A Tale of Tailless

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Abstract

\textit{Drosophila} Tailless (Tll) and its vertebrate homologue Tlx are conserved orphan nuclear receptors specifically expressed in the eye and the forebrain. Tll and Tlx act primarily as transcriptional repressors through their interactions with transcriptional corepressors, Atrophin family proteins, and histone-tail/chromatin-modifying factors such as lysine-specific histone demethylase 1 and histone deacetylases. The functional importance of Tll and Tlx is made apparent by the recent discovery that they are expressed in neural stem cells (NSCs) and are required for self-renewal of these cells in both \textit{Drosophila} and the mouse. This review provides a snapshot of current knowledge about Tll and Tlx and their transcriptional network, which maintains NSCs in developing and adult animals.

Introduction

Nuclear receptors form a large family of transcription factors that are evolutionally conserved in species across the metazoans [1]. The properties of many nuclear receptors are regulated by small hydrophobic molecules such as steroid hormone and by metabolites like oxysterols, bile and fatty acids. Others are called ‘orphan nuclear receptors’ because their corresponding ligands have not been identified. Fly Tailless (Tll) and its vertebrate homologue Tlx are orphan nuclear receptors belonging to the NR2E subclass. Discovered more than two decades ago, Tll is best known for its role in specifying terminal cell fate during \textit{Drosophila} early embryogenesis. Recent research on Tll and Tlx in both \textit{Drosophila} and the mouse has not only shed light on their involvement in brain and visual system development but, most excitingly, has also revealed their involvement in neural stem cell (NSC) maintenance. We here provide an overview of the properties of these two conserved nuclear receptors and highlight their connections with transcriptional cofactors and target genes from the perspectives of visual system development and NSC renewal.

\textbf{Tailless in Drosophila}

\textit{tll} was first identified in a mutant screen as a gene required for the development of terminal structures in the \textit{Drosophila} embryo [2]. As reflected in its name, \textit{tll} mutant lacks the structures posterior to the eighth abdominal segment, including telson and the posterior gut, as well as portions of the head structures and the brain [3–
The \textit{tll} gene, which was identified by Pignoni et al. [5] in 1990, codes for a protein belonging to the nuclear receptor superfamily. In keeping with its phenotype, \textit{tll} transcript is expressed as symmetrical caps at both the anterior and posterior poles of the embryo at the syncytial blastoderm stage 4 [5, 7] (fig. 1a). This transient expression pattern of \textit{tll} is activated indirectly by the maternal Torso receptor tyrosine kinase-signaling pathway at both ends of the embryo [8], in part by relieving the repression effects imposed by the HMG box transcription repressor Capicua and its corepressor Groucho [9, 10], as well as the BTB domain zinc finger protein Tramtrack69 (Ttk69) [11]. For the latter, degradation of Ttk69 in response to the activated Torso pathway has recently been proposed to be a mechanism enabling a dual transcriptional protein complex consisting of Ttk69, heat shock factor, and GAGA factor (a Trithorax group protein), to be converted from a repressor to an activator [12].

During this early phase of its expression, Tll functions to confine the expression of other gap genes, such as \textit{Krüpple} and \textit{knirps}, to the trunk region [13], thereby allowing terminal cell fates to be specified in part by indirectly activating the expression of secondary target genes, including \textit{hunchback}, \textit{brachyverteron}, and \textit{forkhead} [3–6]. Based on this information about \textit{tll}, Moran and Jimenez [14] proposed that Tll primarily acts as a dedicated transcriptional repressor in early \textit{Drosophila} embryos.

While the expression level of \textit{tll} in the posterior region decreases at the gastrulation stage and becomes undetectable by the end of the germband extension stage, its presence in the anterior region remains robust and lasts well into the late stage of embryogenesis, albeit with dynamic changes [5]. At stage 5, the anterior cap of \textit{tll} expression morphs into a horseshoe-like pattern that straddles the dorsal midline in the anterior region from which the anterior portion of the brain, the protocerebrum, arises [6, 7]. Closer examination of \textit{tll} within the protocerebrum revealed its expression in the proneural domains that are positive for \textit{lethal of scute} [6], a proneural gene belonging to the \textit{achaet-scute} complex that is crucial for the development of neuroblasts (NBs) [15, 16]. Correlating with its expression in the protocerebrum, mutation of \textit{tll} causes loss of most NBs in the protocerebrum, but spares those NBs present in the deutocerebrum and tritocerebrum, whose development relies on inputs from other gap genes, including the two homeobox genes \textit{orthodentile} (\textit{otd}) and \textit{empty spiracles} (\textit{ems}) [6, 17]. However, \textit{tll} is required for establishing the boundary between protocerebrum and deutocerebrum. In \textit{tll} mutant embryos, the deutocerebral structure expands anteriorly, consistent with the observation of ectopic expression of \textit{ems} from the posterior brain region into the protocerebral domain [18]. Thus, Tll acts as a transcriptional repressor of \textit{ems}. Regulation of \textit{ems} by Tll is direct, since the promoter region of \textit{ems} contains multiple Tll-binding sites that can be bound by Tll protein in vitro [18].

In many aspects, the Tlx proteins behave like Tll. As described in the rest of this review article, the resemblances not only include the function of Tll/Tlx in cell fate determination and boundary formation, but also the evolutionarily conserved transcriptional network that is deployed to maintain self-renewal of NSCs.

\textbf{Tlx in Vertebrates}

The chick Tlx gene was first cloned Yu et al. [19] by screening cDNA libraries using \textit{RXRβ} as the probe. Around the same time, cloning of mouse \textit{Tlx} gene was reported by Monaghan et al. [20], who used \textit{Drosophila} \textit{tll} as the probe. Sequence comparison revealed that Tll and Tlx proteins are 81% identical in their DNA-binding domains and 41% identical in their ligand-binding domains [19, 20] (fig. 2a). The most conserved sequences were found in the P and D boxes of their DNA-binding domains (fig. 2b), which are involved in the recognition of specific DNA sequences [21]. As shown in figure 2b, the sequences in the P and D boxes of the Tailless-family proteins are distinct from those found in members of the chicken ovalbumin upstream binding protein transcription factor (COUP-TF) family, even though the latter proteins belong to the same NR2 class of nuclear receptors. Thus, the target genes of Tailless-family and COUP-TF-family proteins are likely to be different. The view that Tlx represents the functional homolog of Tll was validated by the demonstration that ectopic expression of chick Tlx in \textit{Drosophila} embryos recapitulates the effects caused by overexpression of Tll [19]. This result, combined with the fact that Tll and Tlx share similarities in their structures and expression patterns (discussed below), led to the view that the genetic programs upstream and downstream of Tll/Tlx, as well as the mechanisms by which Tll/Tlx repress gene expression, are conserved.

Phylogenetic analysis of NR2 subclass nuclear receptors further defines the kinship between Tll/Tlx and other NR2E family members, including Dissatisfaction (DSF, NR2E4) and Photoreceptor nuclear receptor (PNR, NR2E3), whose fly and worm homologs are dHR51 and Defective fasciculation of axons-1 (Fax-1), respectively (fig. 2c) [Nuclear Receptor Signaling Atlas, http://www.
nursa.org). On an evolutionary scale, *Drosophila* DSF, which is involved in regulating adult sexual behavior [22, 23], is closer to Tlx than Tll (fig. 2c). Thus, a gene duplication event took place in arthropods that produced the paired *tll–dsf* genes in the genome of *Drosophila* [24]. Since *Tlx* is the sole *tailless* gene in vertebrates, with properties linked to development of central nervous system (CNS) and adult behavior (described below), the reported in vivo functions of Tlx appear to combine the properties of Tll, which is also involved in visual and nervous system development, and of DSF, which is involved in behavior in flies.

Like its fruitfly counterpart, *Tlx* is also expressed in the developing CNS, including the telencephalon, diencephalon, eye and nasal placode (table 1) [19, 20]. *Tlx* expression in the mouse starts at embryonic day 8 (E8), peaks around E12.5, and then declines from E13.5 through neonate. The expression of *Tlx* increases after birth, with high levels detected in the adult brain [20]. During early mouse brain development, the telencephalic and diencephalic expression of *Tlx* is restricted to the ventricular zone. Closer examination of *Tlx* in this region revealed a graded pattern along the dorsal-ventral axis in...
the telencephalon [20, 25, 26]. At E12.5, *Tlx* is expressed at a higher level in the dorsal-lateral pallium and the lateral ganglionic eminence, at a lower level in the medial ganglionic eminence and the dorsal-medial pallium, but is undetectable in the ventral-medial region of the telencephalon (fig. 3a) [26].

In correlation with the expression of *Tlx* in the developing telencephalon at E12.5, *Tlx* mutant mice exhibit patterning defects, although subtle, in the lateral telencephalic zone. Stenman et al. [26] reported that the pallio-subpallial boundary is disrupted in *Tlx* mutant mice, marked by decreased expression of two ventral pallial markers, *Dbx1* (developing brain homeobox 1) and *Sfrp2* (secreted frizzled related protein 2). Moreover, the expression of *Gsh2* (GS homeobox 2), a subpallial marker, is also expanded into the ventral-most pallial region. Considering that the pallio-subpallial boundary is established by mutual repression of ventrally expressed *Gsh2*...
and dorsally expressed Pax6\cite{27–29}, the same group tested whether the removal of one allele of Pax6 from homozygous Tlx mutant mice affected their phenotype. Indeed, Tlx\textsuperscript{−/−}, Pax6\textsuperscript{+/−} mice displayed a more severe phenotype than Tlx\textsuperscript{−/−}, with the expression of Gsh2 further expanded into the pallial region\cite{26}. This result demonstrates that Tlx and Pax6 cooperate genetically to establish the pallio-subpallial boundary. Since the basolateral amygdala (a part of the limbic system that is involved in processing emotional reactions) derives from the ventral pallium, the disrupted pallio-subpallial boundary found in the developing Tlx mutant mouse brain provides an explanation why adult Tlx mutant mice exhibit behavioral abnormalities\cite{30}.

In spite of these patterning defects found in the CNS of Tlx mutant embryos, Tlx-null mice are viable and appear normal at birth. Mature Tlx knockout mice, in contrast, exhibit severe anatomical deficits in the cerebral
hemispheres [30]. In these mice, late-developing structures such as the upper cortical layers and the dentate gyrus are reduced in size. Moreover, Tlx mutant mice also display severe retinopathies [31–34] and exhibit increased aggressiveness and reduced learning abilities [30, 35, 36]. These observations indicate that Tlx plays a critical role in regulating the development of the visual and nervous systems. Consequently, the properties of Tlx and Tll in connection with the development and functioning of these two systems warrant further discussion.

**Roles of Tll and Tlx in Visual System Development**

The *Drosophila* visual system comprises the adult compound eye, the larval eye (Bolwig’s organ), and optic lobe (an organ relaying the signals from the eyes to the brain), which all originate from a contiguous region in the dorsal head ectoderm at the embryonic stage [37]. During transition from the embryonic to larval stage, a group of primordial cells at the embryonic stage undergo differentiation and give rise to a prominent optic lobe and Bolwig’s organ at the larval stage. At the embryonic stage, tll is expressed in the cells that are destined to become the eye and the optic lobe but not Bolwig’s organ [38]. At the third instar larval stage, tll is expressed in the proliferating NBs located in the optic lobe and the central brain, but not in the ventral nerve cord (fig. 1b). In the third instar larval eye disk, the expression of tll is restricted to proliferating and undifferentiated cells that are located anterior to the morphogenetic furrow (fig. 1c). Based on these expression patterns, it appears that tll is selectively expressed in undifferentiated neural precursor cells.

While the role of Tll in *Drosophila* eye development still waits to be clarified, its involvement in controlling cell fates in the optic lobe is better understood. In tll mutant embryos, the optic lobe is transformed into Bolwig’s organ. Consistently, elevated expression of Tll leads to the opposite effect [38]. Based on these observations, Dumstrei et al. [39] proposed that tll controls the switch of cell fates between optic lobe and Bolwig’s organ at the embryonic stage. They provided evidence showing that the ability of Tll to drive cells to become the optic lobe results from the negative effect of Tll on the output of the epidermal growth factor receptor (EGFR)-signaling pathway in primordial cells, which are capable of developing into either the optic lobe or Bolwig’s organ [38]. The ligand of EGFR, Spitz, is secreted from the nearby Atonal-expressing founder cells [39], which by activating the EGFR-signaling pathway in the primordial cells can direct these cells to adopt the Bolwig’s organ cell fate. This EGFR-directed cell fate, however, is prevented when the primordial cells express Tll (fig. 1d). So far, the mechanism by which Tll antagonizes the EGFR-signaling pathway remains unknown.

Like its fruitfly counterpart, mouse Tlx is also prominently expressed in retinal progenitor cells (RPCs) in the neuroblastic layer throughout the entire period of retinal neurogenesis, as well as in the optic disk [19, 20, 31, 33]. Tlx is first expressed on the innermost surface of the central retina at E11.5, and thereafter is extended toward the periphery of the entire retina. From E13.5 onward, Tlx is expressed uniformly in RPCs in the neuroblastic layer, with prominent expression levels detected within the optic nerve head at E17.5. After the postnatal period, Tlx expression becomes confined to the inner nuclear layer, where Müller glial cells and mature astrocytes are located. Tlx is also transiently expressed in immature astrocytes prior to their migration from the optic nerve to the inner nuclear layer [31], where they, along with the Müller cells, form the characteristic retinal network [40]. In Tlx mutant mice, the specification of each retinal cell type is

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**Fig. 4.** Tlx enables NSCs to undergo self-renewal by repressing the expression of Pten, prospero/Prox1, or Cip/Kip family members such as p21 and p57. Both Pten and p21 function to inhibit G0 → G1 entry and G1/S progression. Their increased expression in Tlx mutant cells leads to the inhibition of proliferation and self-renewal of stem cells. The ability of Tll or Tlx to repress the expression of its target genes depends on inputs from the atrophin (Atro) proteins, HDACs, or LSD1.
initiated normally at the early stage, but the cell numbers in each nuclear layer are progressively reduced at a later stage, followed by malformation of the vascular system surrounding the retina [31]. Thus, it appears that Tlx is not required for specifying a particular retinal subtype, but is essential for the proliferation of RPCs in order to achieve the correct laminar arrangement and thickness. In keeping with this observed role of Tlx in retinal development, 3-week-old Tlx mutant mice show significant retinal and optic nerve degeneration, which also explains why these Tlx mutant mice are visually impaired [33, 34, 36]. Recent studies of conditional disruption of Tlx that spared the visual system indicated that blindness may contribute at least in part to the reduced cognitive abilities and other abnormal behavior observed in these mice [41, 42].

Roles of Tll and Tlx in NSC Self-Renewal

Apart from the involvement of tll in segmentation processes and in specifying optic lobe cell fates, recent study has revealed a further important role of tll in regulating the proliferation of NBs. Drosophila NBs, equivalent to NSCs in vertebrates, undergo asymmetric cell division that produces a larger self-renewing NB and a smaller ganglion mother cell (GMC); the latter give rise to specific nerve cells [43, 44]. Mutation of tll causes loss of most

Table 1. Comparison of Drosophila tll and vertebrate Tlx

<table>
<thead>
<tr>
<th>Spatial expression</th>
<th>Drosophila tll</th>
<th>Vertebrate Tlx</th>
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</thead>
<tbody>
<tr>
<td>Visual system</td>
<td>Larval and adult eye, optic lobe</td>
<td>Developing retina</td>
</tr>
<tr>
<td>Olfactory system</td>
<td>Mushroom body</td>
<td>Nasal placode, olfactory bulb</td>
</tr>
<tr>
<td>Other CNS regions</td>
<td>Protocerebrum</td>
<td>Forebrain, diencephalon</td>
</tr>
<tr>
<td>Temporal expression</td>
<td>Embryonic, larval</td>
<td>Embryonic, postnatal and adult stages</td>
</tr>
<tr>
<td>Interacting partners</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Co-repressor</td>
<td>Atrophin</td>
<td>Atrophin-1, RERE</td>
</tr>
<tr>
<td>Chromatin-modifying factors</td>
<td>Su(var)-3*, Rpd3*, dG9a*</td>
<td>LSD1, HDACs*, G9a*</td>
</tr>
<tr>
<td>Others</td>
<td>Brakeless*</td>
<td>ZNF608*, ZNF609*</td>
</tr>
<tr>
<td>Target genes</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patterning</td>
<td>knirps, ems, otd</td>
<td>Gsh2, Otx, Pax2</td>
</tr>
<tr>
<td>Proliferation</td>
<td>prospero</td>
<td>Pten, p21Cip1, p57kip2, Wnt7a</td>
</tr>
<tr>
<td>Others</td>
<td></td>
<td>microRNAs, GFAP</td>
</tr>
</tbody>
</table>

Target genes in bold have been confirmed by chromatin immunoprecipitation or other assays to confirm that Tll/Tlx bind their promoters directly. * Direct interaction has not been determined.

NBs in the protocerebrum [6], reminiscent of stem cell depletion in Tlx-null mice (discussed below). Kurusu et al. [45] recently showed that loss of tll causes proliferation defects in NBs and elevated apoptosis of GMCs in the mushroom body, the center of olfactory learning in the Drosophila brain. They further showed that overexpression of tll induced marked brain hyperplasia with supernumerary NBs, which again resembles the effect exerted by Tlx in vertebrates [46] (discussed below). In Drosophila, an increased number of NBs is elicited in part by Tll’s repression of prospero, which codes for a homeodomain transcription factor known to be involved in promoting the differentiation of GMCs after asymmetric stem cell division [47, 48]. This mechanism is again analogous to the mechanism by which Tlx prevents differentiation of NSCs by repressing p21 and p57 in the vertebrate CNS (see below) (table 1). Thus, Tll and Tlx play an evolutionally conserved role in maintaining self-renewal of NSCs, at least in part by negatively regulating the expression of differentiation-promoting genes.

In the case of Tlx, in addition to its involvement in establishing the pallio-subpallial boundary in the developing mouse telencephalon [26], emerging evidence has demonstrated that Tlx plays a pivotal role in regulating NSC self-renewal [49]. In early neural development, NSCs within the neural tube undergo a limited number of cell cycles to expand their pool size via symmetric division [50]. During the cell fate specification stage, a subset of
NSCs become determined neural progenitor cells (NPCs) that are destined to produce certain cell types, including neurons, oligodendrocytes and astrocytes; NPCs are characterized by decreased self-renewal potential and pluripotency [51]. In the developing mouse embryo, Tlx is initiated as early as embryonic day 8, when transcript and protein are both found in the ventricular zone (VZ) [20, 25, 52], where NSCs and NPCs reside (fig. 3a). The embryonic expression of Tlx within the VZ peaks at E13 and decreases by E16, coinciding with the generation of cortical neurons between E11 and E17 [53]. In Tlx mutant animals, NPCs proliferate with shorter cell cycles from E9.5 to E12.5, leading to precocious maturation of neurons [25]. By mid-neurogenesis at E14.5, nestin-positive NSCs are largely reduced in number and exhibit lengthened cell cycles when Tlx is mutated [52]. The depletion of NSCs by premature neurogenesis caused by loss of Tlx explains why Tlx mutant mice have altered cortical depth, reductions in dentate gyrus size and a smaller forebrain [54]. Since their limbic system is also impaired, Tlx mice display severe aggressive behavior, cognitive deficits, and decreased copulation activity [30]. Similarly, adult NSCs also require Tlx to remain in a proliferative state, and loss of Tlx causes failure of self-renewal; subsequently, NSCs may differentiate into the 'end state' of glial cells [55]. Thus, although they are heterogeneous, NSCs of both the developing and adult CNS require Tlx to promote proliferation and inhibit differentiation.

Recent studies using fate-mapping and inducible-mutation approaches provide a more lucid view of the role of Tlx in nervous system development (table 2). For at least three out of four stages, Tlx plays essential roles in maintaining NSCs. During the first stage, Tlx acts in developmental NSCs, probably when the pool of NSCs is being established (fig. 3a). Tlx-positive cells express NSC markers, such as nestin and RC2 [52], indicating that these cells possess radial glial characteristics [56]. During this early stage, Tlx prevents NSCs from developing into NPCs prematurely [25]. Transient knockdown of Tlx in NSCs at this phase causes them to exit the VZ and differentiate [52]. The role of Tlx in regulating NSCs and NPCs is reminiscent of the way in which Drosophila Tll controls proliferation and differentiation of the NBs and GMCs. Such similarities again underscore the conservation in the roles of Tll and Tlx in regulating nervous system development.

The reduced number of upper cortical layer neurons and the defective limbic system observed in Tlx-deficient mice are also seen in Pax6 [57] and in T-box transcription factor (Tbr2) mutants [58]. Tbr2 mutants share aggressive behavior with Tlx-deficient mice. Along with the genetic interaction between Tlx and Pax6 that assists in establishing the pallio-subpallial boundary, as noted above [26], these lines of evidence suggest a model in which Tlx and Pax6 cooperate to regulate the transition of early developing NSCs/NPCs (radial glia) into late neurogenic basal NPCs in the subventricular zone (SVZ) that express

Table 2. Phenotypes of Tlx mutant mice

<table>
<thead>
<tr>
<th>Tlx mutation</th>
<th>Phenotype</th>
<th>Behaviors</th>
<th>References</th>
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<tbody>
<tr>
<td>Germline mutation</td>
<td></td>
<td></td>
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<tr>
<td>Targeted disruption</td>
<td>Reduced size of rhinecephalic and limbic structures, decreased cortical thickness, retinal and optical nerve degeneration</td>
<td>Severe aggression, spatial learning deficit, blindness, blunted anxiety and fear-conditioning</td>
<td>25, 30, 33</td>
</tr>
<tr>
<td>Spontaneous mutation</td>
<td>Hypoplasia of cerebrum and olfactory lobes, abnormal eye development</td>
<td>Aggression, deficits in sensorimotor tests, reduced mating</td>
<td>36, 89</td>
</tr>
<tr>
<td>Conditional knockouts</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nestin-Cre</td>
<td>Hypomorphic dentate gyrus, absence of adult neurogenesis</td>
<td>Impaired spatial learning and memory, normal visual function</td>
<td>42</td>
</tr>
<tr>
<td>CaMKII-Cre (BAC)</td>
<td>Similar to germline deletion except for normal eye development</td>
<td>Normal fear conditioning, normal spatial learning and memory</td>
<td>41</td>
</tr>
<tr>
<td>CaMKII-Cre (promoter cre)</td>
<td>No proliferation defect of NSCs in dentate gyrus</td>
<td>No obvious deficit</td>
<td>42</td>
</tr>
<tr>
<td>Induced mutation at postnatal stage</td>
<td>Before P10, loss of adult NSC, after P10, loss of self-renewal ability of NSCs</td>
<td>NA</td>
<td>60</td>
</tr>
<tr>
<td>Induced mutation in adult mice</td>
<td>Reduced adult neurogenesis (67%) in dentate gyrus and absence in SVZ</td>
<td>Impaired spatial learning and memory</td>
<td>42, 60</td>
</tr>
</tbody>
</table>
**Tbr2** (fig. 3a). Loss of Tlx affects the proliferative potential of basal NPCs, but not other characteristics such as migration, which was shown to be unchanged in Tbr2 mutants [58]. Thus, it is conceivable that deletion or mutation of Tlx may alter Tbr2 expression, and disrupted expression of Tbr2 may partly contribute to the defects seen in Tlx mutants.

The second stage of Tlx function begins at postnatal day (P) 9, when radial glial cells become the founders of the adult NSCs [59] (fig. 3b). Tlx is required for this transition: mutation induced before P9 produces a phenotype similar to that of germline mutation because NSCs are similarly lost in both cases. In contrast, loss of Tlx after P9 causes changes similar to those found in induced Tlx mutation at the adult stage, i.e. NSCs exist, but they are proliferation defective [60]. During the third phase at adulthood, Tlx is highly expressed in GFAP-positive cells located at the SVZ [60] (fig. 3c), suggesting that these Tlx-positive adult NSCs are astrocyte-like B-type cells [61]. Tlx is required to maintain NSCs at the sites of adult neurogenesis, including the subgranular zone of the dentate gyrus and the SVZ lining the lateral ventricles. An induced mutation of Tlx at this stage leads to partial and complete loss of neurogenesis in the subgranular zone and SVZ, respectively [42, 60]. These results are consistent with earlier reports that adult stem cells lacking Tlx lose their self-renewal capacity and are prone to differentiating into glial cells [55]. The last stage is when Tlx is found sparsely expressed in mature neurons in the dentate gyrus and part of the cortex. Absence of Tlx at this stage does not cause abnormal proliferation of NSCs or any behavioral defects [42].

In sum, Tlx represents an NSC marker that is expressed at almost all phases of neural development. Based on the phenotypes and behaviors of various mutant mice carrying targeted or spontaneous mutations of Tlx shown in table 2, it can be inferred that severe aggression and reduced anxiety result from defects incurred at an early stage of brain development whereas impaired spatial learning and memory are the consequence of impaired adult neurogenesis taking place in the dentate gyrus.

**Target Genes of Tll and Tlx**

A few target genes of Tll have been identified in *Drosophila*, especially at the embryonic stage. For example, *kni* and *ems* are directly regulated by Tll in early *Drosophila* embryos [18, 62, 63]. In contrast, with the exception of the recently reported *prospero* [45], no information has emerged about which other genes are directly regulated by Tll at later stages.

Because Tlx is involved in NSC renewal, there has been great interest in finding the target genes that contribute to its action. A global gene expression-profiling study has provided valuable information on how Tlx affects the proliferation and differentiation of RPCs [34]. One of the most interesting targets identified by Zhang et al. in this study was the tumor suppressor gene *Pten* (*Phosphatase and tensin homolog deleted on chromosome 10*), which is known to negatively regulate self-renewal and G0→G1 cell cycle entry in NSCs [64]. *PTEN* is frequently mutated in human cancers and plays an important role in brain development [65–68]. Tlx represses *Pten* expression not only in the developing retina [34], but also in the adult brain [60, 69]. Since *PTEN* negatively regulates NSC proliferation [64, 66], it is reasonable to speculate that the ability of Tlx to maintain NSCs in the self-renewal state relies on keeping *PTEN* expression in check.

Cyclin-dependent kinase inhibitors of the Cip/Kip family (including p21 and p57, but not p27) have been reported to be the targets of Tlx in the retina [34] as well as in the cerebral cortex [42, 52]. These findings are in concordance with the observation that p21 and p57 are expressed in more differentiated NPCs in the developing CNS [70]. While increased expression of p21 and p57 provides an appealing explanation for the observed decrease in proliferation among NPCs after mid-neurogenesis and later stages in mutant Tlx animals, these two factors are unlikely to be solely responsible for the premature neurogenesis caused by Tlx mutation since cell cycle arrest by Cip/Kip proteins does not necessarily lead to neural differentiation. Thus, in addition to *Pten*, *p21*, and *p57*, the expression of other NSC regulatory genes, such as *p53* [71] or *p16* [72], may be affected when Tlx is mutated.

Recent studies conducted by Zhao et al. [73] revealed that *microRNA-9* (*miR-9*) is another transcriptional target of Tlx. Interestingly, Tlx is itself a target of *miR-9*. Therefore, Tlx and *miR-9* together form a negative regulatory loop that controls the balance between NSC proliferation and differentiation. Tlx is also subject to regulation by another microRNA: *let-7b* [74]. Therefore, microRNAs appear to serve as differentiation-promoting factors that, by fine-tuning the expression of Tlx in neural precursors, induce their differentiation. Whether or not similar relationships exist between Tll and the corresponding microRNA in *Drosophila* to control NB proliferation and neurogenesis remains to be investigated.

By carrying out gene profile analysis for RNAs isolated from adult brains of wild-type and Tlx mutant mice, Qu
et al. [75] have further identified Wnt7a as another target of Tlx. Wnt proteins are ligands of cell membrane-bound Frizzled receptors. When the Wnt-Frizzled signaling pathways are activated, β-catenin is translocated from the cytoplasm to the nucleus, where it associates with human T-cell factor family transcription factors and activates the target genes [76]. Wnt or β-catenin has been shown to regulate self-renewal of multiple types of stem cells, including hematopoietic stem cells, epidermal and gut progenitors and NSCs in adult brains [77–81]. Intriguingly, Qu et al. [75] characterized Tlx as a direct positive regulator of Wnt7a. They found that the promoter region of Wnt7a contains multiple Tlx binding sites, and their cell-based reporter assays indicate that this promoter region of Wnt7a responds positively to Tlx. This result certainly challenges the current view that Tlx and Tll act as dedicated transcriptional repressors. It would therefore be interesting to investigate which specific factors allow Tlx to activate Wnt7a, and whether additional genes can be activated directly by Tlx. Nevertheless, the finding that Tlx can regulate the expression of a secreted Wnt indicates that Tlx can influence neurogenesis in a non-cell-autonomous manner.

**Mechanisms by Which Tll and Tlx Regulate Gene Transcription**

Since Tll and Tlx are members of the nuclear receptor superfamily, their transcriptional and functional properties are influenced by their associated cofactors. Results from Zhang et al. [34] and our group [63] have shown that transcriptional repression by Tll and Tlx is in part mediated through interactions with Atrophin family proteins. Members of this class of transcriptional corepressors, such as vertebrate RERE (arginine-glutamic acid dipeptide repeats protein) and *Drosophila* Atrophin (also called Grunge), are capable of recruiting diverse histone-modifying enzymes, including histone deacetylases 1 and 2 (HDAC1/2) and a histone H3-K9 methyltransferase (G9a), to repress gene transcription [82, 83]. In cultured NSCs, Sun et al. [69] reported that Tlx interacts with HDAC3 and HDAC5, but not with HDAC1/2. In the retinoblastoma Y79 cell line, Yokoyama et al. [84] reported that the transcriptional repressive effect of Tlx is mediated primarily through its interaction with lysine-specific histone demethylase 1 (LSD1), which is a histone H3-lysine 4 demethylase known to associate with HDAC1/2 and CoREST [85–87]. Using the chromatin immunoprecipitation method, Yokoyama et al. [84] were able to demonstrate that Tlx binds to the Pten promoter, which is also bound by LSD1, CoREST and HDAC1. The ability of LSD1 to regulate the self-renewal of NSCs appears dependent on Tlx because knocking down Tlx blocks the effects on NSC renewal caused by compounds that inhibit the enzymatic activity of LSD1 [88].

These results, taken together, suggest that the associations between Tlx/Tll and specific cofactors or chromatin-modifying factors may be cell type dependent, or perhaps promoter dependent. Since the transcriptional and functional properties of Tlx are influenced by its associated cofactors, each cofactor or chromatin-modifying factor represents a target that allows intrinsic and external signals, and perhaps even chemical compounds, to influence the ability of Tlx or Tll to regulate the expression of its target genes, including *Pten*, *p21* and *prospero* (fig. 4).

**Summary and Prospects**

Tll has been identified for more than 20 years. In the first 15 years, thanks to the in vivo research conducted in both *Drosophila* and the mouse, it became clear that invertebrate and vertebrate Tailless proteins share substantial similarities. The resemblance is apparent not only in their protein structures, expression patterns, and the recruitment of conserved transcriptional cofactors and chromatin-modifying factors, but is also reflected in the parallel roles of both proteins in regulating visual and nervous system development at the functional level. The exciting recent discovery that Tlx is involved in NSC self-renewal and maintenance in vertebrates re-energized the question whether Tll plays a similar role in controlling NSC renewal in *Drosophila*. This question has now been answered by a recent report by Kurusu et al. [45]. They showed that in the mushroom body, mutations of *tll* progressively impair the cell cycle and cause premature loss of NBs. Moreover, overexpression of *tll* induces brain hyperplasia with supernumerary NBs. This behavior of Tll is, once again, shared by Tlx, since Liu et al. [46] recently reported that overexpression of Tlx antagonizes age-dependent exhaustion of NSCs in mice and causes migration of NSCs/NPCs from their natural niche. Moreover, they further determined that several human primary glioblastomas express elevated levels of *Tlx* transcripts. Evidently, uncontrolled Tlx-expressing NSCs contribute to brain tumorigenesis. Thus, caution should be taken when using Tlx-expressing NSCs to treat neurodegenerative diseases.
Because multiple transcriptional cofactors, regulators (such as microRNA) and target genes have been identified for Tlx or Tll, it has now become possible to investigate how these various genetic components respond to local cues (niche) or external stimuli (stress or environmental factors) to influence the activity of Tll and Tlx in regulating brain and eye development and NSC self-renewal. Understanding the respective properties of these modulators of Tlx and manipulating their expression or activities to enhance or decrease the activity of Tlx might contribute to the search for effective therapeutic approaches to treat neurological injury, neurodegenerative disease, or glioblastoma.

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References


