REVIEW ARTICLE

Myc oncogenes: the enigmatic family

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The *myc* family of proto-oncogenes is believed to be involved in the establishment of many types of human malignancy. The members of this family have been shown to function as transcription factors, and through a designated target sequence bring about continued cell-cycle progression, cellular immortalization and blockages to differentiation in many lineages. However, while much of the recent work focusing on the *c-myc* oncogene has provided some very important advances, it has also brought to light a large amount of conflicting data as to the mechanism of action of the gene product. In this regard, it has now been shown that *c-myc* is effective in transcriptional repression as well as transcriptional activation and, perhaps more paradoxically, that it has a role in programmed cell death

(apoptosis) as well as in processes of cell-cycle progression. In addition, particular interest has surrounded the distinct roles of the two alternative translation products of the *c-myc* gene, c-Myc 1 and c-Myc 2. The intriguing observation that the ratio of c-Myc 1 to c-Myc 2 increases markedly upon cellular quiescence led to the discovery that the enforced expression of the two proteins individually showed that c-Myc 2 stimulates cell growth, whereas c-Myc 1 appears to be growth suppressing. Clearly, the disparities in the activities of c-Myc, together with the consistent occurrence of mutations of *c-myc* in human malignancies, means that, although reaching an understanding of the functions of the *myc* gene family might not be simple, it remains well worthy of pursuit.

INTRODUCTION

Since the discovery of *c-myc*, the *myc* gene family and the proteins they encode have probably formed part of the research programme of nearly all the cancer research institutes in the world. A recent survey of the Medline database, for the period 1990–1994, revealed 3343 references which include Myc in their title or abstract and 55 review articles specifically on these genes and proteins. However, in spite of this plethora of information, the role and mechanism of action of Myc proteins still remain enigmatic. In fact, although in recent years there have been considerable advances towards an understanding of the role played by these proteins, the data involved are becoming not only extremely complex, but in many cases highly contradictory. It is therefore the aim of this review to outline aspects of the current literature that are turning the Myc family of proteins into the subject of an intense and interesting debate.

HISTORY

The majority of work on the *myc* gene family has focused on three members which when activated have been shown to be important in the generation of various human malignancies [1]. The most widely studied of these, *c-myc*, was first discovered through its homology with the transforming gene (*v-myc*) of the avian myelocytomatosis virus MC29 [2]. The other two, *N-myc* and *L-myc*, were discovered later through their homology with *v-myc* in the amplified sequences of neuroblastoma cells [3] and a small cell lung tumour [4] respectively. In addition to these three proto-oncogenic family members, two other *myc* genes, *S-myc* and *B-myc*, have also been identified. Despite only having been partially characterized, these two genes appear highly interesting since they differ from *c-myc*, *L-myc* and *N-myc* in that the proteins they encode appear to suppress malignant transformation (Table 1).

In a variety of species the *myc* genes have been shown to be activated by gene amplification [5], chromosomal translocation [6], proviral insertion [7] and retroviral transduction [8] (Table 2). However, it is most frequently reported that the level of the c-Myc protein is elevated in many tumour types by a mechanism that is none of the above and is as yet not understood. Early research into the proteins indicated their expression to be associated with cell-cycle progression [9] and incompatible with terminal differentiation in a variety of cell lineages [10,11]. In addition, *c-myc* was shown to cause cellular immortalization [12] and was able to co-operate with an activated *ras* gene in the transformation of rat embryo fibroblast cells [13]. In 1985, Eisenman et al. [14] established that, in cells containing active c-Myc or v-Myc protein, the majority of the protein was associated with a nuclear fraction, termed the 'matrix lamin'. This observation was later substantiated by the identification of a domain within the c-Myc protein that was found to be effective as a nuclear localization signal [15]. As a result of these findings, two schools of thought were generated as to how the Myc proteins bring about phenotypic change. It was proposed that they either have a direct role in the DNA replication machinery [16] or are involved in the transcriptional control of genes involved in cellular replication [17].

Evidence for a role in the control of gene expression grew when the sequence of the c-Myc protein was shown to contain a series of motifs which were similar to ones previously described for known transcription factors. Leucine zipper motifs, like those found in the oncoproteins v-Fos and v-Jun, were the first to be identified and were shown to be located in the extreme C-terminus of the protein [18] (Figure 1). Subsequently, immediately upstream of the leucine zipper motif, similarity was then found to a second domain termed the 'helix-loop-helix' motif [19]. This domain had already been identified in a number of transcription factors, including the immunoglobulin enhancer binding proteins E12 and E47 [20]. However, although both of these motifs had

Abbreviations used: ODC, ornithine decarboxylase; Rb-1, retinoblastoma susceptibility protein; YY-1, Yin-Yang 1.

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Table 1 Summary of the mammalian *myc* genes described to date

<i>myc</i> gene	Ascribed functions	Amino acid sequence identity with <i>c-myc</i> (%)	Refs.
<i>c-myc</i>	Cellular transformation Apoptosis Continued cell-cycle progression	—	[1,12,19,89]
<i>L-myc</i>	Cellular transformation Continued cell-cycle progression	35	[1,12,19]
<i>N-myc</i>	As for <i>L-myc</i>	38	[1,12,19]
<i>S-myc</i>	Growth suppression Apoptosis	31	[109,110]
<i>B-myc</i>	Inhibition of neoplastic transformation	61	[111]
<i>P-myc</i> , <i>L-myc</i> Ψ	Pseudogenes	Derived from regions of <i>L-myc</i>	[112]

Table 2 Incidence, mechanism and effects of *myc* activation

Abstracted from Marcu et al. [74].

Mechanism of <i>c-myc</i> activation	Effects	Incidence
Amplification	Increased Myc protein abundance	Gastric adenocarcinoma; small cell lung carcinoma; glioblastoma; carcinoma of breast; carcinoma of colon; plasma cell leukaemia; promyelocytic leukaemia; granulocyte leukaemia
Proviral insertion	Deregulated expression of <i>myc</i> by viral long terminal repeat	Leukaemia resulting from infection by avian leucosis virus or Molony murine leukaemia virus
Retroviral transduction	Deregulated expression of viral Gag–Myc fusion protein	Feline leukaemia viruses, e.g. GT3 and FTT; avian leukaemia viruses, e.g. MC29 and MH2
Chromosomal translocations	Deregulated expression of full-length or truncated <i>myc</i>	Burkitt's lymphoma; mouse plasmacytoma

Figure 1 Schematic representation of the structure of the human *c-myc* gene and the two resultant protein products, c-Myc 1 and c-Myc 2

The sites of the gene's promoters are indicated as P0, P1, P2 and P3. The translation initiation sites of the two proteins are shown as the codons of the initial amino acid, i.e. CTG (CUG) and ATG (AUG). NL, nuclear localization; BR, basic region; HLH, helix-loop-helix; LZ, leucine zipper.

previously been shown to be involved in the formation of transcription factor complexes, attempts to detect complexes in which Myc was either homo- or hetero-oligomerized proved fruitless. In spite of this, further studies revealed that the Myc proteins also contained a tract of basic amino acids upstream of the helix-loop-helix motif (Figure 1). This 'basic region' motif had been previously identified in the myogenic transcription

factor MyoD and was found to be the region involved in determining sequence-specific DNA binding [21]. As a final piece of indirect evidence for Myc family members being involved in the control of gene expression, it was shown that a region at the N-terminus of c-Myc (Figure 1) had the ability to act as a transcriptional transactivator. Fusion genes were constructed in which the *c-myc* N-terminal region was fused to the DNA

binding domain of the gene for the yeast transcription factor GAL 4. This construct was then transiently co-transfected into cells, with a reporter construct containing the GAL 4 DNA binding site upstream of the chloramphenicol acetyltransferase gene. The results of these experiments indicated that the transactivation potential of the fusion protein was both potent and highly specific for the N-terminal region of c-Myc [22].

A ROLE AS A TRANSCRIPTION FACTOR

Myc as an activator

As outlined above, many lines of indirect evidence accumulated to indicate that the Myc proteins functioned as transcription factors. However, problems arose in confirming this theory when it was found that Myc proteins could only form complexes with DNA at very high protein concentrations, implying that such interactions may not be physiologically significant [23]. It was therefore considered that Myc might require interaction with a second protein in order to achieve its role. With this in mind, Blackwood and Eisenman [24] screened a baboon expression library with a c-Myc protein labelled with ^{125}I . Subsequent sequencing of hybridizing colonies identified a small, novel, protein which they named Max (Figure 2). Analysis of this protein revealed that it was similar to Myc in that it also contained basic helix-loop-helix and leucine zipper motifs, and it was therefore considered to be a possible dimerization partner. Indeed, when assayed *in vitro* it was found that Max was able to form dimeric complexes with each of the Myc family members and at much lower protein concentrations than had previously been required to achieve Myc homodimerization. In addition, when a complex of c-Myc and Max was used to select preferred DNA sequences from a pool of partially randomized oligonucleotides, it was found that the dimer had specific DNA binding activity for the sequence CACGTG [25]. Further studies of this sequence using electrophoretic mobility shift assays showed that, although homodimers of Max were able to bind without the presence of Myc, appreciable binding of Myc was dependent on it forming a heterodimeric complex with Max [26].

Since the CACGTG motif had previously been identified as the binding site for other transcription factors containing basic region and helix-loop-helix motifs, it seemed encouraging that this might be the transcription target for Myc. This was analysed by a yeast reporter gene assay in which Myc and Max were co-expressed with a β -galactosidase gene under the control of a basal promoter linked to the CACGTG sequence. When Myc and Max were co-expressed in this system there was a considerable increase in the activity of the β -galactosidase gene [27].

The level of activity was dependent not only on the level of Myc but also on the presence of the domains which had previously implicated Myc as a transcription factor. Interestingly, although it was not a surprise that expression of Myc alone did not activate the reporter construct, it was intriguing to find that even though Max:Max homodimers bound to the CACGTG sequence they too did not activate the β -galactosidase gene [27] (Figure 3). Titration experiments revealed that, if the Max protein was expressed at high levels in conjunction with Myc, the β -galactosidase activity observed was less than when Myc and Max were expressed in roughly equivalent amounts. Although it was shown that Max preferentially formed a heterodimer with Myc as opposed to a homodimer [24,26] (Figure 3), this created a situation whereby the activity of genes regulated by Myc would be dependent not only on the levels of Myc but also on the levels of Max. The most likely explanation for the apparent lack of transcriptional activity by Max:Max homodimers has been given by Kato et al. [28]. In experiments designed to identify a transactivation domain in the Max protein, they constructed fusion proteins linking regions of Max with the DNA binding domain of GAL 4. When assayed for their ability to transactivate a reporter gene linked to the GAL 4 DNA binding site it was found that, unlike the Myc transactivation domain, no region of Max was effective in activating the gene's expression.

Although the CACGTG motif is present in the promoter regions of various genes, the search for Myc-activated genes has been somewhat without reward. In an attempt to address this problem Eilers et al. [9] generated a fusion protein between c-Myc and a portion of the oestrogen receptor. When this was introduced into mammalian cells, the activity of the exogenous Myc protein became dependent upon the presence of the steroid hormone β -oestradiol. cDNA libraries were then generated corresponding to mRNA species from control cells and from cells treated with β -oestradiol for 24 h. Comparison of these two libraries identified an mRNA that was induced by Myc as encoding α -prothymosin. Subsequent studies revealed that the gene could be activated by Myc in the absence of protein synthesis and that its activation was dependent upon the integrity of a CACGTG sequence found within the first intron of the gene [29]. As might be expected for a gene regulated by c-Myc, expression of α -prothymosin has been associated with proliferating cells [30,31] and it is expressed in nearly all tissues [32]. However, a role for α -prothymosin is yet to be found, and as a result its involvement in the phenotypes brought about by activated expression of Myc proteins remains a mystery.

A more appealing target for regulation by Myc is the enzyme ornithine decarboxylase (ODC). This enzyme catalyses the rate-limiting step in the production of polyamines [33]. Expression of

Figure 2 Alignment of Myc, Max, Mad and Mxi1

Domains that are common to each protein and are involved in heterodimerization are shown in colour. bHLH, basic helix-loop-helix; LZ, leucine zipper.

Figure 3 Transcriptional consequences of the formation of Myc-associated complexes

Arrow strength indicates preferential complex formation.

ODC is regulated in a cell-cycle-specific manner and is greatly elevated upon cellular transformation [34]. In addition, constitutive overexpression of ODC is able to bring about morphological transformation and its enhanced activity has been shown to be essential for transformation induced by the *v-src* oncogene [35]. The observation that the ODC gene contains two CACGTG sequences led to speculation that it too may be regulated by Myc. Studies using the Myc-oestrogen-receptor chimaeric protein revealed that ODC was indeed activated by Myc [36] and that its activation was dependent on the two Myc:Max binding sites within the first intron [37]. Further to these studies, it must be pointed out that while CACGTG is considered the primary target for the Myc:Max heterodimeric complex, Blackwell et al. [38] have also demonstrated DNA binding at a second canonical site (CATGTG) as well as to a series of non-canonical sequences. However, unlike the CACGTG sequence, the significance of these additional sites to the regulation of any genes by Myc:Max is yet to be established.

Myc as a repressor

The search for direct targets of c-Myc has perhaps been hindered by the relative difficulty of identifying genes which may be inactivated as opposed to activated by the protein. In this regard it is well established that the number of genes which have been shown to be repressed by Myc expression in *in vitro* reporter gene studies [17] or which are repressed in Myc-transformed cells is in excess of those which have been reported to be activated. Upon the discovery that Myc could interact with TFII-I [39] (Figure 4), a transcription initiation factor that activates core promoters through a sequence termed the initiator element [40], studies were undertaken to determine if Myc might participate in transcriptional control involving this protein. *In vitro* transcription assays using the adenovirus major late promoter revealed that, when introduced into a system involving activation by TFII-I, Myc protein was effective in bringing about transcriptional repression (Figure 3). Moreover, these effects were shown to be specific for TFII-I and were not apparent when Myc was introduced into systems being initiated by other factors, e.g. TFII-A. Subsequent analysis of the cyclin D1 gene, which had previously been shown to be repressed in Myc-transformed cells, indicated that the repression of this gene by Myc might also be mediated through TFII-I [41]. One might consider that the apparent repression of cyclin D1 by Myc seems paradoxical, bearing in mind the involvement that the cyclin is known to have in cell-cycle progression. In this regard, it must be pointed out that an independent study has implicated Myc in the activation of cyclin D1 transcription [42]. Further work is therefore required to establish the exact role, if any, that Myc is playing in the regulation of this gene.

Although a model for Myc action involving transcription repression is at first appealing, the probability of it being involved in the establishment of a transformed phenotype appears unlikely. Mutational studies of Myc and Max by Amati et al. [43] have indicated that cell-cycle progression and transformation by Myc proteins requires dimerization with Max. In contrast, inhibition of transcription through interaction with TFII-I occurs independently of Max and is refractory to forced expression of the Max protein [41]. Also, when mutants of the Myc protein were analysed for their ability to repress the promoter of the cyclin D1 gene, a deletion mutant involving amino acids 92–106 was ineffective in bringing about transcriptional repression [41], even though this region was previously shown to be dispensable during Myc-induced transformation of RAT1A cells [44]. However, these observations do not mean that transcriptional repression through TFII-I is artifactual. It would be naive to assume that the only transcriptional targets of c-Myc are those involved in transformation. For example, the ability of Myc

Figure 4 Representation of the sites of interaction of proteins known to bind c-Myc

BR, basic region; HLH, helix-loop-helix domain; LZ, leucine zipper domain; TBP, TATA binding protein component of the transcription initiator TFII-D.

proteins to inhibit processes of differentiation has not as yet been shown to be dependent on Max, and is therefore a possible Myc function in which TFII-I may be involved.

OTHER PROTEINS WHICH BIND TO MYC

An emerging theme arising from the study of transformed cells is that oncoproteins are frequently found to be associated with proteins having tumour suppressor or anti-proliferative function. Classically this observation comes from the analysis of the cellular proteins which co-precipitate with the transforming proteins of DNA tumour viruses. For example, the adenovirus E1A protein and the human papillomavirus type 16 and 18 E7 proteins have been shown to associate with the retinoblastoma protein (Rb-1) [45,46], while the respective E1B and E6 proteins of these viruses are believed to form complexes with p53 [47,48]. In addition, it is now becoming a frequent observation that, within the normal cellular environment, proteins having potential oncogenic activity are often found complexed with those with known tumour suppressor function, e.g. the association between the cyclin-dependent kinase 4 protein and the recently discovered tumour suppressor protein mts1/p16 [49]. It has now become apparent that the c-Myc protein forms such an interaction with the retinoblastoma family member p107 [50,51] (Figure 4). This protein had previously been shown to form complexes *in vivo* with the adenovirus E1A protein [52], the simian virus 40 large T antigen [53] and the human papillomavirus type 16 E7 protein [54]. Although it was known that this interaction formed an important part of the transforming ability of these viruses, its consequences for cellular homeostasis were not understood. Since the interaction between p107 and the N-terminus of c-Myc results in inactivation of the transactivation potential of the Myc protein, it seems feasible that binding of p107 by DNA tumour viruses might facilitate transformation by freeing the N-terminus of c-Myc and allowing the protein to interact with the TATA binding protein [55] (Figure 4). This would then allow Myc to transactivate its target genes. With respect to how this might be important to the establishment of tumours *in vivo*, the study included an analysis of mutated Myc proteins derived from Burkitt's lymphoma cell lines. Although these proteins retained an ability to form complexes with p107, they were unlike wild-type Myc in that their transactivation potential was unaffected by the interaction.

Perhaps more puzzling than the interaction with p107 is the report that c-Myc also interacts with the zinc finger protein Yin-Yang 1 (YY-1) [56] (Figure 4). Depending on the context, YY-1 has been shown to activate [57], repress [58] or initiate transcription [59]. However, when the YY-1 protein is bound to c-Myc both its ability to activate and its ability to repress transcription are reported to be inhibited [56]. Bearing in mind the work summarized above regarding the association between Myc and p107, it is interesting to note that among the targets for repression by YY-1 are the promoters of the adeno-associated virus P5 [59] and the human papillomavirus type 18 [60]. This, therefore, might be a possible reason for a negative regulator of Myc, i.e. p107, being a target for inhibition by human papillomavirus E7 [54]. Although this theory is highly speculative, it is somewhat reminiscent of the effect of the adenovirus E1A protein upon the interaction between Rb-1 and the transcription factor E2F [61]. Binding of Rb-1 by E1A results in the subsequent release of E2F such that it not only activates transcription of cellular genes involved in replication, but also causes activation of the adenovirus E2 promoter [62]. Since little is as yet known about the function and mechanism of action of YY-1, more work is required to establish the significance of its association with c-

Myc and the effect that this may have on the replication of DNA tumour viruses.

OTHER PROTEINS WHICH BIND TO MAX

It has been shown more recently that Max also forms complexes with two other proteins, Mad [63] and Mxi1 [64] (Figure 2). Whereas Mad:Max and Mxi1:Max complexes are similar to Myc:Max in that they have also been shown to bind the CACGTG motif, they are more like the Max:Max homodimer in that they are thought to act as transcription repressors (Figure 3). However, it has more recently been shown that, in contrast to the Max:Max homodimer, Mad:Max and Mxi1:Max are only effective in transcription repression as a ternary complex with homologues of the yeast transcription repressor Sin3 [65,66]. Although the role that these two new complexes may play within the cell is yet to be established, it is reasonable to assume that, since they are in competition with Myc for available Max protein, the levels or activities of Mad or Mxi1 may have a bearing on the ability of Myc to transactivate the promoters of its target genes. In fact, when these two genes were overexpressed in cells transformed by *c-myc* and *ras*, the number of cells scoring positive in an assay of transformation was greatly reduced [67]. In this regard, it is quite possible that loss or mutation of either *mad* or *mxi1* would result in release of Max protein, which would then be available to co-operate with Myc in situations where the abundance of Myc was elevated. However, since these genes have only recently been identified there has only been one report to date that would indicate that they might act as tumour suppressors in the genesis of human cancer. Eagle et al. [68] investigated the possibility that the *mxi1* gene, which maps to 10q24-q25, might be involved in a characteristic deletion of this area that occurs in a small number of cases of carcinoma of the prostate. The study reported that, in five tumours analysed, the *mxi1* locus was indeed reduced to hemizyosity. In addition, in four of the five cases the remaining allele involved was also mutated. This initial finding is highly provocative and it can now be considered an appropriate time to investigate whether *mxi1*, and *mad*, are targets for mutation in other tissues.

REGULATION OF THE ABUNDANCE OF THE MYC PROTEIN

Transcriptional control

It is reasonable to assume that the primary control of the abundance of a protein is largely determined by transcriptional initiation. Workers on Myc have not ignored this fact, and as a result a large amount of information has accumulated on regulation of the *myc* promoter. A complex story has evolved in which the gene is controlled not just by one promoter, but in fact by four. The two major promoters, P1 and P2 (Figure 1), contribute 75–90% and 10–25% of the cytoplasmic *c-myc* mRNAs respectively [69,70]. Approx. 1500 bp downstream of P1 and P2, and close to the translation start site, lies the third promoter P3. This promoter is thought to be less significant than P1 and P2, as it only contributes about 5% of *c-myc* mRNAs [71]. Of similar activity, but only present in the human *c-myc* gene, is a fourth promoter (P0) [72], which is located around 600 bp upstream of the major sites P1 and P2 (Figure 1). The significance of having the four promoters is as yet unknown, but their differential usage has been observed in many cell lineages and upon deregulated expression of the gene [73].

Analysis of this 5' region of the *c-myc* gene for DNase I hypersensitive sites revealed multiple regions of potential protein interaction (reviewed by Marcu et al. [74]). One region of particular note is a binding site which lies –65 to –58 bp

upstream of the P2 promoter. Deletion of this region has indicated that it is essential for the basal activity of P2 [75] and also for the activation of *c-myc* induced by the adenovirus E1A protein [76]. Studies of this region led to the discovery that it is bound by the cell-cycle-regulated transcription factor E2F [77]. In the light of this finding, it is encouraging that it has since been shown that activation of E2F is sufficient to direct cell-cycle progression [78], a characteristic that has also been attributed to the c-Myc protein. However, it is yet to be established whether this function of the E2F protein is, at least in part, mediated through activation of *c-myc* transcription.

Whereas it is perhaps not very surprising that a region of the *c-myc* promoter is controlled by a cell-cycle-regulated transcription factor, studies of other regions have proved to be more intriguing. The analysis of a further DNase I hypersensitive site situated approx. 130 bp upstream of P2 revealed a region that is highly important for P1 activation as well as being modestly involved in the activation of P2 [79]. Partial fractionation of HeLa cell nuclear proteins by Postel et al. [80] revealed a DNA binding activity that was specific for this region and which they termed PuF. The surprising twist came when the binding site for PuF was used to screen a HeLa cell cDNA expression library. The subsequent sequence analysis of a hybridizing clone revealed that it was identical to nm23-H2 [81]. Surprisingly, the protein product of this gene had previously been implicated in suppressing the metastatic spread of certain tumours [82]. Admittedly, it is difficult to comprehend why a suppressor of metastatic disease should be involved in the activation of a gene that is frequently elevated in the late stages of human cancer. Further work is therefore required to establish the role that this protein is playing not only in the regulation of *c-myc* but also in the establishment of a metastatic state.

Other levels of control

Perhaps disheartening to the investigators involved in studying the transcriptional control of *c-myc* was the finding by Bentley and Groudine [83] that the initial control of *c-myc* mRNA levels during induced differentiation of myeloid leukaemic cells was by transcriptional attenuation. Using nuclear run-on analysis they found that the ratio of exon 1 transcription relative to that of exon 2 was increased from 3 to 15 following induction of the differentiation programme. Subsequent analysis of the sequences involved in this process identified a 95 bp segment, located at the 3' end of exon 1 (Figure 1), to be the site of the premature termination [84]. However, when this site was analysed for its ability to attenuate transcription directed by other promoters its effectiveness was highly variable [84]. In this regard, it has since been shown the sequences responsible for the premature termination are not located at the site of attenuation, but are in fact found proximal to the P2 promoter [85]. In this study it was postulated that at this site the RNA polymerase might undergo a pause, at which point it is modified in a way which determines how it will respond at the site of attenuation.

Although a large amount of data has accumulated as to the transcriptional regulation of the *c-myc* gene, it must be pointed out that the mRNA and protein are also subject to post-transcriptional control. Both the mRNA and protein have been shown to have extremely short half-lives, of 15 min [86] and 30 min [87] respectively. However, it is known that these values are by no means absolute. For example, Lacy et al. [88] demonstrated that the half-life of *c-myc* mRNA in an Epstein-Barr virus-negative lymphoma cell line was increased from < 36 to > 70 min following *in vitro* infection with Epstein-Barr virus.

However, the significance that these findings have to the regulation of *c-myc* *in vivo* has yet to be established.

PROMOTER OF CELL GROWTH OR OF CELL DEATH?

Undoubtedly, the most paradoxical revelation from work on Myc was the finding that under certain circumstances the protein is able to induce programmed cell death (apoptosis). Two separate studies, one in fibroblasts [89] and one in haemopoietic [90] cells, revealed that when Myc was constitutively expressed the cells required the presence of exogenous growth/survival factors in order to replicate effectively. If serum in the case of fibroblasts, or interleukin 3 in the case of haemopoietic cells, was withdrawn from the Myc-expressing cultures the cells continued to enter the cell cycle, but then underwent apoptosis.

Two possible explanations for how the cell might make a choice between life and death as a result of Myc expression are outlined in Figure 5. Initially it was thought possible that the absence of exogenous growth/survival factors was having an

Figure 5 Possible mechanisms of Myc-induced apoptosis

(a) Pathway involving two sets of Myc-regulated genes, one set that regulates cell growth and one set that regulates apoptosis. (b) Pathway involving one set of Myc-regulated genes, in which the decision between cell growth and cell death is mediated by p53. G/S, growth/survival factors.

effect upon the transcriptional targets of c-Myc (Figure 5a). As described above, this could perhaps occur through a change in the relative abundances of transcriptional complexes involving the Myc protein, e.g. a change from a predominance of Myc:Max complexes to a predominance of Myc:TFII-I complexes. However, since it has now been shown that the apoptotic cell death induced by Myc requires dimerization with Max [91] and is dependent on an active ODC protein [92], it seems more likely that the same genes are transcribed in both the absence and presence of growth/survival factors and that the decision between life and death is made elsewhere (Figure 5b). The observation that the ability of Myc to induce apoptosis is abrogated in cells lacking a wild-type p53 protein [93] points to an involvement of p53 at some point during the apoptotic programme. In this regard it is interesting to note that apoptosis induced by the adenovirus E1A protein is also mediated by p53 [94]. However, whether the p53 protein acts via this mechanism to eradicate cells that contain an aberrant growth stimulus induced by other oncogenes is yet to be established.

TWO FORMS OF MYC PROTEIN: ONCOGENE VERSUS TUMOUR SUPPRESSOR?

A distinctive feature of the *c-myc* gene is that it encodes two alternatively translated protein products [95–97] (Figure 1). The proteins differ not only in size but also in the mechanism by which their translation is initiated [98]. The shorter and more predominant form of c-Myc, c-Myc 2, is initiated from a standard AUG site, whereas the longer protein, c-Myc 1, is initiated from a non-AUG site (CUG in humans) [98]. As it was noticed that c-Myc 1 was induced to levels comparable with those of c-Myc 2 as cells reached high densities during *in vitro* culture [99], it was considered that the two forms of the Myc protein may perform different roles. In order to investigate this, Hann et al. [100] generated clones of COS cells that expressed high levels of either c-Myc 1 or c-Myc 2. These cells were then transiently transfected with different regions of the Rous sarcoma virus long terminal repeat, to assess the possibility that the two Myc proteins possess distinct transcriptional activities [100]. The study revealed that one specific enhancer element, EFII, was activated by c-Myc 1 but not by c-Myc 2 (Figure 6). Surprisingly, when the sequence of EFII was analysed, it was observed that it did not contain the consensus Myc:Max binding site (CACGTG) and that activation by c-Myc 1 was dependent on a repeat sequence that contained a consensus binding site for a CCAAT/enhancer binding protein.

Subsequent electrophoretic mobility shift assays of this binding site showed, however, that it too was bound by Myc:Max heterodimers but not by Max:Max homodimers. This therefore opens up the possibility of a situation in which Myc:Max and the CCAAT/enhancer binding protein are in competition for the same binding site. However, the consequences that this might have *in vivo* are still unknown.

Although any cellular targets for transcriptional regulation by c-Myc 1 are yet to be discovered, the implications of this protein are extremely interesting, since COS cells in which it is over-expressed display a growth-inhibited phenotype [100]. It is also noteworthy that, in cases of Burkitt's lymphoma in which the *c-myc* gene is translocated to the immunoglobulin locus, the rearrangement frequently occurs such that only c-Myc 2 becomes activated [101,102]. Also, in cases in which the translocation results in both c-Myc 1 and c-Myc 2 activation, it has been reported that the c-Myc 1 protein is frequently mutated [103]. In addition, in all cases of Burkitt's lymphoma that display this reciprocal translocation, the non-translocated *c-myc* allele has been reported to be silenced [104]. In this regard, it is perhaps now worthwhile to re-investigate whether the *c-myc* gene is mutated in other tumours such that only c-Myc 2 becomes activated.

FUTURE PERSPECTIVES

Since the discovery of the Myc protein, much of the effort in cancer research has focused on the ability of oncogenes to induce continued cell-cycle progression. However, we are now aware that the establishment of disseminated disease is dependent not only on proliferative capacity but on a variety of contributing factors. As a result the oncogene field has come full circle, and groups are beginning to re-assess the roles that these genes are playing in processes such as apoptosis and metastasis. While it is now well established that Myc can also function as an inducer of programmed cell death, it would also be of interest to assess any involvement the protein might have in cellular invasion. The observations that the Myc proteins can induce morphological transformation and adhesion-independent growth can be considered as criteria of an invading cell. Coupled with the knowledge that the *myc* gene generally appears to be activated in the late stages of cancer [105], further investigation of a role for Myc in these areas might be particularly rewarding.

One of the major objectives for the further elucidation of the function of the Myc proteins must lie with the identification of

Figure 6 Implications of the different properties of the two Myc proteins, c-Myc 1 and c-Myc 2

C/EBP, CCAAT/enhancer binding protein recognition sequence.

additional target genes. If this is achieved, it will provide a means to establish how all of the phenotypic changes which are attributable to Myc actually occur. For example, at present the role of Myc in the regulation of cellular differentiation is still a contentious issue. It is widely reported that elevated expression of Myc protein can block differentiation in many lineages [10,11]. However, it is not known whether this is through direct interaction with the differentiation machinery or whether it is a result of the continued cell-cycle progression caused by Myc in cells which must undergo growth arrest for terminal differentiation. One report by La Rocca et al. [106] has indicated that the blockage of differentiation of myogenic cells by Myc can be dissociated from the ability of the protein to induce morphological transformation. However, the mechanistic differences that give rise to this observation are yet to be determined.

CONCLUDING REMARKS

In spite of the consistently conflicting data regarding the Myc proteins, the importance of their role in both the normal and the transformed cell cannot be overstated. Most strikingly, studies involving transgenic mice in which Myc was generally over-expressed showed the induction of widespread neoplasia [107], whereas when the gene was disrupted by homologous recombination the mice died before 10.5 days of gestation [108]. When this is considered alongside the frequent reports of *myc* mutation in malignant disease, it becomes obvious that, although the road to an understanding of Myc function may not always be smooth, it is certainly one worth following.

Note added in proof (received 5 February 1996)

Two further Mad-related proteins have since been discovered, Mad3 and Mad4 [113].

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