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Biochemistry

Pur-1, a zinc-finger protein that binds to purine-rich sequences, transactivates an insulin promoter in heterologous cells

(GAGA factor/pancreas/transcription factor)

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ABSTRACT **Purine-rich stretches of nucleotides (GAGA boxes) are often found just upstream of transcription start sites in many genes, including insulin. Mutational analysis suggests that the GAGA box plays an important role in transcription of the rat insulin I gene. We identify here at least four different proteins that bind specifically to the insulin GAGA box. Using a GAGA oligonucleotide, we have isolated a cDNA encoding a sequence-specific protein from a HIT (hamster insulinoma cell line) λgt11 library. This protein, which we designate Pur-1 (for *pur*ine binding), binds to the GAGA boxes of the rat insulin I and II genes and the human islet amyloid polypeptide gene. Pur-1 is a potent transactivator in both pancreatic and nonpancreatic cells. Furthermore, Pur-1 is able to activate an intact insulin promoter in HeLa cells, where it is normally inactive.**

The insulin gene is subject to strict transcriptional controls, both at the level of tissue specificity and in its metabolic regulation. Mutational and *in vitro* protein binding analyses suggest that transcriptional regulation of the insulin promoter is quite complex (1–5). A systematic mutational analysis was used previously to identify nucleotide sequences important for transcriptional activity of the rat insulin I promoter in HIT cells, a hamster insulinoma cell line (1). Among several regions shown to be important for transcriptional activity in HIT cells is a purine-rich region referred to as the GAGA box. Mutation of this sequence, located at −57 to −40 (relative to the transcription start site), results in a 60% loss in transcriptional activity in HIT cells (1). Furthermore, in fetal rat islet cells, mutation of this sequence results in 80% loss of activity and represents the single most deleterious mutation in the 5′ flanking region (M. German and W.J.R., unpublished work). These experiments suggest that the insulin GAGA box is an important transcriptional control region. The mutational sensitivity of the GAGA box could be due to the loss of binding of one or more sequence-specific nuclear factors. We tested this hypothesis by using *in vitro* DNA–protein binding assays to identify proteins binding specifically to the insulin GAGA box. Here we show that a short oligonucleotide comprising the insulin GAGA sequence forms three distinct sequence-specific complexes when incubated with HIT nuclear extract. While all three complexes (numbered 1, 2, and 3) are detected in HIT cell and dog pancreas nuclear extracts, only complexes 1 and 2 are detected in T-cell, Sp2/0 myeloma, and HeLa cell nuclear extracts. In contrast, liver nuclear extract forms only complex 3. These results suggest some degree of tissue selectivity in formation of the insulin GAGA complexes. UV crosslinking analysis reveals that complex 1 contains two proteins, of 80 and 117 kDa. Complex 2 contains a single, 80-kDa protein and complex 3 contains two proteins, 50 and 100 kDa. Thus, there seem to be at least four proteins which bind to the insulin GAGA region.

Molecular cloning of the factors making up the GAGA complexes should enable us to carry out experiments to reveal their function. We therefore set out to isolate clones for the DNA binding components of the complexes. We used the insulin GAGA sequence to screen a HIT cell λgt11 library and identified a cDNA encoding a sequence-specific GAGA binding protein. This protein, which we designate Pur-1 (for its ability to bind to purine-rich sequences), also binds to GAGA boxes from the rat insulin II and human islet amyloid polypeptide (hIAPP) genes. We used transient cotransfection assays to show that Pur-1 is a potent transcriptional activator in both a pancreatic and a nonpancreatic cell line. Pur-1 not only stimulates transcription from multimerized GAGA oligonucleotides but also stimulates transcription from the intact rat insulin I promoter in nonpancreatic (HeLa) cells, where the promoter is virtually inactive.

Recently, the nucleotide sequence of a human zinc-finger protein, MAZ, which binds to two sites in the c-*MYC* promoter, has been reported (6). Comparison of the Pur-1 nucleotide sequence with that of MAZ suggests that MAZ is the human equivalent of hamster Pur-1. We have also isolated a full-length cDNA of Pur-1 from a mouse islet cell line, βTC3 (7); comparison of the hamster, mouse, and human sequences reveals 98.4% identity at the amino acid level.† This strong evolutionary conservation, in concert with the data reported here, suggests that Pur-1 plays an important biologic role.

MATERIALS AND METHODS

Plasmids. HIT72.1 consists of a 1000-base-pair (bp) hamster Pur-1 cDNA cloned into the *Eco*RI site of λgt11. The insert was cloned into pBluescript KS(+) (Stratagene), sequenced, and used to screen a mouse βTC3 cDNA library (a generous gift of Gerhard Christofori, University of California, San Francisco) at high stringency. The resulting positive clone, βTC10.1, was sequenced and determined to be full-length. Pur-1 cDNA was cloned into a cytomegalovirus (CMV) expression vector (8). The −85 insulin promoter–luciferase (−85 Ins–luc) and prolactin promoter–luciferase (Prl–luc) gene constructs are described elsewhere (8). The GAGA oligonucleotides were annealed and multimerized, and eight head-to-tail copies were cloned upstream of the luciferase reporter constructs, as described (5).

Gel Mobility-Shift Assays. The mobility-shift assays, including probe preparations, were as described (9). Nuclear extracts were prepared by the method of Dignam *et al.* (10). HeLa, Sp2/0, and T-cell nuclear extracts were a gift of M. Blanar (University of California, San Francisco). Dog pancreatic and liver nuclear extracts were kindly provided by S. Weinrich (University of California, San Francisco).

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Abbreviations: CMV, cytomegalovirus; hIAPP, human islet amyloid polypeptide.

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†The sequences reported in this paper have been deposited in the GenBank data base (accession nos. L04649 and L06008).

UV Crosslinking. UV crosslinking was performed essentially as described (9), with the following modifications: gels from standard mobility-shift assays were irradiated with UV light (254 nm) for 1 hr, and the radioactive bands visualized overnight at 4°C and subsequently excised. The DNA–protein complexes were transferred to NA45 paper (Schleicher & Schuell) by electrophoresis and eluted in 1.5 M NaCl at 65°C for 1 hr. Samples were loaded directly onto SDS/12.5% polyacrylamide gels, along with protein molecular weight markers. After electrophoresis, gels were dried and analyzed by autoradiography.

Library Screening and Filter Binding Assays. The HIT λgt11 library (11) was screened (12), and positive clones were subjected to four rounds of plaque purification. For filter binding assays, positive cDNA clones were plated at a density of 10^4 per 100-mm Petri dish. Plates were overlaid with nitrocellulose filters impregnated with isopropyl β-D-thiogalactopyranoside, air-dried, and cut into six equal segments, which were hybridized to the indicated probes with identical specific activities and oligonucleotide concentrations, under the same conditions as in the library screening. Filters were exposed to x-ray film at −80°C, and radioactivity was measured in a liquid scintillation counter. Only those filters showing clean backgrounds on autoradiography were chosen for quantitation of binding. A standard curve was obtained by using probes with various specific activities, to ascertain that the filter binding assays fell in the linear range of the assay. Results were expressed as a percentage of binding to the wild-type rat insulin I GAGA sequence. Filter binding assays were carried out on triplicate filter segments and were repeated on two different days with different probe preparations. Results from representative experiments are shown here.

Transient Cotransfection Assays. HIT and HeLa cells were maintained as described (5, 13). Cells were grown to 50% confluency and transfected either by the calcium phosphate method (1) (HeLa) or by using Lipofectin (Bethesda Research Laboratories) (HIT) as recommended by the supplier. Carrier plasmid (pBluescript) was added to the reporter and/or expression plasmids so that the total amount of DNA transfected was constant. Cells were harvested 72 hr after transfection, and luciferase activity was determined (14). All light-unit values reported have been corrected for background activity, which was determined from mock-transfected cells. All transfections were carried out in triplicate and repeated on at least two separate days. Data from representative experiments are shown here.

RESULTS

The Insulin GAGA Region Forms Three Distinct DNA–Protein Complexes in HIT Cell Nuclear Extract. Potential factors which bind to the insulin GAGA region were identified by gel mobility-shift assays. An oligonucleotide corresponding to the insulin promoter from −56 to −33, containing the GAGA sequence, was radiolabeled and incubated with nuclear extract prepared from HIT cells. Three bands, representing DNA–protein complexes 1–3, were observed (Fig. 1). All three complexes were sequence-specific, as judged from the efficient competition by excess unlabeled insulin GAGA oligonucleotide, but not by various purine-containing oligonucleotides (Fig. 1). The same mutation in the insulin GAGA region (S5) which caused decreased transcriptional activity in transfected cells also obliterated formation of all three complexes (data not shown). Further electrophoresis caused a broadening of band 3, resulting in two overlapping bands which were not further resolved. Complex 3 often showed variability in its intensity, whereas complexes 1 and 2 showed a more constant pattern, with band 1 being the most intense. Heat treatment of nuclear extracts resulted in loss of formation of all three complexes (data not shown).

The Insulin GAGA Complexes Show Different Cell-Type Distributions. To determine whether these complexes were pancreas-specific and thus potentially involved in tissue-specific expression of insulin, we assayed their distribution in several cell types (Fig. 2). Complex 3 was absent from HeLa, Sp2/0 myeloma, and T-cell nuclear extracts. Complexes 1 and 2 were absent from dog liver nuclear extract, whereas dog pancreas nuclear extract formed all three complexes. Thus the full complement of all three complexes was found only in nuclear extracts of pancreatic cells, among those tested. Each of the complexes for all cell types tested was shown to be specific by competition with specific and nonspecific competitors (data not shown).

The Three GAGA Complexes Are Composed of at Least Four Proteins That Differ in Molecular Mass. To further characterize the insulin GAGA complexes and determine whether the three complexes might share components, we performed an *in situ* UV crosslinking analysis (Fig. 3). Standard gel mobility-shift assays were carried out with a radiolabeled insulin GAGA oligonucleotide and HIT and dog

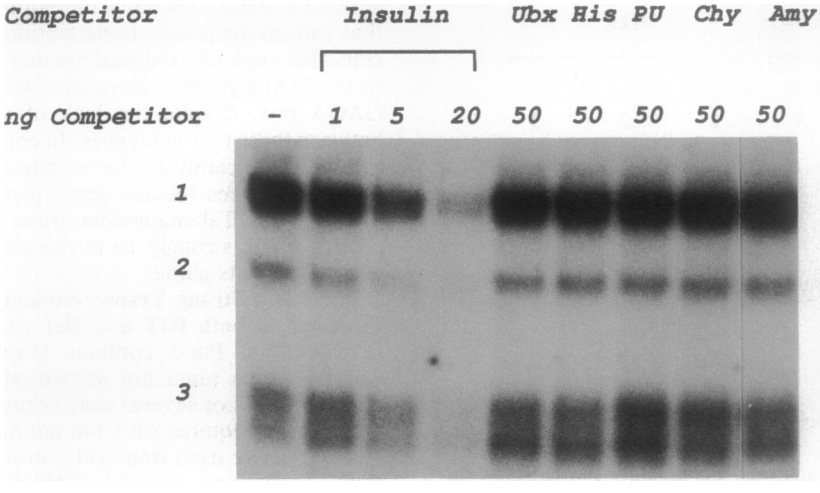

FIG. 1. Gel mobility-shift assay of rat insulin GAGA box with HIT nuclear extract. A 30-bp radiolabeled insulin GAGA synthetic oligonucleotide was added to HIT nuclear extract. Resulting complexes were resolved in nondenaturing 6% polyacrylamide gels. Only the retarded complexes (numbered 1–3) are shown here. For competition studies, the indicated amounts of purine-containing oligonucleotides *Ubx* (15), *His* (16), PU.1 (17), *Chy* (chymotrypsin), and *Amy* (amylase) (18) were mixed with the probe prior to addition of nuclear extract.

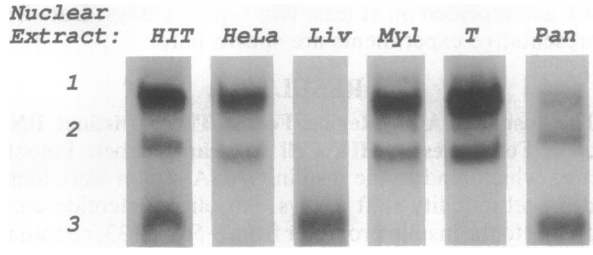

FIG. 2. Distribution of GAGA complexes in various cell types. The insulin GAGA oligonucleotide was incubated with nuclear extract from HIT hamster insulinoma cells, HeLa cells, dog liver (Liv), Sp2/0 myeloma (Myl), a mouse T-cell line (T), or dog pancreas (Pan), and gel mobility-shift assays were performed. Only the retarded complexes are shown. All bands were shown by competition to be specific (data not shown).

liver nuclear extracts. The gels were subsequently irradiated with UV light, and the bands corresponding to the specific complexes were excised. The samples were analyzed by SDS/PAGE, and proteins crosslinked to labeled DNA were visualized by autoradiography. This analysis revealed that complex 1 was composed of two proteins, 80 and 117 kDa; complex 2 contained a single protein, 80 kDa; and complex 3 contained two proteins, 50 and 100 kDa (Fig. 3). Complex 3 from dog liver gave rise to proteins with the same molecular size as those in complex 3 from HIT cells. It is not known whether the 80-kDa proteins are identical in complexes 1 and 2, or whether their similar molecular masses are simply coincidental. Methylation interference studies showed that the DNA contact points for complexes 1 and 2 overlap (data not shown), perhaps due to binding of a protein common to the two complexes.

Pur-1 cDNA Encodes a Sequence-Specific Protein That Binds to the Rat Insulin I and II and hIAPP GAGA Sequences. Oligonucleotide expression screening was used to isolate a cDNA clone encoding a GAGA binding protein. The rat insulin GAGA oligonucleotide was concatemerized and used to screen a HIT cell λgt11 expression library. We screened 800,000 plaques and isolated a 1000-bp cDNA, HIT72.1 (hereafter referred to as Pur-1), whose encoded protein binds strongly to the rat insulin I GAGA box. Northern analysis of a variety of tissues revealed Pur-1 mRNA to be widely distributed (21). Further binding analysis was performed by

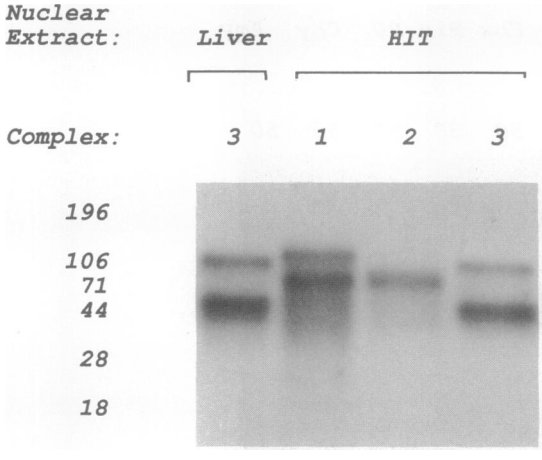

FIG. 3. UV crosslinking analysis. Gel mobility-shift assays with HIT and dog liver nuclear extract were performed as in Fig. 2 and the gels were exposed to UV light. Radioactive bands corresponding to specific complexes were excised, and the complexes were eluted and analyzed in SDS/12.5% polyacrylamide gel. Uncrosslinked probe, which migrates near the bottom of the gel, is not shown. Positions of protein molecular size markers (kDa) are indicated at left.

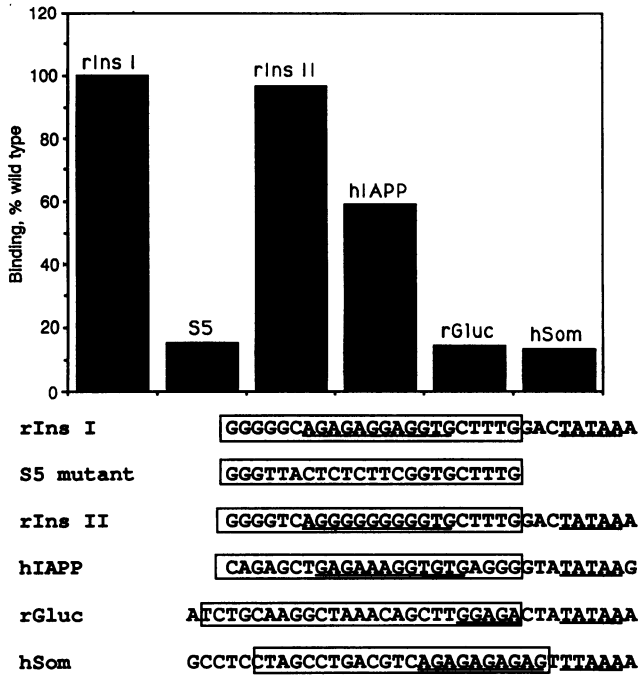

FIG. 4. Analysis of binding of Pur-1 to purine-containing probes. The indicated probes were radiolabeled, concatemerized, and incubated with nitrocellulose filters containing Pur-1-producing λgt11 plaques. Results are expressed as a percentage of binding to the rat insulin I GAGA sequence. Boxed areas indicate regions corresponding to the synthetic oligonucleotides used in this study. The TATA boxes are underlined and shown for reference. rIns, rat insulin; rGluc, rat glucagon; hSom, human somatostatin.

using a semiquantitative plaque binding assay (Fig. 4). Filters containing Pur-1-producing plaques were divided into six equal sections, and each was incubated with a different multimerized probe. Filters were then washed and analyzed by autoradiography, and the amount of radioactivity on the filter was measured. Binding was represented as a percentage of binding to the wild-type insulin GAGA sequence. We tested the ability of Pur-1 protein to bind to several sequences, including the corresponding GAGA regions from the rat insulin II, hIAPP, rat glucagon, and human somatostatin promoters, as well as the rat insulin I block replacement mutant S5 (1). Pur-1 bound equally well to the highly homologous rat insulin II GAGA region. The same mutation in the GAGA region (S5) that caused decreased transcriptional activity in transfected cells also markedly reduced binding to Pur-1 (Fig. 4). Binding to the hIAPP probe was nearly 60% that of the rat insulin I GAGA box, despite the lack of strong overall homology between these two sequences. In contrast, Pur-1 protein failed to bind significantly to the rat glucagon and human somatostatin sequences located just upstream of their respective TATA boxes. Taken together, these results suggest that Pur-1 protein binds strongly to purine-rich sequences, hence the derivation of its name.

Pur-1 Is a Strong Transcriptional Activator of the Insulin Promoter in both HIT and HeLa Cells. Sequence analysis revealed that Pur-1 contains several zinc fingers of the Cys_2His_2 type (data not shown) that comprise the DNA-binding motifs of several transcription factors (19). Furthermore, Pur-1 requires zinc, but not magnesium, for binding to DNA (21). We used transient cotransfection assays with the Pur-1 expression plasmid (CMVPur-1) and luciferase reporter constructs to test whether Pur-1 has an effect on transcription (Fig. 5). For these experiments, we inserted eight copies of the rat insulin I GAGA box upstream of two different promoter–luciferase reporter constructs with minimal promoters: −85 insulin–luciferase (Ins–luc) or prolactin–

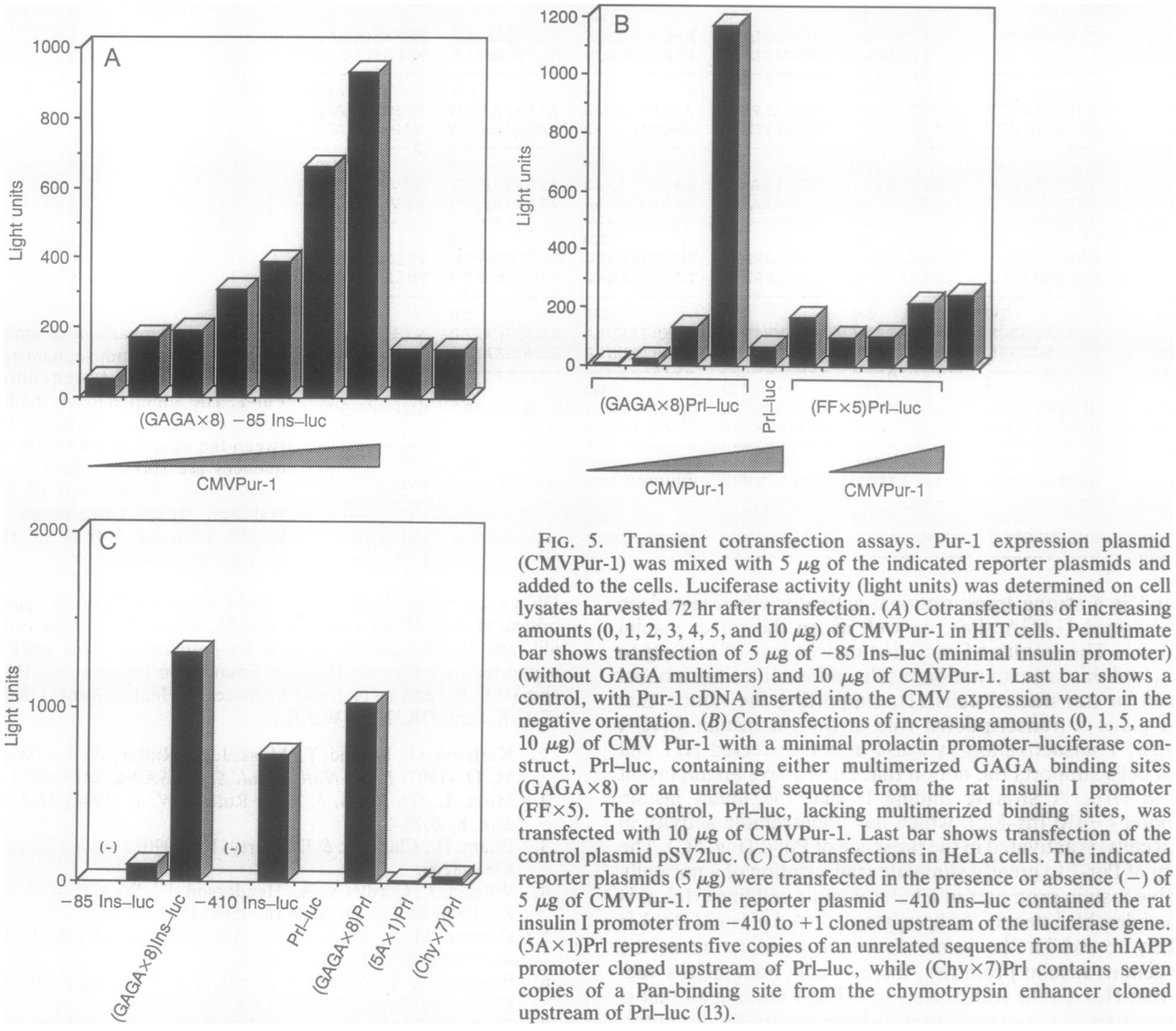

FIG. 5. Transient cotransfection assays. Pur-1 expression plasmid (CMVPur-1) was mixed with 5 μg of the indicated reporter plasmids and added to the cells. Luciferase activity (light units) was determined on cell lysates harvested 72 hr after transfection. (*A*) Cotransfections of increasing amounts (0, 1, 2, 3, 4, 5, and 10 μg) of CMVPur-1 in HIT cells. Penultimate bar shows transfection of 5 μg of −85 Ins–luc (minimal insulin promoter) (without GAGA multimers) and 10 μg of CMVPur-1. Last bar shows a control, with Pur-1 cDNA inserted into the CMV expression vector in the negative orientation. (*B*) Cotransfections of increasing amounts (0, 1, 5, and 10 μg) of CMV Pur-1 with a minimal prolactin promoter–luciferase construct, Prl–luc, containing either multimerized GAGA binding sites (GAGA×8) or an unrelated sequence from the rat insulin I promoter (FF×5). The control, Prl–luc, lacking multimerized binding sites, was transfected with 10 μg of CMVPur-1. Last bar shows transfection of the control plasmid pSV2luc. (*C*) Cotransfections in HeLa cells. The indicated reporter plasmids (5 μg) were transfected in the presence or absence (−) of 5 μg of CMVPur-1. The reporter plasmid −410 Ins–luc contained the rat insulin I promoter from −410 to +1 cloned upstream of the luciferase gene. (5A×1)Prl represents five copies of an unrelated sequence from the hIAPP promoter cloned upstream of Prl–luc, while (Chy×7)Prl contains seven copies of a Pan-binding site from the chymotrypsin enhancer cloned upstream of Prl–luc (13).

luciferase (Prl–luc). In HIT cells, cotransfection of increasing amounts of Pur-1 expression plasmid resulted in strong transcriptional activation of a minimal Ins–luc reporter construct containing insulin GAGA binding sites (Fig. 5*A*). This effect was also observed with a minimal Prl–luc construct (Fig. 5*B*), indicating that the effect is not due to a feature of the minimal insulin promoter itself. We set out to determine whether Pur-1 could activate the whole rat insulin I promoter (from −410 to +1), which contains a single GAGA site in the context of other insulin promoter regulatory elements. The −410 insulin promoter construct had no detectable activity in the nonpancreatic HeLa cell line; however, cotransfection of Pur-1 caused a >100-fold stimulation of transcription (Fig. 5*C*). These results show conclusively that Pur-1 is a transcriptional activator with a capacity to stimulate a normally inactive promoter in a heterologous cell type. Several control experiments were also carried out. Cotransfection of a CMV expression plasmid containing the Pur-1 cDNA cloned in the negative orientation showed little activity (Fig. 5*A*, rightmost bar). Pur-1 did not transactivate a reporter construct containing multimerized copies of unrelated sequences [Fig. 5*B*, (FF×5)Prl–luc; Fig. 5*C*, (5A×1)Prl–luc] or of binding sites for known transcription factors, such as Pan binding sites from the chymotrypsin promoter [Fig. 5*C*, (Chy×7)Prl–luc]. These results indicate that transactivation by Pur-1 requires a functional Pur-1 binding site.

Pur-1 Is a Highly Conserved Protein. While this manuscript was in preparation, the nucleotide sequence of a zinc-finger protein cloned from HeLa cells, termed MAZ, was reported (6). Upon comparison of the human MAZ sequence with our hamster and mouse Pur-1 sequences, we conclude that MAZ is the human equivalent of Pur-1 (Fig. 6). There are only 7 amino acid differences between human and mouse Pur-1 (out of 447 residues), showing a high degree of similarity (98.4% identity). Most of these changes are conservative.

DISCUSSION

We report here the identification and partial characterization of nuclear factors binding to the rat insulin I GAGA box. Gel mobility-shift analysis, coupled with UV crosslinking analysis, allowed us to identify at least four distinct proteins (50, 80, 100, and 117 kDa) that bind sequence-specifically to this region (Figs. 1–3). The relationship of Pur-1 to the four GAGA proteins is unknown. The size predicted from the 447-amino acid sequence of Pur-1 is 52 kDa, which is similar in size to the 50-kDa protein in complex 3. Gel mobility-shift analysis has shown that the hIAPP oligonucleotide forms complexes 2 and 3 but not complex 1 with HIT nuclear extract (data not shown), suggesting that the insulin and hIAPP GAGA regions bind proteins in common. This is consistent with the coexpression of insulin and IAPP in pancreatic β cells (20). Pur-1 binds avidly to the hIAPP oligonucleotide (Fig. 4); therefore, it is possible that Pur-1 represents one of these shared proteins. Antibodies directed against Pur-1 will be necessary to address these questions in a definitive manner.

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            10         20         30         40         50         60
mouse   MFPVFPCTLL APPFPVLGLD SRGVGGLMNS FPPPQGHAQN PLQVGAELQS RFFASQGCAQ
hamster           FPVLGLD SRGVGGLMNS FPPPQGHAQN PLQVGAELQS RFFASQGCAQ
human   ---------- ---------- ---------- ---------- ---------- ----------
            70         80         90        100        110        120
mouse   SPFQAAPAPP PTPQAPAAEP LQVDLLPVLA AAQESAAAAA AAAAAAAAVV TAPPAPAAAS
hamster SPFQAAPAPP PTPQAPAAEP LQVDLLPVLA AAQESAAAAA AAAAAAAAVV TAPPAPAAAS
human   ---------- ---------- ---------- ---------- ---------A A---------
            130        140        150        160        170        180
mouse   TVDTAALKQP PAPPPPPPAV SAPAAEAAPP AAAATIAAAA ATAVVAPTST VAVAPVASVL
hamster TVDTAALKQP PAPPPPPPAV SAPAAEAAPP AAAATIAAAA ATAVVAPTST VAVAPVASVL
human   ---------- --------P- ---------- -S-------- ---------- --------A-
            190        200        210        220        230        240
mouse   EKKTKSKGPY ICALCAKEFK NGYNLRRHEA IHTGAKAGRV PSGAMKMPTM VPLSLLSVPQ
hamster EKKTKSKGPY ICALCAKEFK NGYNLRRHEA IHTGAKAGRV PSGAMKMPTM VPLSLLSVPQ
human   ---------- ---------- ---------- ---------- ---------- ----------
            250        260        270        280        290        300
mouse   LSGASGGGGE AGAGGGTAAV AAGGVVTTTA SGKRIRKNHA CEMCGKAFRD VYHLNRHKLS
hamster LSGASGGGGE AGAGGGTAAV AAGGVVTTTA SGKRIRKNHA CEMCGKAFRD VYHLNRHKLS
human   ----G----- ------A--- ---------- ---------- ---------- ----------
            310        320        330        340        350        360
mouse   HSDEKPYQCP VCQQRFKRKD RMSYHVRSHD GAVHKPYNCS HCGKSFSRPD HLNSHVRQVH
hamster HSDEKPYQCP VCQQRFKRKD RMSYHVRSHD GAVHKPYNCS HCGK
human   ---------- ---------- ---------- ---------- ---------- ----------
            370        380        390        400        410        420
mouse   STERPFKCEK CEAAFATKDR LRAHTVRHEE KVPCHVCGKM LSSAYISDHM KVHSQGPHHV
human   ---------- ---------- ---------- ---------- ---------- ----------
            430        440        450        460        470        477
mouse   CELCNKGTGE VCPMAAAAAA AAAAAAAVVA APPTAVGSLS GAEGVPVSSQ PLPSQPW
human   ---------- ---------- ---------- ---------- ---------- -------
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FIG. 6. Comparison of amino acid sequence of mouse, hamster, and human cDNAs encoding Pur-1. The amino acids in the human sequence which differ between the mouse and hamster sequences are shown. There are 7 amino acid changes out of 447 residues, which corresponds to 98.4% identity. Most of the changes are conservative.

Pur-1 is a strong transcriptional activator when tested on multimerized GAGA sites, as well as on the intact insulin promoter. The insulin S5 mutation causes loss of binding to Pur-1, and this same mutation results in loss of transcriptional activity in transfected cells (1), supporting the notion that Pur-1 plays a transcriptional role at the rat insulin GAGA element. Transactivation by Pur-1 in cotransfected HIT and HeLa cells supports the notion that Pur-1 plays an important role *in vivo*. A striking finding is that the intact insulin promoter (−410 Ins–luc), which has undetectable activity in HeLa cells, is activated in the presence of Pur-1 (Fig. 5*C*). The ability of Pur-1 to greatly stimulate transcription of a normally inactive insulin promoter in a heterologous cell line (HeLa) is of considerable interest. Although transfected plasmid DNA is unlikely to exhibit the same chromatin architecture as the endogenous insulin promoter, these results nonetheless suggest that HeLa cells transfected with Pur-1 contain all that is necessary to carry out transcription from the insulin promoter.

The binding analysis with Pur-1 (Fig. 4) reveals its propensity to bind to purine residues. Pur-1 binds strongly to the rat insulin I and II and hIAPP GAGA boxes but fails to bind to rat glucagon or human somatostatin promoter sequences. Despite any obvious overall homology between these rat insulin and hIAPP sequences, they are all rich in purines. The human equivalent of Pur-1 (MAZ, for *MYC*-associated zinc-finger protein), also recognizes guanine- and adenine-rich sequences in the c-*MYC* promoter (6), further underscoring its binding preference for purines. Beyond this general preference, there is no obvious canonical binding site for Pur-1, and as such, its specificity at the nucleotide level is yet to be revealed. Accordingly, we suggest that the nomenclature for this zinc-finger protein should reflect a more general description of its properties, such as purine binding, rather than its function on specialized genes such as c-*MYC* and insulin. For this reason, we feel that Pur-1 is an appropriate name. The binding of Pur-1 to the rat insulin and hIAPP GAGA regions, along with transcriptional activation of the insulin promoter, suggests that Pur-1 may have a broad role in regulating genes expressed in pancreatic β cells. Indeed, Pur-1 may very well have a more global role in transcriptional regulation, acting on targets in addition to the insulin and c-*MYC* promoters.

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