Lymphoid Enhancer Factor/T Cell Factor Expression in Colorectal Cancer

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Keywords: Colon , HMG, LEF, TCF, Wnt, β-catenin

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Abstract

Genetic inactivation of key components of the Wnt signal transduction system is a frequent event in colorectal cancer. These genetic mutations lead to stabilization of β-catenin, a cytoplasmic•nuclear shuttling protein with a potent transcription activation domain. Stabilization and subsequent nuclear localization of β-catenin produces aberrant, Wnt-independent signals to target genes, an activity tightly linked to the genesis of colon cancers. In the nucleus, the transcription factor family of LEF/TCF proteins transmits Wnt signals by binding to β-catenin and recruiting it to target genes for activation. Such activities are carried out by full-length LEF/TCFs that are thought to be mostly interchangeable and redundant. However, truncated forms of LEF-1 and TCF-1 that do not bind to β-catenin function as dominant negatives and an alternatively spliced TCF isoform with a unique activation function has recently been discovered. The dominant negative forms block Wnt signals because they occupy Wnt target genes and limit β-catenin access; the alternatively spliced TCF isoform activates certain Wnt target promoters whereas other TCF isoforms and LEF-1 do not. A study of LEF/TCF expression and activity in normal intestine and colon carcinomas suggests that the relative amounts of LEF/TCF isoforms may change as tumors progress and this may influence the strength and specificity of Wnt signals in the nucleus. While the underlying mechanism for a change in the LEF/TCF isoform expression is not yet known, recent evidence implicates the Wnt signaling pathway itself as a potential modulator.

Review

I. Wnt Signal Transduction

I.A. Wnt-dependent Signalling

Wnt proteins are secreted morphogens that trigger receptive cells to adopt specific fates or polarities, or to grow and divide. The steps in the signaling pathway and the role of this pathway in cancer are already the subject of several excellent, in-depth reviews [1-8], therefore only a brief overview is presented here[1]. By binding to seven-pass transmembrane receptors at the cell surface called Frizzleds in association with the single-pass transmembrane protein LRP/arrow, Wnt ligands initiate a signaling cascade that reaches the nucleus via rapid movement of cytoplasmic β-catenin through nuclear pores (Fig. 1B). To effect this nuclear localization, the Wnt signal inhibits the activity of the serine/threonine kinase GSK-3β, a ubiquitously expressed and consitutively active kinase [9-11]. In the absence of Wnt signals, GSK-3β cooperates with casein kinases 1α/ε and the proteins Adenomatous Polyposis Coli (APC), axin/conductin, diversin as well as other components to promote the degradation of newly translated β-catenin via the ubiquitin-proteasome pathway (Fig. 1A) [12-22]. This activity is very efficient and in the absence of any incoming Wnt signal, the cytoplasm and nucleus are devoid of β-catenin protein (except for a stable pool of β-catenin in cell adherens junctions where it functions as an essential adaptor protein between the cytoplasmic tail of E cadherin receptors and the cytoskeleton binding protein α-catenin.). Wnt signals inhibit GSK-3β kinase activity to stop degradation and force a rapid increase in the levels of free, cytosolic β-catenin and very soon thereafter, nuclear β-catenin. Nuclear β-catenin binds tightly to LEF/TCF transcription factors and

together they alter transcription of target genes (reviewed recently in [23]). β-catenin does not have a DNA binding domain, but it has a potent transcription activation domain. In general, LEF/TCF transcription factors do not have a strong transcription activation domain, but they have a good DNA binding/bending domain. Thus, when β-catenin binds to a LEF/TCF protein, a potent transcription regulatory complex is formed. When Wnt signals operate properly, the changes in gene expression direct cells to adopt specific cell fates and differentiate, or to grow and divide. Relevant target genes in the colon were recently identified by microarray analysis and include c-Myc, cyclin D1, matrix metalloproteinase-7, EphB and ephrinB, VEG-F as well as many others ([24, 25]; for an extensive, referenced list please see http://www.stanford/edu/~rnusse/pathways/targets.html).

I.B. Wnt-independent signalling in Cancer

Genetic mutation of midstream components can also activate the pathway, but in this case the pathway is turned on constitutively and is independent of Wnt•Frizzled interaction. This problem leads to cancer (Fig. 1C). Loss-of-function mutations in the tumor suppressor APC or axin, or mutations that make β-catenin unable to be degraded (mutation of GSK-3β serine substrates in the N-terminus), allow β-catenin protein to accumulate to very high levels. High levels of β-catenin translates into constitutive Wnt signaling - an activity that has been implicated as a root cause of several different kinds of cancers including colon, prostate, melanoma, hepatocellular carcinomas, and perhaps even certain subsets of breast cancers [1-6, 26]. Presumably, high levels of β-catenin•LEF or β-catenin•TCF complexes form in the nucleus and the target genes of this complex are thus misregulated. Some of the more relevant target genes for cell growth include c-Myc, cyclin D1, etc. That misregulation of Wnt target genes might be a strong transformation signal was tested by

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[1] The following websites are excellent resources for questions about Wnt signaling: http://www.stanford.edu/rnusse/wntwindow.html (free) and http://stke.sciencemag.org (subscription)

overexpression of a LEF-1•β-catenin fusion protein in normal, primary chicken embryo fibroblasts [27]. Three weeks later the fibroblasts exhibited hallmarks of cell transformation: colony formation in soft agar, anchorage-independent growth and loss of contact inhibition. In another system, the highly related γ-catenin (plakoglobin) protein which forms active complexes with LEF/TCFs was shown to upregulate *c-MYC* expression to transform epithelial cells [6]. Finally, overexpression of a stable β-catenin mutant in the skin of mice causes tumors that resemble human pilomatricomas [28]. Although the genes that are misregulated in this system were not identified and shown to be direct targets of LEF/TCFs, knock-out phenotypes of LEF-1 and TCF-3 have profound effects in hair and skin and thus it is likely that aberrant levels of β-catenin•LEF/TCF complexes played a causal role in the formation of tumors. When human pilomatricomas were examined, stabilizing mutations in β-catenin were identified and >75% of the tumors expressed LEF-1 protein in the nucleus [29]. As we will see below, disruption of β-catenin•TCF binding to target genes in colon cancer cells produces a profound and rapid block in the growth of these tumor cells. Thus, β-catenin•LEF/TCF complexes carry oncogenic activities and aberrantly high levels can cause the transformation of many cell types including cells of the colon.

I.C. Modulation of Wnt Signal Strength and Specificity in the Nucleus

Given that β-catenin is capable of oncogenic action, how easy is it for an abundant pool of this protein to gain access to Wnt target genes? First, β-catenin must form a complex with a LEF or TCF protein and second, that complex must be able to occupy Wnt response elements by binding to LEF/TCF binding sites. (Several recent studies have uncovered the potential for LEF/TCF-independent regulation of select target genes by β-catenin. However, the majority of bona fide Wnt response elements have thus far been shown to be LEF/TCF binding sites [30-32]). Numerous inhibitory

pathways limit the activity of β-catenin•LEF/TCF complexes from doing this (ie. the TAK/NLK pathway [33],I-mfa [34], ICAT [35], HBP-1[36], lines [37] and Sox3, Sox 17α/β [38]). Furthermore, once bound to the Wnt response element, β-catenin•LEF/TCF complexes must recruit co-activators of transcription, (Legless [39] and Pygopus [40-42]). In addition to these impinging pathways and requirements, the relative abundance of LEF/TCF isoforms and different family members expressed in the nucleus could also have a profound effect. Normal cells express both activating and inhibiting forms of LEF/TCFs and depending upon the relative abundance of these forms, β-catenin may not have easy access to Wnt targets. Also, LEF/TCFs are not fully interchangeable or redundant with one another. Non-redundancy was noticed in the phenotypes of mice missing one or more LEF/TCF genes, and although this is not fully understood, differences in the functional domains of LEF/TCFs and in alternatively-spliced C-terminal tails produced from the TCF loci have been described. Depending on the abundance or dearth of various isoforms, a subset of Wnt targets may be preferentially activated, selectively ignored or even directly inhibited. It follows then that for Wnt-linked cancers to develop, the signal transduction pathway must overcome any inhibitory pathway, circumvent inhibitory LEF/TCF isoforms, and ensure an abundance of full-length activating LEF/TCFs. Work from several groups supports a model whereby indeed, the pattern of LEF/TCF isoform expression changes as colon cancer progresses to favor stronger Wnt signaling in the nucleus. The remainder of this review focuses on the structure and activity of the functional domains of LEF/TCFs and describes the data that led to this model.

II. LEF/TCF Family

II.A. Family Members

The LEF/TCF family of transcription factors in mammals is comprised of 4 different proteins (Fig. 2). The founding family members, human and mouse LEF-1 and TCF-1, were first discovered in differentiating T and B lymphocytes [43-45]. Two other mammalian LEF/TCF proteins (TCF-3 and TCF-4) were later identified in other tissues by low-stringency cloning procedures and their expression patterns differ [46]. LEF/TCF proteins are expressed in many tissues during embryogenesis in overlapping, but distinct patterns [46, 47]. Shortly after birth, their expression is shut off or down-regulated, presumably when cells of these tissues become terminally differentiated. The known exceptions to this phenomenon are thymus (LEF-1, TCF-1), bone marrow (LEF-1) [48], the skin and dermal papillae at the base of hair follicles (LEF-1, TCF-3) [49, 50], the crypts of colon (TCF-4) [46, 51], intestinal mucosa (TCF-1, TCF-4) [46, 52], testes (LEF-1) [53], and mammary epithelia (TCF-1, TCF-4) [52, 54], and the list is growing. All of these tissues contain sites of continual cell growth and differentiation from stem cell populations.

II.B. LEF/TCF DNA Binding Domain

Of the functional domains that have been mapped in LEF/TCF proteins, four will be described here. The first is the High Mobility Group (HMG) DNA binding domain, an 88 amino acid region near the carboxy terminus containing an HMG box motif and nuclear localization signal. HMG boxes were first recognized in the non-histone, chromatin-associated HMG 1/2 proteins [55], and a hallmark of these motifs is that they bind to the minor groove of the double helix and bend DNA (90°-130°) [56, 57]. Whereas classic HMG proteins do not possess binding specificity for specific DNA sequences, the LEF/TCF family differ because they recognize variants of the core consensus sequence YCTTTGWW [43, 56, 58, 59]. The HMG DNA binding domain is the most highly conserved feature of the LEF/TCF

family (between 93%-99% amino acid sequence identity) which means that LEF/TCFs bind and bend the same consensus DNA sequences. A structural analysis of the binding and bending of a high affinity LEF/TCF site by the HMG DNA binding domain of LEF-1 has been determined by NMR [60].

II.C. β-catenin Binding Domain

The second most highly conserved feature of the LEF/TCF family is a region at the extreme N-terminus. The function of this region was discovered when Behrens et al. and Molenaar et al. identified it as a β-catenin binding domain in yeast two hybrid screens [61, 62]. Genetic experiments in *Drosophila* and *Xenopus* confirmed that this interaction is necessary for Wnt signaling and normal development, establishing that LEF/TCF proteins are bona fide components of the Wnt signal transduction pathway [59, 61, 63]. Since then, all known members of the LEF/TCF family have been shown to bind to β-catenin or one of its orthologues. X-ray crystallographic studies of the interaction between this domain and β-catenin have recently been solved to show that β-catenin binds along an extended region of the extreme N-terminus of each of these TCFs [64, 65]. Binding is proposed to be a multi-step interaction, inducing the formation of alternating β-strand and alpha helix segments between amino acids 12 and 50 to promote extended interactions with several armadillo repeat segments of β-catenin. Importantly, for every LEF/TCF locus examined thus far, this N-terminal region is encoded entirely within the first exon of each gene.

II.D. Alternative C-terminal Tails

A third notable feature of LEF/TCFs is the multiple C-termini generated from alternative splicing (designated by alpha-numeric nomenclature). Different termini were first discovered for TCF-1, but they have since been identified in all family members [66-68]. Although each of these alternative

C-termini has not been very well studied, two important activities have recently been ascribed to the "E" tail of TCFs. One motif that is present in the E-tail of TCF-3 and TCF-4 is recognized by the transcription co-repressor CtBP [69]. While the transcription co-repressing activities of CtBP are best studied, new activities have been attributed to this protein including transcription activation, enzymatic NAD+ dehydrogenase activities and modulation of the cytoskeleton [70-73]. More recently, a region of the E-tail juxtaposed to the HMG DNA binding domain of TCFs has been found to comprise a β-catenin-dependent transcription activation domain [74, 75]. A small, 33 amino acid region is required for activation from Wnt target sites in some promoters but not others. This small region, variously referred to as the CRARF or CR motif is highly conserved among all TCF orthologs and a single amino acid mutation in this domain is embryonic lethal in *Drosophila* [59]. The E-tail may be an extremely important alternative domain necessary for the repression and/or activation of a select subset of Wnt target genes. Interestingly, TCF-1 does not have the CtBP binding elements in its E-tail, but it carries the potent CR motif for transcription activation. TCF-3 has CtBP binding elements but is missing key residues of the CR motif; TCF-4 carries both CtBP binding elements and the CR activation domain. Finally, the *LEF1* locus cannot generate LEF-1 isoforms with an E-tail because the alternative exon needed in the splicing pattern is not present in the locus [68]. Clearly, the alternative splicing feature of the LEF/TCF family can distinguish one member from another by key differences in each of the tails and by the overall unique array of C-terminal activities produced from each locus.

II.E. Transcription Repression Domain

Lastly, a transcription repression domain is located in the central region; the overall function of this domain appears to be conserved for each LEF/TCF even though the primary sequences are not. LEF/TCF proteins do not independently activate or repress target genes, rather they are architectural

proteins that bend DNA to maximize productive interactions between DNA-bound proteins in a larger enhancesosome or repressing complex and they recruit co-activators or co-repressors [76, 77]. While the most well-known role of LEF/TCFs is to recruit the strong transcription activator β-catenin through direct binding to the N-terminus, the central domain of LEF/TCFs can recruit co-repressors of the Groucho family [78]. In addition, LEF-1 can recruit HDAC1, a repressive histone de-acetylase [79], but whether this occurs directly through binding the central domain or via an indirect interaction is not yet known. The central domain in LEF-1 can also engage in protein•protein contacts with the protein ALY [80] or with activating factors that bind to neighboring sites on the T Cell Receptor alpha chain (TCRα) enhancer [81-83]. Nevertheless for many, if not most target genes, it is thought that LEF/TCFs function as transcription repressors in the absence of β-catenin binding, and that this activity is carried out by co-repressor recruitment via the central domain.

III. Dominant negative LEF/TCFs

III.A. Production From Alternative Promoters

For Wnt signals to reach target genes in the nucleus, full-length LEF/TCF proteins with an intact β-catenin binding domain at the N-terminus must be present. Indeed most tissues with normal LEF/TCF expression show abundant levels of full-length protein. However, both the *TCF7* (encoding TCF-1 protein) and *LEF1* genes contain two different promoters for transcription (Fig. 3) [51, 66]. The first promoter in each gene produces mRNA encoding full-length protein with an intact β-catenin binding domain at the N-terminus. But the second promoter is located in the second intron and produces a truncated protein missing the β-catenin binding domain. Truncated LEF-1 and TCF-1 proteins have an intact HMG DNA binding domain and nuclear localization signal and can therefore reside in the nucleus and bind as tightly to Wnt response elements as full-length forms. They cannot

recruit β-catenin as they are missing the N-terminus, and therefore they cannot mediate Wnt signals. They are however, fully capable of recruiting co-repressors such as Groucho through their intact central domain. Thus, if the levels of these truncated forms exceed the levels of full-length, β-catenin binding forms, they could effectively suppress Wnt signaling by occupying regulatory sites and actively inhibiting transcription. These truncated forms are called natural dominant negatives as they are able to prevent β-catenin•TCF complexes from activating reporter genes [51, 61]. Truncated forms are expressed in normal tissues such as thymus, where the *TCF7* locus expresses mostly dominant negative forms, and the *LEF1* locus appears to express equivalent amounts of both (at least at the mRNA level) [43, 44, 66, 84]. In the case of normal and cancerous colon, the relative amounts of full-length and dominant negative proteins may be very important for normal differentiation and tumor progression.

III.B. dnTCF studies in colon cancer cells

Overexpression of dnTCFs has been used to explore the activity of Wnt signals in colon cancer cells. Last year, Clevers and associates created stable colon cancer cell lines in which robust levels of dnTCF-4E and dnTCF-1E could be induced by the addition of tetracycline analogs to the media [24, 25]. Induction of dnTCF-4E caused an abrupt stall in the G1 phase of the cell cycle. In a series of elegant experiments and microarray analyses, it was determined that the stall in the cell cycle was due to downregulation of c-Myc and the subsequent expression of $p21^{CIP1/WAF1}$. Overexpression of the dnTCFs also induced differentiation of the colon cells. The authors of the study concluded that active Wnt signals promote proliferation over differentiation primarily through suppression of $p21^{CIP1/WAF1}$ activity and as such, Wnt signals function as an important switch between a proliferative phase in immature colon cells in the crypt and a non-dividing, differentiation phase of cells as they migrate to

the surface of the intestinal lumen. This study highlights the potent activity of the dominant negative form of TCF and suggests that endogenous dnTCFs may function in immature colon to temper normal Wnt signaling and proliferation and promote differentiation. This may be the case, because mice missing the *TCF7* gene develop intestinal adenomas (see below).

IV. Expression of LEF/TCFs in colon

IV.A.TCF-4

What are the expression patterns of LEF/TCFs in normal colon tissue and colon cancer? Are the dominant negative forms expressed, and if so, is expression altered in cancer? Recent studies have begun to address these questions. Two members of the family, TCF-4 and TCF-1 are expressed in normal gut mucosa and three members, TCF-4, TCF-1 and LEF-1 are expressed in colon cancer. Normal colon tissue requires TCF-4 expression for development and maintenance of its stem cell compartment [46]. Mice missing the *TCF7L2* gene (encodes TCF-4 protein) die shortly after birth with the unusual phenotype of an intestine with a few differentiated villi and no proliferating crypt stem cell compartment. Analysis of TCF-4 expression in adult human colon shows that mRNA and protein is present in all cells of the gut epithelium from the crypt to the tips of the villi [51, 54, 85]. TCF-4 continues to be expressed in colon cancer and western analysis of the protein in human colon cancer cell lines suggests that only a full-length TCF-4 polypeptide is expressed [51]. Thus expression of TCF-4 correlates most closely with maintainance of a mitotically active stem cell population in the intestine.

IV.B. TCF-1

TCF-1 function differs dramatically from TCF-4. TCF-1 is expressed in fetal and adult intestine [52], and its expression has also been noted in mouse small intestine and human colon cancer cell lines [86]. Surprisingly, mice missing the *TCF7* gene (TCF-1 protein) develop intestinal and mammary adenomas [52]. There are dramatic increases in the number of adenomas when the *APC* gene is inactivated in these mice underscoring a genetic link to this tumor suppressor and the Wnt pathway. In this respect, TCF-1 appears to function as a Wnt-linked tumor suppressor rather than a Wnt-linked tumor promoter. While the relative levels of full-length versus truncated TCF-1 isoforms are not known for mouse and human intestine, the expression of TCF-1 in normal thymus tissue shows that the predominate forms are truncated polypeptides missing the β-catenin binding domain [66, 84]. If this is also true for expression of the *TCF7* locus in the intestine, then an abundance of dominant negative TCF-1 could limit Wnt signal transduction by competing with β-catenin•TCF-4 for sites on targets genes, and this could be its normal, essential function. What is the pattern of expression of TCF-1 in primary colon cancer? Again, no definitive answers are yet available, but colon cancer cell lines contain variable amounts of full-length and truncated isoforms [86].

IV.C. LEF-1

LEF-1 function is not considered to be normally relevant to adult colon tissue. The gene is not expressed [51, 87] and no defects in mice missing the *LEF1* gene have ever been reported. However, a recent study has shown that expression of *LEF1* is detected in the intestine of mouse embryos [88]. Perhaps *LEF1* is expressed in the intestine during development and its expression is permanently shut off at birth, like that of many other tissues that express *LEF1* embryonically. If so, LEF-1 may play a heretofore undetected role in embryonic patterning or cell growth of this tissue. Nevertheless, because

LEF1 expression is not detectable in normal adult intestine, it is surprising to find that LEF-1 expression is easily detected in the majority of primary advanced human colon cancers and many colon cancer cell lines [51]. This means that transcription of the *LEF1* locus is activated (or re-activated) by some aberrant mechanism in these tumors. It is not known whether expression of *LEF1* contributes to tumor progression but interestingly, the expression pattern is aberrant as it is skewed entirely towards producing full-length forms that bind to β-catenin. The promoter for the full-length LEF-1 form is activated whereas the intronic promoter that produces the truncated dominant negative form is specifically left silent (or perhaps even repressed).

V. The Potential for Wnt-Linked Autoregulation of Signal Strength and Specificity

Given the tumor suppressive functions of TCF-1 and the aberrantly skewed expression pattern of LEF-1 in colon, it appears that Wnt-linked tumors may progress in part by down-regulating or negating expression of dominant negative LEF/TCF proteins and upregulating the expression of full-length isoforms. Loss of expression of the truncated forms could occur by loss of the gene entirely, and this has been proposed for *TCF7* [52]. Indeed, *TCF7* resides on the same arm of chromosome 5 as the *APC* gene (5q31.1 and 5q22.1 respectively), and loss of both genes in mice causes synergistic increases in number of adenomas [52]. On the other hand, both *TCF7* and *LEF1* upstream promoters contain Wnt response elements (Fig. 3) [51, 52]. *TCF7* is a target of the Wnt pathway, and *LEF1* has also recently been shown to be a bona fide target gene ([51, 75]; J.-H. Ting and M.L. Waterman, in preparation). It is possible that in an early cancer with a growing pool of β-catenin protein, expression of targets such as full-length TCF-1 or LEF-1 could be activated by β-catenin•TCF-4 complexes (without concomitant activation of the intronic promoters). As a result, a greater level of full-length TCF-1 and LEF-1 protein would be available for complex formation with β-catenin.

In an interesting twist, only the E-tail isoforms of TCF-1 or TCF-4 are able to activate the upstream *LEF1* promoter [75]. Two response elements have been mapped ~200 nucleotides downstream of the start sites of transcription. Likewise, the promoter for Cdx-1, a homeobox transcription factor expressed in the endoderm of developing intestine, requires a complex between β-catenin and TCF-4E or TCF-1E binding to elements upstream of the start site of transcription [74]. LEF-1, or TCF isoforms with other C-termini are unable to work with β-catenin to activate these targets. That a subset of Wnt target genes can only be activated by certain alternatively spliced isoforms of TCFs is surprising. The underlying reason for such specificity is not yet known, but the surrounding context of the Wnt target elements in the promoters and/or the presence/absence of neighboring factors and/or subtle differences in the sequence of the Wnt response elements are all possibilities. Although speculative, it is possible that the prevalence of E-tail isoforms increases during tumor progression. Higher concentrations of β-catenin•LEF/TCF complexes in the nucleus could dysregulate normal target genes and perhaps other target genes that should not be expressed in a colon cell (eg. *LEF1*).

VI. Conclusion and Unanswered Questions

In summary, we know of a number of extrinsic inhibitory and stimulatory pathways that can influence the strength of Wnt signals in the nucleus. But we must now include a consideration of the expression pattern of the LEF/TCF proteins themselves, and the role that the Wnt pathway itself may play in modulating these patterns of expression. Results over the past several years emphasize that expression of LEF/TCF loci is complicated, producing isoforms with opposing activities as well as alternatively spliced isoforms that carry selective activities with regard to subsets of Wnt target genes. It is clear that not only can the *strength* of the Wnt signal be modulated by the relative expression

levels of full-length and dominant negative LEF/TCF isoforms, but perhaps even *specificity* of the Wnt signal through alternative splicing of LEF/TCF mRNAs. Current *in situ* hybridization and immunohistochemistry techniques cannot easily distinguish between these various forms and thus can give no information as to whether patterns of expression differ between normal and transformed tissue. Skewed, aberrant expression of the *LEF1* locus to favor full-length activating forms of in colon cancer is the first example that expression patterns may indeed differ. Even though it is not yet clear that the presence of LEF-1 protein promotes tumor progression - TCF-4 and TCF-1 are already expressed in these cells - we now know that these factors are not completely redundant in their actions. The next challenge is to determine whether LEF/TCFs harbor non-redundant functions in colon cancer and whether changing the strength and/or specificity of the Wnt signal through an alteration of expression from LEF/TCF loci plays a bona fide role in the conversion of an early transformation event in a colon cell to an aggressive colon carcinoma years later.

Acknowledgements: The author extends a special appreciation to her colleages Dr. Randall Holcombe and Dr. Larry Marsh and members of the Waterman laboratory for scientific discussion. The author's research is supported by NIH CA8392 and CA096878.

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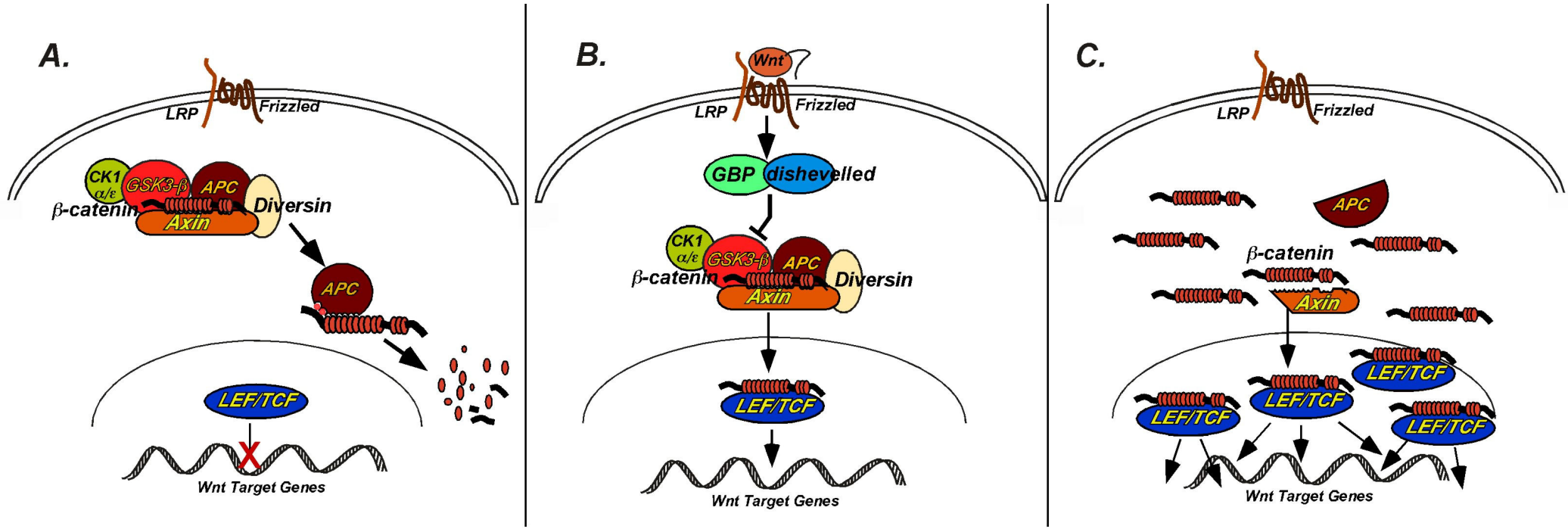
Figure Legends

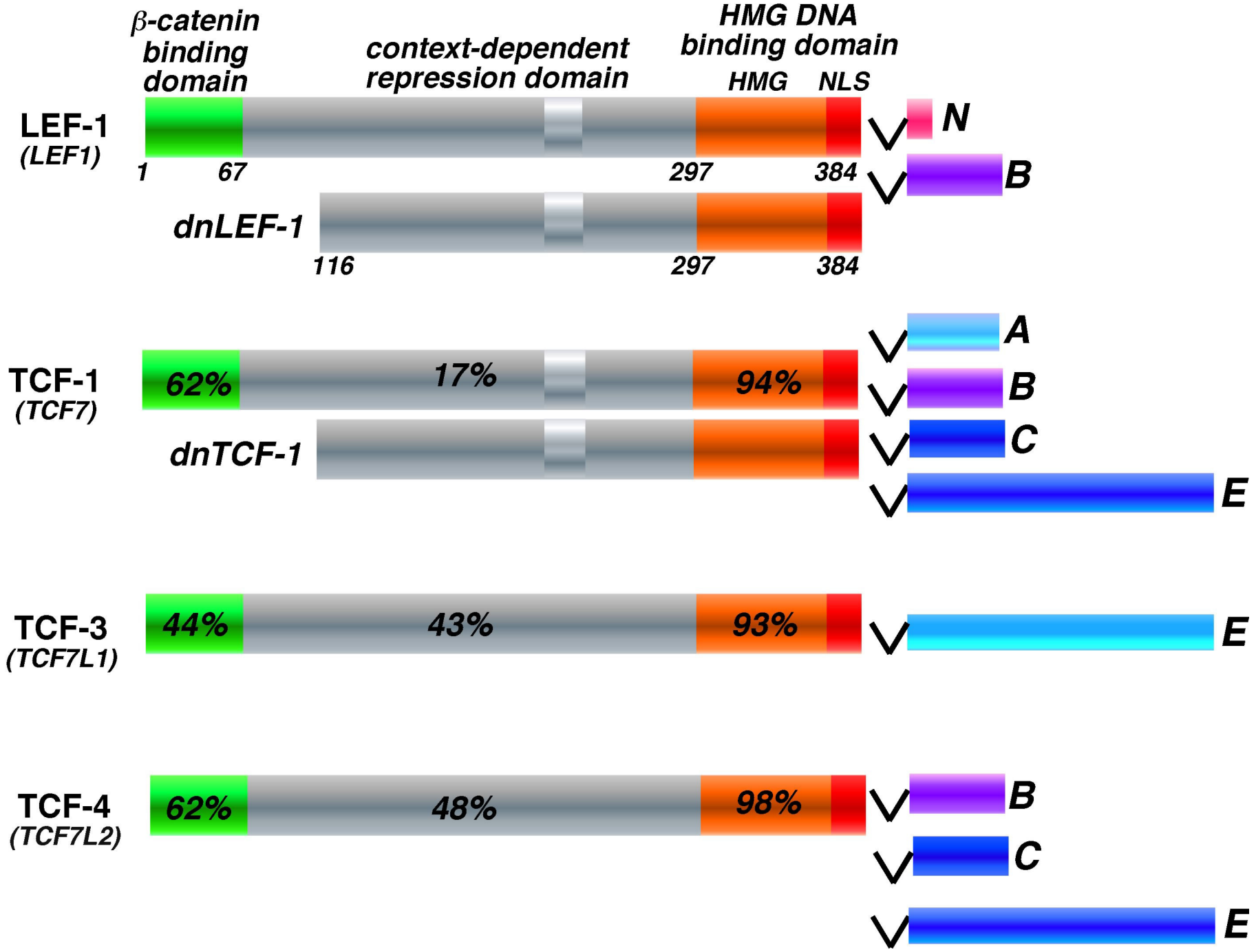
Figure 1. Wnt Signaling in Normal Cells and Cancer. A) In the absence of Wnt signaling, β-catenin is continually degraded by a ubiquitin-dependent mechanism. Capture of β-catenin by the cytoskeletal scaffolding protein Axin in conjunction with the tumor suppressor Adenomatous Polyposis Coli (APC) and Diversin, and its multi-step phosphorylation by Casein Kinase Iα/ε (CKIα/ε) and Glycogen Synthase Kinase 3β (GSK3-β), marks β-catenin for ubiquitination by SCFβ-TrCP E3 ubiquitin ligase (not shown; [89]). LEF/TCF proteins in the nucleus are presumably bound to Wnt target genes in the absence of β-catenin and are recruiting co-repressor complexes to silence transcription. **B)** The Wnt signal transduction cascade is initiated when secreted Wnt ligands bind to any one of a number of Frizzled receptors and Lrp (also known as arrow), on the cell surface. Many of the ensuing steps are not shown for brevity, but signaling inhibits GSK-3β activity via a complex of Dishevelled and GSK-3β Binding Protein (GBP) such that β-catenin cannot be phosphorylated and targeted for ubiquitin-mediated degradation. Unphosphorylated β-catenin is able to translocate through nuclear pores to form a complex with members of the LEF/TCF family and in so doing displaces/inactivates co-repressor factors and activates Wnt target genes through a transcription activation domain in the C-terminus. **C)** Loss of heterozygosity (LOH) of *APC* or *AXIN* loci, or site-specific substitutions in the N-terminus of β-catenin are mutations frequently found in many different cancers. For colon cancer, the most frequent genetic mutation occurs at the *APC* locus where either the entire gene is lost or truncation mutations have been introduced. Genetic inactivation of any one of these three components negates phosphorylation and ubiquitination of β-catenin. High levels of β-catenin protein in the cytoplasm and the nucleus are detected in colon cancers with APC LOH, and APC mutations are detected in $\geq$ 85%

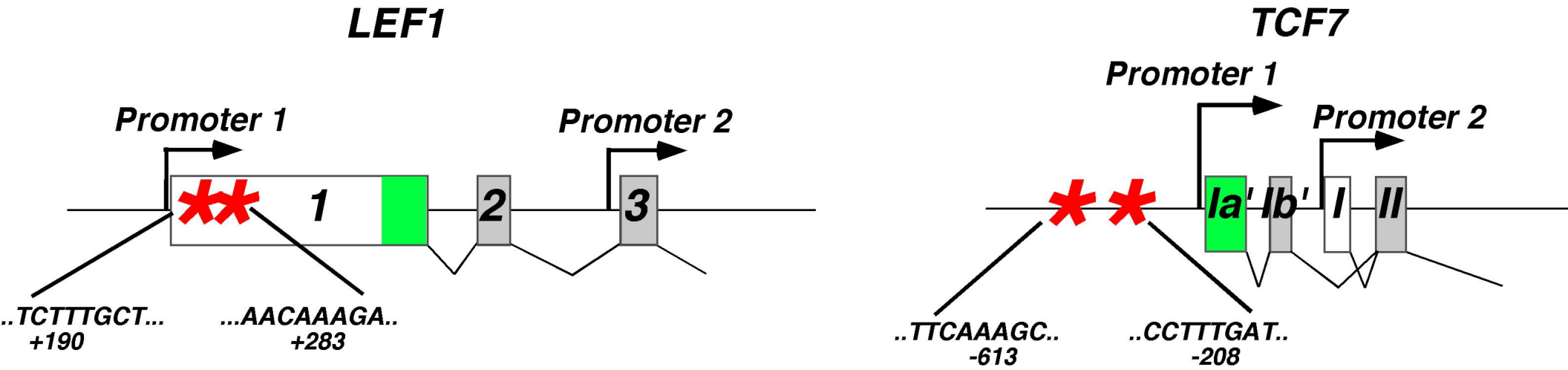
sporadic colon cancers. Strong evidence exists that misregulation of Wnt target gene expression is a crucial component of cell transformation in colon cells.

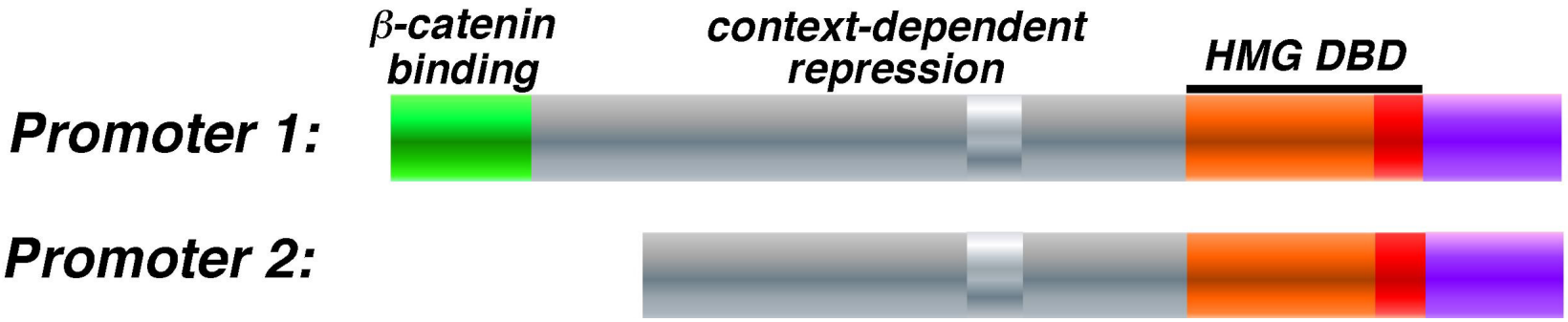
Figure 2. Domain Organization and Isoform Subtypes of the LEF/TCF Family. Structure and alignment of the four human LEF/TCF family members is shown. Italicized names in parantheses refer to the official HUGO name of each of the genes. Percent amino acid identity in select domains is shown relative to the amino acid sequence of LEF-1. Dominant negative isoforms of LEF-1 and TCF-1 are shown as is an alternative exon in the context-dependent repression domain of LEF-1 and TCF-1. Translation initiates in the third exon of each gene (see Figure 3). Alternative C-terminal domains are generated by alternative splicing at the end of each locus. The *LEF1* locus cannot produce a E-tail domain, and the E-tail domain of TCF-3 is divergent from the E-tails of TCF-1 and TCF-4.

Figure 3. Alternative Promoter Usage and Wnt Response Elements in *LEF1* and *TCF7* loci. LEF-1 and TCF-1 proteins exist as full-length activating forms (produced by transcription starting at Promoter 1) and truncated dominant negative forms (transcription at Promoter 2). The N-terminal β-catenin binding domain is encoded in exon 1 of *LEF1* and exon Ia of *TCF7*. The second intron of both genes contains a second promoter. Translation of Promoter 2 transcripts begins at internal methionine residues in the third exon of each gene ("3" for *LEF1* and "II" for *TCF7*). Wnt response elements have been located near Promoter 1 for each locus. In each case, β-catenin•TCF-4 complexes have been shown to activate expression. However, in the case for *LEF1*, only TCF-4 (and TCF-1) isoforms that contain an E-tail at the C-terminus are capable of β-catenin dependent regulation of the promoter.


A.
LRP
Frizzled
CK1
α/ε
GSK3-β
APC
β-catenin
Axin
Diversin
APC
LEF/TCF
Wnt Target Genes
B.
Wnt
LRP
Frizzled
GBP
dishevelled
CK1
α/ε
GSK3-β
APC
β-catenin
Axin
Diversin
LEF/TCF
Wnt Target Genes
C.
LRP
Frizzled
APC
β-catenin
Axin
LEF/TCF
LEF/TCF
LEF/TCF
LEF/TCF
Wnt Target Genes


β-catenin binding domain
context-dependent repression domain
HMG DNA binding domain
HMG
NLS
LEF-1
(LEF1)
1
67
297
384
N
B
dnLEF-1
116
297
384
TCF-1
(TCF7)
62%
17%
94%
A
B
C
E
dnTCF-1
TCF-3
(TCF7L1)
44%
43%
93%
E
TCF-4
(TCF7L2)
62%
48%
98%
B
C
E


LEF1
Promoter 1
Promoter 2
1
2
3
..TCTTTGCT...
+190
...AACAAAGA..
+283
TCF7
Promoter 1
Promoter 2
Ia'
Ib'
I
II
..TTCAAAGC..
-613
..CCTTTGAT..
-208


β-catenin binding
context-dependent repression
HMG DBD
Promoter 1:
Promoter 2: