MRG15, a Novel Chromodomain Protein, Is Present in Two Distinct Multiprotein Complexes Involved in Transcriptional Activation*

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MRG15 is a novel chromodomain protein that is a member of a family of genes related to MORF4. MORF4 (mortality factor on chromosome 4) induces senescence in a subset of human tumor cell lines. Our previous results indicated that MRG15 (MORF-related gene on chromosome 15) could derepress the B-myc promoter by association with Rb. In this study, sucrose gradient analysis demonstrated that MRG15 was present in two distinct nuclear protein complexes, MAF1 (MRG15-associated factor 1) and MAP2. Rb was associated with MRG15 and PAM14 (a novel coil-coil protein) in MAF1, and a histone acetyl transferase, hMOF, was an MRG15 partner in MAP2. Analysis of deletion mutants of MRG15 indicated that the leucine zipper at the C-terminal region of MRG15 was important for the protein association in MAF1 and that the N-terminal chromodomain was required for the assembly of the MAP2 protein complex. Consistent with these data was the fact that a histone acetyltransferase activity associated with MRG15 was lost when the chromodomain was deleted and that both mutant MRG15 proteins failed to activate the B-myc promoter. The various mechanisms by which MRG15 could activate gene transcription are discussed.

Replicative senescence, the final non-proliferative state reached by normal cells in culture, is considered a model for aging at the cellular level. Senescence is dominant over the phenotype of indefinite division exhibited by tumor cell lines (1). In an effort to identify senescence-related genes, MORF4,1 (MORTality Factor on chromosome 4) was cloned as a gene capable of inducing a senescent phenotype in a subset of human tumor cell lines (2). MORF4 is a member of a family of genes, and two of these, MRG15 and MRGX (MORF4-related gene; MAF, MRG15-associated factor; HAT, histone acetyltransferase; msl-3, male-specific lethal 3; HA, hemagglutinin; GFP, green fluorescence protein; Mof, males absent on the first)

high degree of homology to MORF4, are expressed in human cells. Analysis of the deduced amino acid sequences of the three predicted proteins has indicated the existence of helix-loop-helix and leucine zipper domains, features typical of those found in transcriptional regulators, as well as regions thought to be involved in protein-protein interaction. MRG15 has a 96% similarity to MORF4 in amino acid sequence but fails to induce senescence upon introduction into immortal cells. The most striking structural difference between the two proteins is the presence of an N-terminal extension in MRG15 that includes a chromodomain. This region and/or nine single amino acid changes could therefore be responsible for the differential behavior of the two proteins. The fact that MORF4 is a truncated protein raises the hypothesis that it could have a dominant negative effect when transfected into cells, resulting in loss of cell proliferation.

Proteins containing a chromodomain characterized to date have been found to be chromatin remodeling factors involved in causing conformational changes in chromatin by ATP-dependent movement of nucleosomes and modification of histones (3). Although the function of the chromodomain is not completely understood, some evidence suggests it is involved in protein-protein interaction (4–6), and it has also been reported to have RNA binding properties (7). Human MRG15 has overall similarity with the Drosophila msl-3 (male-specific lethal 3) protein, and several recent reports have shown that they are evolutionarily related (8–10). In Drosophila, msl-3 is required for dosage compensation by the transcriptional enhancement of genes on the male X chromosome (11, 12). Although human orthologues of most of the members of the proteins involved in sex dosage compensation in flies exist (8), the processes they are involved in have not yet been elucidated.

We have previously reported that MRG15 interacts with Rb and is involved in the activation of the B-myb promoter under E2F control (13). The action of Rb as a transcriptional repressor of E2F-responsive genes involved in G1/S transition explains in part its function as a tumor suppressor (14). The G1/S Rb-dependent arrest is overcome during the normal cell cycle by phosphorylation of Rb by cyclin-dependent kinases, which results in destabilization of the Rb/E2F complexes. In this report we demonstrate that MRG15 associates with other nuclear proteins in at least two distinct complexes, MAF1 and MAF2 (MRG15-associated factors 1 and 2, respectively). MRG15 interacts with Rb in the MAF1 complex. PAM14, a novel protein isolated by us from a yeast two-hybrid screen using MRG15 and MRGX as bait, is another member of this complex. The MRG15 leucine zipper is necessary for the interaction with Rb as well as for activation of the B-myb promoter (13). These results suggest that MRG15 may disrupt E2F/Rb complexes by direct interaction with Rb. In addition we have found that the hMOF protein, a MYST histone acetylase (15), is a MRG15 partner in MAP2. hMOF is homologous to the Drosophila MOF
protein, which is also involved in the enhancement of transcription of genes on the X male chromosome by acetylation of histone H4 (16, 17). Cells expressing an MRG15 mutant lacking the chromodomain failed to appropriately assemble the MAF2 complex. We detected a histone acetyltransferase (HAT) activity associated with wild type MRG15; however, a mutant construct minus the chromodomain did not have this activity. Additional studies confirm that the chromodomain-minus mutant failed to activate the B-myb promoter, suggesting that this complex is also involved in transcriptional regulation, in this case most likely through chromatin remodeling and histone acetylation. Thus both MAF1 and -2 can affect expression of the B-myb promoter and, thereby, cell cycle progression.

EXPERIMENTAL PROCEDURES

Plasmid Constructs—The MRG15 cDNA was introduced into the BamH1/XhoI sites of pcDNA3.1 to generate pcDNA3.1 MRG15. The MRG15 open reading frame was then amplified using primers that resulted in the incorporation of HindIII and BamHI sites at the 5' and 3'-ends, respectively. Primers were MRG15Hind, 5'-GGAAATTCCTCCACCTGGCGCCGGCAGGAGCC-3', and MRG15Bam, 5'-GAGGATCCGAGGCTCGGCGAACCGATTCTCGAGATG-3'.

After amplification, the amplicons were digested with HindIII and BamHI and cloned directionally into the EcoRI/XhoI-vector backbone cut with the same enzymes. The MRG15 EGFP-N1 chromodomain-minus construct was obtained by amplifying the MRG15 EGFP-N1 construct with primers that amplified all but the chromodomain region (amino acids 26–62) and introduced a PalI site upon religation. Primers were MRG15chr-R, 5'-GCCGTGACGAAAGAGGGCCGCTAGAACG-3', and MRG15chr-R, 5'-GCCGTGACGAAAGAGGGCCGCTAGAACG-3'.

The MRG15 EGFP-N1 leucine zipper-minus was cloned by a similar strategy to the chromodomain-minus mutant. Primers used to generate the MRG15 luminous-EGFP-N1 construct were MRG15leu-F, 5'-GCCCTGAGAAGAAGAGGCCCATGAAGC-3', and MRG15leu-R, 5'-GCCCTGAGAAGAAGAGGCCCATGAAGC-3'.

The MRG15-EGFP construct was obtained using the primers MRGFA-H, 5'-TATGGTGCTTCCAGCGGGCGCGACAGGGG-3', and MRGHA-R, 5'-CTCATTGGTGGCCTTCGACACACCATGGGATGATGCCGAGG-3'. Protein from nuclear extracts was incubated with 40 μl of beads for 1 h at 4°C with constant rotation. These control beads were separated from the remaining soluble proteins with a magnetic holder and washed twice in buffer B diluted 1:3 with 25 mM Tris-HCl, pH 7.5.

For the immunoprecipitations of the sucrose gradient fractions, nuclear extract obtained from EJ-MRG15-HA cells harvested at 32T-150 tissue culture flasks was loaded onto five 5–20% sucrose gradients, which were run simultaneously as described above.

The MRG15-HA construct was cloned by a similar strategy to the chromodomain-minus mutant. Primers used to generate the MRG15 luminous-EGFP-N1 construct were MRG15leu-F, 5'-GCCCTGAGAAGAAGAGGCCCATGAAGC-3', and MRG15leu-R, 5'-GCCCTGAGAAGAAGAGGCCCATGAAGC-3'.

The MRG15-EGFP construct was obtained using the primers MRGFA-H, 5'-TATGGTGCTTCCAGCGGGCGCGACAGGGG-3', and MRGHA-R, 5'-CTCATTGGTGGCCTTCGACACACCATGGGATGATGCCGAGG-3'.

The PCR product was cloned into the BamH1 and XhoI sites of pcDNA3.1 Constructs were verified by sequencing and restriction enzyme analysis.

Cell Lines and Transfections—The immortal cell lines EJ (bladder carcinoma-derived cells) and CMV-MJ-He1 (CMV-transformed human fibroblasts) were maintained in minimum essential medium with Hank's salts supplemented with 10% fetal bovine serum. Cells were transfected by using LipofectAMINE Plus (Invitrogen) according to the manufacturer's instructions. Stable clones expressing PAM14-HA were obtained from EJ and CMV-MJ-He1 parental cell lines as previously described (13). Cells stably expressing MRG15-HA were obtained by transfecting EJ cells with pcDNA3.1 MRG15-HA. 24 h post-transfection, 3,000–10,000 cells were plated into 60-mm dishes in minimum essential medium with Earle's salts supplemented with 10% fetal bovine serum and G418 (1 mg/ml). After a two-week incubation at 37°C in 5% CO2, several clones were isolated and maintained in medium containing G418. MRG15-HA expression was verified by immunoblot analysis.

Antibodies—Rabbit anti-MRG15 polyclonal antibody was raised against a peptide (amino acids 54–64) in the chromodomomain region (Cocalico Biologicals Inc.). Polyclonal rabbit anti-Rb was obtained as previously described (15). Polyclonal rabbit anti-HA and rabbit anti-Rb were purchased from Santa Cruz. Mouse monoclonal anti-Rb and anti-E2F1 were from Neomarkers. Mouse monoclonal anti-HA CA125 and mouse anti-FLAG were from Roche Molecular Biochemicals.

Preparation of Cellular Fractions—Nuclear and cytoplasmic extracts were prepared essentially as described previously (15). Cells were harvested and resuspended in buffer A (25 mM Tris-HCl, pH 7.5, 50 mM KC1, 2 mM Mg Cl2, 1 mM EDTA, 5 mM dithiothreitol), passed through a 29 gauge needle 5 times on ice, and centrifuged at 1800 × g for 10 min. Supernatants (cytoplasmic fraction) were frozen at −70°C or used immediately. Pellets were washed in buffer A and resuspended in buffer B (50 mM Tris-HCl, pH 7.5, 0.42 mM NaCl, 1.5 mM MgCl2, 0.5 mM EDTA, 1 mM dithiothreitol, 25% sucrose), incubated for 30 min on ice, and centrifuged at 1800 × g for 10 min. For sucrose gradient analysis, buffer B was made with 10% sucrose. The supernatants were used immediately or frozen at −70°C. All buffers were supplemented with a protease inhibitor mixture (Calbiochem), phenylmethylsulfonyl fluoride (0.5 mM), and the proteasome inhibitor, MG132 (2 μM).

Analysis by Ultracentrifugation in Sucrose Gradients—300–700 μg of protein from nuclear extracts was loaded on sucrose gradients made in buffer Tris-HCl (25 mM). Samples were diluted with 1.5 volumes of 25 mM Tris-HCl, pH 7.5, before being loaded. Centrifugation was performed in a Beckmann SW50 at 36,000 rpm for 14 h. Fractions of 150-μl-200-μl were collected from bottom to top, and aliquots were analyzed by SDS-PAGE and Western blot. Cytochrome c (1.7 S), peroxidase (3.5 S), alkaline phosphatase (6.3 S), and catalase (11.2 S) were used as sedimentation coefficient standards. Cytochrome c was detected by colorimetry, and the enzymatic activity of peroxidase, alkaline phosphatase, and catalase was determined in the fractions.

Polyclonal rabbit and mouse anti-HA antibodies (Santa Cruz Biotech.) were preincubated with the corresponding antibodies for 30 min on ice. Nuclear extracts were diluted 1:3 in 25 mM Tris-HCl, pH 7.5. 200 μg of protein from nuclear extracts was incubated with 40 μl of beads for 1 h at 4°C with constant rotation. These control beads were separated from the remaining soluble proteins with a magnetic holder and washed twice in buffer B diluted 1:3 with 25 mM Tris-HCl, pH 7.5.

For the immunoprecipitations of the sucrose gradient fractions, nuclear extract obtained from EJ-MRG15-HA cells harvested at 32T-150 tissue culture flasks was loaded onto five 5–20% sucrose gradients, which were run simultaneously as described above. 175–150 tissue culture flasks was loaded onto one 30% sucrose gradient, which were run simultaneously as described above. 175–150 tissue culture flasks was loaded onto five 5–20% sucrose gradients, which were run simultaneously as described above.

Immunostaining and Confocal Microscopy—0.2 × 106 cells were plated on glass coverslips in 35-mm tissue culture dishes in minimum essential medium with Earle's salts plus fetal bovine serum in a 5% CO2 incubator. Transfections with the appropriate constructs were done as described above. Cells were fixed with paraformaldehyde (2%) and permeabilized with Triton-X100 (0.2%) for 10 min. Coverslips were then rinsed with phosphate-buffered saline (PBS) and blocked with 2% goat serum in PBS for 30 min. After incubation with the appropriate primary antibodies, immunostaining was performed using Texas Red-conjugated goat anti-rabbit (1: 500) from Molecular Probes. Staining with 1 mg/ml DAPI (4, 6-diamino-2-phenylindole) was done for 10 min. Immunoreactive proteins were detected using anticytokerin 5/6 (Novocastra) and anti-p16INK4a (Biogenex). Microscopy was performed using an Applied Precision Delta Vision microscope (Issaquah, WA) fitted with an Olympus 1×70 microscope. Images were acquired via wide field sectioning using fluorescent light. The stacked images, usually 20–25 sections, were subjected to point spread function analysis for better image quality on Silicon Graphics software (SGI, Mountain View, CA). Magnification of all cells was maintained at >600. PAM14-HA was seen as red, MRG15-GFP as green, 4,6-diamidino-2-phenylindole nuclear staining as blue.

HAT Activity Assays—EJ cells transfected with either control (pcDNA3.1 MRG15-HA) or Tip60-HA constructs were assayed for histone H4 acetyltransferase activity using the HAT ChromoScreen assay kit (Bachem). The amount of protein was immunoprecipitated using an anti-HA antibody, and the immunoprecipitates were incubated with the first 23 amino acids of histone H4 conjugated to biotin and [3H]acetyl-CoA for one h at 30°C. The mixture was added to streptavidin beads and rocked for 30 min at room temperature. The beads were washed three times with wash buffer and subjected to an alkaline phosphatase immobilization step and a HAT buffer, and 3H activity was measured with a scintillation counter.

Luciferase Assay—These utilized the B-myb promoter reporter constructs and the same experimental approach detailed previously (13). Briefly, expression plasmids were transfected into EJ cells, which were harvested 24 h later with reporter lysis buffer (Promega). The luciferase activity was determined using the Luciferase assay kit (Promega) and a Monolight 2010 luminometer. The data were normalized to the amount of protein in the samples as determined by the Bradford assay. Luciferase assays were performed in triplicate to verify reproducibility, and protein assays were performed in duplicate.
RESULTS

MRG15 Is Present in at Least Two Distinct Nuclear Multi-protein Complexes—From our previous work we had evidence of the presence of MRG15, PAM14-HA, and Rb in independent immunoprecipitates obtained using antibodies against either HA or Rb. This suggested they were present together in the same multiprotein complex (13). Using a cell line stably expressing PAM14 tagged with HA, we immunodepleted the tagged protein in nuclear extracts with an anti-HA antibody and determined that about 30% of MRG15 was still present in the supernatant (data not shown). Because this indicated that MRG15 could be associated with other proteins in more than one complex, we fractionated nuclear extracts from EJ cells stably expressing either MRG15-HA or PAM14-HA and CMV-MJ-Hel 1 cells stably expressing PAM14-HA into 5–20% w/v sucrose gradients. The presence of MRG15, PAM14, and Rb proteins was determined by SDS-PAGE and Western blot analysis. In the stable clone expressing MRG15-HA, the anti-HA antibody demonstrated the existence of at least two different complexes containing MRG15: a more abundant component with a sedimentation coefficient of 3.5 S, MAF1, and a minor component with a sedimentation coefficient of 14 S, MAF2 (Fig. 1a). In all the clones the same distribution of MRG15 was also detected by the anti-HA antibody, and PAM 14 and Rb were found only in the fractions that corresponded to MAF1 (Fig. 1, b and c). The peaks of Rb and MRG15 were not found in the same fraction in EJ-PAM14-HA cells (Fig. 1b), which could be because of clonal variation. Alternatively, there may be more than one Rb/MRG15 complex in this clone.

To eliminate the possibility that these two complexes were the result of the disassembly of a unique multiprotein complex during the preparation of the extract, we investigated the in vivo distribution of MAF1 and -2 by immunostaining and confocal microscopy. CMV-MJ-Hel 1 cells stably expressing PAM14-HA were transfected with a construct encoding MRG15-GFP. GFP was visualized directly and PAM14-HA by immunostaining with an anti-HA primary antibody and a secondary anti-rabbit antibody conjugated to Texas Red fluorescent dye. The images obtained by confocal microscopy demonstrated that MRG15 was present throughout the nucleus (Fig. 2a), whereas PAM14 was more abundant in the perinuclear region (Fig. 2b) where it co-localized with MRG15 (Fig. 2c). The fact that there was no co-localization of MRG15 and PAM14 in the inner part of the nucleus supports the results from the fractionation studies indicating that MRG15 is present in at least two nuclear complexes.

MRG15 Interacts with Both the Phosphorylated and Unphosphorylated Forms of Rb—We have previously established that the MRG15 interaction with Rb in a nucleoprotein complex mediates the activation of the E2F-responsive promoter, B-myb (13), most likely through derepression by the disruption of E2F-Rb complexes. The most common mechanism responsible for this effect is the phosphorylation of Rb by cyclin-dependent kinases, which hyperphosphorylate Rb to the inactive form that loses affinity for E2F (14). Both the phosphorylated and unphosphorylated forms of Rb cosediment with MRG15 at the position of MAF1. To determine whether MRG15 associated preferentially with one of these forms of Rb, nuclear extracts of EJ MRG15-HA cells immunoprecipitated with an anti-HA antibody were analyzed with an antibody that recognizes Rb in both states. Both forms of Rb were present in the immunopre-
cipitates (Fig. 3, lane 3), and some of the phosphorylated molecules were present in the non-precipitated proteins (Fig. 3, lane 1).

**hMOF is an MRG15 Partner in MAF2**—The human MRG15 protein has an overall homology with the msl-3 protein from *Drosophila* (8) that is involved in dosage compensation by transcriptional enhancement of the genes present on the X male chromosome (12). This mechanism requires the recruitment of the multiprotein MSL complex and two associated small RNA molecules, rho1 and rho2, to the X chromosome (9). The MSL complex has multiple activities involved in chromatin remodeling, including histone acetylation via a histone acetyltransferase, MOF. A homologue to the Drosophila protein was recently found in humans, hMOF, a chromodomain protein belonging to the MYST family of acetyl transferases (15). MRG15 and another human protein, the product of the msl-3L1 gene (19), have been proposed as proteins potentially able to interact with hMOF (15). We therefore used an anti-hMOF antibody in Western analysis of EJ-MRG15-HA nuclear extracts immunoprecipitated with either anti-HA or anti-Rb antibodies. The fractions obtained from sucrose gradient sedimentation were also analyzed. hMOF was detected only in the anti-HA immunoprecipitates and the sucrose gradient fractions corresponding to MAF2 but not MAF1 (Fig. 4, a and b) and was absent in the Rb immunoprecipitates (Fig. 4a).

**Immunoprecipitation Analysis of the Proteins in the Sucrose Gradient Fractions**—To demonstrate that the proteins identified by Western analysis in the sucrose gradient fractions (Figs. 1 and 4) were indeed together in the proposed complexes (MAF1 and MAF2), we initially attempted immunoprecipitations of the individual fractions involving MAF1 and MAF2. Western analysis produced no results, most likely because of the low amount of protein present. We then decided to pool the fractions related to MAF1 and MAF2 from five gradients produced simultaneously prior to immunoprecipitation. We were successful in identifying the presence of MRG15 with Rb in MAF1 and hMOF in MAF2 (Fig. 5). We did not identify the presence of PAM14 because it was necessary to run a 7.5% gel to visualize MRG15, Rb, and hMOF, a percentage gel in which

![MRG15-GFP](image1.png)

![PAM14-HA-TEXAS RED](image2.png)

![MRG15-HA](image3.png)

**Fig. 2. Partial co-localization of MRG15 with PAM14 in the cell nucleus.** CMV-MJ-Hel 1 cells stably expressing PAM-HA were transfected with the pEGFP-N1-MRG15 plasmid, fixed, and immunostained using a polyclonal anti-HA antibody and Texas Red-conjugated goat anti-rabbit as secondary antibody. DNA was stained with DAPI. Localization of MRG15-GFP (a), of PAM14-HA (b), and of the merged colocalization of the two proteins (c) is shown.

![Fig. 3. MRG15 binds both the phosphorylated and unphosphorylated forms of Rb.](image4.png)

**Fig. 3. MRG15 binds both the phosphorylated and unphosphorylated forms of Rb.** Nuclear proteins (200 μg) from EJ cells expressing MRG15-HA were immunoprecipitated with the monoclonal anti-HA antibody. Lane 1, half the supernatant following immunoprecipitation; lane 2, proteins that bound nonspecifically to the beads; lane 3, the immunoprecipitated proteins subjected to SDS-PAGE and immunoblotted with a polyclonal anti-HA antibody and a monoclonal anti-Rb antibody.
We demonstrated that MRG15 associates with Rb in MAF1 and hMOF in MAF2.

The MRG15 Leucine Zipper Domain and the Chromodomain Are Necessary for Appropriate Assembly of MAF1 and MAF2, Respectively—Leucine zipper and chromodomain regions are generally thought to be involved in protein-protein interactions. We have previously determined that the MRG15 leucine zipper was required for appropriate interaction with PAM14 and Rb (13). To evaluate the effect of both regions in the constitution of the MAF multiprotein complexes, we analyzed the sedimentation behavior of the protein members of the MAFs when EJ cells stably expressing PAM14-HA were transfected with plasmids containing either wild type MRG15-GFP or deletion mutants lacking either the leucine zipper or chromodomain regions.

PAM14 is lost during electrophoresis. However, the data demonstrate that MRG15 associates with Rb in MAF1 and hMOF in MAF2.

The absence of the leucine zipper caused disassembly of MAF1 by disrupting the association with Rb (Fig. 6) and the displacement of MRG15 to a lower position in the gradient. We did not detect PAM14 in any of the fractions, possibly because of the instability of this protein when it loses association with MRG15. The deletion of the leucine zipper resulted in no significant change in the MAF2 complex, whereas the absence of the chromodomain resulted in complete loss of the MAF2 complex. This indicates that the leucine zipper is necessary for the assembly of proteins in MAF1 and that the chromodomain is required for the assembly of MAF2.

The MRG15 Chromodomain Is Associated with a HAT Activity and Is Also Involved in the Activation of the B-myb Promoter—Because we had detected hMOF in immunoprecipitates as well as sucrose gradient fractions of MRG15, we performed a HAT activity analysis. Nuclear extracts from cells transfected with either empty vector pcDNA3.1 (Invitrogen), pcDNA3.1 MRG15-HA tagged at the C terminus, or a plasmid expressing a HA-tagged Tip60 protein (a known co-activator protein that has HAT activity) (19) were immunoprecipitated with an anti-HA antibody and analyzed for HAT activity. The results, expressed as the percentage of the total HAT activity, indicate that in the case of MRG15-HA there was 2.2-fold increased HAT activity compared with empty vector, similar to the 2.7-fold activity observed with Tip60-HA (Fig. 7a). Interestingly, immunoprecipitates of cells transfected with GFP N-terminal-tagged MRG15 protein did not associate with HAT activity (data not shown), suggesting that the N-terminal region of MRG15 is required for this association.

We had previously demonstrated that MRG15 could activate the B-myb promoter and that the leucine zipper region was required for this derepression, most likely through interaction with Rb. We tested the chromodomain minus mutant in this assay to determine whether HAT activity was involved in affecting the activity of this promoter and indeed observed that the activation was abolished (Fig. 7b). Collectively, these observations suggest that the B-myb promoter activity is dependent on both the interaction of MRG15 with Rb and the resulting inhibition of Rb-mediated transcriptional repression, as well as a HAT-associated activity acting most likely via histone acetylation.
DISCUSSION

The MRG15 gene had been identified (2) as a member of a family of genes related to MORF4, a gene that induces replicative senescence in a subset of immortal cell lines. The MRG15 protein is very similar to the MORF4 protein except for the presence of an N-terminal extension that harbors a chromodomain and nine changes in amino acids, most of which are conserved. This strong homology suggests that MORF4 could have a dominant negative effect on MRG15 action when transfected into cells. Proteins containing a chromodomain are usually involved in chromatin remodeling that leads to transcriptional activation or repression of a large number of genes. This implicates MRG15 as a good target for involvement in the permanent growth arrest elicited by MORF4.

Our previous findings have demonstrated that MRG15 is able to derepress transcription of the luciferase gene under the control of the B-myb promoter and that it associates with Rb and PAM14, a novel protein (13). In this report we have established that MRG15 is present in two distinct nuclear protein complexes, MAF1 and MAF2, in association with proteins known to be involved in the transcriptional activation of genes. MRG15 has a leucine zipper and a chromodomain, motifs known to facilitate interaction with other proteins. The analysis by sucrose gradients of nuclear extracts from cells expressing deleted versions of the MRG15 protein, lacking one or the other of these domains, has shown that the leucine zipper is required for the appropriate assembly of proteins at MAF1 without a significant change in MAF2 constitution. In MAF1, MRG15 associates with Rb, whereas E2F1 is not part of the complex, suggesting a derepressive nature of this association. Thus MRG15 stimulation of the B-myb promoter most likely involves the disruption of Rb-E2F complexes. PAM14 is a novel coiled-coil protein discovered in our laboratory by its ability to interact with MRG15 in the yeast two-hybrid system. Its interaction with MRG15 has been previously demonstrated by GST pull-down and immunoprecipitation (13). In this report we have established that it is part of MAF1. In MAF2 we have found the association of MRG15 with the human hMOF, the Drosophila homologue of which has been shown to have histone acetyl transferase activity (17). In addition, immunoprecipitates of MRG15 have an associated HAT activity. Expression of a chromodomain-minus mutant MRG15 protein resulted in loss of the MAF2 complex and HAT-associated activity. This mutant protein was also unable to positively affect the activity of the B-myb promoter. These results suggest that both MAF1 and MAF2 are needed for B-myb promoter activity, the former involving interaction with Rb through the leucine zipper domain of MRG15 and the latter possibly through relaxation of chromatin around the promoter via histone acetylation. Proteins containing a chromodomain, such as the Drosophila msl-3 protein, are components of multimeric complexes that modify chromatin structure via histone acetylation at target sites specified by the complex (3, 17). Specifically, these msl proteins paint the Drosophila X chromosome in association with the rox RNAs (transcripts specific to the X chromosome) and the histone acetyltransferase MOF to cause hypertranscription in dosage compensation (17). Therefore, the presence of the chromodomain in MRG15 suggests that it possesses the distinct potential to act in transcriptional regulation, perhaps through an interaction with specific RNAs or histone acetyltransferases. The Saccharomyces cerevisiae homologue of MRG15, Eaf3p, is a component of the yeast NuA4 HAT complex that contains Esa1p, the S. cerevisiae homologue of MOF and Tip60 (20). Esa1p and the NuA4 complex are essential for cell division and viability in budding yeast (20). Therefore, the MRG15 protein may be a component of a HAT complex homologous to the yeast NuA4 complex. The role of PAM14 is not clear at this time, but by comparison with other nucleoprotein complexes that have been described, it could be acting as an adaptor protein, facilitating interaction between proteins in the MAF1 complex.

The ability of MRG15 to increase B-myb promoter activity has implications for a potential role for MRG15 in cell cycle
The B-myb protein is an established transcriptional activator that promotes cell cycle progression through stimulation of multiple effectors of cell growth, including cyclin D1 and cdc2 (22, 23). Furthermore, overexpression of the B-myb gene has been demonstrated to promote bypass of p53-induced G1 arrest (23), suggesting that B-myb also induces cell growth through inhibition of growth inhibitory molecules. B-myb expression correlates with cell cycle progression in that it is repressed during G0 and early G1 phases but stimulated in late G1 and S phases (21). Significantly, this mRNA expression pattern is similar to that of MRG15, in that there is a ~2-fold increase in MRG15 mRNA levels at 4–8 h postserum stimulation of normal quiescent fibroblasts, and these elevated mRNA levels persist up to 28 h poststimulation (9). Young fibroblast cells 4–8 h poststimulation are in the early to mid-G1 phase of the cell cycle and enter S phase at 16 h postexposure to serum, with maximal DNA synthesis at 24 h poststimulation. Thus, like B-myb, MRG15 expression correlates with cell cycle progression and therefore represents a putative growth stimulatory factor. It is tempting to speculate that such potential proliferative functions of MRG15 are because of its ability to antagonize one or more activities of Rb in addition to chromatin remodeling.

Western analysis of MRG15 protein levels has indicated that MRG15 protein levels decrease about 2–3-fold with increasing cell population doublings and cell senescence in normal human fibroblasts (data not shown). This further suggests that the function of MRG15 is correlated with cell cycle progression. The fact that the MRG15 transcripts and protein are expressed in a wide variety of tissues and are conserved across a large number of species suggests an essential function for this protein. Future studies aimed at examination of the effect of MRG15 on additional Rb/E2F-repressed promoters and identification of additional cellular factors within the MRG15 protein complexes should provide critical insight into the role of this protein in the cell cycle and other processes.

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REFERENCES
1. Pereira-Smith, O. M., and Smith, J. R. (1983) Science 221, 964–966
2. Bertram, M. J., Berube, N. G., Hang-Swannen, X., Ran, Q., Leung, J. K., Bryce, S., Spurges, K., Bick, R. J., Baldini, Y., Ning, L. J., Clark, E. K., Parkinson, E. K., Barrett, J. C., Smith, J. R., and Pereira-Smith, O. M. (1999) Mol. Cell. Biol. 19, 1475–1485
3. Jones, D. O., Cowell, I. G., and Singh, P. B. (2000) Bioessays 22, 124–137
4. Ball, L. J., Murzina, N. V., Broadhurst, R. W., Raine, A. B., Archer, S. J., Stott, F. J., Murzin, A. G., Singh, P. B., Domaille, P. J., and Lauer, E. D. (1997) EMBO J. 16, 2473–2481
5. Cowell, I. G., and Austin, C. A. (1997) Biochim. Biophys. Acta 1337, 198–206
6. Nielsen, A. L., Oualad-Abdelghani, M., Ortiz, J. A., Remkoutska, E., Chamison, P., and Losson, R. (2001) Mol. Cell, 7, 729–739
7. Akhtar, A., Zink, D., and Becke, P. B. (2000) Nature 407, 405–409
8. Marin, I., and Baker, B. S. (2000) Mol. Biol. Cell 17, 1240–1250
9. Pannuti, A., and Lucchesi, J. C. (2000) Curr. Opin. Genet. Dev. 10, 644–650
10. Bertram, M. J., and Pereira-Smith, O. M. (2001) Gene 266, 111–121
11. German, M., Kurola, M. I., and Baker, B. S. (1995) Cell 72, 39–49
12. German, M., Franke, A., and Baker, B. S. (1995) Development 121, 463–475
13. Leung, J. K., Berube, N., Venable, S., Ahmed, S., Timchenko, N., and Pereira-Smith, O. M. (2001) J. Biol. Chem. 276, 39171–39178
14. Hatakeyama, M., and Weinberg, R. A. (1995) Proc. Cell Cycle Res. 1, 9–19
15. Neal, K. C., Pannuti, A., Smith, E. R., and Lucchesi, J. C. (2000) Biochim. Biophys. Acta 1490, 170–174
16. Hilscher, A., Hilscher-Kreiner, D., Pannuti, A., and Lucchesi, J. C. (1997) EMBO J. 16, 2054–2060
17. Smith, E. R., Pannuti, A., Gu, W., Steurnagel, A., Cook, R. G., Allis, C. D., and Lucchesi, J. C. (2000) Mol. Cell. Biol. 20, 312–318
18. Timchenko, N. A., Wilde, M., Nakanishi, M., Smith, J. R., Darlington, G. J. (1996) Genes Dev. 10, 804–815
19. Ikura, T., Ogryzko, V. V., Grigoriev, M., Groisman, R., Wang, R., Horikoshi, M., Scully, R., Qiu, J., and Nakatani, Y. (2000) Cell 102, 463–473
20. Eisen, A., Utley, R. T., Nourani, A., Allard, S., Schmidt, P., Lane, W. S., Lucchesi, J. C., and Cote, J. (2001) J. Biol. Chem. 276, 3484–3491
21. Lin, D., Fiscella, M., O’Connor, P. M., Jackman, J., Chen, M., Luo, L. L., Sala, A., Travali, S., Appella, E., and Mercer, W. E. (1994) Proc. Natl. Acad. Sci. U. S. A. 91, 10079–10083
22. Sala, A., and Calabretta, B. (1992) Proc. Natl. Acad. Sci. U. S. A. 89, 10415–10419

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