Human host cell factor-1 (HCF-1) is essential for cell cycle progression and is required, in conjunction with the herpes simplex virus transactivator VP16, for induction of viral immediate-early gene expression. We show here that HCF-1 directly binds to the Myc-interacting protein Miz-1, a transcription factor that induces cell cycle arrest at G₁, in part by directly stimulating expression of the cyclin-dependent kinase inhibitor p15INK4b. A domain encompassing amino acids 750–836, contained within a subregion of HCF-1 required for cell cycle progression, was sufficient to bind Miz-1. Conversely, HCF-1 interacted with two separate regions in Miz-1: the N-terminal POZ domain and a C-terminal domain (residues 637–803) previously shown to harbor determinants for interaction with c-Myc and the coactivator p300. The latter functioned as a potent transactivation domain when tethered to DNA, indicating that HCF-1 targets a transactivation function in Miz-1. HCF-1 or a Miz-1-binding fragment of HCF-1 repressed transactivation by Gal4-Miz-1 in transfection assays. Moreover, HCF-1 repressed Miz-1-mediated transactivation of a reporter gene linked to the p15INK4b promoter. Protein/protein interaction studies and transient transfection assays demonstrated that HCF-1 interferes with recruitment of p300 to Miz-1, similar to what has been reported with c-Myc. Our findings identify Miz-1 as a novel HCF-1-interacting partner and illustrate cross-talk between these two proteins that may be of consequence to their respective functions in gene regulation and their opposing effects on the cell cycle.

Human host cell factor-1 (HCF-1) is a transcriptional regulatory protein that was originally identified as an accessory factor required for induction of herpes simplex virus immediate-early genes by the viral transactivator VP16 (1–5). Subsequently, findings demonstrated that HCF-1 is an essential cellular protein that is required for cell proliferation (6). HCF-1 binds directly to VP16 and, in conjunction with the cellular octamer-binding transcription factor Oct-1 promotes the cooperative assembly and stability of a multicomponent protein-DNA transcription complex termed the VP16-induced complex (VIC) on regulatory elements present in the promoter regions of the herpes simplex virus immediate-early genes (reviewed in Refs. 1 and 7). Numerous studies of VP16 and its association with Oct-1, HCF-1, and DNA have provided fundamental insights into mechanisms of transcriptional activation and how combinatorial networks of protein/protein and protein/DNA interactions underpin complexity, specificity, and diversity in transcriptional regulation (1, 7).

Human HCF-1 is a ubiquitously expressed and evolutionarily conserved protein with a number of unusual properties and features (3, 5, 8, 9). HCF-1 is synthesized as a 2035-amino acid long precursor protein that is autocatalytically processed at centrally reiterated 26-amino acid repeat elements (HCF_PRO repeats) to generate a family of N- and C-terminal polypeptides that remain tightly, but noncovalently, associated with each other (10–13). VP16 associates with a discrete 380-residue N-terminal modular domain referred to as HCF_VIC (also called the kelch domain), which is composed of six repeated copies of a kelch-like sequence proposed to form a barrel-like six-bladed β-propeller (14). The HCF_VIC domain is necessary and sufficient to bind VP16, to stabilize VIC, and to promote transactivation (14).

In addition to subserving a role in viral gene expression, HCF-1 is essential for normal cell cycle progression (6). This finding arose from studies of tsBN67 cells, a temperature-sensitive hamster cell line that reversibly arrests at the G₁/S₁ decision point of the cell cycle at the nonpermissive temperature. The defect in tsBN67 cells is due to a single proline-to-serine missense mutation (P134S) at position 134 in HCF-1 (6). Interestingly, HCF-1(P134S) also fails to bind to VP16 (14), foretelling the existence of cellular factors that mimic VP16 in their interaction with HCF-1. The first such HCF-1-interacting cellular factor identified was LZIP (also called Luman) (15–17), a basic leucine zipper protein belonging to the CAMP-responsive element-binding protein/activating transcription factor family of transcription factors. Like VP16, LZIP and a related protein called Zhangfei (18) target determinants in the HCF_VIC domain. More recently, the transcription factors GA-binding protein (19) and Sp1 (20), the nuclear hormone receptor co-regulatory factor PGC-1 (21), and a protein phosphatase (22) have been shown to associate with HCF-1.

The foregoing adds to growing evidence that HCF-1 is an essential, multifunctional, co-regulatory protein that plays a global role in coordinating viral and cellular gene regulation and cell proliferation. However, the cellular role and mechanisms of action of HCF-1 and the identity of its cellular gene targets are essentially unknown. Recent findings show that HCF-1 is chromatin-associated, and it has been postulated that HCF-1 is recruited to DNA through its association with sequence-specific DNA-binding proteins, analogous to what occurs with VP16 and Oct-1 (23). The HCF_VIC domain is neces-
sary and sufficient for chromatin association, and the P134S mutation renders this association temperature-sensitive, suggesting that this detachment is responsible for the growth arrest phenotype in tsBN67 cells (23). However, the minimal region capable of rescuing tsBN67 cells encompasses residues 1–902, which include the HCFV/C domain and a downstream region rich in basic amino acids (6, 14). Moreover, the binding of the cellular proteins LZIP and Zhangfei to HCF-1 is not required to rescue tsBN67 cells and to promote cell cycle progression (23, 24). This indicates that other cellular factors important for cell cycle control that target the N-terminal domain and/or the basic region of HCF-1 may exist. The importance of the basic region in cell proliferation is underscored by recent studies with the HCF-1 family member HCF-2 and the related Caenorhabditis elegans homolog (25, 26). HCF-1, HCF-2, and C. elegans HCF share conserved N- and C-terminal domains; however, HCF-2 and C. elegans HCF lack the basic region and the central HCF PRO repeat. Although HCF-2 and C. elegans HCF are able to support VIC formation, these proteins are unable to rescue the temperature-sensitive cell cycle defect in tsBN67 cells (26). Coexpression of HCF-2 inhibits rescue by HCF-1, however, suggesting that the two factors share a common interacting partner(s) (25). The foregoing indicates that the basic region of HCF-1 provides an additional function that is required, in conjunction with the N-terminal proximal region, to promote cell cycle progression.

To shed light on the cellular and functional roles of HCF-1, we carried out yeast two-hybrid interaction cloning with the HCF-1 basic region as bait to identify putative cellular factors that target this region. We show here that HCF-1 interacts physically and functionally with Miz-1, a recently identified cell cycle regulatory factor that was originally isolated by virtue of its ability to interact with the cellular oncoprotein c-Myc (27–29). Miz-1 is an 803-amino acid long POZ domain/zinc finger transcription factor that modulates transcription by binding directly to initiator elements of target genes (27, 30, 31). Miz-1 derivatives were derived by subcloning PCR-derived XhoI/BamHI subfragments into the corresponding sites of the Gal4 fusion protein expression vector pSG424 by standard procedures. A mammalian expression plasmid for HCF-1 (frames 1–902) was generated by amplifying this fragment from pGNGHC to cloning into the yeast c-Myc plasmid pBGT9 (Clontech). This construct expresses human GAD-DBD-Miz-1 derivatives are able to rescue the temperature-sensitive cell cycle arrest are potentiated by the antimitogenic cyclin D1-associated kinase activity. Recent studies have shown that transcriptional activation of p15INK4b is required to rescue tsBN67 cells and to promote cell cycle progression (23, 24). This indicates that other cellular factors constructed above were verified by DNA sequence analysis. Mammalian expression vectors for Miz-1 derivatives expressing the POZ domain residues 109–308 and residues 637–803 were generated by cloning PCR fragments into pBSUTK. In vitro expression vectors for HCF-1 (residues 1–902) and HCF-1 (residues 1–199) were constructed by cloning appropriate PCR fragments into pBSUTK and pEG565, respectively. The GST-p300-(1572–902) were constructed by cloning appropriate PCR fragments into pBSUTK. In vitro expression vectors for Miz-1 derivatives expressing the POZ domain residues 109–308 and residues 637–803 were generated by cloning PCR fragments into pBSUTK. In vitro expression vectors for HCF-1 (residues 1–902) and HCF-1 (residues 1–199) were constructed by cloning appropriate PCR fragments into pBSUTK and pEG565, respectively. The GST-p300-(1572–2371) plasmid was obtained from R. Ecker, and the pDNA-Myc vector used for in vitro transcription/translation was provided by M. Eilers. Site-directed mutagenesis of pMiz-565 by the following mutagenic primers: 5′-AAAAACGGGCCCCCTTCGTCGCTC-GACTC-3′ and 5′-GAGTCGAGACACGAGGGGCGCTTTTGG-3′ (altered nucleotides are underlined). The authenticity of all clones constructed above was verified by DNA sequence analysis. Mammalian expression plasmid for HCF-1 (residues 1–902) to incorporate the P134S mutation was performed using the QuickChange site-directed mutagenesis kit (Stratagene) with the following mutagenic primers: 5′-AAAAACGGGCCCCCTTCGTCGCTC-GACTC-3′ and 5′-GAGTCGAGACACGAGGGGCGCTTTTGG-3′. In vitro transcription/translation vector for full-length Miz-1 was generated by cloning the Miz-1 open reading frame into the NcoI and SalI sites of pGEM5zf (Promega). In vitro expression vectors for Miz-1 derivatives expressing the POZ domain residues 109–308 and residues 637–803 were generated by cloning PCR fragments into pBSUTK. In vitro expression vectors for HCF-1 (residues 1–902) and HCF-1 (residues 1–199) were constructed by cloning appropriate PCR fragments into pBSUTK and pEG565, respectively. The GST-p300-(1572–2371) plasmid was obtained from R. Ecker, and the pDNA-Myc vector used for in vitro transcription/translation was provided by M. Eilers. 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**Experimental Procedures**

**Plasmids**—Mammalian expression vectors pCGNHC and pFLAG-C1, encoding hemagglutinin/c-Myc and FLAG epitope-tagged derivatives of full-length human HCF-1, respectively, have been described (3, 18) and were provided by W. Herr and T. Kristie, respectively. pCGN-HCF-1FL/pP134S (where FL is full-length) was constructed by isolating a SpeI/Xhol fragment from pCGNHC and inserting this fragment into pCGNHC/H1101/pP134S, supplied by A. Wilson (25). A mammalian expression vector encoding FLAG-epitope-tagged human HCF-1 was provided by R. Tjian (31). Mammalian expression vectors for full-length Miz-1 (pPK7) and human c-Myc (pMNBaseIRESc-Myc) were obtained from M. Eilers (27) and L. Penn (University of Toronto), respectively. pGal4/SXl/Trp, a luciferase reporter vector that contains five upstream copies of the Gal4-binding site, was obtained from J. Hassell (McMaster University). p15INK4b/luc, a luciferase reporter gene that contains sequences spanning −113 to +160 relative to the transcription start site of the p15INK4b promoter, was provided by M. Eilers (30). The in vitro transcription vector pSPUTK was obtained from D. Andrews (McMaster University). The yeast two-hybrid bait plasmid pG7BD-HCF-1 (450–1439), which contains residues 450–1439 of HCF-1 linked to the Gal4 DNA-binding domain (DBD), was constructed by cloning a PCR fragment corresponding to this region into the two-hybrid vector pG7T9 (Clontech). Similarly, pG8BD-HCF-1 (1–380) expresses the HCF-1 N-terminal region and the central HCF PRO repeats. Interaction of HCF-1 and Miz-1

**Two-hybrid Screening**—Two-hybrid analysis was carried out using the Clontech Matchmaker system essentially as described (35, 36). Briefly, yeast strain HF7c (MATa, ura3-2, his3-200, ade2-101, lys2-801, trp1-901, leu2-3,112, can4, gal4-542, gal80-538, URA3:Gal1-loc2) was transformed with pG7BD-HCF-1 (450–1439) and a Helia cDNA library fused to the Gal4 AD (Clontech) by the lithium acetate method (37). Independent transformants (2 × 109) were grown on His−, Leu−, and Trp− plates supplemented with 20 mM 3-amino-1,4-triazole. Library plasmids from His− colonies were selectively recovered from yeast following transformation into Escherichia coli HB101 (leuB5), transformed along with pG7BD-HCF-1 (450–1439) or control Gal4-DBD plasmids into yeast strain Y190 and assayed for β-galactosidase activity at the lacZ overlay assay (36). Colonies that scored positive for β-galactosidase activity in the presence of the HCF-1 bait plasmid, but not with control or irrelevant plasmids, were sequenced. One clone containing a partial cDNA encoding amino acids 269–803 of Miz-1 was selected for further studies.
GST Pull-down Assays—Protein binding assays with GST fusion proteins and [35S]methionine-radioabeled proteins synthesized in vitro using a coupled rabbit reticulocyte transcription/translation system (Promega) were carried out as described previously (38). Briefly, E. coli BL21 cells harboring expression vectors for GST-Miz-1, GST-HCF-1, or GST alone were grown to an A540 nm of 0.6–0.8 and induced with isopropyl-β-D-thiogalactopyranoside ( Gibco BRL) for 3 h. Bacteria were collected by centrifugation and resuspended in buffer containing 0.5% Nonidet P-40, 1 mM EDTA, 20 mM Tris-Cl (pH 8.0), 100 mM NaCl, and one tablet of mini C protease inhibitor (Roche Molecular Biochemicals)/25 μl of buffer, and cell extracts were prepared by sonication. 50 μl of a 50:50 slurry of glutathione-Sepharose 4B was incubated with clarified cell extracts containing GST or GST fusion protein for 1 h at 4 °C. Beads were collected by centrifugation and washed twice with buffer containing phosphate-buffered saline, and beads containing equivalent amounts of bound protein (as determined by Coomassie Blue staining of SDS-polyacrylamide gels) were incubated with 10–20 μl of reticulocyte lysate containing radiolabeled translated protein in buffer A (150 mM KCl, 0.02 mg/ml bovine serum albumin, 0.1% Triton X-100, 0.1% Nonidet P-40, 5 mM MgCl2, and 20 mM Hepes (pH 7.0)) for 2–3 h at 4 °C. Beads were washed extensively with buffer A lacking bovine serum albumin, and bound radiolabeled proteins were eluted from the beads by boiling in SDS sample buffer and analyzed by SDS-PAGE. Competition assays were carried out with unlabeled competitor protein synthesized in vitro as detailed in the figure legends. The total amount of rabbit reticulocyte lysate was kept constant by adding unprogrammed lysate to the binding reactions as required.

Cell Culture, Transfections, and Luciferase Assays—COS-1 cells were cultured at 37 °C in Dulbecco’s modified Eagle’s medium supplemented with 10% fetal bovine serum, 1% g-glutamine, and 1% penicillin/strep-tonycin. Cells were seeded in six-well plates at a concentration of 2–3 × 105 cells/well 1 day prior to transfection to achieve 70–80% confluence. Transfections were carried out using Lipofectamine reagent (4–8 μg/well; Invitrogen) as outlined by the manufacturer. Unless indicated otherwise in the figure legends, transfections typically contained 0.5 μg of luciferase reporter plasmid (pGL4.5Xlac or p15X[lac]) and 0.05–0.5 μg of various effector plasmids (HCF-1, Miz-1, c-Myc, or derivatives thereof). Total DNA and promoter dosage was kept constant with the appropriate amounts of corresponding empty vectors. Luciferase activity was assayed in cell lysates prepared 48 h post-transfection and normalized to protein concentration.

Co-immunoprecipitation Assay—Transfections were carried out as described above. At 48 h post-transfection using Nonidet P-40 lysis buffer (150 mM NaCl, 50 mM Tris (pH 8.0), 1% Nonidet P-40, and 0.2 mM phenylmethylsulfonyl fluoride). 1 ml of clarified supernatant, normalized for protein concentration, was precleared using mouse anti-Gal4 DNA-binding domain monoclonal antibody (Invitrogen) as the primary antibody, followed by horseradish peroxidase-coupled sheep anti-mouse polyclonal antibody (Amersham Biosciences) as the secondary antibody. Proteins were detected by enhanced chemiluminescence with a commercially available kit (ECL, Amersham Biosciences) according to the manufacturer’s instructions.

Immunoblotting—COS-1 cells were transfected as described above, except in Fig. 1, A and B, where the various Gal4-Miz-1 expression plasmids were used. Preparation of cell extracts for immunoblotting using poly-35 nm C pure nitrocellulose membrane (Amer- sham Biosciences) and probed using mouse anti-V5 monoclonal antibody (Invitrogen) as the primary antibody, followed by horseradish peroxidase-coupled sheep anti-mouse polyclonal antibody (Amersham Biosciences) as the secondary antibody. Proteins were detected by enhanced chemiluminescence with a commercially available kit (ECL, Amersham Biosciences) according to the manufacturer’s instructions.

RESULTS

HCF-1 Interacts with Miz-1 via Determinants in the Basic Domain—We carried out yeast two-hybrid screens to identify novel HCF-1-interacting proteins that may provide insights into the cellular functions, targets, and mechanisms of action of HCF-1. Similar approaches by others (15, 16) have used the N-terminal domain (residues 1–450) of HCF-1 as bait because this region is known to be sufficient for interaction with VP16 and for promoting VIC formation (14). We focused our attention on a separate region of HCF-1 (residues 450–1439) that encompasses the basic domain (Fig. 1A) because this region has been shown to be required, along with the HCF-1 VIC domain, to rescue the temperature-sensitive block to cell cycle progression in tsBN67 cells (14). The rationale was that the basic region may target novel HCF-1-interacting proteins that subserve a role in cell cycle regulation and/or other functions of HCF-1 that are independent of the HCF-1 VIC region.

Yeast two-hybrid screens using a Gal4-DBD-HCF-1-(450–1439) fusion protein as bait resulted in the identification of several novel HCF-1-interacting proteins from a HeLa cell Gal4 AD cDNA library. Among the clones identified was a cDNA containing partial cDNAs encoding a zinc finger protein factor called Miz-1, a recently described protein that was originally isolated by virtue of its ability to interact with the c-Myb protein (27, 28). Miz-1 is an 803-amino acid long protein that contains 13 zinc fingers of the C2H2 class and an N-terminal POZ domain (see Fig. 3A). The longest Miz-1 cDNA clone that we isolated from the two-hybrid screen encoded residues 269–803 of Miz-1 and is thus missing the N-terminal POZ domain. As shown in Fig. 1B, HCF-1 bound specifically to Miz-1, as β-galactosidase activity was observed only in the presence of both Gal4-DBD-HCF-1 bait and Gal4-AD-Miz-1 prey plasmids. Significantly, Miz-1 failed to interact with HCF-1 residues 1–380 (Fig. 1B), a domain that is sufficient for interaction with VP16 and the cellular factors LZIP and Zhangfei (14–17).

To determine whether Miz-1 and HCF-1 associate in vivo in mammalian cells, we carried out co-immunoprecipitation experiments with cells cotransfected with expression vectors for V5-tagged full-length Miz-1 and FLAG-tagged full-length HCF-1. Cell extracts were immunoprecipitated with anti-FLAG antibody and probed with anti-V5 antibody. As shown in Fig. 1C, full-length Miz-1 was present in immune complexes precipitated with anti-FLAG antibody from cells that had been transfected with both FLAG-HCF-1-FL and V5-Miz-1FL expression vectors, but not from control cells that were transfected with V5-Miz-1FL alone. Thus, Miz-1 and HCF-1 form a stable complex in vivo in mammalian cells.

To map more precisely the region in HCF-1 that is targeted by Miz-1, we constructed a series of deletions within Gal4-DBD-HCF-1-(450–1439) and tested these for interaction by two-hybrid analysis with Gal4-AD-Miz-1-(269–803). As shown in Fig. 2, a fusion protein containing HCF-1 residues 750–836 was sufficient to bind to Miz-1. Residues between 836 and 1439, which include the HCF-1 POZ repeats, were not necessary for interaction. However, the strongest binding, as reflected by β-galactosidase activity, was observed with HCF-1-(750–902), suggesting that residues between 836 and 902 may contribute to more robust binding and/or stability of the Miz-1-HCF-1 complex in vivo. A fragment spanning residues 836–902 was, however, insufficient to bind with Miz-1. Miz-1 bound directly to HCF-1, as determined by reciprocal GST pull-down assays with radiolabeled proteins synthesized in vitro (Fig. 3B). As shown in Fig. 3B, radiolabeled full-length Miz-1 bound to GST-HCF-1-(750–902), but not to GST alone. Thus, Miz-1 targets part of the basic region of HCF-1 that is required for cell cycle progression (14), with amino acid residues between 750 and 836 being of particular importance for interaction.

HCF-1 Targets Two Separate Domains of Miz-1—As summarized in Fig. 3A, Miz-1 interacts with a number of cellular regulatory proteins, including c-Myb (27), p300 (30), and SMAD proteins (33). c-Myb binds via two separate regions in Miz-1, residues 269–308 and 637–718. Determinants contained within or overlapping these separate regions are also impor-
tant for interaction with p300. SMAD proteins interact with Miz-1 via a region that encompasses the four N-terminal proximal zinc fingers (33). To identify the region(s) of Miz-1 that are required for interaction with HCF-1 and potential relationships to other protein/protein interaction interfaces present in Miz-1, we generated a series of deletions of Miz-1 and tested binding to HCF-1 in vitro in GST pull-down assays. As shown in Fig. 3B, a Miz-1 subfragment spanning residues 637–803 bound very efficiently and specifically to GST-HCF-1-(750–902). In reciprocal binding experiments, radiolabeled HCF-1-(612–902) was unable to bind to overlapping subfragments of Miz-1 corresponding to residues 637–738 and 719–803. c-Myc and p300 also targeted N-terminal residues 269–308 and 190–294 of Miz-1, respectively. Miz-1-(109–308) was, however, unable to bind to HCF-1 (Fig. 3B). These results suggest that Miz-1/HCF-1 interaction is mediated by multiple determinants or an extended region within residues 637–803 of Miz-1 and that HCF-1 targets determinants in Miz-1 that overlap with, but are distinguishable from, those required for interaction with c-Myc, p300, and SMAD proteins.

The Miz-1 fragment isolated in the two-hybrid screen was missing N-terminal residues 1–269, which include the POZ domain (residues 1–108). This indicates that the POZ domain is not required for interaction with Miz-1, at least in the presence of the C-terminal interacting domain. However, because POZ domains have been shown to provide protein interaction interfaces (39–41), we tested whether this region may bind independently to HCF-1. As shown in Fig. 3B, reciprocal GST binding experiments with Miz-1-(1–108) were unable to bind to overlapping subfragments of Miz-1 corresponding to residues 637–738 and 719–803. c-Myc and p300 also targeted N-terminal residues 269–308 and 190–294 of Miz-1, respectively. Miz-1-(109–308) was, however, unable to bind to HCF-1 (Fig. 3B). These results suggest that Miz-1/HCF-1 interaction is mediated by multiple determinants or an extended region within residues 637–803 of Miz-1 and that HCF-1 targets determinants in Miz-1 that overlap with, but are distinguishable from, those required for interaction with c-Myc, p300, and SMAD proteins.

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Thus, as summarized in Fig. 3C, Miz-1 contains at least two modular subdomains that can independently bind to HCF-1. These regions are distinct from those that mediate Miz-1 interaction with its other known interacting partners. The foregoing does not preclude the possibility that subregions of Miz-1
HCF-1 interacts directly with Miz-1 in vitro and targets two separate domains, the POZ domain and the C-terminal region. A, schematic representation of Miz-1 indicating the N-terminal POZ domain and the positions of 13 zinc finger motifs (represented by ovals). Subregions of Miz-1 involved in protein/protein interactions with c-Myc, p300, and SMAD proteins are indicated. Also illustrated is the region of Miz-1 (residues 269–803) encoded in the cDNA retrieved in the two-hybrid screen. B, in vitro GST pull-down assay. [35S]Methionine-labeled protein added to GST alone or with various GST fusion proteins as indicated, and bound material was analyzed by SDS-PAGE. The 1/10 LOAD lanes represent 10% of the [35S]methionine-labeled protein added to the respective binding assay. C, schematic representation of the Miz-1 deletion constructs used above and summary of the in vitro HCF-1 interaction results. +, specific interaction; −, no interaction.

Located between residues 108 and 637, which include the zinc finger cluster, are also important for or influence interaction with HCF-1.

**HCF-1 Targets a Transactivation Function in Miz-1**—Most of the HCF-1-interacting factors identified to date, including Miz-1 described herein, are themselves transcription factors, underscoring the growing evidence that HCF-1 serves as a global transcriptional co-regulatory factor. Recent findings have shown that, in some cases, HCF-1 is recruited directly to the transcriptional activation domains of its interacting partners, including those present in LZIP (42) and GA-binding protein (19), suggesting that one role of HCF-1 may be to directly modulate the activation potential of its interacting partners.

Because a Miz-1 transactivation activation domain had not been identified at the time, we sought to determine whether the HCF-1-binding interface at the C terminus of Miz-1 could serve as a transactivation domain when tethered to DNA. For this purpose, we tested a series of Gal4-Miz-1 fusion proteins by transient transfection assays in mammalian cells using a luciferase reporter gene driven by a promoter containing multiple upstream Gal4-binding sites. As shown in Fig. 4A, full-length Miz-1 (Gal4-Miz-1FL), as well as a derivative lacking the N-terminal POZ domain (Gal4-Miz-1ΔPOZ), resulted in 13- and 31-fold induction of reporter gene activity, respectively, relative to the activity obtained with the Gal4 DBD alone. Thus, full-length Miz-1, as well as the ΔPOZ derivative, activates transcription when tethered directly to DNA.

To determine whether the transactivation function maps to regions required for interaction with HCF-1, we tested the activity of a Gal4 fusion with residues 637–803 of Miz-1. As shown in Fig. 4A, Gal4-Miz-1-(637–803) potently activated transcription, resulting in a 253-fold activation of reporter gene expression relative to the Gal4 DBD alone. Interestingly, transactivation by this derivative was significantly higher than that observed with the full-length protein or the ΔPOZ derivative, a difference that could not be attributed to differences in expression levels of the respective proteins as determined by Western blot analysis (Fig. 4B, lanes 1, 2, and 4, respectively). This suggests that the more robust activation is an inherent property of this isolated domain and that the full activation potential of this region may be masked in the context of the intact protein.

Overlapping subfragments spanning residues 637–718 and 701–803 were marginally active (4–5-fold induction over the control), whereas a fragment containing residues 701–738 was inactive. These subfragments were also unable to bind to HCF-1. As a control, we also tested residues 176–284, a region that encompasses binding determinants for c-Myc (see below) and that has been predicted to harbor an activation function because of its high content of acidic amino acids (28). This region was, however, unable to stimulate expression of the reporter gene.

Thus, the C-terminal HCF-1-interacting domain of Miz-1 harbors an autonomous activation function. The potency of this region in transactivation correlates with its ability to interact with HCF-1. These findings add to the growing evidence that HCF-1 targets activation domains in its interacting partners.

**HCF-1 Represses Gal4-Miz-1-mediated Transcriptional Activation**—To begin to probe the functional consequences of Miz-1/HCF-1 interaction, we carried out transient transfection assays with various Gal4-Miz-1 fusion proteins in the presence or
absence of an expression vector for HCF-1 to determine whether HCF-1 modulates transactivation by Miz-1. As shown in Fig. 5A, cotransfection of full-length HCF-1 led to a dose-dependent repression of transactivation mediated by Gal4-Miz-1FL (Fig. 5A). Similarly, HCF-1 inhibited transactivation by a Miz-1 derivative lacking the POZ domain or a derivative containing only the C-terminal transactivation domain (residues 637–803). In each case, cotransfection of an HCF-1 expression vector led to ∼70–80% inhibition of activity at the highest concentrations of HCF-1 used. The repressive effect was specific to Gal4-Miz-1 derivatives because similar levels of HCF-1 had no effect on transactivation mediated by Gal4-VP16AAD, a Gal4 derivative linked to the potent acidic transactivation domain of VP16. Thus, the repression observed in Miz-1 transactivation is not due to a generalized titration of co-regulatory factors by HCF-1. The observation that HCF-1 repressed Gal4-Miz-1FL and Gal4-Miz-1ΔPOZ to a similar degree suggests that inhibition by HCF-1 is independent of the POZ domain.

We next tested whether the minimal Miz-1-binding domain in HCF-1 (HCF-1-(750–902)) can also affect Gal4-Miz-1-mediated transactivation. As illustrated in Fig. 5B, HCF-1-(750–902) behaved in a manner analogous to full-length HCF-1, inhibiting the transactivation potential of all three Gal4-Miz-1 fusion proteins, but not that of Gal4-VP16AAD. Thus, binding of this subfragment of HCF-1 directly to the Miz-1 transactivation domain is sufficient to inhibit the activity of Miz-1.

**HCF-1 Represses Miz-1 Activation of the p15**<sup>INK4b</sup> **Promoter**—Having shown a repressive effect of HCF-1 on the transcriptional activity of Gal4-Miz-1 fusion proteins, we sought to determine whether a similar effect could be observed with native Miz-1 in the context of a natural Miz-1 target gene. Miz-1 has recently been shown to stimulate transcription of the cyclin-dependent kinase inhibitor p15<sup>INK4b</sup> through direct binding to the initiator element in this promoter (30). To determine whether HCF-1 modulates Miz-1-mediated activation of p15<sup>INK4b</sup> expression, we performed cotransfection assays with a luciferase reporter gene linked to the p15<sup>INK4b</sup> promoter. As shown in Fig. 6, transfection of an expression vector for full-length Miz-1 led to a 7-fold induction of p15<sup>INK4b</sup> reporter gene activity, consistent with recent findings reported by others (30). In contrast, HCF-1 expression had no effect on reporter gene activity. However, coexpression of HCF-1 with Miz-1 inhibited Miz-1 mediated transactivation by ∼70%.

Miz-1 activation of p15<sup>INK4b</sup> expression is also inhibited by interaction with c-Myc (30). Consistent with this, transfection of a c-Myc expression vector, although having no effect on its own, repressed Miz-1-dependent activation of the reporter gene (Fig. 6). The extent of repression by c-Myc was comparable to that observed with HCF-1. Interestingly, coexpression of both HCF-1 and c-Myc led to an additive effect on inhibition of Miz-1 activity, reducing activity to the basal levels observed with the reporter gene alone. Thus, HCF-1, like c-Myc, antagonizes Miz-1-dependent activation of the native p15<sup>INK4b</sup> promoter.

**HCF-1 Interferes with Recruitment of p300 to Miz-1—c-Myc** has been shown to inhibit transactivation by Miz-1 by interfering with recruitment of the p300 coactivator (30). Because HCF-1 targets a region of Miz-1 that is also involved in p300 association, we speculated that HCF-1 might also function in this manner. To determine whether this is the case, we carried out a mammalian two-hybrid assay using Gal4-Miz-1FL and chimeric p300-VP16. As shown in Fig. 7A, transfection of cells with the p300-VP16 expression vector on its own had no effect on reporter gene activity. However, cotransfection expression vectors encoding p300-VP16 and Gal4-Miz-1 led to a 2-fold increase in reporter gene activity compared with cells transfected with Gal4-Miz-1 alone, similar to what has been previously observed (30). Inclusion of an expression vector encoding full-length HCF-1 abrogated the stimulatory effect of p300-
VP16 and further repressed Gal4-Miz-1FL transactivation potential. To determine whether this is the result of HCF-1 interfering with the recruitment of p300 to Miz-1, we used the GST pull-down competition assay with a C-terminal fragment of p300 (residues 1572–2371) that has been shown to interact with Miz-1 (30) fused to GST along with radiolabeled full-length Miz-1 and unlabeled competitor proteins synthesized in vitro. As shown in Fig. 7B, full-length Miz-1 bound to GST-p300 (residues 1572–2371) as expected. Inclusion of equivalent amounts of unlabeled HCF-1-(1–902), c-Myc, or Miz-1 (as a positive control) reduced binding of radiolabeled Miz-1 by 60–70% (HCF-1 did not bind to GST-p300; data not shown). In contrast, inclusion of unlabeled luciferase (negative control) had no effect on binding. Thus, HCF-1 competes with p300 for interaction with Miz-1.

The Causative Mutation in HCF-1 That Leads to Cell Cycle Arrest in tsBN67 Cells Does Not Affect Binding to Miz-1—A subfragment of HCF-1 spanning residues 1–902, including the basic region that is targeted by Miz-1, is necessary and sufficient to rescue the temperature-sensitive cell cycle defect in tsBN67 cells (14). The causative mutation (P134S) in HCF-1 also abrogates interaction with cellular proteins such as LZIP.

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**Fig. 5. HCF-1 antagonizes transactivation by Gal4-Miz-1.** A, COS-1 cells were cotransfected with 0.5 μg of pGal4(5X)luc reporter plasmid and the indicated Gal4-Miz-1 fusion expression vector (50 ng) or Gal4-VP16AAD (50 ng) in the absence or presence of increasing amounts of an expression vector for full-length HCF-1 as indicated. Extracts were prepared 40–48 h later and assayed for luciferase activity. Values shown represent the mean ± S.D. from three independent transfections carried out in duplicate and are normalized to the value obtained from the respective Gal4 fusion effector plasmid alone, which was taken as 100% for each case. B, the minimal Miz-1-binding domain of HCF-1 was sufficient to inhibit transactivation by Gal4-Miz-1. Transfections and measurement of luciferase activity were carried out in an identical manner as described for A, except that the expression vector for HCF-1-(750–902) was used in place of full-length HCF-1.
Fig. 6. HCF-1 represses Miz-1 activation of the p15^INK4b promoter. COS-1 cells were cotransfected with a p15^INK4b luciferase reporter along with expression plasmids for full-length Miz-1, HCF-1, and/or c-Myc as indicated. Values represent the mean activity ± S.D. from three independent transfections done in duplicate and are normalized to the activity obtained from the reporter plasmid alone, which was taken as 1.

Fig. 7. HCF-1 inhibits p300 recruitment by Miz-1. A, COS-1 cells were transfected with a pGal4-binding element luciferase reporter gene in the presence of expression vectors for Gal4-Miz-1FL (50 ng), full-length p300-VP16 (0.25 μg), and/or full-length HCF-1 (0.25 μg) as indicated. Extracts were prepared 40–48 h later and assayed for luciferase activity. Values shown represent the means ± S.D. from two independent transfections carried out in duplicate. B, HCF-1 interfered with p300 binding to Miz-1. [35S]Methionine-labeled full-length Miz-1 synthesized in vitro was incubated with GST or GST-p300-(1572–2371) as indicated in the presence or absence of various unlabeled competitor proteins synthesized in vitro. Unlabeled competitor proteins included HCF-1-(1–902), full-length c-Myc, full-length Miz-1, and luciferase. Band material was analyzed by SDS-PAGE. 5% Load represents 5% of the [35S]Methionine-labeled protein added to the binding assays. Numbers shown at the bottom represent relative band intensities as determined by PhosphorImager analysis and are shown normalized to the reaction lacking unlabeled competitor protein, which was set to 100.

**DISCUSSION**

HCF-1 is a complex, multifunctional protein that is essential for normal cell cycle progression through the G1/S transition (6); however, its cellular roles and mechanisms of action remain poorly understood. Our findings that HCF-1 physically and functionally interacts with Miz-1, a cell cycle regulator that causes cell cycle arrest at G1 (27, 30), and antagonizes Miz-1-dependent activation of the gene encoding a key cell cycle regulatory protein provide a provocative new dimension to the activities of these two proteins that may be of consequence to their respective roles in transcription and their reciprocal effects on cell cycle progression.

Miz-1 targets determinants present in the basic region of HCF-1, a domain that, in conjunction with the N-terminal region, is able to rescue the cell cycle defect in tsBN67 cells (14). Whether Miz-1 is an important effector in this process remains to be determined. Herr and co-workers (14) demonstrated that the minimal fragment of HCF-1 capable of rescuing the cell cycle defect in tsBN67 cells is residues 1–902 and that further C-terminal deletion of this fragment to position 836 abrogates rescue. The minimal Miz-1-binding interface mapped to residues 750–836, and although residues between 836 and 902 enhance binding with Miz-1, they are neither necessary nor sufficient for interaction. Moreover, the causative mutation (P134S) in HCF-1 leading to cell cycle arrest does not affect interaction with or transactivation by Miz-1. The foregoing implies that Miz-1 may not be necessary for HCF-1-mediated cell cycle control and that the phenotypic basis of the P134S mutation may be related to as yet unidentified proteins that target the N-terminal and/or basic regions. However, this does not necessarily negate a role for Miz-1 because cell cycle control by HCF-1 is likely a highly complex process that involves the cooperative interplay with many effector targets that recognize both the N-terminal HCF1_vC domain and the basic region. Recent studies have shown that the basic region of HCF-1 is targeted by several other transcription factors, including GA-binding protein (19) and Sp1 (20). More recently,
10% of the [35S]methionine-labeled protein used in the respective bind-
and bound material was analyzed by SDS-PAGE.

A

FIG. 8. The P134S mutation in HCF-1 does not inhibit binding to or transactivation by Miz-1. A, in vitro synthesized, [35S]methio-

none-labeled HCF-1(1–902) or HCF-1(1–902)(P134S) was incubated with GST, GST-Miz-1(637–803), or GST-VP16(1–404) as indicated, and bound material was analyzed by SDS-PAGE. 1/10 load represents 10% of the [35S]methionine-labeled protein used in the respective binding assays. B, COS-1 cells were cotransfected with a Gal4-responsive luciferase reporter plasmid and the indicated Gal4-Miz-1 fusion expression vector or Gal4-VP16AAD in the absence or presence of increasing amounts of an expression vector for full-length (wild-type [WT]) HCF-1 or HCF-1(P134S) as indicated and assayed for luciferase activity as described in the legend to Fig. 5A.

we have identified a novel zinc finger transcription factor that, like Miz-1, binds to residues 750–902 of HCF-1.2 Thus, the basic region of HCF-1 is targeted by at least four distinct transcription factors. It will be of interest to unravel the combinatorial interplay of these factors with HCF-1 and their potential relationships to the function of HCF-1 in cell cycle regulation and/or transcriptional control.

HCF-1 targets two independent regions of Miz-1: the N-terminal POZ domain and the C-terminal region spanning residues 637–803. The latter region also harbors overlapping but distinguishable determinants for interaction with c-Myc and the coactivator p300, suggesting the possibility of functional interplay among these factors in their interactions with Miz-1 (27, 30). Interestingly, region 637–803 functions as a potent autonomous transactivation domain, and preliminary analysis indicates that the determinants required for binding to HCF-1 and for transactivation are overlapping and/or intimately linked. Thus, as has been shown to be the case with several of its other interacting partners (19, 42), HCF-1 targets a transactivation function in Miz-1. This finding underscores a potential general functional property of HCF-1 whereby it modulates the activity of its partner proteins by directly interacting with their respective transactivation domains (see below). Interes-
terestingly, transcriptional activation mediated by the isolated C-terminal domain was significantly more robust vis-à-vis full-length Miz-1 or the Miz-1POZ derivative, suggesting that, in the context of the full-length protein, other regions of Miz-1 lying between residues 109 and 637 may serve to dampen the activation potential of the C-terminal transactivation domain. The full transcriptional activation potential of Miz-1 may thus be triggered only at times when it has been relieved of this attenuation.

Overexpression of Miz-1 results in G1 arrest via a process mediated in part by induction of the gene for the cyclin-dependent kinase inhibitor p15INK4b at G1 (30), which leads in turn to a reduction in cyclin D1-associated kinase activity. c-Myc binds directly to Miz-1 and inhibits Miz-1 activation of p15INK4b expression, consequently relieving cell cycle arrest and allowing progression through the cell cycle. Conversely, p15INK4b expression is stimulated by transforming growth factor-β, which activates SMAD proteins, which prevent recruitment of c-Myc to Miz-1 and which directly cooperate with Miz-1 in transactivating the p15INK4b promoter (33). Thus, growth inhibi-
tion pathways stimulated by transforming growth factor-β and growth stimulation pathways induced by c-Myc converge through Miz-1 in the regulation of p15INK4b gene transcription (32).

Functional analysis indicated that HCF-1 repressed trans-
activation by Miz-1 both on the natural p15INK4b promoter and on artificial Gal4-responsive promoters in a manner that was associated with recruitment of p300 to Miz-1. This may point to a potential mechanism by which HCF-1 may stimulate cell cycle progression. Thus, as observed with c-Myc, inhibition of Miz-1 activation of p15INK4b by HCF-1 may be expected to result in increased activity of cyclin D1/CDK4 and thus progression through the G1/S restriction point. Interestingly, repression of p15INK4b expression was potentiated in the presence of both HCF-1 and c-Myc, perhaps reflecting an aggregate response due to the fact that both c-Myc and HCF-1 inhibit recruitment of p300 to Miz-1.

Interference with p300 recruitment is one of several possible mechanisms by which HCF-1 inhibits Miz-1 activity because HCF-1 also independently associates with the Miz-1 POZ do-
main. POZ domains are conserved protein/protein interaction motifs and, when found as part of transcription factors, usually function as repressive domains, in part through recruitment of corepressors such as N-CoR (nuclear receptor corepressor) and SMRT (silencing mediator for retinoid and thyroid hormone receptors) (43, 44). The Miz-1 POZ domain is thought to be in a latent repressive state, and it has been postulated that c-Myc serves to convert the Miz-1 POZ domain into an active repressive domain (27). Our findings demonstrate that HCF-1, as well as the minimal Miz-1-interacting subregion (residues 750–902), inhibits transactivation of both Gal4-Miz-1FL and Gal4-Miz-1POZ fusion proteins to a similar degree, suggesting that the POZ domain is not involved in HCF-1-mediated repression. The relevance of HCF-1 interaction with the isolated Miz-1 POZ domain is unclear at present; however, it may be related to results of a recent report showing that Miz-1 is present in the cytoplasm in association with microtubules (31). In the presence of drugs that induce expression of the low density lipoprotein receptor, Miz-1 translocates to the nucleus, where it binds to and activates transcription of the low density lipoprotein receptor gene. Translocation under these circum-
stances requires the integrity of the POZ domain, suggesting that the POZ domain may be required for nuclear import of Miz-1 under certain conditions. HCF-1 has been shown to serve as a nuclear import factor for VP16 (45) and thus could potentially play a similar role with Miz-1. Native HCF-1 is found

2 H. H. L. Wong, D. Piluso, and J. P. Capone, unpublished data.
almost exclusively in the nucleus; however, discrete N-terminal subfragments of HCF-1 have been shown to accumulate in the cytoplasm at G0 (46). It is interesting to speculate that these subfragments may modulate nuclear translocation of Miz-1, thereby potentiating the repressive effects on Miz-1 activity.

In summary, we have identified Miz-1 as a novel interacting partner for HCF-1 and illustrate an intriguing new pathway of regulation that potentially links the opposing effects of these two proteins on cell cycle control. Our findings also represent the first example of HCF-1 inhibiting transactivation of an associated transcription factor. Recently, a novel HCF-1-related protein called HCF-like protein-1 has been identified and shown to inhibit transactivation by LZIP (47). Thus, HCF-1 and its family members may function as both coactivators and corepressors of transcription. We believe that this dual nature can serve HCF-1 well in cell cycle control because HCF-1 could function as a coactivator of genes that are required for cell cycle progression while also functioning as a corepressor of genes (such as p15(INK4b)) that serve to inhibit cell cycle progression. Further studies to elucidate the physiological importance of Miz-1/HCF-1 interaction and functional interplay with other factors involved in cell cycle control and gene regulation will be of interest.

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REFERENCES
1. Herr, W. (1998) Cold Spring Harbor Symp. Quant. Biol. 63, 599–607
2. Xiao, P., and Capone, J. P. (1990) Mol. Cell. Biol. 10, 4974–4977
3. Wilson, A. C., LaMarco, K., Peterson, M. G., and Herr, W. (1993) Cell 74, 115–125
4. Kristie, T. M., and Sharp, P. A. (1993) J. Biol. Chem. 268, 6525–6534
5. Kristie, T. M., Pomerantz, J. L., Twomey, T. C., Paren, S. A., and Sharp, P. A. (1995) J. Biol. Chem. 270, 4387–4394
6. Goto, H., Motomura, S., Wilson, A. C., Freiman, R. N., Nakabeppu, Y., Fukushima, K., Fujishima, M., Herr, W., and Nishimoto, T. (1997) Genes Dev. 11, 726–737
7. Wilson, A. C., Cleary, M. A., Lai, J. S., LaMarco, K., Peterson, M. G., and Herr, W. (1993) Cold Spring Harbor Symp. Quant. Biol. 58, 167–172
8. Kristie, T. M. (1997) J. Biol. Chem. 272, 26749–26755
9. Liu, Y., Hengartner, M. O., and Herr, W. (1999) J. Virol. 74, 99–109
10. Wilson, A. C., Peterson, M. G., and Herr, W. (1995) Genes Dev. 9, 2445–2458
11. Vogel, J. L., and Kristie, T. M. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 9425–9430
12. Hughes, T. A., La Boissiere, S., and O’Hare, P. (1999) J. Biol. Chem. 274, 16437–16443
13. Wilson, A. C., Boutros, M., Johnson, K. M., and Herr, W. (2000) Mol. Cell. Biol. 20, 6721–6730
14. Wilson, A. C., Freiman, R. N., Goto, H., Nishimoto, T., and Herr, W. (1997) Mol. Cell. Biol. 17, 6139–6146
15. Freiman, R. N., and Herr, W. (1997) Genes Dev. 11, 3122–3127
16. Lu, Y., Yang, P., O’Hare, P., and Misra, V. (1997) Mol. Cell. Biol. 17, 5117–5126
17. Lu, R., Yang, P., Padmakumar, S., and Misra, V. (1998) J. Virol. 72, 6209–6218
18. Liu, R., and Misra, V. (2000) Nucleic Acids Res. 28, 2446–2454
19. Vogel, J. L., and Kristie, T. M. (2000) EMBO J. 19, 683–690
20. Gunster, M., Laubhier, M., and Brison, O. (2000) Mol. Cell. Biochem. 210, 131–142
21. Lin, J. Puigserver, P., Donovan, J., Tarr, P., and Spiegelman, B. (2002) J. Biol. Chem. 277, 1645–1648
22. Ajuh, P. M., Brownie, G. J., Hawkes, N. A., Cohen, P. T., Roberts, S. G., and Lamond, A. I. (2000) Nucleic Acids Res. 28, 678–686
23. Wysocka, J., Reilly, P. T., and Herr, W. (2001) Mol. Cell. Biol. 21, 3820–3829
24. Mahajan, S. S., and Wilson, A. C. (2000) Mol. Cell. Biol. 20, 919–928
25. Johnson, K. M., Mahajan, S. S., and Wilson, A. C. (1999) J. Virol. 73, 3930–3940
26. Lee, S., and Herr, W. (2001) J. Virol. 75, 12402–12411
27. Peukert, K., Staller, P., Schneider, A., Carlmark, G., Hanel, F., and Eilers, M. (1997) EMBO J. 16, 5672–5686
28. Schneider, A., Peukert, K., Eilers, M., and Hanel, F. (1997) Curr. Top. Microbiol. Immunol. 224, 137–146
29. Sakamura, D., and Prendergast, G. C. (1999) Oncogene 18, 2942–2954
30. Staller, P., Peukert, K., Kiermaier, A., Seano, J., Lukas, J., Karsunky, H., Morey, T., Bartek, J., Massague, J., Hanel, F., and Eilers, M. (2001) Nat. Cell Biol. 3, 392–399
31. Ziegelbauer, J., Shao, B., Yager, D., Larabell, C., Hoffmann, B., and Tjian, R. (2001) Mol. Cell 7, 439–449
32. Amati, B. (2001) Nat. Cell. Biol. 3, E112–E113
33. Seano, J., Poupounot, C., Staller, P., Schader, M., Eilers, M., and Massague, J. (2001) Nat. Cell. Biol. 3, 400–408
34. Popova, B., Bilan, P., Xiao, P., Faught, M., and Capone, J. P. (1998) Virology 209, 19–28
35. Bae, C., and Elledge, S. J. (1997) Methods Enzymol. 283, 141–156
36. Auge, S., Hare, P., and Misra, V. (1997) Cell Growth & Differ. 8, 3829–3843
37. Amati, B. (2001) BioTechniques 13, 18–20
38. Meertens, L. M., Miyata, K. S., Cochetto, J. D., Rachubinski, R. A., and Capone, J. P. (1998) EMBO J. 17, 6972–6978
39. Bardwell, V. J., and Treisman, R. (1994) Genes Dev. 8, 1664–1677
40. Albagli, O., Dhordain, P., Deweindt, C., Leecq, G., and Leprince, D. (1995) Cell Growth & Differ. 6, 1153–1198
41. Collins, T., Stone, J. R., and Williams, A. J. (2001) Mol. Cell. Biol. 21, 3609–3615
42. Luciano, R. L., and Wilson, A. C. (2000) Proc. Natl. Acad. Sci. U. S. A. 97, 10757–10762
43. Dharhan, P., Albarghumi, O., Lin, R. J., Anseau, S., Queir, S., Leutz, A., Kerck- aert, J. P. Evans, R. M., and Leprince, D. (1997) Proc. Natl. Acad. Sci. U. S. A. 94, 10762–10767
44. Wong, C. W., and Privalsky, M. L. (1998) J. Biol. Chem. 273, 27685–27702
45. La Boissiere, S., Hughes, T., and O’Hare, P. (1999) EMBO J. 18, 480–489
46. Scarr, R. B., Smith, M. R., Beddall, M., and Sharp, P. A. (2000) Mol. Cell Biol. 20, 3568–3575
47. Zhou, H. J., Wong, C. M., Chen, J. H., Qiang, B. Q., Yuan, J. G., and Jin, D. Y. (2001) J. Biol. Chem. 276, 28093–28098
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