The caudal homebox gene Cdx-2 is a transcriptional activator for approximately a dozen genes specifically expressed in pancreatic islets and intestinal cells. It is also involved in preventing the development of colorectal tumors. Studies using “knockout” approaches demonstrated that Cdx-2 is haplo-insufficient in certain tissues including the intestines but not the pancreatic islets. The mechanisms, especially transcription factors, which regulate Cdx-2 expression, are virtually unknown. We found previously that Cdx-2 expression could be autoregulated in a cell type-specific manner. In this study, we located an octamer (OCT) binding site within the mouse Cdx-2 gene promoter. This site, designated as Cdx-2/OCT, is involved in the expression of the Cdx-2 promoter. Both pancreatic and intestinal cell lines were found to express a number of POU (OCT binding) homeodomain proteins examined by electrophoretic mobility shift assay. However, it appears that Cdx-2/OCT interacts only with OCT1 in the nuclear extracts of the intestinal cell lines examined, although it interacts with OCT1 and at least two other POU proteins that are to be identified in the pancreatic InR1-G9 cell nuclear extract. Co-transfecting OCT1 cDNA but not five other POU gene cDNAs activates the Cdx-2 promoter in the pancreatic InR1-G9 and the intestinal Caco-2 cell lines. In contrast, Cdx-2/OCT cannot act as an enhancer element if it is fused to a thymidine kinase promoter. Furthermore, Cdx-2/OCT-thymidine kinase fusion promoters cannot be activated by OCT1 co-transfection. Cell type-specific expression, cell type-specific binding affinity of POU proteins to the cis-element Cdx-2/OCT, and the DNA content-dependent activation of Cdx-2 promoter via Cdx-2/OCT by OCT1 suggest that POU proteins play important and complicated roles in modulating Cdx-2 expression in cell type-specific manners.

Transcription factors are almost never expressed as specific as their downstream target genes (for review see Ref. 1). A given transcription factor is always able to exert multiple biological functions via regulating the expression of different downstream target genes in tissue/cell type-specific manners. This is mainly attributed to the temporal and spatial expression property of a given transcription factor, its ability to bind to cis-elements within different downstream target promoters, and more importantly, its ability to recruit other transcription factors or nuclear co-activators and/or repressors (1–3).

Cdx-2 (CDX2 for humans) is one of the three caudal-related homeodomain (HD) proteins identified in mammals (4–7). Post-embryogenesis, Cdx-2 is expressed in pancreatic islets, differentiated intestinal epithelia including the proglucagon-producing endocrine L cells (6–9). Cdx-2 null mutant mice have been generated recently (10, 11). Cdx-2+/− mutants die between 3.5 and 5.5 days postcoitum, whereas Cdx-2−/− mutants show multiple malfunctions including the formation of multiple intestinal adenomatous polyps. However, malfunctions in the pancreata have not been detected in the Cdx-2 heterozygotic mice. Therefore, Cdx-2 is haplo-insufficient for selected tissues including the intestines but not the pancreas.

Although the physiological and pathological significance of Cdx-2 has been well recognized, the mechanisms, especially transcription factors, which regulate the expression of Cdx-2, remain virtually unknown. We have isolated the mouse Cdx-2 promoter and examined the transcriptional properties of this promoter in pancreatic and intestinal cell lines. We have found that Cdx-2 activates the expression of its own promoter in a cell type-specific manner (12). We have dissected the Cdx-2 gene promoter and demonstrated that Cdx-2 binds to two separated AT-rich motifs, namely the TATA and DBS sites, within a proximal region of the Cdx-2 promoter. The DBS site is critical for autoactivation, whereas the TATA site may serve as an attenuating component for the autoregulatory loop (12).

Members of the POU HD protein family share a common feature of harboring a bipartite DNA binding domain consisting of a POU-specific domain and a POU homeodomain joined by a short variable linker (13). Many POU proteins act as transcriptional regulators via recognizing a conserved 8-base pair octamer (OCT) binding element (13). POU proteins are expressed in early embryogenesis and in diverse organs and/or systems and play fundamentally important roles in the organ development, cell type specification, and growth and differentiation of cells (14, 15).

In this study, we located an OCT binding site in the mouse, Cdx-2 promoter, designated as Cdx-2/OCT. We demonstrate here that Cdx-2/OCT is critical for Cdx-2 promoter expression. OCT1 and other to-be-identified POU proteins are expressed in pancreatic islet and intestinal cell lines examined. It appears that Cdx-2/OCT physically interacts with only OCT1 ex-
pressed in intestinal cell lines, whereas Cdx-2\textsubscript{pOCT} interacts with OCT1 and at least two other yet-to-be-identified POU proteins expressed in a pancreatic A cell line. Although OCT1 was found to bind to and activate Cdx-2 promoter in pancreatic and intestinal cell lines, Cdx-2\textsubscript{pOCT} itself cannot act as an enhancer element when it is fused to a heterologous promoter. In addition, the fusion promoters cannot be activated by OCT1 co-transfection. We conclude that OCT1 and other to-be-identified POU proteins play important and complicated roles in modulating Cdx-2 expression in cell type-specific manners.

**EXPERIMENTAL PROCEDURES**

**Materials**—Radioisotopes were obtained from Amersham Pharmacia Biotech. Oligonucleotides were provided by AGCT Co. (Toronto, Canada). Restriction enzymes and DNA modification enzymes were of molecular biology grade and were purchased from several sources.

**Plasmids**—Construction of the mouse Cdx-2 promoter and the luciferase fusion gene plasmid and its deletion constructs have been described previously (12). Cdx-2-LUC (−138 to +137) and its mutant were constructed by subcloning the correspondent polymerase chain reaction products into a promoter-less luciferase reporter gene plasmid. The forward primers used in polymerase chain reaction are Cdx-2\textsubscript{pOCT} and Cdx-2\textsubscript{pOCT(M)} as indicated in Fig. 1B. The reverse primer represents the mouse Cdx-2 DNA sequence from +119 to +137 (GAGCTTCGTGCCTCCCGGG). OCT1, OCT2A, and OCT2B expression plasmids were kindly provided by Dr. Winship Herr (16), OCT3A was a gift from Dr. Graeme Bell (17), and the Skn-1a and Skn-1i were gifts of Dr. Bogi Andersen (18). Construction of Cdx-2 expression plasmid and its antisense counterpart (Cdx-2(AS)) has been described previously (19). OCT1(AS) was generated by flipping the 1.1-kb EcoRI fragment in the pCG-OCT1 expression vector (16).

**Cell Culture, Transient Transfection, and Luciferase Reporter Gene Analysis**—Human colon cancer cell lines HT-29, COLO-205, SW480, and Caco-2 were obtained from the American Type Culture Collection (ATCC, Manassas, VA) and cultured according to instruction. Cultivation of the baby hamster kidney (BHK) fibroblast, pancreatic islet cell lines INR1-G9, InR1-G9, IN11, RIN-56, and HIT, and the intestinal endocrine L cell lines GLUTag and STC-1 have been described previously (8, 12, 19). BHK, INR1-G9, and Caco-2 cell lines were transfected by a calcium phosphate precipitation method (12). The cells were harvested for the luciferase (LUC) reporter gene analysis 16 h after the transfection.

**Nuclear Protein Extraction and Electrophoretic Mobility Shift Assay (EMSA)**—Nuclear proteins from the cultivated cell lines were prepared as described by Schreiber et al. (21). EMSA and supershifting analysis were performed as described previously (19).

**RESULTS**

**Contribution of Cdx-2\textsubscript{pOCT} to the Expression of the Cdx-2 Promoter**—We located an OCT binding site within the proximal portion of the mouse Cdx-2 promoter, which we designated as Cdx-2\textsubscript{pOCT} (Fig. 1A). To examine the contribution of this OCT binding site to the expression and autoregulation of the Cdx-2 promoter, we generated a pair of Cdx-2-LUC fusion gene constructs, Cdx-2-LUC (−138 to +137 wild type) and Cdx-2-LUC (−138 to +137 mutant). These two fusion genes are identical with the exception that Cdx-2-LUC (−138 to +137 mutant) carries a mutated OCT binding site (Fig. 1B). We then transfected these two fusion genes into the pancreatic INR1-G9 cell line (Fig. 2A) and the intestinal Caco-2 cell line (Fig. 2B). The mutated promoter was found to activate the reporter gene in both cell lines ∼90% compared with the wild type promoter indicating that this OCT binding site is critical for Cdx-2 expression. The wild type promoter could be activated 10-fold by OCT1 co-transfection, whereas the mutant promoter could be activated 5-fold. This result suggests that Cdx-2\textsubscript{pOCT} could also be implicated in Cdx-2 autoregulation (12). In addition, the wild type Cdx-2 promoter could be activated approximately 5-fold by OCT1 cDNA co-transfection, whereas the mutant promoter has no response to OCT1 co-transfection (Fig. 2). Cdx-2 (AS) and OCT1 (AS), which contain Cdx-2 or OCT1 cDNA, respectively, in the antisense orientation, cannot activate Cdx-2 promoter (Fig. 2).

**FIG. 1.** Schematic presentation of the mouse Cdx-2 promoter and the DNA probes/primers utilized. A, CRE, cAMP response element; E box, the consensus sequence CANNTG; TATA and DBS, two Cdx-2 binding sites identified previously (12). The ATGCAAT motif is located between −125 to −116 bp relative to the transcription start site. B, * indicates that this synthetic OCT binding probe and its mutant have been previously utilized for detection of OCT-binding proteins in mammary glands (22).

**Examination of OCT-binding Protein Expression by EMSA**—We then conducted an examination of the expression of nuclear proteins that are able to physically interact with Cdx-2\textsubscript{pOCT} in pancreatic and intestinal cell lines by EMSA. When the pancreatic islet A cell line INR1-G9 was examined, four DNA protein complexes were obtained designated as C1–C4 (Fig. 3, lanes 2 and 10). The formation of these four complexes could be effectively competed by unlabeled native Cdx-2\textsubscript{pOCT} probe (lanes 3 and 4) but not by an unlabeled nonspecific probe (lanes 5 and 6). Therefore, these complexes can all represent specific DNA binding events. Complexes C1, C2, and C3 could be effectively competed by another OCT binding probe (22) (lanes 7 and 8, see below), indicating the likelihood that these three complexes all represent POU protein binding events. When the mouse large intestinal endocrine L cell line GLUTag was examined, only one complex, C3, was clearly observable (lane 11). When the human colon cancer cell line Caco-2 was examined, the only observable complex was C1 (lane 16). The C1 complex was determined to represent the ubiquitously expressed POU protein OCT1 by a supershifting analysis (compare lane 14 with 15 and lane 16 with 17).

The above results would suggest that either the intestinal cell lines express fewer number of POU proteins or most POU proteins expressed in intestinal cell lines cannot bind to Cdx-2\textsubscript{pOCT}. To examine these two possibilities, we then conducted EMSA to examine four human colon cancer cell lines using a synthetic OCT probe (22). DNA sequence of the synthetic OCT probe is shown in Fig. 1B. This artificial probe has been used successfully previously by us to examine the expression of POU proteins in normal and malignant human mammary gland epithelial cell lines (22). When this probe was used, nuclear extracts from four intestinal cell lines were found to form a number of complexes (Fig. 4A). Among these complexes, C1, C2, and C4 were observable for all four colon cancer cell lines examined (lanes 1–4). The formation of C1, C2, and C3 in the Caco-2 cell line was completely competed by the unlabeled OCT probe (lanes 7 and 8) but not by the mutant OCT probe (lanes 9 and 10), indicating that they represent POU protein binding. The native Cdx-2\textsubscript{pOCT} probe was found to inhibit the formation of C1 and C4 but not C2 and C3 (lane 6). This result would suggest that the POU proteins in C2 and C3, expressed by the Caco-2 cell line, are unable to bind to Cdx-2\textsubscript{pOCT}. To further examine this observation, we conducted EMSA using the nu-
tive Cdx-2POCT probe against four colon cancer cell lines. As shown in Fig. 4B, the nuclear extracts from all these cell lines examined were found to form only one major complex, C1 (Fig. 4B). Taken together, our results suggest that with the exception of OCT1, the other to-be-identified POU proteins are also expressed in the intestinal cell lines examined in this study.

However, they are not capable of binding to Cdx-2POCT. When the synthetic OCT probe was applied to the pancreatic islet cell line InR1-G9, three complexes from C1 to C3 were obtained (Fig. 5). Supershifting analyses further revealed that C1 represents OCT1 (lane 2). The identities of C2 and C3 cannot be determined using antibodies against two other POU

![Graph](image)

**Fig. 2. Assessment of the role of Cdx-2POCT in Cdx-2 promoter expression.** SK-LUC, a promoter-less luciferase reporter gene construct; Cdx-LUC(WT) and Cdx-LUC(M), luciferase reporter genes driven by the wild type or the OCT binding site mutated Cdx-2 promoter (-138 to +137), respectively; Cdx-2 and OCT1, cDNAs of these two transcription factors driven by a CMV promoter; Cdx-2(AS) and OCT1(AS), Cdx-2 and OCT1 cDNAs inserted into the expression vector pBAT7 in the antisense orientation (8). Pancreatic InR1-G9 cells (A) or intestinal Caco-2 cells (B) were transfected by the combination of the indicated plasmids (5 μg each). Luciferase activity was measured 16 h after transfection. The data are expressed as mean relative luciferase activity (n = 3) ± S.E. normalized to: 1) the activity obtained after transfection of the promoter-less SK-LUC in the same experiment, and 2) total amount of protein utilized in each assay.

![Graph](image)

**Fig. 3. Detection of nuclear proteins that bind to Cdx-2POCT by EMSA.** NE, nuclear extracts; Comp./S, unlabeled Cdx-2OCT probe; Comp./NS, an unrelated unlabeled 30-bp probe; Comp./OCT, unlabeled OCT probe (Fig. 1B); Ab/OCT1, anti-OCT1 antibody (2 μl); OCT1, the supershifted complex by anti-OCT1 antibody; FP, free probe. Approximately 5 μg of nuclear proteins were utilized in each reaction.
proteins, namely OCT2 and OCT11 (Skn-1a/Skn-1i) (lanes 3–5). We also cannot detect OCT2 and OCT11 expression in the pancreatic and intestinal cell lines used in this study by Western blot analysis (data not shown).

OCT1 Activates Cdx-2 Promoter in Pancreatic and Intestinal Cell Lines—Our observations indicated that OCT1 is expressed in most pancreatic and intestinal Cdx-2-expressing cell lines, and OCT1 physically interacts with the Cdx-2 promoter. We then examined if co-transfection of OCT1 and other POU gene cDNAs would activate Cdx-2 promoter. We found that OCT1 activated Cdx-2 promoter nearly 6-fold in the pancreatic InR1-G9 cell line (Figs. 2A and 6A) and ~4-fold in the intestinal Caco-2 cell line (Figs. 2B and 6B). Among the other five POU genes examined, only OCT2A showed a moderate activation. Because OCT2 is not expressed in any of the Cdx-2-expressing cell lines examined, such a moderate activation may have no biological significance.

Cdx-2POCT Cannot Act As an Enhancer Element When It Is Fused to a Heterologous Promoter—To examine whether the 31-bp native Cdx-2POCT itself could act as an enhancer element, we fused this element with the thymidine kinase (TK) promoter (19) in either 5′ to 3′ (forward) or 3′ to 5′ (reverse) orientations. The Cdx-2POCT-TK-LUC fusion genes together with the wild type TK-LUC were then transfected into the pancreatic InR1-G9 and the BHK fibroblast cell lines. As shown in Fig. 7A, these fusion promoters show a 40–70% activity compared with that of wild type TK promoter when transfected into the InR1-G9 cell line. In contrast, these two fusion promoters showed a negative response to OCT1 co-transfection in both InR1-G9 and BHK cell lines examined. These results suggest that OCT1
activates Cdx-2 promoter through binding to Cdx-2 POCT in a DNA content-dependent manner.

**DISCUSSION**

In 1991, James and Kazenwadel (4) examined the expression of Hox and related homeobox genes in mouse intestines by reverse transcription-polymerase chain reaction using degenerate primers. With the exception of the detection of Cdx-1 (23), they also obtained a portion of a novel caudal cDNA, which they named Cdx-2 (4). Complete cDNA sequences for mouse Cdx-2 were reported in 1994 (6, 7). However, in 1992 German et al. (5) isolated a complete caudal cDNA from a hamster insulinoma cell line, HIT. This cDNA was named Cdx-3. Cdx-2 and Cdx-3 are now considered to be the same gene in different rodents. We and others (8, 9, 12) have used the term Cdx-2/3 previously. To follow the nomenclature regulation for HOX/Hox genes (24), we now use the term CDX2 for this gene in humans and the term Cdx-2 in rodent species.

Cdx-2 is expressed in intestinal epithelia along the entire crypt-villus axis of the small intestine and in crypts of the colon (7). It is also expressed in pancreatic A and B cell lines (5, 8, 9) and in the large and small intestinal endocrine L cell lines (8). More recently, the Cdx-2 expression in human gastric cancer cell lines has been reported (25). CDX2 expression was found to be ceased in the colon cancer cells of patients at later stages, and mutations in the CDX2-coding region has been found in both colon cancer patients and colon cancer cell lines (26–28).

More importantly, Cdx-2<sup>-/-</sup> mice were found to develop multiple malfunctions including a homeotic shift on vertebrae, stunted growth, abnormalities on tails, and the development of multiple intestinal neoplasia within three months of their birth. However, no observable malfunction has been found in the pancreata of these heterozygotic mice (10), and proglucagon...
gene expression appears normal in the Cdx-2<sup>+/−</sup> mice.<sup>3</sup> Such extraneous multiple malfunctions observed in the heterozygotic null mutants suggest that 1) Cdx-2 is haplo-insufficient for selected tissues, and normal cellular phenotype in these tissues requires the expression of both alleles, 2) Cdx-2 has a broad spectrum of downstream target genes, and 3) Cdx-2 requires co-factors to achieve its diverse and cell type-specific biological functions.

Indeed, about a dozen potential downstream target genes of Cdx-2 have been identified including insulin (5), proglucagon (8, 9), sucrase-isomaltase (7), phospholipase A/lysophospholipase (29), lactase-phlorizin hydrolase (30), carbonic anhydrase 1 (31), calbindin-d9K (32), vitamin D receptor (33), and the homeobox gene Hoxc-8 (34). Cdx-2, therefore, provides a very good model system for addressing a fundamentally important biological question: How can a single transcription factor exert its multiple biological functions in tissue/cell type-specific manners?

One of the essential steps for understanding tissue-specific biological functions of Cdx-2 is to investigate mechanisms controlling its expression in different types of cells. For this purpose, we isolated the mouse Cdx-2 promoter and examined the transcriptional properties of this promoter in pancreatic islet, intestinal endocrine, and human colon cancer cell lines. We demonstrated that Cdx-2 is able to activate its own promoter in pancreatic and intestinal cell lines that express CDX2/Cdx-2, but repress its own promoter in fibroblasts (12). This observation, on one hand, provided an explanation for why one wild-type Cdx-2 allele is sufficient for certain tissue such as pancreas. On the other hand, it raised a question why one functional allele is insufficient for other tissues including intestines. We proposed that, except for Cdx-2 itself, other transcription factors and signals might determine whether Cdx-2 expression in a given cell reaches the threshold for triggering the autoregulatory loop. Some of these factors or signals may function only, or more effectively, in one type of cells such as pancreatic cells. If this hypothesis is correct, it would provide an explanation as to why various abnormalities observed among Cdx-2<sup>+/−</sup> mice vary, in degree, time, and location, from one mouse to another.

In this study, we assessed the contribution of Cdx-2<sub>POCT</sub> in Cdx-2 expression. Because the mutant promoter shows ~30% activity compared with that of the wild type, we conclude that this site is important for Cdx-2 expression. In addition, because the mutant promoter loses more than a 2-fold response to Cdx-2 activation, we suggest that this binding site might be also involved in the autoregulation process.

To examine POU proteins that are expressed and capable of binding to Cdx-2<sub>POCT</sub>, we conducted EMSA using both the typical synthetic OCT binding probe (22) and the native 31-bp Cdx-2<sub>POCT</sub> probe. Our results indicated that although Cdx-2<sub>POCT</sub> forms four specific DNA-protein complexes with the pancreatic InR1-G9 cell line, it forms only one complex, C3, with the intestinal endocrine cell line GLUTag and one complex, C1, with the colon cancer cell line Caco-2. Because Cdx-2 haplo-insufficient occurs in intestines but not pancreata, our results would suggest that pancreatic islet cell might express more POU proteins as transcriptional activators for Cdx-2. To further examine this, we need to identify each individual complex formed between the InR1-G9 cell line and the Cdx-2<sub>POCT</sub> probe.

The C1 complex was found to represent the ubiquitously expressed POU protein OCT1. However, four colon cancer cell lines examined do express OCT-binding proteins other than OCT1 detected by EMSA using the synthetic OCT binding probe. These observations would suggest that, except for OCT1, other POU proteins expressed in pancreatic and intestinal cell lines have different binding affinity to the 31-bp cis-element Cdx-2<sub>POCT</sub>. This could be due to the expression of different profiles of POU proteins by pancreatic islets and intestine cells. Alternatively, the same POU protein(s) expressed in pancreatic and intestine cells may have different binding affinities. The latter hypothesis is plausible because the binding ability of POU proteins could be influenced by posttranslational events including phosphorylation status (35–38). Grenfell et al. (35) have demonstrated that Oct-1 and Oct-2 bind differentially to three octamer binding sequences, the one from the H2B promoter (ATGCTAAATAA, similar to the synthetic OCT probe utilized in this study), a TAATGARAT motif, and a perfect consensus overlapping octamer/TAAAGARAT motif (ATGCTAAATAA). The binding activity of these octamer probes could be modulated by PKA, PKC, and casein kinase 2 (35). Inamoto et al. (36) found that MAT1 is able to target cyclin-dependent kinase-activating kinase to Oct factors and lead to their phosphorylation. This may play a role in the recruitment of transcription factor IIH to the preinitiation complex or in subsequent initiation and elongation reactions (36). Zhang et al. (37), however, shown that Oct-1 is a repressor for HLA-DRA. Expression of the tumor suppressor gene Rb leads to the phosphorylation of Oct-1 relieving its repressive effect on HLA-DRA. To assess the overall contribution of POU proteins in regulating Cdx-2 expression in different types of cells, it is necessary to examine the phosphorylation status of these POU proteins in each type of cells in both physiological and pathological conditions.

The expression of POU genes in the gastrointestinal tract has not been actively examined. Hussain et al. (39), however, demonstrated that both InR1-G9 and GLUTag cell lines do express Brn-4, a class III POU gene that acts as a regulator for proglucagon gene expression. Because the C3 complex is observed for both InR1-G9 and GLUTag cell lines (Fig. 3, lanes 10 and 11), it is possible that this complex represents Brn-4. This complex was not observed for the four colon cancer cell lines examined. It will be interesting to examine whether Brn-4 or the protein component for the complex C3 is a tissue-specific transcriptional activator for Cdx-2 in proglucagon producing cells.

Another interesting observation is that although OCT1 activates Cdx-2 promoter via binding to Cdx-2<sub>POCT</sub>, this 31-bp cis-element itself cannot act as an enhancer element for the heterologous TK promoter. Furthermore, the Cdx-2<sub>POCT- TK</sub> fusion promoters do not respond to OCT1 activation. Therefore, OCT1 activates Cdx-2 promoter in a DNA content-dependent manner. Because OCT1 activates both the −769 to +137 and the −138 to +137 Cdx-2 gene promoters, the cis-element(s) critical for this content-dependent activation should be downstream of the Cdx-2<sub>POCT</sub> itself. The to-be-defined cis-element(s) may overlap with Cdx-2 binding sites and the area with which components for general transcription machinery might interact. It is possible that OCT1 and other to-be-identified POU proteins may interact with other transcription factors that bind to yet-to-be-defined cis-element(s) downstream of Cdx-2<sub>POCT</sub> for activating Cdx-2 expression. This contact may promote further protein-protein interactions and recruitment events that interface more effectively with the transcriptional machinery. Indeed, it has been shown previously that OCT1 requires different co-factors such as OCA-B (40), proximal sequence element-binding transcription factor (41), and VP-16 (42) for its diverse biological functions. OCT1 is also able to interact with MAT1, a subunit of cyclin-dependent kinase-activating kinase (36). Furthermore, it has been shown that OCT1 and the glucocorticoid receptor interact synergistically to

---

<sup>3</sup> D. Drucker, personal communication.
activate the transcription of mouse mammary tumor virus and many other cellular genes (43–45). Other steroid hormone receptors including progesterone receptor and androgen receptor, were also found to be able to efficiently recruit POU proteins to adjacent octamer motifs in the cell (45) or to interact with OCT1 independently of DNA (46). To identify co-factor(s) that are essential for OCT1 to activate Cdx-2 expression will add more to our understanding of cell type-specific autoregulation and cell type-specific functions of Cdx-2.

In summary, we demonstrated in this study the complexity of the role of the 31-bp cis-element Cdx-2POCT in regulating Cdx-2 expression and autoregulation. The complexity has been shown at three different levels: 1) expression of POU proteins may occur cell type specifically, 2) binding affinity of POU proteins to Cdx-2POCT also shows cell type specificity, 3) activation of Cdx-2 promoter via Cdx-2POCT by OCT1 depends upon its native DNA content. We suggest that POU proteins play important and sophisticated roles in modulating Cdx-2 expression in a cell type-specific manner.

Acknowledgment—We thank Drs. Winship Herr, Graeme Bell, and Bogi Andersen for providing cDNAs of OCT1, OCT2A, OCT2B, OCT3A, Skn-1a, and Skn-1I and Dr. Vincent Giguere for the TK-LUC luciferase reporter gene.

REFERENCES
1. Graef, I. A., and Crabtree, G. R. (1997) Science 277, 193–194
2. Glass, C. K., Rosenfeld, M. G. (2000) Genes Dev. 14, 121–141
3. Goodman, R. H., Smolik, S. (2000) Genes Dev. 14, 1553–1577
4. James, R., and Kazenwadel, J. (1991) J. Biol. Chem. 266, 3248–3251
5. German, M. S., Wang, J., Chadwick, R. B., and Rutter, W. J. (1992) Genes Dev. 6, 2165–2176
6. James, R., Erler, T., and Kazenwadel, J. (1994) J. Biol. Chem. 269, 15229–15237
7. Suh, E., Chen, L., Taylor, J., and Traber, P. G. (1994) Mol. Cell. Biol. 14, 7340–7351
8. Jin, T., and Drucker, D. J. (1996) Mol. Cell. Biol. 16, 19–28
9. Laser, B., Meda, P., Constanti, I., and Philippe, J. (1996) J. Biol. Chem. 271, 28894–28899
10. Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F., and Beck, F. (1997) Nature 386, 84–87
11. Tamai, Y., Nakajima, R., Ishikawa, T., Takaku, K., Seldin, M. F., Takeo, M. M. (1999) Cancer Res. 59, 2965–2970
12. Xu, F., Li, H., and Jin, T. (1999) J. Biol. Chem. 274, 34310–34316
13. Herr, W., and Cleary, M. A. (1995) Genes Dev. 9, 1679–1693
14. Treier, M., and Rosenfeld, M. G. (1996) Curr. Opin. Cell Biol. 8, 833–843
15. Ryan, A. K., and Rosenfeld, M. G. (1997) Genes Dev. 11, 1207–1225
16. Cleary, M. A., Stern, S., Tanaka, M., Herr, W. (1993) Genes Dev. 7, 72–83
17. Takeda, J., Seino, S., Bell, G. I. (1992) Nucleic Acids Res. 20, 4613–4620
18. Andersen, B., Schonemann, M. D., Flynn, S., E., Pearse, R. V., II, Singh, H., Rosenfeld, M. G. (1993) Science 260, 78–82
19. Jin, T., and Drucker, D. J. (1995) Mol. Endocrinol. 9, 1306–1320
20. Drucker, D. J., Jin, T., Asa, S., Young, T., and Brubaker, P. (1994) Mol. Endocrinol. 8, 1646–1655
21. Schreiber, E., Matias, P., Muller, M. M., and Schaffner, W. (1989) Nucleic Acids Res. 17, 6149
22. Jin, T., Branc, D. R., Zhang, X., Qi, S., Youngson, B., and Goss, P. E. (1999) Int. J. Cancer. 81, 104–112
23. Duprey, P., Chowdhury, K., Dressler, G. R., Balling, R., Simon, D., Guenet, J.-L., and Gruss, P. (1988) Genes Dev. 2, 1647–1664
24. Scott, M. P. (1992) Cell 71, 551–553
25. Bai, Y. Q., Akiyama, Y., Nagasaki, H., Yagi, O. K., Kikuchi, Y., Saito, N., Takeshita, K., Iwai, T., Yuzui, Y. (2000) Mol. Carcinog. 31, 184–188
26. Ee, H. R., Erler, T., Bhathal, P. S., Young, G. P., and Jame, R. J. (1995) Am. J. Physiol. 273, G3–G6
27. Wicking, C., Simms, L. A., Evans, T., Walsh, M., Chawengsaksophak, K., Beck, F., Chenexv/Trench, G., Young, J., Jass, J., Leggett, B., and Wainwright, B. (1998) Oncogene 17, 657–659
28. da Costa, L. T., He, T. C., Yu, J., Sparks, A. B., Morin, P. J., Polyak, K., Laken, S., Vogelstein, B., Kinzler, K. W. (1999) Oncogene 18, 5010–5014
29. Taylor, J. K., Bell, W., Levy, T., Suh, E., Siang, S., Mantle, N., and Traber, P. G. (1997) DNA Cell Biol. 16, 1419–1428
30. Troelsen, J. T., Mitchemmore, C., Spodbsberg, N., Jensen, A. M., Noren, O., and Spstrom, H. (1997) Biochem. J. 322, 833–838
31. Drummond, F., Sowden, J., Morrison, K., and Edwards, Y. H. (1996) Eur. J. Biochem. 236, 670–681
32. Colnot, S., Romagnolo, B., Lambert, M., Cluzeaud, F., Porteu, A., Vandewalle, D. L., and Simon, D. (1999) Mol. Cell. Biol. 19, 2718–2725
33. Chandran, U. R., Warren, B. S., Baumann, C. T., Hager, G. L., DeFranco, D. B. (1996) Genes Dev. 10, 457–465
34. O’Reilly, D., Hanscombe, O., O’Hare, P. (1997) EMBO J. 16, 2420–2430
35. Hussain, M. A., Lee, J., Miller, C. P., and Habener, J. F. (1997) Mol. Cell. Biol. 7, 7186–7194
36. Schubart, D. R., Kolink, A., Kosco-Vilbois, M. H., Botteri, F., Matthias, P. (1996) Nature 383, 538–542
37. Murphy, S., Yoon, J. B., Gerster, T., Roeder, R. G. (1992) Mol. Cell. Biol. 12, 3247–3261
38. Stern, S., Herr, W. (1991) Genes Dev. 5, 2555–2566
39. Chandran, U. R., Jessem, H. M., Drucker, D. J. (1999) J. Biol. Chem. 274, 2372–2378
40. Prefontaine, G. G., Walther, R., Giffin, W., Lemieux, M. E., Pope, L., Hache, R. J. (1999) J. Biol. Chem. 274, 26713–26719
41. Prefontaine, G. G., Lemieux, M. E., Giffin, W., Schild-Poulter, C., Pope, L., LaCasse, E., Walker, F., Hache, R. J. (1998) Mol. Cell. Biol. 18, 3416–3420
42. Song, C. S., Jang, M. H., Kim, S. C., Hassan, T., Roy, A. K., Chatterjee, B. (1998) J. Biol. Chem. 273, 21856–21866
