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Role of *myc* amplification and overexpression in cell growth, differentiation and death

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Genes of the myc family are apparently the most intensively studied of all nuclear oncogenes. This is because their expression is deregulated in many types of human neoplasia due to chromosomal translocation or gene amplification, and because their exact roles in the regulation of cell proliferation have remained poorly known. However, the recent characterization of several domains in Myc proteins that enable sequence-specific regulation of other growth-related genes, and the identification of proteins interacting with Myc proteins have provided insight into the function(s) of Myc proteins in both normal and neoplastic cells. While the natural target genes for Myc remain to be identified, it has become evident that myc overexpression not only promotes cell proliferation, but also increases the rate of programmed cell death.

Key words: amplification / *myc* oncogenes / cell proliferation / apoptosis

Structure and expression of the *myc* genes

The *myc* gene was originally discovered as the transforming gene of a subclass of avian leukemia viruses. The identification of sequences related to v-*myc* in uninfected cells led to the cloning of its cellular counterpart, the c-*myc* gene. Other cellular genes homologous with c-*myc* were later isolated as amplified sequences from tumors: N-*myc* from neuroblastoma; and L-*myc* from small cell lung cancer (SCLC). Besides these three well characterized *myc* genes, several other homologous genes and pseudogenes have been identified (for reviews, see refs 1-3).

The *myc* genes share a similar genomic structure consisting of three exons with long untranslated leader and trailer sequences. In contrast to the partially very highly conserved protein coding regions of the *myc* genes, their regulatory regions differ greatly. This correlates well with their temporally and spatially distinct, although partially overlapping, expression patterns (see ref 1). The c-*myc* gene is expressed in virtually all proliferating cells in both embryonic and adult tissues. By contrast, expression of N-*myc* and L-*myc* is restricted to early stages of differentiation in certain cell lineages. Accordingly, while the c-*myc* gene has been found activated in a wide variety of tumors, the spectrum of tumors with an activated N-*myc* or L-*myc* gene is much more limited (for reviews, see refs 4,5).

The expression of the c-*myc* gene is regulated by several means involving transcription initiation, elongation and termination, translation initiation as well as mRNA and protein stability (for reviews, see refs 6,7). Although *myc* amplification usually results in increased expression of the corresponding mRNA and protein (see ref 5), the amplified gene may still remain under regulation. For example, studies of the HL-60 leukemia cell line and some neuroblastoma cell lines containing multiple copies of the c-*myc* or N-*myc* genes, respectively, have demonstrated normal down-regulation of these genes during differentiation.[8-11]

Proteins encoded by the *myc* genes and their oncogenic potential

All *myc* genes encode short-lived nuclear phosphoproteins (for reviews, see refs 2,3,12). The two major c-Myc polypeptides of 64 and 67 kDa share the same open reading frame within exons 2 and 3. The larger c-*myc* polypeptide contains additional 15 amino acids in its N-terminus due to translation initiation from an unusual CTG codon at the 3′ end of exon 1.[13] Chromosomal translocations of the c-*myc* gene often result in loss of the first exon, and thereby in the production of the shorter polypeptide only. Even in cases where exon 1 has been retained as in some Burkitt's lymphomas, its 3′ end has suffered point mutations that prevent the production of the longer c-Myc polypeptide.[13] Whether the two c-Myc polypeptides have distinct functional or regulatory roles in normal and/or transformed cells, remains to be elucidated.

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Also the N-Myc protein is produced as two distinct polypeptides, whose translation is initiated from two closely spaced AUG codons within the second exon of the N-*myc* gene.[14] By contrast, the heterogeneity of the L-Myc polypeptides is due to differential phosphorylation of a single polypeptide initiated from the second exon.[15] Due to alternative splicing, a shorter L-Myc polypeptide lacking sequences encoded by the third exon may also be produced.[15,16] This minor form of L-Myc resides in the cytoplasm and may thus have functions that differ from those of the full-length protein.

While amplification is a common activation mechanism for all *myc* genes, no chromosomal translocations involving the N-*myc* or L-*myc* genes have been observed.[5] However, a novel type of intrachromosomal rearrangement associated with L-*myc* amplification has recently been identified in two SCLC cell lines, where sequences unrelated to L-*myc* were found immediately upstream of the coding region of the L-*myc* gene.[17] These sequences were shown to be derived from an upstream locus named *rlf*.[18] A similar rearrangement, which results in deregulation of the L-*myc* gene and in production of a Rlf/L-Myc fusion protein, was also detected in a primary SCLC tumor.[19] This suggests that the rearrangement has a role in the development of SCLC.

All three *myc* proteins can transform established fibroblasts such as Rat-1A cells and complement Ras oncoprotein in transformation of primary rat embryo fibroblasts (REFs).[20-23] They can also cause lymphoid malignancies in transgenic mice when expressed under the control of an immunoglobulin enhancer.[24-27] The structural features of Myc proteins important not only for their ability to transform cells, but also for their action in normal cells are discussed in the following chapter (see also Figure 1).

Functional domains of Myc proteins

The Myc proteins contain several domains typical for transcription factors: a basic region that enables sequence-specific DNA-binding, helix-loop-helix and leucine zipper structures that mediate oligomerization as well as several putative transactivation domains (see below and Figure 1).

DNA-binding and oligomerization domains

The helix-loop-helix (HLH) structure, which was originally shown to mediate heterodimerization of MyoD-like myogenic proteins with immunoglobulin enhancer-binding E proteins, is composed of two short amphipathic helices separated by an intervening loop.[28,29] The leucine zipper (LZ) structure, which is found in members of the Fos, Jun and CREB transcription factor families, consists of an α-helix where every seventh amino acid residue positioned on the same face of the helix is leucine.[30] Two leucine zipper proteins can associate with each other by forming a dimer of parallel α-helices in a coiled-coil structure.[31] This structure may be stabilized by additional hydrophobic residues located between the leucines.

Proteins of the Myc family as well as AP-4,[32] TFE3,[33] TFEB[34] and USF[35] contain both helix-loop-helix and leucine zipper structures adjacent to their DNA-binding basic region. These bHLH/LZ proteins do not form heterodimers with each other or with any other HLH or LZ protein. Myc proteins do not even form homodimers when expressed at physiological concentrations.[36,37] This may be explained by the presence of several charged residues at the coiled-coil interface of the c-Myc leucine zipper. The bHLH/LZ structure of Myc proteins is required for their ability to transform REFs in cooperation with Ras oncoprotein.[38,39] The leucine zipper is also essential for the ability of c-Myc to inhibit differentiation of murine erythroleukemia cells.[36]

Non-specific DNA binding may facilitate recognition of specific DNA-binding sites by the Myc basic region. Although the domain of c-Myc that is responsible for non-specific binding (see Figure 1) is dispensable for REF cotransformation ability,[38] it is required for inhibition of differentiation of fibroblasts into adipocytes.[40]

Transactivation domains

The primary structures of activation domains are less precisely defined than the DNA-binding domains. In the N-terminal part of the c-Myc protein there is a putative transactivation domain with multiple glutamine and proline residues;[41] (see Figure 1). This domain contains also consensus sites for recognition by both glycogen synthase kinase 3 (GSK-3)[42] and growth factor-regulated MAP kinases,[43] suggesting that it is subjected to regulation by phosphorylation. Indeed, TPA-induced phosphorylation of the L-Myc protein has been shown to occur at the GSK-3 site.[42] Furthermore,

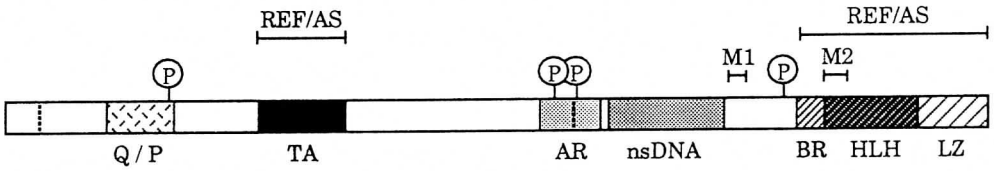

Figure 1. Schematic diagram of the c-Myc protein. Shown are the regions essential for REF cotransformation and for autosuppression (REF/AS); putative phosphorylation sites (P); the M1 and M2 nuclear localization sequences; a glutamine/proline rich (Q/P), an acidic (AR) and an uncharacterized transactivator (TA) region; the regions involved in non-specific (nsDNA) and sequence-specific (BR, basic region) DNA-binding; and the helix-loop-helix (HLH) and leucine zipper (LZ) structures involved in oligomerization. The black-dotted lines indicate the positions of borders between exons.

mutation of a critical serine residue to alanine significantly decreases the transactivating activity of c-Myc in response to serum stimulation.[44] Phosphorylation of the activation domain appears to represent a general mechanism for controlling gene expression, since the activity of several other transcription factors including Jun and CREB is similarly regulated (for a review, see ref 45).

On the C-terminal side of the glutamine/proline-rich region there is another interesting region (marked TA in Figure 1), which is highly conserved between Myc proteins. Also, this region behaves as a transcriptional activation domain, when linked to a heterologous DNA-binding interface.[41] Furthermore, it is required both for REF cotransformation[38] and for negative autoregulation of *myc* expression.[46]

In the middle of the c-Myc protein there is a highly acidic region reminiscent of the acidic activation domains initially identified in c-Jun.[47] Although Kato and coworkers did not observe any significant transactivation by this region,[41] it may prove to have such a role in some other context. The putative cell type-dependent effects of the acidic region are further supported by the observations that this region is not essential in the REF assay,[38] but it is required for transformation of chicken hematopoietic cells.[48,49]

Also N-Myc (see article by M. Schwab, this issue) and L-Myc[50] are capable of transactivation, when fused to a heterologous DNA-binding interface. However, the transactivation activity of L-Myc is considerably lower than that of c-Myc,[50] which may also explain the weaker potential of L-Myc in the REF cotransformation assay.[23] An intriguing question to be resolved is whether the Myc proteins interact with the same cellular factors through their N-termini or whether they have different targets.

Subcellular distribution

Immunofluorescence studies with both normal and transformed cells have revealed that the cellular and viral Myc proteins are relatively evenly distributed in the nucleus excluding nucleoli.[51-53] However, in cells expressing high amounts of either c-Myc or v-Myc, these proteins aggregate into amorphous, phase-dense nuclear structures.[54] More surprisingly, the normally cytoplasmic HSP70 heat shock protein is detected in the same structures. The c-Myc-induced intranuclear distribution of HSP70 is distinct from both its S phase-dependent association with nuclei of unstressed cells[55] and the intranuclear pattern seen in heat-shocked cells.[56,57] Although no physical association between c-Myc and HSP70 has yet been demonstrated, it is interesting that incubation of isolated nuclei with ATP known to solubilize complexes of HSP70 with its target proteins[58] releases HSP70 also from the c-Myc-containing inclusions (P. J. Koskinen, unpublished results). Since very high amounts of the c-Myc protein have been shown to be cytotoxic,[59] the results on the nuclear codistribution of c-Myc and HSP70 raise the possibility that cells have a mechanism to modulate the availability of functional c-Myc protein. Thus, only a certain amount of the c-Myc protein may be maintained in a soluble form, while the rest is stored in nuclear structures in association with HSP70. Furthermore, since cells stably transfected with the c-*myc* gene appear to be more thermosensitive than cells transfected with other types of oncogenes,[60] it is tempting to speculate that *myc* overexpression interferes with the heat shock response by preventing HSP70 from binding to its normal targets.

Two regions with nuclear targeting potential, M1 and M2, have been identified in the c-Myc protein (ref 61; see Figure 1). While deletion of both of them renders c-Myc cytoplasmic, M1 mutants retain partial nuclear localization and REF cotransforming ability. By contrast, M2 mutants have lost this ability, although they reside in the nucleus. Thus, M1 acts as a typical nuclear localization signal being able to carry an otherwise non-nuclear protein into the nucleus, whereas M2 has as yet unknown properties. Interestingly, the M2 region is conserved between all Myc proteins, but the M1 region is absent from L-Myc.

Myc proteins as transcriptional regulators

Sequence-specific DNA-binding by Myc proteins

In cells overexpressing one of the *myc* genes, several genes including the *myc* genes themselves have been shown to be up- or downregulated (see refs 7,12,62). Although Myc proteins have not yet been shown to directly bind to the regulatory regions of these genes, the presence of the conserved DNA-binding and dimerization motifs in the C-termini of Myc proteins suggests that the negative results might have been due to technical problems. For example, preparations of bacterially produced full-length c-Myc protein are usually insoluble.[63] Such problems have recently been circumvented by using more soluble fusion proteins containing C-terminal c-Myc sequences linked to glutathione S-transferase (GST) sequences.[64]

TFE3 and USF transcription factors both bind to the CACGTG-containing sequence of the adenovirus major late promoter.[33,35] By using the technique of selected and amplifed binding (SAAB), Blackwell and coworkers demonstrated that C-terminal sequences of c-Myc fused to GST can recognize the same core sequence.[64] Similar results were obtained by another type of chimera where the c-Myc basic region was linked to the E12/E47 dimerization interface.[65] However, the presence of shared sequence-specificities in several proteins does not imply that they have similar functions. This is exemplified by the ability of USF, but not TFE3 to stimulate transcription from the adenovirus promoter.[33] USF and TFE3 can recognize also other nucleotides within the CAxxTG core consensus, but c-Myc has been shown to bind only to CACGTG, or to a lesser extent to CATGTG.[65,66] Also it should be noted that although the core sequence is necessary for binding by the c-Myc basic region, it may require a certain context conferred by the flanking sequences. In fact, the SAAB technique has been used to define additional nucleotides on both sides of the core hexamer.[66,67]

Interactions of Myc with other proteins

One of the major breakthroughs in Myc research has been the identification of another bHLH/LZ protein, termed Max, as a Myc-binding partner.[68] The *max* gene, which was isolated through a screening of a bacterial expression library with the GST-c-Myc fusion protein, encodes a protein of only 151 or 160 amino acids depending on the absence or presence of optional 9 amino acids in the N-terminal region preceding the bHLH/LZ domain. This region appears to be encoded by an alternatively spliced exon of 27 bp.

Max and its almost identical murine homologue[69] have been shown to form complexes with all Myc proteins, but not with any other bHLH/LZ proteins. The results from *in vitro* association studies have been confirmed by *in vivo* coprecipitations.[70-72] Unlike Myc, Max can also homodimerize, but in the presence of Myc, heterodimerization is preferred.[73] Heterodimers of Myc and Max appear to have a higher affinity to the Myc-binding sequence than either homomer alone.[68,69] Moreover, the DNA-binding ability of Max homodimers, but not of Myc/Max heterodimers has been shown to be negatively regulated by CKII phosphorylation *in vitro*. While no differences in sequence specificity between Myc and Max have been detected, the recent observation that Myc and Max bend DNA in opposite orientations[75] suggests that binding of homo- and heterodimeric complexes may result in distinct interactions with other nuclear proteins.

Besides Max, Myc proteins have been shown to associate with RB.[76] While the physiological significance of this finding remains to be established, it is interesting to note that the binding site of Myc in RB appears to overlap with that of the adenoviral E1A protein.[77] Although the exact sequences of Myc required for RB binding were not determined, the N-terminal region essential for REF cotransformation appears to be involved. Furthermore, this region interacts not only with RB, but also with the TATA-binding factor TFIID.[78,79] Thus, it is tempting to speculate that RB functions by preventing the interactions of transcription factors with the basic transcription machinery. Accordingly, overexpression

of a Myc protein or deletion of the Myc-binding region of RB may overcome the negative effects of RB on cell growth.

Targets of Myc

Putative binding sites for Myc and Max containing the CACGTG sequence have been found within the regulatory regions of some growth factor-regulated genes.[67] These include c-*sis* encoding the B chain of PDGF, and nucleolin, a major nucleolar protein involved in the synthesis and assembly of ribosomes. However, the ability of c-Myc to regulate these genes *in vivo* remains to be tested. Also, the ornithine decarboxylase (ODC) gene, which is maybe one of the most promising candidates for a Myc-regulated gene (S. Tavtigian, personal communication), contains a conserved dyad repeat of the CACGTG motif within its first intron. In transient cotransfection experiments, this region has been shown to be stimulated by either c-Myc or N-Myc.[80] However, here it should be noted that since several other factors also recognize the CACGTG sequence, it may be difficult to determine which of them are the real regulators *in vivo*. Indeed, in the case of p53, which also contains a conserved Myc-binding site,[81] the major DNA-binding activity observed in nuclear extracts represents USF.[82]

To identify *in vivo* target sequences for Myc proteins, one can utilize the same strategy that has successfully been used in the identification of sequences bound by p53.[83] There fragments of total human DNA coprecipitating with the p53 protein were recovered by PCR amplification. Once any Myc-bound DNA sequences will be identified, these can then in turn be used as probes to reveal the adjacent genes whose expression may be regulated by Myc proteins.

Enhancement or suppression of Myc/Ras co-transformation by alternative forms of the Max protein

The *max* gene also gives rise to a truncated form of the Max protein.[84] This is due to introduction of a translation termination codon by an alternatively spliced 101 bp exon in the middle of the *max* coding region. The 103 or 94 amino acid ΔMax polypeptides share the N-terminal DNA-binding and oligomerization domains with the corresponding Max polypeptides, but lack the C-terminal sequences containing the nuclear localization signal identified by Kato and coworkers[73] (see Figure 2). Accordingly, ΔMax resides in the cytoplasm, but is translocated to the nucleus upon association with the c-Myc protein.[84] Interestingly, while overexpressed Max supresses the ability of c-Myc to transform rat embryo fibroblasts in cooperation with Ras, ΔMax enhances it.

Both *max* and Δ*max* mRNAs are ubiquitously expressed, although the observed levels of Δ*max* mRNA as well as protein are much lower than those of *max* in cells and tissues tested so far (ref 84; P. J. Koskinen unpublished results). However, the conservation of the Δ*max* exon in human, mouse and rat suggests that it has a physiologically significant role in some cells, tissues or developmental phases. In contrast to c-*myc*, the expression of either *max* or Δ*max* is not affected by changes in cell growth (refs 66,71; I. Västrick, T. Mäkelä, unpublished results). Also, the levels of *max* are similar in transformed cells expressing an amplifed *myc* gene and in normal cell lines.[66] Unlike Myc, Max and ΔMax are very stable, with half-lives of over 12 h.[71,85] Thus, the formation of Myc/Max and Myc/ΔMax heterodimers appears to be regulated by the levels of the Myc protein.

Recent studies have revealed further complexity in the coding capacity of the *max* gene.[85] As a result of alternative splicing and/or polyadenylation, at least five distinct mRNAs with or without the 27 bp exon can be transcribed, the levels of the minor mRNA forms being dependent on the cellular growth conditions. Two of the mRNAs code for the full-length Max protein, two others for ΔMax and the longest one for another truncated protein with some intron-derived sequences in its C-terminus. Although this protein behaves like ΔMax in the REF cotransformation assay, its biological role remains unclear, since it is very unstable and non-conserved.

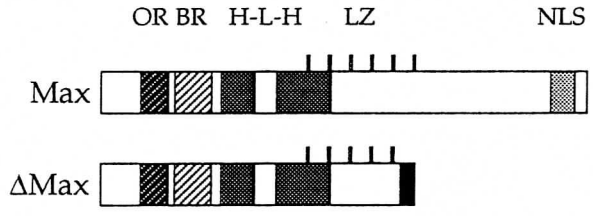

Figure 2. Schematic comparison of the Max and ΔMax proteins. The five ΔMax-specific amino acids are shown in black. OR, optional region of 9 amino acids; H-L-H, helix-loop-helix; LZ, leucine zipper; NLS, nuclear localization signal.

Dual regulation of Myc function(s) by Max and ΔMax

The ability of Max and ΔMax, but not Myc to form homodimers in addition to heterodimers creates a complex situation where the various oligomers can compete for binding to the same DNA sequence. The REF cotransformation results[84] suggest that the heterodimers (at least Myc/ΔMax, but probably also Myc/Max) enhance cell proliferation, while the homodimers of Max suppress cell transformation. This is in accordance with a recent report on the ability of Max to repress Myc-mediated stimulation of reporter constructs containing Myc-binding sequences.[86] By contrast, ΔMax homodimers are incapable of competing for DNA-binding, since they reside in the cytoplasm. Therefore, the critical questions to be tested are whether the observed opposite effects of Max and ΔMax are due to: (1) their differential subcellular distribution; or (2) the C-terminal sequences of Max not included in ΔMax.

In untransformed cells in the absence of substantial amounts of the c-Myc protein, both Max and ΔMax apparently exist as homo-oligomers or—maybe more probably—in complexes with other cellular proteins. Indeed, two laboratories have recently identified novel nuclear proteins associating with Max. Two of these, Mad[87] and MXI-1[88] have identical basic regions linked to HLH/LZ domains and differ in length only by one amino acid residue. Otherwise these proteins are not related to each other or to Myc or Max. Both proteins oligomerize specifically with Max, but not Myc. While the roles of these novel proteins in the regulation of Max—and indirectly also of Myc—remain to be elucidated, it has become clear that the network of interacting proteins around Myc is more complicated than previously anticipated. Yet a third novel gene termed MXI-2 has been isolated.[88] Its product is a protein kinase that associates equally well with Myc and Max. An interesting possibility to be tested is whether either of these proteins is a substrate for MXI-2

Roles for c-*myc* in the cell cycle

Driving of cell proliferation

The c-*myc* gene belongs to the immediate early growth response genes which are rapidly induced upon mitogenic stimulation of quiescent cells. Within 1 to 3 h, there is a transient increase of c-*myc* mRNA and protein levels in the stimulated cells.[53,89,90] Thereafter, c-*myc* expression declines to a basal level, where it remains throughout the cell cycle in continuously proliferating cells.[91,92] If, however, cells are deprived of growth factors or induced to differentiate, their c-*myc* expression is downregulated (see refs 1,7). The critical role of c-*myc* in cell proliferation has been further emphasized by experiments showing that c-*myc* antisense oligonucleotides are able to prevent entry of cells into the S phase, and can even induce differentiation.[93-96] Correspondingly, differentiation can be blocked by constitutive expression of any one of the *myc* genes.[97,98]

Growth factors binding to their cell surface receptors initiate several cascades of biochemical events ultimately leading to DNA synthesis and cell division. Therefore, it is not easy to evaluate the importance of individual nuclear responses such as c-*myc* induction. However, studies on signalling by a colony-stimulating factor 1 receptor (CSF-1R) mutant[99] have demonstrated a central role for c-*myc* in CSF-1-induced mitogenesis. The mutant CSF-1R which lacks one of its cytoplasmic autophosphorylation sites is no longer able to upregulate c-*myc* expression, although its ability to induce *fos* and *jun* genes is unaffected. While cells expressing this mutant failed to grow in serum-free medium containing CSF-1 as the only exogenous growth factor, infection of these cells with a c-*myc* expressing retrovirus restored their ability to proliferate. Quite similarly, two distinct signalling pathways could be distinguished in hematopoietic cells expressing the receptor for interleukin 2 (IL-2R).[100] While in this case induction of *fos* and *jun* required coupling of the IL-2R to a protein kinase of the *src* family, induction of c-*myc* as well as the proliferative response of the cells were dependent on the presence of a serine-rich region of the receptor. Interesting questions to be studied include: (1) what are the second messengers binding to the critical sites of the two cytokine receptors and transducing signals for c-*myc* induction; and (2) does c-Myc regulate those cell cycle genes (cyclins A and B and the $p34^{cdc2}$ kinase) which are induced together with c-*myc* in the IL-2R-expressing cells.

Besides regulating expression of other genes possibly required for progression in the cell cycle, some transcription factors are able to bind to the promoter or enhancer elements found near the

origins of replication and thereby enhance DNA replication.[101] This kind of direct involvement of transcription factors would also explain why transcriptionally active genes are replicated earlier in the S phase than quiescent genes.[102] However, due to conflicting data it is as yet unclear whether also c-Myc can directly participate in DNA replication (see ref 12).

Promotion of apoptosis

The decay of c-Myc after growth factor removal is even more rapid than its appearance after growth factor addition.[53] This has led to the hypothesis that the major function of c-Myc is not to facilitate entry into the cell cycle, but to prevent exit from it. This conclusion is supported by the recent finding that overexpression of c-Myc in IL-3-dependent myeloid cells leads to premature induction of programmed cell death upon growth factor removal.[103] Similar results have been obtained in fibroblastic cells transfected with an oestrogen-inducible c-*myc* construct.[104] In these cells, c-Myc-induced apoptosis could be observed at any stage of the cell cycle. Furthermore, it occured irrespective of the method used to block cell proliferation. c-Myc appears to be involved also in the activation-induced apoptosis of T cell hybridomas, since the death of these cells could be prevented by c-*myc* antisense oligonucleotides.[105] By contrast, glucocorticoid-induced apoptosis of the same cells remained unaffected, confirming previous observations that it proceeds through a distinct pathway.

The loss of c-*myc* overexpressing cells by apoptosis may provide a clue why additional genes are required for cooperation with *myc* genes in the neoplastic transformation of cells. Indeed, such genes include *bcl*-2, c-Ha-*ras* and v-*raf*, all of which have been shown to extend survival of IL-3-dependent cells in the absence of this growth factor.[103,106]

Concluding remarks

Although a prognostic role for *myc* amplification has thus far been demonstrated only in the case of N-*myc* in neuroblastomas (see M. Schwab, this issue, pp 13-18), it has become evident that deregulation of any of the *myc* genes can confer a growth advantage to the affected cells. Since Myc proteins appear not only to promote proliferation, but also apoptosis, other cooperating oncogenes have to be activated or tumor suppressor genes inactivated in order to obtain a viable population of tumor cells.

The recent identification of proteins interacting with Myc has opened up new possibilities for studies on Myc function(s) in both normal and transformed cells. It remains to be determined whether association of Myc with Max is a prerequisite for all observed Myc activities. Since the levels of Max do not appear to be rate-limiting, it does not seem surprising that this gene has not yet been found to be associated with any malignancies. Indeed, results from both transactivation and transformation assays suggest that overexpression of Max (but not ΔMax) could result in a growth disadvantage. The recently identified Max-binding proteins which apparently compete with Myc may prove to have an important role in the restriction of cell growth. Thus, in addition to the crucial question concerning the natural target genes for Myc, the various protein-protein interactions around Myc proteins will apparently be the major issues for future studies.

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