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Nucleotide requirements for CDX2 binding to the *cis* promoter element mediating intestine-specific expression of guanylyl cyclase C

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Abstract Guanylyl cyclase C (GC-C), specifically expressed by intestinal epithelial cells, is the receptor for the *Escherichia coli* heat-stable enterotoxin that causes diarrhea. Tissue-specific expression of GC-C is mediated by the intestinal transcriptional regulator CDX2. This *trans*-activating protein regulates intestine-specific expression by binding to a critical sequence in the proximal promoter of GC-C. The precise nucleotide elements mediating CDX2 binding to promoter elements remain undefined. Several nuclear proteins form complexes with a DNA probe containing the promoter element of GC-C mediating CDX2 binding. The present study examined the nucleotide requirements in the consensus binding site and flanking regions in the *cis* element that mediates specific CDX2 binding to the promoter of GC-C. These studies identified seven core base pairs in the critical promoter element mediating tissue-specific expression of GC-C that are required for CDX2 binding. In addition, base pairs flanking this core sequence contribute to and are required for CDX2 recognition. These studies describe the precise nucleotide sequence within the GC-C promoter that comprises the CDX2 binding site required for intestine-specific expression.

Key words: Guanylyl cyclase C; CDX2; Transcriptional regulation; *cis* Element

1. Introduction

Guanylyl cyclase C (GC-C) is the receptor for the *Escherichia coli* heat-stable enterotoxin (STa), a principal cause of travelers' diarrhea, endemic diarrhea in underdeveloped countries, and neonatal diarrhea in agriculturally important animal herds [1,2]. Binding of STa, or the endogenous ligands uroguanylin or guanylin [3–7], induces the catalytic domain of GC-C to convert GTP to cGMP. Accumulation of cGMP induces net secretion of fluid and electrolytes by enterocytes resulting in diarrhea. In humans, GC-C is specifically expressed only in differentiated intestinal epithelial cells from the duodenum to the rectum [8–11]. Detection of GC-C expression in extra-intestinal sites appears to be a sensitive and specific marker for colorectal cancer metastases [12].

Intestine-specific expression of human GC-C is mediated by a conserved region in the promoter from +118 to −257 bp relative to the start site of transcription [13,14]. DNA footprint analysis of the GC-C promoter identified a protected region from −74 to −82 [14,15], containing a 5 bp consensus binding sequence for the transcription factor CDX2 [14–16]. This region, FP1, is required for intestine-specific expression of a GC-C luciferase construct [15].

CDX2 is a member of the homeobox family of genes related to the *Drosophila melanogaster* caudal gene. Homeobox proteins are critical in processes underlying embryonic development and have been implicated in the maintenance of adult tissue architecture [17–20]. CDX2 plays a role in establishing the differentiated phenotype of intestinal epithelial cells [21–23], and it regulates the transcription of enzymes important for enterocyte function, including sucrase–isomaltase [16,24] and lactase–phlorizin [25,26]. Expression of CDX2 and GC-C is well correlated along the rostral–caudal and crypt-to-villus axes [8,11,27,28].

Caudal family homeodomain proteins, including CDX2, bind DNA in part by recognizing a specific consensus sequence TTTAT/C [16,19,20,29]. Earlier studies demonstrated that the base pair sequence in intestinal promoters, including GC-C, mediating CDX2–DNA recognition includes the TTTAT/C consensus sequence [14,16,25]. However, the precise nucleotide sequences required for specific CDX2–DNA interaction, including flanking stabilization sequences, remain undefined. Conversely, although CDX2 binds to the *cis*-promoter element regulating intestine-specific expression of GC-C, other proteins also form complexes with this portion of the GC-C promoter [13,14]. The present study examined the nucleotide sequence in the consensus and flanking regions that mediates recognition and stabilization of CDX2 binding to the critical *cis* element of the GC-C promoter required for intestine-specific expression.

2. Materials and methods

2.1. Cell culture

Cell lines, obtained from American Type Culture Collection (Rockville, MD, USA), were grown in a 50:50 mixture of Ham's F12 medium and Dulbecco's modification of Eagle's medium (DMEM/F12, Mediatech, Herndon, VA, USA) supplemented with 10% fetal bovine serum (BioWhittaker, Walkersville, MD, USA), 10 000 U/ml penicillin, and 10 000 μg/ml streptomycin (BioWhittaker). Cells were maintained at 37°C in a humidified 5% CO_2/95% air atmosphere.

2.2. Nuclear protein extraction

Nuclear extracts were prepared as previously described [30]. Nuclear protein concentration was determined by BCA protein assay (Pierce, Rockford, IL, USA).

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2.3. Nuclear protein binding reaction

Binding reactions (20 μl) contained 5.0 μg of nuclear extract, 5.0 mM dithiothreitol, 1.0 μg poly-dI·dC-poly-dI·dC (Amersham Pharmacia Biotech, Piscataway, NJ, USA), and 0.5% Nonidet P-40 (Sigma, St. Louis, MO, USA) in binding buffer (4.0% glycerol, 150 mM NaCl, 10 mM Tris–HCl, pH 7.5, 2.5 mM $MgCl_2$). Reactions were pre-incubated at 37°C for 30 min, radiolabeled probe (50 000–100 000 cpm) and/or competitor (μM) added, then incubated for an additional 30 min at 37°C. In supershift studies, 2.0 μl of mouse CDX1 or CDX2 polyclonal antibody (P. Traber, University of Pennsylvania, Philadelphia, PA, USA) were added to reactions after the pre-incubation for 30 min before addition of probe.

2.4. Electrophoretic mobility shift assays (EMSA)

Binding reactions were loaded onto 0.75 mm×16 cm^2 6.0% non-denaturing polyacrylamide gels prepared using 30% 37.5:1 acrylamide:bisacrylamide (Bio-Rad, Hercules, CA, USA) and 0.5×TBE (45 mM Tris borate, 1.0 mM EDTA, pH 8.3, Eppendorf, Westbury, NY, USA), pre-run in 0.5×TBE at 200 V until the current reached 9.0 mA, then electrophoresed at 35 mA in 0.5×TBE buffer for ∼30 min. Some lanes contained free probe and dye only (4.0% glycerol, 0.02% bromophenol blue, 25 mM Tris–HCl, pH 7.5). Gels were dried and exposed to phosphor plates, developed on a PhosphorImager SI (Molecular Dynamics, Sunnyvale, CA, USA), and analyzed by ImageQuant software (Molecular Dynamics).

2.5. Competitor synthesis

Double-stranded oligomers that served as the labeled probe for competitive binding studies were synthesized by the Nucleic Acid Facility, Kimmel Cancer Institute (Thomas Jefferson University, Philadelphia, PA, USA). Wild-type and DC4 probes (Fig. 1) were derived from the GC-C promoter and included FP1 (bold). Wild-type oligomer, with 9 bp flanking each side of FP1, was sequentially mutated [16] to construct six 27 bp competitors (Fig. 1). Three competitors were constructed as negative controls, containing 3 bp disruptions within FP1 (Fig. 1). Nine 27 bp competitors were prepared with single base pair changes in FP1 (Fig. 1). Three competitors were prepared with 3′ or 5′ flanking CT repeats and a single base pair change in, or truncated version of, FP1 (Fig. 1). Two shorter competitors were prepared with CT repeats flanking FP1 or FP1 with TTT mutated to GGG (Fig. 1). Complementary strands were annealed in buffer (10 mM Tris, pH 7.5, 50 mM NaCl, 1.0 mM EDTA) to a final concentration of 1.0 μM on a Hybaid thermal cycler (Teddington, UK) by incubation at 94°C for 10 min then a 1°C/min ramp to 26°C, followed by transfer to ice and storage at 4°C. Alternatively, probes were annealed by boiling in H_2O for 5 min, then slowly cooled to room temperature (∼1°C/min) and stored at 4°C.

2.6. Probe labeling

Annealed oligomers (5.0 pmol) were end-labeled using 1 U of T4 polynucleotide kinase (PanveraTakara, Madison, WI, USA) and 2.0 μl of 7000 mCi/mmol [γ-^{32}P]ATP (ICN, Costa Mesa, CA, USA). Free [γ-^{32}P]ATP was removed using the Qiaquick Nucleotide Removal kit (Qiagen, Valencia, CA, USA) or Micro Bio-Spin chromatography columns (Bio-Rad). Probes were diluted in 10 mM Tris–HCl, pH 7.5, to 50 000–100 000 cpm/μl.

2.7. Cloning of human CDX2

T84 cDNA was reverse-transcribed in a 20 μl RT reaction containing 1.0 μg of T84 total RNA, 0.5 μg of oligo-dT (15-mer, Promega, Madison, WI, USA), 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.0 mM dNTP, 20 U RNasin (Promega), and 5.0 U AMV-RT (Promega) using a Hybaid thermal cycler with the following program: 48°C for 60 min, 94°C for 5 min, 25°C for 5 min. CDX2-specific oligonucleotide primers (*Hin*dIII, forward: 5′-TACT**AAGCTT**TGGTGA-GGTCTGCTCCCG-3′; *Xba*I, reverse: 5′-TAG**TCTAGA**AATTGCT-CTGCCGCTGCAG-3′) [31], designed with 5′ insertions of unique restriction endonuclease sites (bold), were used to amplify human CDX2 from T84 cDNA. PCR was performed using the high fidelity LA PCR Kit (PanveraTakara) in 50 μl reactions containing 10 μl of T84 cDNA product, 1×GC buffer, 2.5 mM $MgCl_2$, 0.5 mM dNTP, 10 pmol each of forward and reverse primers, and 2.5 U of TaKaRa *LA Taq* DNA polymerase (PanveraTakara) using a Hybaid thermal cycler with the following program: 94°C for 60 min, 30 cycles (94°C for 30 s, 58°C for 30 s, 72°C for 2 min), 72°C for 5 min, 25°C for 5 min. Amplified DNA product was digested with *Hin*dIII and *Xba*I, subcloned into the plasmid vector pRc/CMV (Invitrogen, Carlsbad, CA, USA), and sequenced by the Nucleic Acid Facility, Kimmel Cancer Institute (Thomas Jefferson University, Philadelphia, PA, USA).

2.8. In vitro protein expression

Human CDX2 protein was generated by the TNT Quick-Coupled Kit (Promega) with linearized pRc/CMV human *CDX2* as template.

3. Results and discussion

Nuclear extracts from T84 human colon cancer cells formed several complexes with labeled WT, one of which contained CDX2 (Fig. 2A,B) [15]. Binding of labeled WT to T84 cell nuclear extracts was effectively competed by unlabeled WT. In contrast, unlabeled competitor in which the TTT of the consensus CDX2 binding sequence (TTTAT) was converted to GGG (GGGAT; GGG, Fig. 1) did not compete with labeled WT (Fig. 2). The nucleotides in FP1 mediating CDX2 binding were defined by EMSA, examining competition for binding to T84 nuclear extracts of labeled WT and unlabeled competitors

OLIGONUCLEOTIDE SEQUENCES
(SENSE STRAND)

WILD-TYPE AND GGG SEQUENCES

```
WT  5′ TAA TCT CTC TGT TTA TAG CTC TGA CCT 3′
GGG    TAA TCT CTC TGG GGA TAG CTC TGA CCT
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THREE BASE PAIR COMPETITORS
PROTECTED REGION: **TGT TTA TAG**

```
MUT1   GCC
MUT2      GAG
MUT3         AGA
MUT7            GTG
MUT8               GGC
MUT9                  GCT
MUT4                     AGA
MUT5                        GTC
MUT6                           AAG
```

SINGLE BASE PAIR COMPETITORS
PROTECTED REGION: **TGT TTA TAG**

```
PRO1          G
PRO2           T
PRO3            G
PRO4             G
PRO5              G
PRO6               C
PRO7                G
PRO8                 C
PRO9                  T
```

ADDITIONAL COMPETITOR CANDIDATES

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DC1    TTT TTA TAG CTC TCT CTC TCT CTC TCT
DC2    TCT CTC TCT CTC TCT CTC TTT TTA TAG
DC3    TTT ATA GCT CTC TCT CTC TCT CTC TCT
DC4    CTC TGT TTA TAG CTC TCT
DC5    CTC TGG GGA TAG CTC TCT
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Fig. 1. Competitor sequences employed in competitive EMSA. For simplicity, the sense strand of the promoter was selected to describe the sequences and designate the orientations of the mutants, but all alterations represent appropriate changes in both strands.

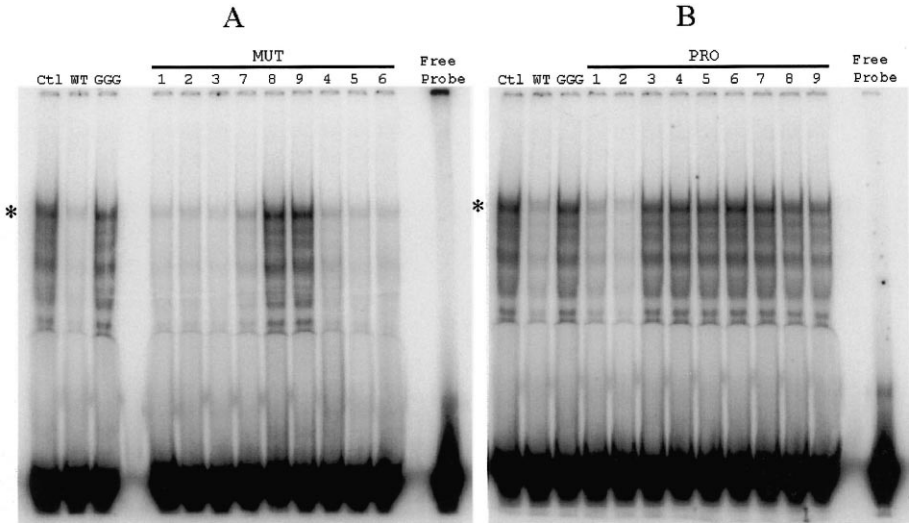

Fig. 2. Competitive EMSA of labeled WT and unlabeled MUT or PRO mutants. A: Nuclear extracts from T84 cells were combined with labeled WT in the absence (Ctl) or presence of a 200-fold excess of unlabeled mutant competitors. Binding reactions were electrophoresed and analyzed as described in Section 2. B: Nuclear extracts from T84 cells were combined with labeled WT in the absence (Ctl) or presence of a 40-fold excess of unlabeled mutant competitors. Binding reactions were analyzed as in A. Data are representative of at least two experiments for each condition. An asterisk represents the CDX2–probe complex.

(Fig. 2). MUT1–6 competed with labeled WT (Fig. 2A); in contrast, MUT8, with three substitutions in the consensus CDX2 binding sequence, did not compete with labeled WT. MUT9 also did not compete, whereas MUT7 partially competed, with labeled WT (Fig. 2A).

These observations suggest that the consensus CDX2 recognition sequence mediates CDX2 binding to the FP1 region of the GC-C promoter. The contribution of each of the nine individual base pairs within FP1 to protein binding was subsequently examined employing mutant constructs PRO1–9 containing single base pair substitutions (Fig. 1). Competitors containing substitutions for bases comprising the consensus CDX2 binding sequence (PRO3, PRO4, PRO5, PRO6, PRO7) did not compete with labeled WT (Fig. 2B). Base pairs in 3′ positions 8 and 9 (PRO8, PRO9) also did not compete for labeled WT (Fig. 2B). In contrast, mutants containing substitutions within FP1, but 5′ to the consensus CDX2 binding sequence, competed with labeled WT (PRO1 and PRO2; Fig. 2B). These results support the suggestion that the consensus CDX2 binding sequence and the two 3′ base pairs mediate association of CDX2 with WT, with little or no contribution by the two 5′-most base pairs of FP1.

The contribution of nucleotides flanking the consensus site was specifically examined. Changing G to T at the second 5′ position (PRO2; Fig. 1) did not alter the efficacy of competition with labeled WT for binding (Fig. 2B); this substitution was employed in some constructs (DC1–3; Fig. 1). Similarly, substituting wild-type flanking regions with dinucleotide repeating sequences (CT; DC4, Fig. 1) did not reduce the efficacy of competition with labeled WT for binding (Fig. 3); this substitution was employed in some constructs (DC1–5, Fig. 1). Removing all 5′ base pairs preceding FP1 (DC1, Fig. 1) did not diminish the efficacy of the mutant competitor to compete for binding (Fig. 3A,B). In contrast, removing all 3′ base pairs following FP1 (DC2, Fig. 1) eliminated the ability of the mutant competitor to compete for binding (Fig. 3A,B). These data suggest that the presence of base pairs flanking the 3′ end of FP1 enhances stability of CDX2 binding to the GC-C promoter.

Although the 3′ seven base pairs of FP1, TTTATAG, are critical for CDX2 binding, the role of the two 5′ base pairs required clarification. Removing the two 5′ base pairs of the FP1 from DC1 (DC3, Fig. 1) completely eliminated the ability of that competitor to compete with labeled WT (Fig. 3A). Thus, the presence of base pairs in the first and second positions at the 5′ end of the FP1 is important, but their identity is not critical, for CDX2 binding.

After identification of the individual sequence requirements for CDX2 binding, the base pairs were incorporated into an

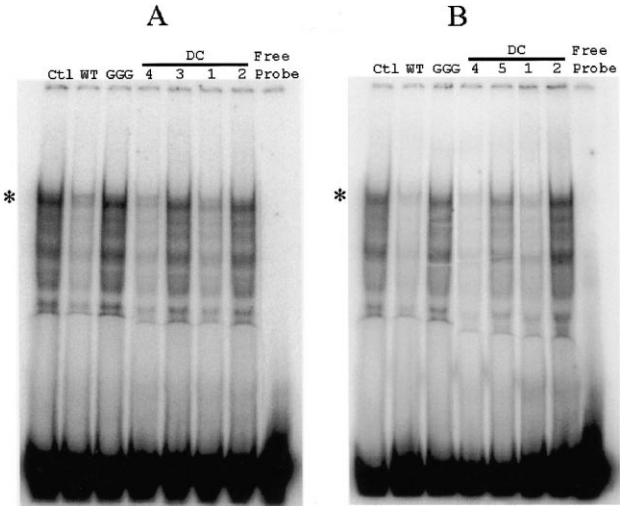

Fig. 3. Competitive EMSA of labeled WT and unlabeled DC mutants. A, B: T84 nuclear extracts were combined with labeled WT in the absence (Ctl) or presence of a 40-fold excess of the indicated competitors. Binding reactions were performed and analyzed as in Fig. 2. Data are representative of at least two experiments for each condition. All lanes shown in each panel are from the same gel; phosphorimages of a given gel were spliced together for clarity.

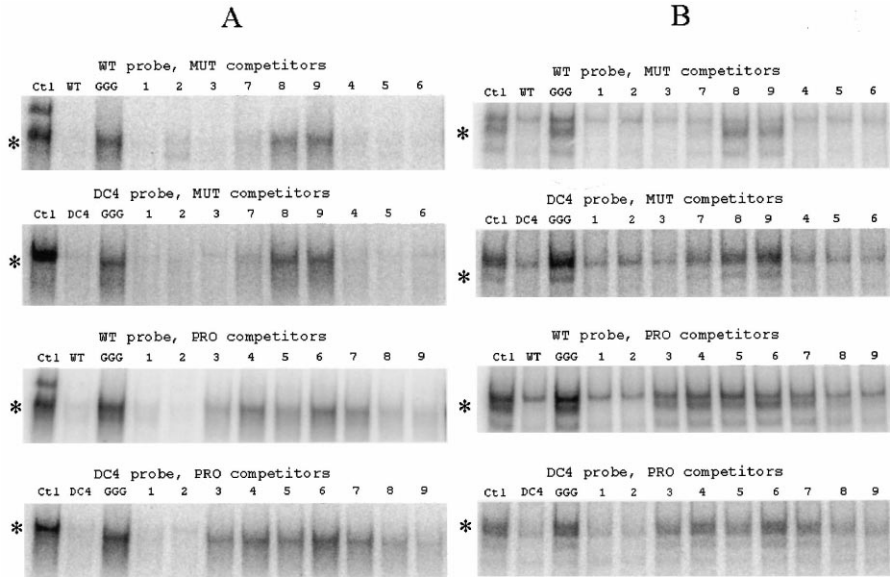

Fig. 4. Competitive EMSA of labeled WT or DC4 and unlabeled MUT or PRO mutants employing intestinal cell nuclear extracts or TNT CDX2. A, B: TNT protein (A) or Caco-2 nuclear extracts (B) were incubated with labeled WT or labeled DC4 in the absence of (Ctl) or presence of a 250-fold excess of the indicated competitors. Binding reactions were performed and analyzed as in Fig. 2. Data are representative of at least two experiments for each condition.

18 bp oligomer. The 18-mer possesses the nine core base pairs of FP1, three additional base pairs on the 5′ end to optimize double-stranded DNA stability, and six additional base pairs on the 3′ end (DC4, Fig. 1) to both optimize oligomer stability and to stabilize CDX2 binding. DC4 effectively competed, whereas DC4 with TTT mutated to GGG (DC5, Fig. 1B) did not compete, for binding with labeled WT (Fig. 3B). The sequence requirements for binding of labeled DC4 to CDX2, determined by competitive EMSA, were identical to those of labeled WT with respect to the core nine base pairs within FP1, employing intestinal cell nuclear extracts (Fig. 4A) or TNT CDX2 (Fig. 4B). Similarly, the requirements of the 5′ and 3′ sequences flanking FP1 for binding of CDX2 to labeled DC4 in intestinal cell nuclear extracts were confirmed (compare Figs. 3 and 5).

The ability of DC4 to bind specifically to CDX2 was examined employing nuclear extracts from T84 and Caco-2 human colon cancer cells, HepG2 human hepatoma cells, HeLa fibroblasts, and TNT human CDX2 (Fig. 6). Extracts from T84 and Caco-2 cells, but not from HepG2 or HeLa cells, formed a single specific complex with labeled DC4, compared to labeled WT which formed specific and non-specific complexes in intestinal and extra-intestinal cells (compare Figs. 2 and 3 with Figs. 4–6) [15]. The major complex formed between labeled DC4 and T84 or Caco-2 cells exhibited a mobility identical to that of the complex formed between DC4 and TNT human CDX2 (Fig. 6). An antibody against CDX2 decreased the mobility of the specific complex formed between DC4 and nuclear extracts from T84 or Caco-2 cells or TNT human CDX2 (Fig. 6). In contrast, an antibody against a related homeodomain transcription factor, CDX1, did not alter the mobility of the intestine-specific complex (Fig. 6). These data demonstrate that DC4 contains a specific binding site for

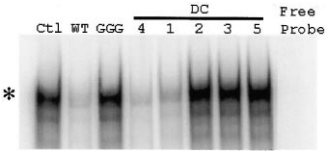

Fig. 5. Competitive EMSA of labeled DC4 and unlabeled DC mutants. T84 nuclear extracts were incubated with labeled DC4 probe in the absence (Ctl) or presence of a 200-fold excess of the indicated competitors. Binding reactions were performed and analyzed as in Fig. 2. Data are representative of at least two experiments for each condition.

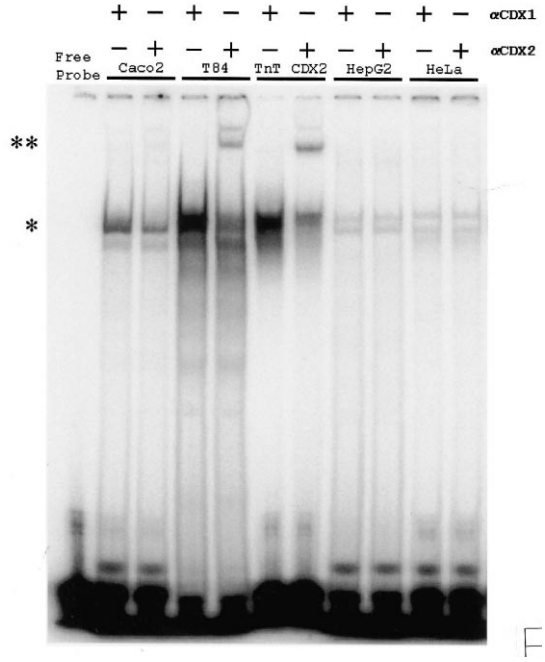

Fig. 6. Interaction of labeled DC4 with CDX2. Nuclear extracts from the indicated cells or TNT protein were incubated with labeled DC4 in the EMSA binding reaction. Binding reactions were electrophoresed and analyzed as in Section 2. Data are representative of at least two experiments for each condition. A double asterisk represents the α CDX2+ CDX2+ probe complex.

CDX2 alone that is sufficient for binding the transcription factor.

Caudal family homeodomain proteins regulate processes central to differentiation and development during ontogeny and the maintenance of differentiated tissues in the adult [16,19,20,29]. The data presented in this study are the first to completely define the requirements of specific base pairs for recognition, and flanking DNA for stabilization, of the direct interaction of CDX2 with a promoter mediating intestine-specific expression of a gene. In addition, they are the first to define the nucleic acid probes required to examine the specific interaction of CDX2 and *cis*-promoter elements by EMSA [15,16,25]. These data will be employed to further examine the role of CDX2 in regulating the expression of GC-C and other genes characteristic of differentiated intestinal epithelial cells.

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References

[1] Cohen, M.B., Jensen, N.J., Hawkins, J.A., Mann, E.A., Thompson, M.R., Lentze, M.J. and Giannella, R.A. (1993) J. Cell Physiol. 156, 138–144.
[2] Giannella, R.A. (1995) J. Lab. Clin. Med. 125, 173–181.
[3] Currie, M.G., Fok, K.F., Kato, J., Moore, R.J., Hamra, F.K., Duffin, K.L. and Smith, C.E. (1992) Proc. Natl. Acad. Sci. USA 89, 947–951.
[4] Hamra, F.K., Forte, L.R., Eber, S.L., Pidhorodeckyj, N.V., Krause, W.J., Freeman, R.H., Chin, D.T., Tompkins, J.A., Fok, K.F. and Smith, C.E. (1993) Proc. Natl. Acad. Sci. USA 90, 10464–10468.
[5] Krause, W.J., Freeman, R.H., Eber, S.L., Hamra, F.K., Fok, K.F., Currie, M.G. and Forte, L.R. (1995) Acta Anat. 153, 210–219.
[6] Li, Z., Taylor-Blake, B., Light, A.R. and Goy, M.F. (1995) Gastroenterology 109, 1863–1875.
[7] Krause, W.J., Freeman, R.H., Eber, S.L., Hamra, F.K., Currie, M.G. and Forte, L.R. (1997) Gen. Comp. Endocrinol. 107, 229–239.
[8] Krause, W.J., Cullingford, G.L., Freeman, R.H., Eber, S.L., Richardson, K.C., Fok, K.F., Currie, M.G. and Forte, L.R. (1994) J. Anat. 184, 407–417.
[9] Carrithers, S.L., Parkinson, S.J., Goldstein, S., Park, P., Robertson, D.C. and Waldman, S.A. (1994) Gastroenterology 107, 1653–1661.
[10] Carrithers, S.L., Parkinson, S.J., Goldstein, S.D., Park, P.K., Urbanski, R.W. and Waldman, S.A. (1996) Dis. Colon Rectum 39, 171–181.
[11] Swenson, E.S., Mann, E.A., Jump, M.L., Witte, D.P. and Giannella, R.A. (1996) Biochem. Biophys. Res. Commun. 225, 1009–1014.
[12] Waldman, S.A., Cagir, B., Rakinic, J., Fry, R.D., Goldstein, S.D., Isenberg, G., Barber, M., Biswas, S., Minimo, C., Palazzo, J., Park, P.K. and Weinberg, D. (1998) Dis. Colon Rectum 41, 310–315.
[13] Mann, E.A., Jump, M.L. and Giannella, R.A. (1996) Biochim. Biophys. Acta 1305, 7–10.
[14] Swenson, E., Mann, E., Jump, M. and Giannella, R. (1999) Am. J. Physiol. 276, G728–G736.
[15] Park, J., Schulz, S. and Waldman, S.A. (2000) Gastroenterology 119, 89–96.
[16] Suh, E., Chen, L., Taylor, J. and Traber, P.G. (1994) Mol. Cell. Biol. 14, 7340–7351.
[17] Scott, M.P., Tamkun, J.W. and Hartzell 3d, G.W. (1989) Biochim. Biophys. Acta 989, 25–48.
[18] Gehring, W., Qian, Y., Billeter, M., Furukubo-Tokunaga, K., Schier, A., Resendez-Perez, D., Affolter, M., Otting, G. and Wuthrich, K. (1994) Cell 78, 211–223.
[19] Gehring, W., Affolter, M. and Burglin, T. (1994) Annu. Rev. Biochem. 63, 487–526.
[20] Freund, J.N., Domon-Dell, C., Kedinger, M. and Duluc, I. (1998) Biochem. Cell Biol. 76, 957–969.
[21] Chawengsaksophak, K., James, R., Hammond, V.E., Kontgen, F. and Beck, F. (1997) Nature 386, 84–87.
[22] Beck, F., Chawengsaksophak, K., Waring, P., Playford, R.J. and Furness, J.B. (1999) Proc. Natl. Acad. Sci. USA 96, 7318–7323.
[23] Tamai, Y., Nakajima, R., Ishikawa, T., Takaku, K., Seldin, M.F. and Taketo, M.M. (1999) Cancer Res. 59, 2965–2970.
[24] Wu, G.D., Wang, W. and Traber, P.G. (1992) J. Biol. Chem. 267, 7863–7870.
[25] Fang, R., Santiago, N.A., Olds, L.C. and Sibley, E. (2000) Gastroenterology 118, 115–127.
[26] Troelsen, J.T., Mitchelmore, C., Spodsberg, N., Jensen, A.M., Noren, O. and Sjostrom, H. (1997) Biochem. J. 322, 833–838.
[27] Li, Z. and Goy, M.F. (1993) Am. J. Physiol. 265, G394–G402.
[28] James, R., Erler, T. and Kazenwadel, J. (1994) J. Biol. Chem. 269, 15229–15237.
[29] Margalit, Y., Yarus, S., Shapira, E., Gruenbaum, Y. and Fainsod, A. (1993) Nucleic Acids Res. 21, 4915–4922.
[30] Abmayr, S.M. and Workman, J.L. (1999) in: DNA-Protein Interactions. Current Protocols in Molecular Biology, Vol. 1 of 3 (Ausubel, F.M., Brent, R., Kingston, R.E., Moore, D.D., Seidman, J.G., Smith, J.A. and Struhl, K., Eds.), pp. 12.1.1–12.1.9, John Wiley and Sons, New York.
[31] Mallo, G.V., Rechreche, H., Frigerio, J.M., Rocha, D., Zweibaum, A., Lacasa, M., Jordan, B.R., Dusetti, N.J., Dagorn, J.C. and Iovanna, J.L. (1997) Int. J. Cancer 74, 35–44.