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Cell-Context Dependent TCF/LEF Expression and Function: Alternative Tales of Repression, De-Repression and Activation Potentials

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Abstract

Wnt signaling controls cell specification and fate during development and adult tissue homeostasis by converging on a small family of DNA binding factors, the T-cell factor/lymphoid enhancer factor (TCF/LEF) family. In response to Wnt signals, TCF/LEF members undergo a transcriptional switch from repression to activation mediated in part by nuclear β -catenin binding and recruitment of co-activator complexes. In mammals, the specificity and fine tuning of this transcriptional switch is also achieved by the cell-context-dependent expression of four members (TCF7, TCF7L1, TCF7L2, and LEF1) and numerous variants, which display differential DNA binding affinity and specificity, repression strength, activation potential, and regulators. TCF7/LEF1 variants are generated by alternative promoters, alternative exon cassettes, and alternative donor/acceptor splicing sites, allowing combinatorial insertion/exclusion of modular functional and regulatory domains. In this review we present mounting evidence for the interdependency of TCF7/LEF1 variant expression and functions with cell lineage and cell state. We also illustrate how the p53 and nuclear receptor family of transcription factors, known to control cell fate and to inhibit Wnt signaling, may participate in the fine tuning of TCF7/LEF1 repression/activation potentials.

Keywords

alternative splicing; cell differentiation; Wnt signaling; transcription factor; nuclear receptor; p53

I. INTRODUCTION

Wnts and Wnt signaling control cell specification and fate during embryonic development and adult tissue homeostasis. Inappropriate spatiotemporal regulation of Wnt signaling results in numerous developmental defects, and its deregulation in adult tissues is highly associated with cancer initiation and progression.^{1–4} Among the different signaling pathways elicited by Wnt proteins,^{5–8} the canonical Wnt/ β -catenin signaling pathway has been directly implicated in the maintenance of adult stem-cell pools and tissue regeneration,⁹ as well as in colorectal and hepatocellular carcinoma.^{2–4} This pathway is

initiated by β -catenin stabilization in the cytoplasm and subsequent accumulation in the nucleus, where β -catenin interacts with T-cell factor/lymphoid enhancer factor (TCF/LEF) DNA binding effectors to modulate the transcription of numerous target genes involved in cell cycle progression, extracellular matrix remodeling, cell adhesion, and cell differentiation. Yet, it has become evident that the transcriptional programs elicited are very diverse, albeit specific in a tissue, cell-type, and cell-state dependent manner, and that consequently the outcomes vary markedly from stem-cell renewal and cancer initiation^{2–4,10–12} to establishment of cell senescence and tissue aging.^{13–17}

Surprisingly, despite these diverse and specific functions, the TCF/LEF family is actually a rather small subset of the high mobility group (HMG) box protein family, with only one member present in *Drosophila* (dTCF/Pan) and *C. elegans* (POP1), and four members in most vertebrates: TCF7 (TCF1), TCF7L1 (TCF3), TCF7L2 (TCF4), and LEF1 (TCF7L3). They are all able to bind with high affinity specific WWCAAAG DNA sequences, also called Wnt-responsive elements (WRE), present in numerous promoters and other transcriptional regulatory regions such as enhancers.^{18,19} Like other HMG box proteins they are devoid of transcriptional activity *per se* but serve as DNA-anchored platforms to recruit transcriptional complexes and chromatin remodeling complexes, and allow by virtue of their DNA bending properties the formation of DNA loops favoring the concentration of distant transcriptional complexes.^{18–21} TCF/LEFs interact with both co-repressor and coactivator complexes, with β -catenin probably the most potent activator identified.^{22–24} A transcriptional switch from repression to activation is the prevailing model for β -catenin-dependent target gene activation.^{25,26} In the absence of Wnt signals, TCF/LEF factors are associated with ubiquitous co-repressors such as the transducin-like enhancer of slip (TLE)/Groucho family members and actively repress transcription of target genes.^{27–29} Upon Wnt signals, β -catenin interacts with TCF/LEF and concomitantly displaces or favors the release of the co-repressor TLE/Groucho and promotes target gene activation by recruiting activator complexes (e.g., pygopus, mediator, p300/CBP) and chromatin remodeling complexes (e.g., Brg1/SWI/SNF, p300/CBP) (see review by Archbold et al.³⁰ and references therein). Although this model provides a mechanistic framework, it is insufficient to fully render the biological diversity and specificity of TCF/LEF activities including β -catenin-independent activities and differential responses to Wnt signaling,^{31–33} which may be mediated by derepression rather than activation.^{34,35}

The differential expression of each TCF7/LEF1 member during development is partly responsible for the specific defects evidenced by loss of functions in animal models (see recent review by Grigoryan et al.¹ and references therein). Nonetheless, phenotypic rescue experiments have revealed that the lack of redundancy was also inherent to differential if not opposite activities,³⁶ with TCF7L1 behaving mainly as a repressor and LEF1 as an activator, while TCF7 and TCF7L2 display dual transcriptional effects depending on the assays and biological systems used^{35,37–42} as well as the isoform studied.^{35,43–49} Indeed, all TCF7/LEF1 factors display complex expression patterns combining various strategies to increase spatiotemporal diversity from a single gene, including tissue-specific enhancers, alternative promoters, alternative exon cassette splicing, and alternative donor/acceptor splicing site (Fig. 1). The combination of regulated transcription and RNA processing allows the versatile expression of a myriad of protein variants with diverse arrangements of both common and unique modular domains. The community is starting to recognize and decipher the biological relevance of these variants in the specificity and regulation of the TCF7/LEF1 transcriptional repression/activation switch. Indeed, all the cellular processes involved from transcription to post-translational modifications are also cell context dependent and under the control of feedback mechanisms and crosstalk with various signaling pathways such as nuclear receptors (NR) and p53 family members, which are known to regulate cell specification^{50–52} and fate,^{53–57} respectively, and to modulate Wnt signaling.^{58–63}

II. TISSUE-SPECIFIC AND CELL CONTEXT-DEPENDENT EXPRESSION AND FUNCTIONS OF TCF7/LEF1 MEMBERS AND VARIANTS

Although TCF7/LEF1 alternatively spliced exons may also affect the rate and/or exon choice for downstream RNA processing, the nuclear retention of partially mature RNA, RNA stability, cytosolic RNA localization to specific RNA-binding complexes (e.g., P-bodies) and miRNA-dependent regulation of translation (Fig. 1), these mechanisms and roles remain to be investigated. In this section, we will primarily review and discuss their roles in the creation of cell context-dependent diversity in TCF7/LEF1 functional and regulatory domains, hence in the TCF7/LEF1 repressor/activator balance. Since TCF7L2 is the most versatile member of the family and the only one able to rescue a POP1-deficient phenotype in *C. elegans* during the first asymmetric mesodermal/endodermal cell specification,⁶⁴ we will particularly focus on the cell lineage dependent expression of TCF7L2 variants and their potential roles in the reinforcement of cell lineage commitments. The organization and exon equivalence of TCF7/LEF1 genes are shown in Fig. 2; for clarity, the latest numbering of *TCF7L2* and *LEF1* exons will be used throughout this review. The exon equivalence between the TCF7/LEF1 members is based upon their sequential organization in the genome starting from the upstream transcription start sites (TSS), sequence homologies, and exon/exon junction conservation (Fig. 2).

A. Tissue-Specific TCF7L2 Enhancers, Common TCF7L2 Polymorphisms, and Individual Susceptibility to Diseases

A nonsynonymous single nucleotide polymorphism (SNP), C888A at the 3'-end of the Exons 3a-d (Fig. 2), a sequence common to all isoforms, has been associated with type-1 diabetes (T1D),⁶⁵⁻⁶⁸ an autoimmune disease characterized by the degeneration of pancreatic β -islet cells.⁶⁹ The resulting Pro-to-Thr conversion is present in all TCF7 variants including the major Δ N-TCF7 variants expressed in activated T lymphocytes,⁷⁰ but the functional consequences are not known. In contrast to TCF7, several common noncoding polymorphisms of the *TCF7L2* locus have been reproducibly associated with increased risk of developing type-2 diabetes (T2D)⁷¹⁻⁷⁴ and its vascular complications, i.e., atherosclerosis and coronary diseases.^{75,76} On the other hand, the association of *TCF7L2* polymorphisms with breast⁷⁷⁻⁷⁹ and colon^{80,81} carcinomas, which are also highly influenced by obesity and T2D,⁸²⁻⁸⁴ remain controversial. The major T2D-associated TCF7L2 SNP, rs7903146 C/T, is located within a region of the large intron-4 corresponding to a pancreatic β -islet-specific open-chromatin region with enhancer activities that are increased in *in vitro* reporter assays when the T2D-risk allele (T) is present.^{85,86} The existence of pancreatic-specific TCF7L2 enhancers within the 90 kbps of the T2D-susceptibility locus has been confirmed *in vivo* by BAC enhancer trapping scan in mouse transgenic reporter lines, though TCF7L2 expression was lost in adult mouse pancreatic β islet.⁸⁷ In the same screen, additional tissue-specific enhancer activities were revealed in the brain, intestine, stomach, and limbs,⁸⁷ confirming earlier studies of *Tcf7l2* expression during mouse embryonic development, and in adult intestine and brain.⁸⁸⁻⁹⁰ However, no significant changes in *TCF7L2* overall expression were found associated with *TCF7L2* polymorphisms in pancreatic tissues and isolated β islets in most studies.⁹¹⁻⁹⁵ In contrast, a significant decrease in the levels of a TCF7L2 alternative exon, Exon 17 (Fig. 2), which is enriched in neuro-endocrine tissues,⁹⁶ was noted in pancreatic tissues and isolated pancreatic β islets by Prokunina-Olsson et al.⁹² but not in other studies.^{93,94} Osmark et al. reported an increase of the alternative Exon-4 form together with up-regulation of *TCF7L2* expression in T2D patients independently of *TCF7L2* polymorphic genotypes.⁹³ These conflicting results can be explained by a complex and cell-type-specific expression of TCF7L2 variants that cannot easily be investigated in most tissues and will likely require analysis at the single-cell level. Whether *TCF7L2* tissue-specific enhancers influence TCF7L2 alternative exon inclusion/exclusion during

transcription-coupled alternative splicing events remains an interesting possibility warranting further investigation.

B. Cell-State-Dependent Usage of Alternative Promoters and β -Catenin Interactions

Most TCF7/LEF1 family members display alternative promoters (Fig. 2), which are responsible for the expression of RNA isoforms with or without at least the first exon.^{31,97–100} The relative levels of these Δ Exon-1 RNA isoforms appear more prominent for TCF7^{100,101} and LEF1^{97,98,102,103} than TCF7L2 in different tissues and cell types,^{41,49,92,104} including breast (Boyechko, submitted) and colon⁴⁷ cancer cell lines. The usage of TCF7 and LEF1 alternative promoters in different cell types is linked to their cell state, with in general a switch from full-length variants in proliferating immature cells to Δ N-variants during cell differentiation and associated cell cycle arrest in the thymus, peripheral T lymphoid organs, intestine, and colon.^{2,47,70} In bone, LEF1 is expressed in proliferating immature osteoblasts, where it inhibits osteogenesis, but upon differentiation its expression is down-regulated¹⁰⁵ and Δ N-LEF1 variants are up-regulated in response to BMP2 signals through RUNX2-dependent but SMAD4-independent activation of the P2 alternative promoter.¹⁰² In contrast, both LEF1 and TCF7 are re-expressed in colon carcinoma cells, where they amplify the deregulation of Wnt signaling.^{47,98} This specific up-regulation of LEF1 depends on the presence of TCF7/LEF1 DNA-binding sites within Exon 1 that participate in local chromatin remodeling at the upstream P1 promoter in a β -catenin-dependent manner,⁹⁹ while the downstream P2 promoter located between Exon 3 and 4 is repressed by the transcription factor Yin Yang (YY)-1 even though it contains also TCF7/LEF1 DNA-binding sites.¹⁰⁶ Of note, one important conserved feature of all TCF7/LEF1 genes across species is the presence of functional TCF7/LEF1 DNA-binding sites in their transcriptional regulatory sequences, which mediate feedback and crosstalk between them.^{35,42,107–109}

Exon 1 encodes the N-terminal domain involved in high-affinity binding of nuclear β -catenin (Figs. 2 and 3).^{110–112} Deletion of this domain is sufficient to produce Δ N-variants that prevent β -catenin-dependent activation of Wnt-target genes in many biological systems.^{30,31,37,98,113} However, extended interactions of β -catenin have been identified in LEF1, within the conserved N-terminal domains encoded by Exon 2 and 3 and within the conserved central region and HMG box domain when bound to DNA (Fig. 2).²⁵ These extended areas of low-affinity β -catenin/TCF interactions overlapping with TLE/Groucho binding domain²⁵ and the absence of detectable β -catenin/TCF/TLE ternary complexes^{25,114} are at the basis of the current model of a repression/activation switch by β -catenin-induced displacement of TLE/Groucho co-repressor complexes.^{25,30} Another low-affinity β -catenin binding site, encompassing a conserved TCF7/LEF1 domain within the context-dependent regulatory region, was recently identified in Δ N-LEF1 based upon its ability to still interact with β -catenin *in vitro*, and to enhance the expression of differentiation Wnt-target genes in mesenchymal and osteoblastic cell lines.¹⁰³ Although the latter implicates that β -catenin-dependent activation of Δ N-LEF1 might still occur, the net effect of bone-targeted Δ N-LEF1 expression in the mouse is to promote osteogenesis.¹⁰³ Similarly, the absence of β -catenin/LEF1 interaction, via mutations of LEF1 within the N-terminal β -catenin binding domain, is associated with increased sebaceous cell differentiation and development of sebaceous tumors in humans.^{115,116} In the mouse, the targeted expression of Δ N-LEF1 in the skin also promotes sebaceous tumor formation and alteration of the tumor-suppressor p53 cascade.¹¹⁵ Moreover, these *in vivo* data emphasize the crucial roles of Wnt signaling in cell lineage specification and their dose dependency,^{51,115} which may both rely on the balance of TCF7/LEF1 repressor/activator members and variants expressed at a given time during lineage specification.

C. Cell-State-Dependent Insertion/Exclusion of TCF7L2, TCF7L1, and LEF1 Alternative Exon Cassettes Within the Cell Context-Dependent Regulatory Region

TCF7L1 and TCF7L2 share an additional conserved exon, Exon 4 and Exon 5 respectively, which is mainly nonalternative and responsible for the bulk of their divergence in the context-dependent regulatory region from TCF7 and LEF1 (Fig. 2). Additional and highly alternative exon cassettes, Exon 4 and Exon 6 (Fig. 2), occur in human, mouse, and bovine TCF7L2 transcripts (Refs. 35, 92, 93, 104, Boyechko et al., submitted), while only the alternative Exon-4 cassette has been described in zebrafish¹¹⁷ and xenopus *Tcf7l2* transcripts.⁹⁰ In mammalian cells the levels of Exon-4 variant are highly correlated with the differentiated states of the cells independently of their cell type (Ref. 35 and Boyechko et al., submitted). TCF7L2 Exon 4 is present in >50% of the transcripts in primary macrovascular endothelial cells and epithelial committed/differentiated carcinoma cells, at intermediate levels (25–30% of the transcripts) in adipocytes, myocytes, blood cells, and microvascular endothelial cells, and <10% of the transcripts in breast cancer cell lines with stem-like properties, in some hepatocarcinoma cell lines, and in the embryonic stem-like HEK293 cells (Refs. 35, 49, 93, and Boyechko et al., submitted). Lower levels of *Tcf7l2* Exon 4 were also noted in heart, brain, and mES cells.⁴³ In contrast, TCF7L2 Exon 6 is a minor alternative exon both in embryonic and adult cells (Refs. 35, 92, Boyechko et al., submitted). A *Tcf7l1* alternative exon cassette, msExon 5 (Fig. 2), was recently identified in mouse embryonic stem cells (ESC) on the basis of its decreased insertion levels during mESC differentiation.¹¹⁸ *Tcf7l1* Exon5 encodes a small (14 AAs) domain, which confers an increased ability to repress Oct4,¹¹⁸ a pluripotency core transcription factor,¹¹⁹ and a specific repression of AP1 family members such as Jun and ATF3 in differentiated mES cells.¹¹⁸ Although this alternative cassette appears not conserved in human ES cells,¹¹⁸ it is worth noting that the alternative Exon-6 cassette in human TCF7L2 encodes a similar though larger domain (Figs. 2 and 3) with low levels of expression in all human embryonic and adult cells tested thus far (Ref. 35, Boyechko et al., submitted). In view of their regulated expression during cell differentiation and also in response to p53 activation for TCF7L2 (see below), it is of interest to define how they may regulate the transcriptional repression-to-activation switch of TCF7L1 and TCF7L2.

Compared to LEF1, the domains in TCF7L2 encoded by the no alternative Exon 5 (37AA) and the alternative Exon 4 (23 AA) and Exon 6 (47AA) further extend the context-dependent regulatory region between the N-terminal β -catenin-binding domain and the low-affinity β -catenin-binding site and TLE/Groucho-binding domain²⁵ (Figs. 2 and 3). The Exon-4 insertion was shown to dampen the transcriptional activity of stable β -catenin⁴³ and to repress³⁵ several Wnt-target gene-promoter reporters. This extension may thus be sufficient to alter the stability of β -catenin/TCF interaction and/or to promote the binding of novel cofactors by providing some organized structures (e.g., small α -helices) to the otherwise disorganized context-dependent regulatory region.^{25,120} Indeed, only the conserved TCF7/LEF1 HMG-Box domains fold in organized structures favorable to protein-protein interactions, when bound to DNA.^{21,25} Accordingly most interacting proteins for which the domain of interaction is known, interact with the HMG-box domain and surrounding sequences (Fig. 2),^{28,29,121–131} except β -catenin, which binds the N-terminal domain even in an extended and flexible conformation.^{25,111,112} Another exception is the LIM-domain protein HIC5, a focal adhesion and stress regulated nucleocytoplasmic shuttling protein, which was identified as an interacting partner of TCF7/LEF1 factors depending on the presence of the alternative Exon 7 in LEF1 (Fig. 2).¹³² The Exon 7–encoded domain is conserved and necessary for β -catenin-dependent activation of TCF7/LEF1-reporter and -target genes such as fibronectin.^{38,132} HIC5 interaction with TCF7/LEF1 members and variants containing this conserved domain inhibits β -catenin/TCF transcriptional activity.¹³² In view of the overlaps between the HIC5 interaction domain and

the low-affinity β -catenin binding site (Fig. 2),²⁵ the HIC5 interaction may prevent and/or weaken β -catenin binding since the LEF1 alternative Exon 7 was shown dispensable for TLE/Groucho repression in reporter assays.²⁹

Interestingly, like the alternative TCF7L2 Exon 4, the alternative insertion of LEF1 Exon 7 is also a regulated event during maturation of T-lymphocytes.¹³³ At the pre-T-cell receptor (TCR) signaling stage, the insertion of LEF1 Exon 7 is increased, which allows the expression of LEF1 variants with greater transcriptional activity on the TCR enhancer, a crucial step in T-lymphocyte maturation.¹³⁴ Moreover, the splicing factor implicated in the insertion of LEF1 Exon 7 during this specific differentiation step was identified as CELF2, a splicing factor recognizing UG-rich intronic sequences flanking Exon 7 and whose expression was also up-regulated during the pre-TCR signaling stage.¹³³ This is the first splicing factor directly implicated in TCF7/LEF1 RNA processing but likely not the only one, in view of the biological relevance of TCF7/LEF1 alternative expression at discrete steps of cell specification and differentiation. Future investigations aiming at delineating a map of the splicing events and associated splicing factors are warranted.

D. Alternative Splicing Events Within TCF7/LEF1 Context-Dependent Regulatory Domains and Signal-Regulated Post-Translational Modifications

The disordered structures of TCF7/LEF1 context-dependent regulatory regions provide the flexibility that is needed for transient interactions and modifications by regulatory enzymes such as kinases and ubiquitin and SUMO ligases.¹³⁵ It is likely that all alternative domains and sequences are necessary to either provide additional and specific regulatory sites or to modulate common regulatory sites. All known post-translational modifications of TCF7/LEF1 factors have been mapped to the N-terminus and context-dependent regulatory region (Figs. 2 and 3). CK1 δ and CK2 phosphorylations of LEF1 on S40 in the N-terminal β -catenin binding domain^{136,137} are dispensable for β -catenin/LEF1 interaction *in vitro*,¹²⁰ and thus their regulatory roles on LEF1 activity remain to be confirmed.

The small SxxSS sequence flanking the HIC5 interacting domain¹³² and juxtaposing the specific TLE/Groucho interaction motif,²⁹ which is always (TCF7L1), alternate (TCF7L2), or never inserted (TCF7, LEF1) via usage of alternative splicing donor sites (Figs. 2 and 3), has been recognized for a long time as a main determinant in the repression/activation potential of TCF7/LEF1 factors.^{37,38,46,49} Although it is dispensable for TLE/Groucho interaction²⁹ and DNA binding affinity,⁴⁶ it affects in a phosphorylation-dependent manner the formation of β -catenin/TCF/DNA complex and subsequent transcriptional activation.⁴⁶ Surprisingly, there are no reports of a direct phosphorylation of the SxxSS sequence in the repressor TCF7L1 and TCF7L2-SxxSS variants, and the possibility advanced by Pukrop et al.⁴⁶ that this sequence may be a kinase interacting domain is a reasonable alternative.

Three kinases and their phosphorylation sites have been identified in TCF7/LEF1 context-dependent regulatory regions, both in common and specific modular domains (Figs. 2 and 3): Nemo-like kinase (NLK),^{138,139} homeodomain interacting protein kinase (HIPK)2,^{34,140} and Traf2/Nck-interacting kinase (TNIK).^{141,142} Their respective Wnt-signaling cascade and outcomes on TCF7/LEF1 repression/activation switch are summarized in Fig. 4. They are all likely to occur simultaneously and concurrently within the same cells, in particular in response to canonical Wnts, in a gradient-dependent manner.¹⁴³ These three kinases interact directly with TCF7L2 and/or TCF7L1 *in vitro* and *in vivo*, but β -catenin interactions are also required for TNIK nuclear localization and for HIPK2 interaction with TCF7L1/TCF7L2.^{34,140–142,144} In the most divergent part of the context-dependent regulatory region, three HIPK2 phosphorylation sites (in P2 and P3) (Fig. 5A)³⁴ are common, with either the two identified NLK sites¹⁴⁵ located within a conserved domain containing a low affinity β -catenin binding site¹⁰³ or with the identified TNIK site¹⁴² located within the specific and

nonalternative TCF7L2/TCF7L1 domain (Figs. 3, 5: P2 site). Of note, both NLK and HIPK2 are proline directed kinases belonging to the large mitogen-activated protein (MAP) kinase family, while TNIK belongs to a small subfamily of the Ste20 kinase family.

In agreement with the conservation of phosphorylation sites, both LEF1 and TCF7 are also phosphorylated by NLK,^{47,145} while TCF7 is not a target of HIPK2 within the context-dependent regulatory domain.¹⁴⁰ Although phosphorylation of LEF1 by HIPK2 on a P2 site has also been proposed,¹⁴⁰ from the sequence and exon/domain localizations (Fig. 5A), it is more likely an equivalent of the P1 site in TCF7L1/TCF7L2, and thus P2 is specific for TCF7L1 and TCF7L2 repressors. An equivalent TCF7L1/2 P2 site (SPSP) is also missing in dTCF, and only armadillo/ β -catenin, but not dTCF/Pan, was found phosphorylated by HIPK2.¹⁴⁶ Nonetheless, the number of xTCF3 phosphorylation sites appears critical for the full response to canonical Wnt8 in *xenopus* animal cap explants, with four major sites (P2 to P4) located in the context-dependent regulatory region,³⁴ while LEF1 contains only two sites and is accordingly less phosphorylated in mammalian cells.¹⁴⁰ In the same region, the major TCF7L2 variants contain three sites, and the insertion of the alternative TCF7L2-Exon 6 domain may provide an additional “SP” phosphorylation site adjacent to P2, increasing the density of target sites for HIPK2 (Fig. 5A). Since HIPK2-dependent phosphorylation of TCF7L1 and TCF7L2 results in their dissociation from DNA and de-repression of target genes,¹⁴⁰ increased or decreased susceptibility of TCF7L2 variants to undergo HIPK2-dependent phosphorylation is likely to affect their occupancy on target gene promoters/enhancers. This will not only affect the basal repressive state of the target gene, but will also create a primed state for further activation from adjacent transcription factor response elements and/or, as proposed by Hikasa et al., by a repressor (TCF7L1/2) to activator (TCF7) switch on TCF7/LEF1 binding sites.¹⁴⁰ Both models of activation after de-repression are nonetheless highly cell-context dependent and also gene-context dependent. At this point it is worth noting that with only one activated kinase like HIPK2, the overall outcome on Wnt target gene and activity will directly depend not only on the balance of TCF7/LEF1 repressor vs. activator members but also their variants.

A Tcf7l1/Tcf7 switch has recently been implicated in the control of Wnt3a-induced stem-cell renewal.³⁶ In this setting, the release of Tcf7l1 repression from specific stem-cell Wnt-target genes, like Nanog and the orphan nuclear receptor Nr5a2, is mediated firstly in a Tcf7l1- β -catenin-dependent manner, followed secondly by a β -catenin-Tcf7-dependent activation.³⁶ Although a role for a kinase and/or Tcf7l1 phosphorylation in Tcf7l1-target gene de-repression in embryonic stem-cells remains to be established, the first event is, however, reminiscent of Wnt3a-induced de-repression of xTCF3-target genes during axis specification in early *xenopus* embryos.³⁴ Unlike TCF7L2 Exon 6, the mouse alternative Tcf7l1 Exon 5, which confers increased repressor activity in embryonic stem cells,¹¹⁸ encodes a domain lacking a putative S/TP site (Fig. 5A). Whether this small 14AA sequence modulates Tcf7l1 de-repression by altering β -catenin/Tcf7l1 interaction, by preventing changes in Tcf7l1 conformation, or by its involvement in the formation of a specific repressor complex, awaits further investigation.

Nonetheless, this switch from TCF7L1 repression to TCF7 activation^{36,140} depends also on the availability of TCF7, which may be not expressed, competing with TCF7L2 repressor variants, or even exported from the nucleus.⁴⁷ Indeed, Wnt-dependent activation of the CAMKII/NLK cascade in colon cancer cells results in the nuclear export of Δ N-TCF7E variants, which alleviates their growth inhibitory effects.⁴⁷ Similarly, the availability of AP1 after TNIK/JNK activation¹⁴⁷ and the proximity of AP1 and TCF7L2 binding sites in 15% of TCF7L2 target genes⁴² can drive strong activated states of Wnt target genes after derepression priming (Fig. 4), as seen, for example, in the co-requirement of both AP1 and β -catenin/TCF complexes for full activation of matrix-metalloproteinase-7 gene

expression.¹⁴⁸ Those availabilities and crosstalk may explain the different outcomes of TCF7L2 phosphorylation by TNIK (activation), HIPK2 (de-repression), and NLK (inhibition) (Fig. 4). However, it is still unclear whether TNIK and NLK also drive de-repression of Wnt target gene to the same extent as HIPK2, and thus there are questions about the importance of multiple HIPK2 phosphorylation sites in TCF7L1 and TCF7L2 for their dissociation from DNA, and the fates of β -catenin/TCF and/or TCF/TLE complexes.

TNIK, like HIPK2, requires β -catenin for its interaction and phosphorylation of TCF7L2 in normal intestinal crypt¹⁴¹ and in colon carcinoma cells,¹⁴² and phosphorylates also the P2 site (Fig. 5A),¹⁴² but no Wnt-dependent changes in TCF7L2 native promoter occupancy and *in vitro* DNA-binding affinity have been reported.^{141,142} On the other hand, NLK phosphorylates all TCF7/LEF1 members on some “S/TP” sites overlapping with HIPK2 sites (P3) (Figs. 2 and 3) and also induces a decrease of the DNA-binding activity of β -catenin/TCF complex *in vitro*,¹⁴⁵ indicating similar conformational changes. Unlike HIPK2, all these NLK-driven events are β -catenin independent,^{138,139,145} and thus *a priori* TCF7/LEF1 members are phosphorylated when in repressor complex with TLE/Groucho and also with CtBP for TCF7L1 and some TCF7L2 variants. Both co-repressors are also phosphorylated by various MAP kinases, including HIPK2, whereby their co-repressor activities are attenuated, though the mechanisms may differ.^{149–151} HIPK2-dependent phosphorylation of TLE/Groucho can lead to its dissociation from the repressor Eyeless/Pax6 during drosophila eye morphogenesis,¹⁴⁹ while CtBP is mainly targeted to proteosomal degradation.¹⁵¹ β -Catenin is also a target of HIPK2-dependent phosphorylation, but various outcomes have been observed, from a β -catenin stabilization and promotion of β -catenin/TCF activity¹⁴⁶ to an increased β -catenin proteosomal degradation.¹⁵² Moreover, TCF7/LEF1 factors may also be targeted to proteosomal degradation after NLK-dependent phosphorylation via the NLK-associated ring finger ubiquitin ligase NARF.¹⁵³ Although further investigation is clearly needed, HIPK2 activation appears to dissociate altogether TCF7/LEF1 repressor complexes, and thus the amplitude of specific target gene de-repression is likely to reflect the differential DNA binding affinities and repressor strengths of the TCF7/LEF1 members and variants. All of those are dependent upon alternative TCF7/LEF1 domains, including the SxxSS sequence^{38,46} and C-terminus tails.^{30,31,38}

E. Alternative Splicing and TCF7/LEF1 DNA-Binding Affinity and Specificity and Tcf7/Lef1 Repression/Activation Potentials

Of note, although the HMG box DNA binding domain is itself highly conserved, with 99% homologies among the TCF7/LEF1 members, it is a key determinant in the stability of the ternary DNA/TCF/TLE repressor complexes. Indeed, the HMG box complete folding occurs when bound to the specific WWCAAAG DNA sequence, which dictates its interactions with the co-repressor TLE/Groucho and consequently further locks its conformation.²⁵ The newly identified HIPK2 phosphorylation sites are not overlapping with the TLE/Groucho interacting domain (Figs. 2 and 3),^{27,29} and thus the conformational changes involved in the DNA/TCF dissociation need to spread through the entire TLE/Groucho interacting domain, suggesting that the strength of TCF7/LEF1 interaction with TLE/Groucho may modulate these changes. Needless to mention, depending also on the assembled repressor complexes and corresponding chromatin remodeling, neither TLE/Groucho nor the repressor TCF7/LEF1 members may be easily accessible to kinases.¹⁵⁴ This would also be in agreement with de-repression/activation of specific subsets of target genes in response to Wnt signaling in a cell state (e.g., stem cell vs. differentiated) but also in a cell lineage (e.g., epithelial vs. fibroblast) dependent manner. Besides TLE/Groucho, other transcriptional activators and repressors are also able to interact with the HMG box domain (see Fig. 2),³¹ and they are competing in most cases with the formation of TCF7/LEF1-DNA complexes *in*

vitro.^{28,29,121–131} It is conceivable that when bound to adjacent sites, they may participate in the de-repression mechanism by favoring the dissociation of TCF7/LEF1 from DNA.

It is clearly established that the diverse C-terminus tails of the TCF7/LEF1 members and variants, which are generated by alternative exon cassettes and alternative splicing (Figs. 2 and 3) are directly implicated in their differential DNA-binding specificity and repression/activation potentials.^{31,35,37,38,43,49,113,128,155} In particular, seminal studies have shown that deletion of the CtBP binding motifs in the xTCF3 C-terminus tail was sufficient to convert it from repressor to an LEF1-like activator,³⁸ and that the addition of an “E” tail (c-clamp) from TCF7 or TCF7L2 to LEF1 C-terminus was sufficient to confer upon it another DNA binding specificity and affinity.^{128,156} The c-clamps are highly conserved sequences in the C-terminus tails of the invertebrates dTCF/Pan and POP1, and vertebrates TCF7-“E” and TCF7L2-“E” variants (Fig. 5B), which are necessary for their binding to bipartite DNA Wnt-responsive element: the classical WRE WWCAAAG plus an adjacent GC-rich sequence, the auxiliary RCCG sequences in mammals,¹⁵⁵ and the helper sequences in *drosophila*.^{30,157} The c-clamp itself is a bipartite cystrich domain, containing the sequences “CRARF” and “WCXXCRRKKKCIRY,” which stabilizes (clamps) the TCF/DNA complex.¹⁵⁵ The original term “E-tail” has become somewhat improper since the “E” tail of TCF7L2 also contains CtBP binding motifs but not TCF7-E tail (Fig. 3). Moreover, in TCF7L2 there are two alternative versions of the c-clamp: the highly conserved “CRARF” c-clamp is encoded by the alternative Exon-15 cassette, while a slightly divergent “CRALF” c-clamp is encoded by the alternative Exon-16 cassette (Fig. 5).⁴³ Based upon the crucial roles of the “CRARF” c-clamps in DNA recognition, the “CRALF” c-clamp may provide another DNA binding specificity and/or modulate DNA-binding affinity. In support of this assumption, in addition to their tissue-specific expression (see below), is the slight increases in β -catenin-dependent activation of the *cdx1*-promoter reporter with TCF7L2-Ex16 (CRALF) variant as compared to TCF7L2-Ex15 (CRARF).⁴³

Only CtBP binding motifs have been identified so far in the nonalternative and large C-terminal tail of the “repressor” TCF7L1 to explain its transcriptional activity (Figs. 2 and 3).^{44,158} However, it is possible that specific auxiliary DNA binding domains, interacting partners, and/or regulatory sequences are required for its unique repressor functions and specific target genes in ES cells and early embryonic development.^{36,37,159} The “activator” LEF1 lacks both the c-clamps and CtBP binding motifs (Fig. 3), but can still repress transcription via its interaction with the co-repressor TLE/Groucho,²⁸ and its target genes are either activated or down-regulated *in vivo*.^{30,31} There are nonetheless two alternative LEF-1 C-termini for which differential activities have not been reported yet. The skipping of the alternative TCF7-Exon 10 (TCF7- Δ Ex10) and TCF7L2-Exons 14 to 17 (TCF7L2- Δ Ex14-17) results in the expression of LEF1-like activator variants, i.e., with short C-termini, although other regulatory functions and/or interacting partners may also exist (Figs. 2 and 3). While TCF7 Δ Ex10 behaves as an LEF1-like activator,^{30,31} the “activator” potential of TCF7L2- Δ Ex14-17 is not obvious, since in concert with stable β -catenin forms it has been shown to increase artificial β -catenin/TCF-reporters⁴⁹ but not Wnt target gene-promoter reporters, irrespective of the presence/absence of the auxiliary RCCG sites in the promoter sequences.^{35,43} All the TCF7 and TCF7L2 variants with half c-clamps, i.e., with only the “CRARF” motif, have lost the “E” tail property (Fig. 5),^{43,155} further stressing the crucial roles of the DNA/TCF complex stability in the repression/activation potential of TCF7/LEF1 members and consequently in their de-repression potential as well.

Nonetheless, reporter assays are not as informative as expected for analyzing subtle differences in DNA-binding affinity and repressor activity, since the chromatin structure is only partially reconstituted on artificial promoters and some Wnt target reporters display even the opposite regulation of that observed *in vivo*.³⁰ Also, preformed β -catenin/TCF

complexes are the key players in reporter assays conducted with overexpression of activated β -catenin and TCF7/LEF1 members and variants, thereby bypassing important steps in the regulation of Wnt target genes, e.g., de-repression and/or displacement of the repressor complex (Fig. 3). *In vitro* DNA-binding assays have previously shown that β -catenin/Tcf7l2 complexes display higher DNA-binding affinity for the classical WRE and cannot grasp differences in the DNA-binding affinity of Tcf7l2 variants, even in the presence of the auxiliary RCCG sequence, while clear differences in native promoter occupancy by ChIp assays can be observed among ectopically expressed Tcf7l2 variants.⁴³ Unlike TCF7L1, which is mostly nonalternative, the other TCF7/LEF1 members are expressed with alternative N- and C-termini in at least four different versions for LEF1 and up to at least 10 different versions for TCF7L2. Therefore, the upcoming challenges are to develop specific tools distinguishing each TCF7L2 variant, like the recently developed specific TCF7–“E” tail antibodies,⁴⁷ to further define their biological roles *in vivo* and determine whether the complex regulation of TCF7L2 alternative splicing participates in a transcriptional repression/activation switch in response to Wnt signaling but also in response to other differentiation and/or stress cascades.

F. Cell Lineages and Insertion/Exclusion of the Alternative Exon Cassettes Encoding TCF7L2 C-Termini

TCF7L2, but not TCF7L1 and LEF1, is able to rescue *POP1(zu189)* phenotype in *C. elegans*.⁶⁴ POP1 activities are crucial for the transition between the first asymmetric division and the specification of the single endodermal precursor and single mesodermal precursor in response to Wnt signals at the blastula stage. A POP1 transcriptional repression-to-activation switch governs the specific up-regulation of endodermal genes in the Wnt-sensitive endodermal precursor, while the POP1-dependent repression of endodermal genes is reinforced in the Wnt nonresponsive mesodermal precursor. The *POP1(zu189)* mutant is defective in the reinforcement of endodermal gene repression in the mesodermal precursor, and thus both precursors undergo an endodermal fate in response to Wnt signaling. Interestingly, the TCF7L2 variant used in these studies corresponds to TCF7L2-Ex15 (CRARF) variant, which thus can repress the expression of endodermal Wnt target genes and promote a mesodermal fate like POP1.⁶⁴ From thorough analyses of TCF7L2 alternative exon expression in various cell types, a tissue-specific expression of TCF7L2 variants has recently emerged (Refs. 35, 43, 89, 92, 93, 96, 160, and Boyechko et al., submitted). TCF7L2-Ex15 (CRARF) variant is predominantly expressed in mesenchymal-derived cells and tissues such as adipocytes, myoblasts, and skeletal muscle.^{93,94,160} Based on the conservation of TCF7L2 expression and impact on cell differentiation across species, TCF7L2-Ex15 (CRARF) variant has likely retained its POP1 ancestral developmental property, i.e., to repress specifically endodermal/epithelial genes and features. It will be interesting to see how other TCF7L2 variants may substitute POP1 for the activation of endodermal genes and/or repression of mesodermal genes.

The alternative Exon 15 and Exon 16 are mutually exclusive exons, i.e., only rare TCF7L2 RNA isoforms have both Exon 15 and Exon 16 inserted, while the alternative in frame Exon 14 is more frequently inserted with either of them (Figs. 3 and 5). This mutually exclusive insertion is in agreement with—and also the cause of—their tissue-specific expression and cell lineage-dependent expression (Boyechko et al., submitted). The alternative TCF7L2-Ex16 (CRALF) is the major variant in epithelial lineage committed cells, such as breast cancer cell lines with myo-epithelial and luminal cell phenotypes, while the alternative Exon 15 is predominantly inserted in breast cancer cell lines with mesenchymal phenotypes (Boyechko et al., submitted). The predominance of the alternative Exon 16 was also noted in normal epithelial tissues such as isolated pancreatic β -islets,⁹³ but not in hepatocellular carcinoma cell lines, in which the alternative Exon 15 is instead prominent.⁴⁹ However, in

the latter study, higher levels of TCF7L2-Ex16 insertion were observed in cell lines with more epithelial differentiated features.⁴⁹ Activation of the canonical Wnt3A signaling pathway decreases the Exon-16 insertion in embryonic stem-like HEK293 cells, while the noncanonical Wnt13A¹⁶¹ and stable β -catenin forms have no effect (Boyechko et al., submitted). The promotion of mesenchymal-to-epithelial transition in breast cancer stem-like cells via either down-regulation of TAp63 or increased cell density results in the specific up-regulation of TCF7L2-Ex16 insertion (Boyechko et al., submitted). Collectively, these studies corroborate the epithelial specific expression of TCF7L2-Ex16 (CRALF) variants, which in mammary epithelial committed cells is also accompanied by the inclusion of the alternative Exon 4. The combined Exon 4/Exon 16 signatures clearly distinguish epithelial breast cancer cell lines from stem-like and mesenchymal-like breast cancer cells (Boyechko et al., submitted). Of note, normal mesenchymal-derived cells display combined Exon 4/Exon 15, while cancer cell lines with a stem-like/mesenchymal phenotype have reduced Exon 4 insertion, which is consistent with the alternative Exon 4 insertion in a cell state-dependent manner but cell type-independent manner. As opposed to TCF7L2-Ex15 (CRARF) variant and its role in repressing endodermal/epithelial genes, TCF7L2-Ex16 (CRALF) variant may participate either in the repression of mesodermal/mesenchymal genes or in the activation of endodermal/epithelial gene expression. The latter possibility is supported by the increase of cdx1-promoter reporter activation by Tcf7l2-Ex16 variant,⁴³ since cdx1 is an homeobox transcription factor implicated in endodermal tissue development such as small and large intestine, and required for the maintenance of epithelial cell differentiation.¹⁶²

The alternative Exon 17 is rarely present because of its neuro-endocrine tissue-specific insertion in some regions of the brain, pancreas, and gut.^{43,89,92,96} On the other hand, the inclusion of the alternative Exon 14 is predominant in microvascular and plastic endothelial cells³⁵ and in cell lines with neural/neuronal differentiation potentials, such as the embryonic stem-like HEK293 cells and the melanocytic MDA-MB-435 cancer cell line (Boyechko et al., submitted). Although *Tcf12* expression is detected in mouse ES cells,^{36,43} it is specifically up-regulated during the neural stages, including in zebrafish and *xenopus* embryos.^{87,117} All the “xTCF4” variants originally cloned during *xenopus* gastrulation correspond in fact to TCF7L2-Ex14 variants, suggesting again that the adult tissue-specific expression of TCF7L2 alternative Exon 14 may have retained the developmental regulation by neural-specific splicing factors, and perhaps the function as well. These TCF7L2-Ex14 variants lack both the c-clamps and CtBP binding motifs, but surprisingly can substitute for TCF7L1-dependent repression of mesodermal genes in *xenopus* embryos.³⁷

TCF7L2- Δ Ex14-17 variants, generated by the skipping of all alternative exon cassettes (Figs. 2 and 3), are up-regulated in all the cell lines and primary cells in culture as compared to normal tissue. TCF7L2- Δ Ex14-17 variants are also strongly expressed in numerous stem-like cancer cell lines (Ref. 104, Boyechko et al., submitted) and are increased in the embryonic stem-like HEK293 cells in response to lithium,³⁵ an activator of both canonical Wnt signaling¹⁶³ and p53 cascade.¹⁶⁴ Canonical Wnt3A signaling decreases only the alternative Exon 16, and p53 up-regulation decreases mainly the alternative Exon 14, and thus neither provokes by itself an overall Ex14-17 skipping like lithium (Boyechko et al., submitted). Nonetheless, while the inclusion of one specific alternative Exon 14-17 is favored during lineage commitment and further differentiation, their skipping in concert with reduced Exon 4 insertions characterizes inversely undifferentiated cancer cells with stem-like cell properties. In mouse ES cells, the Tcf7l2 RNA isoform profile defined by Weise et al.⁴³ is also consistent with the expression of TCF7L2 variants lacking altogether the alternative Exon 4 and the alternative Exons 14 to 17. It is conceivable, since the resulting TCF7L2- Δ Ex4 Δ Ex14-17 variant lacks significant transcriptional activity towards

a variety of Wnt target genes,^{35,43} that it may constitute a permissive/nonrepressive form participating in the maintenance of undifferentiated states (Fig. 6).

A decrease of TCF7L2 Exon 4 insertion was detected by exon-junction microarray in human prostate epithelial cells undergoing an epithelial-to-mesenchymal transition caused by the knockdown of the epithelial-specific splicing factors ESRP1/2.¹⁶⁵ TAK1, a TGF β and Wnt-signaling upstream activator of TNF α (Fig. 4), also displays differentially expressed variants in these ESRP1/2 knockdown cells, but surprisingly, changes in TCF7L2-Exon 14–17 alternative splicing, and more particularly their skipping, were not detected in the same screen.¹⁶⁵ This may be explained by an incomplete coverage of TCF7L2 exon junctions in the microarray. Nonetheless, changes in TCF7L2-Exon 4 levels were not detected in the reverse experiments with ESRP1/2 ectopic expression in mesenchymal-like breast cancer MDA-MB-231 cells, while several epithelial markers and splice variants were re-expressed.¹⁶⁵ Whether increased TCF7L2 exon skipping, including Exon 4 skipping, is also a response to stress such as a DNA damage-like cascade initiated by lithium, for example, and p53 activation and/or an anoikis-like cascade initiated by disruption of cell-cell adhesion, remain to be answered. But in view of the important crosstalk of Wnt signaling with p53 cascade via HIPK2 activation,^{34,140,166} and its dependency on p53 status, regulation of TCF7L2 alternative splicing will certainly be the subject of future investigations. Indeed, regulation of TCF7L2 alternative splicing is a key component in the control of the balance of TCF7L2 variants with differential repressor/activator potentials, and hence cell context-dependent response to Wnt signaling. Moreover, TCF7L2 alternative splicing is likely a direct or indirect target of deregulation in cancer, in agreement with its tumor suppressor role in colon and breast carcinoma,^{33,167} but also in all chronic diseases with which TCF7L2 polymorphisms have been associated.^{71–76}

III. Alternative RNA Splicing and Crosstalk of Wnt Signaling and Nuclear Receptors in the Control of Cell Fate

Like transcriptional control of gene expression, regulation of constitutive and alternative RNA splicing relies on cis-acting core and auxiliary elements respectively located in flanking intronic sequences and even in exons for some splicing inhibitory events. During transcription, the core elements are recognized by the spliceosome machinery, while the auxiliary elements recruit in a sequence-specific manner RNA-binding factors and associated proteins, the splicing regulators, which display either ubiquitous or tissue-specific expression.^{168,169} Alternative splicing is regulated by the availability of the splicing factors—e.g., CELF2 up-regulation precedes LEF1-Exon 7 inclusion during T-lymphocyte differentiation¹³³—but also, more generally, by the balance of splicing factors and regulators, which enhance or inhibit splicing events and their intricate auto- and crossregulation by alternative splicing.^{169,170} Alternative promoter and rate of RNA polymerase II elongation activity also parallel the choice of alternative splicing events.^{171–173} As such, all transcription factors involved in the control of cell fate and regulating TCF7/LEF1 transcription are prone to affect concomitantly their alternative RNA splicing. Moreover, coordinated regulations of factors involved in the same biological processes apply also for RNA processing.^{168,169} For instance Wingless signaling in *Drosophila* S2 cells is accompanied by the usage of an alternative promoter for its own receptor dFz2 and by alternative exon skipping of unfulfilled/hr51, an NR critical for neuron development in the mushroom body and axon guidance, which further emphasizes the interconnections between Wnt and NR signaling during cell differentiation.¹⁷⁴ In this section we explore the known and possible crosstalk of NRs and Wnt signaling at the level of alternative RNA splicing.

Various NR signaling pathways control cell specification and fate during development in a spatio-temporal-dependent manner, as well as diverse physiological functions in the adult, e.g., regulation of circadian rhythm, maturation of the mammary glands, and inflammation.^{62,63} The biological diversity and specificity of the NR family of Zn²⁺ finger transcription factors is conferred by 48 different members able to bind specific repeated DNA sequences either as homodimers, e.g., estrogen receptor (ER/NR3A1), or as heterodimers with the retinoid x receptors (RXR α - γ /NR2B1-3), e.g., vitamin D receptor (VDR/NR1I1). The NRs recognize and are activated by specific ligands, which are mostly lipophilic compounds, e.g., steroid hormones, vitamin D, and retinoids.⁶² Most of the NRs are nuclear, bound to their cognate DNA-response element and associated with the ubiquitous co-repressors NCoR and SMRT in the absence of ligands.¹⁷⁵ Additional tissue-specific and developmentally regulated co-repressors, such as HR (Hairless) for VDR, also provide spatio-temporal NR repression.¹⁷⁶ Upon ligand binding, the NRs undergo a transcriptional switch from repression to activation via ligand-induced conformational changes, leading to the release of the co-repressor complexes and subsequent binding of co-activators—e.g., SRC-1-3, which will further recruit chromatin remodeling complexes, histone acetyltransferase, e.g., p300/CBP and the basal transcription machinery through the mediator.¹⁷⁷

Crosstalk between the NR and Wnt signaling can occur indirectly through competition for co-activators and co-repressors and through Wnt-dependent activation of signaling kinase cascades, for example JNK (Fig. 4), Akt, GSK3 β , and PKA, to cite a few, which will regulate the activity of the NRs, co-activators, and co-repressors.⁶² On the other hand, estradiol can also initiate the Akt/GSK3 β and MAP kinase cascades in an ER-dependent manner at the plasma membrane and cytoplasm, as well as a redox-dependent cascade by an intrinsic antioxidant activity.¹⁷⁸ In fact, the NRs intersect with Wnt signaling at multiple levels and for most, not surprisingly, the outcomes are cell-context dependent. Detailed reviews of direct crosstalk by the NR-dependent regulation of Wnt signaling component expression, NR interaction with β -catenin and/or TCF7/LEF1, and their synergistic effects on NR transcriptional activity, but in general repression of β -catenin/TCF activity, can be found in Beildeck et al. and Mulholland et al. (Refs. 62, 63, and references therein).

A. Crosstalk During the Coupling of Transcription and RNA Splicing

The search for NR co-activators and/or interacting partners has led to the discovery that numerous transcription elongation factors (e.g., 7SK RNA) and splicing factors associated with different small nuclear ribonucleoprotein particles (snRNP) of the spliceosome were present in the complexes.¹⁷⁹ Several of these splicing factors contain either a LxxLL or FxxLF motif whereby they interact directly with NRs: the dead-box helicases DDX5 (p68) and DDX17 (p72), the core factor SF1, which recognizes the branch point sequence of the 3' splice site, Fus/TLS protein, the p54nrb(NONO)/PSF heterodimer, and the prp4 kinase/SRPK1.¹⁸⁰ An interaction of ER α with SF3a p120 of the U2-snRNP complex involves the N-terminal ligand-independent AF1 transactivation domain in a S118 phosphorylation-dependent manner.¹⁸¹ The splicing factor SKIP (Ski-interacting protein), which was also identified as a VDR coactivator (NCOA62), interacts preferentially with NR heterodimer, e.g., VDR/RXR, in a ligand-enhanced manner by an unusual binding site involving the α -helix 10 of the ligand-binding domain.^{182,183} This allows the formation of ternary complexes NR/SKIP with coactivators, e.g., SRC1, but also with other splicing factors, which bind the C-terminal ligand-dependent AF2 transactivation domain.^{182,183} NR coactivators and co-repressors themselves serve as platforms for additional complexes such as Sin3A/HDAC repressor complexes but also the coactivator CARM1/PRMT4, which is a methyltransferase implicated in the coupling of RNA transcription and splicing.¹⁸⁴

CARM1 was shown to interact also with β -catenin,¹⁸⁵ and its recruitment to a subset of Wnt target genes allows histone H3-R17 dimethylation and activation of transcription.¹⁸⁶ It is not known yet whether β -catenin is methylated upon CARM1 interaction like most of CARM1 substrates and whether RNA splicing is also activated. CARM1 auto-methylation is the coupling event with RNA splicing regulation¹⁸⁷ and in the NR case, CARM1 auto-activation is dependent upon its interaction with SRC1 (p160 co-activator) and promotes exon skipping.¹⁸⁸ High CARM1 expression has been found in colon carcinoma cells,¹⁸⁶ which display altered RNA splicing, including alternative promoter and splicing of TCF7/LEF1.^{47,98,189} Similarly, TCF7L2 expression is up-regulated in colon carcinoma, and alteration of splicing events have been reported.^{44,104} TCF7L2 transcription is dependent on both β -catenin/TCF and VDR regulations,^{190,191} which makes CARM1 an interesting candidate to mediate the alternative splicing events occurring in colon carcinoma cells for TCF7/LEF1 members.

Although less documented, there is some evidence for an association of β -catenin and/or TCF7/LEF1 with RNA splicing factors associated with the spliceosome. Fus/TLS, DDX5, and p54nrb/NONO have been found associated with active β -catenin forms in colon carcinoma cells, and overexpression of Fus/TLS has been shown to inhibit β -catenin/TCF activity but to increase ER β alternative splicing.¹⁹² These ER β splicing variants result in a partial deletion of the ligand binding domain, and similar ER α splicing variants are also highly expressed in breast cancer.¹⁹³ Fus and its interacting partner FusIP are also targets of the GSK3 β -degradation complex, and their stability is increased by lithium.¹⁹⁴ Abnormal stability of both Fus/TLS and TAR-DNA binding protein (TDP)-43 and their cytoplasmic aggregation is responsible for the development of a neurodegenerative disease, amyotrophic lateral sclerosis (ALS).¹⁹⁵ Fus is also a target of the DNA-damage cascade via ATM phosphorylation and inhibits cell cycle progression.¹⁹⁶ Its androgen-dependent down-regulation may participate in prostate cancer development.¹⁹⁷

In intestinal epithelial cells, the expression of SF1 is dependent upon their differentiated states and participates in the shutoff of β -catenin/TCF signaling during differentiation of crypt stem cells to mature villous epithelial cells.¹⁹⁸ SF1 expression is also increased by the ectopic expression of the dominant negative Δ N-TCF7L2- Δ Ex14-17 and by butyrate, a PPAR γ ligand and differentiating agent in colon carcinoma cells.¹⁹⁸ Similarly to Fus/TLS, SF1 up-regulation is accompanied by increased ER β alternative splicing,¹⁹⁸ and whether SF1 and Fus/TLS are also involved in the control of endogenous TCF7L2, LEF1, and TCF7 alternative variants expressed in colon carcinoma cells is of particular interest in view of their relationship with epithelial differentiation and deregulation in cancer. Of note, several splicing factors, including Fus/TLS and DDX5, were found in an RNA complex with the IRES sequence of LEF1, indicating that they may also affect translation of full-length LEF1 variant.¹⁹⁹

DDX5 is a nucleocytoplasmic shuttling protein, which is phosphorylated by the nonreceptor tyrosine kinase c-abl downstream of the PDGF and TNF α signaling cascades.²⁰⁰ When phosphorylated, DDX5 prevents β -catenin GSK3 β -dependent phosphorylation and degradation, allowing β -catenin/TCF activity in a Wnt-independent manner.²⁰¹ Phosphorylated DDX5 is also found associated with the promoter sequences of *cyclin-D1* and *c-myc*, two Wnt target genes, and increases their expression.²⁰² DDX5 couples transcription and splicing via its interaction with RNA Pol-II carboxyl-terminal domain (CTD) and its RNA helicase activity, which is necessary for unwinding RNA secondary structures and nascent RNA pairing.²⁰⁰ The helicase activity of DDX5 and DDX17 is dispensable for NR co-activation, while their RNA binding domain is required for the recruitment of the noncoding RNA, SRA, to the activation complex such as with ER α .²⁰³ ER α interaction with DDX5 and DDX17 is also crucial for estrogen-induced alternative

promoter switch of target genes even when the global expression is not significantly affected.²⁰⁴ The usage of alternative promoters appears dependent upon the presence of ER α response elements with adjacent binding sites for CTCF,²⁰⁴ a factor implicated in gene insulation and hetero-/euchromatin boundary formation and remodeling, and the recruitment of both DDX5/DDX17 heterodimers and the RNA coactivator SRA.²⁰⁵ The alternative promoters of ER α coactivator NCOA7/ERAP140, RAR α , GR, and also TP73, are among the novel ER α -target genes in breast cancer MCF-7 cells.²⁰⁴ Whether such a mechanism may apply also to TCF7/LEF1 binding sites in the proximity of CTCF binding sites, e.g., around enhancers, is an intriguing possibility especially for the regulation of LEF1 and TCF7 alternative promoters in colon carcinoma cells,^{47,98} in which DDX5 and DDX17 are also highly expressed.²⁰⁰

B. Crosstalk During Regulation of Constitutive and Alternative Splicing Events

ALY, a component of the TREX complex, which couples RNA splicing and RNA export,^{206,207} was found associated with LEF1 and behaving as a coactivator for LEF1 transcriptional regulation of the TCR α enhancer in a β -catenin-independent manner.^{208,209} Interestingly, the RNA export activity of ALY is also enhanced by Akt1, but not Akt2, phosphorylation, which allows binding of nuclear phosphosinositides onto ALY Gly/Arg (GR) dipeptide repeats and its localization to nuclear speckles.²¹⁰ Of note, nuclear speckles are thought to be the storage, assembly, and post-translational modifications of splicing factors that are released and recruited onto the nascent pre-mRNA localized in perichromatin fibrils, in which transcription elongation and RNA splicing take place.²¹¹

Pinin, a nucleocytoplasmic shuttling protein, was found associated with the co-repressor CtBP2 in nuclear speckles. Pinin attenuates the activity of various repressor/CtBP complexes, leading to derepression of the E-cadherin gene and increase of E-cadherin RNA splicing in a promoter-dependent manner.^{212,213} Pinin down-regulation up-regulates TCF7L2 expression in intestinal and corneal epithelial cells as well as increases active β -catenin forms.^{214,215} A Pinin/CtBP2/ β -catenin complex has been found present on cdx2 promoter and proposed to mediate cdx2 expression in intestinal epithelial cells.²¹⁴ The effects of Pinin on the expression of TCF7L2 variants and alternative RNA splicing were not tested, but future studies are warranted. Indeed, while both ALY and Pinin are transiently present in the exon-junction complexes,²¹⁶ only Pinin is directly involved in pre-mRNA splicing activation.²¹⁷ In particular, Pinin contains Ser/Arg (SR) dipeptide repeats like numerous splicing regulators, e.g., SRp20 and the co-activator SKIP, whereby they can form heterodimers in a phosphorylation-dependent manner.²¹⁸ Pinin has been shown to interact directly with splicing factors associated with the spliceosome, e.g., DDX5,²¹⁶ which regulates both constitutive and alternative RNA splicing, as well as with spliceosome regulatory complexes, e.g., the general activator RNPS1, which forms the apoptosis- and splicing-associated protein complex (ASAP) with SAPI8, a component of the Sin3a/HDAC complex, and Acinus.^{219,220} Acinus interacts also with the RAR α N-terminal AF1 transactivation domain and represses RAR target genes in a ligand-independent manner by recruiting co-repressors such as CtBP2.^{221,222} Its phosphorylation by SR protein kinase (SRPK)2 within the SR domain favors its release from the nuclear speckles and its transcriptional/splicing activities.²²³ On the other hand, Akt1-dependent phosphorylation prevents Acinus C-terminal cleavage by caspase-3,²²⁴ a crucial event for the activation of the ASAP complex during apoptosis.²²⁵ Whether Acinus participates in DNA condensation or in DNA fragmentation during cell death is still debated.^{225,226} Intriguingly, SAPI8 homolog in *drosophila* is associated with the endocytic compartment, where it may play a role in autophagy,²²⁷ and thus SAPI8 via its recruitment to the ASAP complex may also participate in the autophagic-dependent cell-death cascade. In the same line, Pinin is a desmosome-associated protein when not in the nucleus,²²⁸ and thus all these RNA-

processing auxiliary regulators are thought to constitute diverse stress sensors and effectors of stress-induced gene expression programs, including alternative splicing.¹⁸⁹

Pinin is also present with SKIP in a complex with Smad nuclear interacting protein (SNIP1), TRAP150/THARP3, and the cell-death promoting-transcription repressor BCAFL1/Btf.²²⁹ This SNIP1/SKIP complex controls the stability of cyclin-D1 spliced RNA prior to its nuclear export, but not *per se* pre-mRNA splicing.²²⁹ SNIP1 also controls p53 expression in response to UV stress and modulates the activity of the DNA-damage ATR kinase on numerous stress-related targets including p53.²³⁰ Moreover, SKIP splicing activity is also involved in *CDKN1/p21^{cip}* exon splicing during p53-dependent DNA-damage stress response.²³¹ In this case, the spliceosome U2AF65 factor is specifically recruited by SKIP on the *p21^{cip}* gene in proximity to an elongation RNA pol-II pause site where it binds the nascent and unspliced premRNA and favors subsequent splicing.²³¹ In the same line, VDR is rather a pro-apoptotic NR, and its induction of *p21^{cip}* expression may use a similar SKIP-dependent mechanism, since alteration of SKIP activity has been shown to increase unspliced RNA in a VDR-dependent manner.²³² Also, since the composition of SKIP complexes defines its transcriptional and splicing activity, the competitive availability of its partners, like pinin with β -catenin, is likely to influence the response to stress from survival to apoptosis. Interestingly, SKIP has also been identified as a novel effector of β -catenin/TCF signaling and proposed as a possible scaffold for the TCF7/LEF1 repression-to-activation switch.²³³ SKIP interacts with its central SNW domain with TCF7/LEF1 members and recruits HDAC1, and the ternary repressor complex is present on *c-myc* promoter in the absence of Wnt signals.²³³ SKIP also recruits nuclear β -catenin via its C-terminal domains including NLS, and is indispensable for Wnt signaling in *in vitro* reporter assays and *in vivo* for neural crest induction in *xenopus* embryos.²³³ Retinoic acid is another crucial factor for neural crest induction, and its receptor RAR α interacts in a ligand-dependent manner with the SKIP central SNW domain as well.²³⁴ One can speculate that crosstalk between RA and Wnt signaling is likely to occur at the level of SKIP, and whether synergistic activation or competition for SKIP binding is the main outcome during neuronal cell differentiation remain to be seen.

C. Crosstalk and Regulation of Splicing Factor Expression

Since both Wnt signaling and NR pathways affect cell state and cell differentiation, they certainly affect the expression of numerous splicing factors and regulators as well as tissue-specific splicing factors such as epithelial ESRP1/2.¹⁶⁵ Yet few have been reported and studied so far. This can be explained in part by the complex expression pattern of the splicing factors themselves, with multiple alternative splicing and exon cassettes, but also by the fact that these splicing events can occur without overall changes in the RNA levels. Deep mRNA sequencing is going to fill this knowledge gap of the changes in gene expression in different tissues and conditions. However, changes in splicing factors have been reported after activation of Wnt signaling. In addition to SF1, discussed above,¹⁹⁸ the splicing regulator SRp20/Srsf3 has also been identified as a β -catenin/TCF target gene in colon cancer cells,²³⁵ in which it participates in the aberrant splicing associated with cancer cells.¹⁸⁹ A two-fold up-regulation of SRp20 by ectopic expression of β -catenin was sufficient to alter the RNA splicing of a CD44-minigene and produce the metastatic CD44v5 RNA isoform.²³⁶ On the other hand, another metastasis-associated splicing event, Rac1 exon3b inclusion, was inhibited by the SRp20/ β -catenin cascade but increased by the ASF/SF2(Srsf1)/Akt cascade.²³⁶ These somewhat opposite functions of Srp20/Srsf3 on two metastasis markers exemplify the challenges of RNA splicing regulation and outcomes.

Indeed, none of these splicing regulators function alone, but participate in small complexes in the enhancement or inhibition of RNA splicing events. The auxiliary complex composition will depend on both the RNA target sequences and splicing factor availability.

The former implies that each splicing event on a pre-mRNA may be regulated by a specific complex, and the final mature spliced mRNA thus represents the combinatorial activity of different complexes proportionally to their complexity of alternative splicing and alternative exon cassettes. The latter relies also on the expression of tissuespecific splicing factors to confer tissue-specific expression of splice variants that in turn concur in the cell context-dependent responses to stress, Wnt signaling, but also to therapy such as the tissue-specific Fox2/RBM9/RTA splicing factor. The repressor for tamoxifen transactivation (RTA) was identified on the basis of its interaction with the ligand-independent AF1 transactivation domain of ER α , its ability to repress tamoxifen partial agonist transactivation, and its differential expression in sensitive vs. resistant breast cancer cells to tamoxifen, a therapeutic ER antagonist in breast cancer.²³⁷ Although the mechanisms of Fox2/RBM9/RTA-mediated NR repression are not fully understood, they likely involve co-repressor recruitment and transcription/splicing coupling in a cell context-dependent manner. Indeed, Fox2/RBM9/RTA is particularly expressed in breast epithelial cells and is also partly responsible for the expression of the epithelial FGFR2–Exon IIIb vs. mesenchymal FGFR2–Exon IIIc variants, and Fox2 expression changes are sufficient to mediate also epithelial/mesenchymal transitions²³⁸ like ESRP1/2.¹⁶⁵ As such, Fox2 expression is considered as a valuable complement of ER status in breast cancer tissues both for prediction of tamoxifen-therapy resistance and for prognosis of metastatic progression.

IV. CONCLUSION

Despite all the challenges, the biological relevance of alternative splicing in development, but also its crucial role in the maintenance of adult tissue homeostasis in response to stress and its deregulation in numerous age-related diseases such as cancer¹⁸⁹ and neurodegenerative diseases,¹⁹⁵ ensures future investigations of the factors and mechanisms involved for each key gene and each key splicing event, including for TCF7/LEF1 members.

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ABBREVIATIONS

BD	binding domain
CtBP	C-terminal binding protein
LEF	lymphoid enhancer factor
NLS	nuclear localization signal
NR	nuclear receptor
RA	retinoic acid
RAR	retinoic acid receptor
SNP	single nucleotide polymorphism
TGF	transforming growth factor
TLE	transducin-like enhancer of split
TSS	transcription start site
VDR	vitamin D receptor

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FIGURE 1.

Cell context-dependent mechanisms generating TCF7/LEF1 diversity in expression and functions. The expression of TCF7/LEF1 members and variants with differential transcriptional activity and specificity is controlled (i) at the transcriptional level by local chromatin states, enhancers, and alternative promoters,^{85–87} and (ii) at co-transcriptional and post-transcriptional RNA processing levels by alternative splicing of alternative exon cassettes and alternative donor/acceptor splicing sites.^{30,31,113} Although TCF7/LEF1 RNA isoforms are likely to be differentially regulated at other post-transcriptional levels, e.g., RNA isoform stability, they are not documented so far. Similarly, differential translation mechanisms have been shown only for LEF1 RNA isoforms, which use either a cap-dependent or a cap-independent internal ribosome entry site (IRES) mechanism.²³⁹ The resulting TCF7/LEF1 protein variants display differential DNA binding specificity and transcriptional activity both by changes in intrinsic domains and interacting partners^{30,31,113,155,157} and are subjected to differential post-translational modifications^{47,140} and cellular localizations.⁴⁷ All these steps are highly dependent on cell states and cell types, which are controlled by various signaling pathways and their associated family of transcription factors such as the nuclear receptor and p53 families as well as the TCF7/LEF1 members and variants themselves.³⁰

FIGURE 2.

Schematic representation of human TCF7/LEF1 transcripts organization and isoforms generated by alternative promoter and transcription start sites (TSS) (light blue), alternative in frame exon cassettes (red), alternative in-frame splicing (blue), alternative out of frame splicing and alternative exon cassettes changing the open-reading frames (yellow), and stop codon locations (St). Mouse (ms) alternative exon cassettes not conserved¹¹⁸ or not yet identified in humans are represented. The locations of the corresponding translation initiation AUG codon are indicated as well as the functions of the encoded domains. In particular the correspondence between exon and the delineated interaction domains of TCF7/LEF1 with other transcriptional effectors is specified. The small encoded sequences LVPQ and SxxSS present in TCF7L1, absent in TCF7 and LEF1, and generated by alternative splicing in TCF7L2 are indicated. The SNPs in the TCF7 coding region and in the TCF7L2 noncoding regions that have been associated with diseases are represented as well as the location of the 90-kbps region of TCF7L2 in linkage disequilibrium (LD) in T2D and containing tissue-specific enhancer activities.^{85–87}

FIGURE 3.

Schematic representation of the modular structure of TCF7/LEF1 members and variants, and their association with the b-catenin activator complexes and TLE/Groucho repressor complexes. The alternative N-terminal β-catenin binding domain (light blue), the alternative domain (red) and alternative short sequences (blue) within the context-dependent regulatory region are indicated, as well as the conserved HMG box DNA binding domain and NLS (dark blue). The alternative C-termini with difference in sizes, presence of half and full c-clamps (yellow), and presence of CtBP binding motif (green) are indicated for each TCF7/LEF1 member as well as their specific DNA recognition sequence. The mechanisms of increased repression via CtBP and activation via displacement of TLE/Groucho are represented, as well as cooperation or transrepression on adjacent DNA-bound transcription factor. Some of the post-translational modifications of TCF7/LEF1 factors, β-catenin, and TLE/Groucho known to affect the transcriptional outcome are color coded as indicated.

FIGURE 4.

Wnt signaling kinase cascades converging on TCF7/LEF1 phosphorylation and influencing their transcriptional repression-activation switch. Noncanonical Wnts⁵ as well as low canonical Wnt gradients¹⁴³ activate a Ca^{2+} calmodulin-dependent kinase (CAMK) II/TGFB-activated kinase (TAK) I cascade that activates Nemo-like kinase (NLK) in the nucleus, which phosphorylates all TCF7/LEF1 factors on two conserved sites in the context-dependent regulatory region and inhibits canonical Wnt activity by preventing formation of β -catenin/TCF complex^{138,139,145} and triggering nuclear export of TCF7⁴⁷ and/or proteosomal degradation via the NLK-associated ring finger (NARF) ubiquityl-ligase.¹⁵³ Canonical Wnts also activate two parallel signaling cascades dependent on β -catenin stabilization and its subsequent nuclear translocation in association with two upstream kinases, the Traf2-Nck-interacting kinase (TNF1K)^{141,142} and homeodomain interacting protein kinase (HIPK)2,^{34,140} which can interact and differentially phosphorylate TCF7/LEF1 factors depending on the site conservation. TCF7 is an unlikely target of both HIPK2 and TNF1K, while LEF1 is an unlikely target of TNF1K. Multiple HIPK2 phosphorylation sites with variable numbers are present on LEF1<TCF7L2>TCF7L1.^{34,140} The TNF1K phosphorylation site identified overlaps with one of the HIPK2 sites, which suggests that the amplitude of the transcriptional outcome may result from a balance between both TCF7/LEF1 repressor vs. activator and HIPK2 vs. TNF1K activation, since only TNF1K-dependent TCF7L1/TCF7L2 phosphorylation increases β -catenin/TCF activity^{141,142} and also AP1 via activation of JNK.¹⁴⁷ In contrast HIPK2-dependent phosphorylation of TCF7L1, TCF7L2, and LEF1^{34,140} results in the release of TCFs from their cognate DNA binding site and allows derepression of target genes. This release may be mediated by targeted degradation of TCFs as well as by the degradation of the co-activator β -catenin^{152,240} and co-repressors TLE/Groucho^{149,241} and CtBP,²⁴² which are all HIPK2 targets too.

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FIGURE 6.

Schematic representation of the working model of TCF7L2 alternative exon tissue-specific expression and of corresponding TCF7L2 variant activity on differentiation programs.