GCIP, a Novel Human Grap2 and Cyclin D Interacting Protein, Regulates E2F-mediated Transcriptional Activity*

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Regulation of mammalian cell growth and proliferation is governed through receptor-mediated signaling networks that ultimately converge on the cell cycle machinery. Adaptor proteins play essential roles in the formation of intracellular signaling complexes, relaying extracellular signals from the plasma membrane to the nucleus of a cell. The leukocyte-specific adaptor protein Grap2 is a central linker protein in immune cell signaling and activation. Using Grap2 as bait protein, we identified a novel human protein, GCIP (Grap2 cyclin-D interacting protein). We found that GCIP bound to Grap2 in both yeast two-hybrid assays and in mammalian cells through binding to the COOH-terminal unique domain and SH3 domain (designated QC domain) of Grap2. GCIP also associated with cyclin D both in vitro and in vivo. The expression of GCIP was found in all human tissues examined with the highest level of expression in the heart, muscle, peripheral blood leukocytes, and brain. Furthermore, phosphorylation of retinoblastoma protein by cyclin D-dependent protein kinase was reduced and E2F1-mediated transcription activity was inhibited in cells transfected with GCIP. High level expression of GCIP in terminally differentiated tissues and the inhibition of E2F1 transcription activation suggest that GCIP could play an important role in controlling cell differentiation and proliferation.

In response to extracellular mitogenic and growth signals, cells initiate different signaling pathways that ultimately lead to the transcriptional activation of downstream genes and cell proliferation. One set of the genes that respond to mitogenic signals encodes the D-type cyclins (D1, D2, and D3). These cyclins assemble with their catalytic partners, CDK4 and CDK6, as cells progress through the first gap phase (G1) to the initiation of DNA synthesis (S phase) (1). Assembled cyclin D-Cdk complexes then enter the cell nucleus where they are phosphorylated and activated by CDK-activated kinases. The active cyclin D-Cdk complexes participate in the phosphorylation of the retinoblastoma retinoblastoma protein (Rb), resulting in the functional inactivation of Rb and the progression of the cell through the late G1 restriction point into the S phase (2, 3). Rb exerts its growth regulatory functions at least in part by inhibiting the transcriptional activity of E2F, a family of transcription factors that play a major role in cell proliferation, differentiation, apoptosis, and cell cycle progression (4, 5, 7). It has been known that E2F proteins mediate the transcription of a set of genes that controls cellular progression through G1 into the S phase. By binding to the activation domain of E2F, Rb protein actively represses transcription from promoters containing E2F-binding sites, resulting in the arrest of cell cycle progression (3, 6–8). Therefore, disruption of the Rb gene by deletion, mutation, or inactivation by phosphorylation or viral oncoproteins causes the release of free, transcriptional active E2F family of proteins, leading to the unrestricted cell proliferation (4, 5, 8). Understanding the regulation of cyclin D-dependent protein kinases, their phosphorylation of Rb, and their activation of E2F-mediated transcriptional activation will provide insight into the molecular mechanism of cell differentiation and proliferation.

Recently, adaptor proteins or scaffold proteins have been shown to play important roles in the regulation of signaling networks and in determining the specificity and selectivity of signaling pathways (9, 10). Examples include the mitogen-activated protein kinase complexes coordinated by the scaffold proteins S6k and Pbs2p in yeast Saccharomyces cerevisiae (11), the c-Jun NH2-terminal kinase signaling complexes by JIP-1 (12), and the signaling complexes mediated by T-cell-specific LAT proteins (13). Recently, we identified a novel leukocyte-specific adaptor protein, Grap2, in immune tissues (14). Like Grb2, Grap2 contains two SH3 domains and one SH2 domain in its structure. However, Grap2 has a 120-amino acid glutamine/proline-rich domain between the SH2 domain and the COOH-terminal SH3 domain. The expression of Grap2 is highly tissue- and cell-specific, found only in immune tissues and in T lymphocytes and monocytes/macrophages. Although the roles of Grap2 in immune cell signaling and activation is not clear at this moment, it can form signaling complexes with a number of signaling molecules in T cells and leukemia cells to

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¶¶ The abbreviations used are: Rb, retinoblastoma protein; SH, Src homology domain; RT-PCR, reverse transcriptase-polymerase chain reaction; HA, hemagglutinin; GST, glutathione S-transferase; CAT, chloramphenicol acetyltransferase; HLH, helix-loop-helix; Cdk4, cyclin D-dependent protein kinase; PAG, polyacrylamide gel electrophoresis.
 relay extracellular signals from the plasma membrane to the nucleus of the cells. In this report, we describe the cloning of a novel human protein, GCIP, that interacts with Grap2 and cyclin D. In yeast two-hybrid assays and in mammalian cells, GCIP interacts with full-length Grap2 protein and with the COOH-terminal unique and SH3 domains (designated QC domain) of Grap2. Unlike the highly restricted expression of Grap2, GCIP is ubiquitously expressed in all human tissues examined with a high level of expression in the heart, muscle, peripheral blood leukocytes, kidney, and brain, where cell differentiation and proliferation is limited. Furthermore, we found that GCIP associates with cyclin D1 both in vitro and in mammalian cells. In the presence of GCIP, phosphorylation of the retinoblastoma (Rb) protein by cyclin D-dependent Cdk4 kinase was partially inhibited. Overexpression of GCIP in mammalian cells suppressed the E2F1-mediated transcriptional activity, which is required for the transition of the G1 phase to the S phase in cell cycle progression. Together these data suggest that GCIP is a novel human protein potentially involved in the regulation of cell differentiation and proliferation through Grap2 and cyclin D-mediated signaling pathways.

EXPERIMENTAL PROCEDURES

Plasmid Construction for the Yeast Two-hybrid System—The unique proline/glutamine-rich domain and the COOH-terminal SH3 domain (QC domain) of Grap2 cDNA (14) was subcloned into yeast plasmid pAS2-1 (CLONTECH, Palo Alto, CA) to create an in-frame fusion with the GAL4 DNA-binding domain gene. The pAS2-Grap2QC was transformed into yeast strain PJ69-2A using the lithium acetate procedure and plated onto synthetic complete (SC) media lacking tryptophan and adenine. Pretransformed human bone marrow cDNA library (CLONTECH) was mated with the bait strain for 20 h, and then plated on SC medium plates without tryptophan, leucine, adenine, and histidine. The transformed yeast cells were grown at 30 °C for 3–7 days. Transformants grown on the quadruple dropout plates were assayed for β-galactosidase activity. Library plasmid DNA was recovered by transformation into Escherichia coli KC8 cells and sequenced on both strands.

GCIP and Grap2 Plasmid Constructs—Full-length GCIP was cloned into mammalian expression vectors pCMV-HA (CLONTECH) or pCMV-Tag (Promega, WI) by PCR using two oligonucleotide primers. The oligonucleotide sequences used were the following: 5′-AGGATCTCTAGATGGCGAGCTGACA-3′ and 5′-GGGGTACCTCATAATTCAAGTTCACT-3′. PCR products were digested with Bgl II and Kpn I, and cloned into pCMV-HA vector with an in-frame hemagglutinin (HA)-epitope sequence or Flag-epitope sequence at the 5′ end. For construction of glutathione S-transferase (GST)-GCIP protein, full-length GCIP or the unique domain of Grap2 was subcloned into the pMAL-c2 vector (Novagen) (15). Plasmid construction for the yeast two-hybrid system. A number of clones were identified that contain various fragments of Grap2. The unique and SH3 domains (QC domain) of Grap2 was used as a bait to screen a pretransformed yeast two-hybrid library for the existence of a protein that interacts with full-length Grap2 protein. The interaction was confirmed by automatic DNA sequencing.

Northern Blot Analysis and RT-PCR—Multi-tissue poly(A)+ RNA from 16 different human tissues were obtained from CLONTECH. Specific primers (5′-AGCGCAACTGCA-3′ and 5′-GGGGTACCTCATAATTCAAGTTCACT-3′) corresponding to the COOH-terminal region of GCIP were used in the RT-PCR reaction. Positive control and the standard for the amount of cDNA used in the PCR.

Cell Lines and Cell Transfection—Different cell lines were cultured as described in appropriate culture medium. COS-7 and 293T cells (1 × 10^6 cells) were transfected with plasmids as indicated in LipofectAMINE according to the manufacturer’s recommendation (Life Technologies, Bethesda, MD). Jurkat T cells and K562 cells were transfected by electroporation at settings of 960 microfarads and 250 volts. 12–16 h after transfection, the medium was replaced with fresh medium. 36–48 h after transfection, the cells were harvested and lysed in lysis buffer (150 mM NaCl, 20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM of glycerophosphate, 1% Triton X-100, 0.5% Nonidet P-40, 10% glycerol, 0.5 μM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 3 μg/ml apro tin). Kinase assays for cyclin D-Cdk4 were performed using GST-Rb as a substrate as described previously (15).

Expression vectors, pCE2F1, pCMV2E2F1VP16, and the pG5E1BCAT are kind gifts from Dr. David Johnson (University of Texas, M.D. Anderson Cancer Center) (16). For GAL4 and VP16 fusions, a total of 2 μg of plasmids was used in transfections for CAT reporter assays and 10 μg of plasmid was used in luciferase transfection assays. pCMV-LacZ vector was used as an internal control for normalization in all transfections.

In Vitro Binding Assays, Commaunoprecipitations, and Western Blots—GST-Grap2 and GST-GCIP fusion proteins were purified as described in the manual provided with glutathione S-transferase immobilization kit (Amersham Pharmacia Biotech). 1 μg of the in vitro protein binding assay. Briefly, COS-7-transfected cell lysate was incubated with comparable amounts of resin-bound GST fusion proteins. The beads were washed three times in the lysis buffer (150 mM NaCl, 20 mM HEPES, pH 7.4, 2 mM EGTA, 50 mM glycerophosphate, 1% Triton X-100, 0.5% Nonidet P-40, 10% glycerol, 0.5 μM phenylmethylsulfonyl fluoride, 5 μg/ml leupeptin, 3 μg/ml apro tin) and twice in phosphate-buffered saline, pH 7.4. Proteins bound were separated by SDS-PAGE and immunoblotted using an anti-HA monoclonal antibody (CLONTECH), and visualized by chemiluminescence (ECL) (Amersham Pharmacia Biotech). For coimmunoprecipitation, COS-7 cells were cultured to approximately 70% confluency before they were co-transfected with cDNAs encoding HA-tagged GCIP and Grap2 or Flag-tagged Grap2. Proteins were precipitated with specific antibodies and immunoblotted with anti-HA or anti-cyclin D antibodies (Santa Cruz Biotechnology, Santa Cruz, CA).

CAT Assays—Transfected cells were harvested, and extracts were prepared by three freeze-thaw cycles and heat inactivation. Cell lysates were then quantified for total protein, and an equivalent amount of cell lysate (normalized for total protein) from each transfaction was assayed for CAT activity as described in the manual provided with the CAT reporter assay kit (Promega, Madison, WI). Briefly, 50 μg of total cellular protein (COS-7 transfected cells) were incubated in a reaction mixture containing [3H]-labeled chloramphenicol and n-butyltryryl coenzyme A. The reaction products are extracted with a small volume of xylene. The n-butyltryryl amcinol in the xylene phase is mixed with scintillant and counted in a scintillation counter. Conversion to acetylated forms was also analyzed by thin-layer chromatography and visualized by autoradiography.

RESULTS

Molecular Cloning of Human GCIP—To identify proteins that interact with human Grap2, the COOH-terminal unique glutamine/proline-rich domain and the SH3 domain (QC domain) of Grap2 was used as a bait to screen a pretransformed human bone marrow cDNA library using the yeast two-hybrid system. A number of clones were identified that contain various lengths of cDNA sequences derived from a novel gene. One of the clones designated A5 contains a 1.5-kilobase insert and has a full-length open reading frame of 360 amino acids with a predicted molecular mass of 40 kDa (Fig. 1A). We designated the novel PCR using two oligonucleotide primers: GCIP2-cyclin-D-interacting protein, for its association with Grap2 and cyclin D1 in the cell. As shown in Fig. 1B, GCIP has distinct domain structures. Secondary structural analysis revealed a putative helix-loop-helix (HLH) motif in the middle region of the protein without the HLH motif in the middle region of the protein without the two-hybrid systems. A number of clones were identified that contain various lengths of cDNA sequences derived from a novel gene. One of the clones designated A5 contains a 1.5-kilobase insert and has a full-length open reading frame of 360 amino acids with a predicted molecular mass of 40 kDa (Fig. 1A). We designated the novel PCR using two oligonucleotide primers: GCIP2-cyclin-D-interacting protein, for its association with Grap2 and cyclin D1 in the cell. As shown in Fig. 1B, GCIP has distinct domain structures. Secondary structural analysis revealed a putative helix-loop-helix (HLH) motif in the middle region of the protein without the basic DNA-binding domain, suggesting a potential role in the regulation of transcription factors. A comparison with bHLH transcription factors and the Id family of proteins reveals the existence of a conserved second helix domain (Fig. 1C). However, the first helix domain is very divergent compared with
The consensus sequence of the bHLH region was derived from Murre et al (38), eHAND (39), MyoR (40), neurogenin (41), Id1 and Id3 (27). The other bHLH and dnHLH proteins: c-Myc (36), sleraxis (37), myogenin of 360 amino acids are deduced from the open reading frame.

B, The potential domain structure of human GCIP. A tentative helix-loop-helix domain is followed by a Asp/Glu-rich acidic domain. A potential leucine zipper domain is also found with one amino acid substitution (Leu-Cys) in the consensus sequence indicates hydrophobic amino acids.

C, comparison of the potential motif of HLH of GCIP with other bHLH and dnHLH proteins: c-Myc (36), sleraxis (37), myogenin (38), eHAND (39), MyoR (40), neurogenin (41), Id1 and Id3 (27). The consensive sequence of the bHLH region was derived from Murre et al. (42). θ in the consensus sequence indicates hydrophobic amino acids.

FIG. 1. Deduced amino acid sequence and domain organization of human GCIP. A, amino acid sequence of human GCIP. A total of 360 amino acids are deduced from the open reading frame. B, the potential domain structure of human GCIP. A tentative helix-loop-helix domain is followed by a Asp/Glu-rich acidic domain. A potential leucine zipper domain is also found with one amino acid substitution (Leu-Cys) in the consensus sequence indicates hydrophobic amino acids. An aspartic/glutamic acid-rich domain is found between the HLH domain and the potential leucine zipper motif. Data base searches found that the new gene shares 65% homology with mouse Maid, a maternally transcribed gene that encodes a potential negative regulator of basic transcription factors in the mouse egg and zygote (17). However, the human GCIP protein has extra 50 amino acids at its amino terminus (Fig. 1A). To confirm the predicted reading frame of GCIP cDNA, HA-GCIP plasmids were transfected into COS-7 cells or 293T cells, proteins were separated by SDS-PAGE and detected by an anti-HA antibody. As shown in Fig. 2C, a single band with anticipated molecular mass (approximately 42 kDa) of HA-GCIP was observed on the immunoblot.

Expression of Human GCIP in Normal Human Tissues and in Immune Tissues—To examine the expression of the GCIP gene, we performed Northern blot analysis of mRNAs isolated from different human tissues. As shown in Fig. 2A, one major transcript of 1.3 kilobases was detected in all the human tissues, including brain, heart, muscle, colon, thymus, spleen, kidney, liver, small intestine, placenta, lung, and peripheral blood leukocytes. A higher level of GCIP mRNA expression was observed in heart, muscle, peripheral blood leukocytes, kidney, and brain when compared with other tissues, suggesting a potential negative role of GCIP in cell differentiation and proliferation. Probing mRNA isolated from different immune tissues by RT-PCR demonstrated the expression of GCIP in all immune tissues with relatively low expression in bone marrow (Fig. 2B). An extra band was found in leukocytes, but not in other immune tissues. Whether the upper band is an alternate splicing isoform is under investigation.

Interaction of GCIP with the QC Domain of Grap2 in Yeast Cells—To confirm the binding specificity of GCIP with Grap2, we co-transformed yeast cells with Gal4-AD/GCIP and one of the following expression plasmids: Gal4-AD, Gal4-AD/Grap2QC, and Gal4-AD/Grap2-SH2. The human p53 and large T-antigens serve as a positive control pair in the study. As shown in Fig. 3A, only yeast cells co-transformed with GCIP/Grap2 QC plasmids were able to grow on the selective medium (SD/-Leu/-Trp/His) other than the positive control. No growth was observed in cells transformed with GCIP/Grap2 SH2 domain and other plasmids, suggesting that GCIP interacts specifically with the Grap2 QC domain in yeast cells. To quantitate the interactions between GCIP and Grap2, we measured the β-galactosidase activity of the lacZ reporter gene and compared that with the interaction between p53 and large T-antigens. As shown in Fig. 3B, co-transformation of GCIP with the QC domain of Grap2 in yeast cells strongly activated the expression of the lacZ reporter gene. These results clearly demonstrated that in vivo binding of GCIP to Grap2 is highly specific in yeast cells.
GCIP Binds to Adaptor Protein Grap2 in Vitro and in Vivo—

To verify the binding specificity detected in the yeast two-hybrid assays, we examined the in vitro binding of GCIP to Grap2. GST fusion proteins corresponding to different domains of Grap2 (Fig. 4A) were expressed in E. coli and purified by affinity purification. B, HA-tagged GCIP binds to full-length GST-Grap2 full-length protein and GST-Grap2-QC fusion protein, but not the SH2 domain. C, GCIP co-precipitated with Grap2 and Grap2-QC domain in mammalian cells. cDNAs encoding HA-tagged GCIP and Flag-tagged Grap2 or Grap2-QC domain were co-transfected into COS-7 cells. Proteins were precipitated using anti-Flag monoclonal antibody, M2. Proteins bound to Flag-Grap2 were detected by Western blot analysis using specific anti-HA monoclonal antibody.

Fig. 4. Binding of GCIP to Grap2-QC domain in vitro and in vivo. A, schematic diagram of GST-Grap2 fusion protein. GST fusion proteins corresponding to the full-length (FL), the SH2 domain, and the QC domain were constructed, expressed in E. coli, and purified by affinity purification. B, HA-tagged GCIP binds to full-length GST-Grap2 full-length protein and GST-Grap2-QC fusion protein, but not the SH2 domain. C, GCIP co-precipitated with Grap2 and Grap2-QC domain in mammalian cells. cDNAs encoding HA-tagged GCIP and Flag-tagged Grap2 or Grap2-QC domain were co-transfected into COS-7 cells. Proteins were precipitated using anti-Flag monoclonal antibody, M2. Proteins bound to Flag-Grap2 were detected by Western blot analysis using specific anti-HA monoclonal antibody.

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To examine whether GCIP and Grap2 can interact in mammalian cells, we co-transfected HA-GCIP with Flag-tagged Grap2 in COS-7 cells. Protein complex was isolated by immunoprecipitation using anti-Flag monoclonal antibodies (M2). The association of GCIP with Flag-tagged Grap2 was detected by Western blot using HA-specific monoclonal antibodies. As shown in Fig. 4C, HA-GCIP coprecipitates with the full-length Grap2 protein and the QC domain of Grap2, but not the Grap2-SH2 domain. Together, these results indicated that GCIP specifically interacts with the QC domain of Grap2.

GCIP Interacts with Cyclin D1 Protein—To test whether GCIP is involved in cell proliferation, we examined the binding specificity of GCIP to the QC domain of Grap2 fusion protein.

To examine whether GCIP and Grap2 can interact in mammalian cells, we co-transfected HA-GCIP with Flag-tagged Grap2 in COS-7 cells. Protein complex was isolated by immunoprecipitation using anti-Flag monoclonal antibodies (M2). The association of GCIP with Flag-tagged Grap2 was detected by Western blot using HA-specific monoclonal antibodies. As shown in Fig. 4C, HA-GCIP coprecipitates with the full-length Grap2 protein and the QC domain of Grap2, not the Grap2-SH2 domain. Together, these results indicated that GCIP specifically interacts with the QC domain of Grap2.

FIG. 3. Interaction of GCIP with the QC domain of Grap2 in yeast two-hybrid system. A, yeast cells co-transformed with Gal4-AD/GCIP and Gal4-BD/Grap2-QC domain can grow on selective plate (SD-Leu/Trp-His), indicating in vivo interaction of GCIP and Grap2-QC domain in yeast cells. p53 and large T-antigen are known proteins that interact each other in the cell and used as a positive control. B, activation of lacZ reporter gene activity in cells co-transformed with GCIP and Grap2-QC domain. Like p53 and large T-antigen, co-transformation of GCIP with Grap2-QC domain greatly increased lacZ gene expression (hence the β-gal activity). 1, Grap2 QC alone; 2, p53 and large T-antigen; 3, GCIP and Grap2-QC domain; 4, GCIP and Grap2-SH2 domain; 5, GCIP alone.
cell extracts from other cell types (COS-7, Jurkat, and K562 cells).

To further analyze the in vivo binding of GCIP and cyclin D, we transfected HA-tagged GCIP cDNA into different cells (Jurkat, K562, COS-7, and 293T). The cells were starved in serum-free medium initially and subsequently cultured in 10% serum medium. Cells were lysed 48 h after transfection. Proteins bound to GCIP were immunoprecipitated using an anti-HA antibody. Protein complex was separated by SDS-PAGE and subsequently blotted with an anti-cyclin D1 antibody. As shown in Fig. 5B, cyclin D1 was coimmunoprecipitated with HA-GCIP in cells transfected with HA-GCIP, confirming our in vitro observation that GCIP physically interacts with cyclin D1.

**GCIP Regulates Cyclin D-Cdk4 Kinase Activity**—Since GCIP can interact with cyclin D in cellular extracts, we examined the effects of GCIP overexpression on the activity of cyclin D-dependent protein kinase (Cdk4). Cyclin D1-Cdk4 complex decreased the cyclin D-dependent kinase activity approximately 50% when the cyclin D-Cdk4 complex was precipitated with anti-Cdk4 antibodies. On the other hand, GCIP has less inhibitory effect on the phosphorylation of GST-Rb (30%) by the cyclin-D-Cdk4 complex purified by anti-cyclin D1 antibodies. Similar inhibitory effect (~30–40%) was observed by adding purified GST-GCIP protein into the phosphorylation reaction of GST-Rb (data not shown). These data suggest that interaction of GCIP with cyclin D1 reduced the kinase activity of the cyclin D-Cdk4 complex either by disrupting the complex formation or by changing the conformation of the complex.

**GCIP Regulates E2F-mediated Transcriptional Activity**—Since phosphorylation of Rb by cyclin D-dependent protein kinases leads to the activation of E2F transcriptional activation by releasing E2F from Rb (18, 19), we examined whether binding of GCIP to cyclin D has any effect on the activation of E2F-mediated transcription. First, a chimeric E2F protein with the carboxyl-terminal transcription activation domain of E2F1 was fused to a GAL4 DNA-binding domain for its ability to respond to the overexpression of GCIP in transient-transfection assay as described previously (20–22). As shown in Fig. 7A, the GAL4-E2F1 expression vector and the control vector GAL4VP16 could induce transcription activation from a pG5E1BCAT reporter. Co-transfection of GCIP greatly inhibited the transcriptional activity of the GAL4-E2F1 protein, but has little effect on the transcription induced by the GAL4-VP16. Similar results were obtained with the CAT assay on the thin layer plates where co-transfection of GCIP inhibited the CAT activity (Fig. 7B). These results suggested that GCIP directly or indirectly inhibited the transcriptional activation mediated by E2F1 protein.

**DISCUSSION**

We have cloned a novel human protein from bone marrow library, GCIP, that bound to the leukocyte-specific adaptor protein Grap2 and the key cell cycle protein, cyclin D. Expression of GCIP mRNA was found in most of the human tissues, especially abundant in the heart, muscle, peripheral blood leukocytes, brain, and kidney. From the two-hybrid system and in vitro binding assays, we found that GCIP interacted with the QC domain of Grap2. We also showed that GCIP protein could interact with cyclin D both in our in vitro assays and in GCIP-transfected cells in which GCIP can be coimmunoprecipitated with cyclin D. Furthermore, the presence of GCIP in cyclin D-Cdk4 complex decreased the cyclin D-dependent kinase ac-
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Chloramphenicol and E2F1-mediated CAT activity. Cell extracts were incubated with thin layer chromatography assay. Co-transfection of GCIP inhibited by human GCIP affecting the indicated GAL-4 fusion proteins. Co-transfection of 2 m each column.

Wittmann et al. Cell 99: 50-60 (1999) 24, 27-30). However, the amino acid sequence of GCIP shares little identity with the Id proteins (Id1, Id2, Id3, and Id4). Therefore, GCIP may represent a new class of dominant negative HLH proteins, similar to the mouse maternally transcribed gene, Maid (17). Following the putative HLH domain is a region enriched with aspartic and glutamic acids in GCIP. Domains with high acidic amino acid content are considered to be involved in protein-protein interactions. Immediately downstream of the negatively charged domain of GCIP is a potential leucine zipper (leucine-rich) domain, suggesting that these regions may be involved in interactions with other proteins. Due to the structural features of GCIP, it will be of great interest to examine whether GCIP could interact with HLH domain-containing transcription factors and act as a negative regulator in cell differentiation and proliferation.

Fig. 7. Repression of E2F1-mediated transcriptional activity by human GCIP. A. A pG5E1BCAT reporter was induced by transfecting the indicated GAL-4 fusion proteins. Co-transfection of 2 μg of cDNAs encoding GCIP or Rb dramatically represses Gal4-E2F1 protein induced CAT activity. Much less effect of GCIP on the inhibition of Gal4-VP16 was observed. COS-7 cells were transiently transfected with pG5E1BCAT reporter and expression vectors indicated beneath each column. B. repression of E2F1-activated CAT activity by GCIP in thin layer chromatography assay. Co-transfection of GCIP inhibited E2F1 mediated CAT activity. Cell extracts were incubated with [14C]chloramphenicol and n-butyryl coenzyme A. The reaction mixture was extracted and run on thin layer chromatography. The CAT activity was visualized by exposing to x-ray film for 2-7 days.
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brain where cell differentiation and proliferation is limited in normal tissues. Therefore, it is tempting to hypothesize that GCIP, together with other transcriptional inhibitors, is the underlining mechanism that regulates cell proliferation and differentiation. Studies on how the expression of GCIP is regulated by cell cycle signals and during cell proliferation will shed new light on the molecular mechanism of cell differentiation and proliferation.

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