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Reconstitution of an E box-binding Myc:Max complex with recombinant full-length proteins expressed in *Escherichia coli*

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Abstract

The c-Myc oncoprotein (Myc) is a DNA sequence-specific transcription factor that regulates transcription of a wide variety of genes involved in the control of cell growth, proliferation, differentiation, and apoptosis and its deregulated expression is implicated in many types of human cancer. Myc has an N-terminal transcription activation domain (TAD) that interacts with various coactivators and a C-terminal basic-helix-loop-helix-leucine zipper (bHLHZip) domain required for E box-specific DNA-binding and heterodimerization with its obligatory bHLHZip protein partner Max. The analysis of the mechanisms by which the Myc:Max complex regulates transcription at the molecular level in vitro has been hampered by the difficulty in obtaining highly pure recombinant Myc:Max heterodimers that contain full-length Myc with its complete TAD domain and that have sequence-specific DNA-binding activity. Here, we describe a simple method to reconstitute recombinant Myc:Max complexes from highly purified full-length proteins expressed in *Escherichia coli* that are soluble and highly active in E box-specific DNA-binding in vitro. The reconstituted Myc:Max complexes are stable and lack Max:Max homodimers. This procedure should facilitate the characterization of the DNA-binding and transcription activation functions of full-length Myc:Max complexes in vitro and in particular the role of Myc TAD-interacting cofactors and Myc:Max post-translational modifications.

Keywords

Recombinant human Myc and Max proteins, bacterial expression; Reconstitution of Myc:Max heterodimers; Sequence-specific DNA-binding

The c-Myc protein (Myc) is a broadly expressed member of a small family of highly conserved cellular oncogenic transcription factors that also includes the more tissue-restricted N-Myc and L-Myc proteins. Myc is essential for early mammalian embryogenesis and is expressed after birth primarily in undifferentiated and/or proliferative cells and tissues. In normal cells, Myc levels are tightly controlled by growth factors and mitogens at the transcriptional and post-transcriptional levels. Myc is a very unstable protein in normal cells and its rapid turnover is regulated by various covalent post-translational modifications. Myc controls cell growth, proliferation, differentiation, and apoptosis and its deregulated

expression (or overexpression) has been shown to be tumorigenic in mice and is associated with most types of cancers in humans (reviewed in [1]).

Myc effects its normal and oncogenic functions via directly regulating transcription of a surprisingly large number of genes ([2–4], and references therein). The transcription regulatory functions and oncogenic transformation activities of Myc require its heterodimerization with Max, a stable and constitutively expressed ubiquitous protein. Myc and Max heterodimerize via their respective basic-helix-loop-helix-leucine zipper (bHLHZip) domains and bind to E box DNA sequences having the core consensus sequence CACGTG (reviewed in [1,5]). Myc appears to activate transcription, at least in part, by modifying the structure of chromatin. Indeed, Myc recruits histone acetyltransferases (HATs) to promoters in vivo and induces hyperacetylation of core histone H3 and H4 N-terminal “tails” at specific target genes [6–9]. Myc N-terminal transcription activation domain (TAD) interacts with various transcription cofactors, including histone H3- and H4-specific HATs. Several of these HATs including human GCN5 and TIP60 are part of large transcription coactivator complexes that contain the Myc TAD-interacting transformation cofactor TRRAP ([9–13], and references therein). In contrast to Myc, Max lacks a transcription activation domain and can efficiently homodimerize. The Max:Max homodimer is thought to antagonize Myc functions by recognizing the same core E box DNA sequences as the Myc:Max complex. Max also heterodimerizes with other bHLHZip proteins including Mnt(Rox) and members of the Mad family of transcriptional repressors which bind to Myc E boxes and actively antagonize Myc functions via recruitment of mSin3-containing corepressor complexes with histone deacetylase (HDAC) activity (reviewed in [1,5]).

Comparison of the X-ray co-crystal structures of Myc:Max, Max:Max, and Mad:Max bHLHZip dimers bound to a perfect palindromic E box element indicates that Myc, Mad, and Max basic regions within these complexes make essentially identical contacts with each E box half site and that structural differences between the leucine zippers of Myc and Max specify the preferential formation of Myc:Max heterodimers over Max and Myc homodimers and allow selectively Myc:Max to form higher order tetramers ([14], and references therein). Besides binding to E box elements, the Myc:Max complex is also recruited to certain target promoters indirectly via interactions of Myc with other DNA-binding proteins as, for instance, with the regulators Miz1, Sp1, and Smads, which generally results in repression of genes involved in cell cycle/growth arrest (reviewed in [15]).

The binding of Myc and Max to DNA has been extensively analyzed in vitro by using recombinant proteins expressed in bacteria [16–27], in transcription/translation systems in vitro [28–30], and in eukaryotic cells by transfection and transduction with recombinant baculovirus and vaccinia viruses [25,31–35]. In vitro transcription/translation systems while rapid generally give low amounts of Myc/Max proteins, which are contaminated with the various proteins in the transcription/ translation lysate, which include other E box-binding factors. Eukaryotic cell transfection and viral transduction approaches to overexpress and purify active Myc:Max dimers are relatively expensive, time consuming, and require extensive purification steps to remove contaminating proteins and to enrich in DNA-binding competent Myc:Max complexes [25,33,34]. In addition, Myc:Max complexes purified from

eukaryotic cells can be heterogeneous due to alternative Myc translation initiation sites [36,37] and/or to various Myc and Max post-translational modifications [22,38–51]. Indeed, Myc proteins overproduced in mammalian CHO cells and in baculovirus-infected insect cells are phosphorylated and heterogeneous in their apparent molecular mass [31–33]. Most studies have used bacterial expression systems to produce recombinant Myc and Max proteins with sequence-specific DNA-binding activity. However, because full-length recombinant Myc is mostly insoluble, easily degraded, and has by itself only very weak DNA-binding activity [16,21,24], these studies have generally analyzed soluble Myc proteins that lack the N-terminal Myc TAD domain. Indeed, the bacterially expressed C-terminal bHLHZip domain of Myc can efficiently homodimerize and heterodimerize with Max and binds E box elements in a sequence-specific manner. In a few cases, full-length recombinant Myc expressed in bacteria was shown to bind to E box DNA elements in a sequence-specific manner when mixed in molar excess over recombinant Max. In these studies, recombinant Myc was either only partially purified using conventional biochemical techniques [21] or highly purified as a hexahistidine (6His)-tagged protein by metal-affinity chromatography under denaturing conditions [24,26]. In the latter cases, however, large amounts of Myc (in molar excess over Max) were required for dimer exchange and to observe formation of Myc:Max heterodimers on E box DNA elements. In addition, 6His-Myc preparations contained partial Myc products possibly resulting from Myc proteolysis and/or incomplete translation and generated heterogeneous complexes with Max in DNA-binding assays [26].

Here, we describe an improved procedure for the expression and purification of full-length recombinant Myc from *E. coli* that yields homogeneous Myc preparations that lack any proteolytic or partial translation product. We describe further a simple refolding method to pre-assemble stable recombinant Myc:Max complexes that are highly active in E box-specific DNA-binding and contain full-length Myc and Max proteins. Significantly, the reconstituted Myc:Max complexes also appear homogeneous in DNA-binding assays and lack detectable Max:Max dimers. The methods described here will facilitate the analyses of Myc:Max transcription functions at the molecular level in vitro.

Materials and methods

Plasmids

The bacterial expression vector for 6His-tagged human Max (pET-His-Max) was a gift by H.T.M. Timmers and was described previously [34]. The bacterial expression vector for 6His-tagged human full-length c-Myc was kindly provided by M. Eilers and was described previously [26]. The original 6His-Myc expression plasmid carried 2 point mutations within human c-Myc coding sequence, which resulted in 2 amino acid changes: leucine 61 to proline (L61P) and glutamate 385 to glycine (E385G). To restore the wild type c-Myc sequence (P61[CCG]→L61[CTG]; G385[GGA]→E385[GAA]), mutagenesis was carried out with the Quick Change Site-Directed Mutagenesis kit (Stratagene) and sequenced to confirm the wild type genotype.

Expression of recombinant c-Myc and Max in *Escherichia coli*

pRSET-6His-Myc expression vector was transformed into BL21-CodonPlus(DE3)-RP competent cells (Stratagene). pET-His-Max was transformed into BL21(DE3)-pLysS competent cells (Stratagene). Bacteria were grown in LB broth plus 100 µg/ml ampicillin at 30 °C and protein expression was induced with 0.5 mM IPTG at 0.3–0.4 OD_{600 nm}. Induction was performed for 3 h at 30 °C. Bacteria were collected and washed with cold washing buffer (10 mM Tris–HCl, pH 7.9, at 4 °C, 100 mM NaCl, and 1 mM EDTA), and cell pellets were either used directly for purification or stored frozen at –20 °C until further use.

Purification of recombinant human Max

The bacterial pellet from a 500 ml culture (about 2 g wet weight) was resuspended in 15 ml cold lysis buffer (20 mM Hepes, pH 7.9, at 4 °C, 500 mM NaCl, 10% (v/v) glycerol, 0.1% (v/v) NP-40, 10 mM of 2-mercaptoethanol, and 1 mM PMSF) and sonicated on ice (Branson Sonifier 450, VWR Scientific). The lysate, which contained 1 g total protein as determined by Bradford assay (“Bio-Rad” protein assay reagent with BSA as a standard), was centrifuged at 12,500 rpm at 4 °C for 15 min (SS34 Sorval rotor) and the supernatant containing soluble 6His-Max protein (6 mg, 0.6% total protein, determined by semi-quantitative Western blot) was collected and incubated with 250 µl “Talon” resin (Clontech) in the presence of 5 mM imidazole for 2–4 h under constant rotation at 4 °C. The resin was washed successively (in batch) 3 times with 5 ml cold lysis buffer containing 5 mM imidazole, 3 times with 1 ml buffer BC500 (20 mM Tris–HCl, pH 7.9, at 4 °C, 20% (v/v) glycerol, 500 mM KCl, 0.05% (v/v) NP-40, 10 mM of 2-mercaptoethanol, and 0.2 mM PMSF) complemented with 5 mM imidazole, once with 1 ml BC100 (same as BC500 but with 100 mM KCl) containing 15 mM imidazole, and once with 1 ml BC100 containing 30 mM imidazole. Recombinant 6His-Max was eluted from the resin with 300 mM imidazole in BC100 at 4 °C. Typical yield was about 2 mg of 6His-Max protein/500 ml bacterial culture (i.e., about 30% of total expressed 6His-Max). The 6His-Max protein was >95% pure as judged by SDS–PAGE and Coomassie blue staining (Fig. 1A, lane 5).

Purification of recombinant human c-Myc

Bacterial culture, induction, and lysis were performed as above. Recombinant 6His-Myc in the lysate represented about 0.01% of total bacterial protein as determined by semi-quantitative Western blot. After centrifugation of the lysate, the pellet was used to purify insoluble 6His-Myc. The pellet was resuspended with 10 ml E-buffer (50 mM Hepes, pH 7.9, 5% (v/v) glycerol, 0.5 mM of 2-mercaptoethanol, 0.05% (w/v) Na-deoxycholate, and 1% (v/v) NP-40) and homogenized with a Dounce homogenizer and a B-pestle. Extracted inclusion bodies were then recovered by centrifugation (25 min, 14,000 rpm at 4 °C, SS34 Sorval rotor). The 6His-Myc in the pellet was solubilized with 10 ml S-buffer (10 mM Hepes, pH 7.9, 6 M guanidine–HCl, and 5 mM of 2-mercaptoethanol) on a shaker overnight at room temperature. Denatured 6His-Myc protein in S-buffer was adjusted to 5 mM imidazole and bound in batch to 75 µl Talon resin (Clontech) on a rotator for 2–4 h at 4 °C. The resin was washed 3 times with 5 ml each of S-buffer containing 5 mM imidazole, 3 times with 1 ml BC500 containing 7 M urea and 5 mM imidazole, once with 1 ml BC100

containing 7 M urea and 15 mM imidazole, and once with 1 ml BC100 containing 7 M urea and 30 mM imidazole. Recombinant 6His-Myc was eluted from the resin with 300 mM imidazole in BC100 containing 7 M urea. Typical yield was about 20–25 µg of 6His-Myc full-length protein/500 ml bacterial culture (i.e., about 25% of total expressed 6His-Myc). The 6His-Myc protein was >95% pure and lacked any detectable partial product as judged by SDS–PAGE and Coomassie blue staining (Fig. 1A, lane 4).

Reconstitution of Myc:Max complexes

To co-renature a significant amount of Myc:Max heterodimers over Max:Max homodimers and to minimize formation of soluble Myc homodimers, a 3:1 molar ratio of Myc to Max was used during a slow stepwise dialysis. 1.5 µg of purified Max protein was mixed with 15 µg purified Myc protein in 150 µl BC100 containing 7M urea and dialyzed in “Mini Dialysis Tubes” (nominal molecular weight cutoff at 8 kDa, Amersham) in 7 successive steps of 2 h each against BC500 (as above, but with 0.1% NP-40) containing 4M urea (BC500—4 M urea), BC500—2 M urea, BC500—1 M urea, BC500—0.5 M urea, BC500, and twice with BC100. First four dialysis steps were done at room temperature, while the last three were done at 4 °C. After the last dialysis step, the dialyzed proteins were centrifuged at 13,000 rpm for 1 h at 4 °C in an Eppendorf microfuge to remove insoluble aggregates. An aliquot (1 µl) was analyzed by SDS–PAGE and Coomassie staining (Fig. 1B, lane 1) and bovine serum albumin (BSA, 500 ng/µl final concentration) was added to the remaining Myc:Max preparation and stored in small aliquots at -80 °C. No noticeable decrease in DNA-binding activity was observed after up to 10 freeze–thaw cycles. Typically 100% of Max and about 50–60% of total Myc were recovered in the soluble fraction after dialysis. When Myc was dialyzed alone (in the absence of Max), only about 16% of total Myc was recovered as soluble protein. Thus, after dialysis we estimate that more than 50% of renatured Myc is in a soluble heteromeric complex with Max.

Electrophoretic mobility-shift assays

DNA-binding reactions were performed with a Klenow-filled [α -³²P]dGTP-labeled E box oligonucleotide 5-GCTCAGGGACCACGTGGTCGGGGATC-3'. Typical specific activity after radio-labeling was 5×10^5 cpm/pmol. The mutant E box used for competition assays contained the sequence 5'-GCTCAGGGACCAGCTGGTCG GGGATC-3' (mutations underlined) and was blunt ended. Typical binding reactions were done in 10 µl final volume and contained 15 mM Tris–HCl (pH 7.9), 15% glycerol, 100 mM KCl, 0.15 mM EDTA, 0.075% NP-40 (v/v), 7.5 mM of 2-mercaptoethanol, 80 ng poly(dA–dT):(dA–dT), and 375 ng/µl BSA (except where specified). For competition assays unlabeled oligos were mixed with the radio-labeled probe before protein was added. For assays with antibodies, proteins were pre-incubated with antibodies for 15 min at room temperature before addition of the probe. Antibodies used were affinity purified rabbit polyclonal c-Myc N262 (1 µl), Max C-17 (1 µl of 1:20 dilution), and p130 C-20 (1 µl of 1:20 dilution) from Santa Cruz Biotechnology. Purified recombinant USF1 was a kind gift from P. Pognonec. All DNA-binding reactions were performed at room temperature for 30 min and analyzed on a pre-run 6% native PAGE in 0.5 × TAE (Tris–acetate 20 mM, EDTA 0.5 mM). Electrophoresis conditions were 150 V for 45 min at room temperature.

Results and discussion

Expression and purification of full-length recombinant 6His-tagged Myc and Max proteins in *Escherichia coli*

We originally expressed 6His-tagged full-length human c-Myc and Max proteins from inducible T7 expression vectors in the widely used bacterial strain BL21(DE3)pLysS. As previously reported [34], 6His-Max was well expressed (about 0.6% of total bacterial protein) and could be purified to homogeneity by metal (cobalt)-affinity chromatography on “Talon” resin from the soluble fraction with essentially no degradation products (Fig. 1A, lane 5). However, consistent with previous results by others (see Introduction) 6His-Myc proteins in the soluble fraction were always highly degraded and 6His-Myc purified under denaturing conditions from inclusion bodies also contained significant amounts of truncated Myc proteins (data not shown). We found by Western blot analyses with the 9E10 anti-Myc antibody that recognizes an epitope within the C-terminal leucine zipper that most partial products observed in Myc preparations purified under denaturing conditions are truncated at their C-terminus (data not shown). We suggest that these truncated Myc proteins result from incomplete translation due to codon bias and shortage of specific tRNAs. Indeed, Myc is relatively rich in proline residues (8% overall) and its C-terminus is enriched in arginines (about 14% of the C-terminal 124 amino acids), two amino acids for which there is strong bias in codon usage between bacteria and human cells. We therefore expressed 6His-Myc in a bacterial strain (BL21-CodonPlus (DE3)-RP, Stratagene) that contains extra copies of the *argU* and *proL* genes encoding tRNAs for the proline codon “CCC” and the arginine codons “AGA/AGG” which are most often used in human genes.

The expression of 6His-Myc was only about 0.01% of total protein in both BL21(DE3)pLysS and BL21-CodonPlus-RP bacteria. However, consistent with the above observations, 6His-Myc could be purified under denaturing conditions from inclusion bodies of BL21-CodonPlus-RP bacteria to apparent homogeneity as a single band on SDS-PAGE with no detectable partial products (Fig. 1A, lane 4). The integrity of the N- and C-termini of Myc was confirmed further by Western blot with specific antibodies that recognize epitopes within the extreme N-terminal and C-terminal ends of Myc (data not shown).

Reconstitution and characterization of a recombinant full-length Myc:Max complex

Previous in vitro studies on the DNA-binding activity of bacterial full-length Myc and Max and cognate heteromeric Myc:Max complexes utilized Myc and Max proteins that were separately refolded from denatured protein preparations and then mixed together and heated (at 37 or 42 °C, for 15–30 min) to allow Max dimers to dissociate and Myc:Max heterodimers to form (i.e., dimer exchange) just before addition of the DNA probe (e.g., see [21,24,26]). A disadvantage of this dimer exchange protocol is the poor efficiency of active Myc:Max heterodimer formation which requires microgram amounts of Myc and the use of a large molar excess of Myc over Max to minimize the formation of ternary Max:Max–DNA complexes.

To pre-form stable Myc:Max heteromeric complexes, we used a co-refolding approach. Purified denatured Myc and Max proteins in urea were mixed at a molar ratio of 3 (Myc) to

1 (Max) and co-renatured by slow stepwise dialysis (see Materials and methods). Under these conditions, Myc alone does not efficiently refold into soluble homodimers but requires the presence of Max (see Materials and methods, and data not shown).

To verify that the full-length renatured Myc:Max complexes are correctly refolded and active in sequence-specific DNA-binding, we performed electrophoretic mobility-shift assays (EMSAs) with a radio-labeled E box-containing DNA oligonucleotide probe. As shown in Fig. 2, titration of recombinant Myc:Max with a fixed amount of probe resulted in a dose-dependent formation of a protein–DNA complex (lanes 5–7) that migrated more slowly than the protein–DNA complex formed with soluble Max:Max homodimers (lanes 2–4). Significantly, no protein–DNA complex with a mobility corresponding to a Max:Max homodimer-containing complex was observed with our Myc:Max preparation, even under conditions in which most of the probe was bound (lane 7). This demonstrates that the co-renatured Myc:Max complex is highly active in DNA-binding and does not contain significant amounts of Max:Max homodimers. We noted that in contrast to Max:Max homodimers, Myc:Max complexes had a tendency to retain part of the DNA probe in larger aggregates in the well (Fig. 2 asterisk, lanes 5–7, see also below). This was not due to a small proportion of Myc:Max homodimers in our preparations as under identical concentrations renatured Myc alone did not bind to the DNA probe under the conditions used here (data not shown). It is possible, however, that this reflects the ability of Myc:Max heterodimers to form higher order complexes (i.e., tetramers) as observed previously with and without DNA ([14], and references therein).

We analyzed further the optimal salt concentration for DNA binding by Max:Max and Myc:Max complexes. As shown in Fig. 3, optimum binding by Max:Max homodimers was achieved between 70 and 100 mM KCl while Myc:Max complexes had an optimum binding between 100 and 150 mM KCl. Thus, we selected for all our DNA-binding analyses a salt concentration of 100 mM KCl, which approximates the physiological salt concentration. Note that the amount of probe retained in the wells by higher order Myc:Max complexes (asterisk) had a slightly different salt dependence and a broader peak with an apparent optimum at about 70 mM KCl.

To demonstrate further that both Myc and Max proteins were present in the protein–DNA complex observed with our Myc:Max preparation and that DNA-binding competent Myc contains its TAD domain, the Myc:Max and Max:Max complexes were pre-incubated with specific antibodies that recognize the C-terminus of Max and the N-terminal TAD of Myc and then used in EMSA as above. Both the Myc and Max antibodies “supershifted” the Myc:Max–DNA ternary complex and the resulting antibody/Myc/Max–DNA complexes (Ab-complexes) remained in the wells or barely entered the gel (Fig. 4A, lanes 6 and 7). In contrast, only the Max antibody supershifted the protein–DNA complex obtained with Max:Max homodimers (compare lanes 2 and 3). These antibody-dependent effects were specific as antibodies against an unrelated protein, p130, did not influence the binding of Max:Max and Myc:Max complexes (lanes 4 and 8). Furthermore, the Myc and Max antibodies did not interact with the DNA probe by themselves (Fig. 4B, lanes 1 and 2) and did not influence the E box-binding activity of the bHLHZip transcription factor USF1 (lanes 4 and 5). Thus, we conclude that the protein–DNA complexes observed with the

Myc:Max preparation contain both Myc and Max proteins including an intact Myc TAD domain.

To determine whether the recombinant Myc:Max complex binds to the E box element in a sequence-specific manner unlabeled oligonucleotides containing an E box wild-type (E wt, same sequence as the labeled probe) and an E box mutant (E mut, Fig. 5) were used as competitors in EMSA. The E box mutant competitor is identical to the E box wild type DNA except for a two base pair substitution in the center of the E box, which reduces binding by native Myc:Max and Max:Max complexes isolated from mammalian cells [35]. As shown in Fig. 5, E wt but not E mut competitor oligo efficiently inhibited binding of Max:Max (lanes 2–4 vs. 5–7) and Myc:Max (lanes 9–11 vs. 12–14) complexes to the DNA probe at 5- to 20-fold molar excess. Note that all binding reactions in addition contained 80 ng of poly(dA–dT) as non-specific DNA, which is about a 30-fold mass excess over the probe. Therefore, we conclude that binding of the Myc:Max complex to DNA is E box sequence-specific. It appears, however (from the results of Figs. 2 and 5), that the recombinant Myc:Max complex has about 5- to 7-fold lower relative affinity for the E box probe than the Max:Max homodimer and also recognizes weakly the E box mutated sequence (Fig. 5, lane 14). This contrasts with the similar affinities of Myc:Max and Max:Max complexes isolated from Cos7 cells [35]. It will be interesting to address in the future whether modifications of the recombinant Myc:Max complex by enzymes that post-translationally modify Myc and/or Max in mammalian cells can regulate its binding affinity for E box sequences. Note also that the probe bound by higher order Myc:Max aggregates within the wells was also specifically competed with the E wt but not with the E mut oligo (data not shown), suggesting that high order Myc:Max complexes (i.e., tetramers) can also bind specifically to E box sequences as seen in the Myc:Max–DNA co-crystal structure [14].

Concluding remarks

We have presented an improved method to express and purify bacterial recombinant 6His-tagged Myc to apparent homogeneity. The use of BL21CodonPlus-RP bacteria and single-step metal-affinity purification from washed inclusion bodies under denaturing conditions has allowed the isolation of highly pure full-length Myc that lacks degradation or partial translation products. We have described further a simple co-renaturation method that allows the reconstitution of soluble recombinant Myc:Max complexes which are stable and lack any detectable Max:Max homodimer. We have demonstrated that the recombinant full-length Myc:Max complexes bind to E box DNA sequences at nanomolar concentration (Fig. 2) and in a sequence-specific manner (Fig. 5). The relatively simple methods described here will allow the functional analyses in vitro of Myc transcription functions with native promoters and chromatin templates and the study of post-translational modifications on Myc:Max interactions with free DNA and chromatin [25], independently of their emerging role in Myc protein turnover/stability in vivo [42,46,50,51].

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Fig. 1.

Purified Myc and Max proteins and co-renatured Myc:Max complex. (A) SDS-PAGE stained with Coomassie brilliant blue of 6His-Myc (100 ng, lane 4) purified under denaturing conditions from inclusion bodies of BL21CodonPlus(DE3)-RP cells and of soluble Max dimers (600 ng, lane 5). Lanes 1–3 contain BSA 25 ng (lane 1), 100 ng (lane 2), and 250 ng (lane 3). (B) SDS-PAGE stained with Coomassie brilliant blue containing renatured Myc:Max complexes after dialysis (1 μ l, lane 1) and a molecular weight standard (lane 2) with the corresponding masses in kDa.

Fig. 2.

EMSA analysis of soluble Max:Max and renatured Myc:Max complexes. Lane 1 contains the labeled E box probe only (0.2 pmol). In lanes 2–4 increasing amounts of Max:Max homodimers were incubated with the labeled probe: 0.04 pmol (lane 2), 0.16 pmol (lane 3), and 0.50 pmol (lane 4). Similarly, in lanes 5–7 increasing amounts of Myc:Max complex were used: 0.04 pmol (lane 5), 0.16 pmol (lane 6), and 0.50 pmol (lane 7). The positions of the free probe (F) and the Max:Max–DNA and Myc:Max–DNA complexes are indicated on the right. The asterisk (*) points to some probe retention in the wells of the gel (lanes 5–7).

Fig. 3.

Influence of KCl concentration in binding of recombinant Max:Max and Myc:Max complexes to E box DNA. EMSA was performed as described in Materials and methods but with 0.4 pmol of the labeled E box probe and either 0.04 pmol Max:Max or 0.16 pmol Myc:Max complexes in binding reactions containing the indicated KCl concentrations. The asterisk (*) points to the wells where some Myc:Max–DNA aggregates are retained.

Fig. 4.

Both Myc and Max proteins participate in the protein–DNA complex obtained with reconstituted Myc:Max complexes. (A) EMSA analyses with an E box probe and either Max:Max (0.04 pmol, lanes 1–4) or Myc:Max (0.16 pmol, lanes 5–8) complexes pre-incubated with specific antibodies for Myc, Max, and p130, as indicated. Lanes 1 and 5 contain products of “mock” pre-incubations with buffer alone. Antibody/protein–DNA complexes (Ab-complexes) retained in the wells in lanes 3, 6, and 7 are indicated. (B) EMSA analyses were performed with the labeled E box probe incubated with anti-Myc and anti-Max antibodies alone (lanes 1 and 2) or by pre-incubating anti-Myc and anti-Max antibodies with 2 ng recombinant USF1 (0.02 pmol of dimer) before addition of the E box probe (lanes 4 and 5).

E wt 5' GCTCAGGGAC CACGTG GTCGGGGGATC 3'

E mut 5' GCTCAGGGAC CAG[•]CTG GTCGGGGGATC 3'

Fig. 5.

E box sequence-specific binding by reconstituted Myc:Max complexes. EMSA analyses of Max:Max (0.04 pmol, lanes 1–7) and

Myc:Max (0.16 pmol, lanes 8–14) complexes binding to the E box (E wt) labeled probe in the absence (lanes 1 and 8) or presence of increasing amounts (5-, 10-, and 20-fold molar excess) of competitor unlabeled E wt (lanes 2–4 and 9–11) and E mut (lanes 5–7 and 12–14) DNA oligonucleotides. The DNA sequence of the E wt and E mut competitors is shown. The two base pair substitutions in E mut are indicated with two dots above the sequence.