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VIEWPOINT

Evolution of patterning

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Developing tissues are patterned in space and time; this enables them to differentiate their cell types and form complex structures to support different body plans. Although space and time are two independent entities, there are many examples of spatial patterns that originate from temporal ones. The most prominent example is the expression of the genes *hunchback*, *Krüppel*, *pdm*, and *castor*, which are expressed temporally in the neural stem cells of the *Drosophila* ventral nerve cord and spatially along the anteroposterior axis of the blastoderm stage embryo. In this Viewpoint, we investigate the relationship between space and time in specific examples of spatial and temporal patterns with the aim of gaining insight into the evolutionary history of patterning.

Time and space in development

Living organisms are dynamic entities that are patterned in time and space. These two axes of patterning are necessary to generate the organismal cell type complexity.

Time is a fundamental parameter of life measured at very different scales, from the milliseconds or seconds of biochemical reactions to the minutes of the cell cycle, the daily circadian rhythms and finally the millions of years of evolution. In developmental biology, dynamic entities, such as cells and tissues, develop over time relying largely on intrinsic timers that allow for a cell-, tissue-, and species-specific developmental pace [1]. Such timers can be based on oscillating gene expression patterns that allow cells to change function and role periodically over time [2,3] or on the successive expression of different genes [4]. An extreme, but very efficient, example of this change is the temporal patterning of neural progenitors, where they temporally express a series of transcription factors that allows them to generate progeny with different fates depending on their developmental age [5–7] (Fig. 1A).

The position of a cell in space, that is, along the anteroposterior (AP), dorsoventral (DV), and left–right (LR) axes of a complex organism, is another fundamental parameter, as the cell has to integrate its environment and interact with its neighbors. Space in and of itself, unlike time, cannot be intrinsic; every cell's position in space is defined by its environment and neighboring tissue. During development, the influence of the environment on a cell or tissue is called spatial patterning. This is often defined by the exposure to different signals, such as morphogenetic cues. An elaborate example of spatial patterning is the patterning of neuronal progenitors along the DV axis of

Abbreviations
AP, anteroposterior; BMP, bone morphogenetic protein; Cas, Castor; Dll, *Distalless*; DV, dorsoventral; Erm, earmuff; Hb, hunchback; Hbn, homeobrain; Hes, hairy/E(spl)-related; Hth, homothorax; Kr, Krüppel; LR, left–right; PSM, presomitic mesoderm; SAZ, segment addition zone; Scro, scarecrow; Shh, Sonic Hedgehog.

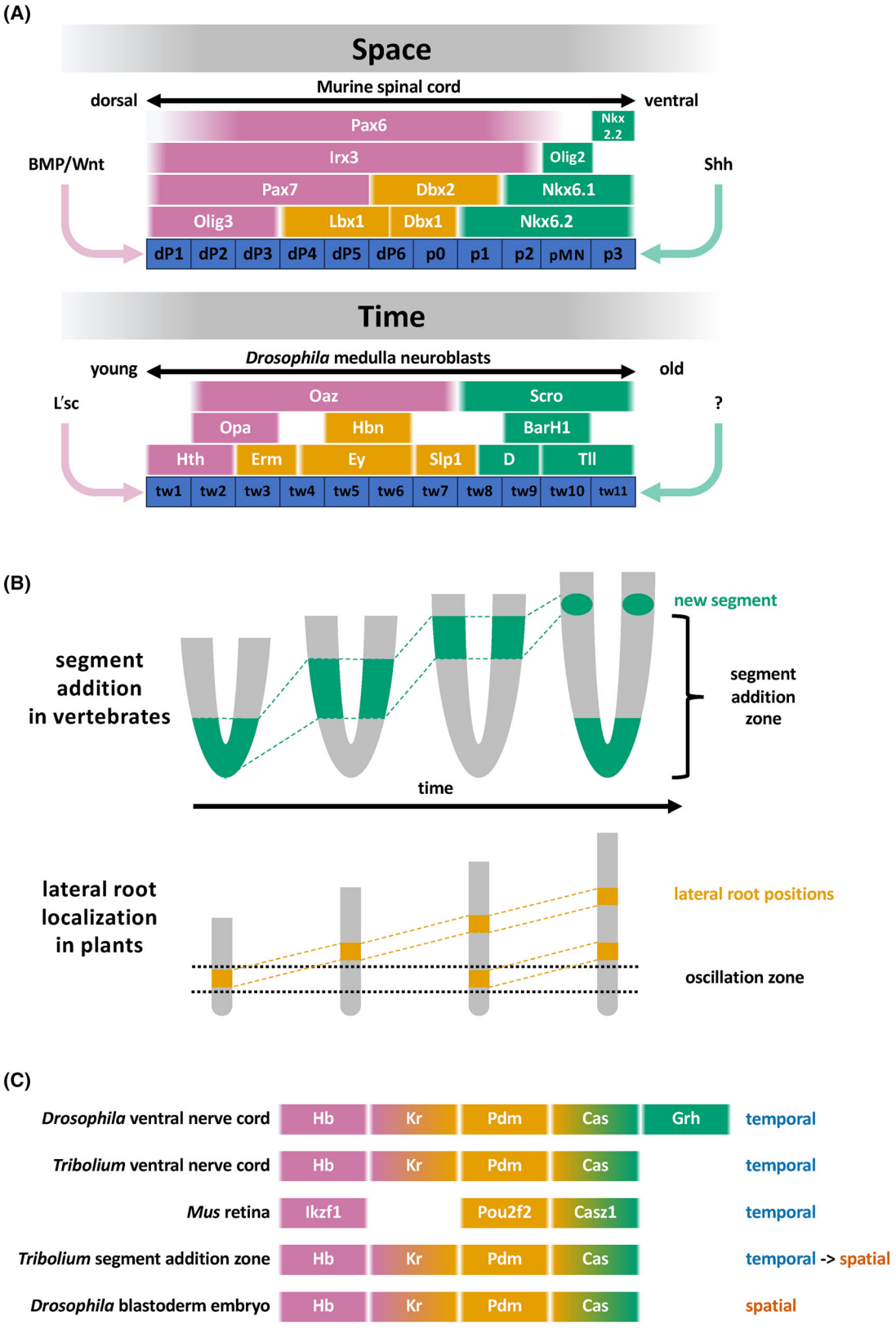

Fig. 1. Temporal and spatial patterning in animals and plants. (A) Spatial patterning: The expression of BMP and Wnt from the dorsal roof plate and Shh from the ventral floor plate leads to the generation of 11 spatial compartments (blue) along the dorsoventral axis of the murine spinal cord. Each of these domains expresses a unique combination of transcription factors (dorsal: pink, medial: orange, ventral: green) that allows it to generate different neuronal types. Temporal patterning: In a similar manner, the life of a *Drosophila* optic lobe neuroblast is divided into (at least) 11 different temporal windows (blue). Each of these temporal windows expresses a unique combination of transcription factors (early: pink, medial: orange, late: green) that allows it to generate different neuronal types. (B) Segmentation in vertebrates uses molecular clocks to generate new segments along the anteroposterior axis of the animal. Similarly, in plants, gene expression oscillation determines the periodic localization of lateral roots. Green signifies the cyclic expression of genes such as *hairy*, while orange illustrates the cyclic activity of the auxin responsive promoter DR5. (C) The transcription factor sequence Hb/Kr/Pdm/Cas is expressed in a temporal and/or spatial pattern in different tissues, such as the *Drosophila* ventral nerve cord and blastoderm embryo, the *Tribolium* ventral nerve cord and segment addition zone, and the mouse retina. The color code of the transcription factors follows the one from (A).

the vertebrate spinal cord, where opposing gradients of Wnt and bone morphogenetic protein (BMP) from the roof plate and Sonic Hedgehog (Shh) from the floor plate, lead to the generation of spatially distinct progenitor domains that can give rise to very different neuronal types [8,9] (Fig. 1A).

Temporal and spatial patterning are two independent modes of regulation; however, spatial patterns can be products of molecular timers [10–12]. For instance, segmentation in vertebrates and invertebrates uses molecular clocks and morphogens to generate spatial patterns that define the animal anteroposterior axis [3,13]. Similarly, in plants, gene expression oscillation determines the periodic localization of lateral roots [14] (Fig. 1B). While the importance of temporal sequences in the formation of spatial patterns is undoubted, very few examples have been recorded in the literature where temporally expressed factors display also spatial patterns in a different context.

One remarkable example of this is the transcription factor series of Hunchback (Hb), Krüppel (Kr), Pdm, and Castor (Cas): this series is expressed in a temporal sequence in the neuronal stem cells of the *Drosophila* ventral nerve cord [15], but is also expressed in a spatial sequence along the anteroposterior axis of the *Drosophila* embryo at the cellular blastoderm stage [15]. In addition, the same genes are expressed in a temporal sequence in the segment addition zone of *Tribolium castaneum* to regulate segment identity along the anteroposterior axis [16–18], as well as in the neural stem cells of the ventral nerve cord (like in *Drosophila*) [19]. Finally, the vertebrate orthologs of three of these genes (Ikzf1/4, Pou2f2, and Casz1) are expressed in the same temporal manner in the vertebrate retina neural stem cells specifying the descendant neuronal types [20] (Fig. 1C).

Is this series a remarkable exception or the rule? Have other temporal sequences been co-opted for spatial patterning? In this Viewpoint, we will examine two prominent examples of spatial patterning (one from vertebrates and one from insects) and their relation to temporal events, as well as present a more contemporary view of morphogen activity in tissue patterning, based on the duration of their expression. Conversely, we will examine the expression of *Drosophila* temporal genes in space and, finally, we will envisage an evolutionary scenario that links temporal and spatial patterning.

The Hox clock

The most famous example of spatially distributed genes is the Hox genes. In most bilaterian animals, Hox genes form clusters of at least seven members, organized genomically in a conserved order and transcribed collinearly along the AP axis [21–23]. The "Hox clock" operates during axial elongation in the posterior embryonic growth zone of vertebrate embryos; Hox genes are transcribed sequentially according to their genomic position in the Hox cluster (temporal collinearity) [24]. As the "Hox clock" coincides with axial elongation, this temporal collinearity is translated into a set of spatial coordinates from anterior to posterior (spatial collinearity) [25]. This positional information is then transferred to the differentiated progeny, which contribute to tissue expansion along the growth axis. The translation of sequential Hox gene activation into spatial patterns in developing axial tissues occurs in all vertebrates studied to date [26].

Notably, the "Hox clock" provides also the mechanism that ensures the spatial distribution of the patterning information by regulating the timing of mesodermal cell ingression. Progenitor cells that express anterior Hox genes ingress earlier and occupy more anterior positions along the body axis [27], while, when the last genes of the cluster are expressed, they inhibit the function of earlier HOX proteins, leading to a slower extension of the axis, until extension is terminated [28]. Importantly, after the progenitor cells have ingressed, they maintain their Hox gene expression, allowing the establishment of stable spatial

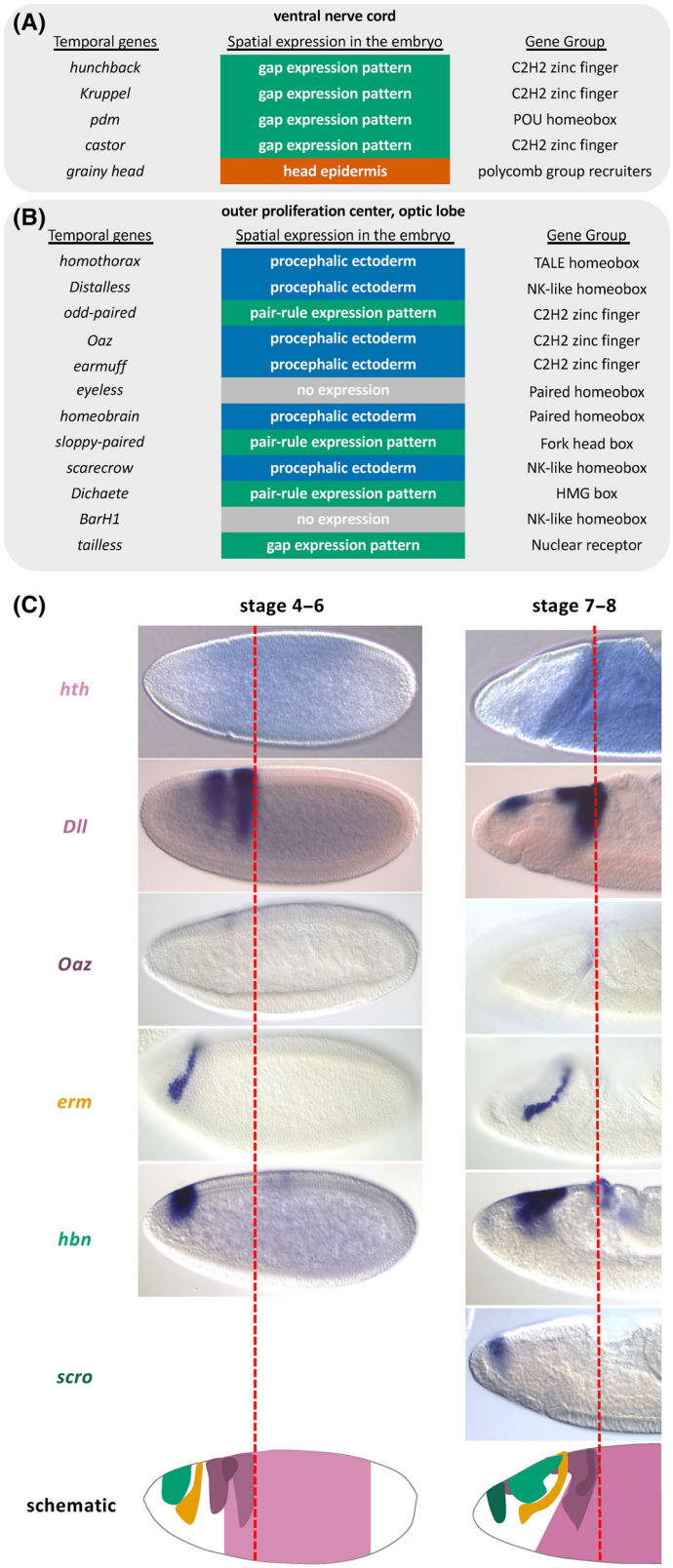

Fig. 2. Spatial expression of neuroblast temporal transcription factors in the blastoderm embryo. (A) The temporal transcription factors of the *Drosophila* ventral nerve cord neuronal stem cells are expressed in a gap-like spatial pattern (green) in *Drosophila* blastoderm embryo. A marked exception is the last temporal transcription factor, *grainy head* [55], which does not follow this pattern and is expressed in the head epidermis instead. (B) The *Drosophila* optic lobe temporal transcription factors can be divided in three categories: (1) *eyeless* (*ey*) and *BarHI* are not expressed in the blastoderm embryo and start to be expressed later. (2) *odd-paired* (*opa*), *Dichaete* (*D*), and *tailless* are expressed in a spatially sequential pattern along the AP axis. Interestingly, *D* is expanded posteriorly in *tailless* (*tll*) mutants in a genetic interaction reminiscent of the optic lobe neuroblasts, where in a *tll* mutant, *D* expression is extended in time [56]. *sloppy paired* (*slp1*) is also expressed in a spatially restricted pattern acting as a pair-rule gene [57]. (3) Finally, *hth*, *Dll*, *Oaz*, *erm*, *hbn*, and *scro* are expressed in the procephalic ectoderm. (C) Interestingly, the temporal genes *hth*, *Dll*, *Oaz*, *erm* (early), *hbn* (middle), and *scro* (mid-late) are expressed in the same spatial order along the anteroposterior axis of the procephalic ectoderm in embryonic stages 4–8. The early temporal gene *hth* is expressed posteriorly in the procephalic ectoderm (as well as in the trunk). The early temporal genes *Dll* and *Oaz* are also expressed posteriorly. Then, the later temporal genes *erm*, *hbn*, and *scro* are expressed progressively more anteriorly. Images from the Berkeley Drosophila Genome Project (https://insitu.fruitfly.org/) [58–60].

domains [27]. Recent studies have provided insight into how the proper timing of Hox gene expression is regulated by enhancers that receive temporal signals at the initial side of the cluster, and subsequent colinear Hox gene expression is achieved by changes in the restrictions imposed by chromatin structure [24,29].

Finally, it is possible that the "Hox clock" is molecularly linked to the segmentation clock of the presomitic mesoderm (PSM), which is driven by oscillations of the Hes (Hairy/E(spl)-related) family of transcription factors to give rise to the future vertebral column (Fig. 1B) [2]. The oscillating expression of several Hox genes in the PSM is directly related to the segmentation clock, and a shift in somite boundaries can lead to a repositioning of Hox boundaries [30]. Furthermore, loss of Hoxb6 in the mouse embryo has been shown to lead to defects in somite formation and the segmentation clock [31]. On the other hand, evidence from zebrafish mutants in which axis identity is maintained, but segmentation proceeds slowly, challenges the idea of a direct link between the two processes [10,32].

Pair-rule and gap genes in insects

A prominent example of spatially expressed genes are the gap, pair-rule, and segment polarity genes of insects. First, gap genes drive AP axis determination and later regulate the periodic expression of pair-rule genes that mediate the segmentation along the AP axis [33]. Segmental boundaries and regional identities are finally maintained by the expression of the downstream segment polarity and Hox genes.

Insects were initially divided into long-germ and short-germ insects depending on whether segment formation occurs simultaneously or sequentially during embryogenesis [34]. In the long-germ insect *Drosophila melanogaster*, segments are formed during the blastoderm stage under the regulation of morphogen gradients that cover the entire AP axis of the embryo. These gradients are formed by the localization of maternally deposited mRNAs, such as *bicoid* and *hunchback* anteriorly and *nanos* and *caudal* posteriorly. During this process, gap and pair-rule genes are seemingly simultaneously expressed in their respective domains along the AP axis. However, in the short germ beetle *Tribolium castaneum*, with the exception of a few anterior segments, most segments form sequentially during germ band elongation from a posterior growth zone called the segment addition zone (SAZ) [33]. During this process, gap and pair rule genes are expressed in a temporally sequential manner in the SAZ, and only when individual cells exit the SAZ, gap gene expression is stabilized, leading to a spatial pattern of expression along the AP axis. Other insects, such as *Nasonia vitripennis*, employ an intermediate mode of segmentation [35,36], suggesting that the long- and short-germ strategies presented above may in fact be the two extremes of a continuum, rather than two distinct mechanisms [34].

Indeed, modeling, live imaging and *in situ* stainings of gap gene expression in the *Drosophila* blastoderm have shown that gap gene expression regions are not static over time but shift from posterior to anterior [37–39]. The cells at the shifting boundary between two domains switch from expressing one gene to another, similar to their sequential expression in the SAZ of *Tribolium*. Based on this, a non-periodic oscillator [37] involving the *Drosophila* gap genes has been proposed to underlie their spatial sequential expression, supporting further the idea that what has been considered as spatial patterns in *Drosophila* has essentially evolved from ancestral molecular clocks [17,40]. In addition, computational models in which segmentation and the pair-rule network were studied through simulations, showed that segmentation in *Drosophila* relies on pair-rule gene expression progressing across cells over time, supporting previous quantitative studies and challenging the notion of pre-structured spatial patterns at the whole-tissue level [41].

Temporal interpretation of morphogen gradients

Morphogen gradients, despite being well-established spatial organizers, have also been suggested repeatedly to act in a temporal manner. Morphogen gradients can pattern tissues based on the time of exposure of the cells to the morphogen rather than based on the morphogen concentration, as the traditional morphogen gradient model suggests [42]. For example, chick neuronal cells can interpret different concentrations of Sonic Hedgehog as if there were proportionally different time periods of Sonic Hedgehog signal transduction [43]. Moreover, the timing and sequence of spatial gene expression in the mouse spinal cord depends not only on the concentration of the external signal, but also on the exposure time [44].

Similarly, the duration of exposure (and not concentration) of human pluripotent stem cells to BMP signaling determined whether cells will remain pluripotent or whether they will differentiate to mesodermal or extra-embryonic states [45]. Another prominent example of a morphogen gradient is Bicoid; cells seem to be interpreting duration of exposure to Bicoid on top of morphogen concentration [46]. Interestingly, this temporal mode of operation of morphogen gradients can lead to pattern formation in unicellular organisms too. Colonies of *Escherichia coli* can generate self-organized ring patterns in the absence of a morphogen gradient, where the morphogen serves as a timer to allow for the bacteria to respond to environmental differences [47].

The speed regulation model was recently proposed to reconcile the spatial and temporal activities of a morphogen gradient; this model posits that different concentrations of a morphogen can differentially modulate the speed of a temporal sequence leading to the establishment of spatial patterns [10,17].

Evolution of spatial from temporal patterns

Temporal transcription factor series have been described in different contexts [48], but nowhere nearly as comprehensively as in the *Drosophila* developing optic lobe [49,50]. As mentioned earlier, the transcription factors that are expressed temporally in the *Drosophila* ventral nerve cord neuronal stem cells are also expressed along the AP axis of the blastoderm embryo like gap genes (Fig. 2A). Interestingly, many of the *Drosophila* optic lobe temporal genes are also expressed spatially along the AP axis of the developing procephalic ectoderm (Fig. 2B,C), where early temporal genes (*homothorax* – *hth*, *Distalless* – *Dll*, *Oaz*, and *earmuff* – *erm*) are expressed more posteriorly than middle (*homeobrain* – *hbn*) and later temporal genes (*scarecrow* – *scro*).

The above raises a few questions and hypotheses:

First, how did the spatial patterns evolve from a temporal series (or vice versa)? Notably, in the developing *Drosophila* optic lobe, static images of temporal transcription factors expression appear as distinguishable spatial gene expression patterns, which are then lost in the adult structure [49,50]. This suggests that this temporal mode of patterning is conceptually analogous to the clock-based patterning of the anteroposterior axis in vertebrates and insects. In this case, neuronal fate is fixed upon division of the progenitors in a similar manner to the fixation of AP fate of the cells that exit the SAZ or the PSM. This type of patterning has been described as a wavefront-based model [10,17]. The emerging possibility is that the ancestral temporal gene expression was "translated" into a spatial pattern, in a manner similar to the gap/pair-rule gene spatial pattern in *Drosophila* that originated from the temporal profile of a sequentially segmenting ancestor.

Second, how has it occurred that the same genes that are involved in temporal patterning of neuronal stem cells pattern also the anteroposterior axis of the embryo? Do these tissues share a common evolutionary origin or were the gene regulatory networks co-opted to regulate the expression of these transcription factors in space? To answer this question, we first need to understand how the temporal series of the stem cells and the gap/pair-rule gene network in the AP axis evolved and how the respective gene regulatory networks were assembled [51,52]. Unfortunately, only minimal information regarding these two networks exists beyond the traditionally studied animal systems.

Finally, we would like to propose a scenario for the evolution of tissue patterning: we have described above how spatial patterns can arise from temporally expressed genes, which makes the evolutionary trajectory from temporal to spatial patterning particularly compelling. Moreover, it is obvious that single-celled organisms can pattern themselves in time, but not in space (in the absence of a three-dimensional tissue structure), in a manner similar to the aforementioned patterning of *E. coli* [47]. To achieve this more efficiently, it is possible that they used the sequential expression of series of transcription factors that allowed them to change their phenotype quickly and drastically (since transcription factors can regulate the expression of many gene batteries at the same time). This would have allowed them to change their

function over time, changing essentially their cell type (or state), while adapting to a changing environment. As more complex organisms evolved, existing temporal sequences of transcription factors were used to pattern the more complex tissues in space, allowing the organism to have multiple different cell types at the same time. This model of temporal-to-spatial transition has been proposed before to explain the evolution of different animal cell types [53,54]. We believe that the discovery of the same series of transcription factors in different organisms (mice, beetles, and flies) and tissues (retina, embryo, and brain) is in favor of this hypothesis.

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Conflict of interest

The authors declare no conflict of interest.

Author contributions

KF and NK conceived the idea and wrote the manuscript.

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