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T-cell factors: turn-ons and turn-offs

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Introduction

The family of T-cell factor (Tcf) and lymphoid enhancer factor (Lef) proteins, which in mammals comprises Tcf1, Lef1, Tcf3 and Tcf4, form a subgroup of the high mobility group (HMG) box-containing superfamily of transcription factors. Alternative splicing and promoter usage give rise to multiple Tcf isoforms, possessing diverse functional domains (Figure 1) (van de Wetering *et al.*, 1996; Korinek *et al.*, 1998; Duval *et al.*, 2000; Hovanes *et al.*, 2000, 2001). Tcfs bind DNA as monomers. The 80 amino acid HMG box mediates sequence-specific binding to a core consensus sequence AGATCAAAGGG through contacts made predominantly within the minor groove of the DNA helix (Giese *et al.*, 1991; van de Wetering *et al.*, 1991; van Beest *et al.*, 2000). Tcfs, like other HMG box-containing transcription factors, have been described as architectural proteins due to their ability to induce substantial bends in DNA, potentially facilitating the formation of large nucleoprotein complexes and thereby promoting transcription (Giese *et al.*, 1992). However, Tcf molecules by themselves are incapable of modulating transcription. Instead they bind a number of auxilliary proteins, thereby recruiting essential functional domains to the regulatory regions of target genes. This review will focus mainly on the identity of these Tcf co-factors.

Tcfs are highly conserved through evolution (Figure 2). Experiments in a diverse range of species, including *Hydra* (Hobmayer *et al.*, 2000), nematodes (Lin *et al.*, 1995; Thorpe *et al.*, 1997), flies (Brunner *et al.*, 1997; Riese *et al.*, 1997; van de Wetering *et al.*, 1997), fish (Kim *et al.*, 2000), frogs (Behrens *et al.*, 1996; Molenaar *et al.*, 1996) and mice (van Genderen *et al.*, 1994; Galceran *et al.*, 1999), have revealed several critical roles for Tcfs during embryogenesis. Thus, Tcfs are required for establishing the embryonic body plan, for specifying cell fate, and for regulating cell proliferation and survival. At subsequent stages of development, Tcfs continue to regulate cell survival, proliferation and differentiation, especially in rapidly self-renewing tissues, such as the skin (van Genderen *et al.*, 1994; Merrill *et al.*, 2001), lymphoid compartment (Verbeek *et al.*, 1995; Reya *et al.*, 2000; Ioannidis *et al.*, 2001) and intestinal mucosa (Korinek

et al., 1998; Roose *et al.*, 1999). Intriguingly, Tcfs participate in these cellular responses in a bimodal fashion: activating processes in one subset of cells, while simultaneously repressing the same functions in a different subset (Brannon *et al.*, 1997; Cavallo *et al.*, 1998; Merrill *et al.*, 2001). This bimodality manifests itself at the level of target gene expression, with Tcfs either activating or repressing expression of the same target gene in different subsets of cells. For example, Tcf activates expression of the target gene *XSiamois* in dorsal marginal blastomeres of *Xenopus* embryos, but represses its expression in ventral blastomeres, contributing to the establishment of the dorso-ventral body axis (Brannon *et al.*, 1997). Understanding how Tcfs can either activate or repress transcription has become a central topic of enquiry for investigators of Tcf function.

Tcfs are the ultimate mediators of Wnt/Wg signalling

The first breakthrough in resolving this issue came with the identification of β -catenin as a specific binding partner of Tcfs (Behrens *et al.*, 1996; Molenaar *et al.*, 1996). Binding occurs between the first 50 amino acids of Tcfs and Armadillo repeats 3–10 of β -catenin (Figures 1 and 3). Fly β -catenin (also known as Armadillo) had already been identified as a component of the canonical Wnt/Wingless (Wg) signal transduction cascade. This signalling pathway (Figure 4) is one of the primary pathways utilized during metazoan development. The placement of β -catenin as a downstream signalling component in this pathway had perplexed many in the field and not without reason, considering that β -catenin was first characterized as an adherens junction (a specialized epithelial cell intercellular junction) component that links the adhesion molecule E-cadherin with the cortical actin cytoskeleton. Nevertheless, it became apparent that in association with Tcfs, β -catenin could supply essential transcriptional transactivation domains, activating target gene expression (van de Wetering *et al.*, 1997). The crystal structures of β -catenin in complex with *Xenopus* Tcf3 and mammalian Tcf4 have subsequently been solved (Graham *et al.*, 2000, 2001; Poy *et al.*, 2001). The structures are broadly similar. The Armadillo repeat region of β -catenin forms a superhelix comprising 12 repeats, with each repeat (except repeat 7) consisting of three α -helices. The β -catenin binding domain of Tcf is an extended modular structure that snakes its way in an antiparallel fashion along a positively charged groove in the β -catenin superhelix, whose surface is composed of amino acid residues in the third helices of Armadillo repeats 3–10. While Tcfs and β -catenin interact extensively, Lys312 and Lys435 of β -catenin form particularly strong and specific anchors that fasten the two molecules together (Figure 5).

Fig. 1. Schematic representation of Tcf splice variants and their most conserved domains. Short forms of Tcf1 and Lef1 lack the N-terminal domain, which interacts with β -catenin. The CAD domain in Lef1 is required for context-dependent activation of the TCR α enhancer. The HMG box mediates sequence-specific DNA binding. The most divergent region of the Tcf family members is the C-terminus, which in certain longer isoforms contains a conserved motif, CRARF, whose function is presently unknown, and two CtBP binding sites.

Tcfs as harbingers of death

Simultaneously, a firm link between Tcfs and tumorigenesis was forged. It is now well appreciated that deregulation of the Wnt pathway through mutation of various of its components results in malignant transformation. Mutated components include, notably, adenomatous polyposis coli (APC; mutated in up to 80% of familial and sporadic colon tumours) and axin/conductin, two tumour suppressor proteins, and β -catenin itself—in the guise of an oncoprotein. Regardless of the protein target, such mutations result in constitutive transcriptional transactivation by Tcf/ β -catenin (Korinek *et al.*, 1997; Morin *et al.*, 1997; Rubinfeld *et al.*, 1997). The inappropriate activation of a programme of Tcf target genes is thus presumed to be responsible for malignant transformation; defining this programme would greatly enhance our understanding (and potentially our treatment) of cancer. To date, very few Tcf target genes have been identified in mammals (a comprehensive list can be viewed at the Wnt website: www.ana.ed.ac.uk/rnusse/wntwindow.html). The most obvious candidates among them for mediating malignant transformation are the oncogenes *cyclin D1* (Tetsu and McCormick, 1999) and *c-myc* (He *et al.*, 1998). *Tcf1* and *Lef1* also appear themselves to be Tcf target genes (Roose *et al.*, 1999; Hovanes *et al.*, 2001). Ablation of *Tcf1* by gene targeting in mice results in a mild tumour predisposition phenotype. Such loss also greatly enhances the tumour susceptibility phenotype resulting from co-inheritance of a mutant APC allele (Roose *et al.*, 1999). The majority of expressed Tcf1 isoforms lack a β -catenin binding domain, and are thus incapable of activating transcription (van de Wetering *et al.*, 1996). It can be inferred therefore that such isoforms ordinarily function as tumour suppressors, perhaps through competing for DNA binding with full-length Tcf molecules, and that the

induction of *Tcf1* expression by an activated Wnt pathway constitutes a negative-feedback mechanism. In contrast, activation of the Wnt pathway in colon tumours leads to the selective ectopic induction of full-length Lef1 isoforms, constituting a positive-feedback loop (Hovanes *et al.*, 2001). It also highlights another aspect of bimodal behaviour by Tcfs (oncogenic transformation versus oncosuppression). Although mutations in the Wnt signalling pathway are mostly associated with colon cancer, a survey of the literature implies its involvement in many other diverse tumour types, including desmoid tumours, hepatocellular carcinoma, melanoma and pilomatricomas.

The Tcf merry-go-round

A major advance in explaining how Tcfs repress transcription followed the discovery that Groucho homologues (or Grgs) are specific binding partners of Tcfs (Roose *et al.*, 1998). Binding occurs between the Q-domain of Groucho and a conserved region in Tcfs between the β -catenin binding domain and the HMG box (Roose *et al.*, 1998) (Figures 1 and 3). Groucho behaves as a transcriptional corepressor in *in vitro* assays, and *Drosophila* Groucho, the founder of this family of molecules, inhibits various developmental signalling pathways *in vivo*, including the Wnt/Wg signalling pathway (Cavallo *et al.*, 1998).

While a competitive antagonism between β -catenin and Groucho for Tcf binding would provide the simplest molecular mechanism to account for a switch in function of Tcfs as transcription factors, biochemical evidence in support of such a model has yet to be provided. This neat model is further challenged by reports claiming that Tcfs can bind various other transcriptional co-regulators. For instance, Tcfs can interact with the adherens junction and desmosomal component plakoglobin, also known as γ -catenin and which is highly related to β -catenin (Simcha *et al.*, 1998). Like β -catenin, plakoglobin possesses putative glycogen synthase kinase 3 (GSK3) phosphorylation sites in its N-terminus and a transactivation domain in its C-terminus (Hecht *et al.*, 1999; Zhurinsky *et al.*, 2000). Plakoglobin levels also appear to respond to Wnt signalling and are regulated by the destruction complex (Figure 4) (Papkoff *et al.*, 1996; Kodama *et al.*, 1999). Plakoglobin mimics β -catenin in assays of Wnt activity such as primary axis duplication in *Xenopus* oocytes (Karnovsky and Klymkowsky, 1995) and also as a dominant transforming oncoprotein (Kolligs *et al.*, 2000). However, β -catenin and plakoglobin appear to play very distinct roles during embryogenesis: a deficiency of β -catenin, unlike plakoglobin, results in very early embryonic lethality (Haegel *et al.*, 1995; Bierkamp *et al.*, 1996; Huelsken *et al.*, 2000).

Lef1 was originally identified by its ability to bind and activate expression from the T-cell receptor α enhancer (Travis *et al.*, 1991; Waterman *et al.*, 1991). Subsequently, this activation was shown to be independent of β -catenin, requiring instead binding of ALY, a ubiquitously expressed nuclear protein that specifically associates with the context-dependent activation domain of Lef1 (Bruhn *et al.*, 1997) (Figure 1). Smads, transcriptional co-activators that are essential components of signalling responses elicited by members of the TGF- β superfamily of growth factors (including TGF- β , activin and BMPs) have been shown to

bind the HMG box of Tcfs (Labbe *et al.*, 2000; Nishita *et al.*, 2000). This direct interaction may largely account for the cooperation between Wnt signalling and TGF- β signalling in establishing Spemann's organizer during amphibian embryogenesis. The HMG box of Lef1 has also been shown to bind the intracellular signalling domain of notch1 (Ross and Kadesch, 2001). This transmembrane receptor mediates lateral inhibition required for refinement of tissue patterning during embryogenesis. Ligand binding to notch triggers proteolytic cleavage of the intracellular domain, which is then free to translocate to the nucleus where it functions as a transcriptional co-activator. Interaction with Lef1 activated a set of target genes distinct from that activated by β -catenin binding. In addition, certain Tcfs (*Xenopus* Tcf3 and mammalian Tcf3 and Tcf4E) bind the transcriptional co-repressor CtBP

through six-amino-acid motifs found in the C-terminus (Brannon *et al.*, 1999).

The purported ability of Tcfs to interact with transcriptional co-factors other than β -catenin and Groucho could allow for the convergence of multiple signalling pathways, while diversifying the choice of target genes expressed in response to a stimulus. But while the above studies suggest a spectrum of possible Tcf interacting co-factors, it is important to stress that no genetic evidence has been advanced to date to support the actual occurrence of these interactions *in vivo*. Thus their biological significance remains undetermined.

In addition to the above transcriptional co-factors, Tcfs also interact transiently with several other classes of molecules that regulate their stability, their subcellular localization or their ability to target DNA. The HMG box of Lef1 was shown to interact with the N-terminus of protein inhibitor of activated STAT γ (PIASy). This interaction results in the covalent modification of Lef1 through conjugation of multiple small ubiquitin-like

Fig. 2. Multiple alignment of Tcfs from various species. Protein sequences for human, mouse, chicken (partial) and frog Tcf4, fish Tcf3, and fly, worm, sea urchin and *Hydra* Tcfs were aligned using the Clustal_W algorithm. Black shading indicates identity, grey shading indicates conserved residues. Note that the highest regions of homology include the N-terminal β -catenin interacting domain (amino acids 1–53 of human Tcf4), the HMG box (amino acids 318–409 of human Tcf4) and a motif (KKCRARFGLDQXXWCXPCRRKKKC) distal to the HMG box found in certain Tcf isoforms.

modifier (SUMO) peptides, suggesting that PIASy functions as an E3 SUMO ligase. Sumoylation of Lef1, which inhibits its function as a transcription factor, does not interfere with DNA or β -catenin binding, but instead leads to sequestration of Lef1 in PML nuclear bodies (Sachdev *et al.*, 2001). Tcfs are further covalently modified by mitogen activated protein kinases (MAPKs). In both *Caenorhabditis elegans* and mammalian cells, homologous MAPKs (Lit1 and NLK, respectively) have been identified that can phosphorylate Tcfs (Pop1 in *C.elegans*). Phosphorylation inhibits Tcf signalling by disrupting DNA binding and by inducing redistribution of Tcfs from the nucleus to the cytoplasm, accompanied by the down-regulation of Tcf protein levels (Ishitani *et al.*, 1999; Meneghini *et al.*, 1999; Rocheleau *et al.*, 1999; Shin *et al.*, 1999). In *Drosophila*, evidence has been provided that acetylation of a conserved lysine residue in the Armadillo binding domain of DTcf (also known as pangolin) by the CREB binding protein (CBP) inhibits Wg signalling, potentially through weakening the interaction between Tcf and Armadillo (Waltzer and Bienz, 1998). Tcf binding to target DNA sequences also appears to be suppressed by association with HMG box repressor protein 1 (HBP1) (Sampson *et al.*, 2001) and I-mfa domain proteins (Snider *et al.*, 2001).

Co-co-factors and the chromatin connection

But it is not only the Tcfs that lead a complex social life: β -catenin and Groucho (the two principal Tcf interacting proteins) are themselves multi-domain molecules capable of binding numerous protein partners (Figure 3). Several transcriptional transactivation domains exist within β -catenin, although the principal activity maps to the C-terminus (van de Wetering *et al.*, 1997; Hecht *et al.*, 1999). In turn, these domains allow β -catenin to make independent associations with the basal transcriptional machinery (Hecht *et al.*, 1999) and other transcriptional co-factors, including CBP/p300 (Hecht *et al.*, 2000; Miyagishi *et al.*, 2000; Takemaru and Moon, 2000) and Brg1 (Barker *et al.*, 2001). Likewise, multiple domains within Groucho appear important for repression, and various proteins, including several transcription factors other than Tcfs, have been described to bind Groucho. To date though, only histone deacetylases (HDACs) related to yeast Rpd3 have been identified as Groucho binding proteins capable of mediating transcriptional repression (Chen *et al.*, 1999).

CBP/p300, Brg1 and HDACs share a common mode of function when modulating transcription: they all target chromatin. Chromatin was originally conceived of as the packaging material of DNA, whose primary function was the solution of the 'space problem', namely: how to condense several metres of DNA into a single cell spanning <100 μ m. The demonstration that chromatin, especially that fraction associated with the regulatory regions of inducible genes, is dynamically reorganized in a manner dependent on gene expression, generated further interest in the topic.

The basic unit of chromatin is the nucleosome, which comprises eight core histones in very tight association, around which 146 bp of double-stranded DNA helix makes approximately two supercoils. Ultrastructurally,

this gives rise to the now celebrated 'beads-on-a-string' appearance of uncondensed chromosomal DNA. This secondary structure is further compacted through nucleosome and nuclear matrix associations mediated by core histones, as well as histone H1, and non-histone chromatin binding proteins. Investigators have been able to show that the precise spacing of individual nucleosomes on gene

Fig. 3. Domain structure of the two principal Tcf binding partners: β -catenin and Groucho/Grg. Protein-protein interaction domains are depicted schematically for β -catenin (A) and Groucho (B). Shown above and below the line diagrams are the names of proteins capable of binding to the indicated domains. (A) The N-terminal domain of β -catenin contains four conserved serine/threonine residues that, when phosphorylated, are recognized by the F-box protein β -Trcp, targeting β -catenin for ubiquitylation and subsequent proteolytic degradation (see Figure 4). Alternatively, the unphosphorylated N-terminus of β -catenin can be recognized by the F-box protein Ebi, resulting in the destruction of β -catenin in response to p53-mediated stress signals (Liu *et al.*, 2001; Matsuzawa and Reed, 2001). The central domain of β -catenin comprises 12 imperfect 42-amino-acid repeats (denoted Armadillo repeats 1–12; note the presence of an insertion within repeat 10). These repeats mediate most of the interactions between β -catenin and its binding partners, including the destruction complex components axin/conductin and APC (also Figure 4), Tcfs, and the intercellular adhesion molecule E-cadherin. The newly identified Wnt/Wg signalling component Legless (Lgs)/BCL9 recruits an additional novel cofactor Pygopus (Pygo), which appears essential for β -catenin activity, although its mechanism of action is as yet undetermined (Kramps *et al.*, 2002). Activity of the peptidyl-prolyl *cis-trans* isomerase Pin1, which isomerizes the peptide bond between phosphorylated Ser246 and the neighbouring Pro residue of β -catenin, inhibits the interaction between APC and β -catenin, resulting in β -catenin stabilization (Ryo *et al.*, 2001). In addition, β -catenin binds the highly related Reptin52 and Pontin52 molecules, DNA-dependent ATPases with helicase activity that also bind the TATA-box binding protein (TBP). While Reptin52 represses Tcf/ β -catenin-mediated gene activation, Pontin52 can stimulate it (Bauer *et al.*, 2000). The *Xenopus* HMG box-containing transcription factors Sox3 and Sox17 α and β are also capable of binding β -catenin and inhibiting its function as a transcriptional transactivator (Zorn *et al.*, 1999), as is the novel 81-amino-acid peptide ICAT (Tago *et al.*, 2000). The C-terminal domain of β -catenin contains potent transcriptional transactivation elements. These elements bind TBP *in vitro*, and may thus recruit the basal transcription machinery (Hecht *et al.*, 1999). (B) Groucho family members contain five protein domains: an N-terminal Q (glutamine-rich) domain, followed by a GP (glycine- and proline-rich) domain, a CcN domain (containing putative casein kinase II/cdc2 phosphorylation sites and nuclear localization signal), an SP (serine- and proline-rich) domain and four WD40 repeats (protein interaction domain). Tcfs (and various other transcription factors including NF- κ B and NK4) bind to the Q-domain as well as to other Groucho domains (e.g. Engrailed, Goosecoid, Hairy and HES-1). HDACs, transcriptional repressor molecules that deacetylate histone proteins, bind to the GP domain of Groucho molecules (Chen *et al.*, 1999).

Fig. 4. The Wnt/Wg signalling cascade. In unstimulated cells, APC shuttles β -catenin from the nucleus (Henderson, 2000; Rosin-Arbesfeld *et al.*, 2000) to a so-called 'destruction complex' in the cytoplasm, comprising axin/conductin and the serine (S)/threonine (T) kinases CKI α (casein kinase I α) and GSK3. CKI α phosphorylation of β -catenin on S45 primes the subsequent sequential phosphorylation of T41, S37 and S33 residues by GSK3 (Behrens *et al.*, 1998; Ikeda *et al.*, 1998; Kishida *et al.*, 1998; Liu *et al.*, 2002). Phospho-S33 and -S37 residues are recognized by the F-box protein β -TrCP, which comprises part of an E3 ubiquitin ligase complex (Jiang and Struhl, 1998; Hart *et al.*, 1999; Liu *et al.*, 1999; Winston *et al.*, 1999). Ubiquitylation of β -catenin induces its rapid degradation by the proteasome (Orford *et al.*, 1997). At low levels of free β -catenin, Tcfs bind Groucho proteins (Grgs), and repress transcription (Cavallo *et al.*, 1998; Roose *et al.*, 1998). Wnt/Wg proteins constitute a large family of secreted cysteine-rich glycoproteins that function as ligands for members of the Frizzled (Fz) family of serpentine receptors (Bhanot *et al.*, 1996) and the low density lipoprotein receptor-related proteins 5 and 6 (LRP5/6). The canonical Wnt signalling pathway is only activated when Wnt binds the extracellular domains of both proteins (Wehrli *et al.*, 2000). This results in inhibition of GSK3 by Dishevelled (Dvl) (Yanagawa *et al.*, 1995) and also in sequestration of axin by LRP5/6 (Mao *et al.*, 2001). Consequently, β -catenin is no longer phosphorylated and can accumulate in the cytoplasm and the nucleus, where in partnership with Tcfs it activates target gene expression (Molenaar *et al.*, 1996; Brunner *et al.*, 1997; Riese *et al.*, 1997; van de Wetering *et al.*, 1997).

promoters and enhancers excludes the binding of transcription factors (Adams and Workman, 1993; Blomquist *et al.*, 1996) and the basal transcription machinery (Laybourn and Kadonaga, 1991; Godde *et al.*, 1995). During the induction of gene expression such nucleosomes are somehow displaced—'remodelled'—allowing access of transcription molecules to promoter/enhancer regions (Zaret and Yamamoto, 1984; Carr and Richard-Foy, 1990).

Two cellular activities have so far been identified which function synergically to remodel chromatin during transcription. Histone acetyltransferases (HATs) modify the N-terminal domains of core histone proteins, among other targets, by catalysing the transfer of acetyl groups to lysine residues (HDACs perform the reciprocal function). SWI/SNF-like multiprotein complexes that contain a single DNA-dependent ATPase component homologous with the yeast SWI2 and Rsc proteins then mediate the actual displacement of acetylated nucleosomes. CBP/p300, while being able to mediate contact with the basal transcription machinery, also has intrinsic and, through binding other HATs such as GCN5/PCAF, associated HAT activity. β -catenin binds the CREB binding or CH-3 domains of CBP/p300 through direct interaction with a region spanning Armadillo repeat 10–C-terminus. This association was shown to enhance Tcf/ β -catenin target gene expression in mammalian cells and *Xenopus* embryos (Hecht *et al.*, 2000; Miyagishi *et al.*, 2000; Takemaru and Moon, 2000), but did not require the intrinsic HAT activity of CBP/p300 (Hecht *et al.*, 2000). In mammals, two SWI2 orthologues exist: *Brahma* and *Brahma related gene-1*

(*Brg1*). Barker *et al.* (2001) recently demonstrated that Brg1 is a binding partner of β -catenin. Armadillo repeats 7–10 of β -catenin bind a conserved domain in the N-terminal half of Brg1. Similarly to CBP/p300, co-transfection of Brg1 enhances Tcf/ β -catenin-mediated transcription. Conversely, inhibition of Brg1 by over-expression of an ATPase-dead dominant-negative mutant suppressed expression of Tcf/ β -catenin target genes, including *c-myc*, in colon carcinoma cell lines. Reduction of endogenous Brahma in *Drosophila* was shown to enhance developmental phenotypes due to loss of Wg signalling activity, while suppressing phenotypes due to overstimulation of the pathway. Intriguingly, Treisman and co-workers have shown that in flies, *osa*-containing SWI/SNF complexes inhibit ectopic activation of Tcf target genes in cells lacking a Wg signal (Collins and Treisman, 2000). In such cells, SWI/SNF cooperates with Groucho, possibly mediated through a biochemical interaction with HDACs (Zhang *et al.*, 2000), to suppress Tcf target gene expression. Although seemingly contradictory findings, it is none the less conceivable that SWI/SNF-like complexes can mediate either activation or repression of a given Tcf target gene since both SWI/SNF and Rsc can catalyse forward and reverse nucleosome remodelling reactions (Logie and Peterson, 1997; Lorch *et al.*, 1998).

In conclusion, a change in expression of a Tcf target gene from a basal level to either an induced or repressed level is likely to require the recruitment of multiple chromatin remodelling complexes (see Figure 6). These complexes are directed to target gene promoters not by Tcfs directly, but through association with their primary

Fig. 5. Crystal structure of the extended region of *Xenopus* Tcf3 β -catenin binding domain interacting with the surface of β -catenin. The surface of β -catenin is coloured according to the relative electrostatic potential, with red denoting negative charge while blue shows positive charge. Two semi-buried charged buttons, Lys435 and Lys312, within β -catenin make critical salt bridges with residues in Tcf. XTcf3 residues are labelled in yellow and β -catenin residues are labelled in white. [Reproduced from Graham *et al.* (2000) with the kind permission of Elsevier Science © 2000.]

binding partners, namely β -catenin and Groucho. While Groucho mediates stable transcriptional repression through binding histone deacetylases, and also indirectly SWI/SNF-like complexes; β -catenin solicits the help of both CBP/p300 and Brg1-containing complexes during activation of target gene expression. However, the sequence and kinetics of deployment of these co-factors remains to be precisely established, as does the identity of the HAT activity presumed to be necessary to fully reverse the repressive effects of Groucho/HDAC function as well as for the optimal recruitment of a SWI/SNF-like chromatin remodelling complex. One recent study using a synthetic Tcf target gene promoter reconstituted with chromatin *in vitro* has already provided further insights into the connection between Tcf regulation of transcription and chromatin remodelling (Tutter *et al.*, 2001). In this study, the authors confirmed that chromatin suppresses the basal transcription of a Tcf target gene. They further demonstrated that Tcfs in complex with β -catenin could bind to chromatin templates without requiring additional factors. β -catenin appears to mask residues present within the N-terminus of Tcf that otherwise interfere with the loading of Tcf on chromatin. This system also reproduced the requirement for CBP/p300 and an ATP-dependent chromatin remodelling fraction for optimal transcription, and such an approach might, therefore, in future allow the full sequence of steps from repressed state to activated state of a Tcf target gene to be fully reconstituted. Finally, the identification of chromatin remodelling enzymes required for Tcf function *in vivo* provides novel drug targets to tackle diseases related to aberrant Wnt signalling such as cancer.

Fig. 6. Multiple steps for activation of a Tcf target gene. In the 'off' state, target gene expression is repressed by a complex comprising Tcf and Groucho (certain Tcf isoforms may also utilize CtBP for additional repression). Groucho mediates repression primarily through recruitment of HDACs, that can in turn enlist *osa*-containing SWI/SNF-like complexes. Acting in concert, these two chromatin remodelling complexes strategically position nucleosomes on the promoter of target genes, in order to exclude assembly of the basal transcription machinery. For induction of target gene expression—the 'on' state—accumulation of β -catenin resulting from Wnt/Wg signalling is presumably sufficient to displace Groucho or at least HDACs (Billin *et al.*, 2000). On recruitment of a HAT activity (X), CBP/p300 and a SWI/SNF-like complex, critical nucleosomes are remodelled, and the basal transcription machinery can now assemble on the promoter and initiate transcription.

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