A Tale of Tailless

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Key Words

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Abstract

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 *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.

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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 *Drosophila* early embryogenesis. Recent research on Tll and Tlx in both *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.

Tailless in *Drosophila*

tll was first identified in a mutant screen as a gene required for the development of terminal structures in the *Drosophila* embryo [2]. As reflected in its name, *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–

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6]. The *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, *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 *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 *Krüppel* and *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 *hunchback*, *brachyenteron*, and *forkhead* [3–6]. Based on this information about *tll*, Moran and Jimenez [14] proposed that Tll primarily acts as a dedicated transcriptional repressor in early *Drosophila* embryos.

While the expression level of *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 *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 *tll* within the protocerebrum revealed its expression in the proneural domains that are positive for *lethal of scute* [6], a proneural gene belonging to the *achaet-scute* complex that is crucial for the development of neuroblasts (NBs) [15, 16]. Correlating with its expression in the protocerebrum, mutation of *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 *orthodentile* (*otd*) and *empty spiracles* (*ems*) [6, 17]. However, *tll* is required for establishing the boundary between protocerebrum and deutocerebrum. In *tll* mutant embryos, the deutocerebral structure expands anteriorly,

consistent with the observation of ectopic expression of *ems* from the posterior brain region into the protocerebral domain [18]. Thus, Tll acts as a transcriptional repressor of *ems*. Regulation of *ems* by Tll is direct, since the promoter region of *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 evolutionally conserved transcriptional network that is deployed to maintain self-renewal of NSCs.

Tlx in Vertebrates

The chick *Tlx* gene was first cloned by Yu et al. [19] by screening cDNA libraries using *RXRβ* as the probe. Around the same time, cloning of mouse *Tlx* gene was reported by Monaghan et al. [20], who used *Drosophila 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 *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 transcription, 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.nrsat.org>).

Fig. 1. *tll* is expressed at the terminal regions in early embryos and in undifferentiated neural precursors in tissues at the third instar larval stage. In situ hybridization experiments were performed on stage 4–5 embryos (**a**), on optic lobes (**b**), and on antenna/eye imaginal disks (**c**) isolated from third-instar larvae, using digoxigenin-labeled anti-*tll* RNA probes. The enlarged image shown in **b** represents a lateral view of the optic lobe (OL) where *tll* is specifically expressed in two crescent-shaped clusters of proliferating NBs. **d** A schematic diagram showing the role of Tll in specifying optic lobe cell fate. Founder cells secrete a ligand of EGFR, called ‘Spitz’, which binds EGFR located on the surface of the nearby primordial cells, which have the capacity to become either optic lobe or Bolwig’s organ (light-sensing larval eye) cells. By antagonizing EGFR signaling, Tll directs primordial cells to adopt the optic lobe cell fate. Those primordial cells that lack Tll, in response to the activated EGFR signaling, become Bolwig’s organ. VNC = Ventral nerve cord; MF = morphogenetic furrow.

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

Fig. 2. Comparison between Tll, Tlx, and other NR2 class of nuclear receptors. **(a)** Tll and Tlx are members of the nuclear receptor superfamily. Common features of nuclear receptors include DNA-binding domains (DBD) and ligand-binding domains (LBD). Interactions with corepressors, such as atrophin family proteins, are mediated through the ligand-binding domain, while association with LSD1 is mediated through both the DNA-binding and the ligand-binding domains. **(b)** Alignments of multiple sequences corresponding to the DNA-binding domain of Tailless proteins and other NR2E and NR2F subclasses proteins. P, D and T/A boxes are involved in DNA binding and dimerization. **(c)** Phylogenetic tree showing the relationship among Tailless, Tlx, and

other NR2 family proteins across species using the maximum-parsimony method. The bootstrap consensus tree inferred from 1,000 replicates is taken to represent the evolutionary history of the taxa analyzed. The maximum-parsimony tree was obtained using the Close-Neighbor-Interchange algorithm with a search level of 5, in which the initial trees were obtained with the random addition of sequences (10 replicates). The tree is drawn to scale, with branch lengths calculated using the average-pathway method and are expressed in terms of the number of changes over the whole sequence. Phylogenetic analyses were conducted in MEGA4 [90]. Mu = Mouse; Hu = human.

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 telence-

phalic 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 subpallium 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*

Fig. 3. *Tlx* maintains NSC self-renewal at all stages in the mouse brain. The upper diagram in each panel shows the expression pattern of *Tlx* in the mouse brain at the indicated stage; the lower diagram in each panel is an enlarged view of the ventricular region where *Tlx* is expressed. **a** At the embryonic stage, *Tlx* forms a graded pattern in the telencephalon along the dorsal-ventral axis. Its expression level is high in the dorsal-lateral region, but is low in the ventral-medial region. Its expression in NSCs in the VZ prevents them from undergoing premature neurogenesis and losing self-renewal ability. NSCs at the early stage generate deep-layer cortical neurons by asymmetrical division and later make a transition to *Tbr2*-positive basal progenitors (BP, marked with yellow nucleus) in the SVZ, which give rise to upper-layer neurons

(marked with red nucleus). NSCs that are *Tlx* positive (*Tlx*+) are marked with blue in their nuclei, and nestin-positive (*nestin*+) cells are marked with magenta in their cytoplasm. **b** At the postnatal stage, *Tlx* is expressed in the VZ and is essential for radial glia (RG) to become astrocyte-like B cells, which are the source of adult NSCs. Induced mutation of *Tlx* at this stage causes loss of adult NSCs or decreased proliferative ability. Radial glia at this stage (*Tlx*+/*nestin*+) are pluripotent, giving rise to neurons, oligodendrocytes and astrocytes. **c** During adult stages, *Tlx* is also found in astrocyte-like B cells (GFAP+) at the subependymal zone of the adult CNS. Mutation of *Tlx* causes complete loss of the self-renewal ability of adult NSCs. LGE = Lateral ganglionic eminence; MGE = medial ganglionic eminence.

and dorsally expressed *Pax6* [27–29], the same group tested whether the removal of one allele of *Pax6* from homozygous *Tlx* mutant mice affected their phenotype. Indeed, *Tlx*^{−/−}, *Pax6*^{−/+} mice displayed a more severe phenotype than *Tlx*^{−/−}, with the expression of *Gsh2* further expanded into the pallial region [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 [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

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 $G_0 \rightarrow G_1$ entry and G_1/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.

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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* mu-

tant 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

Table 1. Comparison of *Drosophila tll* and vertebrate *Tlx*

	<i>Drosophila tll</i>	Vertebrate <i>Tlx</i>
<i>Spatial expression</i>		
Visual system	Larval and adult eye, optic lobe	Developing retina
Olfactory system	Mushroom body	Nasal placode, olfactory bulb
Other CNS regions	Protocerebrum	Forebrain, diencephalon
<i>Temporal expression</i>	Embryonic, larval	Embryonic, postnatal and adult stages
<i>Interacting partners</i>		
Co-repressor	Atrophin	Atrophin-1, RERE
Chromatin-modifying factors	Su(var)3-3*, Rpd3*, dG9a*	LSD1, HDACs*, G9a*
Others	Brakeless*	ZNF608*, ZNF609*
<i>Target genes</i>		
Patterning	<i>knirps</i> , <i>ems</i> , <i>otd</i>	<i>Gsh2</i> , <i>Otx</i> , <i>Pax2</i>
Proliferation	<i>prospero</i>	<i>Pten</i> , <i>p21^{Cip1}</i> , <i>p57^{kip2}</i> , <i>Wnt7a</i>
Others		<i>microRNAs</i> , <i>GFAP</i>

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.

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

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 evolutionarily 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

Table 2. Phenotypes of *Tlx* mutant mice

<i>Tlx</i> mutation	Phenotype	Behaviors	References
<i>Germline mutation</i>			
Targeted disruption	Reduced size of rhinencephalic and limbic structures, decreased cortical thickness, retinal and optical nerve degeneration	Severe aggression, spatial learning deficit, blindness, blunted anxiety and fear-conditioning	25, 30, 33
Spontaneous mutation	Hypoplasia of cerebrum and olfactory lobes, abnormal eye development	Aggression, deficits in sensorimotor tests, reduced mating	36, 89
<i>Conditional knockouts</i>			
Nestin-Cre	Hypomorphic dentate gyrus Absence of adult neurogenesis	Impaired spatial learning and memory, normal visual function	42
CaMKII α -Cre (BAC)	Similar to germline deletion except for normal eye development	Normal fear conditioning, normal spatial learning and memory	41
CaMKII α -Cre (promoter cre)	No proliferation defect of NSCs in dentate gyrus	No obvious deficit	42
Induced mutation at postnatal stage	Before P10, loss of adult NSC, after P10, loss of self-renewal ability of NSCs	NA	60
Induced mutation in adult mice	Reduced adult neurogenesis (67%) in dentate gyrus and absence in SVZ	Impaired spatial learning and memory	42, 60

NSCs become determined neural progenitor cells (NPCs) that are destined to produce certain cell types, including neurons, oligodendrocytes and astroglia; 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

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 informa-

tion 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 $G_0 \rightarrow G_1$ 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 dem-

onstrate 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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