



Genetically engineered zebrafish as models of skeletal development and regeneration

Katrin Henke^{a,*}, D'Juan T. Farmer^{b,c,**}, Xubo Niu^{d,1}, Jessica M. Kraus^{e,1}, Jenna L. Galloway^d, Daniel W. Youngstrom^{e,***}

^a Department of Orthopaedics, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA

^b Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095, USA

^c Department of Orthopaedic Surgery, University of California, Los Angeles, CA 90095, USA

^d Center for Regenerative Medicine, Department of Orthopaedic Surgery, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114, USA

^e Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT 06030, USA

ARTICLE INFO

Keywords:

Zebrafish
Skeleton
Development
Regeneration
Genetic engineering
Transgenesis

ABSTRACT

Zebrafish (*Danio rerio*) are aquatic vertebrates with significant homology to their terrestrial counterparts. While zebrafish have a centuries-long track record in developmental and regenerative biology, their utility has grown exponentially with the onset of modern genetics. This is exemplified in studies focused on skeletal development and repair. Herein, the numerous contributions of zebrafish to our understanding of the basic science of cartilage, bone, tendon/ligament, and other skeletal tissues are described, with a particular focus on applications to development and regeneration. We summarize the genetic strengths that have made the zebrafish a powerful model to understand skeletal biology. We also highlight the large body of existing tools and techniques available to understand skeletal development and repair in the zebrafish and introduce emerging methods that will aid in novel discoveries in skeletal biology. Finally, we review the unique contributions of zebrafish to our understanding of regeneration and highlight diverse routes of repair in different contexts of injury. We conclude that zebrafish will continue to fill a niche of increasing breadth and depth in the study of basic cellular mechanisms of skeletal biology.

1. Introduction

Animal models have played important roles in uncovering the genetic basis of skeletal development, disorders, homeostasis, and diseases. Traditionally, mice have been the model organism of choice due to the large number of available genetic tools and the ability to induce targeted genetic modifications in embryonic stem cells. The recent development of a wide array of new techniques for genetic manipulation has broadened the range of model organisms that can efficiently be genetically modified. Among these, the zebrafish has become a powerful vertebrate model system due to its external fertilization, rapid early development, high fecundity, and transparency at embryonic stages [1]. Most importantly, zebrafish share 70 % of genes with humans, making it

an ideal system to model human development and diseases [2]. While the zebrafish has predominantly been used to study early developmental processes, more recently this small freshwater fish has also been used to interrogate post-embryonic stages, especially with regard to the musculoskeletal system [3–12].

A broad range of methods are available to genetically modify zebrafish and are being used in both forward and reverse genetic approaches. The recent adaptation of the clustered, regularly interspaced, short palindromic repeat (CRISPR)/CRISPR-associated (Cas) system for genome editing significantly expanded the options for genetic modifications in zebrafish. This has led to a large increase in the number of generated targeted mutants and transgenic lines. Currently, there are 53,522 mutant alleles and 31,964 transgenic lines recorded in the

* Correspondence to: K. Henke, Department of Orthopaedics, Department of Human Genetics, Emory University School of Medicine, Atlanta, GA 30322, USA.

** Correspondence to: D. T. Farmer, Department of Molecular, Cell and Developmental Biology, University of California, Los Angeles, CA 90095, USA.

*** Correspondence to: D. W. Youngstrom, Department of Orthopaedic Surgery, University of Connecticut Health Center, Farmington, CT 06030, USA.

E-mail addresses: khenke@emory.edu (K. Henke), djuanfar@mcdb.ucla.edu (D.T. Farmer), xniu@mgh.harvard.edu (X. Niu), jkraus@uchc.edu (J.M. Kraus), jenna.galloway@hms.harvard.edu (J.L. Galloway), dwyongstrom@uchc.edu (D.W. Youngstrom).

¹ Authors contributed equally to this work.

Zebrafish Information Network (<https://zfin.org/>) [13]. These genetically modified animals can now be readily combined with single cell genomic technologies at both a tissue and whole organism level to define pathways important in skeletal development, regeneration and disease. In this review, we will highlight the methods available for genetic manipulation and how these tools have been used to study skeletal development and regeneration.

2. Tools for genetic manipulation in zebrafish

The zebrafish gained popularity as a model system because of its amenability to genetic manipulation, especially as a means to perform large scale forward genetic screens in a vertebrate. Forward genetic screens are a powerful approach to identify genes important for a specific developmental process in an unbiased manner. Here, mutations are randomly induced throughout the genome and mutant progeny are identified by their altered phenotype, followed by mapping to identify the underlying genetic change. On the other hand, targeted mutagenesis allows for the genetic manipulation of a specific gene of interest to study its function. In this section we describe the different methods available for genetic modification of zebrafish and the approaches they are used in.

2.1. Random mutagenesis

In random mutagenesis, mutations are induced throughout the genome to discover gene function. This technique has been used in both forward and reverse genetic approaches. Several methods have been shown to be effective in zebrafish, including induction of mutations through gamma-rays and ultraviolet (UV) light [14–16]. The two major forms of mutagenesis employed in zebrafish today are: (1) chemical and (2) insertional mutagenesis and are discussed in more detail in the sections below.

2.1.1. Chemical mutagenesis

The chemical N-ethyl-N-nitrosourea (ENU) is a highly potent mutagen and is widely used for the random induction of point mutations. In zebrafish, point mutations can be generated at a frequency of about 1 in 500–1000, although with varying frequency for different loci [17–19]. Multiple large scale forward genetic screens using chemical mutagenesis have been performed in zebrafish, including screens for

early craniofacial development and changes in adult skeletal morphology [20–30]. They have led to the discovery of many genes and gene networks that play a role in skeletal development and diseases. Identification of the underlying genetic changes used to be very time consuming and labor intensive, but the recent adaptation of next-generation sequencing methods to map mutations has sped up this process significantly [31–40]. However, due to the high number of polymorphisms in the zebrafish genome and the lack of inbred strains, some phenotype-causing mutations, especially in non-coding regions, remain challenging to identify. Therefore, insertional mutagenesis tools have been developed which allow for the rapid identification of the integration site and thereby of the affected gene (see Section 2.1.2).

In addition to forward genetic screens, ENU mutagenesis has also been employed in reverse genetic approaches, such as Targeting Induced Local Lesions in Genomes (TILLING). Here, progeny of mutagenized fish are screened for mutations in genes of interest by amplification of the region of interest followed by either Cel1 digestion or sequencing, before phenotyping [41]. Initially this approach was used to target a single gene. However, taking advantage of next-generation sequencing technologies allowing for the sequencing of entire exomes and genomes, the Zebrafish Mutation Project used exome sequencing of a large population of F1 progeny from mutagenized fish to identify ENU induced coding mutations genome-wide [42]. Using this approach, knockout alleles in over 60 % of zebrafish genes were identified and are available to the community (Table 1) (<https://www.sanger.ac.uk/collaboration/zebrafish-mutation-project/>, <https://zmp.buschlab.org/>).

2.1.2. Insertional mutagenesis

The drawback of chemical mutagenesis is the potential difficulty in identifying the underlying gene mutation, which can be alleviated through the use of insertional mutagenesis methods. In insertional mutagenesis, random integration of DNA into the genome is used as a mutagen, which enables rapid identification of the mutated region as the donor DNA works as a tag for the integration site [51]. The first insertional screens in zebrafish have been performed by injection of pseudotyped retrovirus into blastula stage embryos [52,53]. Subsequently, the viral DNA integrates into the genome and, when integrated into the germline, is transmitted to the progeny. These retroviral insertion screens have achieved efficiencies of mutagenesis of about one ninth of that observed with ENU [53]. As mutations are caused by integration of DNA, the type of mutations that are being created are typically restricted

Table 1
Databases and collections of genetically modified zebrafish.

Zebrafish Mutation Project (ZMP)	ENU induced mutants, predicted loss-of-function mutations in >60 % of genes of the zebrafish genome	https://zmp.buschlab.org/ [42]
Zebrafish Insertion Collection (ZInC)	Retroviral insertion lines, over 3000 mutated genes	https://research.nhgri.nih.gov/ZinC/ [43]
Zebrafish Gene Trap and Enhancer Trap Database (zTRAP)	GFP and Gal4FF expressing gene and enhancer traps	https://ztrap.nig.ac.jp/ztrap/ [44]
NIGKOF Knock Out Fish Project	Knockout lines generated through transposon insertions in the zTRAP project	https://ztrap.nig.ac.jp/knockout/faces/insertrion/FindInsertion.jsp [45]
ZETRAP 2.0	GFP and KillerRed expressing enhancer trap lines	https://fliptrap.usc.edu/static/contact.html [46]
FlipTrap	Cre mediated switch between protein and gene trap, constructs are flanked by FRT sites allowing replacement of the FlipTrap cassette	https://dresden-technologieportal.de/en/services/view/id/209 [47]
CreZoo	Gene trap lines expressing mCherry-T2A-CreER ^{T2}	https://amfermin.wixsite.com/website [48]
trap-TRAP	Enhancer trap lines for Translating Ribosome Affinity Purification (TRAP)	http://www.zfish.cn/TargetList.aspx
ZAKOC	Zebrafish All-Genome KO Consortium for Chromosome 1	http://zfin.org/action/publication/ZDB-PUB-171002-4/feature-list [49]
zfishbook	International Zebrafish Protein Trap Consortium database, lines generated with gene-break transposons	http://www.zfishmeta.org/ [50]
Zebrafish Information Network (ZFIN)	The most comprehensive database of genetic and genomic data for zebrafish, including mutant alleles and transgenic lines	https://zfin.org/ [13]
Zebrafish International Resource Center (ZIRC)	A central repository for zebrafish wildtype and mutant strains, as well as zebrafish related materials.	http://zebrafish.org
European Zebrafish Resource Center (EZRC)	Repository for zebrafish lines from European researchers and materials	https://www.ezrc.kit.edu/
China Zebrafish Resource Center (CZRC)	Repository for zebrafish lines, resources and technology	http://en.zfish.cn/

to loss-of-function or hypomorphic alleles, while ENU can also induce neomorphic and antimorphic alleles. Several large-scale insertional mutagenesis experiments have been performed [43,53–56] and a large number of mutants have been identified, including mutants with defects in the development of the skeleton [57]. With adaptation of Illumina sequencing methods, it is possible to identify the integration sites quickly and efficiently. Over 6000 F1 progeny carrying integrations were archived and mapped, with over 3700 carrying integrations predicted to cause null or hypomorphic alleles [43] (Table 1).

While retrovirus-mediated mutagenesis has proven to be a very efficient method, handling and preparation of the retrovirus is challenging. Therefore, alternative methods have been explored, of which transposon-mediated random integration of DNA into the zebrafish genome was found to be the most efficient [58]. Transposon systems have been used successfully to deliver genetic material into plants and invertebrates. Several of these transposon systems have been found to work in zebrafish, including the synthetic Sleeping Beauty, the maize Ac/Dc, zebrafish TC1/mariner (ZB) and medaka Tol2 systems, with the Tol2 system being the most widely used with observed evidence of germline integration in about 50 % of injected fish [59–63]. Transposases recognize terminal inverted repeats (TIR), excise the TIR-flanked DNA and insert it into a different location in the genome, with different transposases showing preferences for integrations in specific regions of the genome [64]. Similar to the retrovirus insertions, depending on the location, the insertions can inactivate genes or change gene expression. For mutagenesis, TIRs are cloned into transposon donor-plasmids to facilitate integration of the insert DNA into the genome when injected with transposase mRNA. Coding sequences of fluorescent proteins or transcriptional activators can be cloned into the donor-plasmid and enable visual screening of insertion events into enhancer or coding regions, which at the same time enables investigation of expression patterns of the affected gene.

The flexible design options of the transposon donor plasmid have expanded the utility of the approach beyond the generation of loss-of-function alleles [65]. A wide variety of constructs has been used in enhancer, gene or protein trap screens, including flippase (Flp) or Cre recombinase activatable and switchable conditional cassettes [46–48,66–69]. This has led to the generation of a large collection of available enhancer, gene and protein trap lines and new lines using new versions of donor-plasmids with novel functionalities are constantly being added (Table 1). In addition, transposon-based mutagenesis methods have been extensively used for the generation of reporter, overexpression and conditional lines (see Section 2.3).

2.2. Targeted mutagenesis

While random mutagenesis is a very powerful approach, this strategy requires the generation of a large number of mutagenized fish to screen and phenotype in order to identify mutations in a specific gene of interest. In addition, not every gene is mutated with the same efficiency, and thereby, finding a mutation in a specific gene even in a large population is not guaranteed. Target specific DNA nucleases circumvent this problem and can be used to induce sequence specific double-strand breaks which can result in the formation of small INDELS at the cut site due to error prone repair during non-homologous end joining (NHEJ) [70]. In addition, homology-directed repair (HDR) mechanisms enable precise, target-site specific integration of donor templates with homology arms. While the induction of INDELS using nucleases is highly efficient, methodologies for HDR in zebrafish are still evolving, with many different strategies succeeding at variable efficiencies [71].

The first nucleases shown to be effective in zebrafish were the zinc finger nucleases (ZFNs) [72,73] and the transcription activator-like effector nucleases (TALENs) [74,75]. However, after the discovery of the CRISPR/Cas system for genome editing and its successful application in zebrafish, ZFNs and TALENs have been largely replaced by the CRISPR/Cas system due to easier design and synthesis and higher

versatility [76–79]. The CRISPR/Cas system for genome engineering is based on an adaptive defense mechanism in bacteria [80,81]. It uses synthetic guide RNAs to direct a Cas protein to a target site for genome editing. Target sites consist of 17–24 nucleotides which are being integrated into the guide RNA and are restricted to loci adjacent to short protospacer-adjacent motifs (PAM), which are required for Cas protein function. The length of the target site as well as the PAM sequences are protein specific and new variations of Cas proteins are constantly added to the repertoire. Available Cas proteins include nucleases for the induction of double-strand breaks, nickases inducing single strand cuts, base editors for precise induction of base changes, transposase fusions to facilitate DNA integration and prime editors for induction of mutations through reverse transcription [82]. Many of these have been shown to be active in zebrafish, expanding the application of CRISPR/Cas beyond its utility to generate double stranded breaks for knock-outs and knock-ins [83,84]. CRISPR/Cas tools have been developed to generate global and tissue specific knock-outs, knock-ins and point mutations and to trace individual cells through generation of CRISPR barcodes (Table 2).

One advantage of the CRISPR/Cas system over ZFNs and TALENs is the relative ease of guide RNA design and synthesis and the ability to simultaneously target multiple genes, which has been used in several high-throughput mutagenesis experiments in zebrafish [49,79,85,86]. Besides generating large mutant collections, these large-scale screens have provided important information on the efficiency of INDEL induction, accessibility of target sites, and off-target effects which has aided in the development of easy-to-use guide RNA design tools (Table 2). Now, with commercially synthesized guide RNAs, Cas mRNA or protein, the CRISPR/Cas system is widely accessible in zebrafish [87,88]. CRISPR/Cas genome editing has successfully been used to generate models of human skeletal diseases [8] and due to the high efficiency in the induction of mutations, skeletal phenotypes can readily be detected and analyzed in the F0 generation, further accelerating the discovery process [89–91].

In addition to its gene editing applications, the CRISPR/Cas system, like the Tol2 integration system, has emerged as an important tool to make targeted reporters, enabling the quick and reliable creation of new reporter lines and Cre drivers, as reviewed in detail in the next section.

2.3. Transgenesis

One main advantage of the zebrafish is the ability to perform *in vivo* labeling and imaging due to its small size and transparency during early developmental stages. Optically clear mutants (casper, crystal) have been generated by combining different pigment mutants to enhance *in vivo* imaging capabilities beyond larval stages [143,144]. For the generation of transgenic fish, a number of different techniques have been explored for the delivery of foreign DNA, including retroviral infection, electroporation, particle bombardment and microinjection [145–149]. To date, microinjection is the most widely used technique for the generation of transgenic zebrafish due to its fast and easy application.

Initially, only circular or linearized DNA had been injected into fertilized eggs, which resulted in relatively low transgenesis rates [146,150,151]. Modifications of the donor DNA and addition of I-SceI meganuclease or Tol2 transposase to the injection mix did increase the transgenesis rate significantly [152,153]. Meganuclease or Tol2 recognition sequences are integrated into the donor DNA to allow linearization and integration of the DNA into the genome. To facilitate easy design of Tol2 plasmids for transgenesis, plasmid collections for modular design of donor-plasmids using the Gateway cloning system have been generated and are constantly expanded [154]. Combination of identified, tissue specific promoters with conditional expression systems and fluorophores targeted to different subcellular compartments provide limitless options for the design and generation of transgenic zebrafish for *in vivo* imaging and analysis as well as manipulation of gene function (Table 3) [155].

The ability to target transgene expression to a specific cell type is

Table 2
Subset of available zebrafish CRISPR/Cas tools.

Design tools	CRISPRscan	Guide RNA design tool and off target prediction https://www.crisprscan.org/	[87]
	CRISPRon/ CRISPRoff	On-target and off-target predictions for CRISPR-Cas9 gene editing https://rth.dk/research/crispr/	[92,93]
	Breaking-Cas	Variable design of gRNAs for CRISPR/Cas for all eukaryotic genomes available on Ensembl https://bioinfogp.cnb.csic.es/tools/breakingcas/	[94]
	CCTop/CRISPRater	Tunable CRISPR/Cas target online predictor and effectivity predictor https://cctop.cos.uni-heidelberg.de/index.html	[95,96]
	ACEofBASEs	sgRNA design and off-target prediction tool for adenine and cytosine base editors https://aceofbases.cos.uni-heidelberg.de/index.html	[97]
	CHOPCHOP	CRISPR and TALEN design tool https://chopchop.cbu.uib.no/	[88,98,99]
	CRISPOR	Guide RNA design tool and off target prediction http://crispor.org	[100]
	CRISPRdirect	Guide RNA design tool and off target prediction http://crispr.dbcls.jp/	[101]
	CRISPRseek	Software package for gRNA design	[102]
	GT-Scan appsuite	gRNA and HDR template design tools (GT-Scan, TUSCAN, CUNE) https://gt-scan.csiro.au/	[103–105]
	CRISPR RGEN Tools	Tools for RNA-guided endonucleases, including off target prediction, Cas gRNA design, base editing and primer editing tools http://www.rgenome.net/cas-designer/	[106–110]
	Gene Sculpt Suite	Tools for genome editing including design of short oligonucleotides for homology-based gene editing (GtagHD), prediction of extend of microhomology-mediated end joining repair (MEDJED) and prediction of locations with just 1–2 MMEJ alleles (MENTHU)	[111]

Table 2 (continued)

	Mojo Hand	http://www.genesculpt.org/ CRISPR and TALEN design tool https://talendesign.org/	[112,113]
	CRISPR-ERA	gRNA design tool for genome editing, repression and activation http://crispr-era.stanford.edu/	[114]
	CRISPR-SKIP	Design of exon skipping mutations using single-base editors https://knoweng-0.igb.illinois.edu/crispr-skip/	[115]
Databases	CRISPRz	Database of validated CRISPR targets in zebrafish https://research.nhgri.nih.gov/CRISPRz/	
	iSTOP	Database of sgRNAs for generating STOP codons http://www.cicciolab-database.com/istop	[116]
SpCas9 alternatives	Spg	NGN Pam	[117]
	SpRY	NRN PAM	[117]
	LbCpf1 (Cas12a)	TTTV PAM	[118]
	SpCas9 VQR	NGAN, NGNG PAM	[119]
	SpCas9 EQR	NGAG PAM	[119]
	SpCas9 VRER	NGCG PAM	[119]
	SpCas9 KKH	NNNRRT PAM	[120]
	SauCas9	NNGRRT PAM	[121]
	AsCpf1	TTTV PAM	[121]
	Nme2Cas9	NNNNCC PAM	[121]
	ErCas12a	YTTN PAM	[122]
	ScCas9	NNG PAM	[123]
Transgenic lines	<i>4xUAS:NLS-Cas9, myl7:RFP</i>	Gal4 effector line for Cas9 expression with red heart transgenesis marker	[124]
	<i>4xUAS:NLS-Cas9, cryaa:EGFP</i>	Gal4 effector line for Cas9 expression with green eye transgenesis marker	[124]
	<i>hsp70l:LOXP-DsRed-LOXP-Cas9-GFP, rnu6-32:CRISPR1-tyr efl1a:Cas9-NLS</i>	Cre and heat shock controlled Cas9-GFP fusion expression	[125]
	<i>actb2:NLS-zCas9-NLS, cryaa:TagRFP</i>	Ubiquitous expression of Cas9	[126]
	<i>hsp70l:LOXP-mCherry-LOXP-NLS-zCas9-NLS ubb:NLS-zCas9-NLS, myl7:EGFP</i>	Ubiquitous expression of Cas9 with red eye transgenesis marker	[127]
	<i>hsp70l:LOXP-mCherry-LOXP-NLS-zCas9-NLS, myl7:EGFP</i>	Cre and heat shock controlled Cas9 expression	[127]
	<i>hsp70l:Cas9-IRES-EGFP, myl7:EGFP</i>	Ubiquitous expression of Cas9 with green heart transgenesis marker	[127]
	<i>hsp70l:Cas9-IRES-EGFP, myl7:EGFP</i>	Heat shock inducible co-expression of Cas9 and EGFP with green heart transgenesis marker	[128]
	<i>hsp70l:Cas9-P2A-mCherry, myl7:EGFP</i>	Heat shock inducible co-expression of Cas9 and mCherry with green heart transgenesis marker	[128]
	<i>lyzC:Cas9, cryaa:GFP</i>	Neutrophil-specific expression of Cas9 with	[129]

(continued on next page)

Table 2 (continued)

		green eye transgenesis marker	
	<i>hsp70l:zCas9-T2A-GFP, 5 × (U6:sgRNA)</i>	Heat shock inducible simultaneous expression of Cas9 and GFP, ubiquitous expression 5 guide RNAs for lineage tracing using GESTALT	[130]
	<i>hsp70l:DsRed-v7, myl7:EGFP</i>	CRISPR array, with green heart transgenesis marker for lineage tracing using GESTALT	[130]
	<i>UAS:Cas9T2AGFP; U6:sgRNA1; U6:sgRNA2</i>	Gal4 effector line for simultaneous Cas9 and GFP expression	[131]
Base editors (BE)	BE4max	cytosine BE	[132]
	zABE7.10	adenine BE	[133]
	BE, BE-VQR, dBE-VQR	cytosine BE	[134]
	zAncBE4max	cytosine BE	[135]
	BE4-Gam	cytosine BE	[97,136]
	ABE8e	adenine BE	[97]
	ancBE4max-SpymacCas9	cytosine BE	[136]
	ancBE4max	cytosine BE	[97,132,136]
	evoBE4max	cytosine BE	[97]
	CBE4max-SpRY	cytosine BE	[137]
Transcriptional regulators	zAncBE4max	cytosine BE	[135]
	dCas9-KRAB	repressor	[138]
	dCas9-VP160	activator	[138]
	dCas9-Eve	repressor	[139]
Lineage tracing	GESTALT (genome editing of synthetic target arrays for lineage tracing)	Lineage tracing through modification of a CRISPR/Cas target array	[140]
	scGESTALT (single-cell GESTALT)	GESTALT combined with transcriptome profiling	[130]
	LINNAEUS (lineage tracing by nuclease-activated editing of ubiquitous sequences)	Lineage tracing through Cas9 induced INDELS in a multicopy transgene combined with transcriptome profiling	[141]
	ScarTrace	Lineage tracing through Cas9 induced INDELS in a multicopy transgene combined with transcriptome profiling	[142]
Screening tools	MIC-Drop (multiplexed intermixed CRISPR droplets)	Microfluidics based generation of a library of droplets containing different RNP complexes together with DNA barcodes for injection	[86]

dependent on the availability and identification of specific promoter and/or enhancer elements. Due to their capability to contain large fragments of genomic DNA (up to 300 kb), modified bacterial artificial chromosomes (BACs) have been used to drive transgene expression in zebrafish [156,157]. BACs modifications include the addition of I-SceI or Tol2 sites to increase transgenesis efficiency and addition of reporter genes through homologous recombination. While BACs efficiently capture most regulatory elements of a gene, their handling and modification is not trivial. The identification of smaller and easier to handle regulatory elements alleviates this problem but can be laborious and time consuming. Due to the more compact genome and conserved regulatory mechanisms, regulatory elements from another freshwater teleost, medaka (*Oryzias latipes*), are commonly used to drive gene expression in zebrafish [158]. One example is the use of the medaka *osterix* promoter

Table 3

Subset of available transgenic tools.

Commonly used genetically encoded fluorophores	tagBFP Ex 402, Em 457 [186] mCerulean Ex 433, Em 475 [187] CFP Ex 456, Em 480 [188] mTFP1 Ex 462, Em 492 [189] EGFP Ex 488, Em 507 [190] lanYFP Ex 513, Em 524 [191] mVenus Ex 515, Em 527 [192] E2-Orange Ex 540, Em 561 [193] tdTomato Ex 554, Em 581 [194] dsRed Ex 558, Em 583 [195] mCherry Ex 587, Em 610 [194] RFP Ex 587, Em 637 [196] mKate2 Ex 588, Em 633 [197] For a more comprehensive list of available fluorophores see: https://www.fpbases.org/ [198]
Localization signals	Cell membrane: Lyn myristoylation and palmitoylation sequence [199–201] CAAX motif [202] Nucleus: histone H2A.F/Z fusion [202] histone H2B fusion [203] hmgbl fusion [204] SV40 nuclear localization signal (NLS) [205] nucleoplasmin NLS [206] Actin cytoskeleton: Lifeact [207] Microtubules: ensconsin microtubule-binding domain fusion [208]
Transcriptional activator systems	Gal4-UAS [209] QF-QUAS [210] KalTA4-UAS [210] Cre-loxP/lox2272/loxN [211,212] Flp-FRT [213] Dre-Rox [214]
Conditional expression systems	Cre-loxP/lox2272/loxN [211,212] Flp-FRT [213] Dre-Rox [214]
Inducible gene expression systems	Cre-ERT2 + Tamoxifen [215] LexPR + mifepristone [216] TetA-GBD + doxycycline and dexamethasone [217] TetA-EcR + doxycycline and tebufenozide [217] hsp70l promoter + heat shock [218] GV-EcR + tebufenozide [219] IQ-Switch + tebufenozide [220] GAVPO + blue light [219]
Cell ablation systems	Nitroreductase + metronidazole [221–223] KillerRed + intense green or white light [224] M2 ^{H37A} [219] Kid + Kis anti-toxin [225] PhoCl-Bid [225]
Signaling pathway activity reporter systems	Wnt signaling: TOPdGFP [226] Tcf/Lef-mini:dGFP [227] TCFsiam [228] BMP signaling: BMP-responsive element [229–232] dominant negative <i>Bmpr1a-GFP</i> [233] FGF signaling: <i>dusp6:d2EGFP</i> [234] dominant negative <i>hsp70:dn-fgfr1</i> [235] Notch pathway: <i>Tp1bglob</i> [204] <i>her12-Kaede</i> [236] Hedgehog signaling: <i>ptc1-Kaede</i> [237] <i>ssh-GFP</i> [238] Erk signaling: <i>osx:Erk KTR-mCerulean</i> [239] TGF-beta signaling: <i>12xSBE:EGFP</i> [240] <i>12xSBE:nls-mCherry</i> [240] GFP-aequorin [241,242] GCamp [243] CaMPARI [244]
Genetically encoded Ca sensors	GFP-aequorin [241,242] GCamp [243] CaMPARI [244]

(continued on next page)

Table 3 (continued)

Apoptosis marker	AnnexinV-YFP [245] NES-DEVD-mCardinal-NLS [246] genetically encoded death indicators (GEDIs) [247]
Autophagic flux sensor	GFP-LC3-RFP-LC3DG [248]
FUCCI (Fluorescent Ubiquitination-based Cell Cycle Indicator)	hGeminin [162,249] zCdt1 [162,249]
Lineage tracing tools	Photoconvertible proteins: Dendra Ex 488, Em 505 > Ex 556, Em 575 [250] Kaede Ex 508, Em 518 > Ex 572, Em 580 [251] mEos2 Ex 506, Em 519 > Ex 573, Em 584 [252] Kikume Ex 507, Em 517 > Ex 583, Em 593 [253] Recombinase mediated multicolor expression cassettes: ZebraBrow, priZm (Lox2272-LoxP-RFP-Lox2272-CFP-LoxP-YFP) [254,255] Palmskin (LoxN-Lox2272-LoxP-H2B-EBFP2-LoxN-palm-mCherry-Lox2272-palm-mEYFP-LoxP-palm-mCerulean) [256] FRaeppli-nls (UAS-attB-Lox2272-STOP-Lox2272-phiC31-cmlc2-mTurquoise-attP-nls-E2-Orange-attP-nls-mKate2-attP-nls-mTFP1-attP-nls-TagBFP) [257] FRaeppli-caax (UAS-attB-Lox2272-STOP-Lox2272-phiC31-cmlc2-mTurquoise-attP-E2-Orange-CAAX-attP-mKate2-CAAX-attP-mTFP1-CAAX-attP-TagBFP-CAAX) [257] Tol2 kit (http://tol2kit.genetics.utah.edu) [154] CRISPR/Cas9 vector system for tissue-specific gene disruption [258] Addgene (https://www.addgene.org/)
Plasmid collections	

to drive gene expression in zebrafish preosteoblasts and osteoblasts (Table 4) [159–163].

While meganuclease and transposase based transgenesis methods are very efficient, they often lead to the integration of multiple copies of the transgene and integration site-specific effects on expression [164,165]. This can be avoided through integration of the expression plasmid into a predefined landing site using the phiC31 integrase system [166]. Alternatively, to circumvent the need for identification of regulatory elements, integration of expression constructs can now be targeted to specific locations in the genome to capture the surrounding regulatory region to drive gene expression. Here ZFNs, TALENs or CRISPR/Cas are being used to create double strand breaks at the desired integration site and co-injected donor DNA is integrated into the cut site. A large number of different knock-in strategies are being explored and show different levels of efficiency, often leading to imprecise integration into the genome [71,167,168]. For target integration using homology directed repair, a number of different template designs have been used. Designs differ in the length of the homology arms as well as the type of DNA template used (single stranded oligonucleotides, double stranded linear, *in vivo* linearized or circular DNA) [169–177]. In addition to different template designs, simultaneous inhibition of the non-homologous end joining DNA repair pathway has been explored to improve precise integration [178–180].

However, not all transgenesis strategies require homology arms, and donor DNA without any homology can successfully be integrated through homology-independent DNA repair [181,182]. DNA constructs similar to those used in enhancer, gene and protein trap screens can be used to generate reporter lines for genes of interest.

In the skeletal field, live imaging and manipulation of transgenic zebrafish has been used to capture crucial events during development and regeneration of tendon/ligament, bone and cartilage and is discussed in the sections below. The zebrafish is also an excellent vertebrate model for high-throughput chemical screening using whole organisms.

Table 4

Commonly used musculoskeletal transgenic lines.

Cell type	Musculoskeletal transgenes and papers of origin	Origin of regulatory element used
Osteoblast lineage	(1) <i>osx:EGFP</i> [259] (2) <i>osx:kaede</i> [162] (3) <i>osx:nuGFP</i> [160] (4) <i>osx:Venus-hGeminin</i> [162] (5) <i>osx:mCherry-zCdt1</i> [162] (6) <i>osx:mCherry-NTR</i> [163] (7) <i>osx:H2A-mCherry</i> [162] (8) <i>osx:mTagBFP-2A-CreER</i> [163] (9) <i>entpd5a:pkRED</i> [260] (10) <i>runx2:eGFP</i> [161] (11) <i>osc:eGFP</i> [161] (12) <i>osc:GFP</i> [261] (13) <i>osx:CreERT2-P2A-mCherry</i> [161] (14) <i>entpd5a:YFP</i> [262] (15) <i>entpd5a:Citrine</i> [263] (16) <i>entpd5a:Kaede</i> [264] (17) <i>runx2:GAL4-VP16_2A-mCherry</i> [265] (18) <i>osx:Lifeact-mCherry</i> [266] (19) <i>col10a1:mCitrine</i> [267] (20) <i>col10a1:GFP</i> [268] (21) <i>sp7:luciferase</i> [269]	(1) BAC containing the <i>osterix</i> locus (CH73-243G6) (2), (3), (4), (5), (6), (7), (8), (13), (18), (21) 4.1 kb upstream regulatory region of medaka <i>osterix</i> gene (9), (14), (15), (16) BAC containing the <i>entpd5</i> locus (10), (17) 557 bp enhancer from the last intron of human <i>RUNX2</i> fused to the mouse <i>cFos</i> minimal promoter (11) 3.7 kb upstream promoter sequence of medaka <i>osteocalcin</i> (12) 3.5 kb upstream regulatory region of medaka <i>osteocalcin</i> [270] (19) BAC containing the <i>col10a1</i> locus (20) 2.2 kb upstream promoter of zebrafish <i>col10a1</i>
Cartilaginous cells	(1) <i>col2a1a:eGFP</i> [271] (2) <i>col2a1a:CreERT2</i> [272] (3) <i>col9a2:GFPCaaX</i> [273] (4) <i>col2a1a:mCherry</i> [267]	(1), (2) R2 element upstream the transcription initiation site (358 bp enhancer) (3) 2048 bp of zebrafish <i>col9a2</i> promoter upstream of the start of translation (4) BAC containing the <i>col2a1a</i> locus
Tendon/ligament cells	(1) <i>scxa:mcherry</i> [274] (2) <i>scxa:gal4-vp16</i> [275] (3) <i>col1a2:loxP-mCherry-NTR</i> [276] (4) <i>nkx3.1:gal4-vp16</i> [277] (5) <i>col1a2:Gal4-VP16</i> [277] (6) <i>col1a2:GFP</i> [277]	(1), (2) BAC CH211 251 g8 containing the entire <i>scxa</i> genomic locus (3), (5), (6) BAC CH211–122 K13 containing <i>col1a2</i> locus (4) BAC zC21G15 from the CHORI-211 library containing the <i>nkx3.1</i> locus
Joint cells	(1) <i>irx7:GFP</i> [278] (2) <i>trps1:GFP</i> [279]	(1), (2) SAGp11A, Tol2-mediated gene trap insertions into the endogenous locus [61]
Osteoclast	(1) <i>ctsk:FRT-dsRed-FRT-Cre</i> [280] (2) <i>trap:GFP-CAAX</i> [266] (3) <i>ctsk:EGFP</i> [281] (4) <i>ctsk:Citrine</i> [263]	(1), (3) 3 kb upstream regulatory region of medaka <i>cathepsin K</i> gene (2) 6 kb upstream regulatory region of the zebrafish <i>trap</i> (<i>acp5a</i>) gene (4) BAC containing the <i>cathepsin K</i> locus
Neural crest-derived skeletal cells	(1) <i>sox10:ERT2-Cre</i> [282] (2) <i>sox10:GFP</i> [283] (3) <i>sox10:CreERT2</i> [272] (4) <i>sox10:dsRED</i> [284]	(1) 4.9 kb of zebrafish <i>sox10</i> promoter sequence upstream of the start of translation (2) 4725 bp of zebrafish <i>sox10</i> promoter upstream of the start of translation (3), (4) 4.9 kb of zebrafish <i>sox10</i> promoter sequence upstream of the start of translation

Chemical libraries are commercially available or can be accessed through screening centers at some academic institutions or commercially. Many libraries are assembled to contain known biologically active compounds, for which there is some information about the molecular target, and more specific chemical libraries targeting particular molecular processes, such as kinase or chromatin targeting libraries, can be selected. Although some chemical screens were performed using morphology or staining methods as assays, transgenic fish have enabled automation of the screening platform, enabling larger scale screening efforts [183–185]. These studies highlight the major opportunities available to use chemical screens to interrogate skeletal development and repair, which will serve as an important platform to inform both basic and translational research. Table 3 summarizes available expression and cell labeling systems and musculoskeletal system specific transgenic lines are listed in Table 4.

3. The zebrafish skeleton

The adult zebrafish skeleton can be divided into the endoskeleton, consisting of the cranial, axial and appendicular skeleton and the dermoskeleton, including scales, teeth, and fin rays. Despite a divergence in the number of skeletal elements and the shape of the skeleton in zebrafish, decades of research have demonstrated the deeply conserved mechanisms that control skeletal development in vertebrates. Like mice and humans, the adult zebrafish skeleton is composed of bones joined by fibrous or cartilaginous joints, ligaments that connect neighboring bones, and tendons that attach muscles to bones. While the craniofacial skeleton derives from both neural crest and mesoderm, the axial skeleton arises from mesoderm.

Despite some differences from their mammalian counterparts, the zebrafish skeletal system provides powerful models to understand skeletal development and model human diseases. In this section, we summarize the basic building blocks of the zebrafish skeleton and highlight some of the studies that illustrate the conserved genetic requirements for skeletal system development. As a detailed discussion of specific skeletal elements, like the vertebral column or the paired pectoral fins, is outside the scope of this review, we refer the reader to several excellent reviews [4,285–290]. These reviews carefully summarize the homology of specific skeletal elements to their mammalian counterparts and highlight discoveries and recent advances made using zebrafish models. Likewise, although there is a wealth of skeletal research using the other teleost model, medaka, and most of the methods for genetic modifications described also work in this model, our review will specifically focus on studies in the zebrafish. We refer interested readers to recent work and reviews describing medaka as a model of human skeletal diseases and illustrating how innovative imaging methods can be used to gain new insight into skeletal biology processes such as bone remodeling [7,291–293].

3.1. Cartilage

The earliest chondrocytes in the zebrafish skeleton emerge from neural crest cells within the craniofacial skeleton by 56 hours post fertilization (hpf) [294]. By 72hpf, the craniofacial cartilaginous template is fully formed in the craniofacial skeleton, robustly expresses classic chondrocyte markers like *col2a1a* [271,295], and can be readily detected by Alcian blue staining of glycosaminoglycans and glycoproteins (Fig. 1A). These cartilages are typically 1–3 chondrocyte layers in thickness, with perichondrium surrounding the cartilage. In contrast to cartilages found in the craniofacial skeleton, the cartilages in the axial skeleton, including those along the vertebrae and at the base of fins, emerge later in larval development [296]. Along vertebrae 1–5, basidorsal cartilages form that will later become the neural arches [296]. However, unlike mammalian vertebral bodies which form through endochondral ossification and are patterned following somite boundaries, zebrafish vertebrae are pre-patterned by notochordal signals that induce ossification of notochord sheath cells (chordacentra). These provide a template for autocentrum formation by sclerotome derived osteoblasts and only the patterning of the hemal and neural arches is somite dependent [297].

The mechanisms that govern cartilage specification, differentiation, and growth have been extensively researched using zebrafish, especially within the craniofacial skeleton [298–300]. Chondrocytes in the craniofacial skeleton derive from *barx1+* condensations that express *sox9* [294,301,302], like the gene expression patterns characterized in mice [303,304]. Due to the teleost-specific whole genome duplication, *sox9* in zebrafish, like many other genes, has two orthologs, *sox9a* and *sox9b* [2,240,305,306]. While one copy of 50–90 % of genes duplicated through a whole genome duplication tends to mutate to a pseudogene, the genes maintained as functional pairs provide unique advantages for the study of gene function [307]. Gene pairs evolve and one gene copy can develop new functions (neofunctionalization) or the expression domains or function of the original gene can be subdivided between pairs (subfunctionalization) [308]. The subfunctionalization of *sox9* for example, allows for the analysis of aspects of *sox9* function not possible in mice where haploinsufficiency of *sox9* leads to early lethality [309]. Like in mouse models [310,311], zebrafish chondrocyte differentiation and maturation rely on Sox transcription factors. Mutant alleles for *sox9a* were independently generated using ENU (*jeftw37*) and retroviral (*hi1134*) mutagenesis, and mutant fish displayed severely reduced and absent cartilages throughout the craniofacial skeleton and fins [53,312,313]. In contrast, *sox9b* (*b971*), mutated using gamma radiation, is predominately required in the lower jaw cartilages, with many neurocranium and fin cartilages intact. Simultaneous deletion of *sox9a* and *sox9b* eliminates all cartilages throughout the skeleton, highlighting the conserved requirement for the Sox family to properly establish cartilages in vertebrates [314].

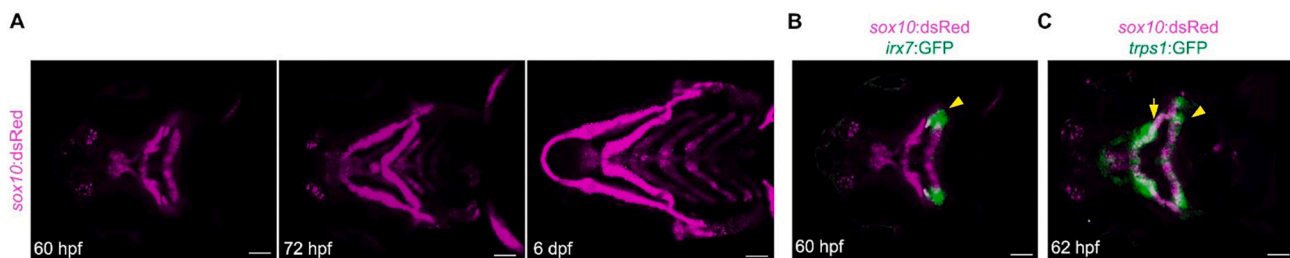


Fig. 1. Cartilage and joint development in zebrafish. (A) A time series of cartilage development in the zebrafish lower jaw skeleton using the *sox10:dsRed* reporter to label chondrocytes. Cartilage formation is clearly apparent within the craniofacial skeleton at 60 hpf. (B) The hyoid joint (arrowhead) that connects the hyomandibular and ceratohyal cartilage expresses *irx7:GFP*. (C) *trps1:GFP* is enriched at joints in the craniofacial skeleton, including the hyoid joints (arrowhead) and jaw joints (arrows). Scale bars, 50 μ m.

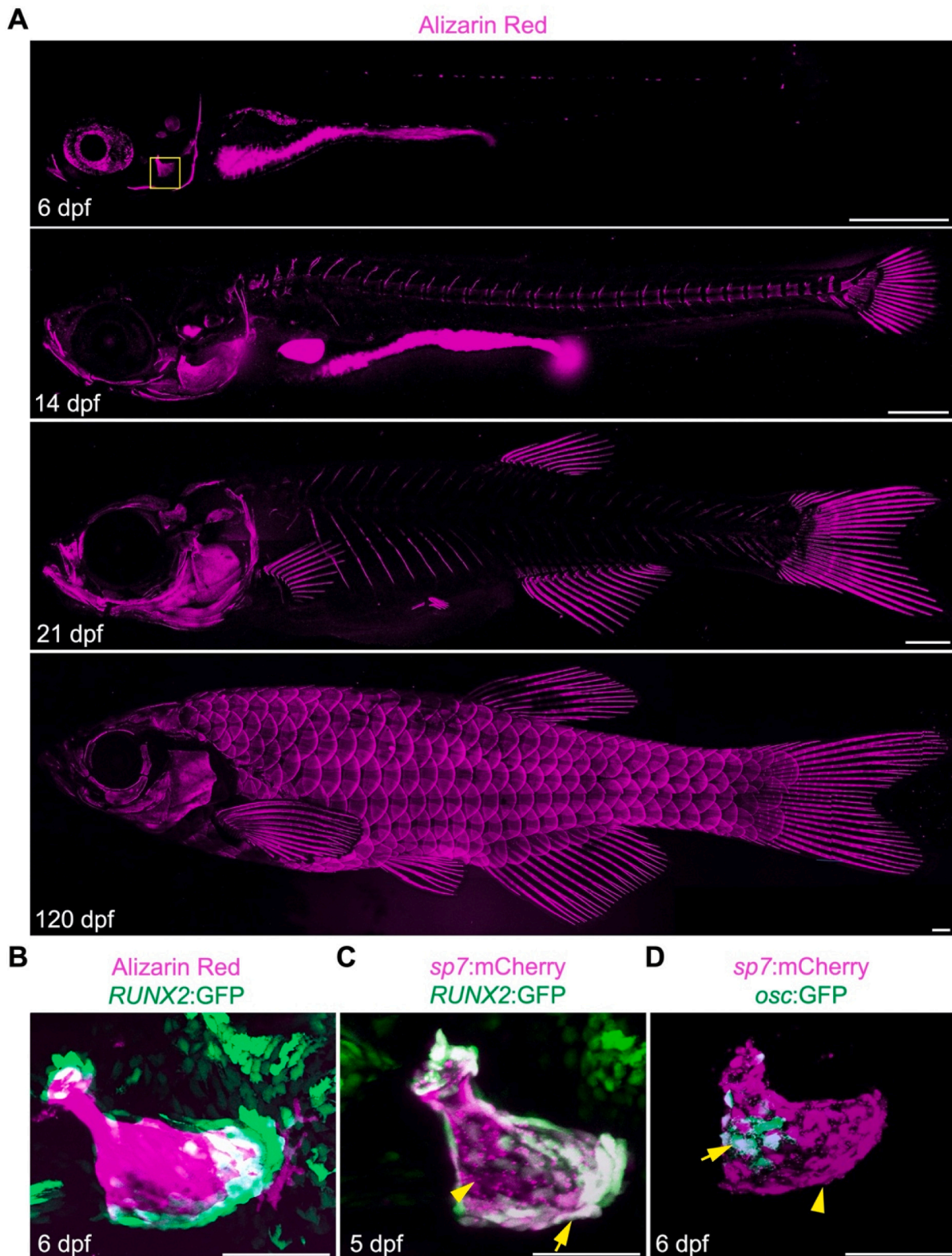


Fig. 2. Bone development in zebrafish. (A) Developmental series of alizarin red stained zebrafish from larval, juvenile and adult stages. Mineralized bones are detected earliest in the craniofacial skeleton and later appear within the axial and fin skeleton. Scales emerge later in development. Scale bars, 500 μ m. (B–D) Osteogenesis in the larval opercula (boxed in A at 6 dpf). (B) *RUNX2:GFP* preosteoblasts are enriched at the tips of the mineralized larval opercular. (C) *sp7:mCherry* high cells are enriched along the surface of the opercular, while *sp7:mCherry*; *RUNX2:GFP* pre-osteoblast are abundant at the bone tips. (D) *osc:GFP*+ osteoblasts are restricted to the most mature osteoblasts away from the edges of the opercula. Scale bars for B–D, 50 μ m. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.2. Bone

Bone formation takes various forms in the zebrafish skeleton, and includes intramembranous, perichondral, and endochondral ossification. The earliest bones develop *via* intramembranous ossification, directly differentiating into osteoblasts from mesenchymal progenitors, independent of cartilage formation [315]. A mineralized opercle is readily detectable by 3 days post fertilization (dpf) [316]. Thereafter, dermal bones will continuously emerge throughout larval and juvenile development within both the craniofacial and axial skeletons (Fig. 2A) [296,317]. Perichondral ossification is apparent around cartilaginous elements between 5-7dpf, particularly around the developing Meckel's and ceratohyal cartilages [316], while endochondral ossification arises later at juvenile stages of development [272,318]. Like endochondral bones in other vertebrates, endochondral bones in the zebrafish contain growth plates composed of proliferative and hypertrophic chondrocytes, are surrounded by perichondrium that forms cortical bone, and have a marrow space [272,318]. However, unlike mammals, the endochondral marrow space does not contain hematopoietic tissue, but instead contains adipocytes distributed within the marrow cavity, as hematopoiesis occurs in the zebrafish head kidney [272,319].

Generally, the adult zebrafish skeleton is composed of four major classes of bone: compact bone, spongy bone, tubular bone and chondroid bone [318]. These bones exist as both cellular bone and acellular bone, with many dermal bones (like the frontal and parietal bones) lacking the many embedded osteocytes that are pervasive in mouse and human bone. Across all bones, osteoblast differentiation takes a similar path as in mice and humans, with osteoblasts expressing *runx2a/b* [320], *osterix (osx/sp7)*, and eventually *osteonectin (osn)*, *osteocalcin (bglap)* and *col10a1a* (Fig. 2B-D) [315]. Like in mice [321], mutants for *sp7 (hu2790)*, isolated through TILLING, develop skeletal defects driven by impaired osteoblast differentiation [322,323]. Zebrafish models also uncovered mechanisms of bone mineralization. The characterization and mapping of two mutants identified in a forward genetic screen led to the discovery of reciprocal roles for *enpp1* and *entpd5* for phosphate/pyrophosphate regulation and bone mineralization, with *enpp1 (hu4581)* mutants displaying ectopic mineralization and *entpd5a (h3718, hu5310)* mutants lacking mineralized bone [262].

As in mammals, normal development, homeostasis and repair of the zebrafish skeleton is dependent on the presence and function of osteoclasts. Osteoclasts are hematopoietic cells of the monocyte/macrophage lineage that possess the ability to not only resorb bone, but also to regulate bone formation [324]. Osteoclasts can be identified by the expression and activity of the lysosomal enzyme tartrate resistant acid phosphatase (TRAP), encoded by the *acp5* gene. Detailed histological analysis in combination with TRAP staining showed that bone resorption predominantly occurs through mononucleated osteoclasts in juvenile zebrafish, while predominantly multinucleated cells are found in adults [325]. Analysis of TRAP expression in the zebrafish lower jaw captured TRAP+ mononucleated osteoclasts at 20 dpf, and multinucleated osteoclasts by 40 dpf, although the emergence of osteoclast types is likely site specific [319,325]. This presence of mono- and multinucleated osteoclasts was confirmed by fluorescent activated cell sorting (FACS) of cells isolated from *ctsk:DsRed* transgenic fish, taking advantage of the unique possibility in zebrafish to isolate primary osteoclasts from entire fish [280]. In both mammals and zebrafish, the ETS-domain transcription factor Pu.1 as well as signaling through colony stimulating factor 1 receptor (Csf1r) play critical roles in myelopoiesis. Analysis of ENU generated mutants in *pu.1 (G242D)*, as well as *csf1ra (j4e1, j4blue)*, showed a significant decrease in myeloid cells and therewith osteoclasts [281,326]. Double mutant analysis revealed a predominant function of *pu.1* in osteoclastogenesis, while *csf1ra* function is important during osteoclast maturation. In mammals, loss of osteoclast function leads to osteopetrosis, however the consequences of loss of Csf1r function to the zebrafish skeleton are milder and can be detected as shape changes of vertebral bodies, hemal and neural arches, as well as an increase in bone

mineral density [280,281,327]. While a lot of research in zebrafish has focused on defining the different stages and sites of hematopoiesis, as well as defining developmental trajectories of hematopoietic cell lineages, the precise origins of osteoclasts in zebrafish remain an active field of investigation [328–331].

3.3. Joints

Articulations between neighboring bones play critical roles in modulating growth, movement and flexibility. Like in other vertebrate models, the zebrafish contains a variety of joint types, including fibrous joints and cartilaginous joints. Fibrous joints typically form between two dermal bones in the craniofacial skeleton. One example are the zebrafish cranial sutures, which display conserved requirements during skull development as zebrafish carrying mutations in *cyp26b1 (t24295)*, generated through ENU mutagenesis) or combinatorial deletions in *twist1b (el570)* and *tcf12 (el548)*, both generated using TALENS, fail to form select sets of cranial sutures, similar to mice and humans [160,332,333]. Cartilaginous joint specification has been extensively researched in zebrafish, with several mutant lines or morpholino treated embryos leading to the loss or gain of cartilaginous joints, including the *Iroquois homeobox factor* family, *nkx3.2*, and *barx1* [278,279,334–336] and transgenic reporters to label developing joints (Fig. 1B-C). Furthermore, recent work has demonstrated the presence of synovial joints in the zebrafish jaw and fins and a conserved requirement for Prg4 (also known as Lubricin), demonstrated by deleting *prg4b (el594)* using TALENS to reveal arthritis phenotypes in mutants [337]. Another recent study identified a highly conserved *nkx3.2* enhancer that is essential for jaw joint formation in zebrafish [338]. These findings highlight the zebrafish as a powerful system to interrogate joint degenerative and developmental diseases.

3.4. Tendons and ligaments

Conserved with that of chick and mouse [339,340], zebrafish tendon development is marked by early expression of *scleraxis a (scxa)* (Fig. 3A), a transcription factor found in tendon cells at all stages, in the cranial regions after 40 hpf and the axial regions after 30 hpf [274,275,277,341–343]. Around 60 hpf, tendon cells express the matrix genes, *tenomodulin (tmmd)*, *col1a1a*, *col1a1b*, and *col1a2*, signifying their differentiation and maturation (Fig. 3B-G) [341]. Zebrafish tendon cells also express a second *scleraxis* paralog, *scleraxis b (scxb)* [341,343]. Generally, the genes expressed by tendons, including *scxa/b*, also label ligaments, making it difficult to molecularly distinguish them although they have unique characteristics at the structural and functional level and can readily be identified based on their attachment points [341,344]. In zebrafish, the majority of cranial tendons originate from the cranial neural crest [341,345], with the exception of the sternohyoideus tendon which is contributed by two germ layers, the neural crest and the mesoderm [275]. Zebrafish pectoral fin tendons are derived from lateral plate mesoderm [275,285] and axial tendons arise from the sclerotome compartment in the somites [277].

Zebrafish tendons rely on conserved regulatory programs found in mice and humans. Similar to *Scx* mutant mice [346], knockout of *scxa (kg170)* or *scxa* and *scxb (kg107)* together using CRISPR/Cas in zebrafish resulted in decreased *tmmd* expression. Notably, the zebrafish mutants also had defects in cranial tendon and ligament development and differentiation, resulting in detached muscle fibers, abnormal swimming behavior, and reduced numbers of intermuscular bones [343,347]. Proper matrix assembly is important for the formation and integrity of the tendon attachment as morpholino knock-down of *thrombospondin 4b (tsp4b)* resulted in muscle detachment [348]. Consistent with research in mice [349] and chicken [350], studies in zebrafish have shown that muscles are required for axial tendon induction but dispensable for induction of cranial and pectoral fin tendon progenitors. However, muscles are necessary for the maintenance of *scxa* expression in the cranial

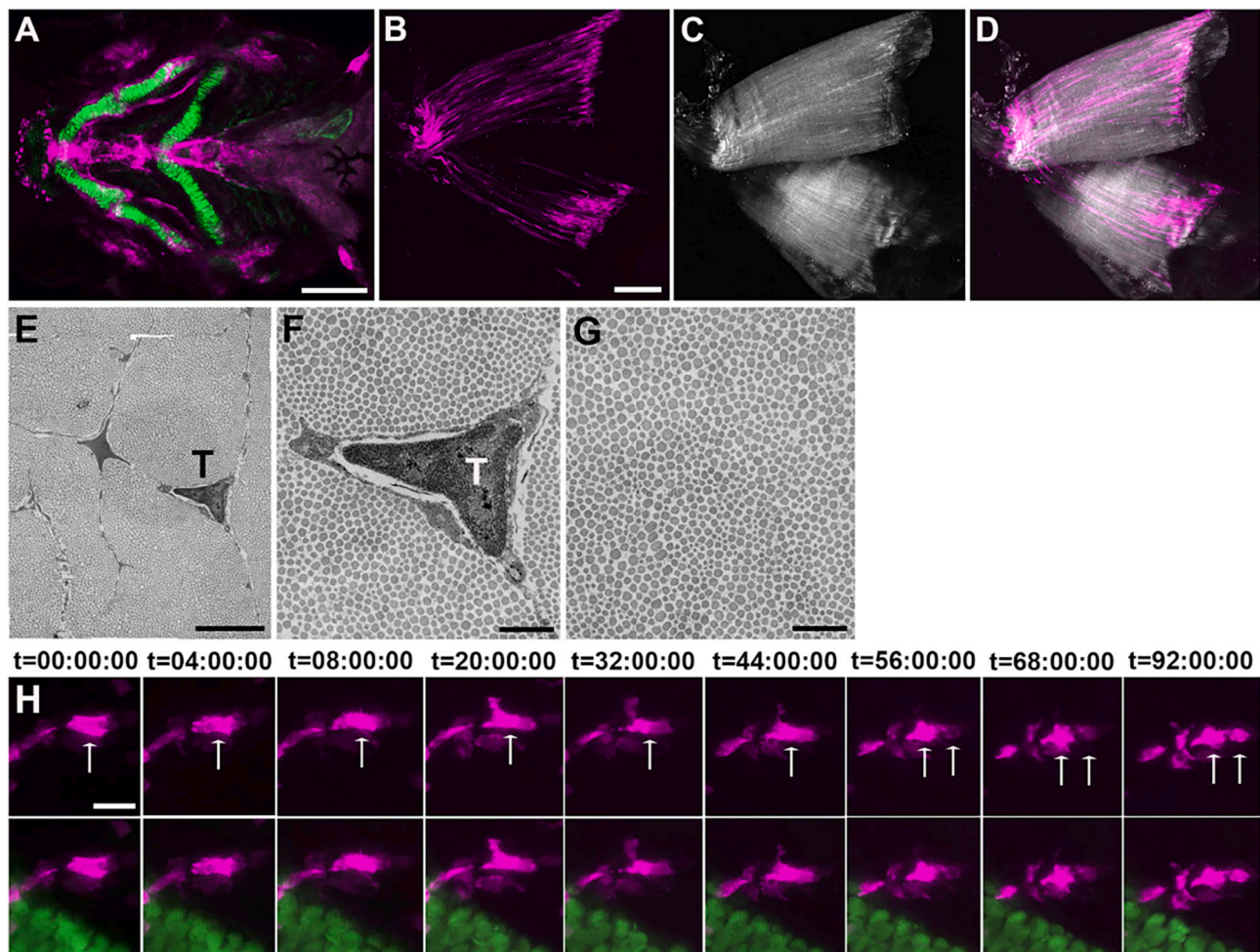


Fig. 3. Zebrafish craniofacial tendon and tendon cell division during tendon regeneration. (A) A double transgenic line shows *scxa:mcherry*; *col2a1a:eGFP* expression at 3dpf. Scale bar, 50 μ m; (B–D) Multiphoton images of sternohyoideus tendon at 180 dpf show *scxa:gal4-*vp16**; *uas:epNTR-RFP*+ tendon cells (B, D) and second harmonic generation (SHG) of collagen fibril (C, D). Scale bar, 100 μ m; (E) Ultrastructural examination of tendon cells and collagen fibrils by TEM. Scale bar, 2 μ m; (F, G) Magnified examination of tendon cells and collagen fibrils in (E). Scale bar, 0.5 μ m; (H) Time lapse imaging of ceratohyal cartilage region shows tendon cell division during tendon regeneration. *scxa:gal4-*vp16**; *uas:epNTR-RFP*+ labels tendon cells (white arrows indicate a cell undergoing division) and *col2a1a:eGFP* labels ceratohyal cartilage. Scale bar, 25 μ m. In (H), tendon cells were ablated at 3–5 dpf, $t = 00:00:00$ was immediately after the cell ablation. T, tendon cell. Images A–D, H, anterior to the left. Images E–G, transverse section.

and pectoral fin tendons at later stages [341]. In turn, problems with the myotendinous matrix can affect the muscle, resulting in movement impairment and muscle weakness [351]. In contrast, cartilage does not appear to be required for the induction of tendon progenitors in the cranial, pectoral fin and axial regions [341]. Several pathways have conserved requirements for tendon development in zebrafish, including the Fibroblast Growth Factors (FGF) [341] and Transforming Growth Factors β (TGF- β) pathways [341,352]. More recently, the zebrafish model has been used to identify novel signaling pathways regulating tendon cell formation, such as Bone Morphogenetic Protein (BMP) [275], Hedgehog [277], and mevalonate [277] pathways. Additionally, a recent study showed mechanical force regulates the cellular projections from tendon cells and extracellular matrix (ECM) production [352]. These studies highlight the conserved requirement of tendon formation across development, as the number of studies using zebrafish as models for tendon biology grow.

3.5. Teeth

Zebrafish have pharyngeal teeth located at the fifth ceratobranchial bone [353]. These teeth have many morphological similarities to vertebrate teeth, including the presence of enameloid, dentin, pulp, and

odontoblasts. They are shed and replaced throughout the animal's lifespan, making them a compelling model for tooth regeneration [353]. Using TRAP staining and *ctsk:DsRed* transgenic lines, large numbers of osteoclasts can be detected at the base of teeth, where resorption of attachment bone is required for tooth replacement. Consequently, loss of osteoclasts in *csf1ra/b* double mutants (*mh5*, and *mh108*, *mh112*) leads to an increase in tooth number due to tooth retention [280,319]. The signaling pathways involved in the development and mineralization of zebrafish teeth overlap significantly with vertebrates [354–357], and several genes have been shown to be essential for tooth formation, including *ext2* [358] and the *foxf* family [300]. Zebrafish teeth are a largely untapped model for biomineralization, tooth development and regeneration and future research is warranted to expand the mechanisms of tooth biology using the zebrafish model.

3.6. Scales

Although absent in mammalian and avian models of skeletal development, zebrafish scales have proven to be a rich resource to understand bone biology. Scales are ectoderm-derived calcified organs that cover the body. The external side of each individual scale has four discrete zones, all of which contain mineralized circuli of various stages.

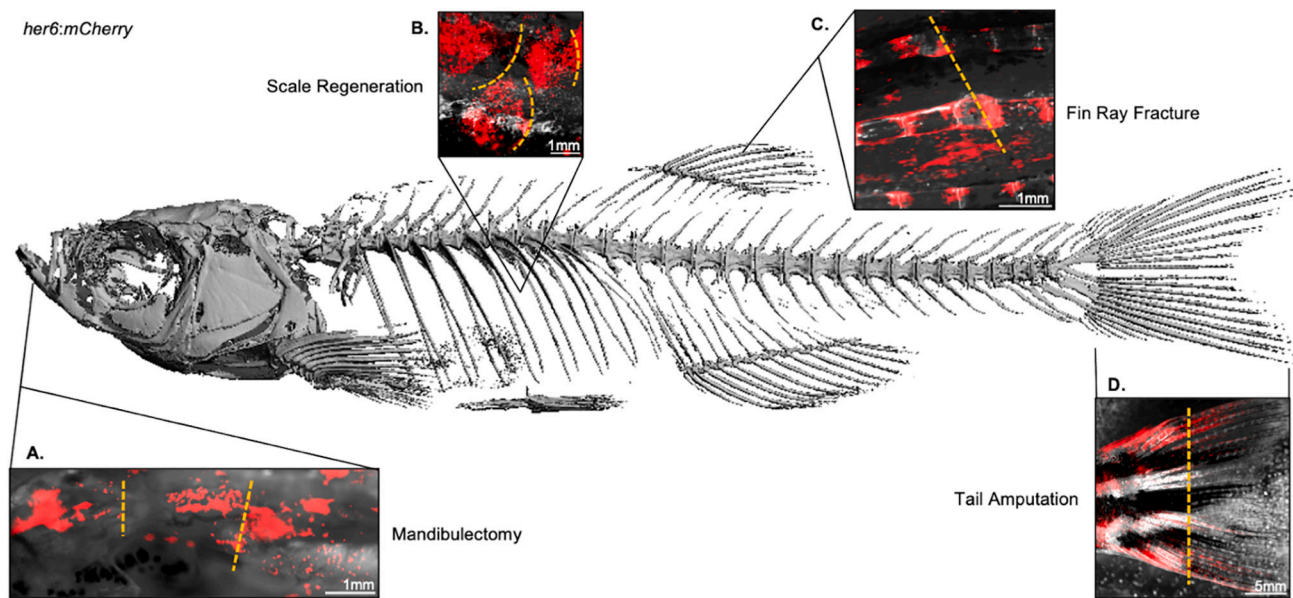


Fig. 4. Diverse regenerative processes activate notch signaling in the zebrafish skeleton. Micro-computed tomography reconstruction of the adult zebrafish skeleton, obtained in a Scanco μ CT40 at $12\ \mu\text{m}^3$ resolution with a lower threshold of 250 mgHA/ccm. Microscopy of zebrafish expressing the Notch signaling reporter *her6:mCherry* demonstrates ability to perform longitudinal intravital imaging of fluorescent transgenes under anesthesia using a stereomicroscopy. Representative images are shown 10 days following each of the following model injuries: (A) unilateral mandibulectomy; (B) scale plucking; (C) dorsal fin ray fracture; and (D) partial tail amputation, which were performed in different animals. Yellow dashed lines in A-D represent approximate injury margins. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Dynamic mineral labeling using calcein and alizarin red show that one circulus is added to each scale per week in growing young adult fish, and alkaline phosphatase (ALP) activity is seen in differentiating osteoblasts along the margins of new circuli, but not old circuli [359]. Like other bones, scales are remodeled by osteoclasts and are often utilized as a proxy to assess osteoclast number and activity in mutant models, using TRAP staining for osteoclasts and von Kossa staining to detect mineralized tissue and assess resorptive activity [280,360–363]. For example, analysis of scales from *csf1ra/b* double mutants did show a complete loss of TRAP staining and no signs of bone resorption, confirming the importance of *Csf1r* function for osteoclastogenesis [280]. Recent transcriptional analysis of CRISPR generated *low-density lipoprotein receptor-related 5 (lrp5)* mutants showed an enrichment in genes involved in both osteoblast signaling and function and osteoclast differentiation, suggesting a novel role for *Lrp5* during osteoclastogenesis. Detailed analysis of osteoclast behavior in mutant scales supported a role for *Lrp5* in osteoclastogenesis as mutants displayed an increase in TRAP staining and an increase in resorbed area, suggesting an increase in osteoclast number and/or activity [363].

Zebrafish scales can be easily harvested from adult zebrafish and cultured for up to 72 h *in vitro* [359]. Several studies have utilized *ex-vivo* scale culture systems to uncover basic interactions between osteoblasts and osteoclasts, to model skeletal changes in pathological conditions, and to perform chemical screens to identify novel regulators of osteoblast and osteoclast activity [364–366]. For example, ontogenetic scales from *sp7:luciferase* transgenic fish have been cultured in osteogenic media to successfully screen for novel drugs with osteogenic potential [269]. These tissue explants also show endogenous interactions between osteoblasts and osteoclasts, something not attainable in monolayer cell cultures. Thus, the zebrafish model provides novel mechanisms to understand skeletal development through the utility of structures absent from alternative models.

4. Regeneration in the zebrafish skeleton

Zebrafish have profound regenerative capacity. Numerous examples show that tissue repair in zebrafish exceeds its mammalian counterparts

[367,368]. The zebrafish is also one of the earliest model organisms used to study musculoskeletal regeneration [369]. Bone regeneration employs multiple distinct cellular methods based on the anatomical location and the extent of injury. Among the diverse routes towards regeneration, fin amputation is the only injury that results in a classical blastema, which is evolutionarily conserved with axolotl [370]. However, fin crush injuries undergo intramembranous repair, and mandible resection heals by endochondral ossification. This diversity of regenerative programs means that an injury can be selected to model a specific mammalian repair mechanism or to examine mechanisms distinct to the zebrafish. The use of transgenic lines together with *in vivo* imaging allows for the observation of cell behavior during the regeneration process in real time.

The different regeneration models (Fig. 4) will be further elaborated in the following section. In addition, this section will also highlight the regenerative strategies for the skeletal components that support bone, including cartilage and tendons/ligaments.

4.1. Fin amputation

Epimorphosis is a process by which complex structures are regenerated by a highly organized cluster of proliferative, undifferentiated cells called a blastema. Amputation of the zebrafish caudal fin is a classic example of this phenomenon, and has been known to science for more than two centuries [369]. The advent of transgenesis has greatly accelerated our understanding of this process. There is an immense body of literature describing caudal fin regeneration [371], and this section will summarize in brief and highlight specific emerging transgenic techniques that have contributed to the field.

The caudal fin faithfully regenerates its native structure and function following serial amputations [372]. This remarkable epimorphic regeneration process is believed to largely mirror both the cell types and signaling mechanisms present during development [373]. Within 24 h of amputation, small clusters of mesenchyme known as blastemas aggregate at the distal ray stumps enveloped by a wound epithelium. Outgrowth of bone and supporting tissues then occurs over a period of weeks until new rays achieve their original sizes and organization [371].

Regenerative osteoblasts derive from multiple proliferative populations including cells derived from intra-ray mesenchyme [163], and to a lesser extent, dedifferentiated osteoblasts activated from the remaining bone tissue [161,374]. With the multitude of available transgenic lines and lineage tracing techniques, these processes can easily be traced and visualized by time lapse imaging *in vivo* in the easily accessible and relatively thin (less than 200µm thick) zebrafish caudal fin. We refer readers to existing reviews that describe this process in further cellular detail [374,375].

Signaling cascades important for regenerative outgrowth following caudal fin amputation include Notch [376], FGF [377], TGFβ [378], Wnt [379], Hedgehog [380], and numerous others. Much of this work has been performed using traditional gene knockouts and reporters, with a more recent trend towards incorporating transcriptomics, epigenetic profiling, and inducible genetic approaches. One example is identification of a tissue-specific *leptin b*-linked enhancer element as essential to the regenerative response of intraray mesenchyme [381]. Live clonal analysis using *tph1b:CreER*; *priZm* animals indicate these activated mesenchymal cells exhibit significant positional and functional heterogeneity that is acquired within the first 2–3 days post-amputation [382]. The transient wound epidermis has also been tracked using *fn1b:creERT2*-mediated fate mapping [383], and found to orchestrate early osteoblast patterning [384].

4.2. Fin ray fracture

Crush injury to the caudal fin hemirays, including intra-ray arteries and nerve fibers, produces a callus that is reminiscent of those seen in mammalian fractures [385]. The gene expression profile of the callus shares many markers in common with caudal fin amputation including *msxb* (early blastema marker), *osterix*, *collagen 1*, *osteonectin*, *osteopontin* and *Tenascin C*. Similar to mammalian fracture repair, the fin ray fracture involves the FGF, retinoic acid, and Wnt signaling pathways [264]. *Osteocalcin:GFP* and *runx2:GFP* fish were leveraged to show that mature fin ray osteoblasts decreased *osteocalcin* expression and increased *runx2* expression around fracture sites, suggesting dedifferentiation of osteoblasts in this model. *Entpd5:Kaede* and *osterix:CreERT2-p2a-mCherry*; *hsp70l:loxP-DsRed2-loxP-nlsEGFP* transgenic animals have shown that dedifferentiated osteoblasts migrate towards the fracture site from adjacent fin ray segments, proliferate and then mineralize the injury site [264].

The fin ray fracture has also been used to study fracture healing in a zebrafish osteogenesis imperfecta (OI) disease model, which showed that similar to human patients, OI zebrafish suffered non-union in about 30 % of fractures, whereas non-union essentially never occurs in wild type animals. Bisphosphonates reduced osteoclast activity and therefore healing in wild-type animals, while reducing incidence of spontaneous fractures in the OI fish. Additionally, bacterial infection by *S. aureus* was shown to impair fracture healing in zebrafish using this model [386]. Furthermore, the fin ray fracture model has been used to show that osteocytes are responsible for the improved fracture healing observed with low-intensity pulsed ultrasound therapy [387], and *wnt16* mutant (*bi667* and *bi451*) zebrafish showed increased vulnerability to spontaneous fractures and delayed osteoblast recruitment and bone mineralization during fracture healing [388]. The fin ray fracture is an accessible and clinically relevant model of intramembranous regeneration, with increased reliance on osteoblast differentiation *versus* what is currently understood in mammals.

4.3. Calvarial trepanation

Surgical defects of the cranial vault—trepanation of the frontal and/or parietal bones—are commonly used to model intramembranous regeneration in rodents. These models have been adapted to zebrafish; a 0.5 mm defect regenerates in 14 days [264]. Reporter animals including *runx2:GFP*, *sp7:GFP*, and *bglap:GFP* bred on a casper double-mutant

background (lacking pigment) have been used for serial intravital imaging of the differentiation process [264]. This group used *sp7:CreERT2*-mediated lineage tracing to reveal contribution of resident calvarial osteoblasts to the repair process. Intravital optical coherence tomography has also been recently applied to monitor small molecule-induced changes to defect regeneration [389].

4.4. Mandibulectomy

The mandible is developmentally derived from neural crest cells of the first pharyngeal arch, and ossification occurs around a central cartilage template (Meckel's cartilage) that persists through adulthood [390]. Mandibulectomy is the only endochondral bone repair model in zebrafish. There are two approaches: bilateral amputation of the distal aspect including the mandibular symphysis [391], and unilateral resection of the mandibular body including the Meckel's cartilage [392].

Amputation of the distal 1/3 of the mandible results in endochondral repair that fully ossifies and does not restore the cartilaginous mandibular symphysis [391]. RNA sequencing of regenerating tissue at day 4 revealed upregulation of numerous genes associated with neural crest cells and their differentiating progeny. Using a follow-up knock-down approach, *foxi1* was identified as regulating *sox9a* + progenitor cells adjacent to the Meckel's cartilage that are required for bone outgrowth from the amputated stumps [393]. The bone formed in this model is believed to primarily derive from mesenchymal cells of the mandible located adjacent to the injury. Some genes representative of epimorphic regeneration of the fin, such as *msxb*, are expressed in this model, but the primary mechanism is believed to be endochondral regeneration [394]. Interestingly, this process is not dependent on Wnt signaling [394].

Regenerative cartilage formed after resection of the mandibular body is continuous with Meckel's cartilage, if any remains following the procedure [394], yet the regeneration process is distinct from developmental endochondral ossification [392]. The repair cartilage cells, lineage-traced with *col2a1a:GFP*, stain for osteoblast-specific *spp1* and embed themselves in alizarin-labeled new bone, and this process requires *ihha* signaling [392]. Zebrafish mandible regeneration is therefore believed to incorporate highly regenerative hybrid cartilage-bone cells with enhanced plasticity relative to most mammalian models [395]. A *her6:mCherry* transgenic reporter shows that Notch signaling is active during the early phases of regeneration, and regenerate bone volume is proportional to early Notch signaling levels [396] (Fig. 4). *hsp70l:Gal4*; *UAS:myc-Notch1a-intra* mediated overexpression of the Notch1a receptor's intracellular domain accelerates the conversion of cartilage to bone [396].

4.5. Scale regeneration

Adult zebrafish scales regenerate following loss. RNA-seq of both ontogenetic and regenerating scales shows conservation of osteogenesis programs between zebrafish and mammals [397]. A prednisolone-induced osteoporosis model was found to impact scale regeneration by inhibiting the size of the new scale and increasing osteoclast activity [361]. Osteoblast-associated genes like *sp7* and *runx2a* are expressed very early in scale regeneration, and Hh signals *shha*, *ihha*, and *ptch1* are all expressed by day 2 of scale regeneration. Small-molecule inhibition of Hh signaling results in a reduction in the number of central and marginal osteoblasts by impacting the recruitment of precursors and osteoblast differentiation [398]. Using *osx:H2A-mCherry* fish, osteoblasts can be seen as early as 24 h post scale plucking (hpp). Genetic ablation of homeostatic osteoblasts using *osx:mCherry-NTR* animals shows similar rates of scale regeneration as control animals, indicating *de novo* osteoblasts contribute to scale regeneration. This was confirmed by using *osx:kaede* photoconvertible animals, which shows that new osteoblasts do not result from proliferation of pre-injury osteoblast populations. *Osx:Venus-hGeminin* and *osx:mCherry-zCdt1* animals enable

discrimination between cell cycle phases, which helped determine the proliferation patterns of osteoblasts in the regenerating scales. *fgf20a:EGFP* transgenic fish have also been used to show *fgf20a* is expressed in the osteoblasts of regenerating scales, and *hsp70l:dnfgfr1-GFP* animals have been used to induce heat-shock controlled inhibition of *fgf* receptors, which resulted in decreased scale regeneration. The poor regeneration in these animals was found to result from decreased proliferation of osteoblasts [162].

Erk activity, dependent on Fgf receptors and MAPKK in scale osteoblasts, is activated in repeated waves that travel from the center to the periphery of regenerating scales once every two days. Osteoblast growth, and therefore scale growth, correlates with the speed and number of Erk waves [239]. To investigate the intercellular interaction between osteoblasts and osteoclasts during osteoclast differentiation, *trap:GFP; osterix:mCherry* double-transgenic fish were generated to visualize both osteoclasts and osteoblasts in a fracture scale model in zebrafish. Flow cytometry sorting followed by confocal microscopy revealed that most *trap:GFP+* cells had mCherry+ particles in their cytoplasm, which were thought to be osteoblast-derived extracellular vesicles. This was confirmed after transplantation of kidney marrow cells from *trap:GFP* fish into irradiated *osterix:mCherry* fish resulted in GFP+/mCherry+ double positive cells at fracture sites, as well as after live imaging of fractured scales showed osteoclasts actively interacting with osteoblasts by engulfing their mCherry+ particles. These osteoblast-derived extracellular vesicles were determined to promote osteoclast differentiation and fusion in cell culture [266].

4.6. Tendon and ligament regeneration

Over 14 million tendon injuries are reported in the United States each year [399], yet their lasting repair remains a significant clinical challenge. Most studies of tendon injury mechanisms employ mammalian models such as mouse and rat, which are characterized by non-regenerative healing through scar formation. These studies have explored the molecular and cellular mechanisms underlying mammalian tendon injury and repair and have improved our understanding of the cell types and pathways involved in the repair process [400–405]. Neonatal mice, which have been found to undergo regenerative tendon healing, have been used to dissect the cellular, mechanical, and structural mechanisms and have shown that Scx-lineage cells contribute to functional repair [403,406]. The zebrafish provides a new system to study tendon and ligament regeneration; however, the model is in its infancy and much work is needed to fully understand the mechanisms guiding tendon and ligament regeneration. Recently, genetic tools were developed to ablate *scxa* + tendon cells in zebrafish and it was discovered that the larvae were able to fully regenerate their tendon composition and pattern (Fig. 3H) [275,407]. Using live imaging and lineage tracing, they established that the new cells were recruited from *sox10*+ perichondral cells and *nkx2.5*+ cells surrounding the muscle and identified BMP signaling as an essential regulator of tendon regeneration. Interestingly, they found ablation in adults also resulted in regeneration of the *scxa*+ tendon cells. Using a similar ablation methodology in the zebrafish, another group depleted tenocytes in the trunk myotendinous junction via *nkx3.1:Gal4; UAS:NTR-mCherry* and *col1a2:Gal4; UAS:NTR-mCherry* transgenic lines and found disrupted muscle morphology [277]. In contrast to tendon cell ablation in zebrafish which caused defective myotendinous junction function, Scleraxis-lineage cell depletion in mice however improved the biomechanical properties of the tendon after injury [408]. It is unclear why in some contexts Scx-lineage cells are important for proper healing and regeneration, but not in others. Clearly, more work is necessary to dissect the complexities of tendon healing in regenerative and non-regenerative contexts. The zebrafish provides a key platform for *in vivo* examination of tenocyte regeneration in the future.

More recently, a zebrafish model of ligament injury was established with the goal of testing jaw joint cartilage regeneration [409]. The jaw

joint damage was introduced by transecting the interopercular–mandibular ligament that can cause jaw joint destabilization. They showed, through transgenic reporter imaging, genetic ablation and single-cell RNA sequencing, multiple cellular populations including *sox10*+ and *grem1a* + cells contributed to jaw joint cartilage regeneration. However, the ability of the zebrafish to regenerate the ligament in this context remains unexplored. Certainly, this is an exciting direction given the frequency of ligament tears in humans, particularly for the anterior cruciate ligament, which cannot reattach without surgical intervention. The emerging models for tendon and ligament regeneration often focus on the cranial or axial tendons and ligaments as the adult pectoral fins consist of distal fin rays connected by muscles to the proximal pectoral girdle, but without long tendinous attachments as found in mammalian limbs [410–412]. These recent works lay a critical foundation for employing zebrafish to understand tendon and ligament regeneration, and the zebrafish model will very likely emerge as a powerful model for discoveries into the cellular and molecular mechanisms of tendon and ligament development and repair.

5. Discussion and future directions

Research in the zebrafish model has grown exponentially in the past two decades. The feasibility of genetic manipulations, imaging, and lineage tracing techniques along with a relatively low cost to maintain zebrafish lines compared to mammalian housing has made it an accessible model for many labs. Technological advancement in genetic editing tools and next generation sequencing strategies have further accelerated work in this system. With high gene homology to humans and conserved developmental processes between vertebrates, the zebrafish is an attractive model for both understanding foundational mechanisms underlying developmental and regenerative biology and also in modeling human diseases. As described throughout this review, these studies include generating zebrafish mutants to model human disease, but they can also be predictive of human disease loci [9]. For example, Frank Eames and colleagues identified mutants with reduced cartilage matrix and increased perichondral bone formation in an ENU mutagenesis screen [413]. Positional cloning revealed mutations in enzymes in the proteoglycan synthesis pathway, *fam20b* and *xylosyltransferase1* (*xylt1*), as phenotype causing, highlighting the critical function of proteoglycan synthesis in endochondral ossification. Several years later, studies showed that mutations of *FAM20B* and *XYLT1* in humans are associated with Desbuquois skeletal dysplasia (DBSD), a chondrodysplasia characterized by skeletal changes including increased ossification with short limbs and stature [414,415]. Complementing these studies, the robust regenerative abilities of the fish have been examined in cellular and molecular detail, providing a blueprint for improving healing mechanisms in humans.

The zebrafish model has also emerged as a prominent model for understanding developmental biology at a single cell resolution. A number of groups have utilized single-cell technologies to understand embryogenesis in zebrafish, including studies focused on neural crest, which gives rise to most of the craniofacial skeleton [416–423]. In the context of skeletal development, a recent study carefully mapped neural crest derivatives in the craniofacial skeleton across zebrafish development, generating both scRNA-seq and scATAC-seq datasets for a range of timepoints as early as 36 hpf and as late as 6 months of age. The authors showed that skeletogenic identities within neural crest are progressively prefigured at an epigenetic level and identified transcriptional signatures for putative multipotent skeletal progenitors and metabolically unique fibroblasts that support the craniofacial skeleton [423]. These datasets will undoubtedly be a staple for zebrafish researchers interested in the development and regeneration of skeletal tissues, and it will be very interesting to understand how the programs and cell types captured in these datasets relate to programs that are activated during regenerative processes. Such work is already underway in the zebrafish fin with recent reports describing fin regeneration at single cell resolution and

demonstrating the pathways required within macrophages, epithelia and osteoprogenitors during fin regeneration [424–426]. Single cell genomics is also now being integrated with lineage tracing tools, enabling gene expression and epigenetic analyses to be combined with lineage data in a single experiment [130,140–142,417]. These tools will drive discovery across species and tissues, and the ease of transgenesis and mutagenesis will make zebrafish a leader in developmental and regenerative biology.

As indicated throughout this review, the zebrafish has been a powerful model to understand skeletal development, and specifically bone and cartilage development. Research investigating osteoclasts, tendons and ligaments, and teeth remain growing areas in the zebrafish community. As these emerging research areas mature, the zebrafish's strengths with respect to imaging and genetics will enable researchers to investigate the complex tissue interactions that drive skeletal shape and function. Complemented with new genomic technologies and precise genetic editing, and considering the relatively low cost to maintain zebrafish, the growing number of disease models, and the advent of multiplex single cell experiments [427–429], the zebrafish model will be a critical discovery model for skeletal development and regeneration.

CRedit authorship contribution statement

All authors contributed to the writing and editing of this manuscript. Conceptualization: D.T.F, K.H. and D.W.Y. Figs. 1 and 2 were contributed by D.T.F; Fig. 3 was contributed by X.N and J.L.G; Fig. 4 was contributed by J.M.K and D.W.Y.

Data availability

No data was used for the research described in the article.

Acknowledgements

DTF was supported by the HHMI Hanna Gray Fellows Program. X.N. and J.L.G. were supported by the NIH/NIAMS AR074541 and the Conine Family Fund.

References

- [1] C.B. Kimmel, *Genetics and early development of zebrafish*, *Trends Genet.* 5 (8) (1989) 283–288.
- [2] K. Howe, et al., The zebrafish reference genome sequence and its relationship to the human genome, *Nature* 496 (7446) (2013) 498–503.
- [3] F. Tonelli, et al., Zebrafish: a resourceful vertebrate model to investigate skeletal disorders, *Front. Endocrinol. (Lausanne)* 11 (2020) 489.
- [4] K. Dietrich, et al., Skeletal biology and disease modeling in zebrafish, *J. Bone Miner. Res.* 36 (3) (2021) 436–458.
- [5] M.T. Valenti, et al., Zebrafish: a suitable tool for the study of cell signaling in bone, *Cells* 9 (8) (2020).
- [6] D.J.M. Bergen, E. Kague, C.L. Hammond, Zebrafish as an emerging model for osteoporosis: a primary testing platform for screening new osteo-active compounds, *Front. Endocrinol. (Lausanne)* 10 (2019) 6.
- [7] L. Lleras-Forero, C. Winkler, S. Schulte-Merker, Zebrafish and medaka as models for biomedical research of bone diseases, *Dev. Biol.* 457 (2) (2020) 191–205.
- [8] N. Wu, et al., The Progress of CRISPR/Cas9-mediated gene editing in generating Mouse/Zebrafish models of human skeletal diseases, *Comput Struct Biotechnol J* 17 (2019) 954–962.
- [9] M. Mari-Beffa, A.B. Mesa-Roman, I. Duran, Zebrafish models for human skeletal disorders, *Front. Genet.* 12 (2021), 675331.
- [10] M. Carnovali, G. Banfi, M. Mariotti, Zebrafish models of human skeletal disorders: embryo and adult swimming together, *Biomed. Res. Int.* 2019 (2019) 1253710.
- [11] R.Y. Kwon, C.J. Watson, D. Karasik, Using zebrafish to study skeletal genomics, *Bone* 126 (2019) 37–50.
- [12] B. Busse, et al., Zebrafish: an emerging model for orthopedic research, *J. Orthop. Res.* 38 (5) (2020) 925–936.
- [13] Y.M. Bradford, et al., Zebrafish information network, the knowledgebase for Danio rerio research, *Genetics* 220 (4) (2022).
- [14] C. Walker, G. Streisinger, Induction of mutations by gamma-rays in pregonial germ cells of zebrafish embryos, *Genetics* 103 (1) (1983) 125–136.
- [15] S. Chakrabarti, et al., Frequency of gamma-ray induced specific locus and recessive lethal mutations in mature germ cells of the zebrafish *Brachydanio rerio*, *Genetics* 103 (1) (1983) 109–123.
- [16] D.J. Grunwald, G. Streisinger, Induction of mutations in the zebrafish with ultraviolet light, *Genet. Res.* 59 (2) (1992) 93–101.
- [17] D.J. Grunwald, G. Streisinger, Induction of recessive lethal and specific locus mutations in the zebrafish with ethyl nitrosourea, *Genet. Res.* 59 (2) (1992) 103–116.
- [18] M.C. Mullins, et al., Large-scale mutagenesis in the zebrafish: in search of genes controlling development in a vertebrate, *Curr. Biol.* 4 (3) (1994) 189–202.
- [19] L. Solnica-Krezel, A.F. Schier, W. Driever, Efficient recovery of ENU-induced mutations from the zebrafish germline, *Genetics* 136 (4) (1994) 1401–1420.
- [20] W. Driever, et al., A genetic screen for mutations affecting embryogenesis in zebrafish, *Development* 123 (1996) 37–46.
- [21] P. Haffter, et al., The identification of genes with unique and essential functions in the development of the zebrafish, *Danio rerio*, *Development* 123 (1996) 1–36.
- [22] S.C. Neuhauss, et al., Mutations affecting craniofacial development in zebrafish, *Development* 123 (1996) 357–367.
- [23] T. Piotrowski, et al., Jaw and branchial arch mutants in zebrafish II: anterior arches and cartilage differentiation, *Development* 123 (1996) 345–356.
- [24] T.F. Schilling, et al., Jaw and branchial arch mutants in zebrafish I: branchial arches, *Development* 123 (1996) 329–344.
- [25] P. Haffter, et al., Mutations affecting pigmentation and shape of the adult zebrafish, *Dev. Genes Evol.* 206 (4) (1996) 260–276.
- [26] S. Fisher, P. Jagadeeswaran, M.E. Halpern, Radiographic analysis of zebrafish skeletal defects, *Dev. Biol.* 264 (1) (2003) 64–76.
- [27] P.C. Yelick, M.H. Connolly, A forward genetic screen for genes regulating mineralized tooth and bone formation in zebrafish (*Danio rerio*), *J. Appl. Ichthyol.* 26 (2) (2010) 192–195.
- [28] V. Andreeva, et al., Identification of adult mineralized tissue zebrafish mutants, *Genesis* 49 (4) (2011) 360–366.
- [29] K. Henke, et al., Genetic screen for postembryonic development in the zebrafish (*Danio rerio*): dominant mutations affecting adult form, *Genetics* 207 (2) (2017) 609–623.
- [30] R.S. Gray, et al., Postembryonic screen for mutations affecting spine development in zebrafish, *Dev. Biol.* 471 (2021) 18–33.
- [31] W.S. Talbot, A.F. Schier, Positional cloning of mutated zebrafish genes, *Methods Cell Biol.* 60 (1999) 259–286.
- [32] N. Bahary, et al., The zon laboratory guide to positional cloning in zebrafish, *Methods Cell Biol.* 77 (2004) 305–329.
- [33] R. Geisler, et al., Large-scale mapping of mutations affecting zebrafish development, *BMC Genomics* 8 (2007) 11.
- [34] M.E. Bowen, et al., Efficient mapping and cloning of mutations in zebrafish by low-coverage whole-genome sequencing, *Genetics* 190 (3) (2012) 1017–1024.
- [35] I. Leshchiner, et al., Mutation mapping and identification by whole-genome sequencing, *Genome Res.* 22 (8) (2012) 1541–1548.
- [36] M.L. Voz, et al., Fast homozygosity mapping and identification of a zebrafish ENU-induced mutation by whole-genome sequencing, *PLoS One* 7 (4) (2012), e34671.
- [37] N. Obholzer, et al., Rapid positional cloning of zebrafish mutations by linkage and homozygosity mapping using whole-genome sequencing, *Development* 139 (22) (2012) 4280–4290.
- [38] A.C. Miller, et al., RNA-seq-based mapping and candidate identification of mutations from forward genetic screens, *Genome Res.* 23 (4) (2013) 679–686.
- [39] J.T. Hill, et al., MMAPP: mutation mapping analysis pipeline for pooled RNA-seq, *Genome Res.* 23 (4) (2013) 687–697.
- [40] K. Henke, M.E. Bowen, M.P. Harris, Perspectives for identification of mutations in the zebrafish: making use of next-generation sequencing technologies for forward genetic approaches, *Methods* 62 (3) (2013) 185–196.
- [41] C.B. Moens, et al., Reverse genetics in zebrafish by TILLING, *Brief Funct. Genomic Proteomic* 7 (6) (2008) 454–459.
- [42] R.N. Kettleborough, et al., A systematic genome-wide analysis of zebrafish protein-coding gene function, *Nature* 496 (7446) (2013) 494–497.
- [43] G.K. Varshney, et al., A large-scale zebrafish gene knockout resource for the genome-wide study of gene function, *Genome Res.* 23 (4) (2013) 727–735.
- [44] K. Kawakami, et al., zTrap: zebrafish gene trap and enhancer trap database, *BMC Dev. Biol.* 10 (2010) 105.
- [45] I. Kondrychyn, et al., Zebrafish enhancer TRAP transgenic line database ZETRAP 2.0, *Zebrafish* 8 (4) (2011) 181–182.
- [46] A. Trinh le, et al., A versatile gene trap to visualize and interrogate the function of the vertebrate proteome, *Genes Dev.* 25 (21) (2011) 2306–2320.
- [47] P. Jungke, S. Hans, M. Brand, The zebrafish CreZoo: an easy-to-handle database for novel CreER(T2)-driver lines, *Zebrafish* 10 (3) (2013) 259–263.
- [48] J. Corbacho, et al., Trap-TRAP, a versatile tool for tissue-specific translatomics in zebrafish, *Front. Cell Dev. Biol.* 9 (2021), 817191.
- [49] Y. Sun, et al., Systematic genome editing of the genes on zebrafish chromosome 1 by CRISPR/Cas9, *Genome Res.* 30 (1) (2020) 118–126.
- [50] N. Ichino, et al., Building the vertebrate codex using the gene breaking protein trap library, *elife* (2020) 9.
- [51] A. Amsterdam, Insertional mutagenesis in zebrafish, *Dev. Dyn.* 228 (3) (2003) 523–534.
- [52] N. Gaiano, et al., Insertional mutagenesis and rapid cloning of essential genes in zebrafish, *Nature* 383 (6603) (1996) 829–832.
- [53] A. Amsterdam, et al., A large-scale insertional mutagenesis screen in zebrafish, *Genes Dev.* 13 (20) (1999) 2713–2724.
- [54] A. Amsterdam, et al., Identification of 315 genes essential for early zebrafish development, *Proc. Natl. Acad. Sci. U. S. A.* 101 (35) (2004) 12792–12797.
- [55] G. Golling, et al., Insertional mutagenesis in zebrafish rapidly identifies genes essential for early vertebrate development, *Nat. Genet.* 31 (2) (2002) 135–140.

- [56] D. Wang, et al., Efficient genome-wide mutagenesis of zebrafish genes by retroviral insertions, *Proc. Natl. Acad. Sci. U. S. A.* 104 (30) (2007) 12428–12433.
- [57] R.M. Nissen, A. Amsterdam, N. Hopkins, A zebrafish screen for craniofacial mutants identifies *wdr68* as a highly conserved gene required for endothelin-1 expression, *BMC Dev. Biol.* 6 (2006) 28.
- [58] K. Kawakami, Transposon tools and methods in zebrafish, *Dev. Dyn.* 234 (2) (2005) 244–254.
- [59] A.E. Davidson, et al., Efficient gene delivery and gene expression in zebrafish using the sleeping beauty transposon, *Dev. Biol.* 263 (2) (2003) 191–202.
- [60] A. Emelyanov, et al., Trans-kingdom transposition of the maize dissociation element, *Genetics* 174 (3) (2006) 1095–1104.
- [61] K. Kawakami, et al., A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish, *Dev. Cell* 7 (1) (2004) 133–144.
- [62] D. Shen, et al., Enhancer trapping and annotation in zebrafish mediated with sleeping beauty, piggyBac and Tol2 transposons, *Genes (Basel)* 9 (12) (2018).
- [63] D. Shen, et al., A native, highly active Tol2/mariner transposon from zebrafish (ZB) offers an efficient genetic manipulation tool for vertebrates, *Nucleic Acids Res.* 49 (4) (2021) 2126–2140.
- [64] M. Munoz-Lopez, J.L. Garcia-Perez, DNA transposons: nature and applications in genomics, *Curr Genomics* 11 (2) (2010) 115–128.
- [65] A. Trinh le, S.E. Fraser, Enhancer and gene traps for molecular imaging and genetic analysis in zebrafish, *Develop. Growth Differ.* 55 (4) (2013) 434–445.
- [66] K.J. Clark, et al., In vivo protein trapping produces a functional expression codex of the vertebrate proteome, *Nat. Methods* 8 (6) (2011) 506–515.
- [67] T.T. Ni, et al., Conditional control of gene function by an invertible gene trap in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 109 (38) (2012) 15389–15394.
- [68] C. Liu, et al., Generation of an enhancer-trapping vector for insertional mutagenesis in zebrafish, *PLoS One* 10 (10) (2015), e0139612.
- [69] Y. Zhong, et al., Improved Tol2-mediated enhancer trap identifies weakly expressed genes during liver and beta cell development and regeneration in zebrafish, *J. Biol. Chem.* 294 (3) (2019) 932–940.
- [70] M.R. Lieber, The mechanism of double-strand DNA break repair by the nonhomologous DNA end-joining pathway, *Annu. Rev. Biochem.* 79 (2010) 181–211.
- [71] K. Prill, J.F. Dawson, Homology-directed repair in zebrafish: witchcraft and wizardry? *Front. Mol. Biosci.* 7 (2020), 595474.
- [72] Y. Doyon, et al., Heritable targeted gene disruption in zebrafish using designed zinc-finger nucleases, *Nat. Biotechnol.* 26 (6) (2008) 702–708.
- [73] X. Meng, et al., Targeted gene inactivation in zebrafish using engineered zinc-finger nucleases, *Nat. Biotechnol.* 26 (6) (2008) 695–701.
- [74] P. Huang, et al., Heritable gene targeting in zebrafish using customized TALENs, *Nat. Biotechnol.* 29 (8) (2011) 699–700.
- [75] J.D. Sander, et al., Targeted gene disruption in somatic zebrafish cells using engineered TALENs, *Nat. Biotechnol.* 29 (8) (2011) 697–698.
- [76] W.Y. Hwang, et al., Efficient genome editing in zebrafish using a CRISPR-cas system, *Nat. Biotechnol.* 31 (3) (2013) 227–229.
- [77] N. Chang, et al., Genome editing with RNA-guided Cas9 nuclease in zebrafish embryos, *Cell Res.* 23 (4) (2013) 465–472.
- [78] L.E. Jao, S.R. Wente, W. Chen, Efficient multiplex biallelic zebrafish genome editing using a CRISPR nuclease system, *Proc. Natl. Acad. Sci. U. S. A.* 110 (34) (2013) 13904–13909.
- [79] G.K. Varshney, et al., High-throughput gene targeting and phenotyping in zebrafish using CRISPR/Cas9, *Genome Res.* 25 (7) (2015) 1030–1042.
- [80] M. Jinek, et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity, *Science* 337 (6096) (2012) 816–821.
- [81] G. Gasunas, et al., Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria, *Proc. Natl. Acad. Sci. U. S. A.* 109 (39) (2012) E2579–E2586.
- [82] A.V. Anzalone, L.W. Koblan, D.R. Liu, Genome editing with CRISPR-cas nucleases, base editors, transposases and prime editors, *Nat. Biotechnol.* 38 (7) (2020) 824–844.
- [83] K. Liu, et al., Expanding the CRISPR toolbox in zebrafish for studying development and disease, *Front. Cell Dev. Biol.* 7 (2019) 13.
- [84] J.T. Flavia De Santis, Vincenzo Di Donato, Chapter 18 - The CRISPR/Cas system in zebrafish, in: *Behavioral and Neural Genetics of Zebrafish*, 2020, pp. 293–307.
- [85] J.A. Gagnon, et al., Efficient mutagenesis by Cas9 protein-mediated oligonucleotide insertion and large-scale assessment of single-guide RNAs, *PLoS One* 9 (5) (2014), e98186.
- [86] S. Parvez, et al., MIC-drop: a platform for large-scale in vivo CRISPR screens, *Science* 373 (6559) (2021) 1146–1151.
- [87] M.A. Moreno-Mateos, et al., CRISPRscan: designing highly efficient sgRNAs for CRISPR-Cas9 targeting in vivo, *Nat. Methods* 12 (10) (2015) 982–988.
- [88] T.G. Montague, et al., CHOPCHOP: a CRISPR/Cas9 and TALEN web tool for genome editing, *Nucleic Acids Res.* 42 (Web Server issue) (2014) W401–W407.
- [89] C.J. Watson, et al., Phenomics-based quantification of CRISPR-induced mosaicism in zebrafish, *Cell Syst.* 10 (3) (2020) 275–286, e5.
- [90] J.W. Bek, et al., Lrp5 mutant and crisprant zebrafish faithfully model human osteoporosis, establishing the zebrafish as a platform for CRISPR-based functional screening of osteoporosis candidate genes, *J. Bone Miner. Res.* 36 (9) (2021) 1749–1764.
- [91] B.H.M. Carolina Medina-Gomez, Alessandra Chesi, Vid Prijatelj, John P. Kemp, Chen Shochat-Carvalho, Katerina Trajanoska, Carol Wang, Raimo Joro, Tavia E. Evans, Katharina E. Schraut, Ruiyang Li-Gao, Tarunveer S. Ahluwalia, M. Carola Zillikens, Kun Zhu, Dennis O. Mook-Kanamori, Daniel S. Evans, Maria Nethander, Maria J. Knol, Gudmar Thorleifsson, Ivana Prokic, Babette Zemel, Linda Broer, Natasja van Schoor, Sjur Reppe, Mikolaj A. Pawlak, Stuart H. Ralston, Nathalie van der Velde, Mattias Lorentzon, Kari Stefansson, Hieab H.H. Adams, Scott G. Wilson, M.Arfan Ikram, John P. Walsh, Timo A. Lakka, Kaare M. Gautvik, James F. Wilson, Eric S. Orwoll, Cornelia M. van Duijn, Klaus Bonnelykke, Andre G. Uitterlinden, Unnur Stykarsdóttir, Timothy D. Spector, Jonathan H. Tobias, Claes Ohlsson, Janine F. Felix, Hans Bisgaard, Struan F.A. Grant, J.Brent Richards, David M. Evans, Bram van der Eerden, Jeroen van de Peppel, Cheryl Ackert-Bicknell, David Karasik, Erika Kague, Fernando Rivadeneira, Genome Wide Association Metanalysis of Skull Bone Mineral Density Identifies Loci Relevant for Osteoporosis and Craniosynostosis, medRxiv, 2021.
- [92] X. Xiang, et al., Enhancing CRISPR-Cas9 gRNA efficiency prediction by data integration and deep learning, *Nat. Commun.* 12 (1) (2021) 3238.
- [93] F. Alkan, et al., CRISPR-Cas9 off-targeting assessment with nucleic acid duplex energy parameters, *Genome Biol.* 19 (1) (2018) 177.
- [94] J.C. Oliveros, et al., Breaking-cas-interactive design of guide RNAs for CRISPR-cas experiments for ENSEMBL genomes, *Nucleic Acids Res.* 44 (W1) (2016) W267–W271.
- [95] M. Stemmer, et al., CCTop: an intuitive, flexible and reliable CRISPR/Cas9 target prediction tool, *PLoS One* 10 (4) (2015), e0124633.
- [96] M. Labuhn, et al., Refined sgRNA efficacy prediction improves large- and small-scale CRISPR-Cas9 applications, *Nucleic Acids Res.* 46 (3) (2018) 1375–1385.
- [97] A. Cornean, et al., Precise in vivo functional analysis of DNA variants with base editing using ACEofBASEs target prediction, *elife* (2022) 11.
- [98] K. Labun, et al., CHOPCHOP v2: a web tool for the next generation of CRISPR genome engineering, *Nucleic Acids Res.* 44 (W1) (2016) W272–W276.
- [99] K. Labun, et al., CHOPCHOP v3: expanding the CRISPR web toolbox beyond genome editing, *Nucleic Acids Res.* 47 (W1) (2019) W171–W174.
- [100] M. Haeussler, et al., Evaluation of off-target and on-target scoring algorithms and integration into the guide RNA selection tool CRISPOR, *Genome Biol.* 17 (1) (2016) 148.
- [101] Y. Naito, et al., CRISPRdirect: software for designing CRISPR/Cas guide RNA with reduced off-target sites, *Bioinformatics* 31 (7) (2015) 1120–1123.
- [102] L.J. Zhu, et al., CRISPRseek: a bioconductor package to identify target-specific guide RNAs for CRISPR-Cas9 genome-editing systems, *PLoS One* 9 (9) (2014), e108424.
- [103] A. O'Brien, T.L. Bailey, GT-scan: identifying unique genomic targets, *Bioinformatics* 30 (18) (2014) 2673–2675.
- [104] L.O.W. Wilson, et al., High activity target-site identification using phenotypic independent CRISPR-Cas9 Core functionality, *CRISPR J.* 1 (2018) 182–190.
- [105] A.R. O'Brien, et al., Unlocking HDR-mediated nucleotide editing by identifying high-efficiency target sites using machine learning, *Sci. Rep.* 9 (1) (2019) 2788.
- [106] J. Park, S. Bae, J.S. Kim, Cas-designer: a web-based tool for choice of CRISPR-Cas9 target sites, *Bioinformatics* 31 (24) (2015) 4014–4016.
- [107] S. Bae, J. Park, J.S. Kim, Cas-OFFinder: a fast and versatile algorithm that searches for potential off-target sites of Cas9 RNA-guided endonucleases, *Bioinformatics* 30 (10) (2014) 1473–1475.
- [108] S. Bae, et al., Microhomology-based choice of Cas9 nuclease target sites, *Nat. Methods* 11 (7) (2014) 705–706.
- [109] G.H. Hwang, et al., Web-based design and analysis tools for CRISPR base editing, *BMC Bioinformatics* 19 (1) (2018) 542.
- [110] G.H. Hwang, et al., PE-designer and PE-analyzer: web-based design and analysis tools for CRISPR prime editing, *Nucleic Acids Res.* 49 (W1) (2021) W499–W504.
- [111] C.M. Mann, et al., The gene sculpt suite: a set of tools for genome editing, *Nucleic Acids Res.* 47 (W1) (2019) W175–W182.
- [112] K.L. Neff, et al., Mojo hand, a TALEN design tool for genome editing applications, *BMC Bioinformatics* 14 (2013) 1.
- [113] A.C. Ma, et al., TALEN-mediated mutagenesis and genome editing, *Methods Mol. Biol.* 1451 (2016) 17–30.
- [114] H. Liu, et al., CRISPR-ERA: a comprehensive design tool for CRISPR-mediated gene editing, repression and activation, *Bioinformatics* 31 (22) (2015) 3676–3678.
- [115] M. Gapinske, et al., CRISPR-SKIP: programmable gene splicing with single base editors, *Genome Biol.* 19 (1) (2018) 107.
- [116] P. Billon, et al., CRISPR-mediated base editing enables efficient disruption of eukaryotic genes through induction of STOP codons, *Mol. Cell* 67 (6) (2017) 1068–1079 e4.
- [117] J. Vicencio, et al., Genome editing in animals with minimal PAM CRISPR-Cas9 enzymes, *Nat. Commun.* 13 (1) (2022) 2601.
- [118] M.A. Moreno-Mateos, et al., CRISPR-Cpf1 mediates efficient homology-directed repair and temperature-controlled genome editing, *Nat. Commun.* 8 (1) (2017) 2024.
- [119] B.P. Kleinstiver, et al., Engineered CRISPR-Cas9 nucleases with altered PAM specificities, *Nature* 523 (7561) (2015) 481–485.
- [120] B.P. Kleinstiver, et al., Broadening the targeting range of Staphylococcus aureus CRISPR-Cas9 by modifying PAM recognition, *Nat. Biotechnol.* 33 (12) (2015) 1293–1298.
- [121] P.R. Takasugi, et al., Orthogonal CRISPR-cas tools for genome editing, inhibition, and CRISPR recording in zebrafish embryos, *Genetics* 220 (1) (2022).
- [122] W.A. Wierson, et al., Expanding the CRISPR toolbox with ErCas12a in zebrafish and human cells, *CRISPR J.* 2 (6) (2019) 417–433.
- [123] Y. Liu, et al., Genome editing in zebrafish by ScCas9 recognizing NNG PAM, *Cells* 10 (8) (2021).
- [124] D. Ratnayake, et al., Macrophages provide a transient muscle stem cell niche via NAMPT secretion, *Nature* 591 (7849) (2021) 281–287.
- [125] S. Hans, et al., Cre-controlled CRISPR mutagenesis provides fast and easy conditional gene inactivation in zebrafish, *Nat. Commun.* 12 (1) (2021) 1125.

- [126] Z. Yang, et al., Generation of Cas9 transgenic zebrafish and their application in establishing an ERV-deficient animal model, *Biotechnol. Lett.* 40 (11–12) (2018) 1507–1518.
- [127] L. Yin, et al., Multiplex conditional mutagenesis using transgenic expression of Cas9 and sgRNAs, *Genetics* 200 (2) (2015) 431–441.
- [128] Y.C. Wu, L.J. Wang, Heat-shock-induced tyrosinase gene ablation with CRISPR in zebrafish, *Mol. Gen. Genomics* 295 (4) (2020) 911–922.
- [129] Y. Wang, et al., A robust and flexible CRISPR/Cas9-based system for neutrophil-specific gene inactivation in zebrafish, *J. Cell Sci.* 134 (8) (2021).
- [130] B. Raj, et al., Simultaneous single-cell profiling of lineages and cell types in the vertebrate brain, *Nat. Biotechnol.* 36 (5) (2018) 442–450.
- [131] V. Di Donato, et al., 2C-Cas9: a versatile tool for clonal analysis of gene function, *Genome Res.* 26 (5) (2016) 681–692.
- [132] B. Carrington, R.N. Weinstein, R. Sood, BE4max and AncBE4max are efficient in germline conversion of C: G to T: a base pairs in zebrafish, *Cells* 9 (7) (2020).
- [133] W. Qin, et al., Precise A*T to G*C base editing in the zebrafish genome, *BMC Biol.* 16 (1) (2018) 139.
- [134] Y. Zhang, et al., Programmable base editing of zebrafish genome using a modified CRISPR-Cas9 system, *Nat. Commun.* 8 (1) (2017) 118.
- [135] Y. Zhao, et al., An optimized base editor with efficient C-to-T base editing in zebrafish, *BMC Biol.* 18 (1) (2020) 190.
- [136] M. Rosello, et al., Precise base editing for the in vivo study of developmental signaling and human pathologies in zebrafish, *elife* (2021) 10.
- [137] M. Rosello, et al., Disease modeling by efficient genome editing using a near PAM-less base editor in vivo, *Nat. Commun.* 13 (1) (2022) 3435.
- [138] L. Long, et al., Regulation of transcriptionally active genes via the catalytically inactive Cas9 in *C. elegans* and *D. rerio*, *Cell Res.* 25 (5) (2015) 638–641.
- [139] X. Dong, et al., Zebrafish Znf1 proteins control the expression of *hox1b* gene in the posterior neuroectoderm by acting upstream of *pou5f3* and *sall4* genes, *J. Biol. Chem.* 292 (31) (2017) 13045–13055.
- [140] A. McKenna, et al., Whole-organism lineage tracing by combinatorial and cumulative genome editing, *Science* 353 (6298) (2016), aaf7907.
- [141] B. Spanjaard, et al., Simultaneous lineage tracing and cell-type identification using CRISPR-Cas9-induced genetic scars, *Nat. Biotechnol.* 36 (5) (2018) 469–473.
- [142] A. Alemany, et al., Whole-organism clone tracing using single-cell sequencing, *Nature* 556 (7699) (2018) 108–112.
- [143] R.M. White, et al., Transparent adult zebrafish as a tool for in vivo transplantation analysis, *Cell Stem Cell* 2 (2) (2008) 183–189.
- [144] P. Antinucci, R. Hindges, A crystal-clear zebrafish for in vivo imaging, *Sci. Rep.* 6 (2016) 29490.
- [145] K. Ozato, et al., Production of transgenic fish: introduction and expression of chicken delta-crystallin gene in medaka embryos, *Cell Differ.* 19 (4) (1986) 237–244.
- [146] G.W. Stuart, J.V. McMurray, M. Westerfield, Replication, integration and stable germ-line transmission of foreign sequences injected into early zebrafish embryos, *Development* 103 (2) (1988) 403–412.
- [147] K. Inoue, et al., Electroporation as a new technique for producing transgenic fish, *Cell Differ. Dev.* 29 (2) (1990) 123–128.
- [148] S. Lin, et al., Integration and germ-line transmission of a pseudotyped retroviral vector in zebrafish, *Science* 265 (5172) (1994) 666–669.
- [149] M. Yamauchi, et al., Introduction of a foreign gene into medakafish using the particle gun method, *J. Exp. Zool.* 287 (4) (2000) 285–293.
- [150] G.W. Stuart, et al., Stable lines of transgenic zebrafish exhibit reproducible patterns of transgene expression, *Development* 109 (3) (1990) 577–584.
- [151] P. Culp, C. Nusslein-Volhard, N. Hopkins, High-frequency germ-line transmission of plasmid DNA sequences injected into fertilized zebrafish eggs, *Proc. Natl. Acad. Sci. U. S. A.* 88 (18) (1991) 7953–7957.
- [152] C. Grabher, J.S. Joly, J. Wittbrodt, Highly efficient zebrafish transgenesis mediated by the meganuclease I-SceI, *Methods Cell Biol.* 77 (2004) 381–401.
- [153] K. Kawakami, A. Shima, N. Kawakami, Identification of a functional transposase of the Tol2 element, an ac-like element from the Japanese medaka fish, and its transposition in the zebrafish germ lineage, *Proc. Natl. Acad. Sci. U. S. A.* 97 (21) (2000) 11403–11408.
- [154] K.M. Kwan, et al., The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs, *Dev. Dyn.* 236 (11) (2007) 3088–3099.
- [155] T. Weber, R. Koster, Genetic tools for multicolor imaging in zebrafish larvae, *Methods* 62 (3) (2013) 279–291.
- [156] Z. Yang, et al., Modified bacterial artificial chromosomes for zebrafish transgenesis, *Methods* 39 (3) (2006) 183–188.
- [157] M.L. Suster, K. Sumiyama, K. Kawakami, Transposon-mediated BAC transgenesis in zebrafish and mice, *BMC Genomics* 10 (2009) 477.
- [158] M. Kasahara, et al., The medaka draft genome and insights into vertebrate genome evolution, *Nature* 447 (7145) (2007) 714–719.
- [159] J. Renn, C. Winkler, Osterix-mCherry transgenic medaka for in vivo imaging of bone formation, *Dev. Dyn.* 238 (1) (2009) 241–248.
- [160] K.M. Sporendonk, et al., Retinoic acid and *Cyp26b1* are critical regulators of osteogenesis in the axial skeleton, *Development* 135 (22) (2008) 3765–3774.
- [161] F. Knopf, et al., Bone regenerates via dedifferentiation of osteoblasts in the zebrafish fin, *Dev. Cell* 20 (5) (2011) 713–724.
- [162] B.D. Cox, et al., In toto imaging of dynamic osteoblast behaviors in regenerating skeletal bone, *Curr Biol* 28 (24) (2018) 3937–3947, e4.
- [163] S.P. Singh, J.E. Holdway, K.D. Poss, Regeneration of amputated zebrafish fin rays from de novo osteoblasts, *Dev. Cell* 22 (4) (2012) 879–886.
- [164] J.O. Bishop, Chromosomal insertion of foreign DNA, *Reprod. Nutr. Dev.* 36 (6) (1996) 607–618.
- [165] K. Kawakami, Tol2: a versatile gene transfer vector in vertebrates, *Genome Biol.* 8 (Suppl 1) (2007) S7.
- [166] C. Mosimann, et al., Site-directed zebrafish transgenesis into single landing sites with the phiC31 integrase system, *Dev. Dyn.* 242 (8) (2013) 949–963.
- [167] T.O. Auer, F. Del Bene, CRISPR/Cas9 and TALEN-mediated knock-in approaches in zebrafish, *Methods* 69 (2) (2014) 142–150.
- [168] A. Boel, et al., CRISPR/Cas9-mediated homology-directed repair by ssODNs in zebrafish induces complex mutational patterns resulting from genomic integration of repair-template fragments, *Dis. Model. Mech.* 11 (10) (2018).
- [169] Y. Zu, et al., TALEN-mediated precise genome modification by homologous recombination in zebrafish, *Nat. Methods* 10 (4) (2013) 329–331.
- [170] W.A. Wiersom, et al., Efficient targeted integration directed by short homology in zebrafish and mammalian cells, *elife* (2020) 9.
- [171] J. Shin, J. Chen, L. Solnica-Krezel, Efficient homologous recombination-mediated genome engineering in zebrafish using TALE nucleases, *Development* 141 (19) (2014) 3807–3818.
- [172] K. Hoshijima, M.J. Jurynec, D.J. Grunwald, Precise editing of the zebrafish genome made simple and efficient, *Dev. Cell* 36 (6) (2016) 654–667.
- [173] Y. Hisano, et al., Precise in-frame integration of exogenous DNA mediated by CRISPR/Cas9 system in zebrafish, *Sci. Rep.* 5 (2015) 8841.
- [174] G. Kesavan, et al., Targeted knock-in of CreEr (T2) in zebrafish using CRISPR/Cas9, *Cell Tissue Res.* 372 (1) (2018) 41–50.
- [175] J.J. Luo, et al., CRISPR/Cas9-based genome engineering of zebrafish using a seamless integration strategy, *FASEB J.* 32 (9) (2018) 5132–5142.
- [176] S.E. DiNapoli, et al., Synthetic CRISPR/Cas9 reagents facilitate genome editing and homology directed repair, *Nucleic Acids Res.* 48 (7) (2020), e38.
- [177] D.S. Levic, et al., Knock-in tagging in zebrafish facilitated by insertion into non-coding regions, *Development* 148 (19) (2021).
- [178] Y. Zhang, Z. Zhang, W. Ge, An efficient platform for generating somatic point mutations with germline transmission in the zebrafish by CRISPR/Cas9-mediated gene editing, *J. Biol. Chem.* 293 (17) (2018) 6611–6622.
- [179] Y.A. Aksoy, et al., Chemical reprogramming enhances homology-directed genome editing in zebrafish embryos, *Commun Biol* 2 (2019) 198.
- [180] E. de Vrieze, et al., Efficient generation of knock-in zebrafish models for inherited disorders using CRISPR-Cas9 ribonucleoprotein complexes, *Int. J. Mol. Sci.* 22 (17) (2021).
- [181] T.O. Auer, et al., Highly efficient CRISPR/Cas9-mediated knock-in in zebrafish by homology-independent DNA repair, *Genome Res.* 24 (1) (2014) 142–153.
- [182] Y. Kimura, et al., Efficient generation of knock-in transgenic zebrafish carrying reporter/driver genes by CRISPR/Cas9-mediated genome engineering, *Sci. Rep.* 4 (2014) 6545.
- [183] J.T. Rosa, et al., Screening of mineralogenic and osteogenic compounds in zebrafish-tools to improve assay throughput and data accuracy, *Pharmaceuticals (Basel)* 15 (8) (2022).
- [184] A. Fleming, M. Sato, P. Goldsmith, High-throughput in vivo screening for bone anabolic compounds with zebrafish, *J. Biomol. Screen.* 10 (8) (2005) 823–831.
- [185] G. Takemoto, et al., Meclozine attenuates the MARK pathway in mammalian chondrocytes and ameliorates FGF2-induced bone hyperossification in larval zebrafish, *Front. Cell Dev. Biol.* 9 (2021), 694018.
- [186] O.M. Subach, et al., Conversion of red fluorescent protein into a bright blue probe, *Chem Biol* 15 (10) (2008) 1116–1124.
- [187] M.A. Rizzo, D.W. Piston, High-contrast imaging of fluorescent protein FRET by fluorescence polarization microscopy, *Biophys. J.* 88 (2) (2005) L14–L16.
- [188] R. Heim, D.C. Prasher, R.Y. Tsien, Wavelength mutations and posttranslational autoxidation of green fluorescent protein, *Proc. Natl. Acad. Sci. U. S. A.* 91 (26) (1994) 12501–12504.
- [189] H.W. Ai, et al., Directed evolution of a monomeric, bright and photostable version of clavularia cyan fluorescent protein: structural characterization and applications in fluorescence imaging, *Biochem. J.* 400 (3) (2006) 531–540.
- [190] B.P. Cormack, R.H. Valdivia, S. Falkow, FACS-optimized mutants of the green fluorescent protein (GFP), *Gene* 173 (1 Spec No) (1996) 33–38.
- [191] N.C. Shaner, et al., A bright monomeric green fluorescent protein derived from *Branchiostoma lanceolatum*, *Nat. Methods* 10 (5) (2013) 407–409.
- [192] G.J. Kremers, et al., Cyan and yellow super fluorescent proteins with improved brightness, protein folding, and FRET forster radius, *Biochemistry* 45 (21) (2006) 6570–6580.
- [193] R.L. Strack, et al., Noncytotoxic orange and red/green derivatives of DsRed-Express2 for whole-cell labeling, *BMC Biotechnol.* 9 (2009) 32.
- [194] N.C. Shaner, et al., Improved monomeric red, orange and yellow fluorescent proteins derived from *discosoma* sp. Red fluorescent protein, *Nat. Biotechnol.* 22 (12) (2004) 1567–1572.
- [195] M.V. Matz, et al., Fluorescent proteins from nonbioluminescent anthozoa species, *Nat. Biotechnol.* 17 (10) (1999) 969–973.
- [196] S. Kredel, et al., Optimized and far-red-emitting variants of fluorescent protein eqFP611, *Chem Biol* 15 (3) (2008) 224–233.
- [197] D. Shcherbo, et al., Far-red fluorescent tags for protein imaging in living tissues, *Biochem. J.* 418 (3) (2009) 567–574.
- [198] T.J. Lambert, FPbase: a community-editable fluorescent protein database, *Nat. Methods* 16 (4) (2019) 277–278.
- [199] M.N. Teruel, et al., A versatile microportation technique for the transfection of cultured CNS neurons, *J. Neurosci. Methods* 93 (1) (1999) 37–48.
- [200] R.W. Koster, S.E. Fraser, Tracing transgene expression in living zebrafish embryos, *Dev. Biol.* 233 (2) (2001) 329–346.
- [201] D.A. Zacharias, et al., Partitioning of lipid-modified monomeric GFPs into membrane microdomains of live cells, *Science* 296 (5569) (2002) 913–916.

- [202] K.M. Kwan, et al., A complex choreography of cell movements shapes the vertebrate eye, *Development* 139 (2) (2012) 359–372.
- [203] T. Kanda, K.F. Sullivan, G.M. Wahl, Histone-GFP fusion protein enables sensitive analysis of chromosome dynamics in living mammalian cells, *Curr. Biol.* 8 (7) (1998) 377–385.
- [204] M.J. Parsons, et al., Notch-responsive cells initiate the secondary transition in larval zebrafish pancreas, *Mech. Dev.* 126 (10) (2009) 898–912.
- [205] P. Collas, H. Husebye, P. Alestrom, The nuclear localization sequence of the SV40 T antigen promotes transgene uptake and expression in zebrafish embryo nuclei, *Transgenic Res.* 5 (6) (1996) 451–458.
- [206] P. Hu, et al., Comparison of various nuclear localization signal-fused Cas9 proteins and Cas9 mRNA for genome editing in zebrafish, *G3 (Bethesda)* 8 (3) (2018) 823–831.
- [207] J. Riedl, et al., Lifeact: a versatile marker to visualize F-actin, *Nat. Methods* 5 (7) (2008) 605–607.
- [208] M. Wuhr, et al., A model for cleavage plane determination in early amphibian and fish embryos, *Curr. Biol.* 20 (22) (2010) 2040–2045.
- [209] N. Scheer, J.A. Campos-Ortega, Use of the Gal4-UAS technique for targeted gene expression in the zebrafish, *Mech. Dev.* 80 (2) (1999) 153–158.
- [210] A. Ghosh, M.E. Halpern, Transcriptional regulation using the Q system in transgenic zebrafish, *Methods Cell Biol.* 135 (2016) 205–218.
- [211] R. Thummel, et al., Cre-mediated site-specific recombination in zebrafish embryos, *Dev. Dyn.* 233 (4) (2005) 1366–1377.
- [212] J. Livet, et al., Transgenic strategies for combinatorial expression of fluorescent proteins in the nervous system, *Nature* 450 (7166) (2007) 56–62.
- [213] A.C. Wong, B.W. Draper, A.L. Van Eenennaam, FLPe functions in zebrafish embryos, *Transgenic Res.* 20 (2) (2011) 409–415.
- [214] J.T. Park, S.D. Leach, TAILOR: transgene activation and inactivation using lox and rox in zebrafish, *PLoS One* 8 (12) (2013), e85218.
- [215] R. Feil, et al., Regulation of cre recombinase activity by mutated estrogen receptor ligand-binding domains, *Biochem. Biophys. Res. Commun.* 237 (3) (1997) 752–757.
- [216] A. Emylanov, S. Parinov, Mifepristone-inducible LexPR system to drive and control gene expression in transgenic zebrafish, *Dev. Biol.* 320 (1) (2008) 113–121.
- [217] F. Knopf, et al., Dually inducible TetON systems for tissue-specific conditional gene expression in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 107 (46) (2010) 19933–19938.
- [218] M.C. Halloran, et al., Laser-induced gene expression in specific cells of transgenic zebrafish, *Development* 127 (9) (2000) 1953–1960.
- [219] K. Mruk, et al., Targeted cell ablation in zebrafish using optogenetic transcriptional control, *Development* 147 (12) (2020).
- [220] J. Hong, et al., IQ-switch is a QF-based innocuous, silencing-free, and inducible gene switch system in zebrafish, *Commun. Biol.* 4 (1) (2021) 1405.
- [221] S. Curado, et al., Conditional targeted cell ablation in zebrafish: a new tool for regeneration studies, *Dev. Dyn.* 236 (4) (2007) 1025–1035.
- [222] K.M. Tabor, et al., Direct activation of the mauthner cell by electric field pulses drives ultrarapid escape responses, *J. Neurophysiol.* 112 (4) (2014) 834–844.
- [223] J.R. Mathias, et al., Enhanced cell-specific ablation in zebrafish using a triple mutant of *Escherichia coli* nitroreductase, *Zebrafish* 11 (2) (2014) 85–97.
- [224] C. Teh, et al., Optogenetic in vivo cell manipulation in KillerRed-expressing zebrafish transgenics, *BMC Dev. Biol.* 10 (2010) 110.
- [225] Z. Labbaf, et al., A robust and tunable system for targeted cell ablation in developing embryos, *Dev. Cell* 57 (16) (2022) 2026–2040 e5.
- [226] R.I. Dorsky, L.C. Sheldahl, R.T. Moon, A transgenic *Lef1*/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development, *Dev. Biol.* 241 (2) (2002) 229–237.
- [227] N. Shimizu, K. Kawakami, T. Ishitani, Visualization and exploration of *Tcf/Lef* function using a highly responsive *Wnt*/beta-catenin signaling-reporter transgenic zebrafish, *Dev. Biol.* 370 (1) (2012) 71–85.
- [228] E. Moro, et al., In vivo *wnt* signaling tracing through a transgenic biosensor fish reveals novel activity domains, *Dev. Biol.* 366 (2) (2012) 327–340.
- [229] D.W. Laux, J.A. Febbo, B.L. Roman, Dynamic analysis of BMP-responsive *smad* activity in live zebrafish embryos, *Dev. Dyn.* 240 (3) (2011) 682–694.
- [230] O. Korchynskiy, P. ten Dijke, Identification and functional characterization of distinct critically important bone morphogenetic protein-specific response elements in the *Id1* promoter, *J. Biol. Chem.* 277 (7) (2002) 4883–4891.
- [231] M.C. Ramel, C.S. Hill, The ventral to dorsal BMP activity gradient in the early zebrafish embryo is determined by graded expression of BMP ligands, *Dev. Biol.* 378 (2) (2013) 170–182.
- [232] R.F. Coltery, B.A. Link, Dynamic *smad*-mediated BMP signaling revealed through transgenic zebrafish, *Dev. Dyn.* 240 (3) (2011) 712–722.
- [233] U.J. Pyati, A.E. Webb, D. Kimelman, Transgenic zebrafish reveal stage-specific roles for *bmp* signaling in ventral and posterior mesoderm development, *Development* 132 (10) (2005) 2333–2343.
- [234] G.A. Molina, S.C. Watkins, M. Tsang, Generation of FGF reporter transgenic zebrafish and their utility in chemical screens, *BMC Dev. Biol.* 7 (2007) 62.
- [235] Y. Lee, et al., *Fgf* signaling instructs position-dependent growth rate during zebrafish fin regeneration, *Development* 132 (23) (2005) 5173–5183.
- [236] C.T. Jacobs, P. Huang, Notch signalling maintains hedgehog responsiveness via a gli-dependent mechanism during spinal cord patterning in zebrafish, *elife* (2019) 8.
- [237] P. Huang, et al., Attenuation of notch and hedgehog signaling is required for fate specification in the spinal cord, *PLoS Genet.* 8 (6) (2012), e1002762.
- [238] A. Shkumatava, et al., Sonic hedgehog, secreted by amacrine cells, acts as a short-range signal to direct differentiation and lamination in the zebrafish retina, *Development* 131 (16) (2004) 3849–3858.
- [239] A. De Simone, et al., Control of osteoblast regeneration by a train of erk activity waves, *Nature* 590 (7844) (2021) 129–133.
- [240] A. Casari, et al., A *Smad3* transgenic reporter reveals TGF-beta control of zebrafish spinal cord development, *Dev. Biol.* 396 (1) (2014) 81–93.
- [241] E.A. Naumann, et al., Monitoring neural activity with bioluminescence during natural behavior, *Nat. Neurosci.* 13 (4) (2010) 513–520.
- [242] S. Knafo, et al., Bioluminescence monitoring of neuronal activity in freely moving zebrafish larvae, *Bio Protoc* 7 (18) (2017), e2550.
- [243] A. Muto, et al., Genetic visualization with an improved GCaMP calcium indicator reveals spatiotemporal activation of the spinal motor neurons in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 108 (13) (2011) 5425–5430.
- [244] B.F. Fosque, et al., Neural circuits. Labeling of active neural circuits in vivo with designed calcium integrators, *Science* 347 (6223) (2015) 755–760.
- [245] T.J. van Ham, et al., Live imaging of apoptotic cells in zebrafish, *FASEB J.* 24 (11) (2010) 4336–4342.
- [246] X. Lu, et al., Photocleavable proteins that undergo fast and efficient dissociation, *Chem. Sci.* 12 (28) (2021) 9658–9672.
- [247] J.W. Linsley, et al., Genetically encoded cell-death indicators (GEDD) to detect an early irreversible commitment to neurodegeneration, *Nat. Commun.* 12 (1) (2021) 5284.
- [248] T. Kaizuka, et al., An autophagic flux probe that releases an internal control, *Mol. Cell* 64 (4) (2016) 835–849.
- [249] M. Sugiyama, et al., Illuminating cell-cycle progression in the developing zebrafish embryo, *Proc. Natl. Acad. Sci. U. S. A.* 106 (49) (2009) 20812–20817.
- [250] N.G. Gurskaya, et al., Engineering of a monomeric green-to-red photoactivatable fluorescent protein induced by blue light, *Nat. Biotechnol.* 24 (4) (2006) 461–465.
- [251] R. Ando, et al., An optical marker based on the UV-induced green-to-red photoconversion of a fluorescent protein, *Proc. Natl. Acad. Sci. U. S. A.* 99 (20) (2002) 12651–12656.
- [252] S.A. McKinney, et al., A bright and photostable photoconvertible fluorescent protein, *Nat. Methods* 6 (2) (2009) 131–133.
- [253] H. Tsutsui, et al., Semi-rational engineering of a coral fluorescent protein into an efficient highlighter, *EMBO Rep.* 6 (3) (2005) 233–238.
- [254] Y.A. Pan, et al., Zebrow: multispectral cell labeling for cell tracing and lineage analysis in zebrafish, *Development* 140 (13) (2013) 2835–2846.
- [255] V. Gupta, K.D. Poss, Clonally dominant cardiomyocytes direct heart morphogenesis, *Nature* 484 (7395) (2012) 479–484.
- [256] K.Y. Chan, et al., Skin cells undergo asymmetric fission to expand body surfaces in zebrafish, *Nature* 605 (7908) (2022) 119–125.
- [257] S. Caviglia, et al., FRaepli: a multispectral imaging toolbox for cell tracing and dense tissue analysis in zebrafish, *Development* 149 (16) (2022).
- [258] J. Ablain, et al., A CRISPR/Cas9 vector system for tissue-specific gene disruption in zebrafish, *Dev. Cell* 32 (6) (2015) 756–764.
- [259] A. DeLaurier, et al., Zebrafish *sp7:EGFP*: a transgenic for studying otic vesicle formation, skeletogenesis, and bone regeneration, *Genesis* 48 (8) (2010) 505–511.
- [260] S. Wopat, et al., Spine patterning is guided by segmentation of the notochord sheath, *Cell Rep.* 22 (8) (2018) 2026–2038.
- [261] J. Vanoevelen, et al., *Trpv5/6* is vital for epithelial calcium uptake and bone formation, *FASEB J.* 25 (9) (2011) 3197–3207.
- [262] L.F. Huitema, et al., *Entpd5* is essential for skeletal mineralization and regulates phosphate homeostasis in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 109 (52) (2012) 21372–21377.
- [263] J. Bussmann, S. Schulte-Merker, Rapid BAC selection for *tol2*-mediated transgenesis in zebrafish, *Development* 138 (19) (2011) 4327–4332.
- [264] K. Geurtzen, et al., Mature osteoblasts dedifferentiate in response to traumatic bone injury in the zebrafish fin and skull, *Development* 141 (11) (2014) 2225–2234.
- [265] M. Uemura, A. Nagasawa, K. Terai, Yap/Taz transcriptional activity in endothelial cells promotes intramembranous ossification via the BMP pathway, *Sci. Rep.* 6 (2016) 27473.
- [266] J. Kobayashi-Sun, et al., Uptake of osteoblast-derived extracellular vesicles promotes the differentiation of osteoclasts in the zebrafish scale, *Commun Biol* 3 (1) (2020) 190.
- [267] R.E. Mitchell, et al., New tools for studying osteoarthritis genetics in zebrafish, *Osteoarthr. Cartil.* 21 (2) (2013) 269–278.
- [268] Y.I. Kim, et al., Establishment of a bone-specific *col10a1:GFP* transgenic zebrafish, *Mol Cells* 36 (2) (2013) 145–150.
- [269] E. de Vrieze, et al., Identification of novel osteogenic compounds by an ex-vivo *sp7:luciferase* zebrafish scale assay, *Bone* 74 (2015) 106–113.
- [270] K. Inohaya, Y. Takano, A. Kudo, The teleost intervertebral region acts as a growth center of the centrum: in vivo visualization of osteoblasts and their progenitors in transgenic fish, *Dev. Dyn.* 236 (11) (2007) 3031–3046.
- [271] R.M. Dale, J. Topczewski, Identification of an evolutionarily conserved regulatory element of the zebrafish *col2a1a* gene, *Dev. Biol.* 357 (2) (2011) 518–531.
- [272] D. Giovannone, et al., Programmed conversion of hypertrophic chondrocytes into osteoblasts and marrow adipocytes within zebrafish bones, *elife* (2019) 8.
- [273] J. Garcia, et al., Sheath cell invasion and trans-differentiation repair mechanical damage caused by loss of caveolae in the zebrafish notochord, *Curr. Biol.* 27 (13) (2017) 1982–1989 e3.
- [274] J.W. Chen, et al., The mevalonate pathway is a crucial regulator of tendon cell specification, *Development* 147 (12) (2020).

- [275] X. Niu, et al., Tendon cell regeneration is mediated by attachment site-resident progenitors and BMP signaling, *Curr. Biol.* 30 (17) (2020) 3277–3292, e5.
- [276] H. Sanchez-Iranzo, et al., Transient fibrosis resolves via fibroblast inactivation in the regenerating zebrafish heart, *Proc. Natl. Acad. Sci. U. S. A.* 115 (16) (2018) 4188–4193.
- [277] R.C. Ma, et al., Stereotypic generation of axial tenocytes from bipartite sclerotome domains in zebrafish, *PLoS Genet.* 14 (11) (2018), e1007775.
- [278] A. Askary, et al., Iroquois proteins promote skeletal joint formation by maintaining chondrocytes in an immature state, *Dev. Cell* 35 (3) (2015) 358–365.
- [279] J.C. Talbot, S.L. Johnson, C.B. Kimmel, *hand2* and *dlx* genes specify dorsal, intermediate and ventral domains within zebrafish pharyngeal arches, *Development* 137 (15) (2010) 2507–2517.
- [280] J. Caetano-Lopes, et al., Unique and non-redundant function of *csfl1* paralogs in regulation and evolution of post-embryonic development of the zebrafish, *Development* 147 (2) (2020).
- [281] M. Chatani, Y. Takano, A. Kudo, Osteoclasts in bone modeling, as revealed by in vivo imaging, are essential for organogenesis in fish, *Dev. Biol.* 360 (1) (2011) 96–109.
- [282] L.J. Rochard, et al., Visualization of chondrocyte intercalation and directional proliferation via zebrafish clonal cell analysis in the embryonic Meckel's cartilage, *J. Vis. Exp.* 105 (2015), e52935.
- [283] E. Curtin, et al., Zebrafish *wnt9a* is expressed in pharyngeal ectoderm and is required for palate and lower jaw development, *Mech. Dev.* 128 (1–2) (2011) 104–115.
- [284] A. Das, J.G. Crump, *Bmps* and *id2a* act upstream of *Twist1* to restrict ectomesenchyme potential of the cranial neural crest, *PLoS Genet.* 8 (5) (2012), e1002710.
- [285] N. Mercader, Early steps of paired fin development in zebrafish compared with tetrapod limb development, *Develop. Growth Differ.* 49 (6) (2007) 421–437.
- [286] M. Mari-Beffa, C. Murciano, Dermoskeleton morphogenesis in zebrafish fins, *Dev. Dyn.* 239 (11) (2010) 2779–2794.
- [287] T. Yano, K. Tamura, The making of differences between fins and limbs, *J. Anat.* 222 (1) (2013) 100–113.
- [288] A. Fleming, et al., Building the backbone: the development and evolution of vertebral patterning, *Development* 142 (10) (2015) 1733–1744.
- [289] M.P. Harris, G. Arratia, Patterning the spine, *Elife* (2018) 7.
- [290] H. Xie, et al., Zebrafish: an important model for understanding scoliosis, *Cell. Mol. Life Sci.* 79 (9) (2022) 506.
- [291] Q.T. Phan, et al., *Cxcl9l* and *Cxcr3.2* regulate recruitment of osteoclast progenitors to bone matrix in a medaka osteoporosis model, *Proc. Natl. Acad. Sci. U. S. A.* 117 (32) (2020) 19276–19286.
- [292] K. Chowdhury, L.S.a.L.S.-L., Comparative study in zebrafish and medaka unravels the mechanisms of tissue regeneration, *Frontiers in Ecology and Evolution* 10 (2022) 783818.
- [293] T. Yu, et al., Live imaging of osteoclast inhibition by bisphosphonates in a medaka osteoporosis model, *Dis. Model. Mech.* 9 (2) (2016) 155–163.
- [294] L. Barske, et al., Competition between jagged-notch and Endothelin1 signaling selectively restricts cartilage formation in the zebrafish upper face, *PLoS Genet.* 12 (4) (2016), e1005967.
- [295] Y.L. Yan, et al., Expression of a type II collagen gene in the zebrafish embryonic axis, *Dev. Dyn.* 203 (3) (1995) 363–376.
- [296] N.C. Bird, P.M. Mabee, Developmental morphology of the axial skeleton of the zebrafish, *Danio rerio* (Ostariophysi: Cyprinidae), *Dev. Dyn.* 228 (3) (2003) 337–357.
- [297] L. Lleras Forero, et al., Segmentation of the zebrafish axial skeleton relies on notochord sheath cells and not on the segmentation clock, *Elife* (2018) 7.
- [298] C.B. Kimmel, et al., The shaping of pharyngeal cartilages during early development of the zebrafish, *Dev. Biol.* 203 (2) (1998) 245–263.
- [299] K. Schlombs, T. Wagner, J. Scheel, Site-1 protease is required for cartilage development in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 100 (24) (2003) 14024–14029.
- [300] P. Xu, et al., Fox proteins are modular competency factors for facial cartilage and tooth specification, *Development* 145 (12) (2018).
- [301] S.M. Sperber, I.B. Dawid, *barx1* is necessary for ectomesenchyme proliferation and osteochondrogenitor condensation in the zebrafish pharyngeal arches, *Dev. Biol.* 321 (1) (2008) 101–110.
- [302] S. Paudel, et al., Patterning of cartilaginous condensations in the developing facial skeleton, *Dev. Biol.* 486 (2022) 44–55.
- [303] E. Wright, et al., The *sry*-related gene *Sox9* is expressed during chondrogenesis in mouse embryos, *Nat. Genet.* 9 (1) (1995) 15–20.
- [304] J.P. Tissier-Seta, et al., *Barx1*, a new mouse homeodomain transcription factor expressed in cranio-facial ectomesenchyme and the stomach, *Mech. Dev.* 51 (1) (1995) 3–15.
- [305] J.H. Postlethwait, et al., Vertebrate genome evolution and the zebrafish gene map, *Nat. Genet.* 18 (4) (1998) 345–349.
- [306] A. Meyer, M. Schartl, Gene and genome duplications in vertebrates: the one-to-four (-to-eight in fish) rule and the evolution of novel gene functions, *Curr. Opin. Cell Biol.* 11 (6) (1999) 699–704.
- [307] J.H. Nadeau, D. Sankoff, Comparable rates of gene loss and functional divergence after genome duplications early in vertebrate evolution, *Genetics* 147 (3) (1997) 1259–1266.
- [308] J. Postlethwait, et al., Subfunction partitioning, the teleost radiation and the annotation of the human genome, *Trends Genet.* 20 (10) (2004) 481–490.
- [309] W. Bi, et al., Haploinsufficiency of *Sox9* results in defective cartilage primordia and premature skeletal mineralization, *Proc. Natl. Acad. Sci. U. S. A.* 98 (12) (2001) 6698–6703.
- [310] W. Bi, et al., *Sox9* is required for cartilage formation, *Nat. Genet.* 22 (1) (1999) 85–89.
- [311] H. Akiyama, et al., The transcription factor *Sox9* has essential roles in successive steps of the chondrocyte differentiation pathway and is required for expression of *Sox5* and *Sox6*, *Genes Dev.* 16 (21) (2002) 2813–2828.
- [312] S. Burgess, N. Hopkins, Use of pseudotyped retroviruses in zebrafish as genetic tags, *Methods Enzymol.* 327 (2000) 145–161.
- [313] Y.L. Yan, et al., A zebrafish *sox9* gene required for cartilage morphogenesis, *Development* 129 (21) (2002) 5065–5079.
- [314] Y.L. Yan, et al., A pair of *sox*: distinct and overlapping functions of zebrafish *sox9* co-orthologs in craniofacial and pectoral fin development, *Development* 132 (5) (2005) 1069–1083.
- [315] N. Li, et al., Tracking gene expression during zebrafish osteoblast differentiation, *Dev. Dyn.* 238 (2) (2009) 459–466.
- [316] C.B. Kimmel, et al., Modes of developmental outgrowth and shaping of a craniofacial bone in zebrafish, *PLoS One* 5 (3) (2010), e9475.
- [317] C.C. Cabbage, P.M. Mabee, Development of the cranium and paired fins in the zebrafish *Danio rerio* (Ostariophysi, Cyprinidae), *J. Morphol.* 229 (2) (1996) 121–160.
- [318] J. Weigle, T.A. Franz-Odenaal, Functional bone histology of zebrafish reveals two types of endochondral ossification, different types of osteoblast clusters and a new bone type, *J. Anat.* 229 (1) (2016) 92–103.
- [319] P.E. Witten, A. Huyssseune, A comparative view on mechanisms and functions of skeletal remodelling in teleost fish, with special emphasis on osteoclasts and their function, *Biol. Rev. Camb. Philos. Soc.* 84 (2) (2009) 315–346.
- [320] M.V. Flores, et al., Duplicate zebrafish *runx2* orthologues are expressed in developing skeletal elements, *Gene Expr. Patterns* 4 (5) (2004) 573–581.
- [321] K. Nakashima, et al., The novel zinc finger-containing transcription factor *osterix* is required for osteoblast differentiation and bone formation, *Cell* 108 (1) (2002) 17–29.
- [322] E. Wienholds, et al., Efficient target-selected mutagenesis in zebrafish, *Genome Res.* 13 (12) (2003) 2700–2707.
- [323] E. Kague, et al., *Osterix/Sp7* limits cranial bone initiation sites and is required for formation of sutures, *Dev. Biol.* 413 (2) (2016) 160–172.
- [324] M.M. Durdan, R.D. Azaria, M.M. Weivoda, Novel insights into the coupling of osteoclasts and resorption to bone formation, *Semin. Cell Dev. Biol.* 123 (2022) 4–13.
- [325] P.E. Witten, A. Hansen, B.K. Hall, Features of mono- and multinucleated bone resorbing cells of the zebrafish *Danio rerio* and their contribution to skeletal development, remodeling, and growth, *J. Morphol.* 250 (3) (2001) 197–207.
- [326] W. Liu, et al., The synergistic role of *pu.1* and *fms* in zebrafish osteoclast-reducing osteopetrosis and possible therapeutic strategies, *J. Genet. Genomics* 47 (9) (2020) 535–546.
- [327] X.M. Dai, et al., Targeted disruption of the mouse colony-stimulating factor 1 receptor gene results in osteopetrosis, mononuclear phagocyte deficiency, increased primitive progenitor cell frequencies, and reproductive defects, *Blood* 99 (1) (2002) 111–120.
- [328] D.V. Coleman, Efficient use of cervical screening, *Lancet* 2 (8557) (1987) 510–511.
- [329] A.V. Gore, et al., The zebrafish: a fantastic model for hematopoietic development and disease, *Wiley Interdiscip. Rev. Dev. Biol.* 7 (3) (2018), e312.
- [330] J. Xia, et al., A single-cell resolution developmental atlas of hematopoietic stem and progenitor cell expansion in zebrafish, *Proc. Natl. Acad. Sci. U. S. A.* 118 (14) (2021).
- [331] S.J. Wattour, L.I. Zon, Stem cell safe harbor: the hematopoietic stem cell niche in zebrafish, *Blood Adv.* 2 (21) (2018) 3063–3069.
- [332] C.S. Teng, et al., Altered bone growth dynamics prefigure craniosynostosis in a zebrafish model of saethre-chotzen syndrome, *Elife* (2018) 7.
- [333] K. Laue, et al., Craniosynostosis and multiple skeletal anomalies in humans and zebrafish result from a defect in the localized degradation of retinoic acid, *Am. J. Hum. Genet.* 89 (5) (2011) 595–606.
- [334] D.T. Farmer, et al., A comprehensive series of *irx* cluster mutants reveals diverse roles in facial cartilage development, *Development* 148 (16) (2021).
- [335] C.T. Miller, et al., Two endothelin 1 effectors, *hand2* and *bapx1*, pattern ventral pharyngeal cartilage and the jaw joint, *Development* 130 (7) (2003) 1353–1365.
- [336] J.T. Nichols, et al., *barx1* represses joints and promotes cartilage in the craniofacial skeleton, *Development* 140 (13) (2013) 2765–2775.
- [337] A. Askary, et al., Ancient origin of lubricated joints in bony vertebrates, *Elife* (2016) 5.
- [338] L.W. Jake Leyhr, Beata Filipek-Górniok, Hanqing Zhang, Amin Allalou, Tatjana Haitina, An Evolutionarily Conserved Cis-regulatory Element of *Nkx3.2* Contributes to Early Jaw Joint Morphology in Zebrafish, *bioRxiv*, 2021.
- [339] R. Schweitzer, et al., Analysis of the tendon cell fate using scleraxis, a specific marker for tendons and ligaments, *Development* 128 (19) (2001) 3855–3866.
- [340] B.A. Pryce, et al., Generation of transgenic tendon reporters, *ScxGFP* and *ScxAAP*, using regulatory elements of the scleraxis gene, *Dev. Dyn.* 236 (6) (2007) 1677–1682.
- [341] J.W. Chen, J.L. Galloway, The development of zebrafish tendon and ligament progenitors, *Development* 141 (10) (2014) 2035–2045.
- [342] P.D. McGurk, et al., In vivo zebrafish morphogenesis shows *Cyp26b1* promotes tendon condensation and musculoskeletal patterning in the embryonic jaw, *PLoS Genet.* 13 (12) (2017), e1007112.
- [343] E. Kague, et al., Scleraxis genes are required for normal musculoskeletal development and for rib growth and mineralization in zebrafish, *FASEB J.* 33 (8) (2019) 9116–9130.

- [344] L. Bobzin, et al., Development and maintenance of tendons and ligaments, *Development* 148 (8) (2021).
- [345] P. Fabian, et al., Lineage analysis reveals an endodermal contribution to the vertebrate pituitary, *Science* 370 (6515) (2020) 463–467.
- [346] N.D. Murchison, et al., Regulation of tendon differentiation by scleraxis distinguishes force-transmitting tendons from muscle-anchoring tendons, *Development* 134 (14) (2007) 2697–2708.
- [347] C. Nie, S. Wan, Y. Chen, D. Zhu, X. Wang, X. Dong, Z.X. Gao, Loss of scleraxis leads to distinct reduction of mineralized intermuscular bone in zebrafish, *Aquaculture and Fisheries* 6 (2) (2021) 169–177.
- [348] A. Subramanian, T.F. Schilling, Thrombospondin-4 controls matrix assembly during development and repair of myotendinous junctions, *elife* (2014) 3.
- [349] A.E. Brent, T. Braun, C.J. Tabin, Genetic analysis of interactions between the somitic muscle, cartilage and tendon cell lineages during mouse development, *Development* 132 (3) (2005) 515–528.
- [350] E. Havis, et al., TGFbeta and FGF promote tendon progenitor fate and act downstream of muscle contraction to regulate tendon differentiation during chick limb development, *Development* 143 (20) (2016) 3839–3851.
- [351] M. Malbouyres, et al., Lack of the myotendinous junction marker col22a1 results in posture and locomotion disabilities in zebrafish, *Matrix Biol.* 109 (2022) 1–18.
- [352] A. Subramanian, et al., Mechanical force regulates tendon extracellular matrix organization and tenocyte morphogenesis through TGFbeta signaling, *elife* (2018) 7.
- [353] C. Van der Heyden, A. Huyseune, Dynamics of tooth formation and replacement in the zebrafish (*Danio rerio*) (Teleostei, Cyprinidae), *Dev. Dyn.* 219 (4) (2000) 486–496.
- [354] B. Verstraeten, et al., Zebrafish teeth as a model for repetitive epithelial morphogenesis: dynamics of E-cadherin expression, *BMC Dev. Biol.* 10 (2010) 58.
- [355] P. Serittrakul, et al., Retinoic acid expands the evolutionarily reduced dentition of zebrafish, *FASEB J.* 26 (12) (2012) 5014–5024.
- [356] Q. Yuan, et al., Role of WNT10A in failure of tooth development in humans and zebrafish, *Mol. Genet. Genomic Med.* 5 (6) (2017) 730–741.
- [357] Y. Gibert, et al., The first formed tooth serves as a signalling centre to induce the formation of the dental row in zebrafish, *Proc. Biol. Sci.* 2019 (286) (1904) 20190401.
- [358] M.I. Wiweger, et al., HSPG-deficient zebrafish uncovers dental aspect of multiple osteochondromas, *PLoS One* 7 (1) (2012), e29734.
- [359] S. Pasqualetti, G. Banfi, M. Mariotti, The zebrafish scale as model to study the bone mineralization process, *J. Mol. Histol.* 43 (5) (2012) 589–595.
- [360] J.R. Metz, E. de Vrieze, E.-J. Lock, I.E. Schulten, G. Flik, Elasmoid scales of fishes as model in biomedical bone research, *J. Appl. Ichthyol.* 28 (3) (2012) 382–387.
- [361] E. de Vrieze, et al., Prednisolone induces osteoporosis-like phenotype in regenerating zebrafish scales, *Osteoporos. Int.* 25 (2) (2014) 567–578.
- [362] K. Urso, et al., A role for G protein-coupled receptor 137b in bone remodeling in mouse and zebrafish, *Bone* 127 (2019) 104–113.
- [363] I.S.-C.C. Khrystoporova, R. Harari, K. Henke, K. Woronowicz, M.P. Harris, D. Karasik, Zebrafish mutants reveal unexpected role of Lrp5 in osteoclast regulation, *Frontiers in Endocrinology* 13 (2022) 985304.
- [364] M. Carnovali, et al., Effects of bioactive fatty acid amide derivatives in zebrafish scale model of bone metabolism and disease, *Pharmacol. Res.* 104 (2016) 1–8.
- [365] S. Pasqualetti, et al., Alendronate rescued osteoporotic phenotype in a model of glucocorticoid-induced osteoporosis in adult zebrafish scale, *Int. J. Exp. Pathol.* 96 (1) (2015) 11–20.
- [366] I. Torvanger, J.R. Metz, P.A. Olsvik, L. Sjøfteland, K.K. Lie, Benzo(a)pyrene reduces osteoclast and osteoblast activity in ex-vivo scales of zebrafish (*Danio rerio* [Hamilton-Buchanan, 1822]) and goldfish (*Carassius auratus* [Linnaeus, 1758]), *J. Appl. Ichthyol.* 34 (2) (2018) 431–439.
- [367] I.J. Marques, E. Lupi, N. Mercader, Model systems for regeneration: zebrafish, *Development* 146 (2019) 18.
- [368] M. Gemberling, et al., The zebrafish as a model for complex tissue regeneration, *Trends Genet.* 29 (11) (2013) 611–620.
- [369] P.M.A. Broussonet, *Memoir on the Regeneration of Certain Parts of the Bodies of Fishes*, C. Forster, London, 1789.
- [370] S. Darnet, et al., Deep evolutionary origin of limb and fin regeneration, *Proc. Natl. Acad. Sci. U. S. A.* 116 (30) (2019) 15106–15115.
- [371] I.M. Sehring, G. Weidinger, Recent advancements in understanding fin regeneration in zebrafish, *Wiley Interdiscip. Rev. Dev. Biol.* 9 (1) (2020), e367.
- [372] A.S. Azevedo, et al., The regenerative capacity of the zebrafish caudal fin is not affected by repeated amputations, *PLoS One* 6 (7) (2011), e22820.
- [373] M.K. Iovine, Conserved mechanisms regulate outgrowth in zebrafish fins, *Nat. Chem. Biol.* 3 (10) (2007) 613–618.
- [374] S. Sousa, et al., Differentiated skeletal cells contribute to blastema formation during zebrafish fin regeneration, *Development* 138 (18) (2011) 3897–3905.
- [375] C. Pfefferli, A. Jazwinska, The art of fin regeneration in zebrafish, *Regeneration (Oxf)* 2 (2) (2015) 72–83.
- [376] M. Schebesta, et al., Transcriptional profiling of caudal fin regeneration in zebrafish, *ScientificWorldJournal* 6 (Suppl 1) (2006) 38–54.
- [377] G.G. Whitehead, et al., fgf20 is essential for initiating zebrafish fin regeneration, *Science* 310 (5756) (2005) 1957–1960.
- [378] A. Jazwinska, R. Badakov, M.T. Keating, Activin-betaA signaling is required for zebrafish fin regeneration, *Curr. Biol.* 17 (16) (2007) 1390–1395.
- [379] D. Wehner, et al., Wnt/beta-catenin signaling defines organizing centers that orchestrate growth and differentiation of the regenerating zebrafish caudal fin, *Cell Rep.* 6 (3) (2014) 467–481.
- [380] M. Thauvin, et al., An early shh-H2O2 reciprocal regulatory interaction controls the regenerative program during zebrafish fin regeneration, *J. Cell Sci.* 135 (6) (2022).
- [381] J. Kang, et al., Modulation of tissue repair by regeneration enhancer elements, *Nature* 532 (7598) (2016) 201–206.
- [382] V.A. Tornini, et al., Live monitoring of blastemal cell contributions during appendage regeneration, *Curr. Biol.* 26 (22) (2016) 2981–2991.
- [383] E. Shibata, et al., Heterogeneous fates and dynamic rearrangement of regenerative epidermis-derived cells during zebrafish fin regeneration, *Development* 145 (8) (2018).
- [384] B.E. Armstrong, et al., Shh promotes direct interactions between epidermal cells and osteoblast progenitors to shape regenerated zebrafish bone, *Development* 144 (7) (2017) 1165–1176.
- [385] A.S. Azevedo, et al., An amputation resets positional information to a proximal identity in the regenerating zebrafish caudal fin, *BMC Dev. Biol.* 12 (2012) 24.
- [386] M.J. Tomecka, et al., Clinical pathologies of bone fracture modelled in zebrafish, *Dis. Model. Mech.* 12 (9) (2019).
- [387] T. Shimizu, et al., Osteocytes as main responders to low-intensity pulsed ultrasound treatment during fracture healing, *Sci. Rep.* 11 (1) (2021) 10298.
- [388] L.M. McGowan, et al., Wnt16 elicits a protective effect against fractures and supports bone repair in zebrafish, *JBM Plus* 5 (3) (2021), e10461.
- [389] X. Xiang, et al., Study on promoting regeneration of zebrafish skull by phycocyanin characterized by in vivo optical coherence tomography, *J. Biophotonics* 15 (5) (2022), e202100333.
- [390] L. Mork, G. Crump, Zebrafish craniofacial development: a window into early patterning, *Curr. Top. Dev. Biol.* 115 (2015) 235–269.
- [391] X. Wang, et al., Two origins of blastemal progenitors define blastemal regeneration of zebrafish lower jaw, *PLoS One* 7 (9) (2012), e45380.
- [392] S. Paul, et al., Ihha induces hybrid cartilage-bone cells during zebrafish jawbone regeneration, *Development* 143 (12) (2016) 2066–2076.
- [393] H. Zhang, et al., Time point-based integrative analyses of deep-transcriptome identify four signal pathways in blastemal regeneration of zebrafish lower jaw, *Stem Cells* 33 (3) (2015) 806–818.
- [394] S. Ohgo, et al., Tissue regeneration during lower jaw restoration in zebrafish shows some features of epimorphic regeneration, *Develop. Growth Differ.* 61 (7–8) (2019) 419–430.
- [395] S. Paul, J.G. Crump, Lessons on skeletal cell plasticity from studying jawbone regeneration in zebrafish, *Bonekey Rep.* 5 (2016) 853.
- [396] J.M. Kraus, et al., Notch signaling enhances bone regeneration in the zebrafish mandible, *Development* 149 (5) (2022).
- [397] D.J.M. Bergen, et al., Regenerating zebrafish scales express a subset of evolutionary conserved genes involved in human skeletal disease, *BMC Biol.* 20 (1) (2022) 21.
- [398] M. Iwasaki, et al., Epidermal regulation of bone morphogenesis through the development and regeneration of osteoblasts in the zebrafish scale, *Dev. Biol.* 437 (2) (2018) 105–119.
- [399] D.L. Butler, N. Juncosa, M.R. Dressler, Functional efficacy of tendon repair processes, *Annu. Rev. Biomed. Eng.* 6 (2004) 303–329.
- [400] Y. Bi, et al., Identification of tendon stem/progenitor cells and the role of the extracellular matrix in their niche, *Nat. Med.* 13 (10) (2007) 1219–1227.
- [401] L.A. Solchaga, et al., Comparison of the effect of intra-tendon applications of recombinant human platelet-derived growth factor-BB, platelet-rich plasma, steroids in a rat Achilles tendon collagenase model, *J. Orthop. Res.* 32 (1) (2014) 145–150.
- [402] N.A. Dymant, et al., Lineage tracing of resident tendon progenitor cells during growth and natural healing, *PLoS One* 9 (4) (2014), e96113.
- [403] K. Howell, et al., Novel model of tendon regeneration reveals distinct cell mechanisms underlying regenerative and fibrotic tendon healing, *Sci. Rep.* 7 (2017) 45238.
- [404] T. Harvey, S. Flamenco, C.M. Fan, A Tpp3(+)/Pdgfra(+) tendon stem cell population contributes to regeneration and reveals a shared role for PDGF signalling in regeneration and fibrosis, *Nat. Cell Biol.* 21 (12) (2019) 1490–1503.
- [405] A.E.C. Nichols, K.T. Best, A.E. Loiselle, The cellular basis of fibrotic tendon healing: challenges and opportunities, *Transl. Res.* 209 (2019) 156–168.
- [406] H.L. Ansoerg, et al., Mechanical, compositional, and structural properties of the post-natal mouse Achilles tendon, *Ann. Biomed. Eng.* 39 (7) (2011) 1904–1913.
- [407] N. Ramkumar, F. Sun, K.D. Poss, Tissue repair: a tendon-see to regenerate, *Curr. Biol.* 30 (17) (2020) R1001–R1003.
- [408] K.T. Best, et al., Scleraxis-lineage cell depletion improves tendon healing and disrupts adult tendon homeostasis, *elife* (2021) 10.
- [409] J. Smeeton, et al., Regeneration of jaw joint cartilage in adult zebrafish, *Front. Cell Dev. Biol.* 9 (2021), 777787.
- [410] N. Siomava, R. Diogo, Comparative anatomy of zebrafish paired and median fin muscles: basis for functional, developmental, and macroevolutionary studies, *J. Anat.* 232 (2) (2018) 186–199.
- [411] H. Grandel, S. Schulte-Merker, The development of the paired fins in the zebrafish (*Danio rerio*), *Mech. Dev.* 79 (1–2) (1998) 99–120.
- [412] M.B. Hawkins, K. Henke, M.P. Harris, Latent developmental potential to form limb-like skeletal structures in zebrafish, *Cell* 184 (4) (2021) 899–911, e13.
- [413] B.F. Eames, et al., Mutations in fam20b and xylt1 reveal that cartilage matrix controls timing of endochondral ossification by inhibiting chondrocyte maturation, *PLoS Genet.* 7 (8) (2011), e1002246.
- [414] J. Schreml, et al., The missing "link": an autosomal recessive short stature syndrome caused by a hypofunctional XYLT1 mutation, *Hum. Genet.* 133 (1) (2014) 29–39.

- [415] Y. Kuroda, et al., A novel gene (FAM20B encoding glycosaminoglycan xylosylkinase) for neonatal short limb dysplasia resembling desbuquois dysplasia, *Clin. Genet.* 95 (6) (2019) 713–717.
- [416] J.A. Farrell, et al., Single-cell reconstruction of developmental trajectories during zebrafish embryogenesis, *Science* 360 (6392) (2018).
- [417] D.E. Wagner, et al., Single-cell mapping of gene expression landscapes and lineage in the zebrafish embryo, *Science* 360 (6392) (2018) 981–987.
- [418] A.G.T. Howard, et al., An atlas of neural crest lineages along the posterior developing zebrafish at single-cell resolution, *elife* 10 (2021).
- [419] D. Tatarakis, et al., Single-cell transcriptomic analysis of zebrafish cranial neural crest reveals spatiotemporal regulation of lineage decisions during development, *Cell Rep.* 37 (12) (2021), 110140.
- [420] E. Lencer, R. Prekeris, K.B. Artinger, Single-cell RNA analysis identifies pre-migratory neural crest cells expressing markers of differentiated derivatives, *elife* (2021) 10.
- [421] J.M. Mitchell, et al., The *alx3* gene shapes the zebrafish neurocranium by regulating frontonasal neural crest cell differentiation timing, *Development* 148 (7) (2021).
- [422] M. Tambalo, R. Mitter, D.G. Wilkinson, A single cell transcriptome atlas of the developing zebrafish hindbrain, *Development* 147 (6) (2020).
- [423] P. Fabian, et al., Lifelong single-cell profiling of cranial neural crest diversification in zebrafish, *Nat. Commun.* 13 (1) (2022) 13.
- [424] Y. Hou, et al., Cellular diversity of the regenerating caudal fin, *Sci. Adv.* 6 (33) (2020), eaba2084.
- [425] B. Laplace-Builhe, et al., NRG1/ErbB signalling controls the dialogue between macrophages and neural crest-derived cells during zebrafish fin regeneration, *Nat. Commun.* 12 (1) (2021) 6336.
- [426] W.J. Tang, et al., Single-cell resolution of MET- and EMT-like programs in osteoblasts during zebrafish fin regeneration, *iScience* 25 (2) (2022), 103784.
- [427] C.S. McGinnis, et al., MULTI-seq: sample multiplexing for single-cell RNA sequencing using lipid-tagged indices, *Nat. Methods* 16 (7) (2019) 619–626.
- [428] S.R. Srivatsan, et al., Massively multiplex chemical transcriptomics at single-cell resolution, *Science* 367 (6473) (2020) 45–51.
- [429] T. Chari, et al., Whole-animal multiplexed single-cell RNA-seq reveals transcriptional shifts across *Clytia* medusa cell types, *Sci. Adv.* 7 (48) (2021), eabh1683.