B-Myc: N-Terminal Recognition of Myc Binding Proteins†

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ABSTRACT: B-Myc is an endogenous, N-terminal homologue of transcription factor c-Myc that lacks the C-terminal DNA binding and protein dimerization domain of c-Myc. Clinical mutations in the c-Myc N-terminal region, and the subsequent misregulation of Myc, are implicated in the development of numerous human cancers. Myc functions to both activate and repress transcription by associating with multiple binding partners. We investigated the structural and dynamical properties of B-Myc, free or associated with the transactivation inhibitor, MM-1, and the activator, TBP, using NMR spectroscopy. B-Myc has no persistent tertiary structure, yet regions corresponding to Myc homology boxes 1 and 2 (MBI and MBII, respectively) have molten globule-like characteristics. B-Myc binds to MM-1 in a specific manner without becoming highly structured. The local regions of B-Myc involved in binding differ for MM-1 and TBP, and regions not identified by mutagenesis are found to be involved in MM-1 binding. The results provide new insights into Myc N-terminal protein—protein interactions. We propose a model for Myc regulation through differential involvement of MBI and MBII in the binding of Myc interacting proteins.

B-Myc is a murine, N-terminal homologue of c-Myc that is 62% identical in sequence (1–4). Although the physiological role of B-Myc is still under investigation, when coexpressed with c-Myc, B-Myc inhibits transcriptional activation and cellular transformation by c-Myc (5). B-Myc expression is regulated by androgens and thus may function in vivo to control cell proliferation in a restricted subset of adult tissues affected by c-Myc (2, 4).

Changes in Myc expression and mutations in Myc are linked directly to the development of cancers that include lymphomas and cancers of the bladder, breast, colon, skin, ovary, and prostate (6–8). As a contributing factor in tumor development, c-Myc is a key target for therapeutic strategies for controlling this disease (9–11). In normal cells, Myc proteins regulate many aspects of cellular metabolism and direct the decision of cells to proliferate or to undergo apoptosis (12, 13). The diverse cellular roles of Myc are reflected in the identity of the genes that are regulated by Myc transcription complexes, such as ornithine decarboxylase, cdc25A, p53, and others (8, 14–17).

The central members of the mammalian Myc protein family are c-, N-, L-, S-, and B-Myc (6, 18). The cellular functions attributed to Myc proteins are mapped to the structural integrity of highly conserved N- and C-terminal domains (Figure 1A). For all Myc proteins, the N-terminal regions contain two such segments termed Myc homology box I (MBI), residues 45–63 of c-Myc (corresponding B-Myc residues 43–61), and Myc homology box II (MBII), residues 129–141 of c-Myc (corresponding B-Myc residues 119–131) (6, 8). MBI and MBII serve as core motifs through which Myc interacts with a variety of regulatory proteins (19). The C-terminal regions of the Myc proteins contain a basic helix-loop-helix leucine zipper (bHLH LZ) motif that mediates the dimerization of Myc with other bHLHZ proteins (e.g., Mad and Max) and the sequence-specific binding of Myc dimers to DNA (17, 20, 21). The X-ray crystal structure of the C-terminal region of c-Myc was determined recently to be a heterodimer with Max (22), but no atomic-resolution structure of a Myc N-terminal region exists to date.

Many proteins interact with the N-terminal regions of Myc proteins, and the differential binding of these proteins is thought to mediate the function of Myc as a transcriptional activator, transcriptional repressor, inducer of cell proliferation, and initiator of apoptosis (23). Here, we examine the interaction of B-Myc with Myc modulator-1 (MM-1) and TATA-binding protein (TBP). MM-1 is a protein that previously has been shown to interact with the N-terminal region of c-Myc (24). Using c-Myc truncation mutants in yeast two-hybrid, mammalian two-hybrid, and GST pulldown

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1 Abbreviations: MBI, Myc homology box I; MBII, Myc homology box II; MM-1, Myc modulator 1; TBP, TATA-binding protein; NMR, nuclear magnetic resonance.
assays, the interaction of MM-1 with c-Myc was mapped to a region spanning MBII (24). MM-1 exerts a negative influence on the transactivation of target genes by Myc and inhibits the ability of Myc to transform cells in cooperation with activated Ras (24). TBP also interacts with the N-terminal region of c-Myc both in vitro and in vivo but is thought to exert a positive influence on Myc-mediated transactivation (25–28).

Structural and dynamical information used to define the nature of macromolecular interactions and interfaces was reported for the C-terminal DNA binding and dimerization domain of c-Myc (22). For the c-Myc N-terminal region, however, available structural information is limited to a recent NMR structure determination for a complex of a peptide derived from MBI of c-Myc and the SH3 domain of Bin-1 (29). In the association of c-Myc with Bin-1, 12 residues of the c-Myc peptide contact Bin-1 and bind in an extended conformation along the surface in the normal poly-Pro site. Structural analysis of the N-terminal domain of Myc has been hampered by the inability to produce sufficient quantities of soluble protein. We have taken advantage of the structural and functional conservation displayed across all members of the Myc family to investigate the structure of the full N-terminal domain using the soluble B-Myc protein, alone and together with transactivation inhibitor MM-1 and the activator TBP using circular dichroism (CD) and NMR spectroscopy. Our data suggest that B-Myc is largely unstructured in solution and that the interaction of B-Myc with MM-1 is mediated not only by MBII, as previously shown, but also by MBI and possibly the unique C-terminal tail. Moreover, the interaction of B-Myc with transcriptional activator TBP was found to be distinct from that of B-Myc with MM-1. These results support a regulatory model in which differential binding of specific residues in the N-terminal regions of Myc proteins leads to either activation or repression of Myc-mediated gene transcription.

**MATERIALS AND METHODS**

**Protein Expression and NMR Sample Preparation.** Full-length murine B-Myc (residues 2–169) was expressed in *Escherichia coli* BL21-RP cells (Stratagene) grown in M9 minimal medium as an N-terminal GST-tagged construct in the pGEX-5x-3 vector (Amersham). Nine conservative mutations were introduced into the original construct to replace rare *E. coli* codons. The cell pellet was resuspended in 1XPBS (pH 8.0), 5 mM DTT, protease inhibitor cocktail (Sigma), and 5 mM EDTA and lysed. Clarified lysate was passed over a GSTrap column (Amersham), and the protein
eluted with 1XPBS (pH 8.0) and 20 mM GSH. GST was cleaved using Factor XA (Novagen) and separated from B-Myc using a MonoQ column (Amersham) eluted with a linear NaCl gradient on an AktaExplorer-100 instrument (Amersham). Fractions were dialyzed against 20 mM NaH2PO4/Na2HPO4 (pH 6.0), 100 mM NaCl, and 1 mM DTT and concentrated. Approximately 5–10 mg of B-Myc was obtained per liter of cell culture. The original construct of B-Myc was a gift from M. Gregory (Vanderbilt University, Nashville, TN).

Full-length MM-1 (residues 1–166) was expressed in the pGEX-5x-1 vector in E. coli BL21-RP cells in LB. Pelleted cells were resuspended in 50 mM Tris (pH 8.0), 100 mM KCl, 10% glycerol, and protease inhibitor cocktail and lysed. Clarified lysate was passed over a HiTrap Ni Affinity column and eluted with a gradient from 0 to 100 mM imidazole. The eluant fractions were passed over a heparin affinity column (Amersham) and eluted with a gradient from 0 to 1 M NaCl. Fractions containing TBP were dialyzed against 20 mM NaH2PO4 (pH 6.0), 100 mM NaCl, and 1 mM DTT and concentrated. The pET-15b-TBP construct was a gift from H. Ariga (Hokkaido University, Hokkaido, Japan).

TATA-binding protein (TBP, residues 1–182) was expressed in the pET-15b vector in E. coli BL21-DE3 cells in LB. Pelleted cells were resuspended in 50 mM Tris (pH 8.0), 100 mM NaCl, 100 mM KCl, 10% glycerol, and protease inhibitor cocktail and lysed. Clarified lysate was passed over a HiTrap Ni Affinity column and eluted with a gradient from 0 to 100 mM imidazole. The eluant fractions were passed over a heparin affinity column (Amersham) and eluted with a gradient from 0 to 1 M NaCl. Fractions containing TBP were dialyzed against 20 mM NaH2PO4 (pH 6.0), 100 mM NaCl, and 1 mM DTT and concentrated. The pET-15b-TBP construct was a gift from L. Stargell (Colorado State University, Fort Collins, CO).

NMR experiments were conducted in 5 mm Shigemi tubes in a 90% H2O/10% D2O mixture with 20 mM NaH2PO4 (pH 6.0), 100 mM NaCl, 5 mM DTT, and 0.01% NaN3 at final protein concentrations of ~0.5–1.5 mM as determined by UV280 absorbance. The B-Myc/MM-1 and B-Myc/TBP samples were prepared at an approximately 1:3 molar ratio.

**CD Spectroscopy.** CD experiments were conducted using a Jasco J-810 spectropolarimeter at room temperature in 20 mM NaH2PO4/Na2HPO4 (pH 6.0) and 50 mM NaCl at total protein concentrations of ~0.5 mg/mL and B-Myc/MM-1 molar ratios 0.5:1, 1:1, and 2:1. Proteins concentrations were determined by UV280 absorbance.

**NMR Spectroscopy.** Triple-resonance experiments were carried out at 300 K on a Varian Inova 800 MHz spectrometer (National Biological NMR Laboratory of Finland) or a Bruker DRX-500 [Purdue Interdepartmental NMR Facility (PINMRF)]. Assignments for main chain and CB resonances of free B-Myc were made by standard methods with data from CT-HNCA, HN(CO)CA, HN(CA)CAB, and HN(CO)-CACB experiments. For B-Myc in the presence of MM-1 or TBP, the quality of triple-resonance experiments was not adequate for chemical shift assignments.

Relaxation data were measured in duplicate at 300 K with a Bruker DRX-750 MHz spectrometer (National Magnetic Resonance Facility, Madison, WI). T1 spectra were recorded using delay times of 10, 20, 40, 80, 160, 320, 640, and 1280 ms. T2 spectra were recorded using delay times of 8, 32, 48, 72, 96, 112, and 128 ms. 15N heteronuclear NOEs were recorded in an interleaved fashion using a 3 ms saturation pulse and an overall delay time of 5 ms (30). All spectra were processed with NMRPipe and analyzed using SPARKY version 3.106 (31). Only peaks resolved at half-peak height were used in the analysis of the relaxation data. R1 and R2 rates were determined using an exponential fit to the signal decay of the selected Lorentzian integrated peak volumes.

**RESULTS**

**Structural Characterization of Free B-Myc.** Approximately 86% of the backbone N, H2, C, C, and CO resonances were assigned for free B-Myc. The amide proton peak dispersion in the 15N HSQC spectrum (Figure 2A) is less than 1 ppm and spans from only 7.7 to 8.6 ppm, similar to the sequence-corrected random coil range of 7.54–8.56 ppm (32, 33). Chemical shift indexing (CSI) showed no consensus for either helical or strand content (data not shown) (34). In addition, CD data, discussed below, confirm the absence of secondary structural elements. These results indicate that free B-Myc lacks a stable tertiary fold and does not contain persistent secondary structure.

1H–15N heteronuclear NOE intensities were measured to further characterize the structure of B-Myc. The data suggest that some degree of structural organization exists for the regions near MBI and MBII despite the lack of overall stable tertiary structure. The NOE value expected for highly mobile HN groups is <0 and for folded proteins the size of B-Myc is >0.7. For a completely unfolded protein with a polymer-like configuration, the NOE value is expected to increase somewhat toward the central region of the chain and fall off near the termini (35, 36). The individual resonance NOE values observed along the B-Myc chain, as well as the trend characterized with a five-residue moving average of the NOE values, are plotted as a function of residue in Figure 3. For the regions surrounding MBI and MBII, the values tend to be relatively larger, while in the center of the protein, between MBI and MBII, the NOE value tends to be smaller and closer to 0 with some residues showing NOE values approaching ~0.5 indicating relatively greater mobility. Thus, the pattern is consistent with neither a completely unfolded protein nor a folded protein. The NOE values are 0.1–0.2 for the molten globule form of the H helix from the similarly sized apomyoglobin protein (37). Therefore, B-Myc in solution is not a well-ordered globular structure, but the vicinity of MBI and MBII appears to be a more collapsed structure on average, with slower rotational mobility similar to that of molten globule proteins.

In view of the limited structure suggested from the NOE profile, we assessed the level of structure predicted from sequence-based algorithms. Interestingly, both secondary structure (38) and ordered structure (PONDR) (39) are predicted for short structural elements that coincide with the location of MBI and MBII (Figure 1B). The MBI helix is amphipathic (Figure 1C) with polyproline (PP) stretches and regulatory phosphorylation sites flanking the sequence (40, 41). The predicted helices, consisting of only seven residues each, may be present but only transiently.

**B-Myc Association.** The association of B-Myc with MM-1 and TBP is apparent from changes in the 15N HSQC spectrum of B-Myc. Addition of either MM-1 (Figure 2B, red) or TBP (Figure 2C, cyan) differentially affects the HSQC spectrum by altering the frequency or intensity of certain peaks observed for free B-Myc. In addition, new peaks are apparent
outside the region of the shifts for free B-Myc, particularly in the case of TBP. Nonetheless, a large increase in the extent of dispersion expected for formation of tertiary structure (42) was observed with neither MM-1 nor TBP.

We used CD spectropolarimetry to assess potential changes in the secondary structure of B-Myc upon binding of MM-1. Neither α-helical nor β-strand secondary structure is apparent in the free B-Myc spectrum (Figure 4A, black), which resembles that of a random coil protein. The CD spectrum of the GST–MM-1 fusion construct (Figure 4A, gray) exhibits the α-helical content expected for GST. The spectrum of B-Myc in the presence of the GST–MM-1 fusion construct and that calculated by summation of the free B-Myc and GST–MM-1 spectra are shown in Figure 4B. The calculated spectrum (gray) approximates that observed for the mixture of B-Myc and the GST–MM-1 fusion construct (black). From these results, we conclude that association with MM-1 induces neither secondary nor tertiary structure in B-Myc. Together, the limited change in chemical shift dispersion and the absence of an effect on the CD spectrum indicate that B-Myc interacts in a largely extended form, similar to that observed for the interaction of c-Myc with the SH3 domain of Bin-1 (29).

To further characterize intermolecular association of B-Myc, the specific residues affected by addition of the transactivation proteins were identified from the changes in the 15 N HSQC spectrum of B-Myc (43, 44). Comparison of the HSQC spectra of B-Myc in the presence of MM-1 (Figure 2B, red) and TBP (Figure 2C, cyan) indicates that the perturbation to the HSQC spectrum depends on the transactivation protein and is likely the result of intermolecular association rather than nonspecific effects, such as crowding at high protein concentrations. A peak was considered to exhibit a perturbation relative to the free state if in the presence of MM-1 or TBP the peak was observed to shift >0.05 or 0.1 ppm in the 1H or 15 N dimension, respectively. Additionally, a perturbation was considered to occur if the peak height was reduced to less than 40% of the intensity observed with free B-Myc. A high contour level is used to plot the overlaid spectra shown in Figure 2 so that peaks with reduced intensity in the presence of MM-1 or TBP do not appear in the plot.

**Figure 2:** 15 N HSQC spectra of (A) B-Myc and (B) B-Myc with MM-1 (red) or (C) B-Myc with TBP overlaid with that of B-Myc alone (black). Labels indicate assigned free B-Myc peaks that show perturbations upon association with either MM-1 (panel B labels), TBP (panel C labels), or both (panel A labels). For the purpose of clarity, the data are plotted with the minimum contour threshold set higher than the intensity of perturbed peaks with reduced intensity in the presence of MM-1 or TBP (see the text for details). (D) Resonances with reduced intensity or altered frequency in the presence of MM-1 (red), TBP (cyan), or both (green) are mapped onto the sequence of B-Myc. Resonances for which relaxation rates were determined are marked with an asterisk. MBI and MBII are underlined.

**Figure 3:** Individual values (circles) and moving average (five-residue window) (curves) of 1H–15 N NOEs. Free B-Myc values are shown as a solid line with filled circles, and B-Myc–MM-1 values are shown as a dashed line and empty circles in the NOE (|Δδ|) graph. The calculated spectrum (gray) approximates that observed for the mixture of B-Myc and the GST–MM-1 fusion construct (black). From these results, we conclude that association with MM-1 induces neither secondary nor tertiary structure in B-Myc.
NMR reveals distinct sites affected by binding MM-1 or TBP. Deletion studies that show c-Myc residues 104 are required for binding to MM-1 and TBP, respectively. These HSQC perturbation results are consistent with previous association of B-Myc with MM-1; resonances of several residues in MBI and MBII are perturbed by MM-1, which implies that MM-1 association involves both MBI and MBII.

Localization of MM-1 Interaction to MBI and MBII. The association of B-Myc and MM-1 was further characterized by heteronuclear relaxation. Given the clear distinction in the changes in the B-Myc HSQC spectrum upon addition of MM-1 and TBP, the effects on heteronuclear relaxation upon association on individual residues of B-Myc.

Interaction with MM-1 differentially affects the R_2/R_1 ratio for individual residues of B-Myc (Figure 5). The results show that certain regions of B-Myc are strongly affected by association with MM-1 while others are not. A 3–5-fold increase in R_2/R_1 is localized to the regions surrounding MBI and MBII with some effect observed at the unique C-terminal region of B-Myc. Other regions show substantially smaller effects. The estimated r_2^app values for MBI and MBII upon binding MM-1 increase by 3.3 and 5.0 ns, respectively, compared to those of regions outside the Myc homology boxes where r_2^app increases an average of only 1.3 ns (Table 1). The r_2^app values increase in the presence of MM-1 but remain significantly lower than values expected for a folded 36 kDa B-Myc-MM-1 complex. For example, the values of 6–9 ns in Table 1 for r_2^app approximate the 8.3 and 9.0 ns values reported for ~13 kDa, globular proteins (47). B-Myc associated with MM-1, therefore, does not form a well-ordered globular dimer.

Figure 5 identifies B-Myc residues that associate strongly with MM-1. Of particular interest is the fact that the elevated R_2/R_1 values extend ~15 residues N-terminal to MBII starting from residue 102. This “extended” MBII form with residues 102–132, called here MBII*, is highly conserved between B-Myc and c-Myc, but the sequences of these two members of the Myc family have less conservation with N-, S-, and addition of MM-1 are reasonably assumed to be the result of intermolecular association. Changes in NMR dipolar relaxation rates for longitudinal (R_1), transverse (R_2), and nuclear Overhauser effect (NOE) relaxation reflect changes in effective molecular weight and rotational correlation time, τ_c, associated with intermolecular binding. In addition, R_2 is sensitive to a residue existing in more than one magnetic environment, such as free and bound states, and the rate of exchange between multiple environments.

15N relaxation of main chain amide resonances was assessed for B-Myc alone and in the presence of MM-1. R_1 and R_2 were estimated for 57 resolved 15N HSQC peaks from residues along the full length of B-Myc (Table 1). Association of B-Myc with MM-1 is apparent from effects on both R_1 and R_2. R_1 values increase approximately 12–43%, while R_2 values show substantially larger increases of 90 to >400%. The dependence of R_1 on τ_c passes through a maximum as τ_c increases, while R_2 increases continuously with τ_c. The trends in the changes in R_1 and R_2 between free B-Myc and B-Myc associated with MM-1 indicate that τ_c for B-Myc is near the value at the maximum R_1.

The site of intermolecular interaction was localized by monitoring changes in the R_2/R_1 ratio of individual residues. In the absence of chemical exchange, the R_2/R_1 ratio depends on a residue’s τ_c value associated with molecular rotation on a nanosecond time scale and is less sensitive to faster local motions on a picosecond time scale (45, 46). We use the R_2/R_1 ratio to assess the nature of the intermolecular association of B-Myc with MM-1 even though exchange cannot be ruled out and estimate an apparent correlation time, τ_c^app, by residue. In the case where exchange does not contribute to R_2, τ_c^app reflects that exchange rather than an accurate rotational correlation time. Nonetheless, examination of changes in B-Myc τ_c^app values in the presence of MM-1 provides a convenient measure to contrast the effect of MM-1 association on individual residues of B-Myc.

2 A small number of resonances that are not mapped to residues in Figure 2C, because overlap precluded a unique assignment of the resonance, were perturbed by binding.

Figure 4: (A) CD spectra of B-Myc alone (black) and the GST-MM-1 fusion construct alone (gray). (B) CD spectrum for the B-Myc/GST-MM-1 solution calculated from the summation of spectra in panel A (black) and observed (gray).
Table 1: $R_1$ and $R_2$ Values for B-Myc and the B-Myc–MM-1 Complex$^a$

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$^a$ Data for MBI and MBII$^a$ are bold. The apparent correlation time, $r_1^{app}$ (see the text), is calculated from the $R_1/R_2$ ratio by Tensor (72). The percent change is represented as (bound − free)/free.

**Figure 5:** $R_1/R_2$ ratios measured at a field strength of 750 MHz for B-Myc (●) and the B-Myc–MM-1 complex (Δ). MBI and MBII are shaded. MBII$^a$ includes the 15 N-terminal residues and is shown in a dotted box.

**Conclusion:**

NMR spectroscopy was used to characterize the structural order of B-Myc both free and bound to transactivation inhibitor MM-1 and transactivation activator TBP. Free B-Myc is unfolded and flexible over its 171-residue length. Relaxation properties are consistent with a more collapsed but disordered state characteristic of molten globules, with residues forming MBI and MBII showing a slight tendency toward slower mobility. An understanding of the functional advantage of intrinsically disordered proteins (50–52) based largely on the residue-level structural information provided by NMR spectroscopy (53) is starting to emerge. Intrinsically disordered regions of certain proteins are known to adopt ordered structure upon binding (54–58). It has been suggested that linking protein–protein association to folding allows for a specific interaction with low affinity (59) that is important for reversible binding of regulatory proteins, including transcription factors.

An important finding determined from the residue-level information of NMR in this study is that the associations of MM-1 and TBP with B-Myc are distinct. Further, NMR relaxation data of B-Myc with MM-1 clearly show that intermolecular association is localized to specific residues in the more collapsed regions of MBI and MBII, but we find no compelling evidence from CD, chemical shift dispersion, or homonuclear NOEs that binding to MM-1 induces secondary or tertiary structure.

That binding is specific, yet substantial regions remain unfolded in the B-Myc–MM-1 complex, appears to differ somewhat from that in certain other intrinsically disordered protein complexes in which more ordered, folded states are acquired upon binding (53). The binding characteristics of the B-Myc–MM-1 complex are more consistent with the recent description of the interaction of c-Myc with the Bin-1 SH3 domain (29). In contrast to association of Myc with Bin-1, however, association of Myc with MM-1 does not occur via a recognized binding domain, such as an SH2 or SH3 domain, as MM-1 has no such domains as determined by sequence analysis. Specific binding of Myc to one transactivation domain, while much of Myc remains unfolded, provides a mechanism for the requirement that Myc functions by specifically recognizing numerous binding partners, some simultaneously. Regions remaining unfolded in one association complex can provide the specificity determinants for recognition of another binding partner.

The diverse cellular functions of the Myc protein family depend on the N-terminal domain of the protein where many protein–protein interactions are mediated by MBI and MBII (6, 8). HSQC perturbation shows the interactions of B-Myc with MM-1, a transactivation inhibitor, to be distinct from those with TBP, a transactivation activator. MM-1 binding affects both MBI and MBII, but association of TBP with Myc influences mostly MBII and sequences C-terminal to this core motif. Exploiting NMR relaxation to more fully characterize Myc protein–protein associations and the influence of Myc point mutations, such as those that replace c-Myc Trp 135 (corresponding to B-Myc Trp 126), which is important for transformation (60, 61), yet dispensable for transactivation (28, 60), will be of interest.

Our results find that the effect on MBII of the interaction between B-Myc and MM-1 extends approximately 15 residues N-terminal to MBII. The extended region, MBII$^a$, is noteworthy in light of the sequence similarity between...
B-Myc and c-Myc. While MBI and MBII are conserved across all members of the Myc family, only B-Myc and c-Myc share extensive sequence similarity for residues N-terminal to MBII (Figure 2A). The higher degree of similarity between B-Myc and c-Myc is apparent from the observation that B-Myc inhibits c-Myc activity presumably by competing for c-Myc N-terminal interacting proteins (5). Our data are the first to suggest that the Myc proteins utilize amino acids outside of the highly conserved MBI and MBII to control the affinity and/or stability of interactions with other proteins. This trait also holds for the interaction of B-Myc with TBP, which extends to residues C-terminal to MBII and more highly conserved between B-Myc and c-Myc than other Myc family members.

In addition to the interaction of MM-I with amino acids N-terminal to MBII, we report a previously undetected interaction between MM-I and MBI. MBI sits in a structurally intriguing region that is predicted to form a short amphipathic helix (Figure 1B) positioned between two polyproline stretches. MBI is located just N-terminal to two known regulatory phosphorylation sites that play a major role in controlling the overall activity of Myc by triggering protein turnover (40, 62–67). Given the involvement of MBI in MM-I association, phosphorylation may also be important in modulating Myc protein–protein interactions by allowing the N-terminal domain to adopt a structure that differentially affects binding of activators and inhibitors.

From the data reported here, we propose a model for Myc regulation in which MBI and MBII bind differentially to alternative regulatory partners (Figure 6). Transcriptional activation involves association of Myc with activators such as TBP and Amy-I (68) mediated through MBII. Further assembly with additional transcription complex factors could require access to regions of Myc near MBI. On the other hand, transcriptional repressor MM-I associates with an interface of B-Myc extending to both MBI and MBII and thus could inhibit proper association of other protein factors of the transcription complex required for transcription. The extended interfaces that have been described for the interaction of Myc with transcriptional repressors such as MM-I (current study and ref 24), Bin1 (69), and p107 (70) suggest that these proteins may serve as a type of “molecular sponge” that interacts with MBII and now MBI to preclude the formation of a productive multiprotein complex needed to activate transcription. Growth signals that culminate in the partial, or complete, release of the transcriptional repressors allow key activators to bind to Myc and recruit the remaining components of the transcriptional machinery.

REFERENCES


