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A Versatile Transcriptional Effector of Wingless Signaling

Minireview

Roel Nusse
Howard Hughes Medical Institute
Department of Developmental Biology
Beckman Center
Stanford University, Medical Center
Stanford, California 94305-5428

When an extracellular signal determines the fate of a cell, the signal is usually transduced to the nucleus, changing gene expression. In the case of *Wingless* (*wg*) signaling in Drosophila (or the related Wnt proteins in other animals), a transcriptional effector of the signaling pathway has been missing. As far as was known, the most downstream component of Wg signaling was the *armadillo* (*arm*) gene product. The Arm protein is similar to β-catenin and plakoglobin in vertebrates, proteins which bind to E-cadherin and link adhesion complexes to the cytoskeleton. Arm was therefore thought to function in cell adhesion rather than in relaying a signal to the nucleus, but recent evidence supports the unorthodox view that this protein can also act as an activator of transcription, revealing an unexpected versatility. Mutant studies in Drosophila now provide a firm genetic basis for a productive interaction between Arm and DNA binding proteins of the Lef-1/TCF family, generating an active transcriptional complex. This short review will summarize these findings, for simplicity mostly using the following nomenclature: Arm for any β-catenin or Armadillo protein; TCF for any Lef-1, TCF, or Pangolin; and Zw-3 for any zeste white 3 or glycogen synthase kinase 3β (GSK-3β).

wg *Signals through* arm; *Arm Binds to TCFs*

wg and *arm* are both members of the segment polarity class of mutations in Drosophila embryogenesis, sharing many phenotypic changes when mutated (Nüsslein-Volhard and Wieschaus, 1980). The best characterized phenotype is the larval cuticle, where absence of *wg* and *arm* causes a lawn of disoriented denticles, instead of neatly organized rows. Both genes are also required for the correct expression of a large set of *wg* target genes, including *engrailed* in the ectoderm and other homeobox genes in the endoderm and mesoderm (reviewed in Klingensmith and Nusse, 1994). There are nevertheless important differences between *wg* and *arm*: strong *arm* mutations can block cell development altogether, including oogenesis. Only a special class of *arm* mutations, disrupting the carboxyl terminus of the protein but leaving a set of internal repeats intact (Figure 1), causes a segment polarity phenotype similar to *wg* (Orsulic and Peifer, 1996). Adhesion between cells appears normal in these mutants, suggesting additional functions of *arm*. Epistasis experiments show that absence of *arm* blocks Wg signaling to nuclear endpoints, such as *engrailed* (Noordermeer et al., 1994).

Arm is in itself not a DNA binding protein and is, under normal circumstances, not found in the nucleus. The missing link between Arm and its possible transcriptional role proves to be a DNA binding protein related to the mammalian Lef-1 or TCF proteins. These proteins contain an HMG domain and were found originally as enhancer binding factors for T cell–specific genes (Clevers and Grosschedl, 1996). Lef-1 and TCF-1 are encoded by distinct genes in mammals (and have different mutant phenotypes when knocked out in the mouse) but bind to similar DNA elements. They are related to the sex-determining factor Sry and are highly conserved in evolution, as testified by a family member in C. elegans, *pop-1* (Lin et al., 1995). Binding of TCFs to DNA results in bending of the helix (Giese et al., 1992), but by themselves, these proteins are poor transcriptional activators. Last year, several groups reported that yeast two-hybrid screens, using either Arm or TCFs as baits, revealed interactions between these proteins (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996). Moreover, complexes between the TCF and Arm were found to act as potent transcriptional activators of reporter gene constructs containing the DNA element recognized by TCF (Figure 1). Fragments of Arm that are active in the transcription assays map to the carboxyl terminus, the same domain mutated in *arm* alleles that give the segment polarity phenotype similar to *wg*. A biological consequence of this transcriptional effect was sought and found in Xenopus embryos: overexpression of Lef-1 causes an axis duplication, and a dominant negative form of TCF-1 was able to block the formation of the primary axis (Behrens et al., 1996; Huber et al., 1996; Molenaar et al., 1996), phenotypes that are generated by other Wnt signaling components as well (reviewed in Miller and Moon, 1996).

Drosophila TCF Mutants and Transcriptional Targets

In spite of these impressive data, a genetic demonstration that TCF is required for *wg* signaling was lacking. This void is now filled by several recent papers. van de Wetering et al. (1997) started by cloning a Drosophila homolog of *TCF* and subsequently found several mutant alleles. The gene maps on the 4th chromosome. Brunner et al. (1997) performed a genetic screen for modifiers of *wg* signaling, using a dosage-sensitive eye phenotype of *wg* as a starting point. Guessing that one complementation group of suppressors, which they named *pangolin*, could be allelic to TCF, they cloned the Drosophila homolog and found specific mutations in the suppressor mutants. Both groups show binding between Arm and TCF and demonstrate that TCF is genetically downstream of Arm: a constitutively active form of Arm, lacking part of the amino terminus (Figure 1), has no effect in the absence of TCF. Embryos homozygous mutant for TCF display a segment polarity phenotype, similar to weak *wg* and *arm* alleles. Most likely, there is maternal contribution to the TCF pool in the embryo, and one would therefore assume that stronger phenotypes can be uncovered by eliminating both the maternal and zygotic gene activities. However, such experiments are difficult to accomplish for 4th chromosome mutants, and likewise, it is difficult to generate well-marked clones of mutant cells to examine for any later requirement for gene function. This problem is somewhat circumvented

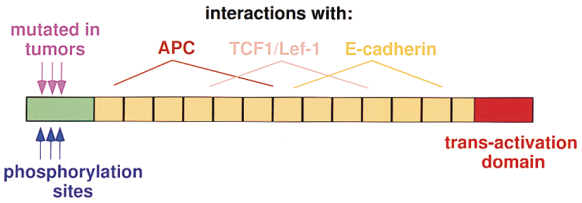

Figure 1. Different Modules on the Armadillo/β-catenin Protein

The middle domain contains 13 imperfect repeats that in different combinations can interact with APC, TCF/Lef1, or E-cadherin. The unique amino-terminal domain can be phosphorylated, which is correlated with down-regulation of Arm. Those residues are sometimes found to be mutated in colon carcinoma or melanoma cell lines. The carboxyl end contains a transcriptional activator domain and is specifically mutated in *arm* alleles affecting Wg signaling.

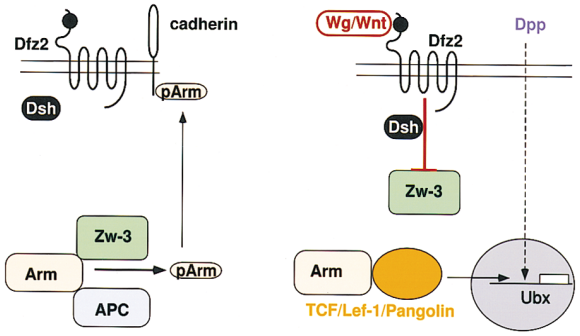

Figure 2. Current Model of the Wg/Wnt Signaling Pathway

In cells not exposed to the *wg* signal (left), Arm levels are kept low through interactions with the protein kinase Zw-3 (GSK-3β) and, as shown in mammalian cells, APC. Most Arm is part of the adhesion complex by binding to E-cadherin. The *wg*/*Wnt* signal (right) leads, through its receptor (Dfz2 or other members of the frizzled family) and *dishevelled* (Dsh), to inactivation of Zw-3. As a consequence, Arm increases in concentration and can bind to TCF1/Lef-1/pangolin. This complex can activate nuclear target genes, including *Ubx*, but *Ubx* expression needs input from *dpp* signaling as well. Loss of APC in mammalian cells can also lead to a critical loss over Arm control, leading to cell transformation, most likely by activation of other target genes.

by the identification of a temperature-sensitive allele of TCF by Brunner et al. (1997). Interestingly, all TCF phenotypes, whether in embryos or in adults, are similar to known *wg* phenotypes, which may imply that TCF is dedicated to the *wg* signal only. This also suggests that TCF, when not used by the *wg* signal, has no other activity; one can imagine that it can act as a repressor of gene expression if not complexed to an activator, but apparently, this is not the case. One should realize, however, that none of these genetic tests have been done in the total absence of TCF function.

These mutant phenotypes show that TCF is in the *wg* pathway. But are any of the *wg* target genes directly activated by the Arm–TCF complex? *wg* controls the expression of many different genes, in a tissue-specific manner, but very few of these target genes and their promoters have been analyzed in sufficient detail. One notable exception is the detailed and long-standing analysis of the function of *Ubx*, a homeobox gene, in the fly midgut by Bienz and colleagues (Bienz, 1994). They had found previously that expression of Ubx in mesodermal cells lining the Drosophila gut is controlled by *wg* and *dpp*, in an intricate way involving autoregulation mediated by these secreted signals. Both *wg* and *dpp* are required for Ubx expression (Figure 2). By fine mapping a control element on the *Ubx* promoter, Riese et al. (1997) have now found a *wg*-responsive element (WRE) adjacent to a *dpp*-responsive element (DRE, a CREB-responsive element). The WRE contains a perfect TCF binding site and can be activated by ectopic expression of a mouse TCF (Lef-1 was used here). In vivo tests for activation of reporter constructs point to a requirement for the dpp element to be immediately adjacent; the WRE by itself is not sufficient for activation by TCF. The requirement for close proximity of the dpp-responsive element and the TCF binding site is reminiscent of earlier work showing that TCFs play an architectural role in activating gene expression. By affecting DNA bending, TCFs may bring together several transcription factors (Giese et al., 1992). Obviously, a combinatorial mechanism of target gene activation—through adjacent enhancers that are both required—provides a simple explanation for the tissue specificity for *wg* or *dpp* signaling. There are many other examples where these two signals cooperate, and in cases where *wg* and *dpp* are not acting together, *wg* may team up with a different signal to elicit yet another response. The specific properties of TCF as a DNA bending protein make it ideally suitable to perform such an integrating function, and it may do so by using Arm as an element in the reconfiguration of transcriptional complexes rather than as a direct activator.

There are of course many questions remaining. What are the transcriptional activators used by TCFs in other cells, including the mammalian lymphocytes where these proteins were originally found? How is Arm directed to its different partners, E-cadherin, or TCF, or yet others? And how does Arm enter the nucleus? It is a fairly big protein (110 kDa) but it lacks a nuclear localization signal. Possibly, cytoplasmic Arm binds to newly synthesized TCF and is carried on to the nucleus. One particular experiment in Drosophila by Riese et al. (1997) gives a hint in this direction: overexpression of Lef-1 causes phenotypes similar to ectopic Wg effects and leads to ectopic expression of a reporter construct containing the Lef-1 binding site. Importantly, these effects are independent of wild-type *wg* function (removing *wg* genetically has no consequence). This may argue that an excess of newly made TCF, while still cytoplasmic, picks up Arm, prevents it from degradation and carries it on to the nucleus to activate gene expression (Figure 2).

During Wg/Wnt signaling, the critical step in activating Arm to become a transcription factor may merely be an increase in cytoplasmic concentration of free Arm molecules. This signal from the Wg receptor is thought to be relayed by the protein kinase Zw-3 (the Drosophila homolog of GSK-3β; Figure 2) (Siegfried et al., 1994). In cells not exposed to a Wnt signal, the Zw-3 kinase is active and may phosphorylate Arm directly. The amino terminus of Arm contains several Zw-3 substrate sites that can become phosphorylated. Eliminating these sites stabilizes Arm sufficiently to activate it (Figure 1).

Based mostly on genetic interactions, but also on biochemical data, the *wg* signal would inactivate Zw-3, leading to an increase in Arm which would then be available for binding to TCF (Figure 2).

Wg Signaling Components in Human Cancer

As found in mammalian cells, the interaction between Zw-3 and Arm is actually more complex. There is yet another player in regulating Arm, the tumor suppressor gene *APC*. The APC protein binds to Arm and accelerates Arm turnover. APC also binds to Zw-3 and is in fact a better substrate for Zw-3 than Arm. A complex between these three proteins may keep Arm levels low (Figure 2) (Rubinfeld et al., 1996). APC, E-cadherin, and TCF can all bind to the middle domain of Arm, the part that contains a stretch of 13-fold repeated sequences, but each partner binds to a specific subset of these repeats (Figure 1). Possibly, there is some competition for binding, and binding by one or the other partners is subject to some regulation.

APC traces its history to tumors found in familial colon carcinomas; it is a tumor suppressor gene that is deleted in tumors or in germ line DNA. The findings on interactions between Arm and TCF have also led to a much better understanding of the mechanism of tumorigenesis by loss of APC. Korinek et al. (1997) show that several colon carcinoma lines, derived from tumors with APC deletions, contain a complex between one of the human TCF homologs (hTCF-4) and Arm. This complex activates expression of reporter constructs, indicating that the loss of APC function in these cells releases enough Arm to team up with TCF to become a transcriptional activator. As predicted from the model in which APC is a gatekeeper of Arm, transfection of full-length APC into those cells inhibits expression of the reporter gene constructs. Mutant forms of APC, when transfected, are incapable of blocking target gene expression.

These experiments provide a plausible explanation of how loss of APC can activate gene expression and lead to cell transformation (although no target genes in oncogenesis are known). A surprise came when Morin et al. (1997) found several colon carcinoma cell lines with normal levels of wild-type APC but nevertheless displaying strong expression of these reporter constructs). It appeared that Arm in those cells had found another way to escape from APC control: by mutations in its own gene. These mutations are also present in several melanoma (Robbins et al., 1996; Rubinfeld et al., 1997) and are of a special class: they eliminate specific amino acids in the amino-terminal domain of the protein, in particular residues that become phosphorylated prior to the proteolytic down-regulation of Arm (Figure 1). Hence, Arm can turn into an oncogene in its own right. A question that remains, however, is whether TCF is required for cell transformation by loss of APC or mutations in Arm. A testable prediction of the models emerging from these papers is that a dominant negative TCF should revert the transformed phenotype.

It is now fairly common to find signal transduction pathways conserved in evolution or to find components in these critical growth-controlling mechanisms mutated in tumors. Nonetheless, the discoveries summarized here form yet another spectacular and exciting example of these principles. Yet, one piece is missing: a genetic function of *APC* in Drosophila. A fly *APC* homolog was recently identified, but based on its expression and a preliminary mutant analysis, *APC* does not participate in Wg signaling (Hayashi et al., 1997). This assumes that the gene is not redundant or maternally provided, but it points to interesting alternative pathways. Further genetic tests of this signaling system in cancer cells, flies, and worms, combined with cell biological experiments, will undoubtedly add more to this rapidly developing story.

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