Contributions of Myc to tumorigenesis

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Abstract

Despite intensive research, the mechanisms by which deregulation of myc gene expression contributes to tumorigenesis are still not fully resolved and many aspects are still enigmatic. Several recent reviews, including one published in this series a few months ago, have summarized recent progress in our understanding of the biochemistry of Myc proteins [Eisenmann, Genes Dev. (2001) in press; Amati et al., Biochim. Biophys. Acta 1471 (2001) 135–145]. Also, the evidence documenting a central role of Myc proteins in human tumorigenesis has been extensively reviewed [Henriksson and Lüscher, Cancer Res. 68 (1996) 109–182]. In this article, we will argue that current progress allows us to present testable hypotheses on how Myc affects specific properties of transformed cells. © 2002 Elsevier Science B.V. All rights reserved.

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1. A brief account of Myc biochemistry

Current models view Myc proteins as transcription factors that exert their biological function through their ability to both activate and repress target genes [1–3]. Early suggestions that Myc proteins have direct roles in DNA replication have not been substantiated, although there is some evidence suggesting an association between Myc and cdc6 and orc-1, proteins involved in the initiation of DNA replication (e.g. [4]).

A dimeric complex with a partner protein, termed Max, mediates transcriptional activation by Myc [5]. Complex formation with Max has been formally proven to be required for most biological activities of Myc [6,7] and essentially all Myc protein in a cell is complexed with Max [8]; unlike expression of c-Myc, expression of max is constitutive or subjected to only minor variation [8]. The heterodimERIC Myc/Max complex binds to specific DNA sequences with a core CACGTG sequence. Many genes that are activated by Myc/Max complexes are now known, either from array projects, educated guesses or from directed searches [9–12]. In some cases, in vivo binding of Myc proteins to promoter or enhancer sequences of these genes has been confirmed by chromatin immunoprecipitation (e.g. [13–15]).

Max can also interact with other bHLH-LZ proteins of the Mad family, namely Mad1, Mxi1, Mad3 and Mad4. Other Mad-related proteins more distantly related are Mnt/Rox and Mga (for review, see [16]). More recently, further complexity has been added to this already intricate network of interacting proteins by the discovery of novel Max-related proteins, Mlx and Mondo, which interact with some Mad proteins but cannot interact with Max [17–19].

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Mad and Max interact via their leucine zippers, so Max-Myc and Max-Mad interactions are mutually exclusive. The Max/Mad complexes bind to the same E-boxes as Myc-Max but repress rather than activate transcription.

Recent work has focused on trying to understand mechanisms of transactivation by the Myc/Max complex and repression by Mad/Max complexes, respectively. The N-terminal domains of Mad proteins recruit the co-repressors mSin3A and mSin3B, which in turn recruit a multi-subunit complex containing the histone deacetylases HDAC1 and HDAC2 [1]. Conversely, activation of endogenous target genes by Myc correlates with the recruitment of TRRAP, a component of at least two histone acetylase complexes [20,21]. Accordingly, Myc directs histone acetylation at target genes in vivo, strongly suggesting that Myc and Mad regulate transcription at least in part through recruitment of histone acetylases and deacetylases, respectively [13,14].

Somewhat paradoxically, Myc proteins also have the ability to repress transcription and several models have been suggested to account for this property. For example, Myc/Max complexes may induce the transcription of transcriptional repressor proteins; in this model, transcriptional repression then occurs as an indirect consequence of gene activation by Myc. Alternatively, Myc has been suggested to associate with several partner proteins other than Max and to inhibit transcriptional activation by these proteins. For example, Myc associates with the zinc finger proteins Sp-1 and Miz-1, which are involved in activation of the p21<sup>cip1</sup> and p15<sup>ink4b</sup> promoters, both of which are negatively regulated by Myc [22–24]. For Miz-1, binding to Myc prevents its association with p300, an obligatory co-factor for Miz-1-mediated activation of p15<sup>ink4b</sup> [22]. Most likely, therefore, repression of at least some genes will turn out to be a bona fide direct biochemical activity of Myc by mechanisms unrelated to its E-box binding and transactivation functions.

2. Models of transformation

Recent reviews have stressed the fact that a number of distinct genetic programs have to be disrupted for a tumor to emerge, including a reduction of growth-factor requirement, inhibition of apoptosis, extension of lifespan and the ability to stimulate angiogenesis [25]. Indeed, the function of individual oncogenes can often be assigned to specific processes, e.g. to extension of lifespan or inhibition of apoptosis. While initial studies stressed the ability of deregulated Myc proteins to stimulate cell proliferation, it is becoming increasingly apparent that deregulation of Myc function may contribute to various properties of tumor cells, defying a simple assignment to a single functional category.

3. Reduction of growth-factor requirements

One common feature of many transformed cells is their reduced dependence on external growth factors and cells expressing deregulated Myc genes share this property [26]. Using conditional Myc-estrogen receptor (MycER) chimeras, it has been shown that activation of Myc often allows cells to enter S-phase and undergo mitosis in the absence of external factors [27]. In many established cell lines, Myc-induced proliferation in the absence of external growth factors is limited solely by Myc-induced apoptosis [28]. Conversely, c-myc<sup>−/−</sup> rat fibroblasts [29] and c-myc<sup>−/−</sup> murine B-cells [30] show a dramatically reduced growth rate with respect to parental cells, mostly due to G1 delay; thus, expression of c-myc appears to be rate-limiting for proliferation in at least some cells.

In response to activation of Myc and also in transgenic systems, in which Myc is expressed in a deregulated manner, at least three distinct genetic programs, which are highly growth-factor-dependent in normal cells, are activated. Activation of any one of these programs may account for the ability of deregulated Myc to reduce the growth-factor dependence. These programs are:

- Activation of cyclin E/Cdk2 kinase [31,32].
- Activation of E2F-dependent transcription [33].
- Activation of cellular growth, resulting in an increase in cell mass [34,35].

The molecular pathway leading to activation of
cyclin E/Cdk2 kinase by Myc in quiescent fibroblasts is well analyzed and at least four direct target genes of Myc appear to be involved (summarized in Fig. 1). Myc regulates cyclin E/Cdk2 kinase by suppressing the function of the cdk2 inhibitor p27 [32,36]; earlier suggestions implying Cdc25A as the mediator of Myc action [37] are not compatible with the failure of Myc to regulate cyclin E/Cdk2 kinase in p27−deficient cells (where p130 substitutes for p27 as inhibitor) [36]. Initially, activation of cyclin D2, Cdk4 and possibly other Myc-target genes induces sequestration of p27 into cyclin D2/Cdk4 complexes; this appears to be sufficient to generate a small amount of active cyclin E/Cdk2 kinase [10,38,39]. This catalyzes phosphorylation of p27 at threonine 187; phosphorylated p27 is then recognized by an E3 ligase complex, which includes Cul-1 as a scaffold protein. Recognition of phosphorylated p27 by the E3 ligase is facilitated by Cks proteins. Both Cul-1 and one of the Cks proteins, Cks2, are target genes of Myc [10,40]. In addition, transcriptional repression of the p15ink4b and p21^cpl^ genes by Myc may further contribute to lowering the total inhibitor level under specific physiological circumstances (e.g. when p53 is activated) [24,41].

From the use of p27-deficient cells, it is clear that the ability of Myc to activate E2F-dependent transcription is independent of its ability to regulate cyclin E/Cdk2 kinase [36]. Also, ectopic expression of cyclin D2 does not substitute for Myc, implying that other targets of Myc are involved. Potential candidates are the E2F2 and E2F3 genes [42]; thus, in Myc expressing cells, the amount of E2F proteins may exceed the amount of pocket proteins required to repress them. Also, the Id-2 gene is a target for transcriptional activation by Myc; Id-2 protein binds to, and inactivates, the retinoblastoma protein, which would result in the activation of E2F-dependent transcription [43].

In addition to the above effects on cell proliferation, Myc also regulates cell growth. For example, enforced expression of the Drosophila c-myc ortholog in wing imaginal discs results in increased cell size [35]. A similar effect has been observed for mammalian c-Myc in murine B-cells [34,44] and developing limb of the chicken (M. Ros and J. León, unpublished). Consistent with these observations, many Myc-target gene products are involved in protein synthesis and ribosome biogenesis (e.g. [9−12]). Specific metabolic pathways are also targeted by Myc. For example, Myc induces glycolytic enzymes, including lactate dehydrogenase, providing a possible molecular explanation for the enhanced rates of glycolysis observed in many tumor cells [45].
doubt exists that stimulation of cell growth and metabolism contributes to cell proliferation; however, how precisely enhanced expression of metabolic genes leads to enhanced flux through metabolic pathways and how this, in turn, could contribute to deregulation of cell proliferation or tumorigenesis, is largely unknown.

Thus, the present array of candidate target genes offers several hypotheses as to how Myc might function to reduce the requirement for external growth factors and enhance proliferation in specific cells and/or tumors. A few genetic studies demonstrating a requirement for a specific target gene in Myc-induced proliferation or tumorigenesis have been performed. Id-2 and cyclin D2 have been shown to be required for Myc to stimulate proliferation of mouse embryo fibroblasts [39,43,46]; conversely, depletion of p27 has been shown to rescue the proliferation defect of c-myc−/− fibroblasts [40]. In addition, lack of E2F2 or E2F3 has been reported to limit the ability of cells to enter S-phase in response to infection with very high titers of adenoviruses expressing Myc [47]; whether this reflects a physiological situation, however, remains to be determined.

To our knowledge, only a single study analyzing Myc-induced tumorigenesis in genetically deficient animals has been published. This study showed that cyclin D1 is not required for MMTV-Myc induced mammary tumorigenesis [48]. Therefore, while there is some certainty that the results presented describe events that are relevant in fibroblasts in culture, there is yet little evidence as to how relevant they are in tumorigenesis. For example, regulation of p27 levels is highly significant during the development of many tumors; whether there is any correlation between deregulation of Myc and p27 levels in human tumors is not clear. At least in human neuroblastoma, this is not the case [49].

4. Immortalization

Most ‘primary’ cells have a finite lifespan in culture. In contrast, being ‘immortal’ is a hallmark of many tumor cells and, as a consequence, the process of ‘immortalization’ is of considerable interest.

The lifespan of primary human cells in culture is largely dictated by telomere length, since expression of the catalytic subunit of human telomerase, htert, can significantly extend lifespan of human primary cells in culture (for review, see [50]). The htert promoter is a target for transactivation by Myc and activation of conditional MycER proteins can induce htert expression and telomerase activity in some primary cells (e.g. [51]). Primary human cells can be converted into tumor cells by the introduction of Ras, htert and the SV40 early region [52]; whether Myc can replace htert in this process remains untested. Activation of telomerase activity is likely to be an important step in the genesis of many, if not all human tumors; for example, telomerase activity can serve as a prognostic marker in childhood neuroblastoma and correlates strongly with amplification of NMYC (e.g. [53]). It is possible, therefore, that activation of the htert promoter (or at least facilitation of htert expression) by Myc contributes to the genesis of human tumors. The hypothesis is hard to test, since telomere length is not a major regulatory factor in rodent tumorigenesis.

In order to achieve immortalization of human cells in culture, mutations in the retinoblastoma pathway are required in addition to maintenance of telomerase activity (e.g. [52,54]). However, manipulation of the culture conditions (for example the use of feeder layers) suggests that these ‘telomere independent’ mechanisms of life span regulation reflect stress responses to the inadequate culture conditions on plastic rather than a counting mechanism of cell divisions per se [55]. If so, deregulated expression of Myc also interferes with such stress responses, since Myc can extend the lifespan of primary human fibroblasts even under conditions when ectopic expression of htert cannot [56].

This situation may be analogous to the situation in primary rodent fibroblasts, since the finite lifespan of primary mouse cells in culture is not dictated by telomere length: arrest in culture is very rapid and occurs without significant shortening of the very long telomeres. Again, senescence appears to be a stress response to the inappropriate tissue culture conditions or unusual environment and thus is likely to be a tissue culture artifact [57]. However, since the genetic circuitry controlling senescence under such conditions involves a number of bona fide oncogenes and tumor suppressor genes, it is likely that related stress situations occur in vivo and that it is important
for an organism that cells respond to them in an appropriate manner. In particular, high-level expression of oncogenic alleles of Ras accelerates senescence; however, in the absence of oncogenic Ras, other stimuli may enhance ARF expression during prolonged passages in culture.

The genetic circuitry underlying this process is well understood (see Fig. 2). Two genetic pathways affect this process: first, both oncogenic Ras and prolonged culture of primary cells induces expression of p19ARF, a negative regulator of mdm-2 (for review, see [60]). Induction of ARF stabilizes p53, thus leading to cell cycle arrest and contributing to induction of apoptosis. Loss of either ARF or p53 allows transformation of primary cells by Ras and immortalizes primary MEFs; therefore, this pathway is necessary for senescence to occur (e.g. [61]).

Second, the concomitant loss of several pocket proteins abolishes induction of senescence by Ras. Introduction of oncogenic Ras into pRb/p107-double knockout or into pRb/p107/p130 triple knockout cells fails to induce senescence, even though both p53 and p19ARF remain both intact and p21 is induced [62,63]. Most likely, therefore, the Cdk4 pathway that controls pocket protein function is a parallel pathway that is independently necessary, but not sufficient to induce senescence in MEFs. This view is supported by observations showing that the loss of p15INK4b, p18INK4c or both also facilitates transformation by Ras alone [64]. However, the effects reported are not as strong as those seen after loss of ARF or p53. Therefore, it is also possible that pocket proteins are downstream of p53 and p21; for example because of the ability of p21 to halt proliferation through its ability to block phosphorylation of pocket proteins (see Fig. 1).

Ectopic expression of Myc immortalizes primary mouse embryo fibroblasts and co-operates with oncogenic alleles of Ras in transformation [65]. What may be the underlying mechanisms?

Paradoxically, as mentioned above for Ras, ectopic expression of Myc induces expression of p19ARF, thus leading to stabilization of p53 [66]. Primary MEFs that are immortalized by Myc have sustained mutations of p53 or have lost expression of ARF. This suggests that induction of ARF limits the immortalizing functions of Myc as well as Myc-induced lymphomagenesis in mice [67]. How can Myc be immortalizing despite its ability to induce ARF?

Before discussing possible models, it is worth remembering that MEFs are by no means clonal cells; thus it is formally possible that expression of Myc selects out a population of cells from the pool that have already sustained ARF mutations and allows their transformation by Ras. In the absence of a clear and validated mechanistic model for Myc’s action, this remains a possibility. However, two models that do not invoke a selection of a cell population from the pool of mouse embryo fibroblasts appear plausible.

4.1. Model I

In one simple model Myc immortalizes primary rodent cells by enhancing the chance of mutations occurring at either the p19ARF or the p53 locus before cells become senescent (see Fig. 3). The model predicts that Myc acts in an irreversible manner and therefore once either mutation has occurred, Myc should no longer be required. How could Myc function in this way? It may affect mutation rates at the p19ARF or p53 loci; this would imply that deregulated expression of Myc is mutagenic. However, there is only limited evidence for this (see below).
Alternatively, Myc may not affect mutation rates per se but extend the time (number of passages) in which mutations can accumulate at either the p53 or ARF locus before cells arrest and become senescent. In this latter view, at least some of the mechanisms by which Myc stimulates cell proliferation would also occur in primary cells (e.g. repression of p21, p15ink4b, induction of Id2 and cyclin D2); via the regulation of these targets, Myc would delay the onset of senescence. Since MEFs are cultivated in highly stressful and potentially mutagenic environments, this delay may well be sufficient to select for ARF- or p53-mutant cells. The findings that both cyclin D2- and Id2-deficient MEFs are resistant to immortalization by Myc strongly argues that such a scenario is plausible [39,43,46].

One prediction from such a model is that the action of Myc is irreversible implying that Myc-induced tumorigenesis is irreversible in vivo. This has been tested several times and there are clear examples of reversible tumor formation, for example in lymphoid cells [68]. However, recent data using a tetracycline-regulated allele of Myc targeted to the breast epithelium show that re-repression of Myc once tumors had established led to tumor regression in only a subset of tumors [69]. Further analysis revealed that the non-regressing tumors had sustained Kras2 mutations, suggesting that once these mutations had occurred Myc was dispensable. Oncogenic mutation of Kras2 did not substitute for Myc function (e.g. by transcriptionally activating endogenous Myc genes), since several target genes of Myc were downregulated when Myc was repressed. Therefore, the data strongly suggest that, in analogy to the proposed model in MEFs, deregulated expression of Myc is required only transiently during mammary tumorigenesis to facilitate the emergence of cell clones that carry mutations in Ras. Whether the emerging tumors have mutations in either ARF or p53 has not been reported; it is not entirely clear, therefore, whether the genes that are mutated to facilitate emergence are identical in both systems.

4.2. Model 2

An alternative model would maintain that Myc interferes with p53-dependent transcriptional activation in primary MEFs at a level distinct from ARF but upstream of p21 and other target genes of p53 (Fig. 3). Thus, Myc may dampen p53 transcriptional responses and thereby facilitate the emergence of immortal clones. In this view, immortalization of MEFs by Myc is the sum of two opposing interactions of Myc with p53: activation of p53 function via ARF and dampening of transcriptional activation by p53 via a mechanistically unknown pathway. At present, there are two pieces of evidence to suggest the existence of such a pathway.

First, at least two genes that are targets for transcriptional activation of p53, namely p21 and gadd45,
are well established targets of transcriptional repression by Myc. Indeed, it has formally been shown that Myc interferes with p53-dependent induction of gadd45 [70]; our own data show the same is true for p21 and other target genes of p53 (J.L. and M.E., unpublished).

Second, further evidence for such a model has been provided by the analysis of K562 cells that harbor both a temperature-sensitive allele of p53 and an inducible allele of Myc [71]. These cells lack ARF and therefore mimic the situation of many human leukemias, which have both wt53 and high levels of Myc. In these cells, a shift in temperature induces both cell cycle arrest and subsequent apoptosis. Activation of Myc in arrested cells suppresses cell cycle arrest by ts53 and this has been traced to its ability to repress induction of p21; similar observations have been made in other cell lines. Surprisingly, however, Myc also reduces p53-induced apoptosis in this situation, unlike what is seen in ARF-positive cells. The p53-target genes which mediate apoptosis in this system have not been identified; however, array analyses show that several target genes of p53 are repressed upon induction of Myc. Together, these data raise the possibility that Myc may dampen p53-mediated signal transduction, maybe by interfering with the function and/or expression of co-activators of p53.

5. Resistance to anti-mitogenic factors

Proliferation of cells in vivo is not only controlled by positively acting growth factors, but is also restrained by anti-mitogenic factors. One of the most prominent examples for such a factor is transforming growth factor β (TGF-β) and a large body of evidence suggests that inactivation of TGF-β signaling contributes to tumorigenesis of colon, skin and breast, among others (for review, see [72]).

Several genetic factors can lead to resistance against TGF-β signaling; for example, mutations in receptor proteins and in Smad proteins, which transmit the TGF-β signal to the nucleus, have been reported in human tumors. However, loss of TGF-β responsiveness can also occur when the TGF-β signaling pathway itself, is intact [73]. In one of these cases, array analysis strongly suggests that the emergence of resistance correlates specifically with a loss of downregulation of the endogenous c-myc gene, which normally occurs in response to addition of TGF-β. Indeed, ectopic expression of c-myc renders keratinocytes resistant to the anti-mitogenic effects of TGF-β; in part, this is due to Myc’s ability to suppress induction of the p15ink4b and p21cip1 genes by TGF-β [74,75]. Repression of p15ink4b is mediated by the Myc-associated protein, Miz-1, which is part of the signaling pathway that mediates induction of the p15ink4b gene by TGF-β; deregulated expression of Myc prevents Miz-1 from functioning properly in the pathway [22,23]. Similarly, repression of p21cip1 appears to be caused by complex formation between Miz-1 and Sp-1 [24].

It should be pointed out that TGF-β may also be a critical part of the mechanism that protects primary cells from transformation by oncogenic Ras, at least in primary keratinocytes (e.g. [76]). In these cells, expression of oncogenic Ras induces expression of TGF-β and secretion of TGF-β is required for induction of premature senescence by Ras. Thus, ‘immortalization’ by Myc (which to our knowledge has not formally been demonstrated in keratinocytes) may reflect its ability to disrupt TGF-β signaling in this cell type.

6. Genomic instability

In order for a tumor cell to emerge, several mutations have to accumulate in a single cell; in addition, most human tumors are characterized by alterations in chromosome numbers. Thus, the genome of tumor cells may be either transiently or irreversibly unstable. The finding that Myc-induced tumorigenesis can be irreversible at least in some cases [69] raises the question as to whether Myc proteins contribute to tumorigenesis by promoting the accumulation of secondary mutations or by destabilizing checkpoint mechanisms that control chromosome number and ploidy.

We are not aware of studies that test whether deregulated expression of Myc affects the appearance of point mutations, although assays to ask this question are available. There is limited evidence to support the suggestion that deregulated expression of Myc can promote gene amplification. For example,
challenge of mammalian cells with the DNA damaging drug N-(phosphonoacetyl)-L-aspartate (PALA) requires amplification of the CAD gene for resistance to emerge and even transient activation of Myc facilitates the emergence of resistant clones upon PALA treatment [77]. Similarly, several genes including cyclin D2 have been demonstrated to be unstable in cells expressing deregulated Myc [78]. Surprisingly, both CAD and cyclin D2 are targets for transcriptional activation by Myc. It is possible, therefore, that the binding of Myc recruits co-activators that increase the likelihood of creating genetic havoc at the binding site. Similarly, there are several pieces of evidence suggesting that Myc can override checkpoint mechanisms that control chromosome number and ploidy. For example, exposure of fibroblasts expressing constitutive Myc to nocodazole led to the accumulation of polyploid cell populations, since cells exiting mitosis did not arrest but instead underwent multiple S-phases [79,80]. A similar effect of Myc is observed in murine keratinocytes [81]. Further, deregulation of Myc enhances the phenotypes of p53 loss when cells are challenged by taxol, leading to an almost complete loss of G2 arrest [79,82].

How does Myc act in these circumstances? One mechanism by which Myc overrides checkpoints is almost certainly an indirect consequence of its mitogenic properties. For example, cells expressing Myc override an arrest imposed by physiological levels of the cdk inhibitor p21, which mediates cell cycle arrest by p53 [32]. Thus, these cells will not respond to activation of p53 (for example in a tsp53 situation) with a cell cycle arrest (e.g. [83]). Similarly, chromosomal abnormalities are observed in cells expressing high levels of cyclin E, suggesting that enhanced proliferation per se may be mutagenic [84].

It is possible that all observed effects of Myc on genomic stability are indirect consequences of its mitogenic properties. The strongest suggestion for alternative pathways comes from the observation that Myc suppresses induction of the DNA-damage inducible gadd45 gene in response to several stress signals [70]. Gadd45-deficient cells are highly unstable suggesting that expression of gadd45 is required for maintenance of correct chromosome number and genomic stability in the G2 phase [85]. Conversely, ectopic expression of gadd45 is sufficient to establish a cell cycle arrest at the G2/M boundary [86]. Expression of gadd45 is induced by a number of different signaling pathways, including p53- and BRCA-dependent pathways, and all seem to be susceptible to inhibition by Myc. If Gadd45 and potentially related proteins are an integral part of mammalian checkpoint responses, it will be important to find out which aspect of checkpoint functions are disrupted by Myc. It is worth pointing out that, in yeast, checkpoints not only function to regulate the cell cycle, but have multiple functions that lead to an integrated repair response. Depending on how Myc interferes with gadd45 induction, it may therefore not only affect cell cycle arrest, but may also interfere with its repair functions.

7. Angiogenesis

All mechanisms discussed so far suggest that Myc acts early in tumor formation. However, several observations suggest that deregulation of Myc can also affect later stages of tumorigenesis, most notably angiogenesis. For example, the reversible activation of conditional alleles of Myc in keratinocytes in vivo induces hyperproliferation, dedifferentiation and angiogenesis in a reversible manner, suggesting that these events may be directly affected by Myc [87]. Similarly, angiogenesis occurs early in the genesis of myc-induced avian lymphomas [88].

Several mechanisms have been suggested to contribute to these observations: for example, suppression of anti-angiogenic factors including thrombospondin and activin A by c-Myc or N-Myc, respectively, has been demonstrated [89,90]. Finally, the finding that there is an overlap between hypoxia-regulated elements and myc-regulated elements in the promoters of glycolytic enzymes suggests additional potential links between Myc- and hypoxia-induced signals.

8. Summary

Recent progress has clarified several key aspects of the biochemistry of Myc proteins, and strongly reinforced the notion that Myc’s biological effects are due to its ability to both positively and negatively affect gene transcription. Current evidence is compat-
ible with the notion that an early and critical contribution of Myc in tumorigenesis is the deregulation of cell growth and proliferation, leading to a clonal expansion of premalignant cells.

In our view, two key issues have now become accessible.

First, work in fibroblasts has identified several genes that appear to be candidates for mediators of Myc’s mitogenic and growth-promoting functions. One might therefore predict, that at least some of these targets are rate-limiting for Myc-induced tumorigenesis, and this seems to us an important and unresolved question.

Second, Myc’s role in tumorigenesis may well go beyond its ability to de-regulate cell proliferation. For example, its ability to immortalize cells may be due to its ability to induce genomic instability and/or to interfere with p53-dependent signal transduction. While much work has focused on Myc’s mitogenic and growth-promoting properties, it now seems critical to understand whether Myc can also promote the accumulation of mutations and, if so, what the pathways are that mediate this effect.

Finally, it is also clear that Myc has been proposed to control or affect other processes, which may be highly relevant for its tumorigenic action. For example, ectopic expression of Myc can block differentiation in several systems and this may be independent of its mitogenic effects [91]. Thus the choice of topics covered in this review is in part due to the preference of the authors, and we apologize to all our colleagues, whose work was not covered in detail.

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