# Structural Aspects of Interactions Within the Myc/Max/Mad Network

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**Abstract** Recently determined structures of a number of Myc family proteins have provided significant insights into the molecular nature of complex assembly and DNA binding. These structures illuminate the details of specific interactions that govern the assembly of nucleoprotein complexes and, in doing so, raise more questions regarding Myc biology. In this review, we focus on the lessons provided by these structures toward understanding (1) interactions that govern transcriptional repression by Mad via the Sin3 pathway, (2) homodimerization of Max, (3) heterodimerization of Myc–Max and Mad–Max, and (4) DNA recognition by each of the Max–Max, Myc–Max, and Mad–Max dimers.

# 1
# Introduction

Mutations of genes of the *myc* family have been shown to be among the most frequently affected in the majority of human malignancies (Nesbit et al. 1999). *Myc* genes were first identified as the transforming agents within chicken retroviruses (Sheiness et al. 1978). Over the last 25 years, compelling evidence has accumulated for the role of *myc* homologs in tumor formation, both in experimental systems and in human cancers (Cole and McMahon 1999; Dang et al. 1999; Eilers 1999; Liao and Dickson 2000; Nesbit et al. 1999).

The Myc gene products are transacting transcriptional regulators containing two independently functioning polypeptide regions: N-terminal transactivating residues and a C-terminal DNA binding segment (for a review see Grandori et al. 2000; Fig. 1). The DNA binding segment tethers Myc family gene products to sequences upstream of the core promoter, thereby enabling activation domains to modulate the efficiency of messenger RNA synthesis (Kato et al. 1990). The initial identification of a DNA binding segment within Myc family genes was based on sequence similarities with other transcription factors possessing a modular DNA binding/dimerization motif consisting of a two amphipathic α-helices (helix H1 and H2) separated by a loop (Murre et al. 1989). Myc family members also contain a basic region preceding the first α-helix and a leucine zipper region carboxy-terminal to the second α-helix. In general, the basic-helix-loop-helix-leucine zipper (bHLHZ) domain specifies dimerization through the helix-loop-helix-leucine zipper (HLHZ) region and DNA recognition through interactions between the basic region (b) and the major groove. However, Myc cannot form homodimers at physiological concentrations in vivo, and is incapable of sequence-specific DNA binding in isolation (Dang et al. 1991).

A better understanding of Myc biology emerged with the identification of a closely related bHLHZ protein Max that serves as an obligate, physiological heterodimerization partner for c-Myc (Blackwood and Eisenman 1991; Prendergast et al. 1991; Fig. 1). While c-Myc is incapable of forming homodimers or interacting specifically with DNA in isolation, the bHLHZ regions of Myc and Max form strong heterodimers, recognize DNA in a sequence-specific manner, and support Myc function in transcriptional activation, cellular transformation, and apoptosis (Amati et al. 1992; Amati et al. 1993). Myc–Max heterodimers recognize a core hexanucleotide element (5′-CACGTG-3′), termed the E-box (Blackwood and Eisenman 1991; Prendergast et al. 1991) and activate transcription at promoters containing E-boxes (Benvenisty et al. 1992; Eilers et al. 1991).
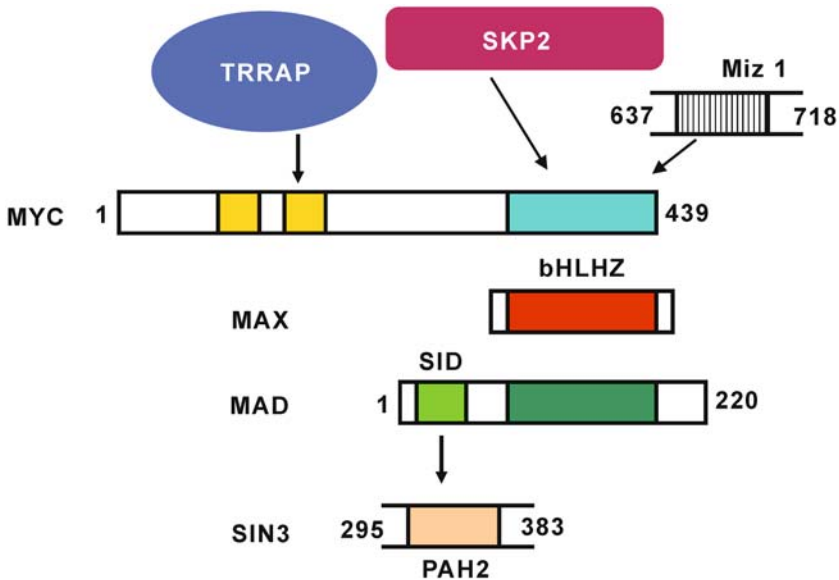
**Fig. 1** Domain organization of c-Myc, Max, and Mad, and schematic organization of proteins involved in transcriptional activation and repression within the Myc/Mad/Max network. The basic-helix-loop-helix-leucine zipper domains of the individual proteins are indicated relative to the full-length (Myc, *cyan*; Max, *red*; Mad, *green*). Transcriptional activation by Myc–Max heterodimers is dependent, in part, on recruitment of TRAAP (*purple*) by Myc transactivating residues (*yellow*). Conversely, transcriptional repression by Mad–Max heterodimers requires an interaction between the Sin3 interacting domain (SID) residues of Mad (*light green*) and a paired amphipathic helix (PAH2) domain of Sin3 (*tan*). The bHLHZ domain of Myc can also recruit the Miz-1 transcriptional repressor (*vertical lines*) and the E2 ubiquitin ligase Skp2 (*pink*)

In addition to acting as a heterodimerization partner for Myc, Max can also form homodimers and bind E-box containing DNA sequences. At present, the biological role or roles of the Max homodimer remain unknown, although there are suggestions that Max can function as a transcriptional repressor (Kretzner et al. 1992). While Max homodimers and Myc–Max heterodimers both recognize the same hexanucleotide element, sequence analyses of putative Myc target genes and the results of in vitro binding assays suggest that nucleotides flanking the E-box can confer binding preferences for Myc–Max heterodimers versus Max homodimers (Grandori et al. 1996; Grandori and Eisenman 1997). In addition, Myc–Max heterodimers recognize a number of noncanonical E-boxes containing variant nitrogenous bases at one or more

sites in the E box hexanucleotide (e.g., 5′-CA**T**G**C**G-3′, 5′-C**AA**CGTG-3′, etc.; Blackwell et al. 1991, 1993; Haggerty et al. 2003).

Shortly after the discovery of Max, a second class of bHLHZ proteins, including Mad1 (Ayer et al. 1993) and Mxi1 (Zervos et al. 1993) were independently identified as additional heterodimerization partners of Max. Mad1, Mxi1, and other Mad family members (Hurlin et al. 1995) inhibit cell growth. High levels of *mad* mRNA and Mad protein are found in growth-arrested, differentiated cells in which c-Myc is not expressed. Each of the Mad family member proteins can recognize the E-box as heterodimers with Max and interfere with the transforming function of Myc (Fig. 1). Hence, Mad1, Mxi1, and related members constitute a family of transcriptional repressors (Hurlin et al. 1994; Larsson et al. 1994, 1997; McArthur et al. 1998). Competition between Myc–Max and Mad–Max heterodimers for a common DNA target appears to control cell fate, determining the choice between proliferation/transformation and differentiation/quiescence.

Myc can also act as a transcriptional repressor at a distinct subset of genes (Li et al. 1994; see chapter in this volume by D. Kleine-Kohlbrecher et al.). At least one pathway of Myc repression has been elucidated through the identification of an association of Myc–Max heterodimers with the BTB-POZ domain protein Miz-1 (Peukert et al. 1997). Association of Myc–Max bHLHZ domains with Miz-1 appears to block the ability of Miz-1 to recruit the p300 co-activator, thereby leading to repression of genes normally activated by Miz-1 (Staller et al. 2001; Fig. 1). There is also some evidence that Myc repression can occur through binding of Myc–Max to core promoter elements (Kwon et al. 1996; Yang et al. 2001); however, the physiological significance of this effect has not been established.

## 2
## Topology of the Amino Terminal Domains

Myc and Mad family members have bipartite structures with separable, independently folded domains. The carboxyl terminal bHLHZ domain dictates sequence-specific DNA recognition, while the amino terminal residues dictate transactivation (Myc) or transrepression (Mad). These amino terminal residues mediate specific biological functions via recruitment of different multiprotein complexes.

Transcriptional repression by Mad–Max heterodimers is mediated by interactions between amino terminal Mad residues and the mSin3 co-repressor (Ayer et al. 1995; Schreiber-Agus et al. 1995), a component of the multiprotein histone deacetylase complex. Mad–Max heterodimers recruit the mSin3

co-repressor to promoter DNA, leading to recruitment of histone deacety-lases, condensation of chromatin structure, and subsequent transcriptional repression (Hassig et al. 1997; Laherty et al. 1997). Conversely, Myc–Max heterodimers activate gene expression by recruitment of multiprotein complexes bearing histone acetyltransferase activity. Myc interacts with TRRAP, a component of the Gcn5 and Tip60 histone acetyltransferase complexes, and this Myc-mediated recruitment of histone acetyltransferase activity results in upregulation of gene expression (McMahon et al. 2000; Saleh et al. 1998).

While detailed structural analysis of protein recruitment by the amino terminal co-activator domain of Myc has not yet been carried out, structures of the interacting domains of Mad and the mSin3 co-repressor have been determined by nuclear magnetic resonance (NMR) spectroscopy (Brubaker et al. 2000; Spronk et al. 2000). All four Mad paralogs contain a 30-residue amino terminal segment, the Sin3 interaction domain (SID), which is both necessary and sufficient for Sin3 association and transrepression (Ayer et al. 1995; Schreiber-Agus et al. 1995). Deletion mapping studies identified a 13-residue peptide within Mad1 that interacts with mSin3A (Eilers et al. 1999). Sin3 contains four repeats of a 100-residue segment, the paired amphipathic helix (PAH) domain; and the second of these repeats (PAH2) serves as the Mad interaction domain (Ayer et al. 1995; Schreiber-Agus et al. 1995).

Heteronuclear NMR spectroscopic studies of the Sin3 PAH2–Mad1 SID peptide complex demonstrate that the Sin3 PAH2 domain forms a left-handed, four-helix bundle containing an extensive, well-defined hydrophobic core (Brubaker et al. 2000; Spronk et al. 2000; Fig. 2a). $\alpha$-Helices 1 and 2 form a hydrophobic pocket, defining the interaction surface for the Mad1 SID peptide. The Mad1 SID peptide forms an amphipathic $\alpha$-helix, and interactions with the Sin3 PAH2 domain engage the nonpolar face of this peptide (Brubaker et al. 2000; Fig. 2a). More recently, the HMG box transcriptional repressor HBP1 has also been shown to interact with the PAH2 of Sin3. The solution structure of the HBP1 SID–Sin3 PAH2 complex demonstrates that the HBP1 SID peptide binds to the PAH2 domain with a reverse orientation relative to that of the Mad1 SID peptide (Fig. 2b). Detailed comparisons of the PAH2–Mad1 SID and PAH2–HBP1 SID structures reveal that both peptides are engaged by the PAH2 domain through similar interactions despite binding in opposite relative helical orientations.

Another intriguing observation that emerges from these structural studies is that both the Mad1 SID peptide and the Sin3 PAH2 domains are partially unfolded in the absence of their respective interaction partners. These mutually induced structural transitions may be representative of a general mechanism for facilitating interactions within multiprotein transcriptional complexes (Dyson and Wright 2002).
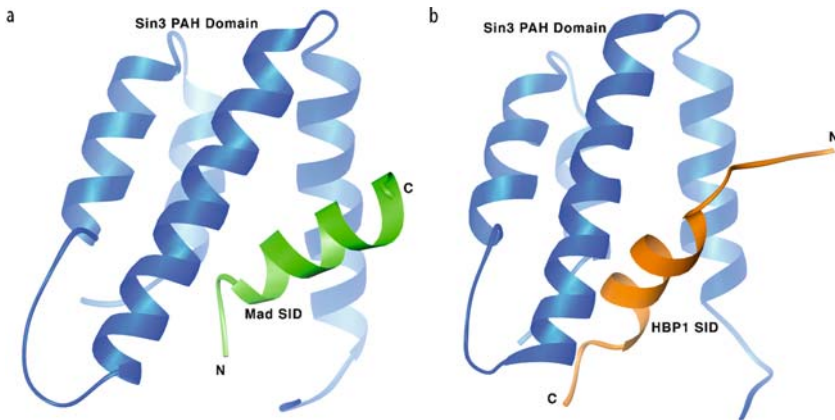
**Fig. 2a, b** Ribbon diagram showing a representative conformer of the second Sin3 PAH2 domain complexed with the Sin3 interacting domain (SID) peptide. **a** The Sin 3 PAH2 domain is colored *tan* and the SID peptide from Mad1 is shown in *green*. **b** The Sin3 PAH2 domain is colored *tan* and the SID peptide from HBP1 is colored in *blue*. Interactions between the two molecules are mediated by the packing of hydrophobic residues from the SID peptide into a hydrophobic pocket created by the α-helices of the PAH2 domain. Note that the Sin3 PAH engages both Mad1 SID and HBP1 SID1 in similar fashions. However, the helical orientations of the SID peptides are completely reversed relative to each other

# 3
# Topology of the bHLHZ Domain

The co-crystal structure of the bHLHZ domain of the Max homodimer bound to DNA revealed the overall topology of this domain and established the structural bases for DNA recognition by bHLHZ domain proteins (Ferre-D'Amare et al. 1993; Fig. 3a). Co-crystal structures of the Myc–Max and Mad–Max heterodimers recapitulate the disposition of secondary structure elements observed within the Max homodimer structure (Nair and Burley 2003). The bHLHZ domains of Myc, Max, and Mad consist of two lengthy α-helices separated by a random coil loop. Residues from the basic region and helix H1 constitute the first continuous α-helical secondary structure element. A conserved proline residue terminates the first α-helix (bH1) resulting in

**Fig. 3a, b** Equivalent views of the Max homodimer and the Myc–Max heterodimer bound to oligonucleotides bearing the E-box (Max, *red*; Myc, *cyan*). The tighter packing within the Myc–Max heterodimer structure is mediated by charge complementarity at residues near the c-terminus of the leucine zipper domain

b

Myc
Max



a

Leucine Zipper/
Helix 2

Helix 1/
Basic Region
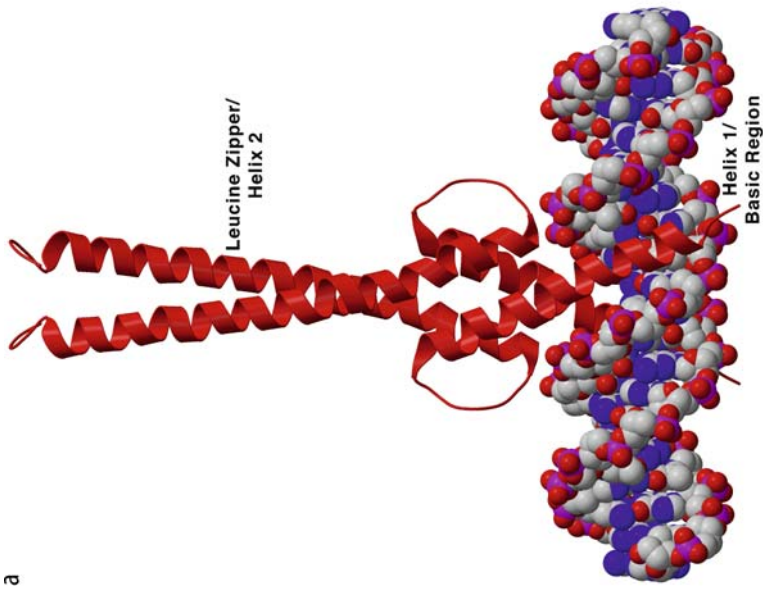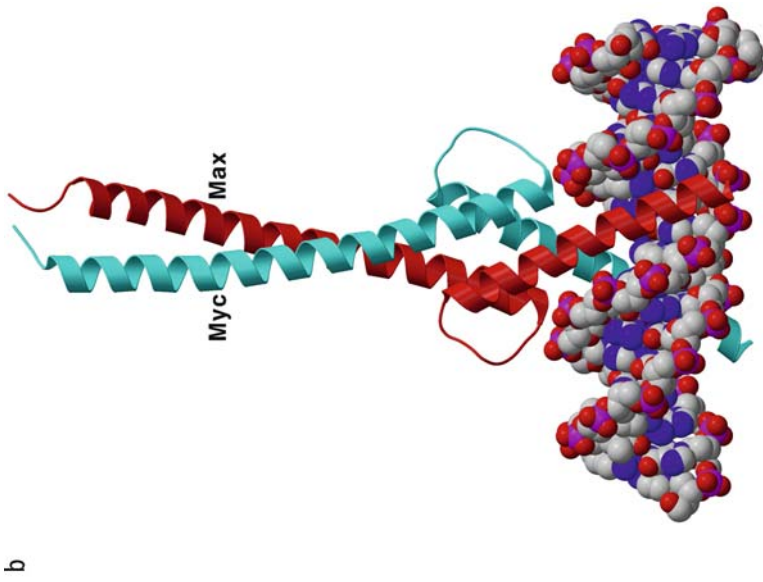
a turn in the backbone structure at the start of the variable loop region (L) that connects the two α-helical segments. The second α-helix is composed of the H2 and leucine zipper regions (Ferre-D'Amare et al. 1993; Nair and Burley 2003).

The Max homodimer and the Myc–Max and Mad–Max heterodimers all consist of two bHLHZ monomers that fold into a globular, parallel, left-handed, four-helix bundle (Fig. 3). Two pairs of α-helices project in opposite directions from the bundle. Two basic regions project from the amino termini of the four-helix bundle and make sequence-specific contacts with cognate DNA. The carboxy-terminal extensions of the four-helix bundle consist of two α-helical segments that form a parallel, left-handed, coiled coil or leucine zipper, similar in structure to the GCN4 homodimer (O'Shea et al. 1991).

The topology of the bHLHZ domain is distinguished from that of purely coiled-coil leucine zipper proteins, such as GCN4, by the presence of a well-defined globular core formed by α-helices H1 and H2 of the four-helix bundle. Hydrophobic residues conserved within the bHLHZ domain form this globular core, which stabilizes the structure of the Max homodimer. Mutagenesis of Myc–Max heterodimers demonstrates that all of the conserved hydrophobic amino acids within H1 and H2 are required for stable association of the dimer (Davis and Halazonetis 1993).

# 4
# Structural Basis for DNA Recognition

In both the Myc–Max and Mad–Max heterodimer co-crystal structures (Nair and Burley 2003), the DNA adopts a modified B-form conformation, characterized by a narrowed major groove and a widened minor groove. Each monomeric component of the heterodimer interacts with half of the 5′-CACGTG-3′ recognition site. The co-crystal structures revealed three portions of the polypeptide chain responsible for DNA contacts: residues from the basic and loop regions, and the first residue of α-helix H2 (Ferre-D'Amare et al. 1993; Nair and Burley 2003).

## 4.1
## Myc–Max Interactions with DNA

Three invariant residues within the basic region make sequence-specific contacts with selected DNA nucleotides within the 5′-Cyt(1)-Ade(2)-Cyt(3)-Gua(4)-Thy(5)-Gua(6)-3′ recognition sequence. In each half of the homo- or heterodimer co-crystal structures, Max residue His-28 participates in a hydrogen bond with the N7 of Gua(3′) (where ′ denotes opposite strand), residue
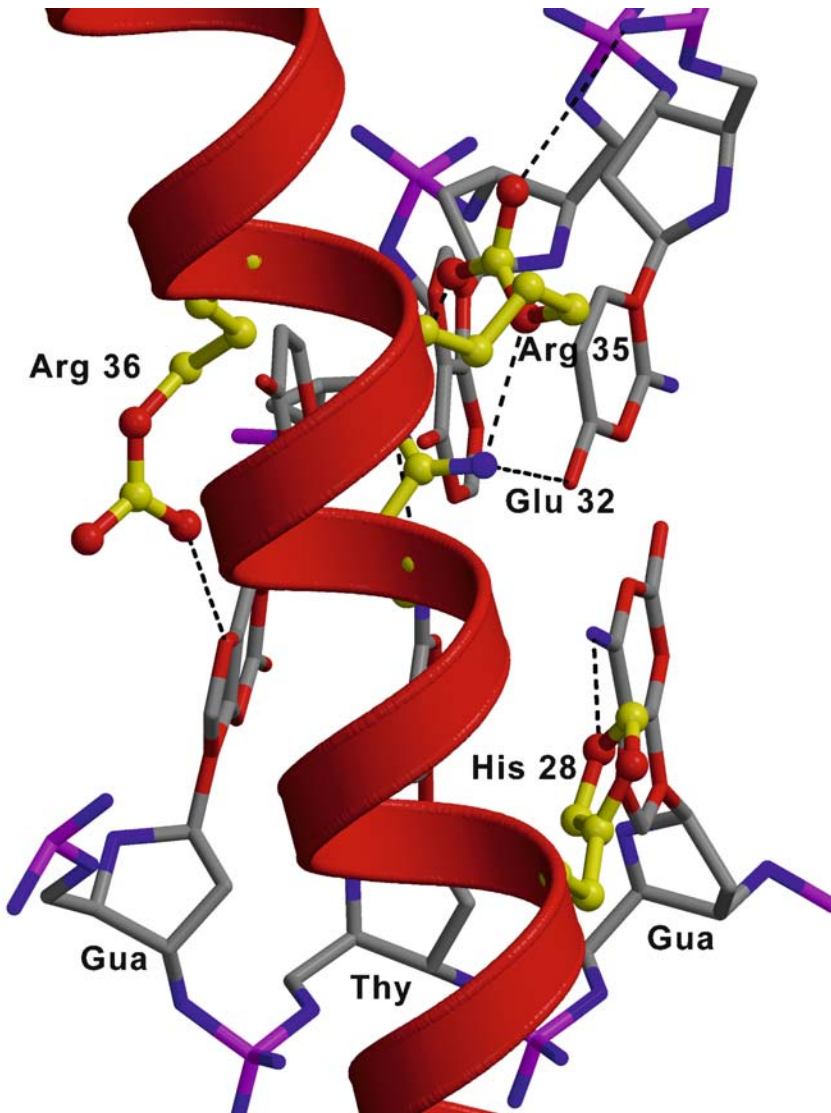
**Fig. 4** Ribbon diagram summarizing the DNA contacts made by the basic region of Max. Equivalent contacts are observed with the basic regions of both Myc and Mad. For clarity, numbering derived from the Max bHLHZ domain has been used. The view is perpendicular to the α-helical axis of the basic region and towards the DNA major groove

Glu-32 participates in hydrogen bonds with N4 of Cyt(3) and N6 of Ade(2), and Arg-36 interacts with N7 of Gua(1′). The hydrogen bond between His-28 and N7 of Gua(3′) dictates specificity for a purine base at this position. An additional interaction between Glu-32 and N4 of Cyt(3) further dictates that His-28 and Glu-32 recognize a G:C base pair (Ferre-D'Amare et al. 1993). The sidechain of Glu-32 is oriented relative to the DNA by a hydrogen bond with Arg-35 (Fig. 4). The corresponding Arg→Lys mutation in the mouse bHLHZ transcription factor *mi* results in small eyes and osteoporosis in the heterozygote, thus underscoring the importance of this Arg residue in bHLHZ-DNA interactions (Steingrimsson et al. 1994).

## 4.2
## Class A Vs Class B bHLHZ Proteins

Proteins of the bHLH (similar in structure but lacking the leucine zipper) or bHLHZ families have historically been divided into two classes according to their DNA binding preferences (Blackwell et al. 1993). Class B bHLHZ proteins recognize the central 5′-CG-3′ dinucleotide of the 5′-CA**CG**TG-3′ hexanucleotide. The specific interaction between Arg-36 (Max numbering) and the purine N7, as seen in the co-crystal structures of the Max homodimer (Ferre-D'Amare et al. 1993) and Myc–Max heterodimer (Nair and Burley 2003) structures, almost certainly dictates the sequence preference for class B bHLHZ proteins. Class A bHLHZ proteins recognize 5′-CA**GC**TG-3′ E-boxes. Sequence comparisons between class A and class B bHLHZ proteins suggest that the preference of class A proteins is due to a hydrophobic residue in place of the conserved arginine at position 36. For example, a single amino acid substitution Arg36→Met suffices to convert some class B proteins into class A (Dang et al. 1992). However, co-crystal structures of the class A proteins E47 (Ellenberger et al. 1994) and MyoD (Ma et al. 1994) show that the corresponding valine or leucine are far from the innermost base pair and do not interact directly with DNA. Thus, the binding specificity of class A proteins cannot be explained in terms of direct sidechain-base contacts in the major groove. It is likely that sequence preference differences between class A and class B bHLHZ proteins involve sequence-dependent DNA deformations and/or solvent-mediated effects. Regrettably, the moderate resolution limits (2.8 Å–2.9 Å) of both the E47 (Ellenberger et al. 1994) and MyoD (Ma et al. 1994) co-crystal structures preclude more rigorous examination of this phenomenon.

## 4.3
## Mad–Max Interactions with DNA

Protein–DNA contacts supported by the basic regions of Myc and Mad are essentially identical to those observed for Max with specificity dictated by residues His-359, Glu-363, and Arg-367 in Myc and His-61, Glu-65, and Arg-69 of Mad. In the Myc–Max heterodimer co-crystal structure, several additional contacts are observed between residues specific to Myc and the phosphate backbone of DNA (Nair and Burley 2003). It is possible that these Myc-specific contacts result in differing affinities between the Myc–Max heterodimer and the Max homodimer for the same 5′-CACGTG-3′ element, but this assertion has not been experimentally confirmed.

## 4.4
## The Loop Region Interacts with DNA

The loop regions connecting helices H1 and H2 vary in sequence, amino acid composition, and length among various members of the Myc family. The loop regions lack sequence conservation, with the notable exception of a lysine residue at position 57 in Max (Lys-389 in Myc; Arg-91 in Mad). In the Max homodimer co-crystal structure, Lys-57 interacts with the DNA phosphate backbone. This interaction is conserved in the structure of the Myc–Max heterodimer in which Lys-389 of Myc also makes similar, presumably nonspecific, contacts with the DNA backbone. Loop-deletion studies of MyoD and DNA affinity studies with synthetic bHLH peptides showed that loop residues contribute to DNA binding. Winston and Gottesfeld estimated a roughly 1.3-kcal/mol contribution to DNA binding by an equivalent lysine residue (Lys-80) of the bHLH protein Deadpan (Winston and Gottesfeld 2000). Binding studies of wild-type and mutant Deadpan bHLH with the major groove binding pyrrole-imidazole polyamides further established that Lys-80 contributes to DNA recognition, via interactions with nucleotides outside the core binding element. Contacts between loop residues and the DNA backbone may represent a mechanism for extending DNA binding selectivity to bases that flank the 5′-CACGTG-3′ core element (Nair and Burley 2000).

## 5
## The Bivalent Myc–Max Heterotetramer

In the Myc–Max co-crystal structure, two Myc–Max/DNA complexes constituting the crystallographic asymmetric unit align in a head-to-tail assembly of
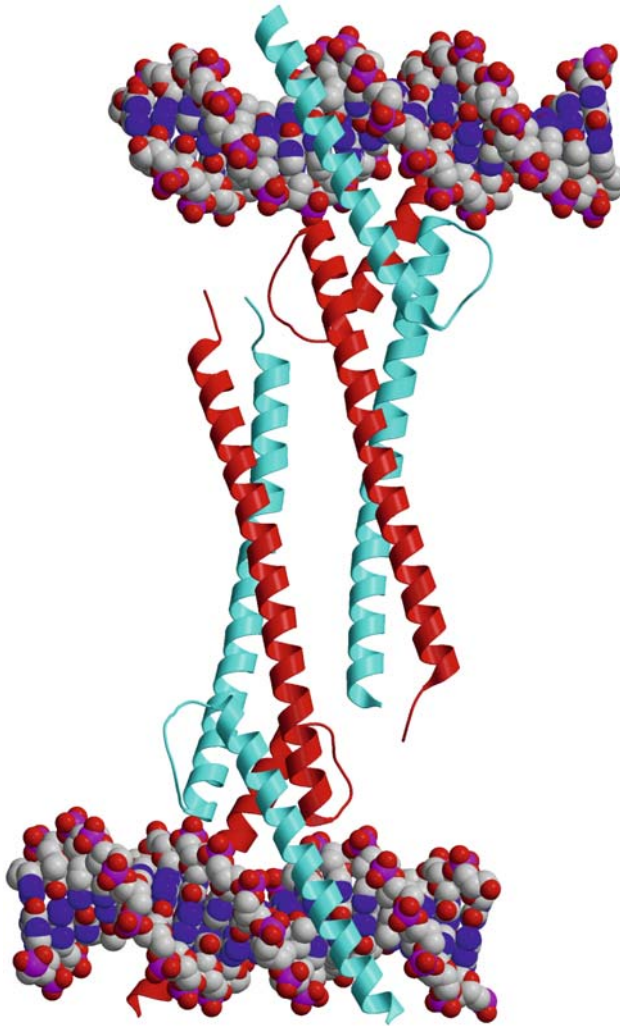
**Fig. 5** Ribbon diagram of the bivalent Myc–Max heterotetramer observed in the Myc–Max/DNA co-crystals (Max, *red*; Myc, *cyan*). This head-to-tail assembly of individual leucine zippers of each heterodimer results in the formation of an anti-parallel four-helix bundle

the leucine zippers of each heterodimer, generating an antiparallel four-helix bundle (Nair and Burley 2003; Fig. 5). This four-helix bundle is topologically similar to α-helical bundles observed in members of the cytokine family and in leukemia inhibitory protein (Hill et al. 1993; Somers et al. 1997).

Previously published in vivo and in vitro studies have shown that Myc–Max heterodimers can form higher order oligomers. Solution studies by Dang and co-workers demonstrated that Myc–Max is capable of forming bivalent heterotetramers, and that tetramerization depends on Myc leucine zipper region (Dang et al. 1989). The physiological relevance of the bivalent heterotetramer observed in the Myc–Max co-crystals is supported by solution experiments that demonstrated Myc–Max tetramerization at submicromolar concentrations and analytical ultracentrifugation studies which yielded a tetramer–dimer equilibrium dissociation constant of approximately 90 nM (Nair and Burley 2003). Given that the measured dissociation constant of the Myc–Max tetramer is lower than estimates of physiologic c-Myc concentrations (Moore et al. 1987; Rudolph et al. 1999), these findings document that c-Myc–Max almost certainly exists as a bivalent heterotetramer in cell nuclei.

The biological relevance of the bivalent Myc–Max heterotetramer is borne out by a wealth of genetic and biochemical data. Genetic characterization of the promoters of putative *myc*-regulated genes has provided further evidence for a physiological role for Myc–Max heterotetramerization. Oligonucleotide microarray analysis has identified several Myc target genes that contain multiple E-boxes within promoters, typically separated by at least 100 nucleotides (Coller et al. 2000; see also Grandori and Eisenman 1997). Given the persistence length of DNA, this separation of Myc–Max binding sites is compatible with DNA looping stabilized by bivalent Myc–Max heterotetramers simultaneously bound to two cognate sequences.

An extensive network of hydrogen bonds and salt bridges mediates the protein–protein interface stabilizing the Myc–Max heterotetramer. Residues that are part of this polar interaction network are unique to the Myc–Max heterodimer. It is remarkable that the polarity of many of the residues that make up the interaction network in the Myc–Max heterotetramer in Myc is altered in Mad. This alteration in polarity of residues that stabilize the interaction of the Myc–Max heterotetramer may explain the lack of tetramer formation by Mad–Max heterodimers both in solution and in the co-crystal structure (Nair and Burley 2003).

It is possible that assembly of Myc–Max into bivalent heterotetramers allows for cooperative regulation at promoters and enhancers containing multiple E-boxes. In vitro site selection experiments and chromatin immunoprecipitation studies have documented that Myc–Max heterodimers can bind to sequences that differ from the canonical E-box (5′-CACGTG-3′) hexanucleotide (Blackwell et al. 1993; Grandori et al. 1996). These sequences are not bound with equal affinities. For example, the noncanonical sequences 5′-CACG**C**G-3′ and 5′-CA**T**GCG-3′ represent low-affinity Myc–Max binding sites (nucleotides that differ from the E-box hexanucleotide are shown in bold).

Given the conservation of amino acids within Myc family proteins, that make direct DNA contacts, this difference in binding affinities of noncanonical sequences is somewhat unexpected. The bivalent heterotetramer observed in the Myc–Max co-crystal structure suggests that cooperative binding may increase the affinity of Myc–Max heterodimers for such noncanonical sites (Walhout et al. 1997). However, this assertion has yet to be validated experimentally.

## 6
## Determinants of Homodimerization Vs Heterodimerization

The bHLHZ segments of Myc, Max, and Mad contain two different dimerization interfaces: the bHLH domain and the leucine zipper domain. Extensive hydrophobic and polar interactions between both of these interfaces stabilize the Max homodimer structure (Ferre-D'Amare et al. 1993) and the quasi-symmetric Myc–Max and Mad–Max heterodimer structures (Nair and Burley 2003). Much of the left-handed coiled-coil that the leucine zipper comprises resembles the structure of canonical leucine zippers, such as the GCN4 homodimer (O'Shea et al. 1991). However, within the Max homodimer structure, a Gln-91–Asn-92–Gln-91–Asn-92 tetrad occurs at the carboxy-terminal end of the zipper region (Ferre-D'Amare et al. 1993). This non-ideal packing scheme results in a flaring of the leucine zipper in the vicinity of the Gln–Asn tetrad.

In contrast, the leucine zipper regions of both the Myc–Max and Mad–Max heterodimers closely resemble the coiled coils found in GCN4 homodimers. The co-crystal structures of both bHLHZ heterodimers demonstrate that the packing defects introduced by the Gln–Asn pairing in Max are compensated for by complementary hydrogen bond interactions with two positively charged Arg–Arg residues located at this position in Myc. Hydrogen bonding between the Max Gln–Asn pair and a Gln–Glu pair at the equivalent position in Mad also results in close packing within the leucine zipper. Mutational analyses documented that residues at these two positions mediate the specificity and avidity for homo- verses heterodimerization within the Myc/Max/Mad network of proteins (Nair and Burley 2003). The packing defects observed in the Max homodimer have been compensated in both Myc–Max and Mad–Max heterodimers. Hence, energetic considerations would suggest that the likely in vivo state for Max polypeptides would be as an obligate *hetero*dimeric species with Myc/Mad.

# 7
# The bHLHZ Domain as an Architectural Scaffold

Work from a number of laboratories has shown that Myc–Max can recruit various cellular factors, such as the zinc-finger protein Miz-1 (Peukert et al. 1997) and the F-box E3 ubiquitin ligase Skp2 (Kim et al. 2003; von der Lehr et al. 2003). Each of these higher order complexes forms as a result of specific interactions with the bHLHZ region of Myc. Given that these proteins are recruited to specific regions of the promoter only in the context of Myc–Max heterodimers, it seems reasonable to suggest that the bHLHZ regions of the Myc–Max heterodimer play an architectural role. Formation of the bivalent heterotetramer observed in the Myc–Max co-crystal structure would provide a substantial platform for recruitment of additional protein factors.

Miz-1 (see chapter by D. Kleine-Kohlbrecher et. al.) encodes a protein of 803 amino acids, bearing 13 putative zinc-finger motifs, which recruits Myc bHLHZ to the core promoter elements of the *P21CIP1* and *P15INK4B* genes (Seoane et al. 2002; Staller et al. 2001; Herold et al. 2002). The interaction between Myc bHLHZ and Miz represses transactivation through competition with the histone acetyltransferase p300 for binding to Miz-1 (Staller et al. 2001). Two-hybrid interaction studies using random mutants of the Myc bHLHZ domain identified several point mutants that retain the ability to heterodimerize Max but do not support interactions with Miz-1. These point mutants of Myc do not repress transcription of *P21CIP1* genes in vivo, thereby demonstrating that residues unique to the bHLHZ domain of Myc support Miz-1-mediated transcriptional repression.

Myc is a target for ubiquitin-mediated proteolysis, and ubiquitination of Myc results in rapid destruction within minutes of Myc synthesis (Salghetti et al. 2001). Thus, turnover plays a fundamental role in the function of Myc and deregulation of this event leads to the onset and development of oncogenic transformations. Recently, two groups independently identified the ubiquitin ligase Skp2 as both a mediator of Myc turnover and a potent stimulator of Myc transcription (Kim et al. 2003; von der Lehr et al 2003). The Skp2 interacting regions have been delimited to two distinct sequences within the Myc polypeptide. The first of these consists of a region within the Myc amino-terminal transactivation domain and the second Skp2 interacting regions consists of the Myc bHLHZ domain. These studies demonstrate that Skp2 is a co-activator of Myc function, and Myc acts to recruit this co-activator activity to target promoters, in part through the bHLHZ domain (Kim et al. 2003; von der Lehr et al 2003).

The assertion that the Myc–Max tetramer is of biological relevance is also borne out by experiments utilizing bHLHZ domain chimeras (Staller

et al. 2001; O'Hagan et al. 2000; James and Eisenman 2002). Several laboratories have constructed such chimeric proteins in which the transactivation domain from Myc is attached to the bHLHZ domain from Mad. Given the conservation of protein–DNA contacts observed in the co-crystal structures of both Myc–Max and Mad–Max heterodimers, such chimeric proteins would be expected to have biological activities similar to those of wild-type Myc. While these Myc/Mad–bHLHZ chimeras can activate E-box dependent transcription, clear differences from the behavior of wild-type Myc are observed. Thus, the bHLHZ domain of Myc supports unique aspects of Myc function. It is possible that the ability of Myc–Max bHLHZ heterodimers (and *only* Myc–Max heterodimers) to form higher order tetramers reflects, at least in part, unique properties of Myc.

# 8
# Conclusions

The structures of several Myc family multiprotein and protein–DNA complexes determined over the past few years have offered a number of insights into the biological functions of Myc/Mad/Max. The structure of the Mad SID–Sin3 PAH complex reveals how a small four-helical domain can mediate selective recruitment of a peptide through mutual induction of disorder-to-order structural transitions. Given the unstructured nature of the activation domains in general, this principle may play a role in recognition by the Myc and Mad transactivation domains.

The co-crystal structures of the Myc–Max and Mad–Max heterodimers recognizing their E-box targets demonstrate how bHLHZ heterodimers mediate specific, high-affinity DNA binding. Tetramerization of Myc and Max is mediated by extensive protein–protein interactions between leucine zipper domains, and the resulting antiparallel four-helix bundle could provide a scaffold for recruitment of additional modulators of transcription. Several of the features observed in these structures are consistent with the biology of Myc family proteins and thus serve as a starting point for further directed biochemical and genetic studies to elucidate the roles played by Myc–Max and Mad–Max in cell-fate determination.

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