

Bin1 is an amphiphysin-related TS that binds to both MBI and MBII of c-Myc [153]. Several Bin1 isoforms exist due to alternate splicing [154], with the major one being able to suppress c-Myc-mediated transcriptional activation [155]. Interestingly, the c-Myc binding domain of Bin1 is dispensible for its antagonistic action against some c-Myc target genes, suggesting that it may operate via two different mechanisms. Bin1 overexpression blocks proliferation, promotes cell cycle arrest, inhibits clonogenic growth and tumorigenicity, and induces apoptosis via as yet incompletely understood pathways that neither require caspases nor are blocked by Bcl-2 over-

expression [154–156]. These effects seem quite specific for c-Myc-transformed cells as Bin1 overexpression has little effect on normal cells or those transformed by SV40 large T antigen [155, 157, 158]. This suggests the intriguing possibility that Bin1 can somehow distinguish normal from inappropriate levels of c-Myc. Greatly reduced or absent Bin1 expression occurs in a variety of primary tumors, including breast and prostate cancer and neuroblastoma [157-159]. In most cases, however, these tumors do not overexpress c-Myc. Why Bin1 should function as a TS in these cells when it exerts no effect on transformed cells in the absence of c-Myc deregulation remains a puzzle. Unfortunately, it has so far not been possible to determine whether Bin 1 functions as a true TS in vivo given that homozygous knockout mice die perinatally as a result of cardiomyopathy [160]. Better evidence for a role of Bin1 in cancer causation will likely come from studies using tissue-specific conditional knockouts and/or by examining additional human tumor material for Bin1 mutations that affect its ability to interact with c-Myc.

A number of c-Myc target gene products govern cell growth and metabolism [161]. In fact, this might be a general feature of c-onc protein deregulation. Consistent with these findings is the observation that c-Myc can control transcription by RNA polymerase III (Pol III), which is essential for the transcription of many short, essential RNA molecules such as 5S RNA, tRNAs and U6 RNAs [162, 163]. Elevated levels of these occur in many tumor cells, including those induced by oncogenic DNA and RNA viruses and chemical carcinogens. Gomez-Roman et al. [162] have found that c-Myc binds to TFIIIB, a core component of the PolII machinery, and that in doing so it enhances the rate of Pol III transcription. This association appears to involve the TRD of c-Myc, as a c-Myc deletion lacking the first 100 amino acids failed to bind to TFIIIB or to promote Pol III transcription. That c-Myc binds to the promoters of these RNA genes indirectly, via its association with TFIIIB, is consistent with the fact that these promoters do not contain consensus E-boxes.

Opposing c-Myc's positive effect on Pol III transcription are p53 and Rb, both of which also interact with TFIIIB [163]. It is not clear whether these regulatory events occur strictly via the common association of these proteins with TFIIIB or whether direct c-Myc-p53 and/or c-Myc-Rb interactions occur as well. If the latter case were true, it would be consistent with earlier reports demonstrating associations between c-Myc and Rb and c-Myc and the Rb-related protein p107 [164, 165].

c-Myc is also under negative regulatory control by a family comprising of several Max-interacting bHLH-ZIP proteins [124, 166]. The four most highly related members of the group, Mad1, Mxi1, Mad3 and Mad4, were the first Max interactors other than the Myc family to be identified. Subsequently, several other, more distantly related members of this family, Mlx, Mnt/Rox and Mga, have been described [124, 166]. In this review, I refer to these collectively as the Mad family.

Mad proteins can be viewed as potential TS's as they antagonize c-Myc function at several levels. First, they directly compete for Max, which is essential for all c-Myc functions. Second, Max-Mad heterodimers directly compete with c-Myc-Max heterodimers for E-box occupancy, although the sites to which the complexes bind are not absolutely identical. Finally, just as c-Myc-Max heterodimers recruit TRRAP and its associated proteins to promote chromatin acetylation and gene activation, Max-Mad dimers recruit histone de-acetylases, via their association with mSin3A and actively suppress transcription [124, 166].

The immediate implication of the above-described pathway is that transformation due to c-Myc overexpression results in an imbalance amongst these various proteins so as to favor c-Myc-Max heterodimers. Conversely, the inactivation of Mad members could also shift this balance without requiring any actual increase in c-Myc levels. To date, there have been some suggestions that select members of the Mad family might function as tumor suppressors, although much of the evidence has been controversial or contradictory, particularly in the case of humans.

Mouse knockout strains for Mad1, Mxi1, Mad 3 and Mnt have been created by several groups [167-170]. Certain primary cells from *mad1–/–* and *mad3–/–* animals do in fact possess properties that might be attributable to functional c-Myc deregulation. These include a reduced ability to withdraw from the cell cycle in the former case and an increased sensitivity to radiation-induced apoptosis in the latter [167,168]. However, the animals themselves appear normal and are not particularly tumor prone. *mxi1–/–* animals, on the other hand, show splenic white pulp expansion, extramedullary hematopoiesis and prostatic dysplasia, and their splenocytes are hyperproliferative in response to in vitro stimulation with anti-CD3 and anti-CD28 antibodies [169]. Primary *mxi1–/–* MEFs also have a proliferative advantage in vitro and are more susceptible to transformation by ras. Although *mxi1–/–* animals spontaneously develop lymphomas at a low incidence and after a long latency period, they are much more susceptible to the development of skin tumors and lymphomas following exposure to 9,10-dimethyl-1,2-benzanthracene and to the development of lymphomas and fibrosarcomas when cross-bred into the tumor-susceptible *Ink4a–/–* background.

More recently, the mammary gland-specific conditional knockout of Mnt has been shown to lead to the development of breast adenocarcinomas [171]. Like *mxi1–/–* MEFs, *mnt–/–* MEFs are also more transformable by ras, and show increased rates of proliferation and apoptosis [172].

The above mouse model studies indicate that loss of certain Mad family members can lead to enhanced cellular

proliferation, increased sensitivity to apoptotic stimuli and in vitro transformation, presumably as a consequence of c-Myc's functional upregulation. Less commonly, this is accompanied by increased tumor susceptibility in vivo. This apparently incomplete deregulation of c-Myc may be in part attributable to Mad family redundancy as well as to the fact that absolute c-Myc levels tend not to change. In addition, the endogenous *c-myc* gene remains subject to normal transcriptional control processes. In a case such as that of the *mxi1* knockout, tumorigenic conversion may require a more accepting background in which the Rb and p53 pathways are also compromised as a result of ink^{4a} inactivation.

Evidence for a role of Mad proteins in human cancers has been somewhat more controversial. A select subgroup of human prostate cancers with deletions of 10q24-q25 (the region to which the *MXI1* gene localizes) have been shown to contain mutations in the non-deleted *MXI1* allele in a small fraction of their tumor cells [173]. The subsequent failure of these findings to be confirmed by other groups may have been due to the fact that polymerase chain reaction (PCR) products were sequenced directly, such that mutations present in a minority of the amplified molecules would not have been detected. It was subsequently demonstrated that over half of prostate cancers with cytogenetically normal chromosome 10's nonetheless possessed submicroscopic single allele deletions of *MXI1* as determined by fluorescence in situ hybridization (FISH) [174]. As before, several such tumors harbored mutations in the non-deleted *MXI1* allele in a minority of tumor cells. Although these findings establish the high frequency with which loss of heterozygosity (LOH) at the *MXI1* locus occurs, the fate of the retained allele, and the consequences of its mutation, remains unclear.

More recently, 10q24-q25 deletions involving the *MXI1* locus have been reported in > 50% of malignant melanomas [175]. One outstanding question, not addressed in any of the studies on either prostate cancer or melanoma, is whether the non-deleted *MXI1* alleles are expressed. Lack of expression, due either to genetic mutation in non-coding regions of the gene or to epigenetic events, would be strong evidence in favor of a bona fide role for *MXI1* as a TS. Simple haploinsufficiency might be more difficult to implicate as a pathogenic lesion [176].

Taken together, the above studies provide some evidence that select Mad family members may be involved in certain types of experimental and naturally occurring tumors. At the very least, the evidence suggests that silencing of these negative regulators results in a partial activation of c-Myc such that some of its overexpression phenotypes emerge (e. g. enhanced proliferation; increased apoptosisis; and, less commmonly, transformation). Perhaps the failure to demonstrate more prominent roles for Mad members as tumor suppressors lies with their high degree of functional redundancy and the fact that overlapping expression patterns are the norm in many tissues [167].

In summary, as a ubiquitously expressed general transcription factor, c-Myc and Mad family members coordinate the regulation of a large assortment of target genes involved in the control of proliferation, growth, cell cycle progression, metabolism and apoptosis. In retrospect, it is not surprising that c-Myc and Mad proteins engage other factors, which, by virtue of their ability to modify chromatin, serve as transcriptional co-activators and co-repressors, respectively. That these responses are properly coordinated and integrated with the least amount of permanent damage is ensured by a second group of c-Myc-interacting proteins which are either bona fide tumor suppressors, candidate tumor suppressors or factors that interact with tumor suppressors. Although some of these proteins appear to serve in the same capacity as those of the first group (e. g. in chromatin remodeling and transcriptional control), they also serve distinct roles. Some appear to restrain c-Myc by limiting its transcriptional activity. Others ensure that ROS, generated as a result of unregulated c-Myc expression, are inactivated via appropriate channels. In cases where ROS generation does produce mutations, these are met with restrictions to cell cycle progression, the recognition of DNA damage and its repair, and, in cases where damage has progressed too far, with cellular demise.

The c-onc-TS balancing act

A recently described experiment of nature further supports the notion that a tumor-free state requires a fine balance between c-onc and TS proteins. Bond et al. [177] have described a common, single-nucleotide polymorphism, termed SNP309, in the first intron promoter of the *MDM2* gene. 50% of normal individuals are homozygous for a T residue at this position, 10% are homozygous for a G residue and the remainder are heterozygotes. SNP309 occurs within a closely spaced cluster of four Sp1 binding sites. In electrophoretic mobility shift assays, oligonucleotides containing the G variant bind recombinant Sp1 two-to-fourfold more avidly than those bearing the T variant. G-variant promoters are also more active in reporter assays, and cells of G/G genotype show, on average, higher levels of endogenous Mdm2 protein and lower levels of p53. G/G individuals who develop sporadic soft tissue sarcomas, or G/G Li-Fraumeni individuals with any type of malignancy, tended to develop their tumors at a significantly earlier age and, in the case of the latter group, more commonly developed multiple tumors. The simplest explanation for these findings is that higher levels of Mdm2 are more efficient at promoting the degradation of p53. In effect, G/G individuals would be Li-Fraumeni-like by virtue of this blunted

p53 response. This model makes several exciting and readily testable predictions. One is that, because many anti-cancer agents work through the p53 pathway, G/G individuals will tend to respond less well to chemotherapy. Another prediction is that *MDM2* gene amplification, which occurs commonly in sporadic soft tissue sarcomas [23], might occur more frequently in T/T individuals who will have relatively higher p53 levels and thus a greater need to suppress its actions during the course of tumor development. A third prediction is that relapse might be more frequent in GG individuals and less commonly associated with mutations in p53 coding regions.

Neuroblastoma is associated with *N-MYC* amplification in approximately 25% of cases [6]. Given that both c-Myc and N-Myc overexpression tends to promote apoptosis [178], the paucity of p53 mutations in these tumors has been somewhat puzzling [179]. At least a partial answer to this riddle may have been provided with the recent finding that *MDM2* is a positive transcriptional target for N-Myc [180], and probably for c-Myc as well [119]. It will be of interest to examine the *MDM2* genotypes of patients with both *N-MYC* amplified and unamplified tumors for SNP309 differences. Perhaps the T/T genotype is more frequent among individuals with *N-MYC*-amplified neuroblastoma, where the relative paucity of Mdm2 protein might benefit from the transcriptional boost provided by the high levels of N-Myc protein.

The Myc-p19^{ARF}-p53 axis serves to underscore once again the intimacy that exists among c-onc and TS proteins. The preceding discussion has provided evidence that p53 levels will be dictated by a variety of factors such as the levels of Myc proteins and the intrinsic sensitivity of the *MDM2* promoter.

Conclusions

The past 3 decades have witnessed a tremendous increase in our knowledge of both oncoproteins and tumor suppressors. A PubMed search performed at the completion of this review (March 2005) revealed over 13,000 references to Rb, nearly 33,000 references to p53 and nearly 15,000 references to Myc. The list goes on and on, with many of the other c-onc and TS proteins discussed here lagging behind in terms of number of references for no other reason than that their discovery and/or the recognition of their function in cancer is more recent. Several generalizations have emerged from these investigations. One is that cancer arises through a multi-step process involving the activation of c-onc genes and the inactivation of TS genes. A second is that the abnormal properties of cancer cells are a corruption of control, signaling and surveillance pathways that normally keep tight rein over such basic cellular functions as the cell cycle, apoptosis, and the recognition and repair of DNA and chromosomal damage. Third, the cancer cell is now known to be highly prone to developing new mutations at an excessively high rate. This allows for its rapid adaptation to a variety of challenging circumstances, whether they be the requirement for oxygen and nutrients, or the need to escape the toxic effects of chemotherapeutic agents. Finally, the relationship between c-onc and TS genes and proteins is one of complex cross-talk that, under normal circumstances, allows the cell to maintain rheostat-like positive and negative control over these important functions. Future endeavors will undoubtedly reveal many more surprising relationships among these different classes of genes and proteins that are likely to underscore further the functional consequences of their interactions. Despite many unanswered questions, our current knowledge is sufficiently well-developed so as to begin to provide new and novel ways to consider how these pathways can be manipulated to provide improved diagnostic and prognostic tools as well as new therapies with the high specifities and low toxicities that have heretofore eluded us.

Acknowledgements. I am grateful to a number a colleagues for their careful and thoughtful critiques of this review. In particular, I thank Steve Hann, Steve McMahon, Misha Nikiforov, George Prendergast and Tom Smithgall. Work from my laboratory was supported by NIH grants RO1-HL33741 and RO1-CA105033.

1 Stehelin D., Varmus H. E., Bishop J. M. and Vogt P.K. (1976) DNA related to the transforming gene(s) of avian sarcoma viruses is present in normal avian DNA. Nature **260:** 170–173
2 Bishop J. M. (1983) Cellular oncogenes and retroviruses. Annu. Rev. Biochem. **52:** 301–354
3 Hayward W. S., Neel B. G. and Astrin S. M. (1981) Activation of a cellular onc gene by promoter insertion in ALV-induced lymphoid leukosis. Nature **290:** 475–480
4 Weinberg R. A. (1983) Transforming genes of tumor cells. Int. Rev. Cytol. Suppl. **15:** 191–202
5 Sukumar S., Notario V., Martin-Zanca D. and Barbacid M. (1983) Induction of mammary carcinomas in rats by nitrosomethylurea involves malignant activation of H-ras-1 locus by single point mutations. Nature **306:** 658–661
6 Nesbit C. E., Tersak J. M. and Prochownik E. V. (1999) MYC oncogenes and human neoplastic disease. Oncogene **18:** 3004–3016
7 Harris H., Miller O. J., Klein G., Worst P. and Tachibana T. (1969) Suppression of malignancy by cell fusion. Nature **223:** 363–368
8 Knudson A. G. Jr (1976) Genetics and the etiology of childhood cancer. Pediatr. Res. **10:** 513–517
9 Friend S. H., Bernards R., Rogelj S., Weinberg R. A., Rapaport J. M., Albert D. M. et al. (1986) A human DNA segment with properties of the gene that predisposes to retinoblastoma and osteosarcoma. Nature. **323:** 643–646
10 Oren M. and Levine A. J. (1983) Molecular cloning of a cDNA specific for the murine p53 cellular tumor antigen. Proc. Natl. Acad. Sci. USA **80:** 56–59
11 Land H., Parada L. F. and Weinberg R. A. (1983) Tumorigenic conversion of primary embryo fibroblasts requires at least two cooperating oncogenes. Nature **304:** 596–602
12 Sherr C. J. (2004) Principles of tumor suppression. Cell **116:** 235–246
13 Nevins J. R. (2001) The Rb/E2F pathway and cancer. Hum. Mol. Genet. **10:** 699–703

14 Chau B. N. and Wang J. Y. (2003) Coordinated regulation of life and death by RB. Nat. Rev. Cancer **3:** 130–138
15 Classon M. and Harlow E. (2002) The retinoblastoma tumour suppressor in development and cancer. Nat. Rev. Cancer **2:** 910–917
16 Frolov M. V. and Dyson N. J. (2004) Molecular mechanisms of E2F-dependent activation and pRB-mediated repression. J. Cell. Sci. **117:** 2173–2181
17 Vousden K. H. (2002) Activation of the p53 tumor suppressor protein. Biochim. Biophys. Acta **1602:** 47–59
18 Kalma Y., Marash L., Lamed Y. and Ginsberg D. (2001) Expression analysis using DNA microarrays demonstrates that E2F-1 up-regulates expression of DNA replication genes including replication protein A2. Oncogene **20:** 1379–1387
19 Muller H., Bracken A. P., Vernell R., Moroni M. C., Christians F., Grassilli E. et al. (2001) E2Fs regulate the expression of genes involved in differentiation, development, proliferation and apoptosis. Genes Dev. **15:** 267–285
20 Harbour J. W. and Dean D. C. (2000) The Rb/E2F pathway: expanding roles and emerging paradigms. Genes Dev. **14:** 2393–3409
21 Sherr C. J. and Roberts J. M. (1999) CDK inhibitors: positive and negative regulators of G1-phase progression. Genes Dev. **13:** 1501–1512
22 Bates S. and Peters G. (1995) Cyclin D1 as a cellular proto-oncogene. Semin. Cancer Biol. **6:** 73–82
23 Xia S. J., Pressey J. G. and Barr F. G. (2002) Molecular pathogenesis of rhabdomyosarcoma. Cancer Biol. Ther. **1:** 97–104
24 Liggett W. H. Jr and Sidransky D. (1998) Role of the p16 tumor suppressor gene in cancer. J. Clin. Oncol. **16:** 1197–1206
25 Johnson D. G., Cress W. D., Jakoi L. and Nevins J. R. (1994) Oncogenic capacity of the E2F1 gene. Proc. Natl. Acad. Sci. USA **91:** 12823–12827
26 Adams P. D. and Kaelin W. G. Jr (1996) The cellular effects of E2F overexpression. Curr. Top. Microbiol. Immunol. **208:** 79–93
27 Lane D. P. (1992) p53, guardian of the genome. Nature **358:** 15–16
28 Vousden K. H. and Lu X. (2002) Live or let die: the cell's response to p53. Nat. Rev. Cancer. **2:** 594–604
29 Quelle D. E., Zindy F., Ashmun R. A. and Sherr C. J. (1995) Alternative reading frames of the INK4a tumor suppressor gene encode two unrelated proteins capable of inducing cell cycle arrest. Cell **83:** 993–1000
30 el-Deiry W. S., Tokino T., Velculescu V. E., Levy D. B., Parsons R., Trent J. M. et al. (1993) WAF1, a potential mediator of p53 tumor suppression. Cell **75:** 817–825
31 Bartek J. and Lukas J. (2001) Pathways governing G1/S transition and their response to DNA damage. FEBS Lett. **490:** 117–122
32 Afshari C. A., Nichols M. A., Xiong Y. and Mudryj M. (1996) A role for a p21-E2F interaction during senescence arrest of normal human fibroblasts. Cell Growth Differ. **7:** 979–988
33 Lowe S. W. and Sherr C. J. (2003) Tumor suppression by Ink4a-Arf: progress and puzzles. Curr. Opin. Genet. Dev. **13:** 77–83
34 Maeda T., Hobbs R. M., Merghoub T., Guernah I., Zelent A., Cordon-Cardo C. et al. (2005) Role of the proto-oncogene Pokemon in cellular transformation and ARF repression. Nature **433:** 278–285
35 Haas K., Johannes C., Geisen C., Schmidt T., Karsunky H., Blass-Kampmann S. et al. (1997) Malignant transformation by cyclin E and Ha-Ras correlates with lower sensitivity towards induction of cell death but requires functional Myc and CDK4. Oncogene **15:** 2615–2623
36 Ortega S., Malumbres M. and Barbacid M. (2002) Cyclin D-dependent kinases, INK4 inhibitors and cancer. Biochim. Biophys. Acta **1602:** 73–87
37 Wingate H., Zhang N., McGarhen M. J., Bedrosian I., Harper J. W. and Keyomarsi K. (2005) The tumor specific hyperactive forms of cyclin E are resistant to inhibition by p21 and p27. J. Biol. Chem, in press
38 Stewart T. A., Pattengale P. K. and Leder P. (1984) Spontaneous mammary adenocarcinomas in transgenic mice that carry and express MTV/myc fusion genes. Cell **38:** 627–637
39 Sinn E., Muller W., Pattengale P., Tepler I., Wallace R. and Leder P. (1987) Coexpression of MMTV/v-Ha-ras and MMTV/c-myc genes in transgenic mice: synergistic action of oncogenes in vivo. Cell **49:** 465–475
40 Kinzler K. W. and Vogelstein B. (1996) Lessons from hereditary colorectal cancer. Cell **87:** 159–170
41 Hanahan D. and Weinberg R. A. (2000) The hallmarks of cancer. Cell **100:** 57–70
42 Hahn W. C. and Weinberg R. A. (2002) Modelling the molecular circuitry of cancer. Nat. Rev. Cancer **2:** 331–341
43 Loeb L. A., Springgate C. F. and Battula N. (1974) Errors in DNA replication as a basis of malignant changes. Cancer Res. **34:** 2311–2321
44 Risinger M. A. and Groden J. (2004) Crosslinks and crosstalk: human cancer syndromes and DNA repair defects. Cancer Cell **6:** 539–545
45 Loeb L. A., Loeb K. R. and Anderson J. P. (2003) Multiple mutations and cancer. Proc. Natl. Acad. Sci. USA **100:** 776–781
46 Kinzler K. W. and Vogelstein B. (1997) Cancer-susceptibility genes. Gatekeepers and caretakers. Nature **386:** 761, 763
47 Goldsby R. E., Lawrence N. A., Hays L. E., Olmsted E. A., Chen X., Singh M. et al. (2001) Defective DNA polymerase-delta proofreading causes cancer susceptibility in mice. Nat. Med. **7:** 638–639
48 Kramer A, Neben K. and Ho A. D. (2002) Centrosome replication, genomic instability and cancer. Leukemia **16:** 767–775
49 Brentnall T. A., Chen R., Lee J. G., Kimmey M. B., Bronner M. P., Haggitt R. C. et al. (1995) Microsatellite instability and K-ras mutations associated with pancreatic adenocarcinoma and pancreatitis. Cancer Res. **55:** 4264–4267
50 Brentnall T. A., Crispin D. A., Bronner M. P., Cherian S. P., Hueffed M., Rabinovitch P. S. et al. (1996) Microsatellite instability in nonneoplastic mucosa from patients with chronic ulcerative colitis. Cancer Res. **56:** 12337–12340
51 Lu N., Hu N., Li W. J., Roth M. J., Wang C., Su H. et al. (2003) Microsatellite alterations in esophageal dysplasia and squamous cell carcinoma from laser capture microdissected endoscopic biopsies. Cancer Lett. **189:** 137–145
52 Chung D. C. and Rustgi A. K. (2003) The hereditary nonpolyposis colorectal cancer syndrome: genetics and clinical implications. Ann. Intern. Med. **138:** 560–570
53 Yao X., Buermeyer A. B., Narayanan L., Tran D., Baker S. M., Prolla T. A. et al. (1999) Different mutator phenotypes in Mlh1-versus Pms2-deficient mice. Proc. Natl. Acad. Sci. USA **96:** 6850–6855
54 Reitmair A. H., Schmits R., Ewel A., Bapat B., Redston M., Mitri A. et al. (1995) MSH2 deficient mice are viable and susceptible to lymphoid tumours. Nat. Genet. **11:** 64–70
55 Toft N. J., Winton D. J., Kelly J., Howard L. A., Dekker M., te Riele H. et al. (1999) Msh2 status modulates both apoptosis and mutation frequency in the murine small intestine. Proc. Natl. Acad. Sci. USA **96:** 3911–3915
56 Hofseth L. J., Khan M. A., Ambrose M., Nikolayeva O., Xu-Welliver M., Kartalou M. et al. (2003) The adaptive imbalance in base excision-repair enzymes generates microsatellite instability in chronic inflammation. J. Clin. Invest. **112:** 1887–1894
57 Spencer F., Gerring S. L., Connelly C. and Hieter P. (1990) Mitotic chromosome transmission fidelity mutants in Saccharomyces cerevisiae. Genetics **124:** 237–249
58 Shih I. M., Zhou W., Goodman S. N., Lengauer C., Kinzler K. W. and Vogelstein B. (2001) Evidence that genetic instability occurs at an early stage of colorectal tumorigenesis. Cancer Res. **61:** 818–822
59 Luft F., Gebert J., Schneider A., Melsheimer P. and von Knebel Doeberitz M. (1999) Frequent allelic imbalance of tumor sup-

pressor gene loci in cervical dysplasia. Int. J. Gynecol. Pathol. **18:** 374–380
60 Rubin C. E., Haggitt R. C., Burmer G. C., Brentnall T. A., Stevens A. C., Levine D. S. et al. (1992) DNA aneuploidy in colonic biopsies predicts future development of dysplasia in ulcerative colitis. Gastroenterology **103:** 1611–1620
61 Tan A. L., Rida P. C. and Surana U. (2005) Essential tension and constructive destruction: the spindle checkpoint and its regulatory links with mitotic exit. Biochem. J. **386:** 1–13
62 Acquaviva C., Herzog F., Kraft C. and Pines J. (2004) The anaphase promoting complex/cyclosome is recruited to centromeres by the spindle assembly checkpoint. Nat. Cell Biol. **6:** 892–898
63 Rieder C. L. and Maiato H. (2004) Stuck in division or passing through: what happens when cells cannot satisfy the spindle assembly checkpoint. Dev. Cell. **7:** 637–651
64 Taylor S. S., Hussein D., Wang Y., Elderkin S. and Morrow C. J. (2001) Kinetochore localisation and phosphorylation of the mitotic checkpoint components Bub1 and BubR1 are differentially regulated by spindle events in human cells. J. Cell Sci. **114:** 4385–4395
65 Howell B. J., Moree B., Farrar E. M., Stewart S., Fang G. and Salmon E. D. (2004) Spindle checkpoint protein dynamics at kinetochores in living cells. Curr. Biol. **14:** 953–964
66 Meraldi P., Draviam V. M. and Sorger P. K. (2004) Timing and checkpoints in the regulation of mitotic progression. Dev. Cell. **7:** 45–60
67 Jallepalli P. V., Waizenegger I. C., Bunz F., Langer S., Speicher M. R., Peters J. M. et al. (2001) Securin is required for chromosomal stability in human cells. Cell **105:** 445–457
68 Arnaud L., Pines J. and Nigg E. A. (1998) GFP tagging reveals human Polo-like kinase 1 at the kinetochore/centromere region of mitotic chromosomes. Chromosoma **107:** 424–429
69 Alexandru G., Uhlmann F., Mechtler K., Poupart M. A. and Nasmyth K. (2001) Phosphorylation of the cohesin subunit Scc1 by Polo/Cdc5 kinase regulates sister chromatid separation in yeast. Cell **105:** 459–472
70 Dai W., Wang Q., Liu T., Swamy M., Fang Y., Xie S. et al. (2004) Slippage of mitotic arrest and enhanced tumor development in mice with BubR1 haploinsufficiency. Cancer Res. **64:** 440–445
71 Michel L. S., Liberal V., Chatterjee A., Kirchwegger R., Pasche B., Gerald W. et al. (2001) MAD2 haplo-insufficiency causes premature anaphase and chromosome instability in mammalian cells. Nature **409:** 355–359
72 Cahill D. P., Lengauer C., Yu J., Riggins G. J., Willson J. K., Markowitz S. D. et al. (1998) Mutations of mitotic checkpoint genes in human cancers. Nature **1392:** 300–303
73 Jallepalli P. V. and Lengauer C. (2001) Chromosome segregation and cancer: cutting through the mystery. Nat. Rev. Cancer **1:** 109–117
74 Pei L. and Melmed S. (1997) Isolation and characterization of a pituitary tumor-transforming gene (PTTG). Mol. Endocrinol. **11:** 433–441
75 Hanks S., Coleman K., Reid S., Plaja A., Firth H., Fitzpatrick D. et al. (2004) Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat. Genet. **36:** 1159–1161
76 Vogelstein B. and Kinzler K. W. (2004) Cancer genes and the pathways they control. Nat. Med. **10:** 789–799
77 Clevers H. (2004) Wnt breakers in colon cancer. Cancer Cell **5:** 5–6
78 Yin X. Y., Grove L., Datta N. S., Long M. W. and Prochownik E. V. (1999) C-myc overexpression and p53 loss cooperate to promote genomic instability. Oncogene **18:** 1177–1184
79 Karlsson A., Deb-Basu D., Cherry A., Turner S., Ford J. and Felsher D/W. (2003) Defective double-strand DNA break repair and chromosomal translocations by MYC overexpression. Proc. Natl. Acad. Sci. USA **100:** 9974–9979
80 Fodde R., Kuipers J., Rosenberg C., Smits R., Kielman M., Gaspar C. et al. (2001) Mutations in the APC tumour suppressor gene cause chromosomal instability. Nat. Cell Biol. **3:** 433–438
81 Kaplan K. B., Burds A. A., Swedlow J. R., Bekir S. S., Sorger P. K. and Nathke I. S. (2001) A role for the Adenomatous Polyposis Coli protein in chromosome segregation. Cell Biol. **3:** 429–432
82 Berrueta L., Kraeft S. K., Tirnauer J. S., Schuyler S. C., Chen L. B., Hill D. E. et al. (1998) The adenomatous polyposis coli-binding protein EB1 is associated with cytoplasmic and spindle microtubules. Proc. Natl. Acad. Sci. USA **95:** 10596–10601
83 Emdad L., Sarkar D., Su Z. Z. and Fisher P. B. (2005) Emerging roles of centrosomal amplification and genomic instability in cancer. Front Biosci. **10:** 728–742
84 Fukasawa K., Wiener F., Vande Woude G. F. and Mai S. (1997) Genomic instability and apoptosis are frequent in p53 deficient young mice. Oncogene **15:** 1295–1302
85 Tarapore P. and Fukasawa K. (2002) Loss of p53 and centrosome hyperamplification. Oncogene **21:** 6234–6240
86 Morris V. B., Brammall J., Noble J. and Reddel R. (2000) p53 localizes to the centrosomes and spindles of mitotic cells in the embryonic chick epiblast, human cell lines and a human primary culture: an immunofluorescence study. Exp. Cell Res. **256:** 122–130
87 Bennett R. A., Izumi H. and Fukasawa K. (2004) Induction of centrosome amplification and chromosome instability in p53-null cells by transient exposure to subtoxic levels of S-phase-targeting anticancer drugs. Oncogene **23:** 6823–6829
88 Patel D., Incassati A., Wang N. and McCance D. J. (2004) Human papillomavirus type 16 E6 and E7 cause polyploidy in human keratinocytes and up-regulation of G2-M-phase proteins. Cancer Res. **64:** 1299–1306
89 Lotti L. V., Ottini L., D'Amico C., Gradini R., Cama A., Belleudi F. et al. (2002) Subcellular localization of the BRCA1 gene product in mitotic cells. Genes Chromosomes Cancer **35:** 193–203
90 Wang R. H., Yu H. and Deng C. X. (2004) A requirement for breast-cancer-associated gene 1 (BRCA1) in the spindle checkpoint. Proc. Natl. Acad. Sci. USA **101:** 17108–17113
91 Jeang K. T., Giam C. Z., Majone F. and Aboud M. (2004) Life, death, and tax: role of HTLV-I oncoprotein in genetic instability and cellular transformation. J. Biol. Chem. **279:** 31991–31994
92 Liu B., Liang M. H., Kuo Y. L., Liao W., Boros I., Kleinberger T. et al. (2003) Human T-lymphotropic virus type 1 oncoprotein tax promotes unscheduled degradation of Pds1p/securin and Clb2p/cyclin B1 and causes chromosomal instability. Mol. Cell Biol. **23:** 5269–5281
93 Liu B., Hong S., Tang, Z., Yu, H. and Giam C-Z. (2005) HTLV-I Tax directly binds the Cdc20-associated anaphase-promoting complex and activates it ahead of schedule. Proc. Natl. Acad. Sci. USA **102:** 63–68
94 Yin X. Y., Grove L., Datta N. S., Katula K., Long M. W. and Prochownik E. V. (2001) Inverse regulation of cyclin B1 by c-Myc and p53 and induction of tetraploidy by cyclin B1 overexpression. Cancer Res. **61:** 6487–6493
95 Sarafan-Vasseur N., Lamy A., Bourguignon J., Pessot F. L., Hieter P., Sesboue R. et al. (2002) Overexpression of B-type cyclins alters chromosomal segregation. Oncogene 21: 2051–2057
96 Moore J. D., Kirk J. A. and Hunt T. (2003) Unmasking the S-phase-promoting potential of cyclin B1. Science 300: 987–990
97 Iacopetta B. (2002) Are there two sides to colorectal cancer? Int. J. Cancer 101: 403–408
98 ryfe R., Kim H., Hsieh E. ., Aronson M.D., Holowaty E. ., Bull S. . et al. (2000) Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. N. Engl. J. Med. 342: 69–77
99 Hemminki A., Mecklin J. P., Jarvinen H., Aaltonen L. A. and Joensuu H. (2000) Chromosomal instability is a favorable prognostic indicator in patients with colorectal cancer receiving chemotherapy. Gastroenterology 119: 921–928

100 Elsaleh H., Powell B., McCaul K., Grieu F., Grant R. and Joseph D. et al. (2001) p53 alteration and microsatellite instability have predictive value for survival benefit from chemotherapy in stage III colorectal carcinoma. Clin. Cancer Res. **7:** 1343–1349

101 Dunican D. S., McWilliam P., Tighe O., Parle-McDermott A. and Croke D.T. (2002) Gene expression differences between the microsatellite instability (MIN) and chromosomal instability (CIN) phenotypes in colorectal cancer revealed by high-density cDNA array hybridization. Oncogene **21:** 3253–3257

102 Marnett L. J., Riggins J. N. and West J. D. (2003) Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. J. Clin. Invest. **111:** 583–593

103 Cooke M. S., Evans M. D., Dizdaroglu M.and Lunec J. (2003) Oxidative DNA damage: mechanisms, mutation and disease. FASEB J. **7:** 1195–1214

104 Loft S. and Poulsen H. E. (1996) Cancer risk and oxidative DNA damage in man. J. Mol. Med. **74:** 297–312

105 Henderson C. J., Smith A. G., Ure J., Brown K., Bacon E. J. and Wolf C. R. (•) Increased skin tumorigenesis in mice lacking pi class glutathione S-transferases. Proc. Natl. Acad. Sci. USA **95:** 5275–5280

106 Ramos-Gomez M., Kwak M. K., Dolan P. M., Itoh K., Yamamoto M., Talalay P. et al. (2001) Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. Proc. Natl. Acad. Sci. USA **98:** 3410–3415

107 Neumann C. A., Krause D. S., Carman C. V., Das S., Dubey D. P., Abraham J. L. et al. (2003) Essential role for the peroxiredoxin Prdx1 in erythrocyte antioxidant defence and tumour suppression. Nature **424:** 561–565

108 Egler, R. A., Fernandes, E., Rothermund, K., Sereika, S., deSouza-Pinho, N., Jaruga, P. et al. Regulation of reactive oxygen species, DNA damage, and c-Myc function by peroxiredoxin I. Oncogene, in press

109 Noh D. Y., Ahn S. J., Lee R. A., Kim S. W., Park I. A. and Chae H. Z. (2001) Overexpression of peroxiredoxin in human breast cancer. Anticancer Res. **21:** 2085–2090

110 Yanagawa T., Iwasa S., Ishii T., Tabuchi K., Yusa H., Onizawa K. et al. (2000) Peroxiredoxin I expression in oral cancer: a potential new tumor marker. Cancer Lett. **156:** 27–35

111 Sulman E. P., White P. S. and Brodeur G. M. (2004) Genomic annotation of the meningioma tumor suppressor locus on chromosome 1p34. Oncogene **23:** 1014–1020

112 Wen S. T. and Van Etten R. A. (1997) The PAG gene product, a stress-induced protein with antioxidant properties, is an Abl SH3-binding protein and a physiological inhibitor of c-Abl tyrosine kinase activity. Genes Dev. **11:** 2456–2467

113 Mu Z. M., Yin X. Y. and Prochownik E. V. (2002) Pag, a putative tumor suppressor, interacts with the Myc Box II domain of c-Myc and selectively alters its biological function and target gene expression. J. Biol. Chem. **277:** 43175–43184

114 Li B., Wang X., Rasheed N., Hu Y., Boast S., Ishii T. et al.(2004) Distinct roles of c-Abl and Atm in oxidative stress response are mediated by protein kinase C delta. Genes Dev. **18:** 1824–1837

115 Nesbit C. E., Tersak J. M., Grove L. E., Drzal A., Choi H. and Prochownik E. V. (2000) Genetic dissection of c-myc apoptotic pathways. Oncogene **19:** 3200–3212

116 Vafa O., Wade M., Kern S., Beeche M., Pandita T. K., Hampton G. M. et al. (2002) c-Myc can induce DNA damage, increase reactive oxygen species, and mitigate p53 function: a mechanism for oncogene-induced genetic instability.Mol. Cell. **9:** 1031–1044

117 Al-Tassan N., Chmiel N. H., Maynard J., Fleming N., Livingston A. L., Williams G. T. et al. (2002) Inherited variants of MYH associated with somatic G:C→T:A mutations in colorectal tumors. Nat. Genet. **30:** 227–232

118 Gu Y., Parker A., Wilson T. M., Bai H., Chang D. Y. and Lu A. L. (2002) Human MutY homolog, a DNA glycosylase involved in base excision repair, physically and functionally interacts with mismatch repair proteins human MutS homolog 2/human MutS homolog 6. J. Biol. Chem. **277:** 11135–11142

119 Fernandez P. C., Frank S. R., Wang L., Schroeder M., Liu S., Greene J. et al. (2003) Genomic targets of the human c-Myc protein. Genes Dev. **17:** 1115–1129

120 Patel J. H., Loboda A. P., Showe M. K., Showe L. C. and McMahon S. B. (2004) Analysis of genomic targets reveals complex functions of MYC. Nat. Rev. Cancer **4:** 562–568

121 Davis A. C., Wims M., Spotts G. D., Hann S. R. and Bradley A. (1993) A null c-myc mutation causes lethality before 10.5 days of gestation in homozygotes and reduced fertility in heterozygous female mice. Genes Dev. **7:** 671–682

122 Oster S. K., Ho C. S., Soucie E. L. and Penn L. Z. (2002) The myc oncogene: MarvelouslY Complex. Adv. Cancer Res. **84:** 81–154

123 Blackwood E. M. and Eisenman R. N. (1991) Max: a helix-loop-helix zipper protein that forms a sequence-specific DNA-binding complex with Myc. Science **251:** 1211–1217

124 Baudino T. A. and Cleveland J. L. (2001) The Max network gone mad. Mol. Cell. Biol. **21:** 691–702

125 Nair S. K. and Burley S. K. (2003) X-ray structures of Myc-Max and Mad-Max recognizing DNA. Molecular bases of regulation by proto-oncogenic transcription factors. Cell **112:** 193–205

126 McMahon S. B., Van Buskirk H. A., Dugan K. A., Copeland T. D. and Cole M. D. (1998) The novel ATM-related protein TRRAP is an essential cofactor for the c-Myc and E2F oncoproteins. Cell **94:** 363–374

127 Dugan K. A., Wood M. A. and Cole M. D. (2002) TIP49, but not TRRAP, modulates c-Myc and E2F1 dependent apoptosis. Oncogene **21:** 5835–5843

128 Nikiforov M. A., Chandriani S., Park J., Kotenko I., Matheos D., Johnsson A. et al. (2002) TRRAP-dependent and TRRAP-independent transcriptional activation by Myc family oncoproteins. Mol. Cell. Biol. **22:** 5054–5063

129 Frank S. R., Parisi T., Taubert S., Fernandez P., Fuchs M., Chan H. M. et al. (2003) MYC recruits the TIP60 histone acetyltransferase complex to chromatin. EMBO Rep. **4:** 575–580

130 Wood M. A., McMahon S. B. and Cole M. D. (2000) An ATPase/helicase complex is an essential cofactor for oncogenic transformation by c-Myc. Mol. Cell **5:** 321–530

131 McMahon S. B., Wood M. A. and Cole M. D. (2000) The essential cofactor TRRAP recruits the histone acetyltransferase hGCN5 to c-Myc. Mol. Cell. Biol. **20:** 556–562

132 Park J., Kunjibettu S., McMahon S. B. and Cole M. D. (2001) The ATM-related domain of TRRAP is required for histone acetyltransferase recruitment and Myc-dependent oncogenesis. Genes Dev. **15:** 1619–1624

133 Eisenman R. N. (2001) Deconstructing myc. Genes Dev. **15:** 2023–2030

134 Patel J. H., Du Y., Ard P. G., Phillips C., Carella B., Chen C. J. et al. (2004) The c-MYC oncoprotein is a substrate of the acetyltransferases hGCN5/PCAF and TIP60. Mol. Cell. Biol. **24:** 10826–10834

135 Biegel J. A., Tan L., Zhang F., Wainwright L., Russo P. and, Rorke L. B. (2002) Alterations of the hSNF5/INI1 gene in central nervous system atypical teratoid/rhabdoid tumors and renal and extrarenal rhabdoid tumors. Clin. Cancer Res. **8:** 3461–3467

136 DeCristofaro M. F., Betz B. L., Wang W. and Weissman B. E. (1999) Alteration of hSNF5/INI1/BAF47 detected in rhabdoid cell lines and primary rhabdomyosarcomas but not Wilms' tumors. Oncogene **18:** 7559–7565

137 Uno K., Takita J., Yokomori K., Tanaka Y., Ohta S., Shimada H. et al. (2002) Aberrations of the hSNF5/INI1 gene are restricted to malignant rhabdoid tumors or atypical teratoid/rhabdoid tumors in pediatric solid tumors.Genes Chromosomes Cancer **34:** 33–41

138. Muchardt C. and Yaniv M. (2001) When the SWI/SNF complex remodels ... the cell cycle. Oncogene **20:** 3067–3075
139 ang W., Cote J., Xue Y., Zhou S., Khavari P. ., Biggar S. . et al. (1996) Purification and biochemical heterogeneity of the mammalian SWI-SNF complex. EMBO J. **5:** 5370–5382
140 Cheng S. W., Davies K. P., Yung E., Beltran R. J., Yu J. and Kalpana G. V. (1999) c-MYC interacts with INI1/hSNF5 and requires the SWI/SNF complex for transactivation function. Nat. Genet. **22:** 102–105
141 Roberts C. W., Leroux M. M., Fleming M. D. and Orkin S. H. (2002) Highly penetrant, rapid tumorigenesis through conditional inversion of the tumor suppressor gene Snf5. Cancer Cell **2:** 415–425
142 Dunaief J. L., Strober B. E., Guha S., Khavari P. A., Alin K., Luban J. et al. (1994) The retinoblastoma protein and BRG1 form a complex and cooperate to induce cell cycle arrest. Cell **79:** 119–130
143 Otsuki T., Furukawa Y., Ikeda K., Endo H., Yamashita T., Shinohara A. et al. (2001) Fanconi anemia protein, FANCA, associates with BRG1, a component of the human SWI/SNF complex. Hum. Mol. Genet. **10:** 2651–2660
144 Venkitaraman A. R. (2004) Tracing the network connecting BRCA and Fanconi anaemia proteins. Nat. Rev. Cancer **4:** 266–276
145 Wang X. and D'Andrea A. D. (2004) The interplay of Fanconi anemia proteins in the DNA damage response. DNA Repair (Amst) **3:** 1063–1069
146 Pagano G. and Youssoufian H. (2003) Fanconi anaemia proteins: major roles in cell protection against oxidative damage. Bioessays **25:** 589–595
147 Qi Y., Gregory M. A., Li Z., Brousal J. P., West K. and Hann S. R. (2004) p19ARF directly and differentially controls the functions of c-Myc independently of p53. Nature **431:** 712–717
148 Eischen C. M., Weber J. D., Roussel M. F., Sherr C. J. and Cleveland J. L. (1999) Disruption of the ARF-Mdm2-p53 tumor suppressor pathway in Myc-induced lymphomagenesis. Genes Dev. **13:** 2658–2669
149 Jacobs J. J., Scheijen B., Voncken J. W., Kieboom K., Berns A. and van Lohuizen M. (1999) Bmi-1 collaborates with c-Myc in tumorigenesis by inhibiting c-Myc-induced apoptosis via INK4a/ARF. Genes Dev. **13:** 2678–2690
150 Yin X., Landay M. F., Han W., Levitan E. S., Watkins S. C., Levenson R. M. et al. (2001) Dynamic in vivo interactions among Myc network members. Oncogene **20:** 4650–4664
151 Grinberg A. V., Hu C. D. and Kerppola T. K. (2004) Visualization of Myc/Max/Mad family dimers and the competition for dimerization in living cells. Mol. Cell. Biol. **24:** 4294–4308
152 Vousden K. H. (2000) p53: death star. Cell **103:** 691–694
153 Sakamuro D., Elliott K. J., Wechsler-Reya R. and Prendergast G. C. (1996) BIN1 is a novel MYC-interacting protein with features of a tumour suppressor. Nat. Genet. **14:** 69–77
154 DuHadaway J. B., Lynch F. J., Brisbay S., Bueso-Ramos C., Troncoso P., McDonnell T. et al. (2003) Immunohistochemical analysis of Bin1/Amphiphysin II in human tissues: diverse sites of nuclear expression and losses in prostate cancer. J. Cell. Biochem. **88:** 635–642
155 Elliott K., Sakamuro D., Basu A., Du W., Wunner W., Staller P. et al. (1999) Bin1 functionally interacts with Myc and inhibits cell proliferation via multiple mechanisms. Oncogene **18:** 3564–3573
156 Galderisi U., Di Bernardo G., Cipollaro M., Jori F. P., Piegari E., Cascino A. et al. (1999) Induction of apoptosis and differentiation in neuroblastoma and astrocytoma cells by the overexpression of Bin1, a novel Myc interacting protein. J. Cell. Biochem. **74:** 313–322
157 Ge K., Minhas F., Duhadaway J., Mao N. C., Wilson D., Buccafusca R. et al. (2000) Loss of heterozygosity and tumor suppressor activity of Bin1 in prostate carcinoma. Int. J. Cancer. **86:** 155–161
158 Ge K., Duhadaway J., Sakamuro D., Wechsler-Reya R., Reynolds C. and Prendergast G. C. (2000) Losses of the tumor suppressor BIN1 in breast carcinoma are frequent and reflect deficits in programmed cell death capacity. Int. J. Cancer. **85:** 376–383
159 Tajiri T., Liu X., Thompson P. M., Tanaka S., Suita S., Zhao H. et al. 2003) Expression of a MYCN-interacting isoform of the tumor suppressor BIN1 is reduced in neuroblastomas with unfavorable biological features. Clin. Cancer Res. **9:** 3345–3355
160 Muller A. J., Baker J. F., DuHadaway J. B., Ge K., Farmer G., Donover P. S. et al. (2003) Targeted disruption of the murine Bin1/Amphiphysin II gene does not disable endocytosis but results in embryonic cardiomyopathy with aberrant myofibril formation. Mol. Cell. Biol. **23:** 4295–4306
161 Dang C. V. (1999) c-Myc target genes involved in cell growth, apoptosis, and metabolism. Mol. Cell. Biol. **19:** 1–11
162 Gomez-Roman N., Grandori C., Eisenman R. N. and White R. J. (2003) Direct activation of RNA polymerase III transcription by c-Myc. Nature **421:** 290–294
163 Felton-Edkins Z. A., Kenneth N. S., Brown T. R., Daly N. L., Gomez-Roman N., Grandori C. et al. (2003) Direct regulation of RNA polymerase III transcription by RB, p53 and c-Myc. Cell Cycle **2:** 181–184
164 Adnane J. and Robbins P. D. (1995) The retinoblastoma susceptibility gene product regulates Myc-mediated transcription. Oncogene **10:** 381–387
165 Gu W., Bhatia K., Magrath I. T., Dang C. V. and Dalla-Favera R. (1994) Binding and suppression of the Myc transcriptional activation domain by p107. Science **264:** 251–254
166 Grandori C., Cowley S. M., James L. P. and Eisenman R. N. (2000) The Myc/Max/Mad network and the transcriptional control of cell behavior. Annu. Rev. Cell Dev. Biol. **16:** 653–699
167 Foley K. P. and Eisenman R. N. (1999) Two MAD tails: what the recent knockouts of Mad1 and Mxi1 tell us about the MYC/MAX/MAD network. Biophys. Acta **1423:** M37–47
168 Queva C., McArthur G. A., Iritani B. M. and Eisenman R. N. (2001) Targeted deletion of the S-phase-specific Myc antagonist Mad3 sensitizes neuronal and lymphoid cells to radiation-induced apoptosis. Mol. Cell. Biol. **21:** 703–712
169 Schreiber-Agus N., Meng Y., Hoang T., Hou H. Jr, Chen K., Greenberg R. et al. (1998) Role of Mxi1 in ageing organ systems and the regulation of normal and neoplastic growth. Nature **393:** 483–487
170 Toyo-oka K., Hirotsune S., Gambello M. J., Zhou Z. Q., Olson L., Rosenfeld M. G. et al. (2004) Loss of the Max-interacting protein Mnt in mice results in decreased viability, defective embryonic growth and craniofacial defects: relevance to Miller-Dieker syndrome. Hum. Mol. Genet. **13:** 1057–1067
171 Hurlin P. J., Zhou Z. Q., Toyo-oka K., Ota S., Walker W. L., Hirotsune S. et al. (2003) Deletion of Mnt leads to disrupted cell cycle control and tumorigenesis. EMBO J. **22:** 4584–4596
172 Nilsson J. A., Maclean K. H., Keller U. B., Pendeville H., Baudino T. A. and Cleveland J. L. (2004) Mnt loss triggers Myc transcription targets, proliferation, apoptosis, and transformation. Mol. Cell. Biol. **24:** 1560–1569
173 Eagle L. R., Yin X., Brothman A. R., Williams B. J., Atkin N. B. and Prochownik E. V. (1995) Mutation of the MXI1 gene in prostate cancer. Nat. Genet. **9:** 249–255
174 Prochownik E. V., Eagle-Grove L., Deubler D., Zhu X. L., Stephenson R. A., Rohr L. R. et al. (1998) Commonly occurring loss and mutation of the MXI1 gene in prostate cancer. Genes Chromosomes Cancer **22:** 295–304
176 Ariyanayagam-Baksh S. M., Baksh F. K., Swalsky P. A. and Finkelstein S. D. (2003) Loss of heterozygosity in the MXI1 gene is a frequent occurrence in melanoma. Mod. Pathol. **16:** 992–995
177 Santarosa M. and Ashworth A. (2004) Haploinsufficiency for tumour suppressor genes: when you don't need to go all the way. Biochim. Biophys. Acta **1654:** 105–122

178 Bond G. L., Hu W., Bond E. E., Robins H., Lutzker S. G., Arva N. C. et al. (2004) A single nucleotide polymorphism in the MDM2 promoter attenuates the p53 tumor suppressor pathway and accelerates tumor formation in humans. Cell **119:** 591–602
179 Prendergast G. C. (1999) Mechanisms of apoptosis by c-Myc. Oncogene **18:** 2967–2987
180 Tweddle D. A., Pearson A. D., Haber M., Norris M. D., Xue C., Flemming C. et al. (2003) The p53 pathway and its inactivation in neuroblastoma. Cancer Lett. **197:** 93–98
181 Slack A., Chen Z., Tonelli R., Pule M., Hunt L., Pession A. et al. (2005) The p53 regulatory gene MDM2 is a direct transcriptional target of MYCN in neuroblastoma. Proc. Natl. Acad. Sci. USA **102:** 731–736