TCF: transcriptional activator or repressor?

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T cell factor, or TCF, has been identified as the transcriptional response factor activated by Wnt-1 and Wingless signalling. TCF is thought to be a potent architectural factor which facilitates assembly of multiprotein enhancer complexes. Its deregulation in the colonic epithelium, and in other cells, leads to cancer. This raises the question of how TCF is kept inactive in unstimulated cells.

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Abbreviations

ALY	ally of LEF-1 and AML-1
AML-1	acute myeloid leukaemia factor 1
APC	adenomatous polyposis coli
BTM	basal transcription machinery
CRE	cAMP response element
Gsk-3	glycogen synthase kinase 3
HMG	high mobility group
LEF-1	lymphocyte enhancer-binding factor
TCF	T cell factor
TCR	T cell receptor
Ubx	Ultrabithorax
Wg	Wingless

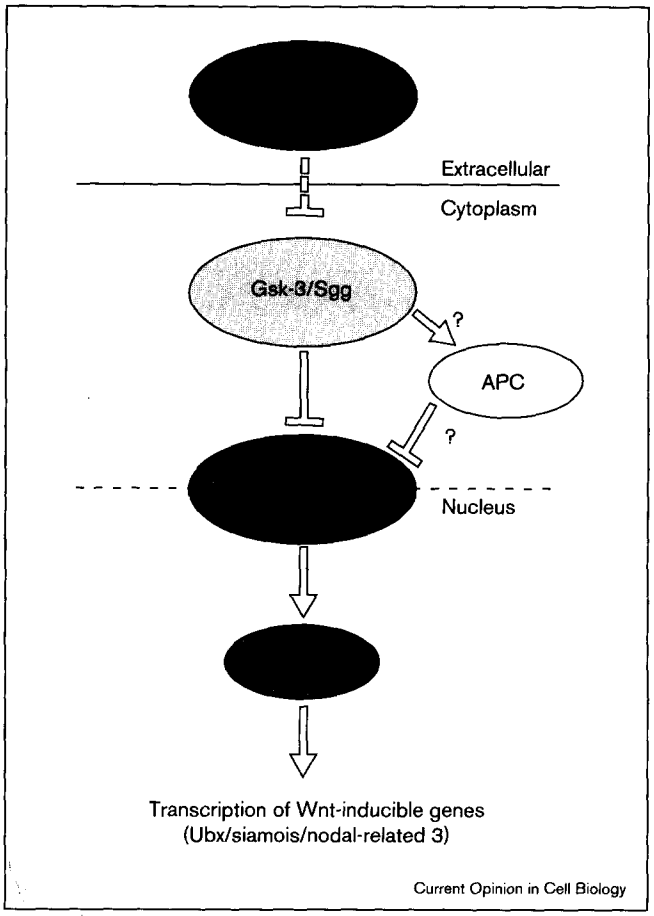
Introduction

About two years ago, the word spread about a physical interaction between β-catenin and lymphocyte enhancer-binding factor 1 (LEF-1). This observation [1••] precipitated feverish work which firmly established that LEF-1, and its closest relative, T cell factor (TCF), are activated by signalling through the Wnt/Wingless pathway [1••–6••]. LEF-1 and TCF are high mobility group (HMG) proteins and were discovered as proteins binding to specific sequences in lymphoid enhancers [7–9]. Their function as ultimate targets for a signalling transduction pathway came as a complete surprise. They thus joined the group of molecules known for their 'other lives' in mammalian cells, such as β-catenin and glycogen synthase kinase 3 (Gsk-3), which *Drosophila* genetics had uncovered as components of the Wingless (Wg) signalling pathway [10–12] (Figure 1). The same components transduce the Wnt-1 signal in vertebrate cells [13].

The current prevailing view is that LEF-1/TCF becomes a transcriptional activator of Wnt/Wingless-inducible genes upon binding to the transcriptional co-activator β-catenin or its fly equivalent, Armadillo [1••–6••]; see also recent reviews [14–18]). Alternatively, LEF-1/TCF may be a transcriptional repressor whose activity is antagonised by β-catenin, a possibility for which there is growing evidence (see below). If so, this would imply that Wnt signalling primarily promotes relief of repression, rather than transcriptional activation, of Wnt target genes.

Here, I shall focus on whether LEF-1/TCF is a transcriptional activator or repressor. If the former is the case, LEF-1/TCF may not have a function in the majority of unstimulated cells (except perhaps a tissue-specific function in a small set of cells; see below). However, should the latter be the case, then LEF-1/TCF may

Figure 1



Downstream components of the Wnt pathway. All known downstream components of the Wnt signalling pathway are shown (dark grey, positively acting; light grey, negatively acting). Regulatory interactions are shown by arrows (stimulatory) and bars (inhibitory) and mark interactions between components that are direct, based on biochemical evidence. The upstream components (see [18]) are left out, as indicated by the interrupted bar between Wnt/Wg and Gsk-3/Sgg. Note that the regulatory relationship between APC and the Wnt pathway is still controversial (indicated by question marks; see text). APC, adenomatous polyposis coli (protein); Arm, Armadillo; β-cat, β-catenin; Gsk, glycogen synthase kinase; Sgg, Shaggy; TCF, T cell factor; Wg, Wingless.

be required to keep Wnt-inducible genes repressed in unstimulated cells. In particular, consider cells that are in a state of prestimulation or poststimulation by Wnt signalling, which almost certainly contain LEF-1/TCF: could there be co-repressors that ensure inactivity of LEF-1/TCF in these cells? If such co-repressors existed, this would be of potential importance in tumourigenesis: recent work has demonstrated that inappropriate activation of LEF-1/TCF in the colonic epithelium, and in other cells, can lead to cancer [19••–21••,22•].

TCF, a Wnt-response factor

Before I can address this question of putative co-repressors, I need to summarise briefly how Wnt/Wingless is thought to bring about changes in transcriptional activation. For simplicity, I shall use 'TCF', meaning all TCF-like factors, including LEF-1 (compare [17]), unless I refer to a particular TCF molecule; likewise, I shall use 'Wnt', meaning Wnt-1 and Wingless, and β-catenin meaning β-catenin and Armadillo (compare [18]).

If a cell is stimulated by Wnt, β-catenin accumulates to high levels in the cytoplasm of this cell and translocates into the nucleus [12,23–25]. How β-catenin gets into the nucleus is still unclear. Although it can apparently piggy-back on TCFs to get there [1••–3••], mutant forms of Armadillo which lack the TCF interaction domain can still translocate to the nucleus [25], suggesting that β-catenin has a constitutive, or possibly Wnt-induced, nuclear transport signal. Once in the nucleus, β-catenin binds to TCF and serves as its co-activator to stimulate transcription of Wnt-inducible genes [1••–6••,19••]. Three Wnt-inducible genes have been identified as direct TCF targets: the *Drosophila* HOX gene *Ultrabithorax* (*Ubx*) [4••], with a key function in endoderm induction [26]; the *Xenopus* homeobox gene *siamois* [27•], with a role in early axis specification [28]; and recently the *Xenopus nodal-related 3* gene [29].

The stability of cytoplasmic ('free') β-catenin is regulated by Wnt signalling [12,25,30] (unlike the membrane-bound β-catenin, stability of which does not increase on Wnt signalling and whose adhesion function is clearly separate from free β-catenin's function in Wnt signal transduction [25,31]). Two proteins keep free β-catenin unstable (Figure 1). One is Gsk-3/Shaggy, whose role as a negatively acting component of the Wnt signal transduction pathway is well established [10–12,24]. Another may be the protein encoded by the tumour suppressor gene adenomatous polyposis coli (APC) [32], which is mutant in the majority of colon cancers [15]. Lack of APC function in colon cells causes the accumulation of high levels of free β-catenin, which activates the resident hTcf-4 [19••,20••] and induces *de novo* LEF-1 expression [22•], suggesting that *Lef1* itself is a target gene for TCF (see also [3••]). APC itself is phosphorylated by Gsk-3, and apparently acts together with Gsk-3 to keep free β-catenin unstable [33•]. Furthermore, a substantial fraction of colon cancer and melanoma cell lines with normal APC contain high levels of free β-catenin which, due to mutation in the Gsk-3 regulation site, escapes destabilisation [20••,21••]. This suggests that APC, like Gsk-3, antagonises Wnt signalling; however, a *Drosophila* APC mutant has failed so far to show a phenotype which would link this gene to the Wingless pathway [34]. More confusingly, genetic work in *C. elegans* indicates that worm APC synergises with Wnt signalling; lack of worm APC function mimics loss-of-function of Wnt or β-catenin [35•]. Finally, overexpression of *Xenopus* APC in early embryos produces a phenotype like that caused by Wnt-1 or β-catenin overexpression, again suggesting a positively acting role of APC [36]. However, the mutant conditions examined so far may not reflect the APC null phenotype, and overexpression of APC (in the presence of normal endogenous APC) may have currently uninterpretable consequences. Thus, the question of whether APC antagonises Wnt signalling (Figure 1), or synergises with it, is still unresolved.

The discovery of TCF as a Wnt-response factor was conceptually pleasing because LEF-1 is thought to be an architectural factor mediating assembly of multiprotein enhancer complexes [37]. LEF-1 cannot stimulate transcription from multimerised LEF-1 sites, but acts strictly in a context-dependent way, that is together with other nearby enhancer-binding factors [37–41]. The same seems to be the case for *Drosophila* TCF (dTCF) whose activity in the *Drosophila* midgut is also strictly context dependent [4••]. This implies that Wnt signalling is not autonomous in altering gene expression, but that it relies on, and co-operates with, other signal response factors or pre-existing transcription factors which may carry positional information (e.g. tissue-specific factors). In T cells, these co-operating factors include a signal response factor that binds to a cAMP response element (CRE), and the lymphoid-specific factors ETS1 and acute myeloid leukaemia factor 1 (AML-1) [38–41]. In the *Drosophila* midgut, one co-operating factor is a protein responding to Decapentaplegic and Ras signalling which together target a CRE [4••,42,43]. This conservation of the CRE as a TCF-co-operative enhancer element suggests that there may be a limited set of TCF partners.

Functional analysis in flies and frogs showed that TCF loss of function mimics loss of function of Wnt or β-catenin in many different cellular contexts [1••–3••,5••,6••,26]. This implies that TCF is an obligatory Wnt-response factor, meaning that every cell stimulated by Wnt signalling responds by activating TCF. Whether TCF is also a dedicated Wnt-response factor, meaning that its sole function is to respond to Wnt signalling, is still unknown (for example, individual TCF factors may also have a cell-specific function independent of β-catenin; see below). It is also unknown whether other Wnts target TCFs. So far, the knockout phenotypes of LEF-1 and TCF-1 in mice have not answered this: *Tcf1* mutant mice show defects in T cell expansion [44], a phenotype

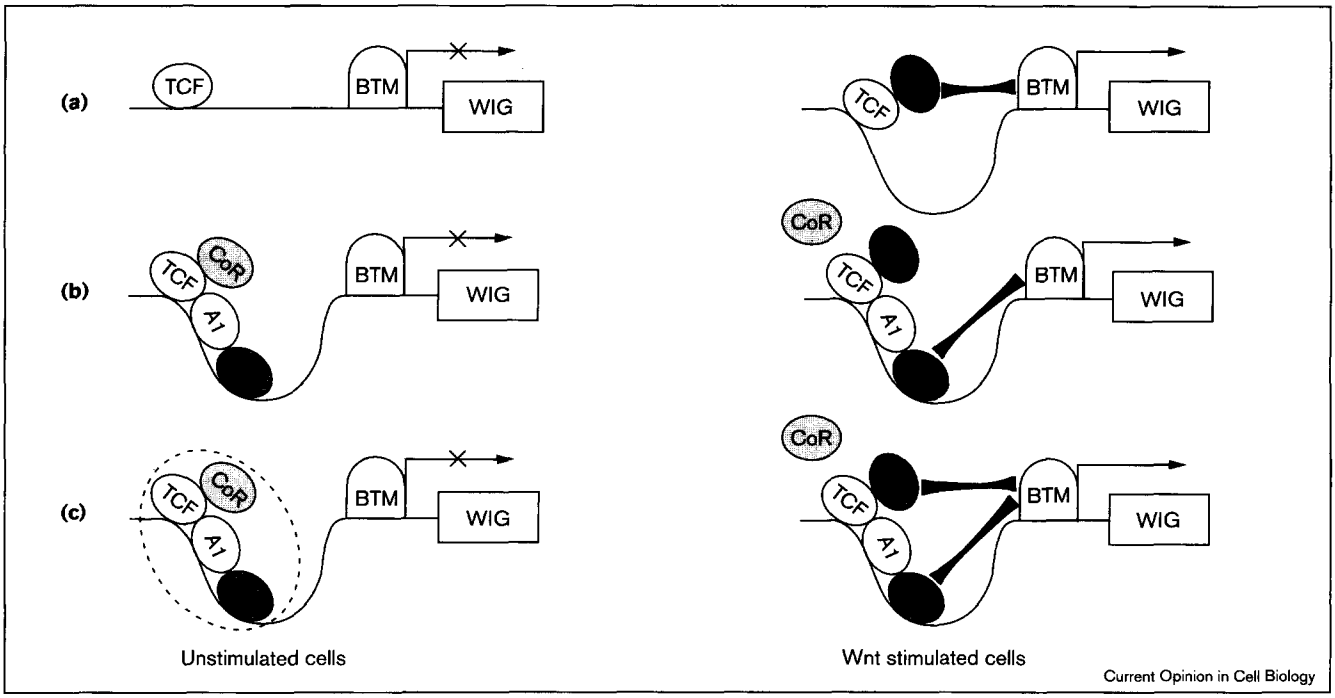
much enhanced in *Tcf1 Lef1* double mutants [45•], whereas *Lef1* mutants show defects in organs that derive from epithelium–mesenchyme interactions [46]. It is still an open question whether Wnt signalling is involved in these processes.

TCF could mediate transcriptional stimulation of Wnt-inducible genes in two ways. Either TCF becomes a transcriptional activator by virtue of binding to its co-activator β-catenin; this bipartite activator may then contact the basal transcription machinery (BTM) assembled at the promoter of Wnt-inducible genes, thereby stimulating their transcription. In this scenario, TCF in unstimulated cells is either not bound to Wnt-inducible genes or, if bound, is neutral regarding their transcription (Figure 2a). Alternatively, TCF is a transcriptional repressor, constitutively bound to Wnt-inducible genes to keep these inactive in unstimulated cells. Upon Wnt stimulation, β-catenin binds to TCF and somehow neutralises its repressive function, thereby allowing other prebound enhancer factors to stimulate transcription (Figure 2b). Note that experiments based on dominant-negative TCFs (which cannot bind β-catenin due to an amino-terminal deletion [1••–3••,5••]) do not distinguish between these possibilities as these truncated TCFs are likely to interfere with either activator or repressor function of endogenous TCF. Below, I shall summarise evidence supporting each of these alternatives.

TCF, a transcriptional activator

Overexpression of LEF-1 stimulates reporter gene transcription in cells that have insignificant levels of β-catenin

Figure 2



Alternative models of TCF function. Bridges indicate direct or indirect contacts between (co-)activators and the basal transcription machinery (BTM). Shading as in Figure 1 (dark grey, positively acting; light grey, negatively acting). **(a)** Wnt signalling mediates transcriptional stimulation. TCF, by binding to its co-activator β-catenin (β-cat), is a transcriptional activator of Wnt-inducible gene(s) (WIG) in Wnt-stimulated cells. This model proposes that TCF has no function in the majority of unstimulated cells (i.e. its putative binding to Wnt-inducible enhancers in these cells is neutral with respect to transcriptional activity). Loss of function of TCF should cause exclusively *armadillo*-like phenotypes. **(b)** Wnt signalling mediates relief of repression. A co-repressor (CoR) binds to TCF in unstimulated cells, keeping the TCF enhancer inactive. In Wnt-stimulated cells, β-catenin displaces this co-repressor, thus allowing other enhancer-bound activators (A1, A2, other proteins [tissue-specific factors, e.g. AML-1 or ETS1, or signal response factors, e.g. CREB, or Decapentaplegic response factor] which bind co-operatively with TCF to the Wnt-inducible enhancer) to contact the BTM. This model proposes an essential function of TCF as a transcriptional repressor in unstimulated cells. Loss of function of TCF should cause *shaggy*-like phenotypes. **(c)** Wnt signalling mediates relief of repression and promotes transcriptional stimulation. This model combines (a) and (b), and has two new aspects. Firstly, TCF earmarks (indicated by dashed circle) Wnt-inducible enhancers for subsequent transcriptional activation in unstimulated cells; it assembles potentially active complexes on these enhancers. Earmarking requires co-operative interactions with other enhancer-binding proteins. Secondly, there is synergy between TCF/β-catenin and other enhancer-bound factors in the transcriptional stimulation of the linked gene in Wnt-stimulated cells. Loss-of-function phenotypes are difficult to predict in this model as the earmarking function of TCF (*armadillo*-like) might mask additional repressive (*shaggy*-like) and activating (*armadillo*-like) functions of TCF. Models (b) and (c) allow for a tissue-specific function of TCF, independent of β-catenin: some tissue-specific factors, once assembled with TCF in a stable enhancer complex, may recruit a co-activator (e.g. ALY, not shown) which is capable of contacting the BTM despite the presence of the co-repressor, thus by-passing the need for β-catenin. Note also that TCF bends DNA [37]; whether this property is critical for TCF's function in stimulating transcription is not known.

[4••,38–40]. This is in contrast to other TCFs, which barely stimulate transcription after overexpression in these cells ([7,47]; X Yu, M Bienz, unpublished data), and which only do so in the presence of high levels of β-catenin [2••,5••,27•]. This suggests a genuine difference between LEF-1 and other TCFs, with LEF-1 being a constitutive (or T-cell-specific) transcriptional activator, and other TCFs being conditional β-catenin-dependent activators. Note, however, that LEF-1 and TCF-1 are redundant regarding their tissue-specific function in T cells [45•].

Alternatively, in these assays, overexpressed LEF-1 might act to relieve repression mediated by another protein bound to the LEF-1 site or elsewhere in the gene. Evidence for this comes from *in vitro* transcription using supercoiled chromatin templates [48]: these experiments suggest that LEF-1 binds to the TCRα enhancer and acts at long range to relieve repression of the linked promoter, which is repressed by HMG I/Y. By doing so, LEF-1 increases the number of active templates rather than the rate of transcription [48].

Unequivocal demonstration that LEF-1 is a *bona fide* transcriptional activator came from experiments based on fusion proteins whereby a domain of LEF-1 was tethered to DNA through a heterologous DNA-binding domain [38,39]. These experiments identified a transactivation domain within the amino terminus of LEF-1, not present in other TCFs, which functions in a context-dependent way, suggesting that this domain may be involved in mediating contacts with adjacently bound transcription factors. Moreover, a co-activator of LEF-1, called ally of LEF-1 and AML-1 (ALY), was identified which binds to this domain (and to the lymphoid-specific factor AML-1), and which itself functions as a context-dependent transcriptional activator when tethered to DNA [49•]. Note that ALY does not bind to TCF-1; also, ALY and β-catenin bind to distinct domains in LEF-1, and the two proteins co-activate LEF-1 independently of one another [49•].

Likewise, the transactivation potentials of β-catenin and Armadillo have been demonstrated by similar tethering experiments, carried out in yeast and in transfected B cells, which identified a transactivation domain within the carboxyl terminus of these molecules [4••,5••]. This domain is essential for the signalling function of Armadillo [50], but it is unknown whether it activates transcription through direct contact with the BTM, or by some other unknown mechanism.

Finally, there is genetic evidence from flies that dTCF (also called Pangolin) may be an activator of transcription. The phenotypes of *dTCF* loss-of-function mutants in the epidermis or in the midgut mimic *wingless* and *armadillo* mutants [5••,6••], implying a positively acting role of dTCF in the Wingless pathway (Figure 2a). If dTCF were a repressor (Figure 2b), *dTCF* mutants should mimic Gsk-3 (*shaggy/zeste-white 3*) mutants, by showing constitutive signalling. So far *shaggy*-like phenotypes have not been observed in *dTCF* mutant embryos; however, these embryos probably contain maternal dTCF which may mask such *shaggy*-like phenotypes. The true loss-of-function phenotype of dTCF still awaits analysis.

TCF may be a transcriptional repressor

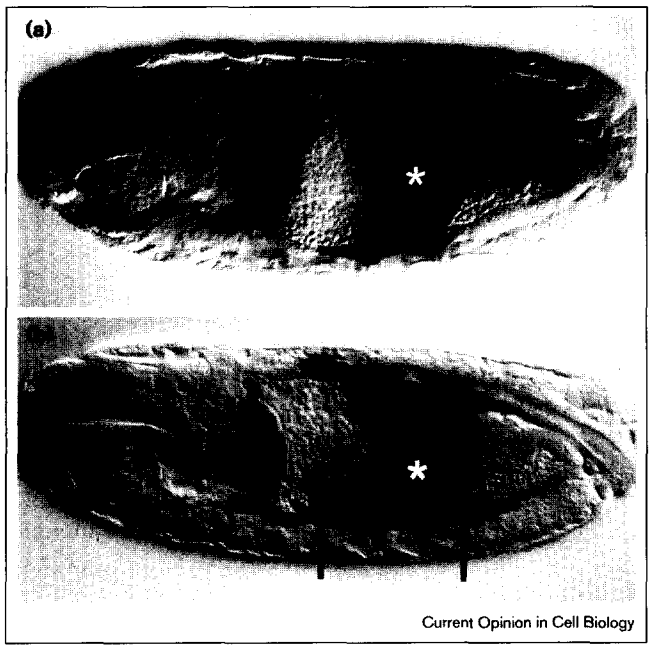
The first evidence that TCF may be a transcriptional repressor came from studies with reporter genes (see Figure 3). Mutation of the TCF binding site in the *Ubx* midgut enhancer not only causes a decrease of *Ubx* expression in cells that are near the Wingless signalling source, but also a gain of expression in cells that are further away from this source (Figure 3b) [4••]. This derepression was caused by three distinct sets of point mutations in the TCF-binding site of this enhancer, making it unlikely that the repression is due to another protein recognising an overlapping site (also, *Drosophila* is thought to contain only one *TCF* gene [17]). Moreover, addition of TCF-binding sites to a minimal synthetic promoter stimulated expression in Wingless-proximal cells, but also reduced expression in Wingless-distal cells [4••], confirming the putative repressor function of dTCF.

A similar observation was made with the *siamois* enhancer [27•]. This enhancer is strongly active dorsally where nuclear accumulation of β-catenin is seen [51], but barely active ventrally where there is very little nuclear β-catenin. Mutation of the three TCF-binding sites within this enhancer reduced expression dorsally, but also caused a dramatic gain of expression ventrally [27•]. This indicates a repressor function of XTcf-3 (a TCF expressed in early frog embryos [2••]) in unstimulated cells, in addition to its activator function in stimulated cells. There is again the caveat that the observed derepression may be due to a distinct protein recognising one of the three mutated sequence motifs.

A further indication that TCF may be a repressor came from an experiment in which a membrane-anchored plakoglobin was overexpressed in frog embryos [52]. Unexpectedly, the resulting phenotype mimicked over-activation of Wnt-1 signalling. The authors suggested that this phenotype may mimic loss of function of TCF since endogenous TCF might be sequestered in the cytoplasm by the membrane-anchored plakoglobin, opening the possibility that the normal function of Wnt-activated plakoglobin (and β-catenin) may be to sequester TCF from DNA [52]. A more likely explanation for this finding is that the membrane-anchored plakoglobin releases membrane-bound β-catenin, thus increasing free β-catenin which in turn activates TCF [53].

Finally, further evidence that TCF may be a transcriptional repressor has come from worm genetics: *pop-1* (a TCF homologue) mutants have the opposite phenotype of Wnt or β-catenin loss-of-function mutants [35•,54•].

Figure 3



Evidence for a repressor function of dTCF. Side views of 14 hours old *Drosophila* embryos, transformed with a *Ubx*/β-galactosidase reporter gene and stained with an anti-β-galactosidase antibody. **(a)** The wild-type *Ubx* midgut enhancer mediates transcription in cells near the Wingless source (white asterisk). **(b)** A mutant enhancer (BG [4••]) whose Wingless-responsive TCF-binding site is mutated mediates less transcription near the Wingless source, but additional transcription further away from this source (indicated by arrowheads). This derepression is seen with three distinct sets of point mutations affecting the TCF-binding site, indicating a repressive function of dTCF in unstimulated cells (note, derepression is spatially limited as the activity of the *Ubx* enhancer also depends on Decapentaplegic signalling, whose spread is limited to the central portion of the midgut [26]).

In other words, loss of Pop-1 is equivalent to β-catenin accumulation or Wnt signalling, indicating that Pop-1 is a repressor whose activity is antagonised by Wnt signalling. Alternatively, Wnt signalling in the early worm embryo may act at two thresholds, in a similar way to Wingless signalling in the *Drosophila* midgut [55]. By analogy to this situation, Pop-1 could be an activator conferring the distal cell fate (i.e. mesoderm, corresponding to Labial expression in the fly midgut) at low signalling levels; this distal fate (and Pop-1 expression itself [35•,54•]) might be repressed by a different factor activated at high signalling levels, thus permitting the proximal fate (i.e. endoderm), which may be the default fate. In this scenario, *pop-1* mutants would reflect complete loss of Wnt signalling, whereas the Wnt or β-catenin mutants would reflect lowering of Wnt signalling (which is consistent with the complete versus incomplete penetrance of the *pop-1* versus the Wnt or β-catenin mutant phenotypes, as observed [35•,54•]).

A function of TCF in unstimulated cells?

As outlined, there are strong indications that TCF may be a transcriptional repressor in addition to being a transcriptional activator. This suggests a function of TCF in unstimulated cells. What might this function be?

I propose that TCF earmarks Wnt-inducible genes such that they can be transcriptionally induced upon Wnt signalling. Furthermore, I suggest that TCF only earmarks a subset of Wnt-inducible genes in each cell type, a selection process that would depend on the availability of partner transcription factors for TCF, for example cell-type-specific factors. This is plausible because it has been shown that LEF-1 binds to the TCRα enhancer co-operatively with the lymphoid-specific factors ETS1 and AML-1 [41] in chromatin templates, an observation that underscores the architectural properties of LEF-1 [37–40]. On the whole, earmarking would not result in transcriptional activation of the linked gene, but would program the transcriptional competence of this gene [56] to respond to Wnt signalling. In exceptional cell types, however, such as T cells, this earmarking process might be sufficient to confer activation of transcription, perhaps due to a TCF partner factor in the same enhancer complex whose ability to contact the BTM, directly or indirectly (e.g. via ALY), by-passes the need for β-catenin.

This putative earmarking function of TCF would predict a critical role of TCF-binding sites for the activity of linked genes. Indeed, expression of reporter genes with mutated TCF-binding sites is much reduced in transient assays [7–9,27•]. The critical role of TCF-binding sites for transcriptional activity is even more apparent when single-copy reporter genes are assayed in stable transformants: a TCF-binding site was found to be essential for efficient and insertion-site-independent transcriptional activity of the adenosine deaminase thymic enhancer when assayed in transgenic mice, albeit not in transiently transfected cells [57]. Furthermore, expression from the TCRα enhancer is severely reduced in immature T cells from *Lef1 Tcf1* double mutants [45•]. Finally, the *Ubx* midgut enhancer contains a second TCF-binding site which, together with the TCF-binding site constituting the Wingless-response element [4••], is absolutely required for transcriptional activity: the double-mutant enhancer is incapable of mediating any reporter gene expression whatsoever in transformed fly embryos (S Eresh, M Bienz, unpublished data).

Conclusions

Current knowledge of TCF function suggests the following plausible scenario (Figure 2c). Before Wnt stimulation, TCF selects a subset of Wnt target genes by binding to these co-operatively with other cell-type-specific factors, thus programming their transcriptional competence to respond to Wnt signalling. While earmarking these genes,

TCF keeps them in a repressed state, presumably with the help of a co-repressor. On Wnt signalling, β-catenin translocates into the nucleus and binds to TCF, thereby somehow 'breaking up' the repressive complexes on TCF-earmarked genes. In addition to relieving repression, β-catenin is likely to increase the rate of transcription, presumably by contacting (directly or indirectly) the BTM assembled at the promoters of Wnt-inducible genes.

Key support for this kind of model would come from the demonstration of a putative function of TCF in unstimulated cells. Further genetic analysis of TCF mutants might reveal this. Equally convincing, and of potential relevance to research on tumourigenesis, would be the identification of putative co-repressors of TCF. The search for these is on!

Acknowledgements

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- • of special interest
- •• of outstanding interest

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These authors discovered the interaction between β-catenin and LEF-1 in a yeast two-hybrid screen, using β-catenin as a bait. They show that a ternary complex forms between β-catenin and DNA-bound LEF-1, and that LEF-1 overexpression in *Xenopus* embryos produces early axis duplication, that is a Wnt-1 overexpression phenotype, in a manner dependent on β-catenin.

2. •• Molenaar M, van de Wetering M, Oosterwegel M, Peterson-Maduro J, Godsave S, Korinek V, Roose J, Destrée O, Clevers H: **XTcf-3 transcription factor mediates β-catenin-axis formation in *Xenopus* embryos.** *Cell* 1996, **86**:391-399.

These authors report the isolation of XTcf-3, a TCF homologue expressed in early *Xenopus* embryos. The authors show that XTcf-3 binds to β-catenin and activates transcription in a β-catenin-dependent way. They also demonstrate that a dominant-negative form of XTcf-3 which cannot bind β-catenin suppresses axis formation in *Xenopus* embryos, implicating a function of XTcf-3 in responding to β-catenin signalling.

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Lef1 was found among genes whose expression was induced in mouse embryonic stem cells with elevated free β-catenin. It is shown that LEF-1 binds β-catenin, that it can import β-catenin into the nucleus and that overexpression of LEF-1 in *Xenopus* embryos causes early axis duplication.

4. •• Riese J, Yu X, Munnerlyn A, Eresh S, Hsu S-C, Grosschedl R, Bienz M: **LEF-1, a nuclear factor coordinating signaling inputs from *wingless* and *decapentaplegic*.** *Cell* 1997, **88**:777-787.

The Wingless response sequence in the midgut enhancer of *Ubx*, a Wingless target gene, is identified as a binding site for LEF-1. The authors demonstrate that LEF-1 activates transcription in the fly embryo, and that it functions in an *armadillo*-dependent way to cause *shaggy*-like phenotypes. Evidence is provided that dTCF acts through the *Ubx* enhancer in a context-dependent way, co-operating with the CRE-binding protein that responds to Decapentaplegic signalling.

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