A new look for 1999

The year 2000 is very close, and there will probably be a lot of changes to coincide with the new Millenium. So we've decided to get in there first and introduce a new design for TIG in 1999! One of the most important aspects of the redesign is that we are including article titles in all reference lists – many readers have expressed a desire for this. In addition, we have renamed some of the article types, and increased the number of words per page. Finally, we have organized the content into three main sections. As ever, the core of each issue is the Reviews and Perspectives section. The Outlook section contains Comment articles (short articles about new trends or recent publications), Meeting Reports, Letters, Journal Club (previously called Monitor), and, new for 1999, Genome Analysis (see recent announcements in TIG). The third section is the Resource section: Internet (previously called Genetwork), Book, CD-ROM, Software, Journal Reviews, Knockout Update (every 3–4 months, previously called It's a Knockout), and Product News (every 3–4 months, and another new feature in 1999). Thanks are due to Craig Santus, Naomi Wright, and John Aspinall (and his colleagues at Mouse in the House) for their contribution to the redesign. We hope you enjoy our new look, and would welcome any feedback. **Happy 1999!**

WNT targets
repression and activation

Several puzzling observations made previously suggested that target genes that are activated by WNT signaling during development were actively repressed in the absence of the signal. Recent work sheds light on how this switch between repression and activation is regulated.

Cells within multi-cellular organisms, at any one point in time, leave the large majority of genes in a silent state, by either actively repressing transcription or withholding positive transcription factors. Repression works by several mechanisms: global repressors, such as the *Drosophila Polycomb* gene products, keep large groups of genes repressed, presumably by interacting with chromatin components[1]; gene-specific repressors bind to regulatory sequences and repress gene expression by interacting with components of the transcriptional machinery.

When cells enter a developmental program and start to express specific genes, repression is relieved. At a global level, chromatin undergoes reconfiguration, including changes in composition and histone acetylation[2]. Gene-specific repressors have to compete with activators, which bind to adjacent regions in the DNA and displace repressors from their binding sites.

In addition, there are several interesting cases of repressors that can themselves turn into activators. One example is the thyroid hormone receptor, which binds to the DNA in a complex containing proteins interfering with transcription. When its ligand, thyroid hormone, enters cells and binds the receptor, it becomes an activator of transcription by exchanging its previous binding partners for transcriptional activators[3]. Such a mechanism is economical: it allows for simultaneous changes in whole sets of genes (often seen during development), and is an optimal design to change gene expression from an off- to on-state. Such switches underlie much of pattern formation and cell differentiation in animal development.

The two faces of TCF

An interesting new variation on the theme of repressors turning into activators is presented in three papers recently published in *Nature*[4–6]. Collectively, these reports show that TCF (a DNA-binding protein) can repress target genes as well as activate those same target genes in cells instructed to change developmental fate. As shown in these papers, there are several mechanisms by which this switch is achieved.

TCFs are HMG box-containing DNA-binding proteins[7]. A couple of years ago, several groups discovered that TCFs (also called *pangolin* in *Drosophila*) mediate signaling by WNT proteins, a family of highly conserved secreted signaling molecules that regulate cell–cell interactions during developmental decisions[8–13]. Insight into the mechanisms of WNT signal transduction has emerged from several systems: genetics in *Drosophila* and *Caenorhabditis elegans*; biochemistry in cell culture; and ectopic gene expression in *Xenopus* embryos[14,15]. As currently understood, WNT proteins bind to receptors of the Frizzled family on the cell surface. Through several cytoplasmic relay components, the signal is transduced to β-catenin (Armadillo in *Drosophila*), which then enters the nucleus and forms a complex with TCF to activate transcription of WNT target genes (Fig. 1).

Several direct targets of WNT signaling are currently known, including *Siamois* in *Xenopus* and *Ubx*, a target of *wingless* (*wg*) in *Drosophila*[11,16]. *Siamois* and *Ubx* have TCF-binding sites in their regulatory sequences. Based on these binding sites, several reporter constructs have been devised that are activated in cell culture assays or in transgenic animals by WNT–β-catenin signaling components. Reporters with normal TCF binding sites are expressed in tissues where WNT is active (*Siamois* in dorsal cells of the *Xenopus* embryo; *Ubx* in the fly embryo's midgut) and reporters with mutant TCF sites are, as expected, downregulated. Surprisingly, however, those mutant reporters are

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ectopically expressed elsewhere in the animals, suggesting that they escape from some repressive influence[11,16].

Gene-dosage interactions between TCF and other WNT-signaling components in *Drosophila* provide additional evidence for a repressive function of TCF. In fly embryos carrying mutations in *wg* or in *armadillo* (*arm*), reduction of TCF suppresses the phenotype: *arm* mutant animals that are also heterozygous for a *TCF* mutation look better than *arm* mutants with normal *TCF* function[6]. This is unexpected if TCF is a positive component of the pathway, because removing its function should enhance the wg or arm phenotype (i.e. make it worse, see Fig. 1).

Biochemical interactions

At the same time as these genetic interactions between TCF and other components of WNT signaling were recognized, several groups found that TCF interacts biochemically with proteins that could mediate repression[4,17]. One such a repressor is the Groucho protein in *Drosophila* (called TLE in vertebrates). Groucho can interact with a variety of DNA-binding proteins, including HLH proteins, such as Hairy in *Drosophila*, and usually confers a repressive effect on the transcription of adjacent genes[18]. Groucho does the same to TCF: it binds to a defined domain on TCF (Fig. 2) and reporter constructs become silenced when Groucho/TLE is co-expressed with TCF, even in the presence of Armadillo/β-catenin[4]. As expected for a repressor, genetic studies in *Drosophila* show that removing *Groucho* suppresses phenotypes of *wg* and *arm*, just like reducing *TCF* suppresses the wg phenotype.

There is yet another protein that interacts with TCF: the *Drosophila* homolog of CBP (Ref. 5; CBP stands for Creb-binding protein). CBP shows up in many transcriptional complexes, and has been implicated in transcriptional activation rather than repression[19]. To some extent, it is

FIGURE 1. WNT signaling
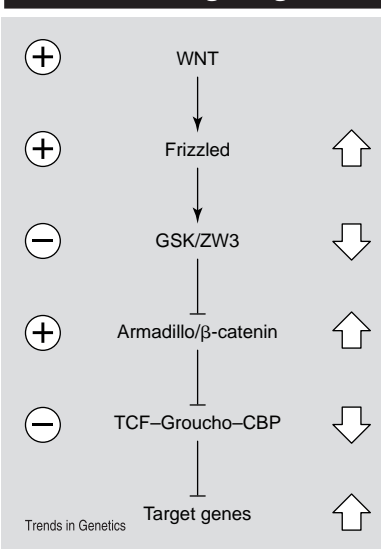
Positive and negative interactions during WNT signaling, simplified in a linear pathway. These are formal genetic interactions that by no means imply similar biochemical interactions. WNT, Frizzled and Armadillo are positive (+) components, activated during signaling (up-arrows). GSK/ZW3 and TCF–Groucho–CBP are negative components (−), that are inhibited during signaling (down-arrows). Most interactions between these components are, in fact, inhibitory. When positive factors, such as WNT and Armadillo are reduced, target genes are downregulated. Further reduction of a negative factor suppresses the phenotype, such that target genes are somewhat upregulated. Complete removal of negative factors (such as GSK/ZW3) leads to strong upregulation of the genes downstream. Further complications arise when the pathway is not linear, that is, when components act in parallel.

FIGURE 2. Domains and binding sites on TCF

The N-terminus of TCF can bind to Armadillo (ARM) but this domain contains a lysine residue (K25) that can be acetylated by CBP, which might interfere with binding (or vice versa). TCF contains a separate binding domain for Groucho, at least in the linear sequence. The HMG box in TCF is important for binding to DNA and to CBP.

understood how CBP works: the protein has a histone acetylation enzymatic activity. In fact, CBP is among those proteins associated with steroid hormone receptors, turning them from repressors into activators, presumably also by acetylating histones and reconfiguring chromatin at the transcription initiation site[20]. CBP is found as a transcriptional co-activator of several other *Drosophila* transcription factors, including Dorsal and CiD, the latter mediating Hedgehog signaling[21,22].

It is, therefore, surprising to find that CBP represses transcription, but the evidence is nonetheless compelling. Mutations in the *Drosophila CBP* gene, called *nejire* (Ref. 19), suppress wg phenotypes in various tissues, which, as argued above, is the expected outcome for a repressor. In addition, Waltzer and Bienz[5] provide solid biochemical evidence for the CBP–TCF interactions. CBP acetylates a particular lysine residue (K25) in the N-terminus of TCF, in a domain of TCF that interacts with Armadillo (Fig. 2). Binding of the Armadillo protein to TCF has the effect that TCF becomes a worse substrate for CBP. In this way, Armadillo antagonizes CBP. It is also possible that the acetylated form of TCF binds less efficiently to Armadillo, although the reported difference (2–3-fold) does not seem to be dramatic[5].

It is not clear yet how Groucho interferes with TCF and Armadillo. Does Armadillo replace Groucho from binding to TCF? As mapped by deletion analysis, the binding sites for Armadillo and Groucho on TCF do not overlap, but it is still possible that TCF in its native state cannot simultaneously bind to these two proteins. An experiment reported by Roose *et al.*[4] suggests however that Groucho interferes with TCF even when Armadillo is present. A chimaeric TCF protein to which a domain of Armadillo is fused is a very potent activator of TCF target genes, and this fusion protein is also inhibited by Groucho. The C-terminus of Groucho, which contains a WD-40 motif, is essential for inhibition. Interestingly, there are natural variant forms of vertebrate genes encoding Groucho that lack this motif and that have the opposite effect to full-length proteins: they stimulate expression of target genes[4].

Perspectives

Collectively, most experimental data support the view that TCF is a repressor when WNT does not convert it into an activator. One bit of evidence is still missing: the absolute null phenotype of TCF in *Drosophila* is not known yet. The gene resides on a chromosome (the fourth) that is difficult to manipulate, which makes it cumbersome to generate embryos totally devoid of maternal gene product. It is also difficult to make mosaic animals with clones of cells
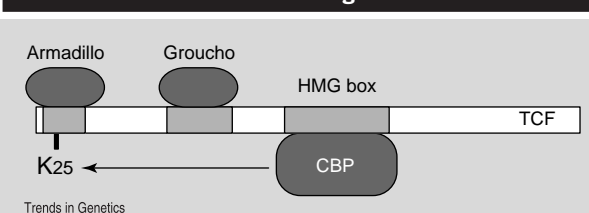
mutant for *TCF*. The genetic tests discussed above are based mostly on dosage interactions, and in the absence of the null phenotype of *TCF* mutations, they should be interpreted with some caution. On the other hand, there are *TCF* mutations in *C. elegans*, in a gene called *pop-1* (Ref. 23). In line with the repressor model, the *pop-1* phenotype is the opposite of the *mom* genes, which are components of WNT signaling in the worm.

The mechanisms by which TCF represses or activates target genes are not only important for understanding pattern formation during development, but also for cancer. The WNT pathway has repeatedly been implicated in tumorigenesis, in mouse mammary cancer and in human colon carcinomas, for example. As recently shown,

the well-known *MYC* oncogene is among the target genes activated in human colon cancer[24]. *MYC* has TCF-binding sites in its promoter, probably subject to the same kinds of regulations as described in other settings. While this observation might suggest that removing TCF would activate *MYC*, and that TCF would therefore be a tumor-suppressor gene product, the mouse knockout phenotype of Tcf4, a gene expressed in colon epithelial cells, points to a more complicated relationship. These animals have an underdeveloped colon rather than tumors[25] and Tcf4 seems to be actually required to maintain the colon stem-cell population. All in all, these are reasons enough to study these intriguing molecules and signaling events in more detail.

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TGFβ inhibitors

new and unexpected requirements in vertebrate development

Analysis of embryonic induction has pointed to the importance of the antagonistic roles played by secreted inducing factors and their soluble inhibitory binding proteins. These interactions have been particularly well characterized in patterning the primary axes of insects and vertebrates. New results implicate similar antagonistic relationships in numerous later events of embryogenesis.

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Pattern formation in animals relies extensively on inductive interactions in which one cell, or group of cells, secretes or displays factors that act upon neighboring cells to change their developmental fate. In the simplest sense, such an inductive pathway need only consist of a ligand produced in the inducing cells and a receptor/transduction mechanism in the induced cell. However given the intricacy and fidelity of ani-

mal development, it is not surprising that many inductive pathways have multiple additional levels of regulation.

For the BMP and activin subgroups of the TGFβ family of ligands, as well as the WNT family of signaling molecules[1], an additional level of control is provided by high-affinity secreted binding proteins. These secreted proteins prevent receptor activation by binding ligand. Many