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1 Evolution of patterning

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10 11 **Running title**

12 Evolution of patterning

13 14 **Abbreviations**

15 AP, anteroposterior; DV, dorsoventral; LR, left-right; PSM, presomitic mesoderm; SAZ, segment
16 addition zone

17 18 **Keywords**

19 spatial patterning; temporal patterning; transcription factors; nervous system; evolution

20 21 **Conflicts of interest**

22 The authors declare no conflicts of interest.

23 24 25 26 **Abstract**

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

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41 **Time and space in development**

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

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

53 The position of a cell in **space**, i.e. along the anteroposterior (AP), dorsoventral (DV), and left-
54 right (LR) axes of a complex organism, is another fundamental parameter, as it has to integrate its
55 environment and interact with its neighbors. Space in and of itself, unlike time, cannot be intrinsic;
56 every cell's position in space is defined by its environment and neighbouring tissue. During
57 development, the influence of the environment on a cell or tissue is called spatial patterning. This is
58 often defined by the exposure to different signals, such as morphogenetic cues. An elaborate example
59 of spatial patterning is the patterning of neuronal progenitors along the DV axis of the vertebrate spinal
60 cord, where opposing gradients of Wnt and BMP from the roof plate and Sonic Hedgehog (Shh) from
61 the floor plate, lead to the generation of spatially distinct progenitor domains that can give rise to very
62 different neuronal types [8,9] (Figure 1A).

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

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

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

85

86 **The Hox clock**

87 The most famous example of spatially distributed genes is the Hox genes. In most bilaterian
88 animals, Hox genes form clusters of at least seven members, organized genomically in a conserved
89 order and transcribed collinearly along the anterior to posterior (AP) axis [21–23]. “Hox clock” operates
90 during axial elongation in the posterior embryonic growth zone of vertebrate embryos; Hox genes are
91 transcribed sequentially according to their 3' to 5' genomic position in the Hox cluster (temporal
92 collinearity) [24]. As the “Hox clock” coincides with axial elongation, this temporal collinearity is
93 translated into a set of spatial coordinates from anterior to posterior (spatial collinearity) [25]. This
94 positional information is then transferred to the differentiated progeny, which contribute to tissue

95 expansion along the growth axis. The translation of sequential Hox gene activation into spatial patterns
96 in developing axial tissues occurs in all vertebrates studied to date [26].

97 Notably, the “Hox clock” provides also the mechanism that ensures the spatial distribution of the
98 patterning information by regulating the timing of mesodermal cell ingression. Progenitor cells that
99 express anterior Hox genes ingress earlier and occupy more anterior positions along the body axis [27],
100 while, when the last genes in the cluster are expressed, they inhibit the function of earlier HOX proteins,
101 leading to a slower extension of the axis, until extension is terminated [28]. Importantly, after the
102 progenitor cells have ingressed, they maintain their Hox gene expression, allowing the establishment of
103 stable spatial domains [27]. Recent studies have provided insight into how the proper timing of Hox
104 gene expression is regulated by enhancers that receive temporal signals at the initial side of the cluster,
105 and subsequent colinear Hox gene expression is achieved by changes in the restrictions imposed by
106 chromatin structure [24,29].

107 Finally, it is possible that the “Hox clock” is molecularly linked to the segmentation clock of the
108 presomitic mesoderm (PSM), which is driven by oscillations of the Hes (Hairy/E(spl)-related) family
109 of transcription factors to give rise to the future vertebral column (Figure 1B)) [2]. The oscillating
110 expression of several Hox genes in the PSM is directly related to the segmentation clock, and a shift in
111 somite boundaries can lead to a repositioning of Hox boundaries [30]. Furthermore, loss of Hoxb6 in
112 the mouse embryo has been shown to lead to defects in somite formation and the segmentation clock
113 [31]. On the other hand, evidence from zebrafish mutants in which axis identity is maintained, but
114 segmentation proceeds slowly, challenges the idea of a direct link between the two processes [10,32].
115

116 **Pair-rule and gap genes in insects**

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

122 Insects were initially divided into long-germ and short-germ insects depending on whether
123 segment formation occurs simultaneously or sequentially during embryogenesis [34]. In the long-germ
124 insect *Drosophila melanogaster*, segments are formed during the blastoderm stage under the regulation
125 of morphogen gradients that cover the entire AP axis of the embryo. These gradients are formed by the
126 localization of maternally deposited mRNAs, such as *bicoid* and *hunchback* anteriorly and *nanos* and
127 *caudal* posteriorly. During this process, gap and pair-rule genes are seemingly simultaneously expressed
128 in their respective domains along the AP axis. However, in the short germ beetle *Tribolium castaneum*,
129 with the exception of a few anterior segments, most segments form sequentially during germ band
130 elongation from a posterior growth zone called the segment addition zone (SAZ) [33]. During this
131 process, gap and pair rule genes are expressed in a temporally sequential manner in the SAZ, and only
132 when individual cells exit the SAZ, gap gene expression is stabilized, leading to a spatial pattern of
133 expression along the AP axis. Other insects, such as *Nasonia vitripennis*, employ an intermediate mode
134 of segmentation [35,36], suggesting that the long- and short-germ strategies presented above may in
135 fact be the two extremes of a continuum, rather than two distinct mechanisms [37].

136 Indeed, modeling, live imaging and *in situ* stainings of gap gene expression in the *Drosophila*
137 blastoderm have shown that gap gene expression regions are not static over time but shift from posterior
138 to anterior [38–40]. The cells at the shifting boundary between two domains switch from expressing
139 one gene to another, similar to their sequential expression in the SAZ of *Tribolium*. Based on this, a
140 non-periodic oscillator [38] involving the *Drosophila* gap genes has been proposed to underlie their
141 spatial sequential expression, supporting further the idea that what has been considered as spatial
142 patterns in *Drosophila* has essentially evolved from ancestral molecular clocks [17,41]. In addition,
143 computational models in which segmentation and the pair-rule network were studied through
144 simulations, showed that segmentation in *Drosophila* relies on pair-rule gene expression progressing
145 across cells over time, supporting previous quantitative studies and challenging the notion of pre-
146 structured spatial patterns at the whole-tissue level [42].
147

148 **Temporal interpretation of morphogen gradients**

149 Morphogen gradients, despite being well-established spatial organizers, have also been suggested
150 repeatedly to act in a temporal manner. Morphogen gradients can pattern tissues based on the time of
151 exposure of the cells to the morphogen rather than based on the morphogen concentration, as the
152 traditional morphogen gradient model suggests [43]. For example, chick neuronal cells can interpret
153 different concentrations of Sonic Hedgehog as if there were proportionally different time periods of
154 Sonic Hedgehog signal transduction [44]. Moreover, the timing and sequence of spatial gene expression
155 in the mouse spinal cord depends not only on the concentration of the external signal, but also on the
156 exposure time [45].

157 Similarly, the duration of exposure (and not concentration) of human pluripotent stem cells to
158 BMP signaling determined whether cells will remain pluripotent or whether they will differentiate to
159 mesodermal or extraembryonic states [46]. Another prominent example of a morphogen gradient is
160 Bicoid; cells seem to be interpreting duration of exposure to Bicoid on top of morphogen concentration
161 [47]. Interestingly, this temporal mode of operation of morphogen gradients can lead to pattern
162 formation in unicellular organisms too. Colonies of *E. coli* can generate self-organized ring patterns in
163 the absence of a morphogen gradient, where the morphogen serves as a timer to allow for the bacteria
164 to respond to environmental differences [48].

165 The speed regulation model was recently proposed to reconcile the spatial and temporal activities
166 of a morphogen gradient; this model posits that different concentrations of a morphogen can
167 differentially modulate the speed of a temporal sequence leading to the establishment of spatial patterns
168 [17,49].

169 **Evolution of spatial from temporal patterns**

170 Temporal transcription factor series have been described in different contexts [50], but nowhere
171 nearly as comprehensively as in the *Drosophila* developing optic lobe [51,52]. As mentioned earlier,
172 the transcription factors that are expressed temporally in the *Drosophila* ventral nerve cord neuronal
173 stem cells are also expressed along the AP axis of the blastoderm embryo like gap genes (Figure 2A).
174 Interestingly, many of the *Drosophila* optic lobe temporal genes are also expressed spatially along the
175 AP axis of the developing procephalic ectoderm (Figure 2B-C), where early temporal genes
176 (*homothorax - hth*, *Distalless - Dll*, *Oaz*, and *earmuff - erm*) are expressed more posteriorly than middle
177 (*homeobrain - hbn*) and later temporal genes (*scarecrow - scro*).

178 The above raise a few questions and hypotheses:

179 First, how did the spatial patterns evolve from a temporal series (or vice versa)? Notably, in the
180 developing *Drosophila* optic lobe, static images of temporal transcription factors expression appear as
181 distinguishable spatial gene expression patterns, which are then lost in the adult structure [51,52]. This
182 suggests that this temporal mode of patterning is conceptually analogous to the clock-based patterning
183 of the anteroposterior axis in vertebrates and insects. In this case, neuronal fate is fixed upon division
184 of the progenitors in a similar manner to the fixation of AP fate of the cells that exit the SAZ or the
185 PSM. This type of patterning has been described as a wavefront-based model [17,49]. The emerging
186 possibility is that the ancestral temporal gene expression was “translated” into a spatial pattern, in a
187 manner similar to the gap/pair-rule gene spatial pattern in *Drosophila* that originated from the temporal
188 profile of a sequentially segmenting ancestor.

189 Second, how has it occurred that the same genes that are involved in temporal patterning of
190 neuronal stem cells pattern also the anteroposterior axis of the embryo? Do these tissues share a
191 common evolutionary origin or were the gene regulatory networks co-opted to regulate the expression
192 of these transcription factors in space? To answer this question, we first need to understand how the
193 temporal series of the stem cells and the gap/pair-rule gene network in the AP axis evolved and how
194 the respective gene regulatory networks were assembled [53,54]. Unfortunately, only minimal
195 information regarding these two networks exists beyond the traditionally studied animal systems.

196 Finally, we would like to propose a scenario for the evolution of tissue patterning: we have
197 described above how spatial patterns can arise from temporally expressed genes, which makes the
198 evolutionary trajectory from temporal to spatial patterning particularly compelling. Moreover, it is
199 obvious that single-celled organisms can pattern themselves in time, but not in space (in the absence of
200 a three-dimensional tissue structure), in a manner similar to the aforementioned patterning of *E. coli*
201 [48]. To achieve this more efficiently, it is possible that they used the sequential expression of series of
202 transcription factors that allowed them to change their phenotype quickly and drastically (since
203

204 transcription factors can regulate the expression of many gene batteries at the same time). This would
205 have allowed them to change their function over time, changing essentially their cell type (or state),
206 while adapting to a changing environment. As more complex organisms evolved, existing temporal
207 sequences of transcription factors were used to pattern the more complex tissues in space, allowing the
208 organism to have multiple different cell types at the same time. This model of temporal to spatial
209 transition has been proposed before to explain the evolution of different animal cell types [55,56]. We
210 believe that the discovery of the same series of transcription factors in different organisms (mice,
211 beetles, and flies) and tissues (retina, embryo, and brain) is in favor of this hypothesis.

212
213

214 **Author contributions**

215 KF and NK conceived the idea and wrote the manuscript.

216
217

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378 **Figure legends**379 **Figure 1: Temporal and spatial patterning in animals and plants**

380 **A) Spatial patterning:** The expression of BMP and Wnt from the dorsal roof plate and Shh from the
381 ventral floor plate leads to the generation of eleven spatial compartments (blue) along the dorsoventral
382 axis of the murine spinal cord. Each of these domains expresses a unique combination of transcription
383 factors (dorsal: pink, medial: orange, ventral: green) that allows it to generate different neuronal types.

384 **Temporal patterning:** In a similar manner, the life of a *Drosophila* optic lobe neuroblast is divided into
385 (at least) eleven different temporal windows (blue). Each of these temporal windows expresses a unique
386 combination of transcription factors (early: pink, medial: orange, late: green) that allows it to generate
387 different neuronal types.

388 **B)** Segmentation in vertebrates uses molecular clocks to generate new segments along the
389 anteroposterior axis of the animal. Similarly, in plants, gene expression oscillation determines the
390 periodic localization of lateral roots. Green signifies the cyclic expression of genes such as *hairy*, while
391 orange illustrates the cyclic activity of the auxin responsive promoter DR5.

392 **C)** The transcription factor sequence Hb/Kr/Pdm/Cas is expressed in a temporal and/or spatial pattern
393 in different tissues, such as the *Drosophila* ventral nerve cord and blastoderm embryo, the *Tribolium*
394 ventral nerve cord and segment addition zone, and the mouse retina. The color-code of the transcription
395 factors follows the one from Figure 1A.

396

397 **Figure 2: Spatial expression of neuroblast temporal transcription factors in the blastoderm**
398 **embryo**

399 **A)** The temporal transcription factors of the *Drosophila* ventral nerve cord neuronal stem cells are
400 expressed in a gap-like spatial pattern (green) in *Drosophila* blastoderm embryo. Marked exception is
401 the last temporal transcription factor, *grainy head* [57], which does not follow this pattern and is
402 expressed in the head epidermis instead.

403 **B)** The *Drosophila* optic lobe temporal transcription factors can be divided in three categories:

404 1) *eyeless* (*ey*) and *BarHI* are not expressed in the blastoderm embryo and start to be expressed
405 later

406 2) *odd-paired* (*opa*), *Dichaete* (*D*), and *tailless* are expressed in a spatially sequential pattern
407 along the AP axis. Interestingly, *D* is expanded posteriorly in *tailless* (*tll*) mutants in a genetic
408 interaction reminiscent of the optic lobe neuroblasts, where in a *tll* mutant, *D* expression is
409 extended in time [58]. *sloppy paired* (*slp1*) is also expressed in a spatially restricted pattern
410 acting as a pair-rule gene [59].

411 3) Finally, *hth*, *Dll*, *Oaz*, *erm*, *hbn*, and *scro* are expressed in the procephalic ectoderm.

412 **C)** Interestingly, the temporal genes *hth*, *Dll*, *Oaz*, *erm* (early), *hbn* (middle), and *scro* (mid-late) are
413 expressed in the same spatial order along the anteroposterior axis of the procephalic ectoderm in
414 embryonic stages 4-8. The early temporal gene *hth* is expressed posteriorly in the procephalic ectoderm
415 (as well as in the trunk). The early temporal genes *Dll* and *Oaz* are also expressed posteriorly. Then, the
416 later temporal genes *erm*, *hbn*, and *scro* are expressed progressively more anteriorly. Images from the
417 Berkeley *Drosophila* Genome Project (<https://insitu.fruitfly.org/>) [60–62].

Figure 1

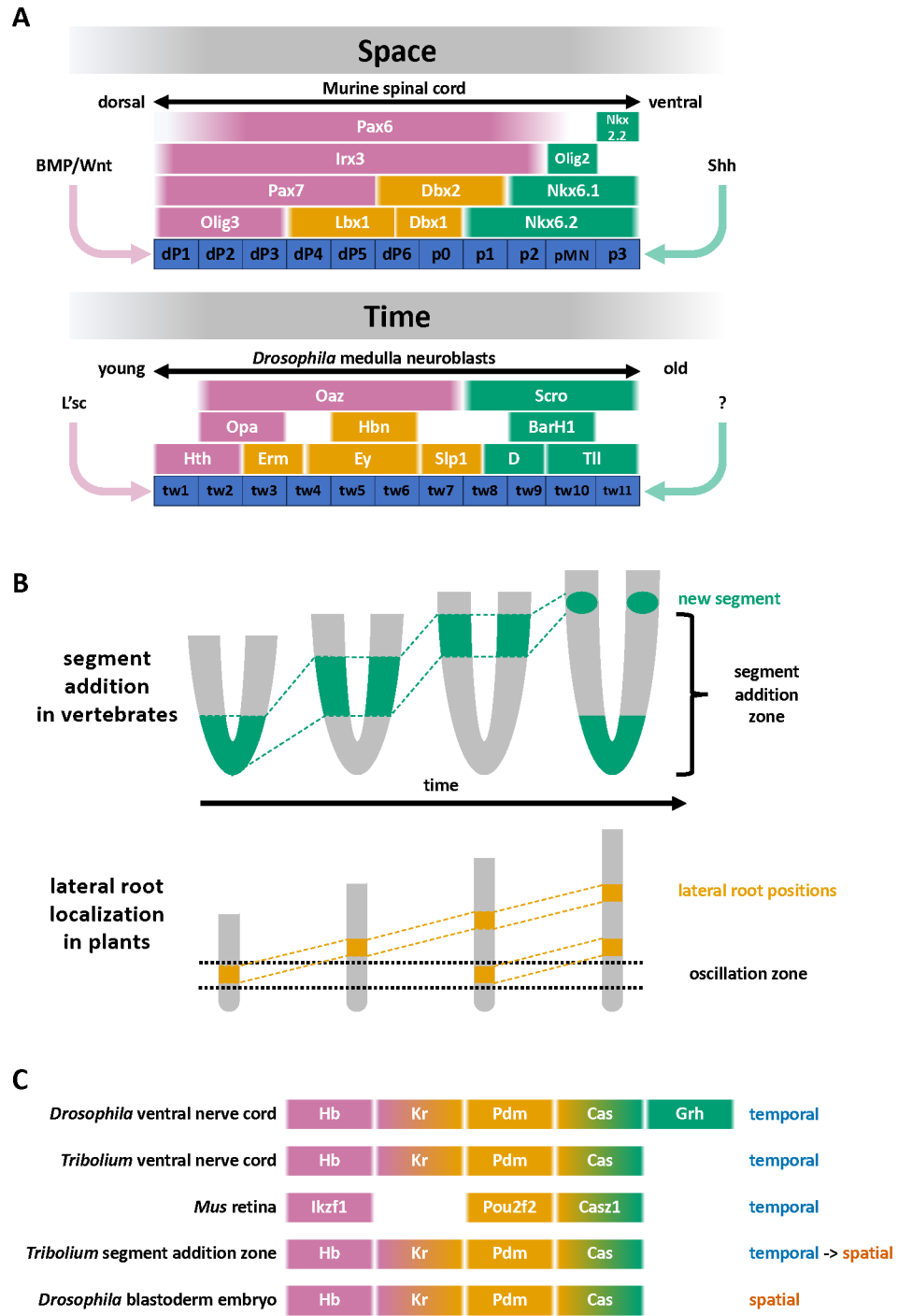


Figure 2

A

ventral nerve cord		
<u>Temporal genes</u>	<u>Spatial expression in the embryo</u>	<u>Gene Group</u>
<i>hunchback</i>	gap expression pattern	C2H2 zinc finger
<i>Kruppel</i>	gap expression pattern	C2H2 zinc finger
<i>pdm</i>	gap expression pattern	POU homeobox
<i>castor</i>	gap expression pattern	C2H2 zinc finger
<i>grainy head</i>	head epidermis	polycomb group recruiters

B

outer proliferation center, optic lobe		
<u>Temporal genes</u>	<u>Spatial expression in the embryo</u>	<u>Gene Group</u>
<i>homothorax</i>	procephalic ectoderm	TALE homeobox
<i>Distalless</i>	procephalic ectoderm	NK-like homeobox
<i>odd-paired</i>	pair-rule expression pattern	C2H2 zinc finger
<i>Oaz</i>	procephalic ectoderm	C2H2 zinc finger
<i>earmuff</i>	procephalic ectoderm	C2H2 zinc finger
<i>eyeless</i>	no expression	Paired homeobox
<i>homeobrain</i>	procephalic ectoderm	Paired homeobox
<i>sloppy-paired</i>	pair-rule expression pattern	Fork head box
<i>scarecrow</i>	procephalic ectoderm	NK-like homeobox
<i>Dichaete</i>	pair-rule expression pattern	HMG box
<i>BarH1</i>	no expression	NK-like homeobox
<i>tailless</i>	gap expression pattern	Nuclear receptor

