Seminars in Cell & Developmental Biology 49 (2016) 102–108

Contents lists available at ScienceDirect

Seminars in Cell & Developmental Biology

journal homepage: www.elsevier.com/locate/semcdb

Subdivision of the lateral plate mesoderm and specification of the forelimb and hindlimb forming domains

Satoko Nishimoto*, Malcolm P.O. Logan**

Randall Division of Cell and Molecular Biophysics, King's College London, Guy's Campus, London SE1 1UL, UK

ARTICLE INFO

Article history:
Received 14 October 2015
Received in revised form
17 November 2015
Accepted 21 November 2015
Available online 28 November 2015

Keywords:
Lateral plate mesoderm
Regionalization
Limb development
Tbx genes
Hox genes
Pitx1

ABSTRACT

The limbs are a significant evolutionary innovation that enabled vertebrates to diversify and colonise new environments. Tetrapods have two pairs of limbs, forelimbs in the upper body and hindlimbs in the lower body. The morphologies of the forelimbs and hindlimbs are distinct, reflecting their specific locomotory functions although they share many common signalling networks that regulate their development. The paired appendages in vertebrates form at fixed positions along the rostral–caudal axis and this occurs as a consequence of earlier subdivision of the lateral plate mesoderm (LPM) into regions with distinct limb forming potential. In this review, we discuss the molecular mechanisms that confer a broad region of the flank with limb-forming potential and its subsequent refinement into distinct forelimb-forming, hindlimb-forming and interlimb territories.

Contents

1. Introduction

Tetrapods form two pairs of appendages, the forelimbs and the hindlimbs, at fixed positions along the rostro–caudal body axis. The axial skeleton in vertebrates consists of several types of vertebrae, cervical (neck), thoracic (chest), lumbar (lower back) and sacral (hip). Forelimbs are formed at the cervical–thoracic boundary and hindlimbs at the lumbar–sacral boundary. This relative position of the limbs and vertebrae is conserved despite of the difference in the number of vertebrae in each region in different species [1]. For example, the chicken has 13 cervical and 7 thoracic vertebrae and the mouse has 7cervical and 13 thoracic vertebrae, however the forelimbs are formed at the cervical–thoracic boundary in both species.

* Corresponding author. Tel.: +44 0 207 848 6886; fax: +44 0 207 848 6435.
** Corresponding author. Tel.: +44 0 207 848 6886; fax: +44 0 207 848 6435.
E-mail addresses: satoko.nishimoto@kcl.ac.uk (S. Nishimoto), malcolm.logan@kcl.ac.uk (M.P.O. Logan).

http://dx.doi.org/10.1016/j.semcdb.2015.11.011

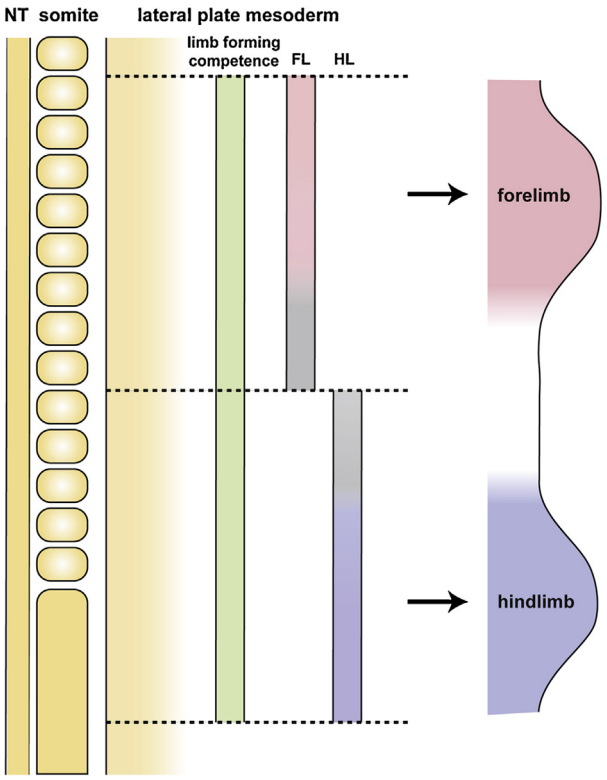

Fig. 1. LPM is divided into subdomains. A broad region of the LPM has a limb forming competence (green), a rostral domain of which has a forelimb forming competence (pink) and a caudal domain of which has a hindlimb forming competence (purple).

The limb developmental programme starts in discrete regions of the LPM (Fig. 1), following an inductive signal from the paraxial mesoderm [2]. Responding to this axial signal, cells in distinct subdomains of the LPM activate genes required to initiate limb outgrowth. These include the T-box transcription factors, *Tbx5* in the forelimb and *Tbx4* in the hindlimb region (discussed in detail in the following sections). *Tbx5* and *Tbx4* establish *Fgf10* expression in the mesenchyme, which subsequently signals to the overlaying ectoderm to activate *Fgf8* transcription. *Fgf8* in turn signals to the mesenchyme to positively regulate *Fgf10* thereby establishing a positive feedback loop [3–7]. This feedback loop of FGF signalling is required and sufficient for both forelimb and hindlimb outgrowth. *Fgf10* mutant mice lack all the limb skeletal elements of autopod, zeugopod and stylopod [4–6] and the phenotype is equally penetrant in forelimb and hindlimb, indicating that while the upstream mechanisms to ensure the establishment of *Fgf10* expression in the forelimb and hindlimb may differ, the objective of establishing *Fgf10* expression and its action are the same. In spite of the common signals shared in forelimb and hindlimb development, limb elements with distinct morphologies are produced. The differences in how forelimb and hindlimb-forming cells will respond to common patterning signals is established early, prior to overt limb bud formation and is a property that is retained even if forelimb cells are grafted into the hindlimb, or vice versa [8]. Here we review the studies that revealed how LPM is divided into subdomains, such as the forelimb forming and the hindlimb forming regions.

2. *Tbx5* and *Tbx4* serve as markers of the subdomains within the LPM

The clearest gene molecular marker of whether cells will produce forelimb, or hindlimb structures are the T-box transcription factors, *Tbx5* and *Tbx4* and a paired-type homeodomain transcription factor, *Pitx1* and a LIM-homeodomain transcription factor, *Islet1* [9–14]. *Tbx5* expression is restricted to the forelimb forming LPM whereas *Tbx4*, *Pitx1* and *Islet1* are restricted to the hindlimb forming LPM.

Tbx5 and *Tbx4* are paralogous genes derived from an ancestral *Tbx5/4* gene. These genes play essential roles in the initiation of limb outgrowth. Both *Tbx5* mutants and *Tbx5* morphants of zebrafish fail to form pectoral fins, the homologous structure of the forelimb [15–17] Furthermore, deletion of *Tbx5* in mouse results in the loss of all the forelimb skeletal elements [18,19]. In human, mutations in *TBX5* are associated with Holt–Oram Syndrome (HOS; OMIM 142900), a dominant disorder characterized by heart and upper limb abnormalities [20,21]. The skeletal abnormalities in the upper limb range from mild triphalangeal thumb to phocomelia in severe cases. These studies demonstrated a conserved role of *Tbx5* in forelimb formation.

Similarly genetic deletion of mouse *Tbx4* leads to outgrowth defects of the hindlimb, although some rudimentary distal structures are formed [22]. This suggests that *Tbx4* is essential for normal hindlimb initiation, however its requirement is not exclusive as *Tbx5* in the forelimb and other factors function redundantly. Mutations in human *TBx4* are associated with Small Patella syndrome (SPS; OMIM 147891), a dominant disorder characterized by dysplasia of patella, pelvis and foot [23].

The restricted expression domains of *Tbx5* and *Tbx4* in the forelimb and the hindlimb, respectively, suggest that these genes could play an active role in determining forelimb and hindlimb morphologies and this was supported by some experiments in the chick [24,25]. Gene deletion–gene replacement experiments in mouse embryos, however, clearly demonstrated that *Tbx5* and *Tbx4* have equivalent roles in the initiation of limb outgrowth and do not control limb-type specific morphology [26]. Ectopic expression of *Tbx4* in the *Tbx5* mutant forelimb can rescue forelimb formation in the absence of *Tbx5* activity demonstrating that *Tbx4* can produce forelimb features and *Tbx5* is not required for forelimb structures to form. There is good evidence, however, that the hindlimb-restricted gene, *Pitx1*, can determine at least some aspects of hindlimb-specific morphology. Forelimbs expressing *Pitx1* ectopically acquire hindlimb-like morphology in chick and mouse embryos [25,27,28]. A similar activity is apparently observed in humans. Liebenberg syndrome (OMIM 186550) is thought to be caused by regulatory mutations in *Pitx1*, causing it to be expressed ectopically in the forelimb. Individuals with Liebenberg syndrome have long arms, elongated metacarpals and dramatically affected elbow joints that have features similar to a knee joint, including a patella [29]. In the mouse, the relatively longer hindlimb metatarsals compared to forelimb metacarpals are generated by increasing the growth rates of the metatarsal primordia during a discrete time-window [30]. This accelerated growth of the metatarsals is regulated by *Pitx1* and the growth rate of metacarpal elements can be made metatarsal-like by ectopic expression of *Pitx1* in the forelimb [30].

The correlation between the expression profile of *Tbx5* and *Tbx4* and the type of limb these cells go on to form has been demonstrated using ectopically induced limbs in the chick inter-limb LPM. A bead soaked with FGF can induce a wing-like structure when placed near the endogenous wing and this ectopic limb bud expresses *Tbx5*, while an FGF bead placed near the endogenous leg can induce a leg-like structure that expresses *Tbx4* [12,13,31]. Perhaps clearest of all are ectopic limbs induced from the middle of the interlimb that have mosaic morphology, the anterior part closest to the wing forms wing digits while the posterior part closest to the hindlimb forms leg digits and this is reflected in the domains of *Tbx5* and *Tbx4*/*Pitx1* expression, which are restricted to the anterior and posterior parts of the ectopic limb buds. These results demonstrate that *Tbx5* and *Tbx4* are markers of the forelimb and hindlimb