Identification of a Promoter-specific Transcriptional Activation Domain at the C Terminus of the Wnt Effector Protein T-cell Factor 4*

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Wnt growth factors control numerous cell fate decisions in development by altering specific gene expression patterns through the activity of heterodimeric transcriptional activators. These consist of β-catenin and one of the four members of the T-cell factor (TCF) family of DNA-binding proteins. How can the Wnt/β-catenin pathway control various sets of target genes in distinct cellular settings with such a limited number of nuclear effectors? Here we asked whether different TCF proteins could perform specific, nonredundant functions at natural β-catenin/TCF-regulated promoters. We found that TCF4E but not LEF1 supported β-catenin-dependent activation of the Cdx1 promoter, whereas LEF1 specifically activated the Siamois promoter. Deletion of a C-terminal domain of TCF4E prevented Cdx1 promoter induction. A chimeric protein consisting of LEF1 and the C terminus of TCF4E was fully functional. Therefore, the TCF4E C terminus harbors a promoter-specific transactivation domain. This domain influences the DNA binding properties of TCF4 and additionally mediates an interaction with the transcriptional coactivator p300. Apparently, the C terminus of TCF4E cooperates with β-catenin and p300 to form a specialized transcription factor complex that specifically supports the activation of the Cdx1 promoter.

Multicellular organisms characteristically employ a limited number of signaling systems in order to generate the panoply of different cell types found in the body. The repeated use of the same growth factor families and signaling pathways poses the question of how the same effector proteins can generate distinct, tissue-specific responses (1). The canonical Wnt/β-catenin signal transduction pathway provides an interesting model system to address this problem. Wnt growth factors constitute a large family of secreted glycoproteins that control numerous developmental processes in a wide range of organisms (2, 3). In order to evoke transcriptional responses, the Wnt/β-catenin signaling cascade utilizes a small number of bipartite transcription factor complexes. These complexes are formed by β-catenin, which provides a transcriptional activation function, and by a member of the T-cell factor (TCF)1 family of DNA-binding proteins, which guides β-catenin to the promoter regions of specific target genes (4, 5). β-Catenin belongs to an evolutionarily conserved family of proteins, which, as a signature motif, carry multiple copies of a 42-amino acid module, the Armadillo repeat (6). In β-catenin, 12 copies of this repeat form the large, central domain of the protein and provide the interaction surface for most of the known binding partners of β-catenin (7–10). The Armadillo repeat domain is flanked by short N- and C-terminal extensions, which harbor the transcriptional activation domains of β-catenin (11–13). In addition to its role in Wnt signaling, β-catenin also performs a function in cell-cell adhesion, where it is crucial for the formation of cadherin-catenin complexes (2, 3).

In mammals, four genes encode the TCF family members TCF1, LEF1, TCF3, and TCF4, which have some structural features in common (5). The extreme N terminus harbors the binding site for β-catenin. The recognition and occupation of specific DNA sequence motifs is mediated by nearly identical HMG-box domains, which are located near the C terminus or in the middle of TCFs. Interspersed between the β-catenin-binding domain and the HMG-box are sequences that interact with Groucho/TLE transcriptional corepressors (14–17). Groucho/TLE factors are histone-binding proteins and additionally interact with a histone deacetylase (18, 19). This suggests that their function is to set up a specialized repressive chromatin structure that prevents inappropriate activation of β-catenin/TCF target genes in the absence of a Wnt signal. Since TCF proteins are functionally neutral on their own, their role in gene regulation is likely to serve as chromosomal docking sites for various interaction partners, thus promoting the formation of different transcription factor complexes with distinct properties.

The participation of Wnt/β-catenin signaling in multiple developmental programs necessitates that β-catenin-TCF complexes induce only subsets of all potential Wnt/β-catenin target genes at any particular time and that different sets of genes are addressed depending on the particular cellular background. How this is achieved is not understood. Several different mechanisms have been described whereby the formation and activity of β-catenin-TCF complexes is controlled by covalent modification of TCFs or competitive binding to β-catenin (3–5). These mechanisms, however, appear to affect the expression of Wnt target genes indiscriminately. Alternatively, β-catenin could employ transcriptional coactivators in a promoter-specific manner. Among the cofactors of β-catenin are BCL9/Legless, Brg-1, p300, and the closely related CBP (20–25). CBP

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1 The abbreviations used are: TCF, T-cell factor; TBE, TCF-binding element; EMSA, electrophoretic mobility shift assay; CBP, CREB-binding protein; HA, hemagglutinin; GST, glutathione S-transferase.
and p300 are widely used transcriptional coactivators that can provide a link to the basal transcription machinery or can target chromatin structure through their intrinsic acetylase activity (26). Both p300 and CBP have been implicated in differential promoter activation by β-catenin (21, 27). In addition, several observations closely link TCF family members to mechanisms that generate promoter-specific transcriptional responses. TCFs can interact with Smad proteins, and this interaction appears to be critically involved in the combinatorial regulation of the Xenopus laevis Tcf3 gene promoter by the transforming growth factor β and Wnt signaling pathways (28, 29). Similarly, an interaction between LEF1 and the basic helix-loop-helix/leucine zipper protein microphthalmia-associated transcription factor has been implicated in the melanocyte-specific expression of Wnt target genes (30). In addition, multiple isoforms with different functionalities arise from TCF genes by way of alternative splicing and the use of dual promoters, and different TCF family members perform distinct tasks in developmental processes (31–36). It thus appears that TCF family members and their isoforms are intrinsically different and can support the execution of different developmental programs.

To better understand the mechanisms whereby Wnt signals selectively activate target genes, we began to compare TCF family members with respect to their ability to support β-catenin-mediated activation of different β-catenin/TCF target gene promoters. Here we report that LEF1 and the TCF4E isoform have opposing capabilities to synergize with β-catenin at the Siamois and the Cdx1 promoters. A promoter-specific transcriptional activation domain was identified at the C terminus of TCF4E, which can interact with the p300 transcriptional adaptor. The interaction between TCF4E and p300 differs from the β-catenin/p300 interaction, because β-catenin induces a posttranslational modification of p300, whereas TCF4E does not. We propose that TCF4E supports the promoter-specific assembly of a dormant transcription factor complex, the transcriptional activity of which is triggered by β-catenin-induced phosphorylation of p300.

**EXPERIMENTAL PROCEDURES**

**Plasmids**—Expression vectors for murine β-catenin, human p300, human TCF4E, and mouse LEF1 were those as previously described (6). All other plasmids were generated by standard cloning techniques or polymerase chain reaction-based strategies (37). Details of the constructs are available upon request.

**Electrophoretic Mobility Shift Assays (EMSA)**—For EMSA, LEF1 and TCF4 proteins were transfected and translated in vitro using the SP6 TNT system (Promega). Translation efficiency was analyzed by Western blotting with anti-HA antibodies (3F10; Roche Applied Science). After boiling for 5 min, one-tenth of each lysate was loaded onto an 8% SDS-polyacrylamide gel. For EMSA, LEF1 and TCF4E fusion proteins were immobilized on glutathione-Sepharose 4B beads and incubated with nuclear extracts at 4 °C for 2 h. Following extensive washing with buffer without bovine serum albumin, material retained on the glutathione-Sepharose matrix was eluted in SDS-PAGE loading buffer, separated by SDS-PAGE on 10% gels, and visualized by fluorography. Radiolabeled p300 for these experiments was transcribed and translated in vitro using the SP6-based TNT system (Promega). For each binding reaction, we used a 4-μl aliquot of a 50-μl TNT reaction.

**Phosphatase Assays**—Transfected 293 cells were lysed for 30 min on ice in 1 ml of IPK buffer (50 mM Tris-HCl, pH 7.6, 75 mM KCl, 1 mM dithiothreitol, 10 mM NaF, 0.1 mM sodium orthovanadate, and “Complete” protease inhibitor mix). After clearance by centrifugation at 20,000 × g for 15 min, 1–2 μg of cell lysate were treated with λ-phosphatase (New England Biolabs) for 20 min at 37 °C in a total volume of 10 μl in the buffer supplied with the enzyme. Untreated or phosphatase-treated samples were loaded onto 6% SDS-polyacrylamide gels and visualized by fluorography. Radiolabeled p300 was visualized by Western blotting as described above.

**RESULTS**

**LEF1 and TCF4E Exhibit Promoter-specific Transactivation Properties**—To test whether downstream components of the Wnt/β-catenin signal transduction pathway contribute to the differential regulation of Wnt target genes, we asked whether LEF1 and TCF4E were equally capable of supporting activation of Wnt-regulated promoters by β-catenin and p300. LEF1 and the TCF4E splice variant are representatives of the short and long isoforms of TCF family members (5). Their main differences are the extended C terminus in TCF4E with binding to OS osteosarcoma cells (ATCC number HTB-96) were cultured as described (21). To monitor TCF protein expression, 2 × 10^4 293 cells, seeded into 35-mm dishes, were transfected with 2.5 μg of expression vector using FuGENE6 reagent (Roche Applied Science). For immunoprecipitations, 293 cells were transfected as described before (21). For reporter gene assays, cells were plated into 24-well plates (5 × 10^4 cells/well) and transfected 4 h later with a FuGENE6/DNA mixture containing 10 ng of pRL-CMV or pCMV-β-galactosidase (Promega) as internal standard, 100 ng of luciferase reporter plasmid, and expression vectors for β-catenin (50 ng), TCF (10 ng), and p300 (250 ng) together with increasing amounts of competitor as indicated. Total amounts of DNA were kept constant by adding empty expression vector where needed. Firefly luciferase reporters were p01234, pcD7-1 Luc, and pcD7 (-350/+72) Luc (39, 40). Luciferase reporter plasmid and β-galactosidase activity were determined as before (21) using 96-well plates and a Labsystems Minoskan Ascent luminometer. To measure Renilla luciferase activity, 100 μl of a solution with 0.5 μM coelenterazine (Calbiochem) in 25 mM Tris/HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA was injected per well of a 96-well plate, and after a delay of 0.5 s, light emission was recorded for 5 s. Reporter gene activities shown are average values and their S.D. values, obtained from at least three independent experiments after normalization against β-galactosidase or Renilla luciferase activities.

**Western Blotting and Immunoprecipitation**—Protein extracts from cells transfected in 35-mm dishes were made by lysing cells in 200 μl of SDS-PAGE sample buffer containing “Complete” protease inhibitor mix (Roche Applied Science). After boiling for 5 min, one-tenth of each lysate was loaded onto an 8% SDS-polyacrylamide gel.
ing motifs for the transcriptional corepressor CtBP and a context-dependent transactivation domain at the N terminus of LEF1 (Fig. 1A). Transactivation properties of LEF1 and TCF4E were compared at the promoters of the mouse Cdx1 gene and the Siamois gene of *X. laevis* (39, 40). Both of these genes are Wnt/β-catenin targets, which contain multiple TCF-binding elements (TBEs) in their promoter regions, and in their natural contexts they are regulated in a highly cell type-specific manner. Combinations of expression vectors for epitope-tagged TCF4E and LEF1, an activated form of β-catenin with Ala substitutions in the N-terminal destruction box (β-catSS3A) (3, 41), and p300 were transfected into the human embryonic kidney cell line 293 and the human osteosarcoma cell line U-2 OS together with the pCdx1–4 Luc and p01234 luciferase reporter constructs (Fig. 1, C and D). When β-catenin, TCF4E, LEF1, and p300 were expressed individually, they had little or no effect on promoter activity. Pairwise combinations of β-catenin and LEF1 or β-catenin and TCF4E only weakly activated the luciferase reporters. Low levels of reporter gene expression were also induced by β-catenin and p300. In the absence of transfected TCFs, β-catenin presumably employs a limited pool of endogenous TCFs (14). In contrast, high levels of reporter gene activation were obtained when β-catenin, p300, and one of the TCFs were expressed simultaneously. Importantly, however, TCF4E and LEF1 clearly differed in their ability to synergize with β-catenin and p300, although they are expressed at similar levels (Fig. 1, B and D). In both 293 and U-2 OS cells, TCF4E supported β-catenin/p300-dependent activation of the Cdx1 promoter but not of the Siamois promoter. Conversely, LEF1 mediated activation of the Siamois promoter but not of the Cdx1 promoter (Fig. 1D). Even when we raised the amount of transfected plasmid, LEF1 did not support activation of the Cdx1 reporter (not shown). Thus, the two TCF family members are functionally different and act in a promoter-specific manner.

**The C Terminus of TCF4E Harbors a Promoter-specific Activation Domain**—To gain insight into the mechanisms underlying its promoter-specific activity, we determined which domain in TCF4E is required for activation of the Cdx1 promoter. A panel of deletion mutants was constructed (Fig. 2A), and Western blotting experiments were performed to confirm that all mutants were expressed at similar levels (Fig. 2B). Functional testing revealed that all mutants that lack the C1.1 domain (amino acids 436–482 of the TCF4E C terminus) are unable to synergize with β-catenin and p300 in Cdx1 promoter activation (TCF4AC, TCF4AC1, and TCF4AC1.1) (Fig. 2D). Constructs that retain this domain but have deletions in other parts of the TCF4E C terminus (TCF4AC2 and TCF4AC1.2) activate the Cdx1 promoter. From these results, we conclude that the C1.1 domain contains at least essential parts, if not all, of a promoter-specific transactivation domain. In addition, because activation of the Cdx1 promoter by TCF4E was seen with the short reporter construct used in these experiments (Fig. 2C), it appears that the proximal promoter region with its TBEs contains all of the determinants that make the Cdx1 promoter specifically responsive to TCF4E.

**LEF1 Forms a Nonproductive Transcription Factor Complex at the Cdx1 Promoter**—To rule out the possibility that LEF1 and the inactive TCF4 mutants were unable to activate the Cdx1 promoter because they could not access it, we performed competition experiments. For this, activation of the Cdx1 promoter by β-catenin, p300, and TCF4E was challenged by coexpressing increasing amounts of LEF1 and TCF4AC. As shown in Fig. 3A, higher levels of both factors progressively inhibited Cdx1 reporter activation. Deletion of the DNA-binding domain of LEF1 (Fig. 3A, LEF1ΔHMG) and, to a lesser extent, removal

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**FIG. 1.** LEF1 and TCF4E differ in their ability to support β-catenin (β-cat)/p300-mediated activation of the Siamois and Cdx1 promoters. A, scheme of human TCF4E and mouse LEF1 constructs. Binding sites for β-catenin (βBD), TLE proteins, CtBP, the context-dependent transactivation domain in LEF1 (CTA), the DNA-binding domains (HMG), the adjacent nuclear localization signals (black boxes), and the C-terminal HA epitope tags are indicated. B, Western blot analyses of LEF1 and TCF4E expression in transfected 293 cells with a monoclonal antibody against the HA epitope tag. Note that the anti-HA antibodies detect TCF4E proteins at additional slower migrating form (marked with an asterisk in lane 3), which arises as the result of an unknown posttranslational modification. Mw, molecular weight standard. C, schematic representation of luciferase reporter gene constructs. In pCdx1–4 Luc, a mouse Cdx1 promoter fragment from position −3600 to +72 drives firefly luciferase expression. In p01234, an 800-bp promoter fragment from the X. laevis Siamois gene controls luciferase expression. Positions of functional TBEs are indicated by black boxes, and nonfunctional TBEs are depicted as light gray boxes. D, 293 (top panels) or U-2 OS cells (bottom panels) were transfected with expression vectors for β-catenin, p300, LEF1, TCF4E, the Cdx1 or Siamois luciferase reporters, and the pCMVβ vector for internal standardization as indicated. A stabilized β-catenin mutant (β-catSS3A) was used. Luciferase reporter gene activities are shown as relative values compared with the activity measured in lysates from cells transfected with only the luciferase reporter (relative activity of 1).
of the β-catenin-binding domain (Fig. 3A, LEF1ΔN) impaired this effect. Accordingly, the dominant negative activity of LEF1, and presumably of the TCF4Δ₁.₁, and the chimeric LEF1-T4C protein readily generated protein-DNA complexes (Fig. 4D, compare lanes 7–9 with lanes 11–13). In contrast, under the conditions used, LEF1 generated primarily a single species of the Cdx1 reporter complexes representing single, double, and triple occupancy of the Cdx1 probe (Fig. 4C, lanes 20–28). In contrast, under the conditions used, LEF1 generated primarily a single species of the Cdx1 probe (Fig. 4C, compare lanes 3–5 with lanes 6–8). Interestingly, this difference was also seen when oligonucleotides with a single TBE (either TBE₃a, -3, or -4) derived from the Cdx1 promoter were used as probes (not shown). The C terminus of TCF4E appears to be at least partly responsible for the reduced DNA binding capacity of TCF4E, as indicated by the complementary increases and decreases in the DNA-binding capabilities of TCF4AC₁.₁ and the LEF1-T4C chimera by EMSA with two different DNA probes (Fig. 4). The TCRα₂₅ probe harbors a single consensus TBE derived from the T-cell receptor α-chain enhancer (38). The second probe was a Cdx1 promoter fragment, which contains three TCF binding motifs: TBE₃a, TBE₃, and TBE₄ (39). The TBE₃a element is a newly discovered TBE that was not reported previously. The TCF proteins were transcribed and translated in vitro, and similar amounts of the various factors, as determined by Western blotting (Fig. 4A), were used. Although both probes were bound by LEF1 and TCF4E, LEF1 exhibited an affinity for the single binding site of the TCRα₂₅ probe that was at least 5 times higher than that of TCF4E (Fig. 4C, compare lanes 3–5 with lanes 6–8). Interestingly, this difference was also seen when oligonucleotides with a single TBE (either TBE₃a, -3, or -4) derived from the Cdx1 promoter were used as probes (not shown). The C terminus of TCF4E appears to be at least partly responsible for the reduced DNA binding capacity of TCF4E, as indicated by the complementary increases and decreases in the DNA-binding capabilities of TCF4AC₁.₁ and the LEF1-T4C chimera by EMSA with two different DNA probes (Fig. 4C, compare lanes 3–5 with lanes 6–8).}

Whereas TCF4E possesses a markedly reduced affinity for a single TBE when compared with LEF1, this difference was not seen in EMSAs with a Cdx1 promoter fragment containing all three TBEs together. TCF4E, TCF4AC₁.₁, and the chimeric LEF1-T4C protein readily generated protein-DNA complexes representing single, double, and triple occupancy of the Cdx1 probe (Fig. 4C, lanes 20–28). In contrast, under the conditions used, LEF1 generated primarily a single species of protein-DNA complexes (Fig. 4C, lanes 17–19). Still, LEF1 can recognize all three Cdx1 TBEs also in the context of the entire promoter fragment as shown by DNase I footprinting experiments. Because the same protein preparations were used for EMSA with the Cdx1 promoter fragment and the single TBEs, the observed differences in DNA binding are inherent properties of the TCF proteins and not due to variable efficiencies of protein folding. In addition, these experiments also confirm the potential regulatory functions of the TCF4E C terminus. As seen in EMSAs with a single TBE, we observed that deletion of

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Fig. 2. The C terminus of TCF4E harbors a promoter-specific activation domain. A, scheme of TCF4E deletion constructs. Amino acid positions of the deletion end points are given. Other features of TCF4E are depicted as in Fig. 1. All constructs contain the C-terminal HA epitope tag. B, expression of the TCF4E deletion mutants was analyzed by Western blotting with an anti-HA antibody as before (Fig. 1B). Mₐ, molecular weight standard. Asterisks mark posttranslationally modified forms of TCF4E and its derivatives. C, schematic representation of the pCdx1(−350/+72)Luc reporter. The promoter fragment in this reporter harbors only TBE₃a, -3, and -4. D, 293 cells were transfected with combinations of expression vectors for the TCF4E deletion mutants, β-catenin, p300, the Cdx1 reporter, and pCMVβ as indicated. Luciferase reporter gene activities were determined as in Fig. 1. Reporter gene activity without β-catenin (β-cat), TCF4E, and p300 was arbitrarily assigned the value of 1. WT, wild type.

Since LEF1 can occupy the Cdx1 promoter construct, we wondered whether the addition of the C-terminal activation domain of TCF4E would endow LEF1 with the ability to stimulate Cdx1 promoter activity. A LEF1-TCF4E chimera was generated (Fig. 3, C and D), and when coexpressed together with either β-catenin or p300 alone, the LEF1-T4C fusion mediated even higher levels of Cdx1 reporter activity than TCF4E (Fig. 3B). In the presence of both p300 and β-catenin, TCF4E and LEF1-T4C activated the Cdx1 promoter equally well. For its function, the chimeric protein required an intact DNA-binding domain. This shows that the LEF1-T4C chimera must be able to bind to the Cdx1 promoter in order to activate and rules out the possibility that LEF1-T4C stimulates the reporter only indirectly. Thus, the presence of the TCF4E C terminus turns LEF1 into an activator at the Cdx1 promoter. Together with the previous results, this demonstrates that the transactivation domain present at the C terminus of TCF4E is necessary and sufficient to confer promoter specificity upon TCF proteins.

LEF1 and TCF4 Possess Different DNA Binding Properties in Vitro—Wnt target gene activation depends on sequence-specific promoter recognition by TCFs and on TCβ-catenin complex formation. However, no differences were seen with respect to the ability of LEF1, TCF4E, or TCF4AC to interact with β-catenin (not shown). Therefore, we compared the DNA binding properties of LEF1, wild-type and mutant TCF4E, and the LEF1-T4C chimera by EMSA with two different DNA probes (Fig. 4). The TCRα₂₅ probe harbors a single consensus TBE derived from the T-cell receptor α-chain enhancer (38). The second probe was a Cdx1 promoter fragment, which contains three TCF binding motifs: TBE₃a, TBE₃, and TBE₄ (39). The TBE₃a element is a newly discovered TBE that was not reported previously. The TCF proteins were transcribed and translated in vitro, and similar amounts of the various factors, as determined by Western blotting (Fig. 4A), were used. Although both probes were bound by LEF1 and TCF4E, LEF1 exhibited an affinity for the single binding site of the TCRα₂₅ probe that was at least 5 times higher than that of TCF4E (Fig. 4C, compare lanes 3–5 with lanes 6–8). Interestingly, this difference was also seen when oligonucleotides with a single TBE (either TBE₃a, -3, or -4) derived from the Cdx1 promoter were used as probes (not shown). The C terminus of TCF4E appears to be at least partly responsible for the reduced DNA binding capacity of TCF4E, as indicated by the complementary increases and decreases in the DNA-binding capabilities of TCF4AC₁.₁ and the LEF1-T4C chimera by EMSA with two different DNA probes (Fig. 4C, compare lanes 3–5 with lanes 6–8). Whereas TCF4E possesses a markedly reduced affinity for a single TBE when compared with LEF1, this difference was not seen in EMSAs with a Cdx1 promoter fragment containing all three TBEs together. TCF4E, TCF4AC₁.₁, and the chimeric LEF1-T4C protein readily generated protein-DNA complexes representing single, double, and triple occupancy of the Cdx1 probe (Fig. 4C, lanes 20–28). In contrast, under the conditions used, LEF1 generated primarily a single species of protein-DNA complexes (Fig. 4C, lanes 17–19). Still, LEF1 can recognize all three Cdx1 TBEs also in the context of the entire promoter fragment as shown by DNase I footprinting experiments. Because the same protein preparations were used for EMSA with the Cdx1 promoter fragment and the single TBEs, the observed differences in DNA binding are inherent properties of the TCF proteins and not due to variable efficiencies of protein folding. In addition, these experiments also confirm the potential regulatory functions of the TCF4E C terminus. As seen in EMSAs with a single TBE, we observed that deletion of

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2 A. Hecht, unpublished observations.
the C1.1 domain increased the ability of TCF4E to bind to the Cdx1 promoter fragment. This is evident from the greater abundance of DNA complexes with all three TBEs present in the immunoprecipitate (Fig. 5A). Apparently, the C terminus of LEF1-T4C complexes migrated at nearly identical positions (Fig. 4C, compare lanes 20 – 22 with lanes 23 – 25). In addition to this inhibitory activity, which seems to be linked to the C1.1 domain, the TCF4E C terminus also appears to harbor an opposing activity, which seems to be linked to the C1.1 domain, the regulatory functions present at its C terminus.

The Promoter-specific Activation Domain of TCF4E Interacts with the Transcriptional Coactivator p300—High level induction of the Cdx1 reporter constructs required simultaneous expression of β-catenin, p300, and TCF4E. However, pairwise combinations of p300 and TCF4E and especially the LEF1-T4C chimera also elicited a significant response of the Cdx1 promoter through protein-protein interactions with p300. Indeed, p300 co-immunoprecipitated with epitope-tagged TCF4E from lysates of transfected 293 cells (Fig. 5A, lane 3). To clarify whether the presence of p300 was simply due to coprecipitation with β-catenin, which was also detected in the immunoprecipitate and which can interact with p300 on its own (21), we performed control experiments with the TCF4ΔN mutant lacking the β-catenin-binding domain of TCF4E. As expected, β-catenin no longer coprecipitated with TCF4ΔN, whereas p300 was still present in the immunoprecipitate (Fig. 5A, lane 4). In contrast, deletion of the C1.1 domain significantly reduced the amount of p300 coprecipitating with TCF4ΔC1.1 even in the presence of β-catenin (Fig. 5A, lane 5). Apparently, the C terminus of TCF4E contributes to an interaction between TCF4E and p300.
Complex formation between TCF4E and p300 was further characterized by GST pull-down experiments with bacterially expressed GST-TCF4E and p300, which was transcribed and translated in vitro. All GST-TCF4E fusions containing the C1.1 domain interacted with p300 (Fig. 5, B and C, constructs A, D, and F), whereas all mutants lacking the C1.1 domain did not (Fig. 5, B and C, constructs B, C, and E). Thus, the C1.1 domain in TCF4E, which is required for promoter-specific transactivation, also mediates an interaction with p300.

**DISCUSSION**

TCFs are multifunctional transcription factors that control the expression status of Wnt target genes by forming multiprotein promoter complexes that incorporate either repressing or activating cofactors (5). Due to a common overall structure and seemingly identical DNA binding specificities, TCF proteins were long considered to be largely interchangeable components of the Wnt signaling cascade. However, recent reports revealed functional differences between TCF family members and between isoforms derived from the same TCF gene. For example, LEF1 appears to mainly act as a β-catenin-dependent transcriptional activator, whereas the repressor activities of TCF3 prevail over its activating function (42–44). The molecular basis for these differences is unknown, but in the case of TCF proteins from X. laevis it was shown that activating properties require the presence of an alternatively spliced exon at their N termini (31, 35). Here we report that also LEF1 and TCF4 have different transactivation capacities, although both factors contain the activating N-terminal exon. Additionally, whereas both LEF1 and TCF4 can act as activators, they appear to have distinct spectra of target genes, and a novel, promoter-specific transcriptional activation domain was identified in the TCF4E variant.

The domain of TCF4E responsible for its promoter-specific activity is located at the C terminus and contains the “CRARF” amino acid motif and a second block of mostly positively charged amino acids. The “CRARF” domain is evolutionarily conserved and specifically present in the “E” variants of TCF1 and TCF4 gene products (5, 34). However, to date, no specific function has been assigned to this domain. Based on our results, it may be involved in at least two different processes, namely DNA binding and selective activation of certain promoters. Although LEF1 and TCF4E recognized the same DNA sequences, their affinities for single or multimerized recognition motifs varied considerably. Pukrop et al. (31) also observed 3–7-fold differences in affinity for single TBEs between LEF1 and other TCF family members. These differences are likely to be of importance, because TCF proteins are not only mediators of Wnt signaling but also contribute to Wnt/β-catenin-independent gene regulation, for example at the TCRα enhancer or at the HIV-1 promoter (38, 45). How can inappropriate activation of these regulatory elements by β-catenin-TCF complexes
be prevented? Perhaps significantly, both the TCRa enhancer and the HIV-1 promoter contain single TCF binding motifs (38, 45), whereas the known Wnt target genes typically contain multiple TCF recognition elements. Under competitive conditions, when TCF levels are low, or when only a particular TCF family member is expressed in a cell, the differential recognition and occupancy of single versus multiple binding sites could be one way to distinguish Wnt target genes from nontarget genes.

Although differences in DNA binding may contribute to the differential activation of the Cdx1 promoter by LEF1 and TCF4E, we believe that the C-terminal activation domain in TCF4 performs additional functions. LEF1 and TCF4 mutants lacking the C1.1 domain displayed differences in promoter

![Fig. 5. The C terminus of TCF4E mediates an interaction with p300. A, TCF4E and p300 co-immunoprecipitate from cell lysates. 293 cells were transfected with expression plasmids for Glu-Glu-tagged p300 (p300-EE, 15 μg) and HA-tagged wild-type (WT) or mutant TCF4E (5 μg) as indicated. Cell extracts were prepared 40 h after transfection and used for immunoprecipitation (IP) with anti-HA antibodies. Immunoprecipitated material and a fraction of each cell lysate were resolved by SDS-PAGE and analyzed by Western blotting (IB) with antibodies as shown. B, the promoter-specific activation domain at the TCF4E C terminus interacts with p300 in vitro. GST or GST-TCF4 fusion proteins shown schematically in C were bound to glutathione-Sepharose beads and incubated with radiolabeled p300. Proteins retained by GST or the GST-TCF4 fusions and 10% of the input material were analyzed by SDS-PAGE and fluorography. C, structure of the GST-TCF4 fusion proteins and, for comparison, of TCF4E. Amino acid end points of the TCF4E fragments are given. Functionally relevant domains and interaction sites for TCF4E-binding factors are indicated as before. Hatched bar, GST.](image)

![Fig. 6. β-Catenin (β-cat), but not TCF4E, induces phosphorylation of p300 in vivo. A, altered electrophoretic mobility of p300 in the presence of β-catenin. 293 cells were transfected with expression plasmids for p300-EE (15 μg), β-catS33A, or HA-tagged TCF4E (5 μg) as indicated. Cell extracts were prepared 40 h after transfection. A fraction of each cell lysate was resolved by SDS-PAGE and analyzed by Western blotting with antibodies against p300. The position of anti-p300 immunoreactive material in the presence or absence of β-catS33A or TCF4E is indicated at the right side of the blot. B, reduced electrophoretic mobility of p300 in the presence of β-catenin is due to phosphorylation. Cell extracts prepared as in A were treated with increasing amounts of λ-phosphatase (λ PPase) for 20 min at 37 °C. Samples were then resolved by SDS-PAGE on 6% gels and analyzed by Western blotting with anti-p300 antibodies. C, specificity of the phosphatase effect. Fractions of the same cell extracts as in B were subjected to phosphatase treatment either in the absence of the essential λ-phosphatase cofactor MnCl₂ or in the presence of the phosphatase inhibitors EDTA and sodium fluoride (NaF). Samples were analyzed by SDS-PAGE and Western blotting as in B.](image)
recognition, but there was no clear correlation between the ability to activate the Cdx1 promoter and the mode of promoter binding in vitro. TCF4ΔC and TCF4ΔC1.1 did not activate the Cdx1 promoter and blocked Cdx1 induction by wild-type TCF4E in our competition experiments, yet they bound to the Cdx1 promoter in vitro even more efficiently than TCF4E or LEF1 (Figs. 3 and 4). Moreover, the pattern of DNA-protein complexes produced by the LEF1-T4C chimera differed from those of all other factors analyzed, although the chimera is functionally equivalent to TCF4E. Thus, an influence on promoter occupation or topology is unlikely to be the only mechanism by which the Cdx1-specific activation domain at the C terminus of TCF4E functions. An additional mode of action could be that the C1.1 domain mediates protein-protein interactions. Indeed, it interacts with p300 in vitro, and p300 co-immunoprecipitated together with TCF4 from cellular lysates. Although p300 may not be the only factor that interacts with the C1.1 domain, the match between the ability to form a complex with p300 and the ability to support activation of the Cdx1 promoter strongly suggests that the interaction between TCF4E and p300 is of physiological relevance.

Expression of the murine Cdx1 gene begins in ectodermal and mesodermal cells of gastrulating embryos around day 7.5 of gestation. It occurs in a graded fashion along the body axis with highest levels of expression in posterior parts. From day 14 of embryonic development onward, Cdx1 is also expressed in the endoderm of the developing intestine (46–48). The latter tissue expresses both TCF4 and LEF1 (49, 50). Based on our results, TCF4E may be responsible for Cdx1 regulation in the intestine. However, TCF4 is not expressed in the early embryonic Cdx1 expression domain (51). We suggest that there activation of the Cdx1 promoter is mediated by an isoform derived from the TCF1 gene. Preliminary results indicate that TCF1E, but not TCF1B, is expressed in the early embryonic Cdx1 promoter (51). Many factors have been identified that interfere with the gene regulatory activity of β-catenin and TCFs and could help to shape specific gene expression patterns. However, inhibitors like ICAT act upstream of target gene promoter activation and cause a global inhibition of Wnt signaling rather than a selective and differential regulation of Wnt target genes (3, 5, 52). More likely, mechanisms that govern tissue-specific Wnt target gene regulation act at the level of individual regulatory elements and their associated transcription factors. For example, a hierarchy of regulatory events permits Wnt effectors to maintain expression of the murine Brachyury gene but only after the initiation of transcription by a different regulatory system (53).

Alternatively, the activation of Wnt target genes may depend on the simultaneous input from multiple signaling pathways such as a combination of Wnt and transforming growth factor β signals (54, 55). At the molecular level, the two pathways are integrated through physical interactions of the
Smad proteins and members of the TCF family, necessitating the presence of DNA recognition motifs for both families of DNA-binding proteins (28, 29). Thereby, only those genes with the proper combination of cis-active DNA elements in their promoter regions will respond to the combinatorial input from Wnt and transforming growth factor β-signaling pathways. This underscores that the DNA sequences of promoter regions and the interaction partners of TCF proteins are critical determinants for the differential regulation of Wnt target genes.

Although p300 and CBP have already been linked to differential gene regulation by β-catenin (21, 27), it was unexpected to find an interaction between TCF4E and a transcriptional coactivator like p300. Intuitively, one would surmise that recruitment of p300 by TCF4E catalyzed transcription even in the absence of a Wnt signal. However, even the low level Cdx1 promoter activation upon expression of p300 and TCF4E was found only if TCF4E contributed by TCF4E is required. What could be the promoter-specific activity of TCF Family Members

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