Bioelectricity in Developmental Patterning and Size Control

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Developmental patterning is essential for regulating cellular events such as axial patterning, segmentation, tissue formation, and organ size determination during embryogenesis. Understanding the patterning mechanisms remains a central challenge and fundamental interest in developmental biology. Ion-channel-regulated bioelectric signals have emerged as a player of the patterning mechanism, which may interact with morphogens.

zebrafish embryonic development long fin short fin

1. Introduction

Embryonic development is a self-autonomous and robust process in which a new body develops from a fertilized egg. This developmental process requires coordinated and complex cellular events such as proliferation, differentiation, and movement. The related patterning mechanisms are essential and instructive elements that eventually guide the body shape and organ sizes [1][2][3]. The morphogen gradient and transcription network are the mainstay theories and have been verified in many organ systems of various organisms [4][5][6]. Recent and past evidence revealed that ion-channel-related bioelectricity is a new component of the regulating mechanism for developmental patterning, regeneration, and cancers [7][8].

Bioelectricity is defined as endogenous electrical signaling across cell membranes and is mediated by the dynamic distribution of charged molecules [7][8][9][10][11][12][13]. This is represented by a difference in the net charge of cations and anions inside versus outside a cell. Many components are involved in electrical potential formation [12][14]. In essence, the semipermeable lipid-based plasma membrane acts as an electrical insulator, but also as a capacitor that can accumulate charge, while specialized passages (ion channels, pumps, connexins/gap junctions, and solute carriers) regulate ion flow from one side to the other, altering the voltage of the cell (**Figure 1**A). All cell types form ionic gradients across their cell membranes because channels exist throughout all living organisms in all domains of life, including plants, fungi, and bacteria [14][15][16][17][18][19][20][21][22][23][24]. Thus, ion regulation and the resulting bioelectricity are considered essential properties of living cells across evolution, and their innate properties can be used for cellular communication [25][26]. Therefore, understanding additional aspects of bioelectricity in cells and organisms is fundamental for modern physiology and ontology.

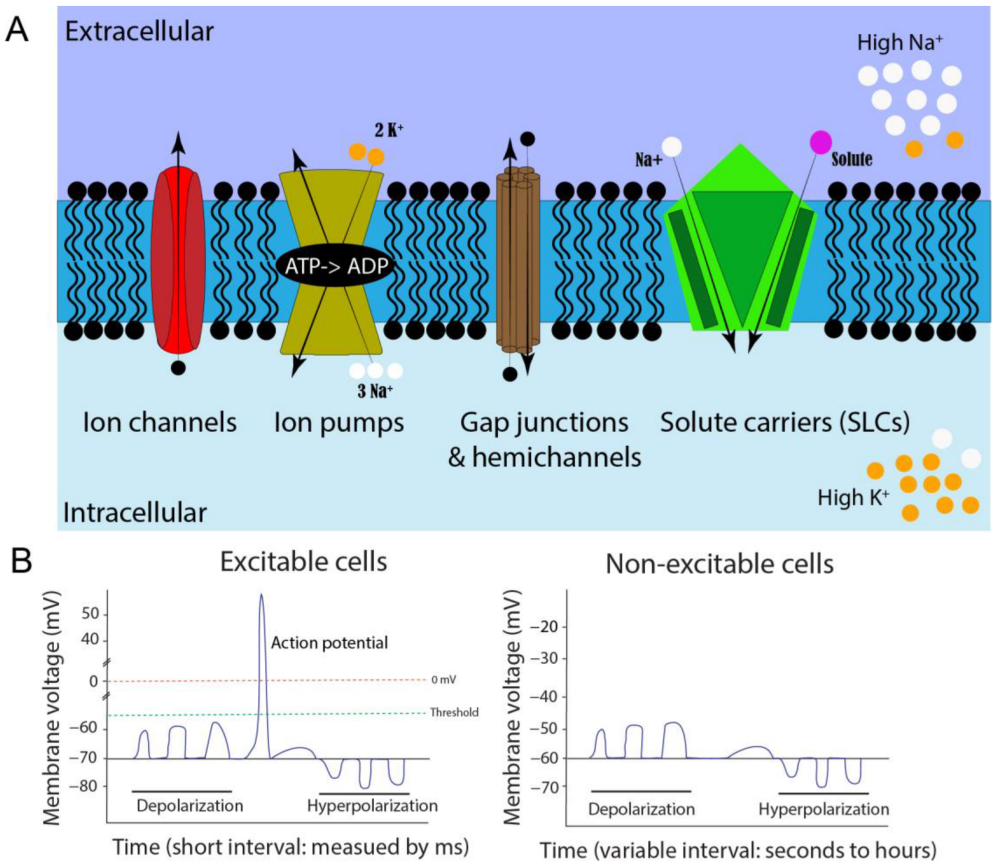

Figure 1. Cell membrane potential formation and comparison of neuromuscular excitable cells and non-excitable somatic cells. (**A**). Illustration of resting membrane potential, ion regulators, and ionic concentrations when the cell is in a non-excitable state. Different shapes represent various ion regulators on a cell membrane (blue region). The arrows indicate the movement of ions when the regulators are open. (**B**). Comparison of neuromuscular excitable and non-excitable somatic cells. Excitable cells usually exhibit action potentials, while the non-excitable somatic cells have membrane potential fluctuations, which vary in their amplitudes and frequencies.

Neuronal and muscular systems have been well investigated for their bioelectric activities. The field of neuromuscular bioelectricity has a relatively long and diverse history [27]. Luigi Galvani first demonstrated the relationship between electricity and animals in 1780 by electrically stimulating frog limbs to cause movement. However, it was almost another hundred years before the first measurements of action potentials, in 1865 by Julius Bernstein, using a differential rheotome [28]. The first intracellular electrical measurements of the resting membrane in the protozoon *Paramecium* were performed in 1934 [29]. Afterward, ion discoveries on neuronal bioelectricity were made by Hodgkin and Katz, using the giant squid axon as an experimental model [30]. Their intracellular recording studies paved the way for neurology and the fundamental understanding of action potentials [31]. One example is the combinational uses of neuronal axons' action potential, voltage-gated Ca^{2+} ion channels, and synaptic neurotransmitters for neural signals [32]. However, the function of bioelectricity remains largely unknown outside of a neuromuscular context. Expanding on these concepts of neuronal bioelectricity and neurotransmitters, it is not inconceivable that other electrical signals could travel across the membranes of non-nerve cells and trigger various responses: to cause other ions to enter the cell (or be released from internal stores); to change

transcriptional regulation of the machinery; to cause protein modifications, such as conformation or phosphorylation, to affect function; as well as to modify plasma membrane molecules such as receptors, kinases, and lipids [33][34][35][36].

2. Cellular Contributors to Membrane Potential and Bioelectricity

2.1. Cell Membrane Potential and Concentration Gradients

Bioelectricity can be exhibited in several different forms in multicellular organisms: on cellular, tissue, and organ levels. For example, cell membrane potential or membrane voltage (Vm) is one of the integral cellular bioelectric properties (**Figure 1**A). Many essential cellular physiological processes rely on Vm. These include cross-membrane transport (e.g., nutrients, salts, water), cell volume control, secretion, the cell cycle, and migration [13][25]. Additionally, Vm allows for cognitive and motor function through neuronal signaling, resulting in organismal, tissue, or cellular sensory detection, and locomotive movement [25].

In typical neuronal signaling, the steady-state baseline voltage is called resting Vm, whereas the excited “signaling” state is called an action potential (AP). Although this generally results in a range between −30 and −80 mV, Vm can even exceed a range of −5 mV to −150 mV, depending on cell type [9]. These resting Vm values can fluctuate in a small or large deviation. Large and rapid depolarization changes from negative to more positive membrane potential are referred to as APs, which are barely reported outside of neuronal and muscular tissues. These APs are triggered by ion channels that respond to changes in voltage that reach a certain threshold. More specifically, depolarizations may merge along a neuron axon or dendrite, eventually pass the Vm threshold for voltage-gated ion channels, and form an AP [37]. These AP waves can propagate from multiple locations, and if two meet from opposing directions, they will annihilate each other [38]. This quick (millisecond) and extreme (≥100 mV difference) swing in voltage, caused by altering intracellular ion concentrations, is unique to excitatory cells. However, increasing evidence shows smaller and longer-duration types of electrical signaling events in other, non-excitable cell types, such as melanocytes, can have significant effects [39]. Changes in Vm of non-excitable somatic cells could come from a variety of factors and would not be classified as traditional AP signals (**Figure 1**B). Smaller and less extreme increases or decreases in Vm can occur within embryonic neural and non-neural tissues over various periods, such as milliseconds, seconds, minutes, hours, or even days. Such subtle bioelectric signals may be essential in cell differentiation and embryonic patterning during development [7][8][9][40].

The electromagnetic force of the differential distribution of ions across the cell membrane generates the electric potential. Thus, the concentration gradient of each ion molecule jointly contributes to Vm value [41]. For example, there is a high level of potassium (K^+) and low levels of sodium (Na^+) within cells at resting Vm. High levels of intracellular K^+ and extracellular Na^+ ions are mainly established by the sodium/potassium ATPase pump (**Figure 1**A). One ATPase pump binds three intracellular Na^+ ions, utilizes ATP to change conformation via phosphorylation, and releases the three Na^+ ions into the extracellular space. Next, two extracellular K^+ ions will bind to this outward-facing conformation, causing dephosphorylation and reversal of conformation that allows potassium ions

into the cell against its concentration gradient [7][42]. This form of active transportation and the resulting electrochemical gradient is responsible for high intracellular potassium.

The electrical potential difference that counteracts or balances the concentration gradient for a given ion is called equilibrium potential. If only one permeant ion species exists in a cell, its resting membrane potential will equal the equilibrium potential for that ion. Potassium and sodium ions are the two main contributors to membrane potential, but Cl^- and Ca^{2+} ions can also affect Vm, in addition to other charged molecules, such as protons (H^+) and organic anions, depending on cell types. Generally, potassium equilibrium potential is close to resting cell membrane potential in many cell types, including glia and neurons. Thus, maintenance of high intracellular potassium is critical for establishing resting Vm [32][41]. This difference in concentration is hard to maintain, and potassium ions can exit the cell through various leak channels, such as K2P potassium channels on the plasma membrane [43]. Removing positively charged K^+ ions from the cell will result in a more negative electrical charge, forcing more positive ions to be pulled back into the cell against the chemical gradient. This constant cycling of potassium being pumped into cells and leaking out helps to establish the electric potential of resting Vm. Eventually, these electric and gradient forces will reach equilibrium. This balance can be mathematically described in the Nernst equation [32][41].

2.2. Membrane Potential Contributors: Ion Channels, Gap Junctions, and Solute Carriers

Ion channels are a group of transmembrane proteins that significantly contribute to overall cellular bioelectricity. Channels are essentially small pores in the cell membrane that alter permeability for specific ions based on selectivity (molecular charge and size) and gating (what is required to open the channel) [44]. Channel conductivity is aligned with the ion concentration gradient, so energy is not required for a high rate of ion-selective transport. However, the channels will only allow ions to flow down their concentration gradient (moving from high to low concentration areas). The composition of these channels on the cell membrane has been compared to an electronic component called a field-effect transistor [45]. In the human genome, more than 400 family members of ion channels are currently characterized, accounting for around 1.5% of the genome [46]. A comprehensive list of human ion channel details can be found on the HUGO Gene Nomenclature Committee website and the IUPHAR/BPS Guide to Pharmacology [44][47].

Based on ion selectivity, ion channels can be classified as sodium (Na^+), calcium (Ca^{2+}), potassium (K^+), chloride (Cl^-), or proton (H^+) channels, as well as non-charged molecules such as aquaporins [44][48]. The most direct Vm-contributing ion channels are K^+ and Na^+, while the others play a minor role or secondary messenger role, such as that of Ca^{2+}. Each ion channel type can then be further categorized by gating mechanism. One group, voltage-gated channels, will open or close when their voltage-sensitive domains detect a specific change in membrane potential, usually a significant depolarization from action potentials in neurons. Another type, ligand-gated ion channels, relies on their receptor binding a particular ligand to cause or prevent ionic flow. A third category, leak channels, continually allows a small amount of sodium or potassium to leave the cell, regardless of Vm state [43][49][50]. This type of channel can profoundly impact Vm because it can heavily affect the ion gradient at different stages of excitatory conditions. There are additional mechanisms to regulate or gate leak channels, such as temperature,

mechanical force, and light [43][49][50]. Another interesting group of channels is that of inwardly rectifying potassium channels (Kir) [51]. These channels allow K+ ions to move more easily into, rather than out of, a cell when the cell membrane is depolarized. This is because the intracellular concentration of potassium is so high at rest, and this type of ion movement occurs against the concentration gradient. Even when these are functioning, it is difficult for K+ ions to enter the cell, and they might leak out. Due to this unique characteristic, the Kir channels will impact concentration gradients, resting membrane potential, and cell excitability [51]. Furthermore, different channels can show distinct levels of rectification (e.g., high or low). The lipid species, such as PIP2 (phosphatidylinositol 4,5-bisphosphate), can further regulate Kir channels, as can Mg^{2+}, polyamines, phosphorylation, or protein–protein interactions [51].

Gap junctions are membrane proteins that physically connect adjacent cells to allow ions, small molecules, and electrical impulses to pass directly by a regulated gate between cells. Like ion channels, their conductance is passive and down an electrochemical gradient. Thus, they do not rely on ATP-like ion pumps. Gap junctions are formed by connecting proteins called connexins and pannexins in vertebrates and innexins in invertebrates (depending on the number of Cys residues in their extracellular loop and glycosylation) [52]. These connexins have unique protein structures, properties for permeability, and gating. Each gap junction comprises six connexin subunits on one cell that oligomerize with another six connexins on an adjacent cell. The connection of the same connexin isoform is called homogenous/homomeric, but these properties can change and become more complex by forming heterogeneous/heteromeric gap junctions [53]. When these connexins are not coupled to form a gap junction, they are known as hemichannels [54]. These hemichannels may serve as an ionic and molecular interchange routes between the cytoplasm and the extracellular environment [55]. Gap junctions and hemichannels play significant roles in cell-to-cell communication by exchanging ions, small molecules, subcellular vesicles, electric impulses, and organelles, due to their relatively larger pores [56]. Thus, they are natural modulators of cellular bioelectricity. Electrical synapses between neurons can be considered a specialized gap junction. In addition, gap junctions have also been found to be needed for direct cell communication in tunneling nanotubules (TNTs) [57][58]. Gap junctions are crucial for many physiological processes, including synchronized depolarization of cardiac muscle and embryonic development [59][60]. One ubiquitous gap junction, connexin 43 (CX43), has been implicated in multiple organisms and diseases and it contributes to electrical signaling [61]. Connexin mutations and misregulations have been shown to cause many diseases, such as neurodegenerative diseases and congenital morphological defects in mice and humans [62][63].

Another group of Vm ion regulators is solute carrier proteins (SLCs). These proteins utilize secondary active transport, where thermodynamically favorable reactions (i.e., ions moving down their concentration gradient) are paired with one or more other molecules to be transported in an unfavorable reaction [64]. The free energy provided by the movement in the favorable direction makes movement in the less favorable direction possible and allows transport without directly consuming cellular energy. These reactions utilizing the electrochemical gradient can occur with both substrates moving in the same direction, known as symporters, or substrates moving in opposite directions, known as antiporters. Thus far, over 450 transporter proteins are found in the plasma membrane of cells and subcellular organelles [64][65][66]. These SLCs have an extensive range of substrate specificity, including ions, organic ions, sugars, vitamins, amino acids, nucleotides, oligopeptides, drugs, and metals. In addition, some SLCs

can transport multiple different biomolecules, others can only transport a single biomolecule, and up to 30% are "orphan" proteins, whose substrates remain unknown. Thus, these SLCs have been involved in many physiological regulations, such as selective barriers, neurotransmitters, nutrition, and metabolic regulation [64][66]. More than 190 diseases have been linked to SLCs, such as thyroid, hearing, neurological, metabolic, and congenital defects [64][66]. Due to the nature of their substrates, the SLCs could be an essential contributor to cellular bioelectricity.

3. Bioelectricity Evidence from Zebrafish Genetics

3.1. Zebrafish as a Superior Model for Bioelectric Research

The zebrafish has become one of the leading model organisms used in research since its debut in the 1970s, due to its unique advantages [67][68][69]. First, zebrafish share vertebrate biology with humans. Zebrafish possess 70% orthologous genes to humans [70]. Second, it is a relatively affordable model, compared to murine models. Third, small body size and external development make zebrafish embryos an ideal in vivo system. Fourth, tractable genetics has been developed in zebrafish, including large-scale forward genetic mutagenesis, CRISPR-based reverse genetics, and Tol2 transposon-based transgenesis [71][72][73]. Furthermore, a significant source of mutation lines is available through the repository ZFIN, and the greater zebrafish research community is highly collaborative [74][75]. All these advantages make zebrafish popular for studying developmental biology, neuroscience, physiology, toxicology, drug screens, and many human diseases such as cancers [67][68][69][76][77]. Zebrafish are also particularly suited to bioelectric research. The combination of excellent and well-established genetic tools with transparent external embryonic development can allow for manageable mutant generation and cutting-edge microscopy to explore previously unattainable information. These attributes can also be useful in bioelectric research. Below, researchers highlight bioelectric-related zebrafish studies that demonstrate the importance of this model as an optimal way to characterize and uncover the as-yet-undetermined bioelectric characteristics and mechanistic properties.

3.2. Zebrafish Mutants with Adult Fin-Size Change

Zebrafish adults have two sets of fins: paired fins (pectoral and pelvic) and unpaired median fins (dorsal, anal, and caudal), aligning their anterior to posterior body margins [78][79][80]. Each fin comprises endoskeletons and external dermal bones: the fin rays, or lepidotrichs. The adult fin size and its proportion to the body are generally unvarying. Zebrafish paired fin development was reported to share similar mechanisms with tetrapod limbs, as corresponding signaling centers such as ZPA (zone of polarization) and AER (apical ectodermal ridge) were characterized in zebrafish [78][81][82]. Although direct evidence of bioelectricity in zebrafish fin development is still lacking, indirect evidence came from several zebrafish fin mutants from large-scale forward genetic screenings.

The first reported zebrafish mutant with elongated fin size is *longfin*(*lof*^{t2}), which is a dominant mutant that occurred in nature and is present in the widely used Tüpfel fish line. The causal mutant gene of the *lof* has remained unknown for decades until recently. Two independent reports pinpointed Kcnh2a, a voltage-gated potassium channel [83][84]. There is a 0.9 Mb chromosomal reversion upstream of the *kcnh2a* gene on chromosome 2 [84]. This

inversion disrupts gene regulation and causes a change of the cis-ectopic expression of *kcnh2a* in zebrafish fins. Similar to the *lof*^{t2}, another longfin (*alf*^{dty86d}), an ENU-induced mutant, possesses elongated fins in adults in a dominant way [85]. This *alf*^{dty86d} mutant was reported to be caused by gain-of-function mutations in *kcnk5b*, a potassium leak channel gene [85]. The authors also reported larva fish overgrowth and cellular voltage change, indicating the Kcnk5b-mediated bioelectricity of fin anlagen could be the underlying mechanism through local overgrowth [85].

The *schleier* is another zebrafish mutant with elongated fins. This mutation is caused by the inactivation of a potassium–chloride cotransporter, *slc12a7a/kcc4a* [86]. This mutant is also genetically dominant, and homozygous adults exhibit broken stripes and pigmentation alternations. A CRISPR mutation experiment revealed that the function levels of Kcc4a correspond to the fin and barbel lengths. In addition, *kcnk5b* knockout in the *schleier* fish embryos can reduce the adult fin lengths, suggesting that Slc12a7a might function together with Kcnk5, and both might be required for bioelectric regulation in wildtype fish. Interestingly, the same research group also identified *slc43a2/lat4a*, an L-leucine amino acid transporter that can modify the *kcnh2a* mutation effect in *lof*^{t2} mutant fish, resulting in a flying-fish-like phenotype [84]. This *lat4a* mutant, *lat4a*^{nr21}, is also dominant and exhibits a short-finned phenotype in heterozygotes. The interactions between Lat4a and Kcnh2a in the flying-fish-like zebrafish suggest they are also involved in bioelectric regulation. Along with this short-finned phenotype, two additional mutants were reported. They are the shortfin (*sof*) mutants (4 alleles: *sof*^{b123} (spontaneous), *sof*^{i7e1}, *sof*^{i7e2}, *sof*^{i7e3} (ENU-induced)) caused by a hypomorphic mutation in the gap junction, Cx43 [87], as well as a fish mutant, *mau*, caused by a dominant missense mutation in *aqp3a* (*aquaporin 3a*) [88]. Like the *lat4a*^{nr21} mutation, the *cx43* mutation in *sof* also reverted the *lof*^{t2} long-finned phenotype [89], suggesting that Cx43 is another bioelectric regulator for zebrafish fin size.

The laboratory recently characterized a dominant long-finned mutant, Dhi2059, which was generated via a large-scale insertional mutagenesis [90]. The *kcnj13* gene's exon 5 was disrupted by a retroviral insertion. Although this exon encodes 5′ UTR (untranslated region), not protein, viral DNA insertion leads to a transient and ectopic expression of *kcnj13* in the somites between 15S (15-somite stage) and 48 dpf (days post fertilization) in Dhi2059 fish embryos. Transgenic fish Tg (−5.4k *pax3a*: *kcnj13*-IRES-EGFP), in which the *kcnj13* gene is under the control of the −5.4k *pax3a* promoter, can phenocopy the long-fin phenotype. Thus, *kcnj13* misregulation resulted in elongated fins in the adult zebrafish, mainly by increasing the length of fin rays [90]. Different from the previously mentioned long-finned mutants (*lof, alf, schleier*), the results suggest that the adult fin size can be determined at the somite stage in early fish embryos. This indicates that bioelectricity is set up early and could serve as a memory for patterning and size regulation in later ontology (see detailed discussion in the prospective section). In addition, researchers showed that transient expression of multiple potassium channels (*kcnj1b, kcnj10a, kcnk9*, human *KCNJ13*) in zebrafish early embryos (by microinjection) could also cause chimeric long fins in injected adult fish. This result suggests it is not a specific potassium channel, but that bioelectricity is the key to the elongated fin phenotype.

Multiple key points can be obtained by comparing these zebrafish mutants. First, all the mutant genes are involved in ion regulation, which is intrinsically linked to bioelectricity. These ion regulators have their own ion type selecting

properties and conductance. It becomes challenging to explain the fin phenotype with a specific channel or ion. Instead, it is more reasonable that electric signaling is the underlying mechanism. Different ion regulators with other properties can be used to construct and modify the bioelectric state of cell groups and tissues. Second, all of these mutations are genetically dominant; most are gain-of-function, ectopically expressed, or neomorphic. Lastly, the specificity of the zebrafish fin-size phenotype may be caused by the spatiotemporal distribution of these ion regulators during embryonic development, as exampled by the Dhi2059 mutant. Taken together, the zebrafish's adult fin size could be regulated at multiple stages. Although most studies reported altered gene expression in fin anlagen or local fins, the experimental data suggested that somites, the embryonic origin of fin ray progenitor cells, can play a critical patterning role.

Consistent with zebrafish mutants, it is also worth noting that different potassium channels were recently identified in other teleosts through genome association studies. The inwardly rectifying channel gene *kcnj15* was mapped to long-finned betta fish [91]. Additionally, the ether-à-go-go (EAG) potassium channel gene, *kcnh8*, was found to be highly expressed in the male caudal fins in Xiphophorus [92]. Like zebrafish, *kcnk5bS* was identified as a candidate for long-tailed goldfish [93]. Together with zebrafish mutants, these data suggest that ion-channel-mediated bioelectricity plays an essential role in fin patterning.

3.3. Zebrafish Mutants with Adult Pigmentation Pattern Alterations

Zebrafish adults exhibit distinct stereotypical stripe patterns along their bodies, with alternating rows of melanophores (dark pigments) and xanthophores (red-orange pigments) mixed with iridophores (iridescent pigments) [94][95][96]. Local and long-range interactions and communication among these different pigment cells during embryonic and larval stages are essential to forming the stripe patterns [57][93][94]. Among many mutant zebrafish lines with altered pigmentation patterns, several are mutations of ion regulators, suggesting that ion-channel-mediated bioelectric signals play important roles in pigmentation patterning. Two of the fish mutants, *albino* and *golden*, resulted from the loss of function of solute carrier genes, *slc45a2* and *slc24a5*, respectively [97][98][99]. The phenotypic results of the two mutants are a complete loss of melanophores and light stripes (melanophores with small and fewer melanin granules), respectively. The two genes are expressed in zebrafish melanophores, and light pigmentation was thought to be mainly caused by reduced melanogenesis due to ion and proton alteration in the melanophores [98][99]. The *transparent* (*tra*) fish possess fewer iridophores, melanophores, and dark spots, instead of stripes, in adults. This *tra* is a loss-of-function mutation of the *mpv17* gene, which encodes a non-selective channel that modulates mitochondria membrane potential [100][101]. Although the loss of Mpv17 was found to cause a reduction in the number of mitochondria and reduced pyrimidine synthesis [101], the bioelectricity of iridophores might also contribute to patterning defects.

In addition to chromophore defects, zebrafish stripe patterns were found to be altered in additional mutants. The *leopard* (*leo*^{t1}) mutation, also known as *tup*, is a spontaneous recessive mutation causing spots in the adult *Tüpfel* fish line. This mutation is caused by the *cx41.8* (*connexin 41.8*) gene, which encodes Gja5b in zebrafish [102]. Similarly, *luchs* (*luc*^{tXA9}) is a mutation of the *cx39.4* (*connexin 39.4*) gene, which encodes Gja4 [102]. Both *cx41.8* and *cx39.4* are required for melanophore and xanthophore development. Both mutants show aggregated dark spot

patterns instead of stripes. Interestingly, it was shown that these two connexins could form heteromeric, in addition to homomeric, gap junctions, which are essential for melanophore and xanthophore cellular communication [102] [103]. Recently, another mutant zebrafish, *schleier*, was reported to be caused by hypomorphic function of another solute carrier, *slc12a7a/kcc4a* [86]. The homozygous mutant fish show broken stripes in the ventral body flank and anal and caudal fins. Gap junctions usually conduct small molecules and ions between neighboring cells. Thus, they can modulate molecular and electrical coupling among the adjacent cells [59][60], and over longer distances [57] [58]. Additionally, the *obelix(obe)/jaguar(jag)* mutants, which are caused by a *kcnj13* loss of function, have fewer stripes compared to wildtype fish [104]. Kcnj13 is an inwardly rectifying potassium channel that regulates cell excitability and membrane potential. Based on the less severe pigmentation phenotype of the *kcnj13* null mutants ($kcnj13^{pu107}$, $kcnj13^{pu109}$) the lab generated, the original alleles (jag^{b230}, $obetc^{271d}$, and obe^{td15}) are most likely dominant negative [90][104]. More recently, *kcnj13* expression was found to underlie the pattern diversification among *Danio* species via the *kcnj13* regulatory changes [105]. This potassium channel gene is expressed in melanophores during development, suggesting that it may regulate melanophore bioelectric properties. Indeed, cellular electrical communication was partially disrupted in this mutant. The dissociated melanophores of *jag* are more depolarized when measured with a voltage-sensitive dye, DiBAC4(3), than the melanophores from wildtype fish. Wildtype melanophores are transiently depolarized when contacted by the dendrites of a xanthophore, and then moved away from the xanthophore. In contrast, jag^{b230} melanophores lost contact-dependent depolarizations and repulsive migration behavior [106].

Three additional zebrafish mutants could also be related to bioelectric regulation, though the related genes are not direct ion regulators. Spermidine is an endogenous polyamine that can regulate ion channels and connexins [107] [108]. The *idefix* (ide^{t26743}) fish is a loss-of-function mutant of the *srm* (*spermidine synthase*) gene [109]. Homozygous ide^{t26743} mutants have fewer narrowed and often interrupted dark stripes in the trunk and fewer strips in the fins. This *ide* mutation can further reduce melanophores when crossed with leo^{t1}, luc^{tXA9}, and obe^{271d} mutants, suggesting that spermidine may modulate connexin and potassium channel functions. Moreover, ectopic expression of spermidine/spermine N1-acetyltransferase (Ssat), a polyamine metabolic enzyme in melanophore, caused broken stripes and a loss of melanophores in the $leo^{t1/t1}$ background, also supporting this idea [110]. Another zebrafish mutant, *schachbrett* ($sbr^{tnh009b}$), is caused by a loss of function mutation of tight junction protein 1a (Tjp1a), which is expressed in iridophore [111]. Like ide^{t1}, the $sbr^{tnh009b}$ mutant exhibits more substantial pigment patterning defects in luc^{t32241} and leo^{t1} background, indicating Tjp1a may interact with connexins. Thus, Tjp1a may indirectly affect the bioelectricity of chromatophores. The third zebrafish mutant, *mau,* also possesses spotted pigments. The underlying gene of the *mau* mutation is *aqp3a,* which is mainly expressed in skin and muscle, but not in chromatophores [88]. Transplantation of $aqp3a^{tVE1/+}$ blastomere cells into wildtype and $Aqp3a^{R220Q}$ in a transgenic experiment revealed that Aqp3a might indirectly influence chromatophores for pigment patterning. Aqp3a is a transporter of non-polar solutes such as glycerol, peroxide, and urea, excluding ions [112]. Thus, Aqp3a can modulate the ion concentrations related to cellular bioelectricity.

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