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Review

Bioelectric signaling coordinates patterning decisions during embryogenesis

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ABSTRACT

All cells generate an electrical potential across their membranes through the unequal distribution of ions. Changes in voltage potentials in non-excitable cells act as key regulators of pattern formation during embryogenesis through integration with the biochemical signaling and genetic hardwiring of an organism. Alteration of these signals leads to incorrect cell fate decisions, ectopic structure formation and overall body mispatterning. Here we review the important advances in bioelectricity as it relates to embryonic development across multiple organisms.

KEYWORDS: bioelectric signaling, membrane voltage potentials, cell fate, patterning, embryogenesis

1. Introduction

Understanding how embryos transform from a single fertilized cell to complex three-dimensional organisms is one of the central questions of developmental biology. Classically, developmental biologists have focused on biochemical signaling, genetic regulatory networks and mechanical forces as underlying control parameters for embryogenesis [1]. Although this 'gene-centric' approach has provided valuable information, it misses the dynamics and complexity of systems interactions during development, in particular physiological parameters that might not easily be captured with classic molecular techniques [2]. One such parameter, whose role in embryogenesis is beginning to be unraveled, is bioelectric signaling.

Bioelectricity is not a novel concept, and its involvement in biological processes has been studied for decades [3]. Bioelectric signaling regulates important cellular behaviors such as proliferation, migration and differentiation through the establishment of voltage gradients across populations of cells. These voltage gradients are not the fast-spiking currents generated in the nervous system, but rather are slow, long-term currents in non-excitable cells. Ion channels and pumps unequally distribute numerous ion species such as sodium, chloride and potassium across cellular membranes, resulting in voltage potentials. In addition, gap junctions allow for the diffusion of small molecules between cells, and can electrically connect entire cell populations. In addition to small ions, larger charged biomolecules such as growth hormones or signaling molecules like serotonin can also be involved in establishing electric gradients [2]. With the advancement of molecular tools, recent studies have revealed that voltage potentials are key modulators during development, regeneration and tumorigenesis [4]. The role of bioelectricity in regeneration and cancer progression has previously been the focus of several reviews [5-7]. This review highlights the role of bioelectric signaling during tissue formation, patterning and organogenesis during embryonic development.

2. Early patterning events: Left-right asymmetry

Voltage gradients have been implicated in the proper establishment of the left-right axis across multiple

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species. Although most vertebrates display a bilaterally symmetric exterior body plan, there is a highly conserved asymmetric orientation of internal organs along the left-right axis [8]. After decades of research, it is commonly accepted that left-right asymmetry establishment occurs in three phases. In the first phase, bilateral symmetry must be broken in such a way that the left-right axis remains correctly oriented with respect to both the dorso-ventral and anterior-posterior axes. This symmetry break is then transduced to differential expression of genes between the left and right sides of the body. Lastly, changes in gene expression alter cellular behavior, leading to asymmetrical organ morphogenesis. Recently, numerous studies have demonstrated that bioelectric signaling acts during both the initial symmetry-breaking event and subsequent downstream events.

Conserved roles for voltage gradients in left-right patterning have been found in species ranging from ascidians to chick [9, 10]. In Xenopus, where early bioelectric signaling has been extensively studied, multiple ion transporters and channels are required for normal left-right patterning during embryogenesis [11-16]. For example, both the maternal mRNA and protein of the H^+/K^+-ATPase ion transporter become asymmetrically localized by the second cell division through cytoskeletal shuttling, suggesting that these biophysical signals act during the initial symmetry-breaking event [11, 14]. Although the H^+/K^+-ATPase transporter itself is electrically neutral, its activity is coupled with multiple potassium channels to generate a voltage gradient between the left and right sides of the body [13, 15]. This gradient allows for maternal serotonin, which is a charged molecule, to pass through gap junctions and become asymmetrically localized to the right ventral blastomeres where it acts as an electrophoretic morphogen [17-19]. Furthermore, abolishment of either H^+/K^+-ATPase function or serotonin signaling causes a randomization of asymmetrically expressed laterality genes such as *nodal*, *lefty* and *pitx-2*, as well as complete heterotaxy of organs. Another channel H^+-V-ATPase also plays a critical role during early cleavage stages to generate an asymmetric H^+ flux [12]. This asymmetry acts to regulate cytoplasmic pH and maintain the voltage gradient between the left and right blastomeres. H^+-V-ATPase inhibition prevents serotonin localization in the right ventral blastomeres and also induces randomization of gene expression and organ placement. Inwardly rectifying potassium channels such as Kir6.1 are yet another class of channels that have been implicated in the establishment and maintenance of voltage gradients in Xenopus [15]. Kir6.1 acts both during cleavage stages and gastrulation, where it acts to maintain tight junctions and membrane voltage. Similar to other channels that have been studied, inhibition during either of these periods randomizes the expression of *nodal* and induces heterotaxia. Importantly, the underlying mechanism linking early biophysical events to asymmetric gene expression has been demonstrated in Xenopus. Carneiro *et al.* showed that epigenetic regulation through histone deacetylase (HDAC) activity modulates the expression of the asymmetric gene *Nodal related 1* (*Nr-1*) [20]. Injection of a dominant negative form of HDAC led to reduction of *Nr*-1 gene expression and organ randomization through the expression of the methylation marker H3K4me2. A proteomic screen revealed that the HDAC association protein Mad3 requires serotonin binding to maintain normal left-right patterning, thus identifying the link between early voltage gradients and asymmetric gene expression.

Although the timing of activity varies, the roles of the ion channels and pumps identified in Xenopus are conserved across multiple species. H^+/K^+-ATPase function is required for proper left-right axis establishment in sea squirts, sea urchins, zebrafish and chick [9, 11, 17, 21, 22]. In the invertebrate species *Ciona intestinalis*, blocking H^+/K^+-ATPase activity with the drug omeprazole randomizes expression of the normally left sided *Ci-Pitx* gene [9]. Inhibition of potassium channels also induces ectopic *Ci-Pitx* expression, indicating the presence of a similar mechanism as observed in Xenopus. However, *C. intestinalis* differs from Xenopus in the fact that H^+/K^+-ATPase activity regulates asymmetry relatively late in the development (during neurulation and tailbud stages), and inhibition of H^+/K^+-ATPase activity never resulted in a complete reversal of organs. Despite the differences between the control of asymmetry observed in Ciona and Xenopus, the fact that such a basal chordate group has conserved mechanisms lends support to a conserved role for ion fluxes in left-right axis determination. Other invertebrates such as sea urchins also require restricted H^+/K^+-ATPase activity during development, with the H^+/K^+-ATPase protein being asymmetrically localized during blastula

stages [21]. As observed in other animals, inhibition of this channel randomized asymmetric gene expression. H^+/K^+-ATPase activity induces several Notch pathway genes in sea urchins, which in turn indirectly control the expression of the laterality gene *nodal* by restricting expression to the right side of the embryo [23]. Abolishing the pump's activity led to complete suppression of Notch target genes, a lack of mesodermally derived tissues and delayed gastrulation. Similar to what is seen in sea urchins, the requirement of both H^+/K^+-ATPase activity and Notch signaling is also present in chick embryogenesis [24]. Although the pump is symmetrically expressed during gastrulation, there is a difference in membrane potential between the left and right side of the primitive streak [11]. This voltage gradient leads to transient accumulations of calcium ions, which in turn induce the asymmetric expression of Notch, which activates *nodal* expression. In addition to Notch signaling, perturbation of multiple ion channels and pumps, including H^+/K^+-ATPase, H^+-V-ATPase and Kir6.1 disrupt the expression of *sonic hedgehog* in chick, which is another critical laterality gene [11, 12, 15]. Overall, these studies highlight ion fluxes and voltage gradients as conserved mechanisms for the early establishment of the left-right axis in multiple species. These biophysical changes are then transduced to changes in the expression pattern of core signaling pathways, which propagate and define the left-right axis during later developmental stages.

3. Late patterning events: Tissue formation and organogenesis

In addition to early patterning events such as left-right axis determination, bioelectric signaling acts in conjunction with transcriptional and biochemical cascades to pattern complex structures such as tissues and organs. Disruption of correct ion fluxes and membrane potential can alter important cell behaviors that ultimately lead to improper tissue patterning and even the formation of ectopic structures across a diverse array of organisms.

3.1. Drosophila and other insects

During oogenesis in *Drosophila melanogaster* (fruit fly) and *Actias luna* (luna moth) voltage gradients between nurse cells and the developing oocyte asymmetrically distribute charged cytosolic proteins [25, 26]. If gradients across intercellular bridges are chemically destroyed or altered, the oocyte/nurse cell protein distribution is completely abolished. In both species, it is the asymmetric distribution of Ca^{2+} ions that generates the correctly oriented voltage gradients. Recently, Krüger *et al.* demonstrated that the spatial distribution of membrane voltage and intracellular pH is correlated with stage-specific developmental processes during *Drosophila* oogenesis [27]. They identified six distinct cell-types based on their membrane potential, intracellular pH, and ion channel and pump composition. There was a similar pattern between the spatial distribution of membrane potential and the activity of voltage-gated calcium channels, which suggests calcium signaling might transduce bioelectric signaling to downstream genetic changes. Krüger and colleagues propose a role for V-ATPase pumps for generating the spatial voltage gradient (similar to observations in Xenopus) and sodium transporters for establishing the pH gradients. An asymmetric distribution of gap junctions, which electrically coupled distinct cell populations, was also observed.

Bioelectric signaling events also regulate proper patterning of *Drosophila* wings. For example, V-ATPases have been shown to regulate both canonical and non-canonical Wnt-signaling pathways during *Drosophila* wing development [28]. V-ATPase function is required for the proper asymmetric distribution of the Frizzled protein and the subsequent planar cell polarity signaling pathway. When the pump's function was inhibited, asymmetric Frizzled localization was lost, resulting in misshapen wing hairs and impairment of anterior-posterior alignment of the hairs. Hermle *et al.* also demonstrated that V-ATPase localization and function affects Wingless morphogen distribution during wing development, thus affecting the canonical Wnt-signaling pathway. Mutations in an inwardly rectifying potassium channel Kir2.1 also affect *Drosophila* wing patterning [29]. Disruption of the *Drosophila* homolog of Kir2.1 (Irk2) with a dominant-negative allele, a p-element allele, or RNAi produced wing-patterning defects. These defects are similar to the ones observed when Decapentaplegic (DPP, the drosophila homolog of Bone morphogenetic protein) signaling is perturbed.

Dahal *et al.* successfully demonstrated the connection between bioelectric signaling and anatomical output by determining that reducing the function of Irk2 decreased DPP signaling in the larval imaginal wing disc [29].

3.2. Amphibians

In *Xenopus laevis*, not only does bioelectric signaling regulate left-right axis formation, it also modulates several transcriptional pathways during embryogenesis that are needed to generate normal tissues and organs [30]. Disruption of the endogenous spatial gradients of membrane potential leads to incorrect gene expression and gross anatomical mispatterning during craniofacial and neural development [31, 32]. Interestingly, these voltage gradients appear immediately prior to the activation of important biochemical pathways, creating a 'prepattern' for downstream craniofacial gene expression and morphogenesis [31]. For example, in developing brain tissue, prepatterns of hyperpolarization activate calcium and Notch pathways to specify cells to neural fates and produce the correct spatial organization [32]. In addition to activating regulatory cascades, bioelectric signals also control the balance between apoptosis and proliferation to achieve the correct morphological pattern of the brain and the nervous system [33]. In another study, Blackiston *et al.* found that growing nerve axons harness distinct areas of differential voltage to read the 'electric topography' of neighboring cells and ensure proper innervation patterning of grafted tissue [34]. Normally, transplanted tissues such as eyes, display little or no innervation with host tissues. Yet, Blackiston *et al.* demonstrated that grafting eye primordia into an artificially depolarized host embryo leads to hyperinnervation of the transplant [34]. Further investigation revealed that membrane potential acts as an upstream modulator of extracellular serotonin that in turn drives this hyperinnervation phenotype. Other cell types, including neural crest cells also respond to changes in membrane potential [35]. A study examining the behavior of melanocytes during development demonstrated that depolarization of endogenous glycine-gated chloride channels causes melanocytes to hyperproliferate and invade areas of the body where they are normally absent [36]. These channels are located in a population of sparsely distributed cells, and act as 'instructor cells' for developing melanocytes. The initial change in membrane potential is transduced through serotonin signaling, cyclic AMP signaling and transcription factors such as Sox10 and Slug to produce an 'all or nothing' hyperpigmentation phenotype [37].

Amazingly, not only can alterations in bioelectric signaling result in the mispatterning of developing tissues, they can also redirect the developmental trajectory of entire tissues and organs, and even induce the formation of ectopic structures. For example, depolarization of a hypersensitive glycine-gated chloride channel (endogenous channels weren't activated), expressed under a muscle-specific promoter, not only generated the hyperpigmented phenotype described above, it also resulted in muscle mispatterning and ectopic muscle formation [38]. Ectopic muscle cells, identified by the expression of mature skeletal muscle markers, were found along the neural tube, far outside of the normal muscle permissive areas of the body. Perhaps the most extreme example of bioelectric patterning control of tissues/organs that has been described to date is the induction of functioning ectopic eyes [39]. Pai *et al.* elegantly showed that misexpression of multiple ion channels resulted in ectopic eye formation in areas along the body – well outside of the normal eye-field [39]. Calcium signaling transduces the change in membrane potential by inducing the expression of the eye-field genes *PAX6* and *Rx1*, leading to fully formed eyes in regions far beyond the anterior neural field (e.g., the tail and the gut). The fact that multiple types of ion channels generated the same phenotypic outcome suggests that ectopic eye formation is a result of voltage change, rather than specific ion channel function.

3.3. Zebrafish

Most studies on the bioelectric control of cell behavior in zebrafish have focused on its roles during the formation of the adult pigment pattern [40-43]. However, ion channels have also been implicated in heart and fin development [44-46]. Two independent genetic screens identified the *small heart* and *heart and mind* mutants, both of which possess mutations in the coding region of the α1B1 isoform of Na^+/K^+-ATPase. *Small heart* mutants have undersized and malformed hearts in addition to other developmental defects in the brain, eyes and kidneys [45]. The *heart and mind* mutants

show defects in heart tube extension, cardiomyocyte differentiation and embryonic heart function [44]. Knockdown with morpholinos and blocking channel function with pharmacological agents such as ouabain also phenocopy these developmental defects, indicating that flux through the channel is required for proper pattern formation of the heart. Although other studies have focused on genetic manipulations of different ion channels during heart development, it is unclear how large a role ion flux plays in cardiogenesis [47]. In addition to studying the role of membrane potentials during heart organogenesis, Perathoner *et al.* demonstrated that flux through a 2-pore domain potassium channel Kcnk5b is necessary for the allometric growth of the fins and barbels of zebrafish [46]. *Another longfin* mutants, who have a mutation in Kcnk5b, have hyperpolarized membrane potentials compared to controls, leading to an increase in cell proliferation and ultimately to the development of oversized fins. Importantly, the increase in proliferation is only observed in the fins, and not in the neighboring tissues, and despite this increase the correct fin organization and patterning is maintained.

3.4. Chick, mouse, and beyond

There are decidedly fewer studies focused on later patterning events during chick and murine embryogenesis, largely due to the technical difficulties of studying ionic fluxes in these systems. However, a few studies have demonstrated roles for bioelectric signaling during tissue development in these model systems. For example, during chick neurulation, endogenous extracellular currents play a causal role in tail development [48]. In fact, when conductive implants were surgically inserted into embryos, extracellular current was decreased by ~30%, resulting in numerous tail defects, ranging from mispatterned tails to completely absent tails. To date, the underlying mechanism connecting the change in endogenous currents to tail phenotypes remains to be elucidated. Similar to the results obtained from examining zebrafish hearts, Na^+/K^+-ATPase was also found to be involved with specifying cardiac progenitor populations and precardiomyocyte differentiation during chick development [49]. Chick embryos that were exposed to ouabain (inhibits Na^+/K^+-ATPase function) during gastrulation (stages 5-8) displayed inhibited heart development and cardiomyocyte differentiation, whereas embryos treated after stage 8 had normal heart morphology, indicating an early role for bioelectric signaling during heart development.

Another example of bioelectric regulation in chick and mice has been observed during limb development. During limb bud formation, there is a switch from inwardly directing currents along the flank of the body to outwardly directing currents in the limb bud [50]. Applying an external counter-current in chick induced abnormal limb formation. Thus, it is likely that endogenous currents are providing spatial and trophic cues for the developing limb.

Not only has bioelectric signaling been observed to play a role in craniofacial patterning in Xenopus, studies suggest it also helps modulate the formation of mammalian head structures [29, 31]. For example, in mice, knockdown of the inwardly rectifying potassium channel Kir2.1 leads to craniofacial defects, similar to what is seen in Anderson-Tawil syndrome and fetal alcohol syndrome [29, 51, 52]. *Kir2.1* knockdown mice have cleft palate due to a reduction in palatine processes and vomer bones. They also display digital defects such as digit duplication. Even heterozygous pups have decreased anterior and posterior palatine processes and decreased ossification. The mouse neurological mutation *weaver* has a mutation in another inwardly rectifying potassium channel GIRK2 [53, 54]. The *weaver* mutation causes degeneration and death of cerebellar granule cells and dopaminergic neurons within the first 3 postnatal weeks. Mutant mice have reduced inward K^+ current through these channels, resulting in excessive neuronal depolarization and excitability, which ultimately leads to granule cell death. Recently, another study demonstrated that human patients with Keppen-Lubinsky disease also have mutation within GIRK2, also known as the *KCNJ6* gene [55]. Keppen-Lubinsky patients display multiple defects including severe developmental delay, microcephaly, large eyes, narrow nasal bridges, a high palate and aged appearance. Interestingly, although all three patients analyzed had distinct mutations in *KCNJ6*, each mutation led to a reduction in ionic conductance through the channel, indicating that its role in the maintenance of membrane potential is required for proper development. These studies are some of the first to link ion channel function and membrane potentials to human developmental diseases, highlighting both the importance of animal

model studies and the conservation of bioelectric signaling across taxa.

4. Stem cells: Insights into bioelectric signaling at the cellular level

Changes in membrane potentials can alter cell behaviors including migration, proliferation and differentiation [56, 57]. Understanding how physiological parameters such as membrane voltage guide and direct stem cells towards specific cell fates is critical for obtaining a holistic view of embryonic development as well as developing cellular based therapies. In fact, much of the knowledge about bioelectrical signaling in mammalian systems is based on studies examining *in vitro* stem cells.

Bioelectric signaling has been extensively studied during neural development and neural stem cell differentiation [58]. Schaarschmidt *et al*. showed that fetal human neural progenitor cells (hNPCs) have a unique profile of ion channels including A-type voltage-gate potassium channels (K_v) and their activity is required for cell proliferation [59]. Interestingly whole-cell patch clamp recordings showed two separate functioning K_v currents I_A and I_k, but no action potentials, suggesting that these channels have a separate developmental function apart from neuronal firing. The I_A current was transient with its amplitude decreasing as differentiation progressed. Conversely, the I_k current amplitude increased during differentiation. Inhibition of each of these currents, individually via exposure to neurotoxins, either inhibited or enhanced proliferation - indicating that each channel contributes differentially to cell fate progression. Based on these results it was hypothesized that depolarization due to I_k inhibition was responsible for the decrease in proliferation. In another study, rat embryonic neural stem cells were artificially depolarized via channelrhodopsin-2 (ChR2) activation [60]. This depolarization also led to a decrease in the cellular proliferation of neural progenitor cells. In contrast, He *et al.* demonstrated that depolarization behaves differently with another neural progenitor population, and actually promotes the differentiation of midbrain dopamine neurons in isolated rat neural precursor cells [61]. Depolarization with a high potassium concentration led to changes in chromatin structure via histone modifications and allowed transcription factors to access dopamine differentiation gene promoter regions. Finally, a recent study implicated depolarization as a driving force for correct neuronal connectivity and aggregations in neuron/glial co-cultures [62]. Combined, these studies highlight the complexity of membrane potential regulation during neurogenesis and indicate that electrical changes such as depolarization differentially regulate the progression into individual cell types.

Similar to the animal models previously discussed, ion channels have also been implicated in the regulation of cardiogenesis *in vitro*. For example, activation of calcium-gated potassium channels (K_{Ca}) drives pluripotent murine stem cells to cardiomyocyte fates [63]. Kleger *et al.* determined that prolonged channel activation resulted in morphological change, loss of pluripotency markers and upregulation of mesodermal and cardiac genes [63]. Additionally, this led to pacemaker-like cell specification through the activation of the sino-atrial gene program. Modulation of membrane potential can also influence cells that are further along in their developmental tracts, such as human cardiomyocyte progenitor cells (hCMPCs) [64]. Hyperpolarization of hCMPCs in culture increased intracellular calcium accumulation and activated calcineurin signaling, activation of cardiac gene expression, and ultimately the differentiation of fully functional cardiomyocytes. Taken together, these data suggest modulation of ion flux in stem cells and progenitor cells can cause genetic changes and induce specific cell fates.

5. Conclusion: Looking towards the future

Bioelectric signaling acts as another layer of control during development that is both essential and integrated with the genomic components of embryogenesis. The examples presented above provide evidence for physiological signals, such as membrane voltage potentials, connecting the environment of cells to the genetic and biochemical cascades required for correct pattern formation. However, despite knowing a lot about ionic currents and how they function, we are just beginning to elucidate how bioelectric signaling works in concert with transcription pathways to modulate cell behavior. Despite recent findings, several questions still need to be addressed in order to advance this field including: how are cells able to compare

bioelectric states across distances, and how is the input stemming from bioelectrical signals, chemical gradients, and physical forces able to direct the formation of complex structures. Also, in addition to connecting ion fluxes with specific downstream events, understanding the differences and similarities between multiple ion channel species remains to be elucidated. Gaining a better understanding of how bioelectricity directs cell behavior may provide novel insights to developing therapeutics to treat, and maybe someday prevent human disease.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

REFERENCES

1. Levin, M. 2014, The Journal of Physiology, 592, 2295-2305.
2. Funk, R. H. and Thiede, C. 2014, Journal of Clinical and Experimental Oncology, S1.
3. Levin, M. 2012, BioEssays, 34, 205-217.
4. Adams, D. S. and Levin, M. 2013, Cell and Tissue Research, 325, 95-122.
5. Levin, M. 2007, Trends in Cell Biology, 17, 261-270.
6. Levin, M. 2009, Seminars in Cell & Developmental Biology, 20, 543-556.
7. Levin, M. 2014, Molecular Biology of the Cell, 25, 3835-3850.
8. Vandenberg, L. N. and Levin, M. 2013, Developmental Biology, 379, 1-15.
9. Shimeld, S. M. and Levin, M. 2006, Developmental Dynamics, 235, 1543-1553.
10. Levin, M. and Mercola, M. 1999, Development, 126, 4703-4714.
11. Levin, M., Thorlin, T., Robinson, K., Nogi, T. and Mercola, M. 2002, Cell, 111, 77-89.
12. Adams, D., Robinson, K., Fukumoto, T., Yuan, S., Albertson, R., Yelick, P., Kuo, L., McSweeney, M. and Levin, M. 2006, Development, 133, 1657-1671.
13. Morokuma, J., Blackiston, D. and Levin, M. 2008, Cellular Physiology and Biochemistry, 21, 357-372.
14. Aw, S., Adams, D., Qiu, D. and Levin, M. 2008, Mechanisms of Development, 125, 353-372.
15. Aw, S., Koster, J., Pearson, W., Nichols, C., Shi, N.-Q., Carneiro, K. and Levin, M. 2010, Developmental Biology, 346, 39-53.
16. Vandenberg, L. N., Morrie, R. D. and Seebohm, G. 2013, Mechanisms of Development, 130, 254-271.
17. Fukumoto, T., Kema, I. and Levin, M. 2005, Current Biology, 15, 794-803.
18. Zhang, Y. and Levin, M. 2009, Developmental Dynamics, 238, 1923-1935.
19. Vandenberg, L. N., Blackiston, D. J. and Rea, A. C. 2014, The International Journal of Developmental Biology, 58, 799-809.
20. Carneiro, K., Donnet, C. and Rejtar, T. Karger, B. L., Barisone, G. A., Díaz, E., Kortagere, S., Lemire, J. M. and Levin, M. 2011, BMC Developmental Biology, 11, 29.
21. Hibino, T., Ishii, Y., Levin, M. and Nishino, A. 2006, Development Genes and Evolution, 216, 265-276.
22. Kawakami, Y., Raya, Á., Raya, R. M. and Rodríguez-Esteban, C. 2005, Nature, 435, 165-171.
23. Bessodes, N., Haillot, E., Duboc, V., Röttinger, E., Lahaye, F. and Lepage, T. 2012, PLoS Genetics, 8, e1003121.
24. Raya, Á., Kawakami, Y., Rodríguez-Esteban, C. and Ibañes, M. 2004, Nature, 427, 121-128.
25. Cole, R. W. and Woodruff, R. I. 2000, Journal of Insect Physiology, 46, 1239-1248.
26. Cole, R. W. and Woodruff, R. I. 1997, Journal of Insect Physiology, 43, 275-287.
27. Krüger, J. and Bohrmann, J. 2015, BMC Developmental Biology, 15, 1.
28. Hermle, T., Saltukoglu, D., Grünewald, J., Walz, G. and Simons, M. 2010, Current Biology, 20, 1269-1276.

29. Dahal, G. J., Gassaway, B., Kwok, B., Tong, Y., Ptáček, L. J. and Bates, E. 2012, Development, 139, 3653-3664.
30. Pai, V. P., Martyniuk, C. J. and Echeverri, K. 2015, Regeneration, in press.
31. Vandenberg, L. N. and Morrie, R. D. 2011, Developmental Dynamics, 240, 1889-1904.
32. Pai, V. P., Lemire, J. M., Paré, J. F. and Lin, G. 2015, The Journal of Neuroscience, 35, 4366-4385.
33. Pai, V. P., Lemire, J. M., Chen, Y., Lin, G. and Levin, M. 2015, The International Journal of Developmental Biology, 59, 327-340.
34. Blackiston, D. J., Anderson, G. M., Rahman, N. and Bieck, C. 2015, Neurotherapeutics, 12, 170-184.
35. Morokuma, J., Blackiston, D., Adams, D., Seebohm, G., Trimmer, B. and Levin, M. 2008, Proceedings of the National Academy of Sciences of the United States of America, 105, 16608-16613.
36. Blackiston, D., Adams, D., Lemire, J., Lobikin, M. and Levin, M. 2011, Disease Models & Mechanisms, 4, 67-85.
37. Lobikin, M., Lobo, D., Blackiston, D. J. and Martyniuk, C. J. 2015, Science Signaling, 8, ra99.
38. Lobikin, M., Paré, J.-F.F., Kaplan, D. L. and Levin, M. 2015, The International Journal of Developmental Biology, 59, 303-311.
39. Pai, V. P., Aw, S., Shomrat, T., Lemire, J. M. and Levin, M. 2012, Development, 139, 313-323.
40. Iwashita, M., Watanabe, M., Ishii, M., Chen, T. and Johnson, S. L. 2006, PLoS Genetics, 2, e197.
41. Watanabe, M., Iwashita, M., Ishii, M. and Kurachi, Y. 2006, EMBO reports, 7, 893-897.
42. Inaba, M., Yamanaka, H. and Kondo, S. 2012, Science, 335, 677-677.
43. Irion, U., Frohnhöfer, H. G., Krauss, J., Champollion, T., Maischein, H. M., Geiger-Rudolph, S., Weiler, C. and Nüsslein-Volhard, C. 2015, eLife, 3, e05125.
44. Shu, X., Cheng, K., Patel, N., Chen, F. and Joseph, E. 2003, Development, 130, 6165-6173.
45. Yuan, S. 2004, Circulation Research, 95, 595-603.
46. Perathoner, S., Daane, J. M., Henrion, U. and Seebohm, G. 2014, PLoS Genetics, 10, e1004080.
47. Keßler, M., Just, S. and Rottbauer, W. 2012, Stem Cells International, 2012, Article ID 462161.
48. Hotary, K. B. and Robinson, K. R. 1992, Development, 114, 985-996.
49. Linask, K. K. and Gui, Y. H. 1995, Developmental Dynamics, 203, 93-105.
50. Altizer, A. M., Moriarty, L. J. and Bell, S. M. 2001, Developmental Dynamics, 221, 391-401.
51. Zaritsky, J. J., Eckman, D. M. and Wellman, G. C. 2000, Circulation Research, 87, 160-166.
52. Tristani-Firouzi, M. and Etheridge, S. P. 2010, Pflügers Archiv-European Journal of Physiology, 460, 289-294.
53. Herrup, K. 1996, Proceedings of the National Academy of Sciences of the United States of America, 93, 10541.
54. Surmeier, D. J. and Mermelstein, P. G. 1996, Proceedings of the National Academy of Sciences, 93, 11191-11195.
55. Masotti, A., Uva, P. and Davis-Keppen, L. 2015, The American Journal of Human Genetics, 96, 295-300.
56. Blackiston, D. J., McLaughlin, K. A. and Levin, M. 2009, Cell Cycle, 8, 3527-3536.
57. Sundelacruz, S., Levin, M. and Kaplan, D. 2009, Stem Cell Reviews, 5, 231-246.
58. Aprea, J. and Calegari, F. 2012, Stem Cells International, 2012, Article ID 816049.
59. Schaarschmidt, G., Wegner, F., Schwarz, S. C., Schmidt, H., Schwarz, J. and Linden, R. 2009, PLoS One, 4, e6168.
60. Wang, S. J., Weng, C. H., Xu, H. W. and Zhao, C. J. 2014, The Journal of Membrane Biology, 247, 493-500.
61. He, X. B., Yi, S. H., Rhee, Y. H., Kim, H., Han, Y. M. and Lee, S. H. 2011, Stem Cells, 29, 1861-1873.
62. Özkucur, N., Quinn, K. P., Pang, J. C., Du, C., Georgakoudi, I., Miller, E., Levin, M. and Kaplan, D. L. 2015, Brain and Behavior, 5, 24-38.

63. Kleger, A., Seufferlein, T., Malan, D., Tischendorf, M. Storch, A., Wolheim, A., Latz, S., Protze, S., Porzner, M., Proepper, C., Brunner, C., Katz, S. F., Varma Pusapati, G., Bullinger, L., Franz, W. M., Koehntop, R., Giehl, K., Spyrantis, A., Wittekindt, O., Lin, Q., Zenke, M., Fleischmann, B. K., Wartenberg, M., Wobus, A. M., Boeckers, T. M. and Liebau, S. 2010, Circulation, 122(18), 1823-36.
64. Vliet, P., Boer, T. P., Heyden, M. A. G., Tamer, M. K., Sluijter, J. P. G., Doevendans, P. A. and Goumans, M.-J. 2010, Stem Cell Reviews and Reports, 6, 178-185.