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REVIEW

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Epigenetic regulation in zebrafish development: the roles of polycomb group proteins in heart and pectoral fin development

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Abstract

Background Polycomb group proteins, known for their role in epigenetic gene silencing, are critical regulators of cell fate and tissue development across species. These highly conserved proteins are assembled into two multi-protein complexes: Polycomb Repressive Complex 1 and Polycomb Repressive Complex 2.

Main body In zebrafish, a prominent vertebrate model, the roles of PRC1 and PRC2 have been extensively studied, particularly in the context of organogenesis. This review focuses on the emerging functions of PcG proteins in zebrafish development, with an emphasis on the involvement of PRC1 and PRC2 in the formation and differentiation of the heart and pectoral fins. By integrating findings from recent studies, we aim to provide a comprehensive overview of how PcG function contributes to the intricate processes underlying zebrafish heart and pectoral fin development.

Conclusion This review highlights the zebrafish model as a powerful system for unravelling the complex roles of PcG proteins in vertebrate development.

Keywords Polycomb proteins, Zebrafish, Heart development, Pectoral fin development

Background

In eukaryotes, DNA is wrapped around a histone octamer, forming nucleosomes, which assemble to constitute chromatin. The terminal regions of histones, including both N-terminal and C-terminal tails, undergo various post-translational modifications that regulate chromatin states, which can exist in an open and transcriptionally active state, called euchromatin, or in a compact and transcriptionally inactive state, known as heterochromatin [1, 2]. Histone modifications influence transcriptional regulation by altering chromatin accessibility. The regulation of chromatin dynamics is influenced by numerous factors, including the Polycomb group (PcG) protein complexes [3]. PcG genes were first discovered through screening for mutants in *Drosophila melanogaster* that exhibited an extra sex comb phenotype. At least 16 PcG genes were identified as transcriptional

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repressors of the homeotic (Hox) genes, which play a role along the body axis [4–6]. The composition and function of these genes are conserved across vertebrates, where they play crucial roles in cellular differentiation, proliferation during early development, tissue integrity, homeostasis maintenance, and stem cell renewal [7–13]. The conserved role of PcG proteins is primarily mediated through their involvement in the multiprotein complexes PRC1 and PRC2, which silence target genes, thereby precisely and dynamically regulating gene expression during development.

Polycomb repressive complexes are formed by a catalytic core and various accessory proteins, classified based on their specific accessory components as shown in Fig. 1. Core component of PRC1, Really Interesting New Gene 1A or B (RING1A/B) catalyses the monoubiquitination of lysine 119 on histone 2A (H2AK119ub) [14–18]. EZH1/2 of PRC2 harbours histone methyltransferase activity, catalysing the di- and trimethylation of lysine 27 on histone H3 (H3K27me2/3) [19, 20]. The functions of PRC1 and PRC2 are closely interdependent. PRC2-mediated histone modification, specifically H3K27me3, is recognised by the CBX subunit of cPRC1. This recognition facilitates the recruitment of cPRC1 to PRC2-occupied target sites, leading to gene repression by catalysing H2AK119ub [15, 21, 22]. Similarly, vPRC1 recruits PRC2 to target loci, where vPRC1 catalyses H2AK119ub, facilitating PRC2 recruitment and subsequent histone methylation and transcriptional repression [23–26].

Researchers often use embryonic stem cells (ESC), *Drosophila melanogaster*, and mice to investigate Polycomb-mediated repression. However, because of the essential role PcG proteins play in development, PcG-deficient mice models frequently do not survive, whereas zebrafish (*Danio rerio*) embryos can survive through the gastrulation stages. In mice, maternal contributions to the embryo cease after the 2-cell stage, whereas in zebrafish, this support extends up to the 1000-cell stage. This extended maternal contribution in zebrafish offers a unique advantage for studying early gene regulation, providing a more suitable model for exploring the mechanisms underlying early development [27, 28]. Moreover, the zebrafish model offers a useful model to investigate the role of PcG proteins in vertebrate development, owing to its distinctive genetic and developmental features.

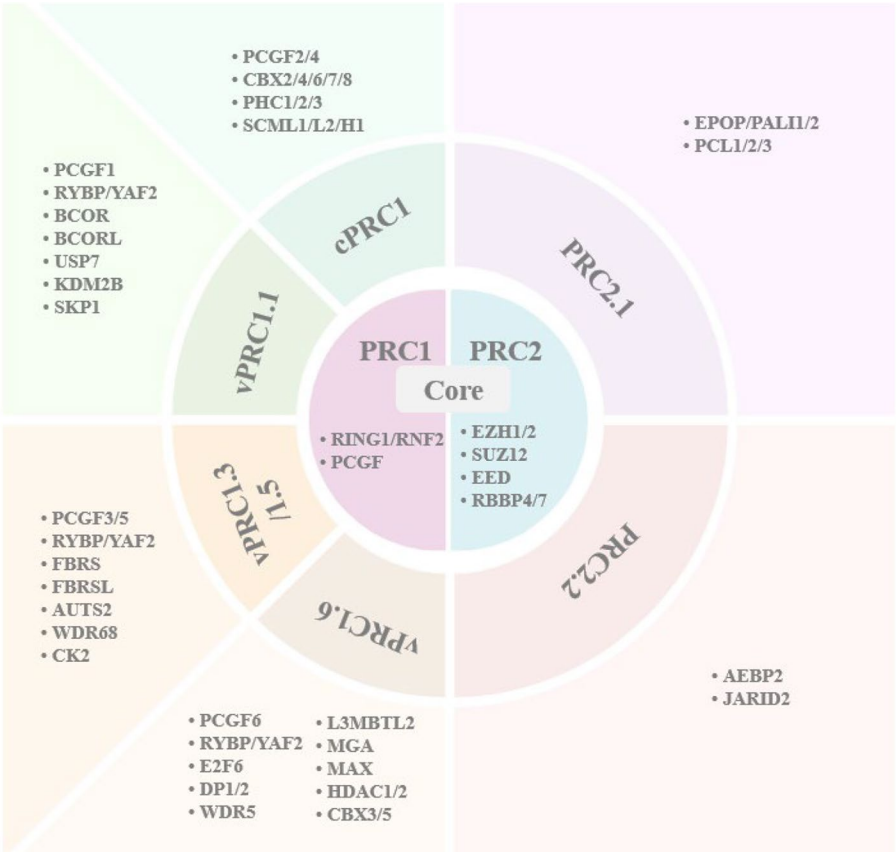

Fig. 1 Overview of PRC classification and composition

In this review, the following sections will summarize the crucial roles of Polycomb proteins in the development of the zebrafish, highlighting the crucial role in the heart and pectoral fin development.

In PRC1, the catalytic core is formed by the heterodimerization of the RING1A/B with one of six Polycomb group RING finger (PCGF) family members, resulting in six distinct PRC1 variants. PRC1 is further divided into canonical PRC1 (cPRC1) and variant/non-canonical PRC1 (vPRC1), depending on whether the core heterodimer associates with chromobox (CBX) proteins or RYBP. cPRC1, involving PCGF2/4, assembles with CBX and other essential subunits like PHC and SCM proteins to mediate gene silencing. In contrast, vPRC1 can form complexes with all six PCGF types and RYBP/YAF2 along with additional accessory proteins. In PRC2, the catalytic core is composed of four subunits: Enhancer of Zeste Homolog 1/2 (EZH1/2), Embryonic Ectoderm Development (EED), Suppressor of Zeste 12 (SUZ12), and Retinoblastoma-Binding Protein 4/7 (RBBP4/7). PRC2 exists in two variants, PRC2.1 and PRC2.2, each associated with different accessory components.

Main text

Zebrafish as in vivo model to study PcG function

The zebrafish emerges as an ideal in vivo model for understanding the complex roles of PcG proteins due to its ortholog counterpart of all PcG components. Introduced as a model organism by, George Streisinger in the 1970s, zebrafish were chosen for their optical clarity, high productive rate and diploid genome. Subsequent forward genetic screenings confirmed their suitability for developmental studies [29]. Zebrafish offer several advantages, including rapid development as they become sexually mature within 12 weeks after fertilization (wpf), and their *ex utero* development facilitates manipulation and observation from the single cell stage, they can produce hundreds of offspring in a single mating, providing a large sample size for experiments [29–33]. The teleost lineage which includes zebrafish underwent a significant genome duplication event around 320 million years ago, known as teleost genome duplication (TGD), which led to the generation of redundant ohnologs. Following this duplication, one of each pair of ohnologs was randomly lost, resulting in the retention of approximately 15–20% of the duplicated genes within the teleost infraclass [34–37]. Genes coding for PRC subunits in zebrafish are mentioned in Table 1. In the zebrafish genome, 13 PcG subunits are present as pairs of duplicated genes. During the rediploidization process, some ohnologs like core PRC1 components RING1A, PCGF2, and PCGF3, the core PRC2 component RBBP7 and the accessory vPRC1 subunit HDAC2 were lost, leading to a reduction in the number of duplicated genes in the zebrafish genome. Despite the absence of several gene orthologs, the diversity and complexity of PRC complexes in zebrafish remain intact, making zebrafish an excellent model for studying Polycomb-mediated gene silencing during development.

Table 1 Genes encoding for PcG components in zebrafish

Type	PRC1		PRC2	
Core	*rnf2*		*ezh1*	
	bmi1a/1b		*ezh2*	
	pcgf1		*eed*	
	pcgf5a /5b		*suz12a/12b*	
	pcgf 6		*rbbp4*	
Subtype	**cPRC1**	**vPRC1**	**PRC2.1**	**PRC2.2**
Accessory subunits	*cbx2*	*rybpa/b*	*skida11*	*aebp2*
	cbx4	*Yaf2*	*lcor*	*jarid2a/2b*
	cbx6a/6b	*kdm2ba/bb*	*lcorl*	
	cbx7a/7b	*auts2a/2b*	*phf1*	
	cbx8a/8b	*l3mbtl2*	*mtf2*	
	phc1	*e2f6*	*phf19*	
	phc2a/2b	*max*		
	phc3	*mgaa/ab*		
		usp7		
		skp1		
		bcor		
		bcol1		
		atrx		
		fbrsl1		
		fbrs		
		wdr68/dcaf7		
		tfdp1a/1b		
		wrd5		
		hdac1		
		cbx3a/3b		

PcG complexes are crucial for embryonic development, primarily by mediating repressive histone modifications that silence inactive developmental regulator genes during early embryogenesis. This gene silencing is critical for the progression of embryos through the gastrulation stage, as knockout studies in mice demonstrate that the absence of PcG function results in embryonic lethality [8, 10, 13, 38, 39]. Various forward and reverse genetic approaches enabled the targeted study of PcG genes. Techniques such as *N*-ethyl-*N*-nitrosourea (ENU) mutagenesis [40], morpholino-based knockdown [41, 42] and advanced gene-editing techniques—like zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs) [43–45] and the clustered regularly interspaced short palindromic repeat (CRISPR)/CRISPR associated protein 9 (CRISPR/Cas9) system [43–45] have been used to investigate the roles of PcG in zebrafish

development. Mariette Hanot and colleagues provide a comprehensive overview of how zebrafish studies have advanced our understanding of Polycomb-mediated gene repression. Their review extensively discusses PcG genes in zebrafish, the effects of PcG deficiency on zebrafish organ development, behaviour, and diseases such as cancer [46]. While their work offers valuable insights into the role of PcG proteins in zebrafish development, it does not extensively address their specific functions in heart and pectoral fin development. Building on these findings, our review focuses on the critical roles of PcG proteins in the development of the heart and pectoral fins.

Role of polycomb-mediated repression in the heart development

Zebrafish embryos do not rely solely on the heart and blood circulation for oxygen supply; they also obtain oxygen through passive diffusion. This characteristic allows zebrafish mutants to survive early development even with cardiovascular defects. Whereas, mammals cannot survive without a functional cardiovascular system [47]. Despite the simpler structure of the zebrafish heart which consists of just two chambers (atrium and ventricle), the genetic and molecular processes guiding heart formation are highly conserved across vertebrates [48–50]. This provides a valuable opportunity to study the genetic and molecular mechanisms underlying heart development. The zebrafish heart begins to develop shortly after fertilization and undergoes various stages, including initiation, heart tube formation, looping, maturation, and specification, as shown in Fig. 2a. During these stages, many key genes and signalling pathways, such as *nkx2.5*, BMP signalling, retinoic acid (RA) and fibroblast growth factor (FGF) pathways play essential roles [51–54]. The development of the zebrafish heart exemplifies a finely tuned orchestration of multiple genes and tightly regulated signalling pathways. This developmental precision ensures the proper alignment and maturation of the cardiac conduction system, which is essential for efficient pumping and circulation [55–57]. Moreover, the genetic conservation of these developmental processes with higher vertebrates makes the zebrafish an invaluable model for studying heart development and disease.

PRC1 in zebrafish heart development

PcG proteins play a crucial role in heart development as both PRC1 and PRC2 deficient embryos display the phenotype 'heart-string' or tubular heart in which the heart fails to loop properly after 48hpf and appears like a string instead of a two-chambered heart. The only zebrafish homolog of the enzymatic subunit of PRC1, *rnf2* upon loss of function displays pericardial edema and stringy heart [58–61]. *rnf2* $^{ibl31/ibl31}$ mutant shows the normal level of early cardiac markers, *nkx2.5, tbx5*, and *hoxb5b* at 10-15ss in lateral plate mesoderm (LPM) which indicates the heart filed specified correctly and PRC1 does not play a role during early development of heart [61]. In Another study, Chrispijn et al. performed the RNA sequencing of individual *rnf2* $^{ibl31/ibl31}$ mutant heart and they observed an increased number of altered gene expressions over time. The transcription factor T-Box proteins *tbx2a, tbx2b*, *tbx3a* and *tbx5* are upregulated in the mutant heart at 48hpf and 72hpf. At the same time, genes involved in the structural development of heart-like *vhmc, myl7, myh6,* and *nppa* are downregulated [58, 62–64]. Together this suggests that PRC1/*rnf2* does not play a role in the early phase of heart development but regulates the gene involved in the structural development of the heart. An additional phenotype of weak contractility was observed in homozygous *rnf2 (f5)* mutant embryos [60]. During atrioventricular canal (AVC) development between two chambers, the transformation of smooth muscle into striated muscle involves the participation of several genes associated with both smooth muscle and skeletal muscle development [32, 65]. These include smooth muscle genes such as *acta2, myl9b, ppp1r12*, and *myh11a*, as well as skeletal muscle-related genes like *acta1a, myl1*, *tnni2b*, and *tnnt3a* [66, 67]. This transition is critical for the proper formation and function of the AVC, ensuring the structural integrity and contractile properties necessary for cardiac performance [57, 68, 69]. In the *rnf2 (f5)* mutant, the expression level of the smooth and skeletal muscle genes was dysregulated resulting in a change in cardiac sarcomere assembly. On TEM analysis, cardiac sarcomere alignment was not like the wild type as the myofibril fibres were closely packed, I-band and Z disc were far from each other compared to the wild type. On mapping cardiac conduction with calcium-sensitive dye, fluo-4 AM, the reduced intensity of the calcium signal in the *rnf2(f5)* mutant heart is observed compared to the wild type. Abnormality in the sarcomere causes the contraction defect, as the cardiac sarcomere is the basic contractile unit of the contraction system in the heart [60]. The core component of the heart contraction system, AVC formation disrupted and expression of AVC-specific genes *activated leukocyte cell adhesion molecule a* (*alcama*) in endocardium, *versican a* (*vcana*) in myocardium and *bmp4* were expanded and diffused. These results indicate that the PRC1 is crucial for cardiac sarcomere assembly, maintaining cardiac contraction and AVC constriction by repressing the non-cardiac sarcomere gene expression [60].

Another component of non-canonical PRC1, *mgaa* knockdown with morpholino fails the heart tube looping with cardiac edema [70]. In *mgaa* morphant heart, *gata4* was upregulated twofold at the 5somite stage

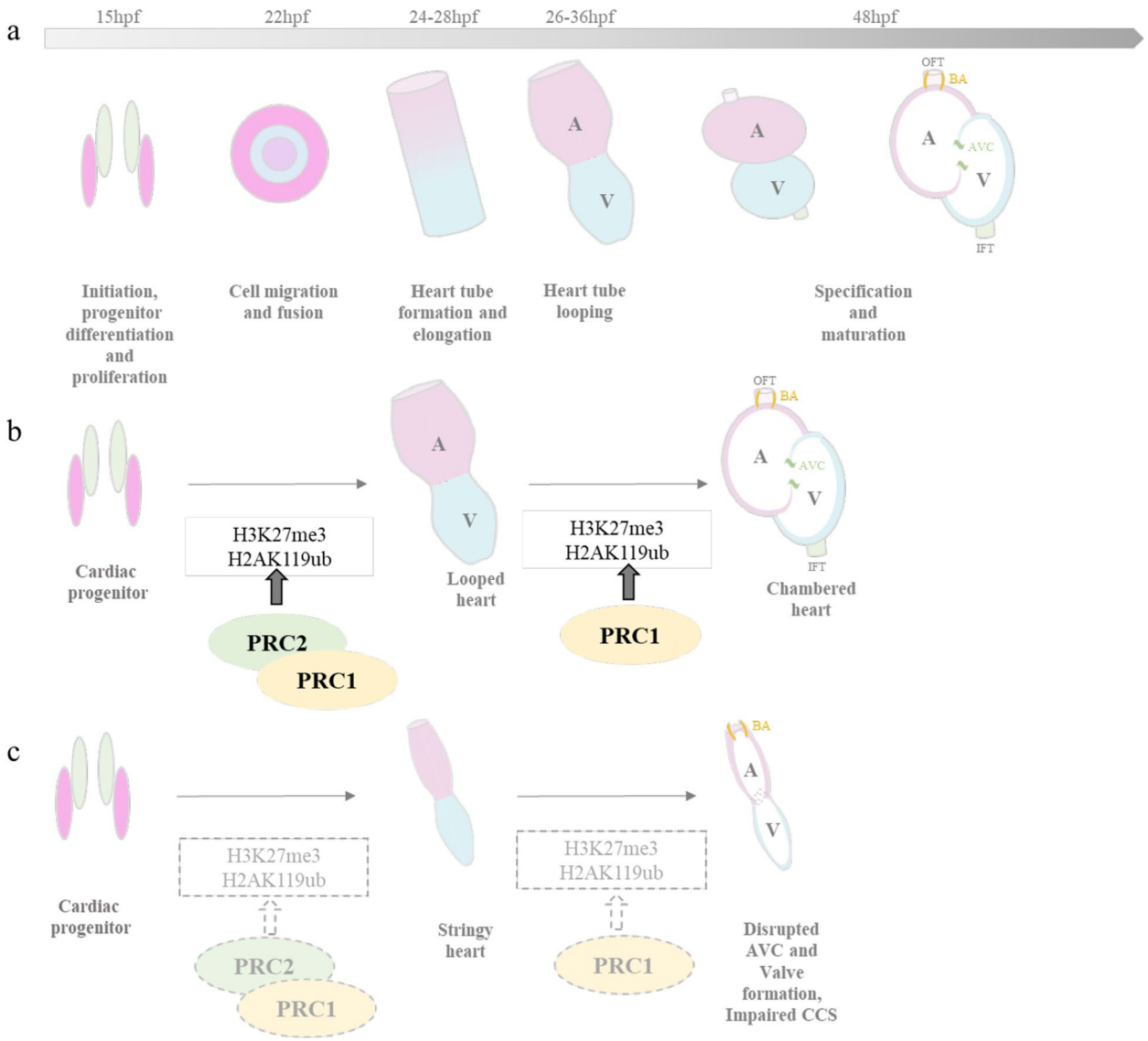

Fig. 2 Zebrafish heart development and role of PRC1/PRC2 in heart development. **a** Heart development in zebrafish begins shortly after fertilization. During gastrulation, cardiac progenitor cells migrate towards the midline and localize in the anterior lateral plate mesoderm (ALPM). By 15 h post-fertilization (hpf), cardiac progenitor cells are established, and by 19 hpf, they migrate and fuse at the midline to form a cardiac disc. By 24 hpf, this disc transitions into a heart tube, which begins beating and initiates circulation throughout the body. At 36 hpf, the heart undergoes leftward bending, forming an "S" shape that defines the future atria and ventricle. Chamber formation and alignment occur during the hatching period (48–72 hpf), when the heart begins to pump blood irregularly. After 48hpf, valve formation at the AV canal, IFT, OFT, and BA development occurs to ensure blood flow from the heart to the gills. **b** PRC2 is involved in regulating the early stages of heart development, while PRC1 contributes to the the formation and specification of the chambered heart from the looped heart structure. **c** In embryos lacking PRC1 and PRC2, the heart fails to undergo proper looping, resulting in a string-like appearance. PRC1 is essential for cardiac sarcomere assembly, the maintenance of cardiac contraction, and the formation of the atrioventricular canal (AVC) and valve structures between the atria and ventricle. In PRC1-deficient embryos, AVC and valve development are disrupted, along with impairments in the cardiac conduction system (CCS). V, ventricle; A, atria; BA, bulbous arteriosus; AVC, atrioventricular canal; OFT, outflow tract; IFT, inflow tract

and fourfold at 24hpf in LPM. On co-injecting *mgaa* and *gata4* to reduce the level of both, rescues the heart looping/ edema defect. vPRC1/*mgaa* controls the *gata4* transcript level required for heart tube looping [70].

PRC2 in heart development

Ezh2 deficient zebrafish embryo displays the common phenotype of cardiac edema, stringy heart, and small ventricle, especially in *maternal-zygotic ezh2* (*MZezh2)*

mutants in which Ezh2 function lost from both maternal and zygotic origin [19, 71–74]. Additionally, the ezh1/2 inhibitor PF-06726304 acetate has been shown to lead to heart edema [75]. Similarly, the ezh2-specific inhibitor GSK126 and 3-deazaneplanocin A (DZNep), which targets several S-adenosylmethionine (SAM)-dependent methyltransferases including ezh2, have also been found to induce heart edema [19, 71]. San and colleagues derived a premature *ezh2 (hu5670)* mutant from ENU-mutagenized library. To investigate the effects of complete *ezh2* loss on early development, they generated *maternal-zygotic ezh2 (MZezh2)* mutants at the same locus by performing germ cell transplantations, effectively eliminating both maternal and zygotic *ezh2* contributions [73, 76]. In *MZezh2* mutant, *nkx2.5* expressing cell number is low at 12 somite stage compared to wild type sibling and *nkx2.5* expression is absent in posterior pharyngeal arch progenitor at 1 and 1.5 dpf whereas another early marker *hand2* remains unaffected. The expression of chamber-specific markers like *vmhc, amhc*, and *nppa* was partially reduced. The reduction in *nkx2.5* expression and low number of cells expressing these chamber-specific markers indicates the initiation of cardiogenesis and terminal differentiation is hampered in the *MZezh2* mutant. GFP-tagged *myl7*-expressing cells were observed to move away from the cardiac tube in the *MZezh2* mutant heart due to which the heart appears small compared to wild type and heterozygous sibling. At 2 dpf, the GFP-positive extra cardial cells lack the *nkx2.5* expression in heterozygous and wild type after heart tube formation but in *MZezh2* mutant due to loss of *ezh2,* these cardial cells fail to repress the *nkx2.5*. Instead of heart jogging to the left like in the wild type, homozygous mutants remain tubular after 24hpf. The heart tube fails to loop and AVC is not properly developed maybe due to ectopic expression of *has2* which facilitates the cardiac cell migration. Similarly, other myocardial markers *mef2cb* and *myl7* show expanded expression rather than being restricted in the myocardial cells. This indicates that PRC2 maintains the cardiac cell integrity which regulates the size and structure of the heart. Overall *ezh2* regulates the gene involved in initiation, terminal differentiation, heart looping, and maintenance of structural integrity during heart development [73]. CHIP-sequencing data showed the absence of both *ezh2* and H3K27me3 at genes involved in heart development such as *tbx3a, tbx5*, and *isl1* loci. Peaks for PRC1/*rnf2* are also not detected at the same loci as PRC2 recruits the PRC1 at the target loci [72]. Different studies showed that the reduction of different epigenetic markers H3K4me3, H3K27me3, and H2AK119ub developed a similar phenotype of stringy heart [60, 73, 77]. Mutants of the *ezh2* homolog *ezh1*, such as $ezh1^{b1394/-}$ or $ezh1^{-/-}$, do not exhibit any heart defects, even though cardiomyocytes in these mutants show a modest reduction in H3K27me3 levels. Additionally, *ezh1; ezh2* double mutants ($ezh1^{b1394/-}$; $ezh2^{b1392/-}$) display phenotypes nearly identical to those of *ezh2* single mutant ($ezh2^{b1392/-}$). This finding suggests that *ezh1* is dispensable for zebrafish heart development and cannot compensate for *ezh2*/PRC2 function [78]. Overall, *ezh2*/PRC2 appears to play key roles in regulating gene expression and maintaining heart structural integrity in zebrafish.

Studies reviewed here indicate that the PcG proteins, PRC1 and PRC2, are critical in zebrafish heart development. PRC2 appears to function primarily in the early stages, contributing to cellular structure and integrity, while PRC1 plays a role in the later stages of heart formation. Despite these insights, many questions remain unresolved, as PcG mutants do not survive to adulthood, leaving gaps in understanding how adult heart cardiomyocytes maintain their identity and function. Much work is still needed to fully integrate PcG function into the broader framework of cardiac transcriptional regulation.

Role of polycomb-mediated repression in the pectoral fin development

Zebrafish pectoral fins are evolutionarily related to the forelimbs of tetrapods, including humans, with both structures governed by similar genetic and molecular mechanisms that guide limb development across vertebrates [79–81]. Despite their differing appearances, zebrafish fins and tetrapod limbs rely on conserved genetic pathways during development. Phylogenetic studies indicate that although fish fins and tetrapod limbs are not morphologically equivalent, the underlying regulatory processes are deeply conserved, suggesting a shared evolutionary origin [79, 81, 82]. The early stages of zebrafish fin development closely resemble tetrapod limb formation, with critical genes such as *shh*, *fgf8*, and *tbx5* showing similar expression patterns and functions [83–85]. This parallel is further supported by the role of retinoic acid (RA) signaling, which is essential in initiating fin and limb formation across various species, including mice, chicks, and zebrafish [86–88]. Detailed zebrafish pectoral fin development in comparison with tetrapod limb development is reviewed by Mercader [89]. The genetic conservation of zebrafish pectoral fin with other models like mice and chick, allows researchers to investigate the roles of various genetic factors, such as PcG proteins, which play critical roles in the regulation of gene expression during development. The development of the pectoral fin in zebrafish is illustrated in the Fig. 3a. Briefly, The initiation of pectoral fin development in zebrafish begins within the LPM around the 2 to 3 somite stage, which closely parallels the early stages of vertebrate

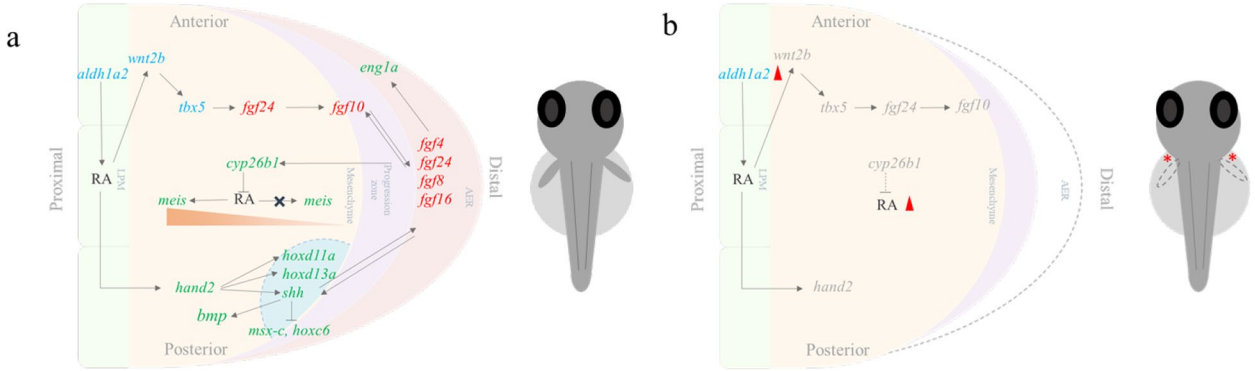

Fig. 3 Zebrafish pectoral fin development and effect of PRC1/PRC2 deficiency on pectoral fin development. **a** Retinaldehyde dehydrogenase 2 (*aldh1a2*) synthesizes RA which initiates expression of downstream target *wnt2b* and *tbx5* (genes involved in pectoral fin initiation are shown in blue) *tbx5* activates *fgf* cascade(Genes involved in pectoral fin outgrowth are shown in red). AP patterning: RA initiates *hand2* expression in the posterior region, which induces *shh*, *hoxd11a*, and *hoxd13a* in the ZPA and governs anterior patterning (ZPA marked in blue, Blunt head arrow shows the repression) DV patterning: *shh* induces the expression of *bmp* in the dorsal region and *fgf4* induces the expression of *eng1a* in the ventral region of AER. PD patterning: Gradients of RA and *fgf* regulate proximodistal patterning. *fgf* induces *cyp26b1* expression, which degrades RA locally, leading to lower RA levels in the distal fin. (Genes involved in patterning are shown in green.) AER, Apical Ectodermal Ridge; AP, Anteroposterior; ZPA, Zone of Polarizing Activity; DV, Dorsoventral; PD, Proximo-distal. **b** In embryos deficient in PRC1/PRC2, the initiation of fin development and the Fgf signalling pathway are impaired, leading to compromised formation of AER. Increased *aldh1a2* expression leads to elevated RA signaling (indicated by the red arrow). A red asterisk marks the absence of a pectoral fin. Genes written in grey indicate downregulation

limb formation [90, 91]. The pectoral fin undergoes significant development during embryogenesis and continues to mature post-hatching. By approximately 3 to 4 wpf, the zebrafish pectoral fin achieves its mature structure, characterized by fully developed fin rays supported by a well-defined skeletal framework [92].

Research involving mutations in core components of the PRC has shed light on how PcG proteins contribute to the formation and development of pectoral fins, offering insights into their broader role in vertebrate limb development.

Role of PRC1 in pectoral fin development

PRC1/*rnf2* deficient zebrafish lack the pectoral fins indicating the role of PcG protein in pectoral fin development [58–61, 93]. In *rnf2*$^{ibl31/ibl31}$ mutant, the expression of early expressing genes in pectoral fin development, *tbx5* and *hand2* is preserved but is lost by 40 hpf in the fin mesenchyme. The downstream target of *tbx5*, *fgf24* and *fgf10* is initiated correctly, it becomes reduced and confined to a small area following the downregulation of *tbx5*. Additionally, with the absence of AER markers such as *dlx2a*, *fgf8*, *fgf24* and *versican*, the formation of the AER is compromised. This indicates that while fin formation begins properly but its outgrowth is hindered in *rnf 2*$^{ibl31/ibl31}$ mutant. Even though exogenous Fgf could rescue *tbx5* expression in the pectoral fin bud of zebrafish embryos, it failed to restore fin bud outgrowth. The RA-synthesizing enzyme *aldh1a2* is overexpressed and extends beyond the posterior LPM region. Simultaneously, the expression of the Wnt target gene *cyp26a1*, which typically converts RA into an inactive form, is reduced at 32 hpf, indicating an excess of RA formation. In response to this accumulation, RA-responsive genes such as *dhrs3*, which converts retinaldehyde back to retinol, are upregulated, reflecting increased RA metabolism in *rnf 2*$^{ibl31/ibl31}$ mutant [61, 93–95]. Elevated RA levels are associated with various developmental abnormalities, including limb deformities, neural tube defects, craniofacial malformations, and cardiac issues [94, 96]. On inhibition of RA signalling using DEAB in *rnf2* $^{ibl31/ibl31}$ mutant at 24 hpf, there was an increase in *tbx5* and *hand2* levels compared to untreated mutants, although these levels did not reach those seen in wild-type embryos. Although the expression of mesenchymal genes *tbx5* and *hand2* is increased, it is still not sufficient to increase *fgf24* expression in the ectoderm, which is necessary for fin outgrowth. Excess RA signalling in *rnf2* $^{ibl31/ibl31}$ mutant led to the ectopic expression of RA-responsive *hox* genes such as *hoxc6a* and *hoxc8a* in the posterior region and throughout the brain, deviating from their typical anterior restriction observed in wild-type embryos. This abnormal expression pattern indicates a disruption in the anterior–posterior axis formation, potentially impacting the overall body plan and function [97, 98]. Additionally, in *rnf2*$^{ibl31/ibl31}$ mutant, the fin-specific expression of *hoxa9b*, *hoxc8a*, and *hoxd9a*, which are normally repressed by PRC1, was impaired, while their axial

expression domains were expanded. The expression of patterning markers such as *shh* and *msxc* for AP patterning, as well as *eng1a* and *wnt7a* for DV patterning, was either completely absent or reduced compared to wild types in *rnf2* $^{ibl31/ibl31}$ mutant. This dysregulation of patterning genes may be due to the absence of an upstream regulator necessary. This suggests that the loss of PRC1/Ring1b-mediated repression in these mutants affects the spatial regulation of *hox* genes, elevates the RA signalling and hampers Fgf signalling contributing to the lack of pectoral fin phenotype [61].

Role of PRC2 in pectoral fin development

In the PRC2 *MZezh2* mutant, pectoral fins are absent, and the expression of *tbx5* is entirely missing in these fins. The *hoxab* gene cluster, a Polycomb target, shows altered expression in mutants, extending beyond its normal anterior–posterior axis domain [72]. PRC2 plays a crucial role in modulating gene expression influenced by retinoic acid signalling [99, 100]. Similar to the *rnf2* $^{ibl31/ibl31}$ mutant, the *hoxa9b* gene is not expressed in the pectoral fin bud region of the *MZezh2* mutant but shows ectopic expression in the posterior region and throughout the brain, contrasting with the anterior restriction seen in wild-type embryos. AP patterning gene, *shh* expression is absent in the pectoral fin bud region of the *MZezh2* mutant at 48 hpf. The expression patterns of the *hoxd9a, hoxc8a*, and *hoxc6a* genes in *MZezh2* mutants reveal an anterior shift in the boundary of *hox* gene expression, similar to what is observed in the *rnf2* $^{ibl31/ibl31}$ mutant [61, 73, 93]. When the Ezh2-specific inhibitor GSK126, which selectively targets Ezh2, is introduced, a delay in pectoral fin development occurs. However, *ezh2(ul2)* mutants do not display this pectoral fin defect, likely due to the maternal contribution of *ezh2*, which supports normal body plan formation [19]. In *ezh1*-deficient mutants, there is a slight increase in *ezh2* expression in the pectoral fin region, suggesting that *ezh2* compensates for the loss of *ezh1* and highlighting the crucial role of PRC2 in pectoral fin formation [101].

The study revealed that mutations in core components of the PRC disrupted normal pectoral fin development in zebrafish. Specifically, PcG-deficient zebrafish embryos exhibited a lack of pectoral fins, which was linked to ectopic expression of *hox* genes, upregulation of RA signalling, impaired FGF signalling, and the absence of key patterning gene expression. These findings underscore the critical role of PcG-mediated gene regulation in the proper development of pectoral fins. However, further research will deepen our understanding of the role of PcG in fin and limb development.

Conclusion

Polycomb group complexes are essential for proper development, playing a significant role in various biological processes, particularly in the regulation of gene activation and repression. These complexes and their products are specifically associated with developmental genes, controlling their expression, which is crucial for development. While several mechanisms for PcG recruitment, such as PREs, recruitment through transcription factors have been identified in other systems, the precise mechanisms governing PcG recruitment in zebrafish heart and pectoral fin development remain incompletely understood. In this review, we focused on the role of PcG protein during the zebrafish heart and pectoral fin development. However, this finding provides the path for researchers to investigate the PcG function during the development which is not yet fully understood as the PcG-deficient mice die before gastrulation. On the other hand, PcG-deficient zebrafish survive gastrulation and reach to embryo stage. However, the role of PcG at the molecular level during development is not yet completely revealed. The comprehension of these at the molecular level not only enhances our knowledge of fundamental biological processes but also offers valuable insights into potential therapeutic approaches for treating congenital heart defects and limb abnormalities in humans. Looking forward, further investigation into the molecular pathways regulated by Polycomb proteins may reveal new therapeutic targets, contributing to advances in regenerative medicine. This review emphasizes the complex relationship between epigenetic regulation and developmental biology, setting the stage for future breakthroughs in both basic science and clinical applications aimed at improving human health.

Abbreviations

PcG	Polycomb group
PRC	Polycomb Repressive Complex
Hox	Homeotic genes
RING1A/B	Really interesting new gene 1A or B
PCGF	Polycomb group RING finger
H2A	Histone 2A
CBX	Chromobox
RYBP	RING1/YY1-binding protein
cPRC1	Canonical Polycomb Repressive Complex 1
vPRC1	Variant Polycomb Repressive Complex 1
ncPRC1	Non-canonical Polycomb Repressive Complex 1
PHC	Polyhomeotic
SCM	Sex Comb on Midleg
YAF2	YY1-associated factor 2
EZH	Enhancer of Zeste Homolog
EED	Embryonic Ectoderm Development
SUZ12	Suppressor of Zeste 12
RBBP	Retinoblastoma-Binding Protein
H3K27me2	Histone H3 lysine 27 demethylation
H3K27me3	Histone H3 lysine 27 trimethylation
EPOP	Elongin BC and Polycomb Repressive Complex 2-Associated Protein
PALI	PRC2-Associated LCOR Isoform

PCL	Polycomb-like
CpG	Cytosine-phosphate-guanine
H3K36me3	Histone H3 lysine 36 trimethylation
JARID2	Jumonji and AT-Rich Interaction Domain Containing 2
AEBP2	Adipocyte Enhancer Binding Protein 2
H2AK119ub	Histone 2A lysine 119 monoubiquitination
KDM2B	Lysine Demethylase 2B
MGA/MAX	MAX gene-associated
AUTS2	Autism susceptibility candidate 2
CK2	Casein kinase 2
EloB/C	Elongin B/C
L3MBTL2	L3MBTL Histone Methyl-Lysine Binding Protein 2
ESC	Embryonic stem cells
KO	Knockout
dpf	Day post fertilization
hpf	Hours post fertlization
ZFN	Zinc-Finger Nucleases
TALEN	Transcription Activator-Like Effector Nucleases
ENS	Enteric Nervous System
CNS	Central Nervous System
MO	Morpholino
nkx2.5	NK2 homeobox 5
Bmp	Bone morphogenetic protein
RA	Retinoic acid
FGF	Fibroblast growth factor
OFT	Outflow tract
IFT	Inflow tract
gata5	GATA binding protein5
hand2	Heart and neural crest derivatives expressed 2
vmhc	Ventricular myosin heavy chain
Amhc	Atrial myosin heavy chain
myh6	Myosin heavy chain 6
myl7	Myosin light chain 7
ltbp3	Latent Transforming growth factor β Binding Protein 3
tbx	T-box transcription factor
shh	sonic hedgehog
vcana	Versican a
alcama	Activated leukocyte cell adhesion molecule a
nppa	Natriuretic peptide A
tnnt	Troponin T type
AVC	Atrioventricular canal
TEM	Transmission electron microscope
LPM	Lateral plate mesoderm
CHIP	Chromatin immunoprecipitation
DZNep	3-Deazaneplanocin A
Wpf	Week post fertilization

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Authors' contributions
C.A, V.C. (Vincenzo Carafa), and L.A. supervised the general concept of this review. P.K., S.S.P, V.C. (Vincenza Capone), and D.C., reviewed the main literature and wrote the manuscript. All authors reviewed the manuscript.

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Data availability
No datasets were generated or analysed during the current study..

Declarations

Ethics approval and consent to participate
Not applicable.

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