Tcf-1-mediated transcription in T lymphocytes: differential role for glycogen synthase kinase-3 in fibroblasts and T cells

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Abstract

β-Catenin is the vertebrate homolog of the Drosophila segment polarity gene Armadillo and plays roles in both cell–cell adhesion and transduction of the Wnt signaling cascade. Recently, members of the Lef/Tcf transcription factor family have been identified as protein partners of β-catenin, explaining how β-catenin alters gene expression. Here we report that in T cells, Tcf-1 also becomes transcriptionally active through interaction with β-catenin, suggesting that the Wnt signal transduction pathway is operational in T lymphocytes as well. However, although Wnt signals are known to inhibit the activity of the negative regulatory protein kinase glycogen synthase kinase-3β (GSK-3β), resulting in increased levels of β-catenin, we find no evidence for involvement of GSK-3β in Tcf-mediated transcription in T cells. That is, a dominant negative GSK-3β does not specifically activate Tcf transcription and stimuli (lithium or phytohemagglutinin) that inhibit GSK-3β activity also do not activate Tcf reporter genes. Thus, inhibition of GSK-3β is insufficient to activate Tcf-dependent transcription in T lymphocytes. In contrast, in C57MG fibroblast cells, lithium inactivates GSK-3β and induces Tcf-controlled transcription. This is the first demonstration that lithium can alter gene expression of Tcf-responsive genes, and points to a difference in regulation of Wnt signaling between fibroblasts and lymphocytes.

Introduction

Armadillo/β-catenin and members of the Tcf/Lef transcription factor family functionally interact to transduce wingless/Wnt signals in Xenopus axis specification (XTcf-3) (1,2) in the establishment of segment polarity in Drosophila (dTCF) (3–5), and in colon carcinoma and melanoma (Tcf-4) (6–8). Wnt signals inhibit the activity of the negative regulatory protein serine kinase glycogen synthase kinase-3β (GSK-3β), which is complexed with cytoplasmic adenomatous polyposis coli proteins and β-catenin (9). This is believed to result in increased pools of free β-catenin, which can subsequently interact with Tcf factors. Whereas Tcf factors can bind DNA by themselves, they do not activate transcription. However, a potent transactivation domain is supplied by the C-terminus of β-catenin (1,3 and see results).

The first identified member of this family, Tcf-1, also interacts with β-catenin (1) and is specifically expressed in T lymphocytes (10). In this report we confirm the physical interaction of Tcf-1 and β-catenin, and demonstrate its functional importance in T cells. Tcf-1 is required for T lymphopoiesis, as evidenced in a targeted gene disruption experiment (11). Tcf-1+/− thymocytes are arrested at the transition of the CD4−CD8− double-negative to the CD4+CD8+ double-positive stage and, unlike wild-type cells at this stage, are not in cell cycle.

How Tcf-1 transcriptional activity is controlled is unclear at the moment. However, Proud et al. have reported that T cell activation by phorbol myristate acetate (PMA) and/or ionomycin, similar to the Wg/Wnt pathway, inhibits GSK-3β activity (12). We have therefore tested if classical T cell activation regimes, such as stimulation with PMA/ionomycin, anti-CD3 antibodies or phytohemagglutinin (PHA) could activate Tcf reporter genes. We show here that these agents do not activate Tcf-dependent transcription, although they do inhibit GSK-3β activity. This prompted us to investigate more closely
the role of GSK-3β in Jurkat T cells and in C57MG cells, which are commonly used for Wnt signaling studies. These cells contain the closely related Tcf-4 transcription factor. Treatment of cells with lithium is a well-known way to inactivate GSK-3β (13–15), and in both Jurkat and C57MG cells GSK-3β activity is rapidly inhibited by lithium. However, only in C57MG cells is Tcf-4-dependent transcription activated by lithium. We therefore conclude that either GSK-3β is not involved in Tcf-mediated transcription in T cells (but another kinase is) or that inhibition of GSK-3β is insufficient to mediate transcriptional activation. Alternatively, another negative regulatory kinase might act in concert with GSK-3β. Thus, GSK-3β activity is differently regulated in fibroblasts versus lymphocytes. Our findings also suggest that there is a difference in regulation of Wnt signaling between fibroblasts and lymphocytes.

Methods

Constructs, transfections and reporter gene assays
β-Catenin and deletion mutants of β-catenin, Tcf-1 p45 E1B, and dominant negative GSK-3β (K → A mutant, kindly provided by Dr Woodgett, Toronto, Canada) were cloned into pCDNA (Invitrogen, Carlsbad, CA) by standard methods. The dominant positive lck expression construct (pCKF505) was kindly provided by Dr T. Mustelin (San Diego, CA). The TKTOP and TKFOP optimal and mutated Tcf luciferase reporter constructs are similar to the previously described pTOPFLASH and pFOPFLASH, except that instead of a minimal Fos promoter, they contain a TK promoter, avoiding the cAMP-responsive element present in the minimal fos promoter. The dominant positive Tcf-1/β-catenin fusion protein consists of the 132 C-terminal amino acids of β-catenin linked to Tcf-1 lacking the first 55 amino acids. The C-terminus of β-catenin was PCR amplified, introducing a Kozak sequence and appropriate restriction sites, and confirmed to be free of mutations by sequencing.

Transfections were done with 3 × 10⁶ Jurkat T cells, growing exponentially, by electroporation with 3 µg luciferase reporter constructs, 10 µg β-catenin constructs, 2 µg pCDNA-TCF-1 and/or 2 µg CAT control constructs. C57MG cells were transfected using lipofectamine, as described (19). pSV40-CAT was used to normalize for transfection efficiency. CAT values were determined as pristane/xylene extractable ¹⁴C-radio labeled, butyrylated chloramphenicol, as described (3). Luciferase values were determined by lysing the cells in 1 mM DTT, 1% Triton X-100, 15% glycerol, 25 mM Tris pH 7.8, and 8 mM MgCl₂. Luciferase activity was measured with a Lumac/3M biocounter using luciferin as a substrate. In some experiments, cells were washed and split into two fractions to be able to determine both luciferase and CAT activity.

Stimulations
Jurkat T cells were grown in RPMI, supplemented with 10% FCS and antibiotics. CT6 cells were grown in RPMI, 10% FCS, supplemented with 10 ng/ml IL-7; MO7 cells were grown in IMDM 20% FCS, supplemented with IL-3 (25 ng/ml) and SCF (10 ng/ml). C57MG cells were grown in DMEM/10% FCS and antibiotics. Cells were washed after transfection and stimulated with PMA (25 ng/ml), PHA (20 ng/ml), 10 ng/ml ionomycin, (2 mM) or antibodies against CD3/CD28, or by the cytokines SCF (20 ng/ml) or IL-7 (10 ng/ml) for 8 h, or for the indicated amount of time. For cytokine stimulations, cells were washed 3 times and grown overnight in 2% serum without cytokines, before stimulating them.

Gel retardation assays
In vitro translated proteins were made using the T7 Tnt reticulocyte lysate coupled transcription and translation system (Promega, Madison, WI). Proteins were incubated with a radiolabeled optimal Tcf probe (double-stranded 15mer CCCCTTGTACCTTACC, from Isogen, Maarssen, Netherlands) in the presence or absence of a anti-β-catenin antibody (Transduction Laboratories, Lexington, KY) or anti-Tcf-1 (7H3).

Binding reactions were done in the presence of 500 ng poly(dl-dC), 10 mM HEPES, 60 mM KCl, 1 mM EDTA, 1 mM DTT and 15% glycerol. Incubations with DNA probe were for 40 min at room temperature (in incubations with antibodies, 20 min extract with probe followed by addition of antibody), after which complexes were analyzed on a native 4.5% polyacrylamide gel.

Western blotting
Jurkat cells (5 × 10⁶) were stimulated with PHA, and lysed in RIPA buffer supplemented with protease and phosphatase inhibitors. Equal amounts of protein (cell equivalents or measured by Bradford method) were loaded on a 10% SDS–PAGE, blotted on nitrocellulose, and stained with Ponceau S to confirm equal loading and transfer of the samples. Blots were probed with a β-catenin antibody (Transduction Laboratories) and visualized using the ECL system (Amersham, Little Chalfont, UK).

Immunoprecipitations and GSK-3β kinase assay
GSK-3β assays were done as described (16). In short, cells were stimulated, washed in ice-cold PBS and lysed in lysis buffer (50 mM Tris, 200 mM NaCl, 10 mM EDTA, 50 mM NaF and 1% Triton X-100, supplemented with phosphatase and protease inhibitors). Lysates were passed through a syringe and incubated with an antibody against GSK-3β and Sepharose-Protein A beads for 2 h at 4°C, while tumbling in a rotator. Beads were washed twice in 50 mm Tris, pH 7.4, 1 mM DTT, 10 mM MgCl₂ and 0.1 % Triton X-100, once in 5 mM Tris, pH 7.4, 0.5 M LiCl, 1 mM DTT and 10 mM MgCl₂, and once in Tris/MgCl₂ buffer. Beads were incubated with the substrate mix (1 mg P-GS1 peptide, 50 mM ATP, 1 mg heparin and [γ³²P]ATP) for 20 min at 37°C. Kinase reactions were stopped by addition of PBS/EDTA and reactions spotted on phosphocellulose filter. Filters were washed 4 times in 75 mM orthophosphoric acid, dried and measured in a scintillation counter. Each stimulation, immunoprecipitation and kinase reaction was done in duplicate, and the mean of these is reported in the results.

Results

Tcf-1 and β-catenin physically and functionally interact in T cells
We previously reported the cloning of β-catenin in a yeast two-hybrid screen as a molecule interacting with Tcf-1 (1).
Tcf-1 signaling and GSK-3β in T cells

**Fig. 1.** Tcf functionally interacts with β-catenin to form a transcriptionally active complex. (A) In vitro translated β-catenin and Tcf-1 interact. In vitro translated Tcf-1 (p45 4A1B form) and wild-type β-catenin were translated in vitro and used in a gel retardation analysis using an optimal Tcf probe. Supershifts were done with the indicated antibodies to confirm the nature of the protein–DNA complex and show that Tcf-1 and β-catenin physically interact. (B) Co-transfection of optimal (TOP) or mutated (FOP) reporter constructs for TCF/Lef with pCNA-Tcf-1 and/or pCNA-β-catenin demonstrates that β-catenin is responsible for Tcf-dependent transcription. (C) The C-terminus of β-catenin is required for transactivation of Tcf, whereas the N-terminus is dispensable. Representative experiments are shown. Duplicate transfections were performed and are shown. Normalization for transfection efficiencies was done using pSV40-CAT. (D) A Tcf-1/β-catenin fusion protein acts as a dominant positive factor. AZU-2 B cells were transfected with pCDNA-DP Tcf-1 and reporter constructs. Duplicate transfections were performed and are shown. Normalization for transfection efficiencies was done using pSV40-CAT.

Co-transfection of both genes results in transcriptional transactivation of Tcf reporter plasmids (3). Gel retardation analysis of in vitro translated Tcf and β-catenin confirmed that Tcf-1 physically interacts with β-catenin (Fig. 1A).

To assess if this interaction was functionally important, we performed a transient reporter gene assay using a luciferase reporter gene containing three optimal Tcf sites (TKTOPflash) (Fig. 1B). Transfection of this reporter into Jurkat T cells, which contain high amounts of Tcf-1 and moderate amounts of the related gene product Lef-1 (17), did not result in active transcription. Co-transfection of the reporter plasmid with β-catenin alone was sufficient to transactivate the reporter gene, indicating that endogenous Tcf-1/Lef-1 was activated by providing β-catenin (Fig. 1B). Overexpression of β-catenin did not activate reporter constructs containing the IL-2 enhancer or multimerized binding sites for Oct-1, AP1 or NF-AT (not shown). Deletion of the C-terminus of β-catenin abrogated the transactivation of Tcf reporter constructs, whereas the N-terminus was dispensable for the effect (Fig. 1C). These observations agreed with earlier experiments showing that Tcf proteins interact with the central region of β-catenin, while the C-terminus of the latter constitutes a transactivation domain (3). β-Catenin molecules lacking the C-terminus (e.g. ΔC β-catenin and ΔNΔC β-catenin) can therefore be regarded as dominant-negative competitors of β-catenin/Tcf signaling in this system. Similar observations have been made in three other T cell lines (CEM, Molt-4 and CT6).

The idea that Tcf-1 provides the DNA binding motif via its HMG box and β-catenin the transactivation domain through its C-terminus was substantiated using a Tcf-1/β-catenin fusion protein. Transfection of this construct into AZU-2 B cells, which
lack Tcf/LEF factors, very strongly activated Tcf-dependent transcription (Fig. 1D). Therefore this fusion protein can be regarded as a dominant positively acting factor (DP Tcf-1).

**T cell activation does not activate Tcf transcription, but decreases GSK-3β activity**

How Tcf-1 transcriptional activity is controlled is unclear at the moment. Welsh et al. have shown that T cell activation by PMA and/or ionomycin, similar to the Wg/Wnt pathway, inhibits GSK-3β activity (12). This makes T cell stimulation by PMA/ionomycin and other T cell activation stimuli, such as anti-CD3 antibodies or PHA, a logical candidate to activate Tcf-dependent transcription. We hypothesized that since these agents decrease GSK-3β activity, they would lead to increased β-catenin levels, resulting in increased Tcf controlled transcription. We first tested if PHA or anti-CD3 stimulation would also result in inhibition of GSK-3β activity. Treatment with PHA leads to a rapid, transient decrease of GSK-3β activity to ~50–60% of unstimulated conditions (Fig. 2A), comparable to the decrease reported for PMA/ionomycin (12). Treatment with anti-CD3 antibodies resulted in a somewhat smaller and more transient inhibition of GSK-3β activity (not shown). However, treatment of Jurkat T cells with PMA/ionomycin or PHA could not activate Tcf reporter genes (see Fig. 2B). Similarly, treatment with anti-CD3 with or without anti-CD28 could also not activate Tcf reporter genes (not shown). PHA also does not activate Tcf transcription in Jurkat clones stably transfected with reporter constructs (see Table 2). These stimulations also did not change the levels of β-catenin in the cell (not shown).

Stimulation through β1 integrin (CD29) activates a protein kinase called ILK (integrin-linked kinase) which reportedly leads to increased β-catenin levels in epithelial cells and could activate Tcf/LeF transcription (18 and S. Dedhar, pers. commun.). Similar to more classical T cell activation stimuli, cross-linking of CD29 also was unable to activate Tcf-dependent transcription (Fig. 2B).

**Table 1.** Activation of Tcf-1 transcription in Jurkat T cells by transient transfection of signaling molecules

<table>
<thead>
<tr>
<th>Transfected Construct</th>
<th>Reporter</th>
<th>Luciferase Valuea</th>
<th>TOP:FOP ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>TOP</td>
<td>263</td>
<td></td>
</tr>
<tr>
<td>GSK3β dominant negative</td>
<td>TOP</td>
<td>1200</td>
<td>0.52</td>
</tr>
<tr>
<td>Lck dominant positive</td>
<td>TOP</td>
<td>1510</td>
<td>0.82</td>
</tr>
<tr>
<td>Wild-type β-catenin</td>
<td>TOP</td>
<td>12,540</td>
<td>1.17</td>
</tr>
<tr>
<td>S33 β-catenin</td>
<td>TOP</td>
<td>94,747</td>
<td>12.3</td>
</tr>
</tbody>
</table>

Jurkat T cells were transfected with indicated expression constructs, optimal (TOP) or mutated (FOP) Tcf reporter luciferase constructs and pSV40-CAT (transfection efficiency internal control), as described in Methods.

aCAT-corrected luciferase values.

**Fig. 2.** T cell activation lowers GSK-3β activity, but does not activate Tcf-dependent transcription. (A) PHA stimulation transiently and rapidly decreases GSK-3β activity. Each symbol represents the mean of two independent determinations. The results from four independent experiments are shown as relative kinase activity. After about 30 min GSK-3β activity has returned to basal (not shown), which is indicated as 1.0. (B) Stimulation of Jurkat T cells with PMA/ionomycin, PHA or antibodies against CD29 does not activate Tcf-dependent transcription. Cells were transfected with optimal (TKTOP) or mutated (TKFOP) reporter constructs, grown for 18 h, washed and stimulated for 8 h with the indicated stimuli. Duplicate transfections were performed and are shown. Normalization for transfection efficiencies was done using pSV40-CAT.

**Dominant negative GSK-3β does not activate TCF-1 transcription, but S33 β-catenin does**

GSK-3β is a negative regulator of Wnt/Wg signaling. A dominant negative GSK-3β would therefore be expected to act as an activator of the Wnt pathway. Indeed a dominant negative GSK-3β activates the Wnt pathway in Xenopus oocytes (13) and prevents GSK-3β-induced apoptosis in PC-12 cells (23). Therefore, to test more directly for a role of GSK-3β in Tcf-mediated transcription, we transiently transfected a dominant negative form of GSK-3β that lacks kinase activity into Jurkat cells. Although luciferase values did increase after transfection (indicating that the dominant negative GSK-3β is active), the transcription from the mutated reporter construct (FOP) increased as much as from the optimal Tcf motif (TOP).
Tcf-1 signaling and GSK-3β in T cells

Fig. 3. Lithium activates Tcf-dependent transcription in C57MG cells, while decreasing GSK-3β activity. (A) C57MG cells were transfected with TKTOP and TKFOP reporters and empty expression vector or S33 β-catenin, or stimulated with 20 mM LiCl or KCl (as control for osmotic effects). Both β-catenin and lithium can activate Tcf transcription. Normalization for transfection efficiencies was done using pSV40-CAT. (B) Lithium decreases GSK-3β activity in C57MG and Jurkat T cells. Cells were stimulated with LiCl, lysed and GSK-3β activity was determined as described in methods. The average of two independent experiments are shown. (C) Dominant negative GSK-3β activates Tcf-dependent transcription in C57MG cells. Cells were transfected with ‘kinase dead’ (K → A) GSK-3β, β-catenin expression vectors, CAT control vector and reporter constructs. CAT-corrected luciferase values of duplicate transfections with TOP and FOP reporters were used to calculate the TOP:FOP ratio. A ratio > 1.0 indicates increased Tcf-dependent transcription.

Table 2. Lithium and PHA do not specifically activate Tcf-1 transcription in stable Jurkat clones

<table>
<thead>
<tr>
<th>Jurkat clone</th>
<th>Treatment</th>
<th>Fold activation</th>
</tr>
</thead>
<tbody>
<tr>
<td>TOP 10</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>0.6</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>1.0</td>
</tr>
<tr>
<td>TOP 33</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>4.9</td>
</tr>
<tr>
<td>TOP 49</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>3.1</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
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</tr>
<tr>
<td>FOP 16</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>6.6</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>0.8</td>
</tr>
<tr>
<td>FOP 23</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>4.5</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>0.9</td>
</tr>
<tr>
<td>FOP 42</td>
<td>none</td>
<td>1.0</td>
</tr>
<tr>
<td></td>
<td>lithium</td>
<td>7.3</td>
</tr>
<tr>
<td></td>
<td>PHA</td>
<td>0.8</td>
</tr>
</tbody>
</table>

Three independent stable TOP and three stable FOP Jurkat clones were treated with PHA or 20 mM LiCl for 8 h and luciferase activity was measured. Data are given as fold activation, were 1.0 is the untreated condition.

Thus, ‘kinase dead’ GSK-3β did not specifically activate Tcf transcription (Table 1). Similarly, a dominant positive Ick(F506) also non-specifically increased both TOP and FOP values. In contrast, either wild-type β-catenin or a β-catenin molecule carrying a point mutation in serine residue 33 strongly and specifically activated Tcf transcription (Table 1). Serine 33 is a potential phosphorylation site for negative regulatory kinases such as GSK-3β (and/or others) and activating phosphatases. Activation of Tcf transcription by S33 β-catenin was stronger and lasted longer (not shown) than by wild-type β-catenin, suggesting that a negative kinase other than GSK-3β may be involved in this pathway.

Involvement of GSK-3β in Tcf transcription in Jurkat and C57MG cells

The above reported results prompted us to test if GSK-3β is involved in Tcf-mediated transcription in established Wnt-responsive systems. The murine breast epithelial cell line C57MG has been used extensively as a model system for Wnt signaling. This cell line contains Tcf-4 and has been used extensively as a model system for Wnt signaling. This cell line contains Tcf-4 and transfection with Wnt-1 leads to increased Tcf-4-dependent transcription (19). In these cells, transfection with β-catenin activates Tcf reporter genes 4- to 6-fold (Fig. 3A). Treatment of cells with lithium chloride inhibits GSK-3β and has been used as a pharmacological agent to activate Wnt signaling (13). Stimulation of C57MG cells with lithium specifically activated Tcf-dependent transcription (Fig. 4A) and in parallel experiments led to decreased GSK-3β kinase activity (Fig. 4B) by 20–40%. In contrast, in Jurkat T cells, lithium also decreased GSK-3β activity (albeit to a somewhat lesser extend than in C57MG cells), but did not specifically activate Tcf transcription (Table 2). The effect of lithium was tested in transient transfections (not shown), but also in the more sensitive system of Jurkat clones stably transfected with TCF reporter genes...
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(Reference page).

In transient transfected Jurkat cells, no increase in Tcf-dependent transcription was observed. In the stable reporter clones, lithium increased luciferase values in some clones; however, it increased both TOP and FOP reporter clones to the same extent. Thus, no effect of lithium was seen in transiently transfected cells, while a non-specific increase was observed in the more sensitive stable transfecteds. A possible explanation for this effect is provided by the fact that GSK-3β inhibits translation elongation factors (12). Lithium treatment could lead to non-specific enhancement of translation, leading to higher luciferase values. This effect is expected to be stronger in the stable transfecteds.

We have also tested if the dominant negative GSK-3β construct could induce Tcf-dependent transcription in C57MG cells. As shown in Fig. 3(C), this ‘kinase dead’ GSK-3β indeed activates the Tcf optimal reporter to comparable levels as overexpression of β-catenin. This in contrast to the situation in Jurkat T cells, as reported above.

Discussion

Recent studies have indicated that β-catenin and members of the Lef/Tcf HMG box transcription factor family functionally interact to affect cell fate in Xenopus embryos (1,2). Thus, ectopic expression of murine Lef-1 or overexpression of endogenous xTcf-3 translocates β-catenin to the nucleus and induces axis duplication. Extending this work in Drosophila, several groups have shown that the fly TCF family member, dTCF functions directly downstream of Armadillo in activating transcription of the engrailed and Ubx genes, thus establishing segment polarity in the Drosophila embryo (3). Another member of this family, Tcf-1, is exclusively expressed in T lymphocytes in adults (10) and is required for T lymphopoiesis, as evidenced in targeted gene disruption experiments (11). Here we have confirmed the interaction of β-catenin and Tcf-1 in T cells, demonstrating that Tcf-1 also interacts with β-catenin to generate a bipartite transcription factor complex.

A central event in the Wingless/Wnt signaling pathway is the inhibition of GSK-3β activity (20). Primary T lymphocytes reportedly down-regulate GSK-3β activity in response to PMA/ionomycin (12). We therefore tested whether GSK-3β activity was modulated upon activation of Jurkat T cells. Treatment of Jurkat T cells with PHA or lithium induces a rapid inactivation of GSK-3β, but, unexpectedly, does not result in increased Tcf-mediated transcription. In C57MG cells, which contain the Tcf/LeF member Tcf-4, lithium treatment inhibits GSK-3β activity and strongly increases Tcf transcription. We therefore conclude that in T cells, inhibition of GSK-3β is not sufficient to activate Tcf-mediated transcription. Given the fact that a S33 β-catenin mutant strongly activates transcription, another kinase might be involved, either alone or in concert with GSK-3β. Our findings are in agreement with concerns raised by other investigators (21) about GSK-3β being the sole negative regulatory kinase involved in Wnt signaling.

It is of course possible that an even stronger inhibition of GSK-3β activity in Jurkat is needed to activate Tcf-dependent transcription. Nevertheless, the fact that a ~50% inhibition (PHA or PMA) of GSK-3β in Jurkat does not elicit a response, while a 20–40% (lithium) inhibition does so in C57MG, already shows that Tcf-controlled transcription is differentially regulated by GSK-3β in Jurkat versus C57MG. In many other systems a modest reduction (~30%) of GSK-3β activity is sufficient to elicit effects, e.g. in insulin, epidermal growth factor and nerve growth factor signaling (24,25).

In C57MG cells inhibition of GSK-3β is apparently sufficient to activate transcription, although it may be that other kinases are involved as well. Lithium can inhibit GSK-3β in two ways, either indirectly by affecting inositol phosphate metabolism subsequently inhibiting GSK-3β or directly affecting GSK-3β kinase activity (14,15,22). Whatever the exact mechanism, it is clear that in C57MG cells lithium can activate Tcf-dependent transcription and therefore expression of target genes. To our knowledge this is the first demonstration that lithium can affect TCF/LEF target gene expression. Treatment with lithium may thus be teratogenic and/or oncogenic, given that (inappropriate) Wnt signaling is involved in differentiation processes and in the development of colon carcinomas (6). Our findings indicate that GSK-3β regulates Tcf/β-catenin signaling differentially in fibroblasts versus lymphocytes and suggest a difference in Wnt signaling between these two cell types.

The studies we report here are the first investigations into the signal transduction pathways that activate Tcf-mediated transcription in T cells. It is clear that TCR–CD3 signaling does not activate Tcf-1 transcription, unlike known transcription factors controlling the IL-2 enhancer. Studies with the IL-7-responsive cell line CT6 and the SCF–responsive line M07 indicate that these cytokines, which are important for thymocyte development, are not involved in Tcf-dependent signaling either (data not shown). The phenotype of Tcf-1 mutant mice indicates that both the DN2 (CD25+CD44+) CD4+ and CD8+ ISP cells are not in cycle (Schilham and Clevers, submitted, and 11). Thus, signaling pathways involved in expansion of early progenitors are likely candidates for activation of Tcf-1 transcription. Initial studies show that members of the Frizzled family, which are receptors for Wnt factors, can be cloned from a thymus cDNA library (Staal and Clevers, unpublished). Thus Wnt factors interacting with Frizzled may compose the first step of a signaling cascade that culminates in activation of Tcf-1 target genes in thymocytes.

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Abbreviations

GSK-3β glycogen synthase kinase-3β
PHA phytohemaggulutinin
PMA phorbol myristate acetate
Tcf-1 T cell factor-1

References

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