Bifacial stem cell niches in fish and plants
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Embryonic development is key for determining the architecture and shape of multicellular bodies. However, most cells are produced postembryonically in, at least partly, differentiated organs. In this regard, organismal growth faces common challenges in coordinating expansion and function of body structures. Here we compare two examples for postembryonic growth processes from two different kingdoms of life to reveal common regulatory principles: lateral growth of plants and the enlargement of the fish retina. In both cases, growth is based on stem cell systems mediating radial growth by a bifacial mode of tissue production. Surprisingly, although being evolutionary distinct, we find similar patterns in regulatory circuits suggesting the existence of preferable solutions to a common developmental problem.

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Growing radially — two systems with similar properties
As an exceptional example for the remodeling of adult body structures, shoots and roots of many plant species grow radially. This is to increase mechanical support for their body and to generate additional vascular tissues harboring long-distance transport capacity. The process is mediated by a stem cell niche called the cambium which produces water-transporting cells (xylem, wood) toward the organ center and carbohydrate-transporting cells (phloem, bast) toward the organ periphery [1] (Figure 1a,a')). Like their differentiated progenies, cells in the cambium are much longer than wide and divide mostly along their longest axis and in parallel to the organ surface. Thereby, shoots and roots not only expand laterally but, at the same time, the diameter of the cylindrical cambium domain enlarges overall. For example, the total cell number as captured in cross sections from the shoot-to-root boundary in Arabidopsis italiana increases by a factor of 20 within 20 days. Concomitantly, there are 14 times more cambium cells by the end of this period [2]. Tangential expansion of the cambium domain is achieved by symmetric cell divisions perpendicular to the organ surface, thereby increasing the size of the stem cell pool. During tissue formation in radial orientation, xylem and phloem cells expand and acquire a specialized morphology facilitating intra-cellular long-distance transport [3].

Like plants, teleost fish such as medaka (Oryzias latipes) harbor the remarkable feature of life-long growth, which is accompanied by continuous growth of their organs. One instructive example is the eye which grows in two phases. During embryogenesis, a lateral out-pocketing of the neural tube forms an optic vesicle which transforms into the bi-layered optic cup (embryonic retina). The lens-facing layer of the optic cup gives rise to the neuroretina (NR) and the lens-averted layer, initially functioning as reservoir for both, NR and retinal pigment epithelium (RPE), eventually forms the RPE (Figure 1b,b')) [4]. The NR represents the light sensitive, multi-layered tissue composed of one glial and six neuronal cell types [5]. The surrounding RPE is crucial for maintaining the proper performance of photoreceptors in the NR and acts as a light barrier [6]. At the end of embryonic development, a distinct marginal zone of the NR and RPE termed ciliary marginal zone (CMZ) has been established that forms a ring surrounding the lens (Figure 1b,b')). Distinct stem cells for NR and RPE reside in the CMZ and facilitate postembryonic growth by producing proliferating tissue progenitors [7]. Importantly, NR and RPE progenitors and differentiated cells are separated by the cavity of the ventricle which extends into the retina (gray line Figure 1b')). Thus, like the cambium, juvenile CMZ-based tissue production follows a bifacial mode adding NR and RPE cells centrally and peripherally to the CMZ (Figure 1b)). Lateral expansion of the eye is accompanied by an increase in retinal stem cell (RSC) number, the activity of which massively increases the size (cell number) of the retina by a factor of 10 within the first 20 days after initial retinogenesis [7,8**].

In plants, extracellular matrices, the cell walls, prevent cell migration and cause a largely invariant cell arrangement. Strikingly, a similar situation is found in the fish
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Figure 1

Schematic overview of the bifacial cambial and retinal stem cell niches of plants and fish.
The geometry of the cylindrical plant stem (here Arabidopsis thaliana) and the hemispherical vertebrate eye (here Oryzias latipes) is provided by a radial niche of stem cells located inside the tissue. From here, coordinated growth occurs in two directions (centrally and peripherally) giving rise to distinct tissues.
(a–a’) For plants, cambial stem cells (CAM, magenta) give rise to central xylem (wood, blue) and to peripheral phloem (bast, green).
(b–b’) In the juvenile fish, retinal stem cells reside in the ciliary marginal zone (CMZ, magenta) producing both, the lens-facing neuroretina (NR, blue) and the lens-averted retinal pigment epithelium (RPE, green) that are physically separated by the cavity of the ventricle (gray line in (b’)). Growth is driven by both, preferential asymmetric cell divisions that result in one stem and one progenitor cell (white arrows, periclinal in plants, radial in fish) and rare symmetric cell divisions to expand the stem cell pool (black arrow, anticlinal in plants, circumferential in fish). Immediate descendants are further subjected to massive proliferation and subsequent differentiation.
(a’) In plants, progenitors (purple) on either side of the cambium undergo differentiation into a variety of specialized cell types. (b’) In contrast, the retinal progenitor cells (RPCs, purple) differentiate into only one cell type in case of the RPE and into six neuronal and one glial cell type for the NR. These are organized in a stereotypical layering (ONL, outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer; RGC, retinal ganglion cell layer; ON, optic nerve).
a’ modified from Ref. [19], with permission from Elsevier.
b drawing of adult medaka reprinted from Ref. [45], with permission from Elsevier.

retina. To maintain its functionality, it is mandatory that cells – at the time of terminal differentiation – take and retain their position in a pseudo-crystalline arrangement. This arrangement readily allowed revealing clonal relationships. In the cambium recent analyses have demonstrated a bipartite organization of the cambium reflected by distinct expression domains of cambium regulators [9]. Similarly, lineage analyses in the fish retina following cell transplantation has uncovered a bipartite stem cell niche in the CMZ that is composed of a mixed population of distinct stem cells for either the NR or the RPE [7]. Clonal analyses in the postembryonic NR using inducible
cell lineageing tools highlight the same behavior also in adult RSCs [8**]. One lineage is formed by predominant asymmetric divisions resulting in the formation of one RSC and one retinal progenitor cell (RPC) rather than symmetric divisions resulting in two RSCs or RPCs as expected in a more stochastic mode of cell division [8**,10]. For the cambium, clonal relationships within and between cambium domains have not been established yet. However, a large body of anatomical evidence argues for the presence of only one bifunctional pool of stem cells producing both xylem and phloem tissues, which seems to contrast the situation in the CMZ (Figure 1) [11]. Collectively, the cambium in plants and the CMZ in fish both represent bifacial stem cell systems with a local fixation of terminally differentiated cells providing opportunities for revealing regulatory constraints.

**Homeobox transcription factors act as key regulators**

In the cambium and the CMZ, homeobox transcription factors promote stem cell activity (Figure 2). One such key factor in Arabidopsis is the homeobox transcription factor WUSCHEL RELATED HOMEBOX 4 (WOX4). WOX4 is exclusively expressed in the cambium and the number of cambial stem cells is reduced in wox4 mutants [12,13]. Yet, no significant increase of cambial cell number or general differentiation deficits is observed upon ectopic WOX4 expression demonstrating that WOX4 alone is not sufficient for promoting stem cell properties [12,13]. In contrast to WOX4, expression of the retinal homeobox gene family Rx is not restricted to the CMZ but found as well in photoreceptors and Müller glia of the differentiated NR [14**]. Still, Rx2 functions as a stem cell marker and a balancer in stem cell fate decisions [14***]. Moreover, loss of Rx2 and its orthologs leads to a lack of eye structures in multiple species, at least partly, due to a reduction in stem cell number [15–18].

Interestingly, intercellular movement of WOX transcription factors is a common concept of stem cell maintenance in plants [19]. In Arabidopsis, the two WOX proteins WUSCHEL (WUS) and WOX5 are specifically expressed in areas with reduced cell division rates within the shoot and root apical meristem, respectively, and travel to the actual stem cells where they promote stem cell activity [20,21]. Whether movement is essential for the function of WOX4 in the cambium and whether WOX4 expressing cells act as a niche to maintain stem cell function in their close vicinity is currently unclear. Looking at the situation in the fish eye, non-cell autonomous activity of homeoproteins has been suggested [22,23]. Considering this observation, a similar mechanism for Rx and WOX4 transcription factors to promote proliferation of stem cells or tissue-specific progenitors is possible. Regulating directionality of lateral movement, which is tightly regulated in the shoot apical meristem [20], would be one elegant way to balance the bidirectional production of tissues in bifacial stem cell niches.

**Boundary formation by mutually inhibitory interactions**

The exceptional importance for radial patterning in the context of a bifacial mode of tissue production is reflected by mutual interactions between intercellular signaling cascades determining cell identities and fate transitions in the CMZ and, most likely, in the cambium (Figure 2). In fact, mutual inhibitory interactions of two signaling cascades, namely auxin and cytokinin signaling, play crucial roles in boundary formation during initial plant vascular development [24**,25*,26]. Cytokinin signaling is active in procambial cells whereas auxin signaling is active in protoxylem cells [26]. Auxin signaling inhibits cytokinin response by stimulating transcription of the cytokinin signaling repressor AHP6 [26]. In turn, via diverse mechanisms, auxin signaling is inhibited in cells with high cytokinin response [24**,26,27]. These mutual inhibitions are thought to build a sharp boundary between

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**Figure 2**

(a) CLE41/44-PXY signaling promotes proliferation through WOX4 and, in parallel, blocks xylem differentiation through GSK activation. Spatial differences in plant hormone signaling may define the boundaries between phloem, cambium and xylem.

(b) Cross-regulation between Wnt and Hh signaling pathways through expression of the antagonists Gl3 and Sfrp-1, fine-tunes proliferative activity of stem cells (SC) and progenitors. CMZ, ciliary marginal zone; NR, neuroretina; RPE, retinal pigment epithelium; white arrow, asymmetric cell division.
two regulatory domains determining respective cell fates. Interestingly, a recent study using Populus trichocarpa, a deciduous tree, revealed that auxin and cytokinin have distinct spatial distribution with auxin peaking in the cambium and cytokinin in the developing phloem [28**, suggesting that similar mechanisms reside in the cambium. Moreover, gibberellic acid, another plant hormone, is enriched in developing xylem cells (Figure 2(a)) [28**]. Connecting hormonal signaling with stem cell control, WOX4 is important for the positive effect of auxin on cambium proliferation [13]. Whether this reflects a requirement of WOX4 for an auxin–cytokinin crosstalk or a more direct role downstream of auxin signaling remains to be determined.

In vertebrates, signaling pathways constraining the proliferative domain of the CMZ have been analyzed in more detail (Figure 2(b)). First, Hedgehog (Hh) signaling has been proposed to promote the transition from slowly cycling stem cells to faster cycling progenitor cells [29,30]. Second, an antagonistic crosstalk between Hh and Wnt signaling pathways controls tissue patterning (Figure 2(b)). Hh transcription is enriched in the RPE and the ganglion cell layer of the NR. From here, the Hh ligand diffuses to the CMZ expressing the Hh receptor Patched 1 (Ptc1) [31]. Overactivation of Hh signaling increases the transcription of the Wnt antagonist Secreted frizzled-related protein 1 (Sfrp-1) and results in the reduction of proliferation in the CMZ [31]. Conversely, the Wnt-dependent Glioma-associated oncogene family zinc finger 3 (Gli3) represses Hh signaling [31]. This antagonistic cross-regulation fine-tunes the proliferation of retinal stem and progenitor cells and thus constrains the CMZ spatially. In light of the life-long activity of both, the CMZ and the cambium, and a possible plasticity of the organs produced [32], a tightly controlled but very dynamic boundary formation may be another inherent feature of both systems.

Control of patterning and differentiation by intercellular signaling

One of the key intercellular signaling pathways controlling cambium patterning is CLAVATA3/EMBRYO SURROUNDING REGION-related/PHLOEM INTERCALATED WITH XYLEM (CLE41/44-PXY) signaling. CLE41 and CLE44 genes are expressed in the phloem from where, after posttranslational processing, CLE41 and CLE44 dodecapeptides are thought to be secreted [33,34]. These peptides bind directly to the PXY receptor-like kinase which is expressed in the cambium (Figure 2(a)) [9,33,34,35**,36*,37]. CLE41/44 application and overexpression experiments show that the CLE41/44-PXY module induces cambium proliferation and, at the same time, suppresses xylem differentiation [33,34]. The effect of the module on cambium proliferation depends fully on the WOX4 and the redundantly acting WOX4 gene [12,38]. As a separate function, CLE41/44-PXY binding activates glycogen synthase kinase 3 proteins (GSK3s) which, in turn, inhibit the transcription factor BR1-EMS SUPPRESSOR 1 (BES1) thereby countering xylem differentiation [39]. Interestingly, in other contexts GSK3s mediate phosphorylation of auxin-related ARF transcription factors in a CLE41/44-PXY-dependent manner [40] providing the possibility to cell-autonomously modulate auxin signaling in cambium stem cells. Together, these characteristics define the phloem as a part of the local niche essential for cambium proliferation and patterning and, together with Hh signaling in the CMZ, exemplify how bifacial stem cell systems communicate with their differentiated descendants.

As an additional feature, CLE41 seems to provide positional information for orienting cell divisions in parallel to the organ surface [34,41]. In the medaka CMZ, orientation of the cell division axis is crucial to maintain the hemispheric structure of the eye during growth. This is accomplished by a tightly coordinated ratio of preferential asymmetric (radial growth) and rare symmetric (circumferential growth, i.e., expansion of the stem cell pool) cell divisions [8]. The same situation holds true for the cambium [42] and mechanical stimuli have been discussed as determinants for alternating between radial and tangential divisions of stem cells in this case [43]. Although a set of auxin-dependent basic helix-loop-helix (bHLH) transcription factors determine the orientation of division axes of vascular cells in other contexts [24**,44], the molecular mechanism coordinating the division axes has been neither addressed in the cambium nor in the CMZ.

Conclusion

The comparison of molecular patterns determining lateral plant growth and the growth of the fish eye reveals striking similarities in their regulatory principles. In both cases, bifacial stem cell niches are regulated by homeobox transcription factors maintaining life-long growth. Also, stem cells are in both cases under the influence of signaling molecules sent by stem cell descendants ensuring stem cell maintenance and balanced production of two tissues at the same time. A dynamic formation of signaling boundaries may be an inherent property of a bifacial stem cell niche allowing the adaptation of the systems over time. Importantly, mechanisms coordinating the production rates of two tissues, determining tissue identity or orienting cell divisions, all essential features of bifacial stem cell niches, are only beginning to emerge. Since the two systems are evolutionary fully distinct, it will be exciting to see how these problems have been solved independently in two different kingdoms of life.

Conflict of interest statement

The authors declare no conflicts of interest.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
◆ of outstanding interest


In this paper, the authors use clonal analysis to show that stem cells in the postembryonic retina of medaka preferentially divide asymmetrically during homeostatic growth.


The authors show that MOL1, a leucine-rich repeat receptor-like kinase represses ciliary activity and is expressed in the distal ciliary domain distinct from PXY expressing cells.


In this paper, the authors show that Rx2 functions as a stem cell marker and balances stem cell fate decisions between neuroretina and retinal pigment epithelium.


The authors show that cytokinin biosynthesis is controlled by auxin signaling through the LOG4 enzyme and that local cytokinin production is important for vascular patterning in vivo and also in computational models.


The authors reveal hormonal and gene expression profiles along radial cambium domains in Populus trichocarpa.


signalling pathways controls post-embryonic retinal proliferation. *Development* 2012, **139**:3499-3509.


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