# Pygopus and the Wnt signaling pathway: a diverse set of connections

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**Summary**

**Identification of Pygopus in *Drosophila* as a dedicated component of the Wg (fly homolog of mammalian Wnt) signaling cascade initiated many inquiries into the mechanism of its function. Surprisingly, the nearly exclusive role for Pygopus in Wg signal transduction in flies is not seen in mice, where Pygopus appears to have both Wnt-related and Wnt-independent functions. This review addresses the initial findings of Pygopus as a Wg/Wnt co-activator in light of recent data from both fly and mammalian studies. We compare and contrast the developmental phenotypes of *pygopus* mutants to those characterized for known Wg/Wnt transducers and explore the data regarding a role for mammalian Pygopus 2 in tumorigenesis. We further analyze the roles of the two conserved domains of Pygopus proteins in transcription, and propose a model for the molecular mechanism of Pygopus function in both Wg/Wnt signaling and Wnt-independent transcriptional regulation.**
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Abbreviations: APC, adenomatous polyposis coli; Arm, Armadillo; BPTF, bromodomain and PHD finger transcription factor; Brg1, brahma related gene 1; CBP, CREB binding protein; Fzd, Frizzled; GSK3β, glycogen synthase kinase 3β; HAT, histone acetyltransferase, HDAC, histone deacetylase; HMT, histone methyltransferase; H3K4me3, histone 3 trimethylated at lysine (K) 4; ING2, inhibitor of growth 2; LEF/TCF, lymphocyte enhancer factor/T cell factor; Lgs/BCL9, legless/B-cell CLL/lymphoma 9; MED12, Mediator subunit 12; MLL/SET1, mixed lineage leukemia/Su(var) enhancer of zeste trithorax 1; NHD, N-terminal homology domain; PHD, plant homeodomain; Pygo, Pygopus; TBP, TATA binding protein; TRRAP, transformation transcription domain associated protein; Wg, Wingless.

## Introduction

During development, canonical Wingless (Wg)/Wnt signaling functions in critical processes such as body axis patterning and tissue morphogenesis. In adults, the Wg/Wnt pathway maintains homeostasis in multiple tissues and regulates stem cell proliferation.[1] Heritable and somatic mutations in genes encoding components of the Wnt cascade are implicated in human diseases, including cancer.[2,3] Understanding the molecular effectors of this pathway is thus crucial to the development of effective therapeutic strategies.

Canonical Wg/Wnt signaling initiates when Wg/Wnt ligands bind Frizzled (Fzd)/LRP receptors.[4] Ligand binding triggers a series of intracellular events that lead to inhibition of the cytoplasmic β-catenin [or its fly homolog, Armadillo (Arm)] destruction complex, which consists in part of adenomatous polyposis coli (APC), Axin and glycogen synthase kinase 3β (GSK3β). In the absence of Wg/Wnt, this complex decreases cytosolic levels of Arm/β-catenin (referred to as β-catenin from here on) by phosphorylation, which earmarks β-catenin for ubiquitination and proteolytic degradation. Inhibition of the destruction complex following Wg/Wnt ligand binding enables accumulation of β-catenin in the cytoplasm. Stabilized β-catenin translocates to the nucleus and interacts with lymphocyte enhancer factor/Tcell factor (LEF/TCF) transcription factors as well as numerous co-factors to elicit changes in gene expression (Fig. 1).[5]

Pygopus (Pygo) proteins, the prototype of which was identified in *Drosophila* several years ago, have emerged as co-factors of special interest because of their apparently specific and devoted role in Wg signaling and the potential insights that they may offer into the molecular mechanism of β-catenin transcriptional activation. Chromatin regulation is a key aspect of transcriptional control, and Pygo proteins contain evolutionarily conserved PHD fingers that are often found in proteins with a chromatin remodeling function. Here, we review literature on nuclear co-factors of β-catenin, specifically highlighting the Wnt-dependent and Wnt-independent involvement of Pygo proteins, and propose chromatin function to be the underlying mechanism that unifies the diverse array of Pygo-Wnt connections reported in the literature.

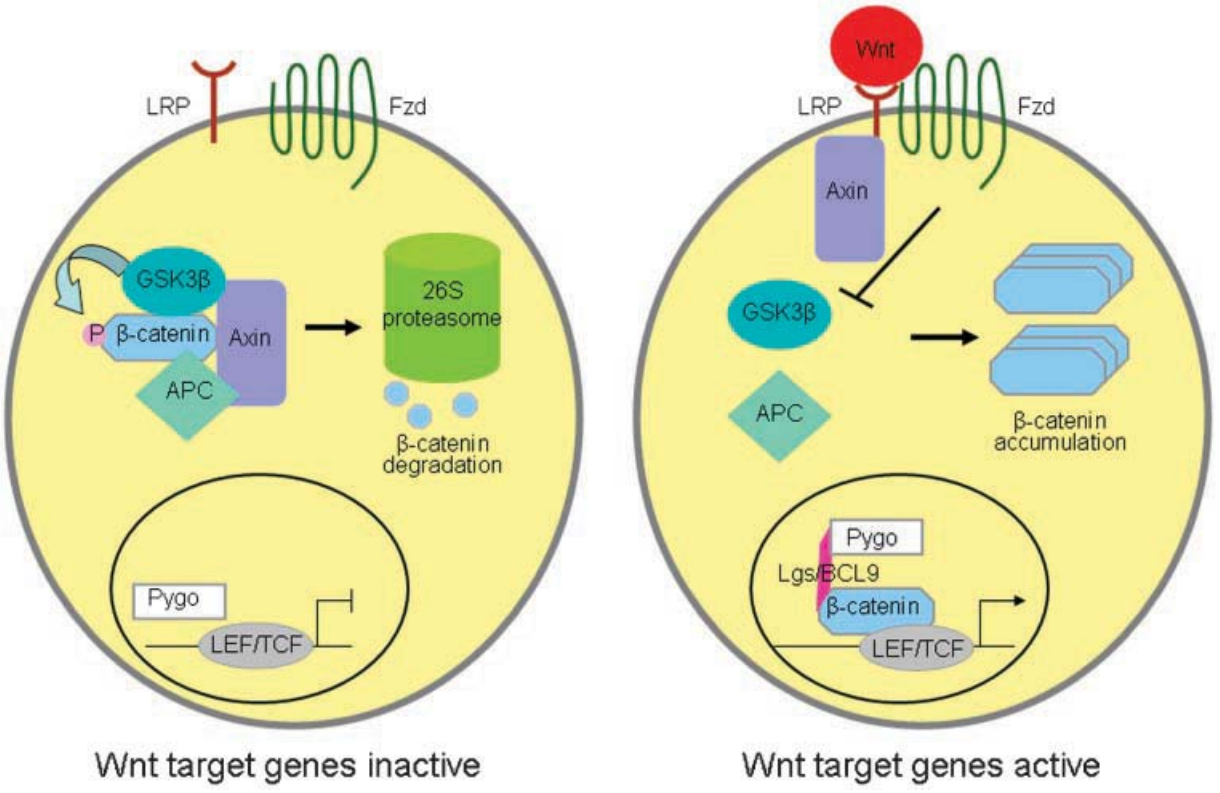


**Figure 1.** Schematic of canonical Wg/Wnt signaling. When Wg/Wnt ligands are not present, a complex of Axin, APC and the serine-threonine kinase GSK3β binds and phosphorylates cytoplasmic β-catenin. This triggers ubiquitination by an E3 ligase complex containing β-TrCP, resulting in proteolytic degradation of β-catenin. Binding of Wg/Wnt ligands inactivates the phosphorylation of β-catenin, enabling its accumulation. Through largely unknown mechanisms, β-catenin then translocates to the nucleus and binds to LEF/TCF transcription factors to regulate gene expression. Pygo and Lgs/BCL9 are recently identified nuclear co-factors of β-catenin.

### β-catenin associates with numerous positive and negative regulators of transcription

Nuclear β-catenin acts as a scaffold with distinct regions to which co-factors bind, either in tandem or in a concerted manner, and alter transcription at Wnt target promoters [Fig. 2 is a nucleus-centric, updated modification of the diagram from an earlier review on the topic;[5] readers are referred to this review for additional discussions]. The C-terminal half of β-catenin generally functions to connect β-catenin to the basal transcription machinery via associations with such proteins as TATA-binding protein (TBP), Mediator subunit 12 (MED12), and Parafibromin/Hyrax, as well as to chromatin remodeling activities as exemplified by associations with CREB-binding protein (CBP), p300, brahma related gene 1 (Brg1, a component of the SWI/SNF complex), the imitation switch chromatin remodeling ATPase ISW1, transformation transcription domain associated protein (TRRAP), and mixed lineage leukemia/Su(var) enhancer of zeste trithorax 1 (MLL/SET1). Proteins known to bind the N-terminal region of β-catenin include Pontin, Reptin, Legless [Lgs, fly homolog of the mammalian B-cell CLL/lymphoma 9 (BCL9) protein] and, indirectly via its interaction with Lgs/BCL9, the plant homeodomain (PHD) finger protein Pygopus.

In addition to LEF/TCF, β-catenin has recently been shown to also serve as a co-activator for other transcription factors such as homeodomain protein Prop1[6] and nuclear hormone receptors.[7–9] Moreover, components of nuclear hormone receptor coactivator complexes such as GAC63 have been shown to enhance LEF/TCF-dependent activation,[8–13] expanding the repertoire of β-catenin/LEF-associated co-activators. The myriad proteins shown to interact with β-catenin demonstrate both the need for exquisite physiological regulation of transcription of Wnt/β-catenin target genes, and the possible context-specificity by which such regulation occurs.

It is widely acknowledged that LEF/TCF proteins interact with co-repressors such as Groucho to keep Wnt target genes silent in the absence of an active signal, and that activation of the Wnt signaling cascade results in displacement of such co-repressors by β-catenin.[14] Recent evidence has also rendered legitimacy to the notion that β-catenin itself can

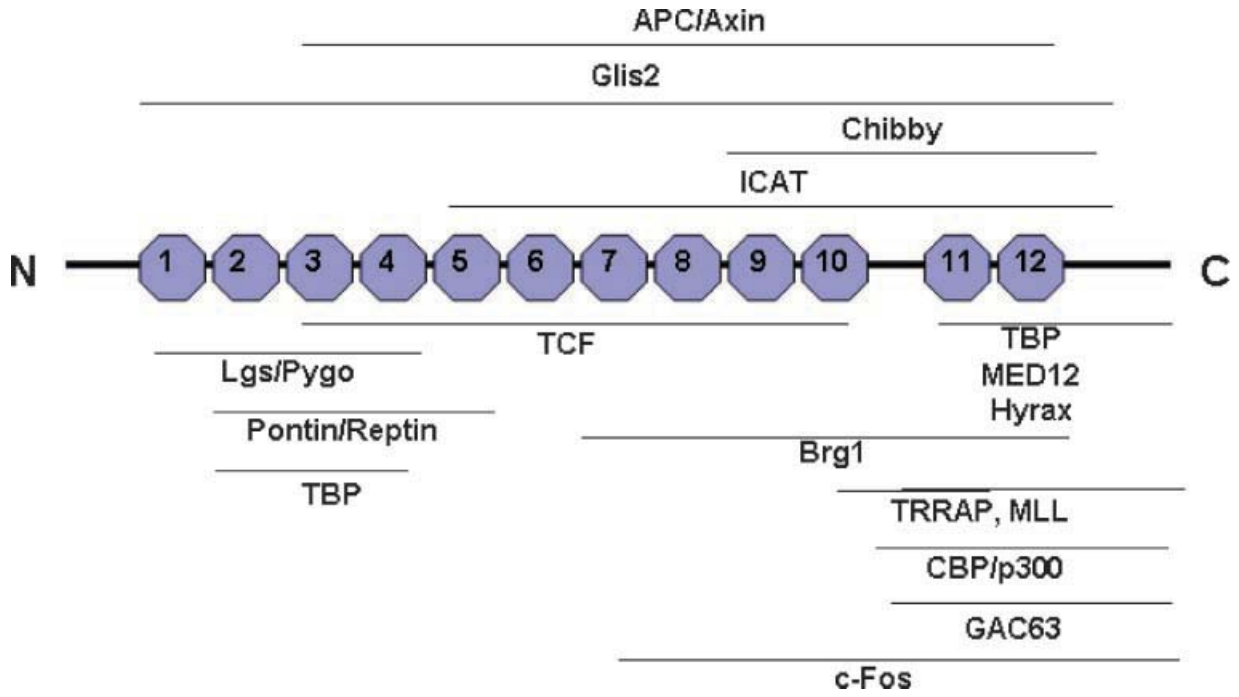


**Figure 2.** Overview of nuclear β-catenin-associating co-factors. Through its armadillo repeat domains, β-catenin acts as a scaffold for binding of co-factors to assemble a transcriptional activation complex at Wg/Wnt target promoters. Horizontal bars indicate the regions of β-catenin to which the various co-factors bind (Adapted from Ref. 5).

interact with co-repressors and transcriptional inhibitors including ICAT, Chibby, Glis2, TIS7 and, possibly, Brinker.[15–22] Interestingly, competition or coordinated binding between co-activators and co-repressors to the β-catenin/LEF complex, resulting in the recruitment of opposite chromatin remodeling activities [histone acetyltransferases (HAT) versus histone deacetylases (HDAC)] to Wnt target promoters, emerges as the underlying mechanism of transcriptional regulation by this complex.

Among the various proteins that interact with β-catenin, Pygo proteins have been the focus of recent efforts to identify molecular effectors of Wnt signaling and their functional mechanisms. *Drosophila* studies identified dPygo as a dedicated nuclear co-activator of Wg signaling.[23–26] Subsequent analyses suggest the mechanism of Pygo function might be nuclear retention of β-catenin;[27–29] however many data suggest Pygo lends essential transcriptional co-activator function to β-catenin in addition to (or in lieu of) anchoring β-catenin in the nucleus.[23–25,30–32] Surprisingly, ablation of *pygpous* genes in mice does not phenocopy mutants with loss of Wnt signaling.[33–35] These studies suggest that mammalian Pygo proteins have evolved to play augmenting instead of essential roles in the Wnt pathway. Below we review the findings on this class of genes in light of these new data, and examine its role in transcriptional regulation.

## Developmental phenotypes of *Wg/Wnt* mutants: where do *pygopus* genes fit in?

*Pygopus in flies*

In 2002, three different laboratories performed three different genetic screens to search for additional components of Wg signaling in flies and identified *Drosophila pygopus* (*dpygo*).[23,25,26] A fourth laboratory identified *legless* in yet another screen for genetic modifiers of Wg signaling in flies, and subsequently discovered dPygo as a Lgs-interacting protein (the gene was thus named *pygopus*, as the *pygopus* genus belongs to the family of legless lizards) using a yeast two-hybrid screen.[24] Although ubiquitously expressed, the loss of *dpygo* function produces defects that are strikingly similar to those caused by loss of *Wg* or *Arm*. In all four studies, mutant flies containing *dpygo* null alleles or hypomorphic alleles lacking the PHD domain exhibit embryonic and adult phenotypes consistent with loss of Wg signaling. For example, cuticles from *dpygo*-deficient embryos exhibit a lawn of denticles, consistent with loss of Wg signaling that normally establishes naked stripes along the ventral cuticle.[36] Genetic epistasis and cell biology experiments place *dpygo* downstream of Axin and Arm stabilization, whereas molecular analyses reveal loss of expression of Wg target genes in multiple developmental sites in the absence of functional

dPygo. Together, these findings, particularly the phenotypic parallels between *dpygo* and *Wg* mutants, led to the prevailing notion that *Drosophila* Pygo is a dedicated core component of Wg/Arm signaling, a feature that sets Pygo apart from other transcriptional co-factors of the β-catenin–LEF complex. However, phenotypes that cannot be explained by attenuated *Wg* transcription have been noted and expression of some Wg target genes are reduced but not completely abolished in *dpygo* mutant flies,[25] reminding us that *dpygo* is not exclusively or ubiquitously required for Wg-mediated processes. A similar theme of Wnt-dependent and Wnt-independent functions was also hinted for *pygopus* in *Xenopus*.[23,37]

*Developmental defects in mammals: pygopus mutants do not completely phenocopy loss of Wnt signaling*

Recent studies have extended the analysis on *pygopus* to mice, with surprising findings regarding the requirement for mammalian Pygopus proteins in Wnt signaling.[33–35] Unlike flies, which have one *pygopus* gene, mammals have two *pygopus* homologs, namely *Pygo1* and *Pygo2*.[23,24,26,38] Mouse *pygopus* genes are expressed in regions where Wnt signaling is known to be important for development as well as where Wnt signaling has no demonstrated function.[33–35,38] Accordingly, mice deficient for *Pygo2* or for both *Pygo1* and *Pygo2* expression exhibit some phenotypes that are consistent with loss of Wnt signaling, as well some characteristics atypical of known Wnt signaling mutants. Because deletion of either *Pygo2* alone or both *Pygo1* and *Pygo2* together in mice results in no obvious differences in development,[34] it appears that the phenotypes reported thus far primarily reflect the role of *Pygo2* in the processes analyzed.

In addition to ablated or impaired formation of select eye and kidney structures, studies in *pygopus* mutant mouse embryos report exencephaly (with incomplete penetrance) and perinatal lethality, reduced hair follicle density and abnormal lung morphology.[33–35] Overall, such developmental phenotypes are considerably less severe than those observed for mice null for β-catenin that display germ layer and axial patterning defects early in embryogenesis.[39,40] Moreover, the early developmental defects such as improper limb bud formation reported in Wnt3a-deficient mice or compound mutant mice lacking both Tcf1 and Lef1 expression[41] are not observed in *Pygo2*-deficient mice. Furthermore, intestinal stem cell formation and hair follicle development, two well-known processes that require active Wnt signaling,[42–44] are not or minimally affected by loss of *Pygo2*.[35] Clearly, mammalian *pygopus* genes are not essential for all Wnt-requiring processes.

Before ''Wnters'' lose interest in *pygopus* genes altogether, it is important to point out that some Wnt-requiring processes do entail a mammalian Pygopus function. The defect in lung morphogenesis in *Pygo2* null embryos[35] is reminiscent of the effect of inducing Dkk1 (a Wnt inhibitor) expression in lung epithelia of developing mice.[45] Lung morphogenesis had previously been shown to require β-catenin,[46] suggesting that loss of *Pygo2* function in this context is consistent with impaired canonical Wnt signaling. Mammary gland development is impaired in mice deficient for Lef1 or over-expressing Dkk1,[47] and mice lacking *Pygo2* exhibit defective mammary gland morphogenesis (unpublished observations). In both developing lung and mammary glands, reduced Wnt signaling is evident by decreased expression of BAT-gal, an in vivo reporter gene for canonical Wnt signaling,[35] (and unpublished observations). Similarly, *Pygo2* is important for canonical Wnt signaling in the developing kidney, where defective ureteric bud morphogenesis is accompanied by loss of Wnt reporter gene expression in *Pygo2*$^{-/-}$ embryos.[34] Interestingly, although multiple steps of a common developmental pathway (e.g. kidney development) are known to require Wnt signaling, the involvement of *pygopus* varies from step to step. While nephron induction and mammary fate induction depends on canonical Wnt signaling, these processes occur normally in the absence of *pygopus* function; instead, additional development is affected[34] (and our unpublished observations). Together, these findings indicate that mammalian Pygopus proteins have evolved to play a context-dependent role in canonical Wnt signaling. This apparent divergence from *Drosophila* may tailor to the needs of complex gene regulation in mammals.

Mammalian *pygopus* genes also have Wnt-independent functions, as exemplified by *Pygo2*'s involvement in eye development. Ablation of *Pygo2* affects lens formation by reducing expression of the Pax6 gene required for induction of lens fate.[33] However, the spatial separation between sites of Pygo2 function and Wnt reporter expression, together with the difference in lens phenotypes between *Pygo2*- and β-catenin mutants led the authors to conclude that *Pygo2*-mediated regulation of lens development is independent of its involvement in Wnt/β-catenin signaling. We note that Wnt-independent functions have also been previously ascribed to β-catenin and TCF/Lef, core effectors of Wnt signaling.[48–50]

## Pygopus and cancer: aberrant expression in Wnt-dependent versus Wnt-independent contexts

The Wnt signaling pathway is important in adult mammals for maintenance of homeostasis in such tissues as breast, intestine and blood.[1,47,51] As such, deregulation of Wnt pathway components is associated with a causal or progressive role in cancer. *Drosophila* studies identifying *pygopus* as an essential component of canonical Wg signaling raise the exciting possibility that Pygopus proteins may be additional targets for cancerous mutations as well as therapeutic interventions. While such enthusiasm is somewhat tapered by the context-dependent nature of *pygopus* function in

mammalian Wnt signaling, there exists experimental evidence that Pygopus proteins may be involved in neoplastic transformation of multiple cell types.

A vast majority of colorectal cancers bear mutations in APC or β-catenin, resulting in abnormally high Wnt signaling activity. Knockdown of *pygopus* genes in colorectal cancer cells containing a mutant APC reduces Wnt reporter gene expression,[26] suggesting that endogenous Pygopus proteins modulate signaling output in these cancer cells. Deregulated Wnt signaling is also linked to breast cancer. The expression of human *Pygo2* is upregulated in some breast cancer cell lines and tumors, and reduction of Pygo2 levels causes decreased growth of breast cancer cells and reduced expression of *cyclin D1*, a known Wnt target gene.[52] Moreover, more than 50% of breast cancers have been shown to display amplifications of chromosomal region 1q21-q22,[53] where the human *Pygo2* gene resides. Epithelial ovarian cancers include subtypes that are Wnt-active, and those that are not, but interestingly *Pygo2* is overexpressed in both subtypes.[54] Knockdown of *Pygo2* disrupts growth of cell lines derived from both Wnt-active and Wnt-inactive tumors. While in vivo functional evidence is still needed to demonstrate the involvement of *Pygo2* in tumorigenesis, these findings are consistent with the developmental theme of Wnt-dependent and Wnt-independent roles of Pygo2. Furthermore, they suggest that Pygo2 should remain a candidate therapeutic target for select if not all cancers irrespective of its involvement in Wnt signaling.

## Transcriptional co-activator function of Pygopus proteins—a closer look

Pygo binds to the N-terminal domain of β-catenin via Lgs/BCL9 and, together, they augment Wnt reporter gene expression via two possible mechanisms that yet have to be reconciled. Thus far, Lgs/BCL9 has been demonstrated primarily as an adaptor between β-catenin and Pygo with no apparent intrinsic co-activator function.[24,32] Pygo proteins have two distinct conserved domains, an N-terminal homology domain (NHD) and a C-terminal PHD zinc finger motif.[23–26] Evidence suggests that both domains are important for the function of Pygo proteins. Below we examine existing findings in order to tease out a possibly unifying theme regarding the mechanism of Pygo function.

### *Requirement for the Pygopus NHD*

The functional importance of the NHD is implicated at both overexpressed and physiological levels. *dpygo* mutant flies with an intact NHD but lacking a functional PHD finger have a less severe cuticle phenotype than null mutants.[26,55] In contrast, ubiquitous expression of a chimeric protein, in which the N-terminal region of dPygo lacking the PHD is fused to a dominant negative dTCF (which lacks the Arm/β-catenin interaction domain), is able to partially rescue the denticle defects in *dpygo* or *Arm* mutant flies.[56] Studies in *Xenopus* embryos injected with mRNA coding only the NHD of xPygo reveal gain of Wnt activity mimicking the effects of injecting dominant-active Wnt RNA.[37] Notably, however, not all Wnt target genes are activated by the overexpressed NHD motif, suggesting that the NHD may function in a promoter-specific manner.

The transactivation capability of the Pygo NHD has been demonstrated in a variety of assays. The N-terminal region of dPygo (including the NHD) is able to activate reporter transcription in mammalian 293T cells when fused to a GAL4 DNA-binding domain or a dominant negative dTCF,[56] indicating that the NHD when directly tethered to a DNA-binding activity can elicit a transcriptional response. Similarly, the NHD when tethered to DNA in *Drosophila* S2 cells activates reporter transcription in the absence of appreciable levels of endogenous Arm, Lgs or dPygo.[32] Furthermore, a full-length Pygo protein expressed off a transgene in clones of cells devoid of endogenous dPygo is able to activate the expression of the Wg target gene *senseless*, whereas a single point mutation at a conserved amino acid within the NHD (F99) abolishes this gene activation without having a significant effect on Lgs nuclear localization.[30] In one study, it has been shown that reporter activation and rescue of fly viability also highly depend on a conserved NPF amino acid motif within the NHD.[32] This appears to contradict the observation that defective cuticle formation is rescued by overexpression of a construct with missense mutations in the coding sequence for the NPF motif.[55] Most likely the NPF residues in Pygo NHD are essential for some but not all Pygopus-related processes, with their contributions varying with developmental/genetic contexts and sometimes masked by high levels of artificial overexpression. Recent data show that the NHD is required for association of dPygo with dTCF in *Drosophila* salivary glands even in the absence of Wg signaling, and that this interaction is dependent on the NPF motif.[57] The discovery of this interaction raises the possibility that a β-catenin-independent Pygo/TCF complex may have the capability to regulate basal promoter activity when Wg signaling is not active, a notion worthy of future investigation. Furthermore, the biochemical mechanism(s) by which the NHD activates transcription in β-catenin-dependent and -independent contexts remains to be elucidated.

### *Requirement for the Pygopus PHD finger*

A single point mutation in the dPygo PHD finger generates a denticle phenotype resembling that caused by loss of essential Wg signaling components.[23] In contrast, ubiquitous expression of the PHD finger restores the segmented cuticle pattern in *dpygo* mutant embryos.[55] Wg target gene activation as assayed in *Drosophila* embryos reveals that the PHD contributes to Arm-mediated transcription. Expression of Wg target genes including *En, Wg* and *Dll*, as well as enhancer activity from the Wg response element in UbxB gene, are

significantly reduced in *dpygo* mutant embryos lacking the PHD.[24,26]

At present, a widely accepted molecular function of the Pygo PHD finger is to mediate interaction with Lgs/BCL9, and consequently β-catenin. Evidence shows that the PHD finger is both necessary and sufficient for Lgs/BCL9 interaction,[24] and that indeed residues within the finger that mediate binding of Pygo to Lgs/BCL9 are important for the rescue of denticle defects in *dpygo* mutant embryos.[55] These residues are also important for the ability of DNA-tethered Pygo to activate reporter gene expression in transient transfection assays.[55] In the context of the full-length protein, the PHD of Pygo may, via its interaction to Lgs/BCL9, function to enhance target gene transcription by (1) providing a means to bring the NHD to the β-catenin/LEF transcriptional complex and possibly target DNA to facilitate transactivation, and/or (2) anchoring β-catenin in the nucleus thereby elevating the level of the nuclear β-catenin/LEF complex. Interestingly, a nearly full-length Pygo protein that contains the PHD but not the NHD stimulates reporter activity when tethered to DNA,[23] indicating that at least in some contexts the PHD can enhance transcriptional activation independently of the NHD.

Does the PHD finger in Pygo proteins serve any additional role besides binding Lgs/BCL9? The observation that both the Pygo PHD and Lgs become dispensable when the NHD of Pygo is brought to β-catenin via alternative means[24,32,56] seems to suggest that the only function of the PHD is to mediate interaction to Lgs/BCL9, and hence β-catenin. However, caution needs to be exercised when interpreting misexpression and overexpression data, as the presence of a high level of a defective protein may bring about the same degree of functional output as a physiological level of the wild type. Several amino acids that are necessary for reporter gene activation are clearly not involved in Pygo–Lgs interaction,[55] suggesting that Pygo proteins indeed are involved in Lgs/β-catenin-independent interactions (see below).

In summary, functional data suggest that both the PHD and the NHD motifs of Pygo proteins are important and required under physiological conditions for optimum activation of Wg/Wnt target genes but, when overexpressed, each domain can compensate to some degree for loss of the other. It appears that Pygo can associate with LEF/TCF-mediated activity both in Wg/Wnt-responsive transcription via PHD binding to BCL9/β-catenin/LEF and possibly in Wg/Wnt-independent transcription via NHD binding to LEF/TCF.

## Mechanism of Pygopus function in transcriptional regulation – a role in chromatin remodeling?

Despite ample experimental evidence demonstrating the importance of Pygo proteins in development, Wg/Wnt signaling, and transcriptional activation, a clear picture of how these functions are achieved is still lacking. PHD proteins have long been implicated in chromatin-mediated transcriptional control.[58,59] The interaction of β-catenin with multiple proteins associated directly or indirectly with HAT or HDAC as well as other chromatin remodeling activities calls attention to the role of chromatin remodeling in regulating Wg/Wnt target gene expression. It has been suggested that during Wg/Wnt signaling, Pygo may exchange or stabilize the co-activators that interact with the C terminus of β-catenin;[5,31] however, a specific role for Pygo in chromatin remodeling has not been proposed.

Is it possible that the primary function of Pygo is to participate in chromatin remodeling? It may be in this chromatin context that Pygo interfaces with Wg/Wnt signaling in a highly specific manner in at least some tissue- and developmental contexts due to the pivotal importance of chromatin remodeling in Wg/Wnt-mediated gene activation. The ''histone code'' hypothesis predicts that multiple histone modifications may occur sequentially,[60] and experimental evidence indeed demonstrates that one type of histone modification (e.g. acetyl-lysines) often constitutes a recognition motif for protein complexes with another chromatin modification activity (e.g. GCN5 and Swi2/Snf2).[61–64] Recent studies show that the PHD fingers of several proteins including inhibitor of growth 2 (ING2) and bromodomain and PHD finger transcription factor (BPTF) bind tri-methylated histone H3 tails, thus linking chromatin remodeling to changes in gene expression.[65–70] Histone methyltransferases (HMT) of the SET1 family have been shown to complex with β-catenin at the *c-Myc* enhancer and to mediate trimethylation of the lysine 4 residue on histone 3 (H3K4me3).[71] Is it possible that the methylated histone serves as a docking site for the PHD of Pygo, and this in turn facilitates histone H3 acetylation, as is the case for the Yng1 protein,[69] as well as other subsequent chromatin remodeling events such as those mediated by the SWI/SNF complex?

The crystal structure of the PHD finger of Pygo1 is reportedly different in electrostatic surface potential than that of other PHD domains;[72] however sequence alignment does reveal the presence of several amino acids known to be required in ING2 and BPTF for H3K4me3 binding (Fig. 3). Our preliminary analysis using in vitro substrates shows that the PHD of Pygo2 is indeed able to directly and specifically bind H3K4me3 (unpublished data). Perhaps Pygo proteins are important enhancers of Wg/Wnt-dependent, and possibly Wg/Wnt-independent, transcription because they confer product (K4-trimetylated H3)-binding ability to a macromolecular protein complex (β-catenin/SET1) that possesses substrate modification activity (H3 K4 trimethylation). Such capacities have been previously described as a positive feedback mechanism for chromatin modifying complexes.[73] A priori, the Pygo PHD may act as a chromatin recognition effector to recruit chromatin remodeling complexes in a manner either dependent upon or independent of β-catenin. However, by

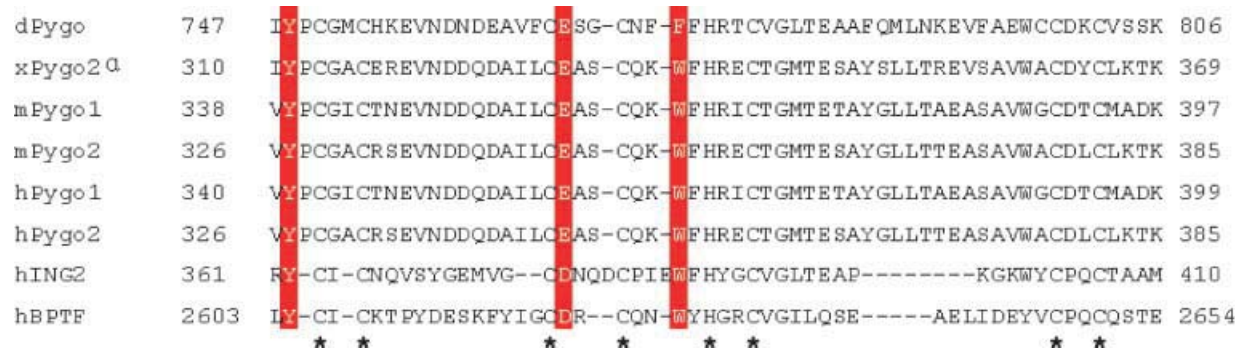


**Figure 3.** Sequence alignment of PHD domains from Pygo proteins and ING2/BPTF. Note that residues critical for H3K4me3 binding of ING2 and BPTF (highlighted in red) are highly conserved at comparable positions of the PHD fingers of Pygopus (*Drosophila, Xenopus*, mouse and human). The asterisks represent the conserved cysteine and histidine residues of the $C_4HC_3$ PHD consensus sequence.

being able to bind both H3K4me3 and Lgs/BCL9 (and hence β-catenin), Pygo may serve as a module of a positive feedback loop to increase the local concentration of β-catenin-associated chromatin-modifying enzymes, such as HMT, HAT and SWI/SNF complexes, to ensure that these enzymes can act in a processive manner to switch a sufficiently long stretch of chromatin to an active state (Fig. 4). The NHD domain of Pygo may bind to LEF/TCF under conditions when cellular β-catenin levels are low to provide an alternative, β-catenin-independent, means to assemble chromatin remodeling complexes at target promoters[57] and/or stabilize/facilitate interactions with other components of the transactivating complexes. As *Pygo* mutant mice share common developmental phenotypes (e.g. defects in brain and eye morphogenesis) and reduced Wnt target gene expression with animals deficient in the SWI/SNF component Brg1,[74,75] this speculative model of Pygo function in chromatin remodeling may not be completely far-reaching. Although these ideas have yet to be experimentally validated, they nonetheless reconcile the sometimes conflicting data regarding the role of Pygo proteins in gene activation, and suggest a possible molecular mechanism by which Pygo-containing complexes may operate.

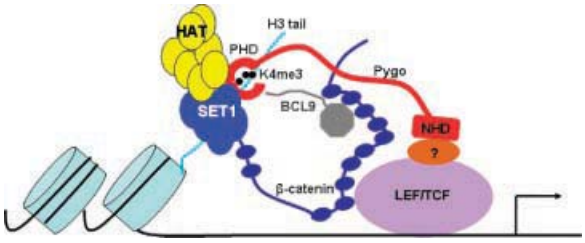


**Figure 4.** Proposed model of Pygopus function in transcriptional regulation. Trimethylation of histone H3 at lysine 4 (K4) may facilitate recruitment of a multi-subunit protein complex and dissociation of promoter DNA from the nucleosome. Note that, in this model, both the NHD and PHD of Pygopus are important for Wg/Wnt-responsive transcription: the NHD mediates binding of Pygopus to LEF/TCF and possibly other unidentified protein(s), while the PHD finger binds β-catenin (via Lgs/BCL9) and H3K4me3. The interaction of the NHD motif with LEF/TCF may enable activation of LEF/TCF-responsive genes independently of β-catenin.

## Conclusion

Current studies reveal an evolutionary divergence in the requirement for *pygopus* genes in Wg/Wnt signaling. Mammalian *pygopus* 2 is important in some but not all Wnt-requiring developmental processes, whereas *pygopus* in *Drosophila* is essential for nearly every Wg/Wnt-mediated process assayed. Pygopus may operate more globally in chromatin remodeling, and its functions in Wg/Wnt signaling are a subset of its chromatin-related activities. A recent commentary regarding the function of PHD fingers reiterated that the interaction of the PHD motif with methylated histone tails is not the primary mode of transcriptional regulation, but rather augments the interface of co-factors and chromatin remodeling complexes upon their recruitment to target promoters.[65] Chromatin remodeling via PHD fingers appears to be an evolutionarily conserved function, as ablation results in loss of histone modifications and improper transcriptional control from plants[76] to mammals.[77] Alterations to the chromatin modifying and transcriptional functions of PHD proteins are implicated in human diseases.[78] Perhaps these studies can provide clues to the molecular functions of mammalian *pygopus* genes in both Wnt signaling and Wnt-independent processes.

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