Supplementary Methods

Gene Constructs

We have described previously (1-5) the following constructs used in this study—pcDNA3-FLAG-rat ARRB1 (wild-type), the FLAG-tag sequence is N-DYKDDDDK-C (N and C represent the amino terminus and carboxyl terminus, respectively, of the peptide; D, Y, and K represent amino acids aspartic acid, tyrosine, and lysine, respectively); pGEX2TK; pGEX2TK-RB1; pGEX5X1-rat ARRB1 (wild-type); pGEX5X1-rat ARRB1 (amino acids 1–163); and pGEX5X1-rat ARRB1 (amino acids 163–418).

We have also described the following constructs in a previous study (4)—pcDNA3-influenza hemagglutinin (HA) epitope-E2F1; pcDNA3-FLAG-E2F2; pcDNA3-myelocytomatosis viral oncogene homolog (Myc)-tagged-E2F3; and pcDNA3-E2F1 (amino acids 103–284). Similarly, the constructs containing DNA-binding transcription factor required for the activation of yeast GAL genes in response to galactose (GAL4 DNA-binding domain) fused to E2F1 amino acids 304-437 (pcDNA3-GAL4-E2F1 [amino acids 304–437]), and E2F1 amino acids 89-304 fused to virion protein 16 (VP16) activation domain (pcDNA3-E2F1 [amino acids 89–304]-VP16) have also been previously published (4, 5).

Primer Sequences for Chromatin Immunoprecipitation Assay

PCR was performed using the following primers:

BIRC5 (Forward) 5′-CGCCTCTACTCCCAGAAG-3′

BIRC5 (Reverse) 5′- TGTAGAGATGCGGTGGTC-3′

CDC6 (Forward) 5′-GGC CTC ACA GCG ACT CTA AGA-3′

CDC6 (Reverse) 5′-CTC GGA CTC ACC ACA AGC-3′

CDC25A (Forward) 5′-TCT GCT GGG AGT TTT CAT TGA CCT C-3′

CDC25A (Reverse) 5′-TTG GCG CCA AAC GGA ATC CAC CAA TC-3′

TYMS (Forward) 5′-GAC GGA GGC AGG CCA AGT G-3′

TYMS (Reverse) 5′- TGG CGC ACG CTC TCT AGA GC-3′

FOS (Forward) 5′-TGT TGG CTG CAG CCC GCG AGC AGT TC-3′

FOS (Reverse) 5′-GGC GCG TGT CCT AAT CTC GTG AGC AT-3′

Primer sequences for Real-time PCR

Real-time PCR was performed with the following primers for *BIRC5*, *TYMS*, *CDC6* and *18S* RNA (6, 7):

BIRC5 (Forward): 5′-ACTTGGCCCAGTGTTTCTTCT-3′.

BIRC5 (Reverse): 5′-GAAAGCGCAACCGGACGAATG-3′.

TYMS (Forward): 5′-′CTGCCAGCTGTACCAGAGAT-3′.

TYMS (Reverse): 5′-ATGTGCATCTCCCAAAGTGT-3′.

CDC6 (Forward): 5′-CCCCATGATTGTGTTGGTAT-3′.

CDC6 (Reverse): 5′-′TTCAACAGCTGTGGCTTACA-3′.

18SRNA (Forward): 5′-CTCAACACGGGAAACCTCAC-3′.

18SRNA (Reverse): 5′-AAATCGCTCCACCAACTAAGAA-3′

In Vitro Glutathione S–transferase (GST) Binding Assays

We have shown previously that the prokaryotic expression plasmids pGEX2TK, pGEX2TK-RB1, pGEX5X1-rat ARRB1 (wild-type), pGEX-5X1-rat ARRB1 (amino acids 1-163), pGEX-5X1-rat ARRB1 (amino acids 163-418) yield purified GST-fusion proteins (3, 8, 9). BL21 strain of *Escherichia coli* (*E. coli*) cells were transformed with the above plasmids and grown in Luria Bertani (LB) medium (Fisher Scientific) containing 100 μg/mL ampicillin

(Fisher Bioreagents, Pittsburgh, PA). Overnight cultures were diluted four-fold in LB medium containing 100 μg/mL ampicillin and incubated at 37°C until the optical density at 600 nm (OD_{600}) reached 0.5. After induction with 0.5 mM isopropylthiogalactoside (IPTG), bacteria were allowed to grow at 30°C for an additional 6 hours. Bacteria were then harvested, washed in PBS, snap-frozen in liquid nitrogen and kept overnight at –80°C. Next day cells were resuspended in lysis buffer (PBS with 0.5% Tween-20, 150 mM NaCl, 1 mM EDTA, 5 mM sarkosyl, 1 mg/mL lysozyme, 0.1 mg/mL DNase1, 1 mM DTT, 0.5 mM sodium orthovanadate, 0.5 mM sodium fluoride, pH 7.4) and lysed by sonication. After centrifugation at 10,000 *g* at 4°C for 15 minutes, the supernatant was incubated with glutathione-Sepharose beads (GE healthcare, Piscataway, NJ) at 4°C for 1 hour. The beads were washed four times in PBS and stored at 4°C as a 15% suspension in PBS in the presence of a mixture of protease inhibitors namely 5 μg/mL leupeptin, 5 μg/mL pepstatin, 5 μg/mL trypsin-chymotrypsin-inhibitor, 5 μg/mL aprotinin and 1 mM PMSF. The amount of GST-tagged proteins bound to the beads was quantified using the Bio-Rad Protein Assay Kit.

In vitro transcription and translation of the following constructs was performed using the rabbit reticulocyte lysate translation system (Promega, Madison, MI), according to the manufacturer's instructions—pcDNA3-E2F1 (amino acids 89-304)-VP16; pcDNA3-GAL4-E2F1 (amino acids 304-437); pcDNA3-E2F1 (amino acids 103-284); pcDNA3-HA-E2F1; pcDNA3-FLAG-E2F2; pcDNA3-Myc-E2F3; pcDNA3-FLAG-rat ARRB1 (wild-type). GST pull-down assays were performed by adding equal amounts of GST fusion proteins to 5 μL of the above-prepared ^{35}S-methionine-labeled rabbit reticulocyte lysates in 200 μL of protein binding buffer (20 mM Tris–HCl [pH 7.5], 50 mM KCl, 0.5 mM EDTA, 0.5% IGEPAL-CA630, 1 mM DTT, 0.5 mM sodium fluoride, 0.5 mM sodium orthovanadate, 5 mg/mL BSA, 5 μg/mL leupeptin, 5 μg/mL pepstatin, 5 μg/mL trypsin-chymotrypsin inhibitor, 5 μg/mL aprotinin and 1 mM PMSF) and rotated on a nutator at 4°C for 2 hours. The beads

were washed four times in binding buffer, and bound proteins were eluted in SDS sample buffer. Proteins were resolved on a 10% SDS–polyacrylamide gel, and visualized by autoradiography on a blue sensitive x-ray film (Midsci, St. Louis, MO). Approximately, one-fifth of the protein amount used in the binding assay was used for control samples (3, 9, 10). The experiment was repeated two independent times.

References

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