Identification of Domains Mediating Transcriptional Activation and Cytoplasmic Export in the Caudal Homeobox Protein Cdx-3*

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The caudal genes have important functions in embryonic development and cell differentiation. The caudal-related protein Cdx-2/3 (the protein designated Cdx-2 in the mouse and Cdx-3 in the hamster) is expressed in the gastrointestinal epithelium and in islet and enteroendocrine cells, where it activates proglucagon gene transcription. We show here that Cdx-3 sequences amino-terminal to the homeodomain (amino acids 1–180) function as a heterologous transcriptional activation domain when fused to the LexA DNA binding domain. A Cdx-3-Pit-1 fusion protein containing only the first 83 amino acids of Cdx-3 linked to the POU domain of Pit-1 markedly stimulated the transcriptional activity of a Pit-1-responsive promoter. Analysis of the transcriptional properties of Cdx-3 mutants in fibroblasts and islet cells revealed distinct amino-terminal subdomains that function in a cell-specific manner. Point mutations within the amino-terminal A domain were associated with reduced transcriptional activity. Furthermore, internal deletions and selected point mutations within domain A, but not the B or C domains, resulted in accumulation of mutant Cdx-3 in the cytoplasm. Unexpectedly, mutation of an Asp-Lys-Asp motif within domain A identified a putative cytoplasmic membrane-associated export signal that mediates Cdx-3 compartmentalization. These experiments delineate unique activities for specific amino-terminal sequences that are functionally important for Cdx-3 biological activity.

The caudal genes, originally identified in Drosophila (1), are expressed early during development in a concentration gradient across the anteroposterior axis of the embryo. Perturbation of caudal function as a result of inactivating mutations (2) or ectopic expression (3) leads to disruption of normal segmentalization and an abnormal body pattern. Caudal genes were subsequently identified and characterized in mammalian species, including mice and humans (4–6). The mammalian caudal homologues cdx-1 and cdx-2/3 are expressed early during embryonic development in the gut, tailbud, neural tube, and mesoderm, following which, expression during adult life is predominantly restricted to the epithelium of the gastrointestinal tract (4, 7). In contrast, the cdx-4 gene, which maps to the X-inactivation center of mouse and human X chromosomes (8), is expressed only transiently during embryonic development, from day 7 to day 10 post coitum (9).

Several lines of evidence suggest that members of the caudal gene family have important roles in the control of cell proliferation and differentiation in cells of endodermal origin. The chicken Caudal protein CHox-Cad is expressed in embryonic but not adult liver, and expression is reactivated in regenerating liver cells following partial hepatectomy, consistent with a role for CHox-Cad in hepatocyte differentiation (10). Conditional expression of murine cdx-2 in undifferentiated intestinal IEC-6 cells leads to cessation of cellular proliferation followed by resumption of growth and development of cellular characteristics reflective of more differentiated intestinal cell types (11). Overexpression of cdx-2 in human colon cancer cells up-regulates molecules involved in cell adhesion and cell-cell interactions, and cdx-2 expression in turn is modulated by basement membrane components (12). Taken together, these experiments provide substantial evidence linking cdx-2 with changes in cell growth and differentiation.

Complementary evidence for the biological importance of caudal genes in pattern formation and intestinal differentiation derives from analysis of loss of function mutations in mice. Disruption of the murine cdx-1 gene by homologous recombination results in anterior homeotic transformation of vertebrae, potentially due to the importance of cdx-1 for transcriptional control of members of the Hox gene cluster (13). Although homozygous cdx-2−/− mice are embryonic lethal, cdx-2+/− heterozygote mice exhibit a complex phenotype, with tail and skeletal abnormalities including a homeotic shift of vertebrae and rib malformations (14). Cdx-2+/− mice also exhibit stunted growth and develop multiple adenomatous intestinal polyps that do not express the remaining cdx-2 allele. These observations demonstrate that even cdx-2 haploinsufficiency leads to striking abnormalities in cellular differentiation pathways in vivo.

Despite the emerging biological importance of Caudal proteins, the structural determinants of caudal function remain poorly understood. Alignment of caudal family members demonstrates significant amino acid homology within subdomains, designated A–C (9, 15) of the amino-terminal region; however, the functional significance of these subdomains has not yet been ascertained. We recently demonstrated that the hamster cdx-2 homologue, designated cdx-3, is an important regulator of proglucagon gene transcription not only in pancreatic islets but also in enteroendocrine cells of the intestine (16, 17). To elucidate the specific regions of the Cdx-2/3 protein (the protein designated Cdx-2 in the mouse and Cdx-3 in the hamster) that are important for transcriptional activation and biological function, we have now carried out studies of the structural deter-
minants of Cdx-3-dependent activation of the proglucagon promoter in vitro.

MATERIALS AND METHODS

Plasmids—The plasmid pBAT7/8 and cdx-3 were kindly provided by Dr. M. S. German (San Francisco, CA). The plasmid pBAT7 is similar to pBAT7 (18), except that additional restriction sites were introduced into the polylinker to facilitate cloning. The pBAT7 expression vector is under the control of the cytomegalovirus promoter. The linker 5′-CTCTAGGACCATGTACGTGAGCTACAAGCTTCTCGAG-3′, containing an optimized Kozak consensus sequence and encoding the first seven amino acids of cdx-3, was ligated into pBAT7 at the Avr II and XhoI sites. To construct cdx-3 deletions, HindIII and XhoI restriction sites were introduced into the linker sequences, resulting in the insertion of a lysine at position 6 after tyrosine at position 5 and a change of aspartic acid to glutamic acid at position 8 of the Cdx-3 amino terminus. The polymerase chain reaction (PCR)2 was used to synthesize various amino-terminal, carboxyl-terminal, and internal deletions of cdx-3. The cdx-3 mutant cDNAs were cloned into the XhoI site of the expression vector pBAT7. The open reading frame for all mutant cdx-3 cDNAs was verified by DNA sequencing to ensure that the DNA and corresponding translated protein sequence was correct and in frame.

The full-length Pit-1 cDNA in the expression vector Pex-3.5 and the LexA/luciferase reporter and LexA-Pit-1(8–80) plasmids were obtained from Dr. H. Elsholtz (University of Toronto) and have been described (19, 20). The chimeric LexA-cdx-3 cDNAs containing various lengths of cdx-3 coding sequences were generated by fusing cdx-3 sequences to a cDNA encoding an amino acid LexA DNA binding domain from amino acids 82–92 of proglucagon promoter-luciferase reporter gene has been previously described (16). Site-directed mutagenesis was carried out using the Amersham Pharmacia Biotech unique site elimination mutagenesis kit, optimized for PCR (21). All mutant cdx-3 cDNAs were subject to DNA sequencing to verify the presence of the correct sequence change.

Cell Culture and Transfections—Babymouse kidney (BHK) fibroblasts and InR1-G9 islet cells (22) were grown in Dulbecco’s modified Eagle’s medium supplemented with 5% calf serum. Cells were transfected using the calcium phosphate method and harvested 18 h after transfection for determination of luciferase activity using a Bertold LB9501 luminometer. All transfections were carried out in triplicate on at least three separate occasions, with 5 μg each of luciferase reporter plasmid and the cdx-3 or LexA-cdx cDNAs.

Antiserum to cdx-3 and Western Blot Analysis—A hamster cdx-3 partial cDNA generated by PCR that encodes the carboxyl-terminal 54 amino acids was ligated in frame downstream of glutathione S-transferase sequences in a pGEX plasmid (Amersham Pharmacia Biotech). The glutathione S-transferase-cdx-3 fusion protein was purified using glutathione-Sepharose beads and used for antibody production in rabbits. For Western analyses, 5–10 μg of nuclear protein was size-fractionated by SDS-PAGE and transferred to a nitrocellulose membrane. The immunoreactive protein was detected by the ECL system (Amersham Pharmacia Biotech) with 1:2000 dilution of the primary Cdx-3 antiserum and 1:5000 dilution of the peroxidase-labeled anti-rabbit IgG as second antibody. Polyclonal c-Jun antibody (1:1000) was from Santa Cruz Biotechnology, Inc. (Santa Cruz, CA).

Nuclear Protein Extract and EMSA—Nuclear protein was isolated from transfected cells as described previously (16). Synthetic oligonucleotides corresponding to the proglucagon proximal promoter G1 sequences (16, 23) were annealed, purified by 5% polyacrylamide gel electrophoresis and 32P-labeled with the Klenow enzyme. EMSA was performed as described previously (16). Site-directed mutagenesis was carried out using the Amersham Pharmacia Biotech unique site elimination mutagenesis kit, optimized for PCR (21). All mutant cdx-3 cDNAs were subject to DNA sequencing to verify the presence of the correct sequence change.

RESULTS

The results of recent experiments suggest that amino acids 1–180, which make up the amino-terminal region of cdx-2, function as a heterologous activation domain (26). To identify functionally important regions of the Cdx-3 protein independent of the Cdx-3 homeobox, we generated a series of LexA-Cdx-3 fusion proteins. The transcriptional properties of these molecules were studied using a cotransfected reporter plasmid containing LexA DNA binding sites adjacent to a minimal promoter upstream of the luciferase cDNA coding sequences. As a control, we first analyzed the transcriptional properties of a LexA-Pit-1 cDNA containing the LexA DNA binding domain fused to amino acids 8–80 of the transcription factor Pit-1 (19). The Pit-1 amino-terminal sequences conferred approximately a 27-fold induction of reporter activity in transfected BHK cells, similar to the magnitude of induction reported with the identical LexA-Pit-1 plasmid in transfected COS-7 cells (19). Fusion of the entire cdx-3 open reading frame adjacent to the LexA DNA binding domain resulted in a 6-fold activation of reporter activity (Fig. 1a).

Although deletion of the sequences carboxyl-terminal to the cdx-3 homeodomain (LexA-cdx 1–250) was associated with a reduction in relative transcriptional activation, the cdx-3 carboxyl-terminal sequences alone, from amino acids 245–313, did not confer transcriptional activation to the LexA DNA binding domain. The greatest activation of luciferase activity was observed with the plasmid containing the cdx-3 amino-terminal sequences, LexA-cdx(1–180), resulting in a 25–40-fold activation of reporter activity (Fig. 1, a and b). Plasmids containing the homeodomain alone, or the homeodomain plus the carboxyl-terminal sequences, displayed no transcriptional activation (Fig. 1a).

The results of these experiments using chimeric proteins in transfected fibroblasts demonstrated that Cdx-3 amino acids 1–180 encode a transcriptional activation domain, in agreement with experiments carried out analyzing the transcriptional properties of the cdx-2 amino-terminal region (26). To determine whether protein subdomains within the 180 amino acid Cdx-3 amino-terminal region might be identified that are sufficient for conferring transcriptional activation, we analyzed the activity of a series of LexA fusions to the carboxyl-terminal regions of Cdx-3 amino-terminal sequences. In contrast to the potent transcriptional activation observed with LexA plasmids containing the intact 180 amino acid amino-terminal region of Cdx-3, LexA-cdx fusion genes containing smaller regions of cdx-3 amino-terminal sequences were transcriptionally inactive (Fig. 1b).

These experiments demonstrated that Cdx-3 amino-terminal sequences display functional activity when fused to the DNA binding domain of a bacterial transcription factor. To determine whether Cdx-3 amino-terminal sequences also mediate transcriptional activation in the context of a heterologous mammalian transcription factor, the first 120 amino acids of Pit-1 were replaced with amino acids 1–83 of Cdx-3. Transfection of BHK fibroblasts with the Cdx-3L–83/Pit-1 chimeric protein produced a 350-fold activation of luciferase activity.
(Fig. 2), significantly greater than the 100-fold activation of reporter activity observed following transfection of full-length wild-type Pit-1 (Fig. 2). In contrast, minimal activation was seen with the control plasmid Pit-1(121–191). These observations define a subregion of the Cdx-3 amino terminus (amino acids 1–83, containing subdomains A and B (Fig. 1c)) as being capable of functionally substituting for an endogenous activation domain in a mammalian transcription factor.

As the above experiments used heterologous DNA binding domains and DNA target sequences for analysis of Cdx-3 transcriptional activation, we next examined whether the amino-terminal Cdx-3 sequences were functionally important for transcriptional activation of the proglucagon promoter, a known cdx-3-responsive target gene (16, 27). A proglucagon promoter-luciferase reporter plasmid was cotransfected with a series of cdx-3 plasmids containing in-frame deletions in the cdx-3 amino-terminal coding sequence. Full-length cdx-3 produced an 8–17-fold activation of the proglucagon promoter (Fig. 3, a and b). Remarkably, internal deletion of Cdx-3 amino acids 84–180, containing sequences comprising subdomain C (Fig. 1c), had no effect on the relative transcriptional induction of the proglucagon promoter (Figs. 3a and 4). In contrast, deletions within the first 75 amino acids containing domains A and B, alone or in combination with more extensive carboxyl-
terminal deletions, were consistently associated with attenuation of the cdx-3-induction of the proglucagon promoter (Fig. 3, a and b). One of the smallest Cdx-3 amino-terminal mutants containing a deletion within domain A, Δ(8–41), displayed a clearly reduced ability to activate the proglucagon promoter. Progressive deletion of additional cdx-3 sequences, as represented by Δ(8–106) and Δ(8–139), produced a further decline in cdx-3-dependent transcriptional activation, whereas deletion of the entire cdx-3 amino-terminal domain (Δ(8–180)) resulted in complete loss of proglucagon promoter activation (Fig. 3b). These observations, taken together with the data demonstrating the functional activity of the first 83 amino acids in the context of a Cdx-3/Pit-1 fusion protein, clearly illustrate the importance of the Cdx-3 amino-terminal region for transcrip-
Transcriptional activation by the Caudal Protein Cdx-3

Fig. 4. Transcriptional properties of cdx-3 plasmids in InR1-G9 islet cells. The cdx-3 plasmids (5 μg) were cotransfected into hamster InR1-G9 glucagon-producing islet cells with 5 μg of the −82 proglucagon-luciferase plasmid, and the luciferase activity obtained was normalized to the background luciferase activity measured following cotransfection of the pBAT8 expression vector alone in the same experiment. Each plasmid was transfected in triplicate, and the data shown represent the mean ± S.E. for three different experiments. **, p < 0.01; ***, p < 0.001 (cdx-3 mutants compared with cdx-3 (wild-type)).
with wild-type Cdx-3, was detected by Western blotting (Fig. 7b).
Similarly, the Cdx-3(D10A) point mutation exhibited a significant reduction in transcriptional activity in both fibroblast and islet cells, without impairment of protein expression or G1 element binding (Fig. 7).

In contrast, the double mutants Cdx-3(D8A/K9M) and Cdx-3(K9M/D10A) and the triple point mutation Cdx-3(D8A/K9M/D10A) resulted in complete elimination of transcriptional activity in islet and fibroblast cells (Fig. 7a). Furthermore, Western blotting demonstrated marked reduction in protein expression of the three mutants, reflected in a corresponding decrease in G1 element binding activity (Fig. 7a). Analysis of the mutationally sensitive Cdx-3 amino-terminal sequences within domain A demonstrated that amino acids 8–10 resembled a diacidic Asp-X-Asp motif (Fig. 1c) that is remarkably similar to a recently described consensus sequence required for protein export from the endoplasmic reticulum (29).

These observations suggested that the reduction in nuclear expression of Cdx-3 mutant proteins harboring double or triple point mutations in a putative membrane export signal might be attributable to defective Cdx-3 trafficking from the cytoplasm to the nucleus. Analysis of the cellular localization of wild-type cdx-3 following transfection of BHK cells demonstrated that the majority of immunoreactive wild-type protein was localized to the nuclear compartment (Fig. 8). In contrast, a marked reduction in the levels of nuclear cdx-3(K9M/D10A) was observed in the same experiment, whereas the levels of cdx-3 and cdx-3(K9M/D10A) in the nuclear membrane fraction were com-

**Fig. 5.** Analysis of cdx-3 expression by EMSA and Western blotting. a and b, Cdx-3 plasmids were transfected into BHK cells, and then nuclear extracts were prepared and nuclear protein was utilized for EMSA experiments with a G1 proglucagon promoter probe (16) and for Western blotting with a Cdx-3-specific primary antibody (16). The numbered arrows to the left of the EMSA panels denote the migration position of the cdx-3 mutant-DNA complexes. NS, nonspecific complexes detected in the absence of transfected cdx-3. c, EMSA analysis of Cdx-3(8–41) and Cdx-3(8–74) mutants transfected into BHK cells, using the Gc proglucagon promoter oligonucleotide probe; complex formation was competed for using excess homologous wild-type Gc, or a 27-base pair heterologous oligonucleotide from the uncoupling protein 2 coding region (UCP). NS, nonspecific complexes; FP, free probe. Arrows denote the migration positions of the complexes formed with wild-type Cdx-3, Cdx-3(8–41), and Cdx-3(8–74).
endogenous wild-type Cdx-3 and transfected Cdx-3 point mutations, cotransfected with the 2/3 activated transcription of the individual cdx-3 plasmids harboring single point mutations, cotransfected with the −82 proglucagon-luciferase plasmid, was normalized relative to the values obtained following transfection of the wild-type cdx-3 plasmid into BHK fibroblasts (a) and InR1-G9 islet cells (b). * p < 0.05 (point mutations versus cdx-3 wild-type). c, Western blot analysis of wild-type Cdx-3, Cdx-3 point mutations, and endogenous c-Jun in transfected BHK cells. Due to the expression of endogenous cdx-3 in InR1-G9 cells, Western blot analysis of the identical transfected plasmids in InR1-G9 cells could not distinguish between endogenous wild-type Cdx-3 and transfected Cdx-3 point mutations (not shown).

Fig. 6. Transcriptional properties of Cdx-3 amino-terminal point mutations in BHK and InR1-G9 cells. a and b, the transcriptional activity of the individual cdx-3 plasmids harboring single point mutations, cotransfected with the −82 proglucagon-luciferase plasmid, was normalized relative to the values obtained following transfection of the wild-type cdx-3 plasmid into BHK fibroblasts (a) and InR1-G9 islet cells (b). * p < 0.05 (point mutations versus cdx-3 wild-type). c, Western blot analysis of wild-type Cdx-3, Cdx-3 point mutations, and endogenous c-Jun in transfected BHK cells. Due to the expression of endogenous cdx-3 in InR1-G9 cells, Western blot analysis of the identical transfected plasmids in InR1-G9 cells could not distinguish between endogenous wild-type Cdx-3 and transfected Cdx-3 point mutations (not shown).

DISCUSSION

The caudal-related gene designated cdx-2 in the mouse and cdx-3 in the hamster is expressed in both the glucagon-producing cells of the islets and the enteroendocrine cells of the intestine and activates the proglucagon promoter in cell transfection studies (16, 17, 27). The importance of Cdx-2/3 for proglucagon gene transcription was emphasized by experiments demonstrating that Cdx-2/3 activated transcription of the endogenous proglucagon gene in its native chromatin context in transfected islet cells in vitro (17). cdx-2/3 also regulates an increasing number of genes expressed in the intestine, including the sucrase isomaltase, carbonic anhydrase 1, lactase-phlorizin hydrolase, calbindin-D9K, apolipoprotein B, and intestinal phospholipase A/lysophospholipase gene promoters (11, 30–35).

Despite the emerging importance of caudal family members for pattern formation and cell differentiation, little is known about the functional relevance of domains outside the homeodomain, such as conserved domains A–C, which are postulated to be potentially important for Caudal protein biological activity (9). Analysis of Cdx-2 structure-function relationships using Cdx-2:GAL4 fusion proteins localized a transcriptional activation domain to the amino-terminal amino acids 15–180 of the Cdx-2 protein (26). Furthermore, the functional activity of the Cdx-2 amino-terminal activation domain was shown to be dependent on the contextual location of promoter binding sites in a cell-specific manner (26). These observations suggested that one or more cellular factors, present in intestinal cells but not fibroblasts, modified the transcriptional activity of Cdx-2 in vitro.

The results of our experiments have defined in more detail the specific and minimal amino-terminal Cdx-2/3 sequences capable of functioning as a heterologous activation domain in vitro. Our data demonstrate that whereas the amino-terminal 180 amino acids function as an activation domain in a LexA: Cdx fusion protein, as little as the first 83 amino acids of Cdx-3 are capable of functioning as a heterologous transcriptional activation domain when fused to Pit-1 sequences in vitro. Furthermore, removal of amino acids 84–180 from the native Cdx-3 protein had no effect on the transcriptional activity of Cdx-3 in BHK fibroblasts or InR1-G9 islet cells. These results provide further evidence that the amino-terminal 83 amino acids in Cdx-3 can function as an activation domain in the context of the native Cdx-3 protein.

Whereas deletions within the first 84 amino acids of Cdx-3 were generally associated with diminution of transcriptional activity in BHK cells, Cdx-3(8–74) did not exhibit a reduction in transcriptional activity in InR1-G9 islet cells. These findings may be potentially explained by the presence of at least two independent modular domains within the first 180 amino acids of Cdx-3 that can functionally compensate for each other in InR1-G9 islet cells, but not in BHK fibroblasts. These observations provide further experimental support for the existence of cell-specific factors (26), differentially expressed in islet versus fibroblast cells, that interact with the Cdx-3 activation domain(s) to modify functional activity in a cell-specific manner.

Alignment of the amino-terminal regions of the Caudal homeodomain proteins reveals a number of highly conserved subdomains, designated A–C (Fig. 1c) amino-terminal to the homeobox (9). One of the conserved regions in Caudal proteins, domain A, is represented by the first 14 amino acids (MYV-SYLLDKDVSAMY) in Cdx-3. Two additional conserved subdomains, designated B (amino acids 68–78) and C (amino acids 161–172) of Cdx-3 (Fig. 1c) are also located within the first 180 amino acids amino-terminal to the homeodomain (9). Chimeric proteins containing either domain B (LexA:Cdx (42–83)) or domain C (LexA:cdx141–180) were not transcriptionally active, suggesting that subdomains B and C alone are not sufficient to confer transcriptional activation in the presence of a heterologous DNA binding domain. Furthermore, deletions in domains B and C, as represented by cdx-3(84–180), did not impair transcriptional activation. In contrast, single point mutations in domain A, as represented by Cdx-3 (D10A), exhibited reduced transcriptional activity despite normal levels of nuclear expression and DNA binding, illustrating the importance of domain A for transcriptional activation.

Our results identify an amino-terminal subdomain that is critical for cdx-3 expression. Classical nuclear localization signals mediating nuclear import are generally characterized by clusters of basic amino acids, in contrast to the mutationally sensitive diacidic motif identified here for cdx-3 (36). Hence, it seems unlikely that sequences within domain A of the Caudal proteins, specifically the DXXE motif, constitute a functional nuclear localization signal. Furthermore, domain A is not hydrophobic and would not be predicted to function as a leader protein.
sequence directing wild-type Cdx-3 to the endoplasmic reticulum compartment (37). Moreover, deletion of the DKD motif from Cdx-3 did not compromise nuclear localization of amino-terminally deleted cdx-3 plasmids. The striking homology of the Cdx-3 DXE sequence to a recently described endoplasmic reticulum export signal (29) raises the possibility that Cdx-3 may normally associate with a cytosolic membrane compartment, perhaps in a transient and/or regulated manner. It thus appears that although a functional DXE motif may be necessary for export from a cytoplasmic compartment, it is not absolutely necessary for cytoplasmic to nuclear translocation.

Despite considerable progress in the elucidation of molecular chaperones and mechanisms important for protein folding and export of proteins from the endoplasmic reticulum to the golgi and plasma membranes (37, 38), the molecular machinery that directs proteins such as transcription factors from the cytoplasm to the nucleus remains less well understood (36). The implication from our findings that wild-type Cdx-3 may be transiently associated with a cytoplasmic membrane fraction is not without precedent. For example, both endogenously expressed and transfected engrailed homeodomain proteins have been localized to cytoplasmic caveolar membrane fractions in subcellular fractionation experiments (39). Furthermore, the sterol regulatory element binding protein-1 transcription factor has also been localized to a cytosolic membrane fraction. Sterol depletion leads to proteolytic generation of a smaller sterol regulatory element binding protein-1 molecule that subsequently translocates from the cytoplasmic membrane compartment to the nucleus (40). Finally, secreted signaling proteins (41) and direct protein-protein interactions (42) may contribute to regulation of cytoplasmic transcription factor sequestration and nuclear import, as shown for the homothorax gene that directly interacts with extradenticle to induce nuclear translocation; in homothorax mutant embryos, extradenticle remains transcriptionally inactive due to cytoplasmic sequestration (42).

Our results demonstrate that sequences within cdx-3 domain

![Figure 7](http://www.jbc.org/)

**Fig. 7.** a, relative transcriptional activity of clustered Cdx-3 amino-terminal point mutations in BHK fibroblasts and InR1-G9 islet cells. The transcriptional activity of the cdx-3 plasmids harboring single, double, or triple point mutations, cotransfected with the −82 proglucagon-luciferase plasmid, was normalized relative to the values obtained following transfection of the wild-type cdx-3 plasmid into BHK fibroblasts or InR1-G9 islet cells. Each plasmid was transfected in triplicate, and the data shown represent the mean ± S.E. of three separate experiments. *, p < 0.05; **, p < 0.05 (transcriptional activity of point mutations versus wild-type cdx-3). b, Western blot analysis of nuclear extracts following transfection of wild-type cdx-3 and cdx-3 point mutations in BHK fibroblasts. c, EMSA of BHK cell extracts transfected with wild-type and cdx-3 amino-terminal point mutations using the proglucagon promoter G1 element probe. † denotes transfection of the expression vector pBAT7 alone. FP, free probe.

**Fig. 8.** Western blot analysis of cytoplasmic and nuclear fractions following transfection of BHK cells with pBAT7 alone or with wild-type cdx-3 or cdx-3(K9M/D10A). N.E., nuclear extracts; N.M., nuclear membranes; C.M., cytoplasmic membranes. Cytoplasmic and nuclear membrane fractions were isolated as described under "Materials and Methods."
Increasing evidence that even partial reductions in cdx-2/3 expression are associated with changes in cell physiology (12), proliferation (14), and tissue development (14), mutations in the Cdx-2/3 amino-terminal region that affect protein compartmentalization or transcriptional activation may be expected to produce a significant biological phenotype in vivo.

REFERENCES
1. Mlodzik, M., Fjose, A., and Gehring, W. A. (1985) EMBO J. 4, 2961–2969
2. Macdonald, P. M., and Struhl, G. (1986) Nature 324, 837–845
3. Mlodzik, M., Gibson, G., and Gehring, W. J. (1990) Development 109, 271–277
4. James, R., and Kazenwadel, J. (1991) J. Biol. Chem. 266, 3246–3251
5. Hu, Y., Kazenwadel, J., and James, R. (1993) J. Biol. Chem. 268, 27214–27225
6. Bonner, C. A., Loftus, S. K., and Wasmuth, J. J. (1995) Genomics 28, 206–211
7. Duprey, P., Chowdhury, K., Dressler, G. R., Balling, R., Simon, D., Guenet, J., and Gruss, P. (1995) Cell 83, 641–653
8. Lorentz, O., Duluc, I., De Arcangelis, A., Simon-Assmann, P., Kedinger, M., and Freund, J.-N. (1997) J. Cell Biol. 139, 1553–1565
9. Subramanian, V., Meyer, B. L., and Gruss, P. (1995) Cell 83, 641–653
10. Chawengsaksophak, K., James, R., Hammond, V. E., Kontgen, F., and Beck, F. (1997) Nature 386, 84–87
11. Joly, J. S., Maury, M., Joly, C., Duprey, P., Boulekbache, H., and Condamine, H. (1992) Differentiation 50, 75–87
12. Jin, T., and Drucker, D. J. (1996) Mol. Cell. Biol. 16, 19–28
13. Jin, T., Trinh, D. K. Y., Wang, F., and Drucker, D. J. (1997) Mol. Endocrinol. 11, 203–209
14. German, M. S., Wang, J., Chadwick, R. B., and Rutter, W. J. (1992) Genes Dev. 6, 2165–2176
15. Lew, A. M., and Elsholtz, H. P. (1995) J. Biol. Chem. 270, 7156–7160
16. Elsholtz, H. P., Lew, A. M., Albert, P. R., and Sundmark, V. C. (1991) J. Biol. Chem. 266, 22919–22925
17. Chen, X., Liu, W., Quinte, L., and Scala, G. (1997) Nucleic Acids Res. 25, 682–684
18. Drucker, D. J., and Mojsis, J. (1988) Endocrinology 123, 1861–1867
19. Wang, M., and Drucker, D. J. (1995) J. Biol. Chem. 270, 12364–12365
20. Marmirol, S., Ognibene, A., Baveloni, A., Cinti, C., Coclo, L., and Maraldi, N. M. (1994) J. Biol. Chem. 269, 13–16
21. Kaufmann, S. H., Gibson, W., and Shaper, J. H. (1983) J. Biol. Chem. 258, 2710–2719
22. Taylor, J. K., Levy, T., Suh, E. R., and Traber, P. G. (1997) Nucleic Acids Res. 25, 2293–2300
