Transgenic fluorescent zebrafish lines that have revolutionized biomedical research

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Abstract
Since its debut in the biomedical research fields in 1981, zebrafish have been used as a vertebrate model organism in more than 40,000 biomedical research studies. Especially useful are zebrafish lines expressing fluorescent proteins in a molecule, intracellular organelle, cell or tissue specific manner because they allow the visualization and tracking of molecules, intracellular organelles, cells or tissues of interest in real time and in vivo. In this review, we summarize representative transgenic fluorescent zebrafish lines that have revolutionized biomedical research on signal transduction, the craniofacial skeletal system, the hematopoietic system, the nervous system, the urogenital system, the digestive system and intracellular organelles.

Keywords: Zebrafish, Transgenic, Fluorescent, Signal, Skeletal, Hematopoietic, Nervous, Urogenital, Digestive, Organelle

Background
Zebrafish (Danio rerio or Brachydanio rerio) are freshwater fish native to South Asia and were first described scientifically in 1822 by the Scottish physician Francis Hamilton in the monograph entitled An account of the fishes found in the river Ganges and its branches, an ichthyological masterpiece [1]. In this monograph, he referred to the zebrafish as “this beautiful fish I found in the Kosi river, where it grows to about two inches in length (Fig. 1).” The genus name ‘Danio’ came from the Bengali word ‘Dhani,’ which means rice or rice paddy. One and a half century later, zebrafish made its debut in the biomedical research fields with a George Streisinger’s milestone paper entitled Production of clones of homoygous diploid zebra fish (Brachydanio rerio) [2]. In 1997, the Lin group reported the creation of a transgenic zebrafish line expressing green fluorescent protein (GFP) in a cell-specific manner. They microinjected into one-cell stage zebrafish embryos promoter sequences of GATA-1, an erythroid-specific transcription factor, fused to GFP, and confirmed expression of GFP in the GATA-1+ cells in F1 and F2 zebrafish [4]. Since then, numerous transgenic zebrafish lines expressing fluorescent proteins in a molecule, intracellular organelle, cell or tissue specific manner (referred to as transgenic fluorescent zebrafish lines hereafter) have been generated. As zebrafish embryos are small and transparent, molecules, intracellular organelles, cells or tissues of interest in the transgenic fluorescent zebrafish lines can be easily visualized and tracked in real time and in vivo. As such, they have revolutionized biomedical research on cell biology, developmental biology, genetics, toxicology, human disease pathobiology and drug development. As of April 29,
2021, the Zebrafish Information Network (ZFIN) [5] displays 8960 transgenic zebrafish lines expressing GFP.

In this review, we summarize the representative transgenic fluorescent zebrafish lines that have been extensively used in biomedical research as well as some of their primary applications.

**Main text**

**Signal transduction**

Early embryonic zebrafish development is tightly regulated by various signaling cascades that should be sequentially activated in restricted domains and time windows [6, 7]. The default state of the fertilized embryos is the ventral fate, determined by the expression of maternal genes related to Transforming growth factor beta (TGF-β) including radar, bone morphogenetic protein 2 (bmp2), bmp4 and bmp7 [7–9]. Ventral tissue specification during early embryonic development is abruptly interrupted at the onset of maternal-zygotic transition stage by the expression of various BMP antagonists such as bozozok, noggin and chordin. The expression of these genes is triggered by the canonical Wnt (Wnt/β-catenin) and Fibroblast growth factor (FGF) signaling, and is restricted to putative organizer regions [7, 10]. Formation of these organizer regions is a prerequisite for the subsequent anterior–posterior (AP) body axis specification. The AP patterning is a complex process triggered by maternal Wnt/β-catenin signaling whose activity is required for the induction of Nodal, another TGF-β related signaling, whereby FGF expression is stimulated in both organizer and blastoderm margin [6, 7]. Ectopically expressed Nodal results in severe embryonic dorsalization as it antagonizes BMP signaling, while the depletion of Nodal disrupts mesendoderm specification, leaving ectoderm unaffected [6, 7]. These signaling pathways also play critical roles in later embryonic development, especially in left–right patterning and in organogenesis.

Various transgenic zebrafish lines have been generated to study the aforementioned signaling pathways as well as the consequences of their hypo- or hyper-activation. To study the role of BMP signaling, Pyati et al. generated a transgenic zebrafish line integrating into its genome a dominant negative BMP receptor fused to GFP under the control of heat shock promoter [Tg(hsp70l:dnBmpr-GFP)] (Table 1). With this transgenic line, they were able to delineate the roles of BMP signaling before and after mid-gastrulation by exposing the line to heat shock at different developmental stages [11]. To visualize BMP signaling in live embryos, several transgenic lines have been generated expressing various fluorescent proteins under the control of BMP responsive element adopted from mouse inhibitor of differentiation-1 enhancer: Tg(hsp70l:dnBmpr-GFP), Tg(BmpRE-AAVmlp:EGFP), Tg(BRE-AAVmlp:d2GFP) and Tg(BRE-AAVmlp:dnKO2) [12–15]. EGFP indicates enhanced GFP. To track Nodal activity, Dubrelle et al. measured the nuclear accumulation of Smad2, the hallmark of Nodal signaling activation. To accurately quantify the nucleo-cytoplasmic ratio of Smad2, they generated a transgenic zebrafish line expressing GFP-Smad2 ubiquitously, and then observed the graded nuclear accumulation of GFP-Smad2 along the diffused Nodal gradient from vegetal to animal axis during blastula stage [16].

Several lines are available to study Wnt/β-catenin signaling in live embryos. In the Tg(top:GFP) transgenic line, the fluorescent reporter is under the control of TOP-Flash comprising four consensus TCF/LEF binding elements juxtaposed to a c-fos minimal promoter [17, 18].
Also developed are transgenic fluorescent zebrafish lines in which GFP or nuclear mCherry is driven by the enhancer region of minimal promoter of Xenopus gene siamois with seven tandem repeats of the TCF/LEF binding elements: Tg(7xTCF-Xla.Siam:GFP), Tg(7xTCF-Xla.Siam:nls:Cherry), Tg(kdr:EGFP), Tg(Tie2:EGFP), Tg(myl7:EGFP), Tg(sox10:mRFP), Tg(hsp70l:dkk1-GFP), Tg(hsp70l:wnt8a-GFP) and Tg(fli1:EGFP) [19]. With these reporter lines, Moro et al. identified zebrafish tissues sensitive to canonical Wnt stimuli including the hypothalamus, the gill arches and rakers, the olfactory bulb and the cloacal aperture. In contrast to the canonical Wnt signaling, transgenic zebrafish lines have not been used to study non-canonical Wnt signaling, to the best of our knowledge. Therefore, it would be challenging to explore zebrafish planar cell polarity governed by the non-canonical Wnt signaling in real time and in vivo.

As the endogenous expression of dual specificity phosphatase 6 (dusp6, also called Mkp3) is directly controlled by FGF signaling throughout zebrafish development, Molina et al. introduced destabilized EGFP under the control of the isolated dusp6 promoter. The resulting Tg(dusp6:EGFP) is highly sensitive to FGF signaling and allows for the visualization of FGF responsive tissues [20]. Moreover, as Tg(dusp6:EGFP) is easily sensitized by specific FGF signaling chemical antagonists, this reporter embryos are very useful to identify compounds that alter FGF signaling. On the other hand, to suppress

| Construct name                                      | Construct ID                                      | Transgenic name                                      | Transgenic ID   | Target                     | References |
|-----------------------------------------------------|---------------------------------------------------|------------------------------------------------------|-----------------|----------------------------|------------|
| Tg(hsp70/dkk1b-GFP)                                 | ZDB-TGCON-STRCT-070403-1                         | Tg(hsp70/dkk1b-GFP)                                  | ZDB-ALT-070403-1| Dkk1b                      |            |
| Tg(hsp70/wnt8a-GFP)                                 | ZDB-TGCON-STRCT-070403-3                         | Tg(hsp70/wnt8a-GFP)                                  | ZDB-ALT-070403-3| Wnt8a                      |            |
| Tg(fli1-EGFP)                                       | ZDB-TGCON-STRCT-07017-94                         | Tg(fli1-EGFP)                                        | ZDB-ALT-011017-8| Pharyngeal arch, endothelial cells and skeletogenic precursors | [19, 20, 23]|
| Tg(dusp6:ZEGFP)                                     | ZDB-TGCON-STRCT-07017-3                          | Tg(dusp6:ZEGFP)                                      | ZDB-ALT-071017-3| FGF signaling              |            |
| Tg(hsp70/dnkfr1(α)-EGFP)                            | ZDB-TGCON-STRCT-07017-101                        | Tg(hsp70/dnkfr1(α)-EGFP)                             | ZDB-ALT-060322-2| FGF signaling              | [21, 22]   |
| Tg(fabp10α/dnkfr1-EGFP)                             | ZDB-TGCON-STRCT-131120-6                         | Tg(fabp10α/dnkfr1-EGFP)                              | ZDB-ALT-131120-24| FGF signaling specifically in liver | [22]       |
FGF signaling in developing embryos, dominant negative FGF receptor (dn-fgfr1) was exploited for the transgenesis. Initially, Hsp70 promoter was adopted to generate Tg(hsp70l:dnfgfr1-EGFP) zebrafish that can be activated by moderate heat stimuli [21]. Later on, Tsai et al. generated transgenic zebrafish in which dn-fgfr1 is expressed specifically in the liver using a hepatocyte specific promoter, a liver fatty acid binding protein (Ifabp) promoter. This adult Tg(fabp10a:dnfgfr1-EGFP) manifested liver pathology such as hepatic steatosis and cholestasis, suggesting that the maintenance of hepatic FGF signaling is required for lipid homeostasis [22].

The craniofacial skeletal system

Introduction

Formation of the craniofacial skeleton is an intricate process that requires a series of developmental events mediated by cells derived from the neural crest (NC), endoderm, mesoderm, and ectoderm. The craniofacial skeleton is largely derived from cranial neural crest (CNC) cells that populate the pharyngeal arches [24, 25]. From there, CNC cells become skeletogenic precursors called ectomesenchyme and then chondrocytes and osteoblasts that will subsequently form facial cartilages and bones, respectively [24–26]. While the CNC is an important transient cell population for the development of craniofacial skeleton, the pharyngeal endoderm, mesoderm, and ectoderm are required for the CNC to form the craniofacial skeleton. During embryogenesis, the head ectoderm forms a series of infoldings, termed clefts, and the pharyngeal endoderm develops a series of outpocketings, termed pouches [27, 28]. The endodermal pouches, together with the ectodermal clefs, segment the pharyngeal arches and have important signaling functions in the survival and differentiation of CNC cells during craniofacial skeleton development [27, 29]. While the head mesoderm of the pharyngeal arches is primarily of myogenic origin, it also contributes to craniofacial skeleton development by establishing and patterning the pharyngeal endoderm [27, 30]. Thus, to better understand craniofacial skeleton development, essentially required is the unprecedented ability to track and manipulate cells derived from the NC, endoderm, mesoderm and ectoderm in vivo. To this end, several transgenic fluorescent zebrafish lines have been generated (Table 2) and widely used for research on craniofacial development.

Ectomesenchyme transgenic lines

The Fli1 transcription factor marks endothelial cells as well as CNC-derived skeletogenic precursors [31, 32]. Although the Tg(fli1:EGFP) transgenic line was originally generated to visualize endothelial cells [23], it has been widely used to image CNC cells within the pharyngeal arches. Lawson and Weinstein identified fragments containing the 5’ end of the fli1 gene by screening a P1-derived artificial chromosome (PAC) library, and subcloned a 15-kb PAC DNA fragment into the pGEM3zf plasmid. EGFP was placed immediately upstream of the fli1 start codon, resulting in the Tg(fli1:EGFP) transgenic construct, which was linearized with NotI and then injected into one-cell stage embryos to generate Tg(fli1:EGFP) zebrafish. This transgenic zebrafish labels the endothelial cells as well as CNC cells. During craniofacial skeleton development, this line expresses EGFP in post-migratory CNC cells in the pharyngeal arches. To understand the early stage of craniofacial skeletal formation, this line, along with the Tg(sox10:EGFP) line that expresses EGFP in NC including the CNC, has been used to visualize the skeletogenic precursors. For example, this line was adopted to study the roles of the variant histone H3.3, BMPs and inhibitor of DNA binding 2a (Id2a) in ectomesenchyme potential of the CNC [33, 34] as well as to correlate defects in pharyngeal pouches with later defects in the condensation of CNC cells to form the craniofacial skeleton [27, 35].

Cartilage and bone transgenic lines

In addition to the transgenic reporters marking the CNC and skeletogenic precursors, a number of transgenic lines have been developed to image different cell types within the differentiated skeleton in vivo. These include Tg(sp7:EGFP), Tg(RUNX2:EGFP) and Tg(osteocalcin:EGFP) for osteoblasts, and Tg(col2a1a:EGFP) and Tg(sox9a:EGFP) for chondrocytes. Sp7 (Osterix) and RUNX2 are expressed in committed osteoblast progenitors, and Osteocalcin (Bone γ-carboxyglutamate protein [Bglap]) in late osteoblasts [36].

Sp7 is a transcription factor that is expressed in osteoblasts but not chondrocytes, making it an excellent marker of osteoblasts [37]. Although a 4.1-kb upstream regulatory region of the medaka sp7 gene was used to drive mCherry or nuclear GFP expression in zebrafish [38], this transgenic line did not completely recapitulate expression pattern of endogenous zebrafish sp7 [39, 40]. To overcome this discrepancy, the Kimmel group wished to generate the transgenic sp7 reporter using a regulatory region of the zebrafish sp7 [39]. Since the regulatory elements necessary for zebrafish sp7 expression were not known, they used bacterial artificial chromosome (BAC) mediated transgenesis to drive EGFP expression under the control of the sequences upstream of sp7. Using an E. coli recombination machinery, they inserted a DNA fragment containing EGFP and a kanamycin resistant cassette into the start codon of the sp7 coding sequence.
in a BAC harboring the full sp7 sequences. The resulting BAC Tg(sp7:EGFP) construct was used to generate a transgenic line via BAC transgenesis technology [41]. In this transgenic line, EGFP expression reproduced endogenous sp7 expression in the otic primordium and skeletal structures, including osteoblasts. In the adult transgenic fish, EGFP was detected in skeletal elements, such as the tail fin bony rays, and regenerating fin rays post-amputation [39]. In vivo imaging of Tg(sp7:EGFP) line revealed that a population of sp7:EGFP− cells is a key source of repair chondrocytes in jawbone regeneration [42]. In addition, this line contributed to produce epigenome maps, including DNA methylation and chromatin accessibility, in sp7:EGFP− osteoblasts from uninjured and regenerating fins [43].

Tg(RUNX2:EGFP) is another transgenic line that has been extensively used for in vivo imaging of osteoblasts. The transcription factor Runx2 along with Sp7 is critical to osteoblast differentiation in zebrafish and mammals, rendering it an excellent marker of osteoblasts [4, 44, 45]. The Weidinger group originally generated the Tg(RUNX2:EGFP) to study the cellular basis of fin regeneration in adult zebrafish [46]. They utilized a 557-bp multispecies-conserved regulatory element located in the last intron of the human RUNX2 gene. They cloned it into the Tol2 transposon vector pGWcfosEGFP, containing the cFos minimal promoter and EGFP, thereby generating the Tg(Hsa.RUNX2-Mmu.Fos:EGFP) construct, with which they created Tg(Hsa.RUNX2:EGFP) line using the Tol2 transposon system. This line expresses EGFP in all

| Table 2  | Transgenic reporter zebrafish lines targeting the craniofacial-skeletal system |
|----------|---------------------------------------------------------------------------------|
| Construct name | Construct ID | Transgenic name | Transgenic ID | Target | References |
| Tg(fli1:EGFP) | ZDB-TGCON-STRCT-070117-94 | Tg(fli1:EGFP)1 | ZDB-ALT-0111017-8 | Pharyngeal arch, endothelial cells and skeletogenic precursors | [23] |
| Tg(sox10:GAL4-VP16-IRES-EGFP) | ZDB-TGCON-STRCT-120523-1 | Tg(sox10:GAL4-VP16-IRES-EGFP)110 | ZDB-ALT-120523-1 | NCs and CNS | [34] |
| Tg(sp7:EGFP) | ZDB-TGCON-STRCT-100402-2 | Tg(sp7:EGFP)1212 | ZDB-ALT-100402-1 | Osteoblasts | [41] |
| Tg(Hsa.RUNX2-Mmu.Fos:EGFP) | ZDB-TGCON-STRCT-120209-6 | Tg(Hsa.RUNX2-Mmu.Fos:EGFP)235 | ZDB-ALT-120209-60 | Osteoblasts | [46–49] |
| Tg(Hsa.RUNX2:EGFP) | ZDB-TGCON-STRCT-110713-1 | Tg(Hsa.RUNX2:EGFP)4008 | ZDB-ALT-110713-1 | Late osteoblasts | [36, 42, 46, 47] |
| Tg(fr.col2a1a.2:EGFP) | ZDB-TGCON-STRCT-111205-2 | Tg(fr.col2a1a.2:EGFP)13 | ZDB-ALT-111205-4 | Chondrocytes | [54] |
| Tg(-1.7col2a1a:EGFP-CAAX) | ZDB-TGCON-STRCT-111205-1 | Tg(-1.7col2a1a:EGFP-CAAX)112 | ZDB-ALT-111205-1 | Chondrocytes | [57] |
| Tg(foxp2.A:EGFP) | ZDB-TGCON-STRCT-100412-4 | Tg(foxp2.A:EGFP)342 | ZDB-ALT-100412-5 | Chondrocytes | [60, 61] |
| Tg(her5PAC:EGFP) | ZDB-TGCON-STRCT-070117-155 | Tg(her5PAC:EGFP)1939 | ZDB-ALT-050919-8 | Mid-hindbrain, pharyngeal endoderm and pouch | [65] |
| Tg(her5PAC:EGFP) | ZDB-TGCON-STRCT-070117-155 | Tg(her5PAC:EGFP)1939 | ZDB-ALT-050919-8 | Mid-hindbrain and pharyngeal endoderm | [65] |
| Tg(nkx2.3:GAL4-VP16,myl7:EGFP) | ZDB-TGCON-STRCT-130610-1 | Tg(nkx2.3:GAL4-VP16,myl7:EGFP)102 | ZDB-ALT-130610-2 | Pharyngeal pouch | [67] |
| Tg(UAS:CFP-Eco.NfsB,myl7:EGFP) | ZDB-TGCON-STRCT-130610-7 | Tg(UAS:CFP-Eco.NfsB,myl7:EGFP)153 | ZDB-ALT-130610-8 | Gal4/UAS system | [67] |
| Tg(nkx2.3:EGFP-dvl2) | ZDB-TGCON-STRCT-130610-3 | Tg(nkx2.3:EGFP-dvl2)2003 | ZDB-ALT-130610-4 | Pharyngeal pouch | [67] |
| Tg(nkx2.3:alcama-EGFP) | ZDB-TGCON-STRCT-130610-4 | Tg(nkx2.3:alcama-EGFP)1904 | ZDB-ALT-130610-5 | Pharyngeal pouch | [67] |
| Tg(nkx2.5:EGFP) | ZDB-TGCON-STRCT-120828-5 | Tg(nkx2.5:EGFP)883 | ZDB-ALT-120828-8 | Cardiogenesis and head mesoderm | [67, 84] |
| Tg(nkx2.5:GAL4-VP16,myl7:EGFP) | ZDB-TGCON-STRCT-130610-2 | Tg(nkx2.5:GAL4-VP16,myl7:EGFP)274 | ZDB-ALT-130610-3 | Cardiogenesis and head mesoderm | [67] |
newly forming bones of the craniofacial and appendicular skeleton from embryo to adult [46], making it suitable to investigate bone development and regeneration. For example, the in vivo imaging of GFP expression or wholemount in situ hybridization (WISH) against gfp RNA in this line demonstrated that mature osteoblasts dedifferentiate to form pre-osteoblasts following fin amputation or bone fracture, and that NF-kB signaling negatively regulates osteoblast dedifferentiation [46–48]. Time-lapse imaging analysis of this line to explore dynamics of skull bone formation showed that Sp7 is required for the maturation and proliferation control of RUNX2:EGFP+ early osteoblasts [49].

Osteocalcin is a late osteoblast marker. To generate an osteocalcin reporter, the Weidinger group first cloned the 3.7-kb promoter sequence of the medaka (Oryzias latipes) osteocalcin and inserted it into the upstream region of EGFP in pBluescript vector containing I-SceI meganuclease sites flanking the insert [50]. The resulting construct was used to create an osteocalcin reporter line Tg(osteocalcin:EGFP) using I-SceI mediated transgenesis [46]. This line was used to demonstrate that zebrafish osteoblasts dedifferentiate into osteogenic precursor cells in the fractured bony fin ray and skull [47], and that repair chondrocytes dedifferentiate into osteoblasts during zebrafish jawbone regeneration [42].

A better understanding of craniofacial skeleton development entails transgenic lines that can visualize cartilage development as well. As such, Tg(col2a1a:EGFP) and Tg(sox9a:EGFP) lines were generated to illuminate chondrocyte formation and distribution during cartilage development. Collagen is the most abundant protein in the extracellular matrix [51, 52] and type II collagen is an important component of cartilage [53]. Zebrafish has two paralogs of col2a1, the col2a1a and col2a1b, of which col2a1a is robustly expressed in all craniofacial chondrocytes, making it an excellent marker for this cell type [54]. Dale and Topczewski identified a regulatory element in the col2a1a locus, termed R2, located from −1720 to −1411 bp of the transcription start site (TSS). Using the Multistep Gateway Recombination system (Invitrogen) and the Tol2kit [55, 56], they fused the R2 element to a minimal promoter containing a carp TATA box and the adeno virus E1b promoter upstream of EGFP, generating the Tg(rr.col2a1a.2:EGFP) construct. They also created the Tg(-1.7col2a1a:EGFP-CAAX) construct with the entire 1.7-kb fragment upstream of the TSS. Both resulting transgenic lines, Tg(rr.col2a1a.2:EGFP) and Tg(-1.7col2a1a:EGFP-CAAX), express EGFP in craniofacial cartilage. These lines helped uncover that Wnt9a is required for palatal extension [57], and that Iroquois transcription factor inhibits chondrocyte maturation at the hyoid joint [58]. The entire 1.7-kb upstream sequence was used to drive guanine nucleotide exchange factor subunit RIC1 and its downstream targets in chondrocytes, which revealed the underlying mechanism of RIC1-linked Mendelian syndrome [59].

The transgenic zebrafish line sox9a zc81Tg, commonly known as Tg(sox9a:EGFP), has been also widely used for in vivo imaging of craniofacial chondrocytes. Interestingly, the Tg(sox9a:EGFP) line was generated inadvertently. To generate a transgenic reporter visualizing the central nervous system (CNS) during development, the Dorsky group isolated a 9.7-kb DNA fragment from the foxP2 locus, and used it to build the Tg(foxp2.A:EGFP) construct via Gateway technology [60]. They then micro-injected the resulting constructs into zebrafish embryos at one-cell stage, and raised the embryos to adulthood. While screening F1 embryos from founder zebrafish, they noted one founder whose embryos expressed EGFP in the pharyngeal arches where endogenous foxp2 is not expressed. This finding suggested that in the genome of this founder, EGFP expression was driven by a nearby promoter rather than by a foxP2 promoter per se. Indeed, further investigation revealed that the Tg(foxp2.A:EGFP) insertion locus in the genome was located approximately 120-kb upstream of the sox9a TSS [61]. As a result, F1 zebrafish from the founder was termed Tg(sox9a:EGFP) line instead of Tg(foxp2.A:EGFP) line. About 55 h post-fertilization (hpf) at which Tg(fli1:EGFP) expression in the CNC-derived ectomesenchyme is abolished in the pharyngeal arches, the Tg(sox9a:EGFP) line expresses EGFP in early cartilage rudiments developing in these arches [61]. This line was used to uncover the role of sox9a-expressing cells in musculoskeletal integration in the zebrafish jaw [62]. Time-lapse imaging of craniofacial skeleton development in this line revealed that the opercle and the adjacent ceratohyal cartilage migrate in a coordinated manner [63], and that gaps in sox9a:EGFP+ cartilage exhibit hallmarks of joint identity during craniofacial skeleton development [64].

Pharyngeal endoderm transgenic lines

Absence of the endoderm or the pharyngeal endoderm derived pharyngeal pouches leads to severe defects in the craniofacial skeleton of zebrafish, indicating that these tissues are critical to the craniofacial skeleton development [30]. To better understand how the pharyngeal endoderm and pouches regulate craniofacial skeleton development, transgenic reporter lines were needed to visualize these tissues during craniofacial development. The first transgenic reporter illuminating pharyngeal endoderm formation was the Tg(hspa:EGFP) transgenic line [65], although this line was originally generated to investigate the development of midbrain-hindbrain (MH) domain of the...
vertebrate embryonic neural tube in vivo. For this purpose, Tallafuss and Bally-Cuif isolated a PAC containing the complete set of regulatory elements of her5, the first reported gene expressed in the MH domain, and inserted EGFP within exon 2 of the her5 genomic region via ET-mediated homologous recombination [66]. The resulting construct was used to establish the Tg(her5PAC:EGFP) line via the PAC-mediated transgenesis [65]. In this line, EGFP expression reproduces endogenous her5 expression in the MH domain and the pharyngeal endoderm, making this line the first transgenic reporter of the pharyngeal endoderm. They also generated the Tg(-3.4her5:EGFP) line harboring EGFP driven by 3.4-kb fragment upstream of the her5 TSS, which mirrors the EGFP expression pattern in the Tg(her5PAC:EGFP) line almost completely [65]. Since then, both lines have been extensively used. For example, time-lapse imaging analysis in the Tg(-3.4her5:EGFP) line revealed that the pharyngeal pouches are formed by the branching morphogenesis and that Wnt11r and Wnt4a are required for this morphogenesis [67]. This line was also employed to correlate defects in the pharyngeal endoderm with later defects in the craniofacial cartilage development [68–70].

Together with the Tg(her5:EGFP) line, the Tg(sox17:GFP) line has been used to illuminate development of the endoderm, including the pharyngeal endoderm. In zebrafish and mammalian embryogenesis, a transcription factor Sox17 is the key regulator of the specification and morphogenesis of endodermal epithelia, making it an excellent pan-endoderm marker [71–73]. Chung and Stainer originally generated the Tg(sox17:GFP) line to identify progenitors of endodermal organs such as the liver, pancreas and intestine [74]. They first isolated the 4.2-kb upstream regulatory region from the pEGFP1 sox17 promoter plasmid constructed by Yutaka Kikuchi and cloned it into a Tol2 GFP transposon vector, thereby building the Tg(sox17:GFP) construct, with which they created the Tg(sox17:GFP) line using the Tol2 transposon system [66]. In this line, GFP is expressed in almost all endodermal cells, including the endodermal cells at 50% epiboly, the progenitors of the liver, pancreas and intestine, the condensing foregut endodermal cells, and the pharyngeal endodermal cells [74–77]. Various studies on the endoderm and craniofacial skeleton development have benefited from the Tg(sox17:GFP) line. For example, in vivo imaging of sox17:GFPGreen pharyngeal endoderm correlated defects in the morphogenesis of pharyngeal endoderm with later defects in craniofacial cartilage morphology [76, 78, 79]. In addition, this line has been used to understand the developmental mechanisms underlying formation of the endodermal organs such as the liver, pancreas, and intestine [74, 80, 81].

During craniofacial development, a population of cells in the pharyngeal endoderm forms a series of pouches that governs the development of pharyngeal arches and craniofacial skeleton [27, 30]. To better understand the role for this specific population of cells in the development of craniofacial skeleton, transgenic lines expressing reporter genes specifically in the pouches were needed. Zebrafish nkx2.3 is specifically expressed in the developing pouches, but not in the pharyngeal endoderm, making it an excellent marker of the pouches [82]. To illuminate the morphogenesis of pouches, the Crump group created a Tg(nkx2.3:Gal4-VP16) line that can drive expression of any gene governed by the UAS promoter specifically in the pouches [67]. They isolated a 5.5-kb regulatory region upstream of the nkx2.3 locus and made the Tg(nkx2.3:Gal4-VP16,myl7:EGFP) construct using the Gateway technology, with which they developed the Tg(nkx2.3:Gal4-VP16,myl7:EGFP) line using the Tol2 transposon system [67]. The Tg(UAS:CFP-Eco.NfsB,myl7:EGFP) line expresses cyan fluorescent protein (CFP) fused to nitroreductase (NTR), an E. coli enzyme that converts the prodrug metronidazole (MTZ) to cytoxin, specifically in the pharyngeal pouches throughout pouch morphogenesis in a pattern consistent with endogenous nkx2.3 expression [67, 82]. The combination of Tg(nkx2.3:Gal4-VP16,myl7:EGFP) line and Tg(UAS:CFP-Eco.NfsB,myl7:EGFP) line revealed the pouch-specific requirements for effectors of Wnt and Dvl signaling during pouch morphogenesis. In addition, a number of transgenic constructs has been generated with the 5.5-kb upstream regulatory region of nkx2.3, including the Tg(nkx2.3:EGFP-dvl2) and Tg(nkx2.3:alcama-EGFP), time-lapse imaging of which showed correlation between the rearrangement of pouch cells and the dynamic subcellular localization of Dishevelled (Dvl) and Alcama during pouch formation [67].

**Pharyngeal mesoderm transgenic lines**

The head mesoderm has been reported as an important source of multiple signaling necessary for pouch formation associated with the craniofacial skeleton development [27, 30]. However, the absence of transgenic reporters that can mark and manipulate the head mesoderm in live animals, has hindered the progress in understanding the cellular and molecular mechanism by which the head mesoderm controls the development of the pharyngeal endoderm and craniofacial skeleton. In zebrafish, nkx2.5 is the earliest heart field marker, and expressed farther posteriorly to the mesoderm adjacent to the pharyngeal arches and pouches during craniofacial skeleton development [83]. Thus, a transgenic
The hematopoietic system

Introduction

The hematopoietic genes and regulatory networks as well as all mature blood lineages and maturation intermediates in zebrafish are evolutionarily conserved [86]. As in mammals, zebrafish hematopoiesis initiates with sequential primitive and definitive waves of blood cells [87]. First, the primitive waves of hematopoiesis produce erythrocytes and macrophages through unipotent precursors from the embryonic mesoderm. Afterwards, the definitive hematopoietic stem cells (HSCs) capable of generating all mature blood cell types arise from the hemogenic endothelium [88]. The transgenic reporter lines labelling each and every type of blood lineages have been introduced over the past two decades, allowing for real time imaging in live zebrafish and purification of blood cells of interest through fluorescence activated cell sorting (FACS) [89]. Here, we summarize the representative zebrafish transgenic reporter lines labeling distinct blood populations (Table 3), which have been used for characterizing various hematopoietic events.

Erythroid transgenic lines

During early embryogenesis, red blood cells (RBCs) arise from the intermediate cell mass (ICM), which is derived from the posterior lateral mesoderm (PLM) [90, 91]. To label RBCs, the Lin group at Medical College of Georgia generated a Tg(gata1a:GFP) zebrafish by injecting the linearized construct G1-GM2 comprising the zebrafish GATA-1 promoter and GFP (GM2) into embryos [92]. The GATA-1 promoter construct is a 5.6-kb DNA fragment from a GATA-1 genomic fragment containing the 5’ upstream region of the GATA-1 TSS. This line has been used to study embryonic erythropoiesis [92], thrombocyte development [93], hematopoietic transplantation [86], leukemia [94] and real-time cardiac pumping dynamics [95].

Myeloid transgenic lines

Myeloid cell populations including macrophages and neutrophils originate from anterior lateral mesoderm [96]. To uncover the molecular and cellular mechanisms regulating neutrophil-mediated inflammation in vivo, the Huttenlocher group isolated a 6-kb upstream regulatory region of the zebrafish zMPO promoter sequences and subcloned an 8-kb fragment of the zMPO 5’-untranslated region (UTR) into the 5’ region of GFP (zMPO-GFP), producing the transgenic line Tg(mpx:GFP) where neutrophils express GFP under the control of myeloperoxidase promoter [97]. The Lieschke group developed a macrophage-specific reporter line to track macrophages and their interactions with neutrophils in real time and in vivo [98]. The promoter of a macrophage-specific marker mpeg1 was utilized to generate the line. The proximal 1.86-kb DNA fragment of mpeg1 5’-UTR was subcloned into the 5’ region of either EGFP or mCherry, and Tol2-mediated transgenesis was performed to generate Tg(mperg1:EGFP) or Tg(mperg1:mCherry) that expresses EGFP or mCherry in the macrophages, respectively [98]. Tg(mpx:GFP), Tg(mperg1:EGFP) and Tg(mperg1:mCherry) lines can address behavior and development of myelopoids such as phagocytosis of senescent neutrophils [98]. These lines have been used to take real-time and in vivo images of responses against inflammation [97], wound detection [99], neutrophil motility [100], the role of eosinophils in immunity [101], tissue regeneration [102, 103], cardiac wound healing[104], metabolic diseases [105], microglia development [106], axon degeneration [107] and HSC development [108].

HSC transgenic lines

The first primitive wave of hematopoiesis is followed by the definitive wave of hematopoiesis, wherein HSCs give rise to distinct types of blood cells. HSCs can both self-renew and differentiate into all mature blood cell types over the lifetime of an individual [109]. Definitive HSCs arise from hemogenic endothelium, a special population of endothelial cells within the ventral wall of the dorsal aorta that transdifferentiates into HSCs [88]. Specified HSCs budding out from the hemogenic endothelium circulate through the vascular system, eventually homing to the caudal hematopoietic tissue (CHT) where HSCs
expand and mature [110]. Afterwards, HSCs migrate to the kidney marrow to act as multipotent blood progenitor cells [111].

To label this HSC population, the Zon group generated three valuable HSC-specific transgenic reporter lines. First, the \( \text{Tg(-6.0itga2b:EGFP)} \) line was originally created to assess zebrafish thrombocytes and prothrombocytes, but turned out to be more specific for the HSC population [86]. The transgenic construct was generated using EGFP driven by the promoter of platelet glycoprotein IIb (\( \text{itga2b} \)) cloned from PAC 166I10. The second transgenic line \( \text{TgPAC(myb:2xmyb-EGFP)} \) was generated by homologous recombination of a 3.7-kb EGFP construct into the UTR precisely before the start site of a PAC clone containing \( \text{cmyb} \) [112]. Combination of \( \text{TgPAC(myb:2xmyb-EGFP)} \) and endothelium-specific \( \text{Tg(lmo2:DsRed)} \) labels HSCs at 36 hpf [112]. Finally, the Zon group generated \( \text{Tg(Mmu.Runx1:EGFP)} \) and \( \text{Tg(Mmu.Runx1:NLS-mCherry)} \), wherein the mouse Runx1 +23 enhancer drives either EGFP or nuclear mCherry. The double transgenic line \( \text{Tg(Mmu.Runx1:EGFP); Tg(Mmu.Runx1:NLS-mCherry)} \) showed consistent expression of both fluorescent proteins, confirming the HSC specific expression of the reporter in the lines [110]. To generate the transgenic construct, the Runx1 +23 enhancer was amplified from C57/BL6 mouse genomic DNA [113]. Subsequently, the resulting Runx1 +23 enhancer DNA fragment and the β-globin minimal promoter were simultaneously cloned into the Tol2 plasmid. These HSC-specific transgenic lines have been used to study embryonic HSC emergence [88, 114–116], thrombocyte development [117], environmental regulation of HSCs [110], mechanosensory regulation of HSC specification [118, 119], brain development related to DNA damage [120] and leukemia [121].

In addition to the aforementioned reporter lines transiently expressing markers of HSC populations, several transgenic lines that permanently label HSC precursors have been also generated to understand the origin of HSCs with lineage-tracing studies. To test whether HSCs
arise from endothelial cells in the zebrafish embryo, the Traver group used a permanent marking system consisting of a floxed reporter transgene and a Cre line driven by the endothelium-specific kdrl upstream promoter/enhancer elements [88]. The transgenic construct was generated by cloning a 6.8-kb DNA fragment of the kdrl promoter/enhancer sequences [122] into the upstream region of a Cre recombinase. The resulting construct Tg(kdrl-Cre) was subcloned into meangnulase plasmid vector, which was then linearized and injected into one-cell-stage zebrafish embryos. In parallel, Tg(actb2:LOXP-STOP-LOXP-DsRedEx), a Cre-mediated genetic switch line, was generated with a 10.5-kb upstream fragment of the β-actin promoter/enhancer followed by a 5.7-kb line, was generated with a 10.5-kb upstream fragment of the β-actin promoter/enhancer sequences [122] into the upstream region of a Cre recombinase. The resulting construct Tg(kdrl-Cre) was subcloned into meangnulase plasmid vector, which was then linearized and injected into one-cell-stage zebrafish embryos. In parallel, Tg(actb2:LOXP-STOP-LOXP-DsRedEx), a Cre-mediated genetic switch line, was generated with a 10.5-kb upstream fragment of the β-actin promoter/enhancer followed by a 5.7-kb floxed STOP cassette and DsRed [88]. DsRed serves as a reporter for Cre-mediated removal of the STOP cassette. The transgenic construct was ligated into the Tol2 vector [123] and the final construct was co-injected with Tol2 mRNA into one-cell stage embryos to produce the transgenic founders. This switch line undergoes excision of the STOP cassette in Cre-expressing cells, permanently marking these cells and their progeny with DsRed expression. Using these combinatorial transgenic lines, Bertrand et al. demonstrated that HSCs originate from embryonic endothelium in the adult kidney marrow. All types of blood cells from zebrafish whole kidney marrow expressed DsRed, suggesting that all the blood lineages arise from endothelial population. In addition, these lines have also been used in endocardial lineage tracing during zebrafish heart regeneration [124].

Furthermore, the Traver group narrowed down the source of HSC populations in the hemogenic endothelium in the ventral wall of the dorsal aorta. Using gata2b as the key marker for this HSC precursor cells, they generated TgBAC(gata2b:KALTA4) to trace cells arising from the hemogenic endothelium [125]. The Gal4 variant KalTA4 was inserted before the start site of gata2b on the BAC through BAC recombineering [126, 127], and the modified BAC plasmid was injected with Tol2 mRNA into Tg(4xUAS:GFP) embryos. Subsequently, the candidate founders were screened for GFP expression in the endothelium. To permanently label gata2b-specific hemogenic endothelium for lineage tracing, Butko et al. used triple transgenic zebrafish lines TgBAC(gata2b:KALTA4); Tg(UAS:Cre, cryaa:Venus); Tg(actb2:LOXP-STOP-LOXP-DsRedEx). In this triple transgenic line, tissue-specifically expressed GAL4 turns on the UAS promoter to induce Cre, which in turn activates permanent DsRed switch in the hemogenic endothelium. The presence of DsRed+ cells in the CHT, thymus and kidney in 3 dpf embryos indicates that HSCs originate from the gata2b-specific hemogenic endothelium in the dorsal aorta [125].

**Lymphoid transgenic lines**

Unlike the primitive wave of hematopoiesis, lymphopoiesis originates solely from definitive HSC progenitor cells [128]. rag1 and rag2 are traditionally used markers for lymphocyte populations during early zebrafish development and are expressed in the thymus and adult kidney where zebrafish lymphocyte maturation occurs [129, 130]. The Lin group generated lymphoid lineage specific transgenic reporter lines using these rag1 and rag2 drivers [131, 132]. First, Tg(rag1:GFP) was generated using reporter constructs containing a 4.7-kb fragment 5’ of the zebrafish rag1 TSS. Chi-induced homologous recombination [133] inserted the GFP gene into the PAC containing the rag1 promoter [131]. In addition, to generate Tg(rag2:GFP) construct, a 6.5-kb fragment of the rag2 TSS was subcloned from the PAC into the 5’ region of a modified GFP reporter gene (GM2) on pBluescript KS(-) plasmid [132]. These lymphoid-specific lines have been used to study lymphocyte development [86], olfactory neuron development [132] and T cell leukemia [134, 135].

**Summary**

Hematopoiesis is highly conserved in zebrafish and mammals. Over the past two decades, diverse blood-specific transgenic reporter lines along with advanced imaging technologies, FACS sorting analysis and various genetic approaches have helped further our understanding of the development and physiological roles of hematopoietic cell lineages. The increasing number of more tailored transgenic fluorescent zebrafish lines is anticipated to widen our understanding of hematopoiesis ontogeny.

**The nervous system**

**Habenula transgenic lines**

The habenula is an evolutionarily conserved brain area of the limbic system that connects the telencephalic nuclei to the brain stem nuclei, such as the interpeduncular nucleus (IPN), ventral tigmental area, and raphe [136]. It is implicated in various behaviors such as pain, stress, anxiety, sleep and reward [137]. Furthermore, the habenulae of many vertebrates show left-right asymmetry with respect to size and neuronal circuits, making it a good model to examine brain asymmetry [138]. To study the physiological roles and asymmetrical development of the habenula, transgenic animal models in which the habenula can be labeled and manipulated are required.

To verify the asymmetrical innervations of the zebrafish dorsal habenula, a homologous region to the mammalian medial habenula [139], Okamoto et al. cloned a 5-kb 5’ upstream sequence and a 0.3-kb intron of the pou4f1 gene that encodes a POU-domain transcription factor into the upstream region of the zebrafish hsp70 promoter.
and EGFP sequences [140], resulting in creation of the Tg(pou4f1-hsp70I:GFP) construct (Table 4). Subsequently, the linearized construct was microinjected into zebrafish embryos to produce transgenic zebrafish [141], in which the medial subdomain and the axonal projections of the dorsal habenula were labeled [140, 142, 143]. The labeled medial subdomain was larger in the right dorsal habenula than in the left [140]. Moreover, the axonal projections from the GFP-expressing neurons specifically terminated in the ventral and intermediate IPN [140]. In addition, Okamoto et al. developed a BAC construct to label and manipulate the zebrafish ventral habenula, a homologous region to the mammalian lateral habenula [139]. The GAL4-VP16 sequence was introduced via BAC recombineering into the BAC clone (CH211-172G6) [144] containing the zebrafish amine oxidase copper containing 1 (aoc1) gene, a ventral habenula specific gene [139], resulting in TgBAC(aoc1:GAL4-VP16) construct [145]. To facilitate its genomic integration, the BAC construct was modified further by introducing an

| Construct name | Construct ID | Transgenic name | Transgenic ID | Target References |
|---------------|--------------|----------------|--------------|-------------------|
| Tg(pou4f1-hsp70I:GFP) | ZDB-TGCON-STRCT-070117-170 | Tg(pou4f1-hsp70I:GFP) | ZDB-ALT-050317-18 | Medial division of the dorsal habenula [140] |
| TgBAC(aoc1:GAL4-VP16) | ZDB-TGCON-STRCT-150312-5 | TgBAC(aoc1:GAL4-VP16) | ZDB-ALT-150312-5 | Ventral habenula [145] |
| Tg[olig2:EGFP] | ZDB-TGCON-STRCT-070117-167 | Tg[olig2:EGFP] | ZDB-ALT-041129-8 | OLs, RG, MNS [150] |
| Tg[elavl3:EGFP] / Tg[HuC:EGFP] | ZDB-TGCON-STRCT-130919-1 | Tg[elavl3:EGFP] / Tg[HuC:EGFP] | ZDB-ALT-130919-2 | Differentiated neurons (motor and sensory) [160] |
| Tg[elavl3:GCaMP3] / Tg[HuC:GCaMP3] | ZDB-TGCON-STRCT-131122-1 | Tg[elavl3:GCaMP3] / Tg[HuC:GCaMP3] | ZDB-ALT-131122-1 | Differentiated neurons (motor and sensory) [162] |
| Tg[1mbp:EGFP] | ZDB-TGCON-STRCT-120103-2 | Tg[1mbp:EGFP] | ZDB-ALT-120103-1 | Myelin sheaths, OLs and Schwann cells [163] |
| Tg[mbp:EGFP-CAXX] | ZDB-TGCON-STRCT-120103-3 | Tg[mbp:EGFP-CAXX] | ZDB-ALT-120103-2 | Myelin sheaths, OLs and Schwann cells [167] |
| Tg[mbpa:GAL4-VP16] | ZDB-TGCON-STRCT-150803-3 | Tg[mbpa:GAL4-VP16] | ZDB-ALT-150803-1 | Myelin sheaths, OLs and Schwann cells [168] |
| Tg(UAS:Eco.NfsB-mCherry) | ZDB-TGCON-STRCT-110215-5 | Tg(UAS:Eco.NfsB-mCherry) | ZDB-ALT-110215-7 | Gal4/UAS-NTR system [168] |
| Tg(-4.9ox10:EGFP) | ZDB-TGCON-STRCT-070117-69 | Tg(-4.9ox10:EGFP) | ZDB-ALT-050913-4 | Neural crest derived cells (Schwann, DRG) and OL lineage cells [180] |
| Tg(gfap:EGFP) | ZDB-TGCON-STRCT-070410-2 | Tg(gfap:EGFP) | ZDB-ALT-060623-4 | RGs in brain, SC, retina and peripheral spinal nerves [182] |
| Tg(gfap:NTR-mCherry) | ZDB-TGCON-STRCT-160630-2 | Tg(gfap:NTR-mCherry) | ZDB-ALT-160630-2 | RGs in brain, SC, retina and peripheral spinal nerves [186] |
| Tg(gfap:Cre-ERT2,cryaa:YFP) | ZDB-TGCON-STRCT-150902-1 | Tg(gfap:Cre-ERT2,cryaa:YFP) | ZDB-ALT-150902-1 | RGs in brain, SC, retina and peripheral spinal nerves [187] |
| Tg(-3.9nesf:EGFP) / Tg(-3.9nesf:GFP) | ZDB-TGCON-STRCT-100308-3 | Tg(-3.9nesf:EGFP) / Tg(-3.9nesf:GFP) | ZDB-ALT-100308-4 | NSCs [183] |
| Tg[fluor:EGFP] | ZDB-TGCON-STRCT-070410-2 | Tg[fluor:EGFP] | ZDB-ALT-070410-12 | RGs in brain, SC, retina and peripheral spinal nerves [183] |
| Tg[BAC(nes:EGFP)] | ZDB-TGCON-STRCT-110309-7 | Tg[BAC(nes:EGFP)] | ZDB-ALT-110309-7 | NSCs [191] |
| Tg(UAS:E1B:DsRed,Hsa.MAPT_F301L) | ZDB-TGCON-STRCT-111028-1 | Tg(UAS:E1B:DsRed,Hsa.MAPT_F301L) | ZDB-ALT-111028-1 | Tauopathic neurons (CNS neurons) [195–199] |
| Tg(slca6a3:EGFP) | ZDB-TGCON-STRCT-111206-9 | Tg(slca6a3:EGFP) | ZDB-ALT-111206-10 | Dopaminergic neurons [202] |
| Tg(slca6a3:CFP-NTR) | ZDB-TGCON-STRCT-160128-1 | Tg(slca6a3:CFP-NTR) | ZDB-ALT-160128-7 | Dopaminergic neurons [204] |
iToL2 cassette via homologous recombination, which was then used to generate transgenic fish [146]. GAL4-VP16 driven GFP+ cells were specifically localized in the ventral habenula, and their axons projected to the median raphe [145]. In this transgenic fish, optogenetics [147] and targeted toxin expression [145] can label or functionally deactivate the ventral habenula.

**Oligodendrocyte transgenic lines**

Multipotent neural progenitor cells produce diverse kinds of neural cells including interneurons, motor neurons and glial cells during vertebrate development. The progenitor cells of the pMN domain in the spinal cord, a part of the CNS, are the representative cell clusters for studying mechanisms underlying early neuroglia development. Since this population is specified by an oligodendrocyte lineage transcription factor 2 (Olig2), Shin et al. generated an olig2 reporter zebrafish line with Tg(olig2:EGFP) construct that was created by inserting EGFP into a BAC clone harboring olig2 regulatory sequences (NCBI accession number NM_178100) via BAC recombiner engineering [148, 149]. This line visualizes progenitor cells and radial glia (RG) of the pMN domain, motor neurons (MNs), oligodendrocyte lineage cells in the spinal cord at early developmental stages, and oligodendrocytes (OLs) in the adult stage [150]. It has been extensively used to study the developmental mechanisms of MNs and OLs from olig2+ progenitor cells [148, 151], regeneration of MNs after injury [152, 153] and the specification and differentiation of OLs [154]. In addition, this transgenic line has been exploited to mark cerebrospinal fluid contacting neurons in the spinal cord [155] and eurydendroid cells in the cerebellum [156].

**HuC transgenic lines**

HuC, a homolog of *Drosophila elav*, is a vertebrate neuron-specific gene [157] that plays important roles in post-mitotic differentiation and maintenance of neuronal cells [158]. As HuC is critical during the early stage of neuronal differentiation, its reporter line was needed to study zebrafish neurogenesis. Thus, Park et al. cloned a 4.6-kb genomic DNA fragment upstream of zebrafish HuC into the 5′ end of the start codon of the GFP gene in CS2A(−) plasmid. The resulting construct Tg(huc:ga4fp) was linearized and microinjected into zebrafish embryos to generate the transgenic line [159], in which all differentiated neurons in the CNS, including motor and sensory neurons, were labeled [159]. This transgenic line was used to determine the developmental status of neurons [159]. The Huc promoter derived from Tg(huc:ga4fp) construct has been extensively used to develop diverse transgenic lines such as Tg(huc:kaede) using phototactivatable fluorescent proteins for lineage tracing [160], Tg(huc:gal4) using GAL4/UAS transactivation system for the targeted gene expression [161], and Tg(huc:GCamp3) for the detection of neuronal activity [162].

**Myelination transgenic lines**

Myelin sheaths are lipid-rich, multilayered membranes around axons, and are generated by OLs in the CNS and Schwann cells in the peripheral nervous system (PNS). They are required for nerve conduction velocity, protection of the axons from environmental risk factors, and supply of some energy sources such as lactate. Upon acute injury, myelin sheaths are lost, yet later on they are regenerated by myelinating glial cells. However, in chronic demyelinating conditions such as multiple sclerosis (MS), regeneration of myelin sheaths is practically impossible. Therefore, treatment of demyelinating diseases entails our deeper understanding of formation of the myelin sheaths. Jung et al. generated transgenic zebrafish to visualize myelinating glial cells and myelin sheaths in the zebrafish CNS and PNS [163]. They constructed Tg(mbp:egfp) by cloning a 2-kb regulatory element of the zebrafish *myelin basic protein (mbp)* into a Tol2-GFP vector lacking the cytomegalovirus (CMV) promoter [163] and established a transgenic line using transposon-mediated integration [164]. In this line, as expected, GFP specifically labeled myelin sheaths and OLs and Schwann cells from embryos to adulthood [163]. This transgenic model has aided in the investigation of the development of OLs in vivo [165] and been used for high-resolution screening of chemical compounds that regulate myelination [166]. In addition, the mbp promoter in Tg(mbp:egfp) was used to develop a Tg(mbp:EGFP-CAX) line using membrane-targeted EGFP to study the dynamics of myelination [167]. Furthermore, using a chemogenetic ablation system, demyelination zebrafish models such as Tg(mbp:gal4vp16;uas:nfsb-mcherry) have been generated [168, 169].

**Neural crest cell (NCC) transgenic lines**

NCCs originate from the neural plate border and migrate to diverse locations to form various cell lineages such as neurons and glia in the PNS, craniofacial cartilage, and smooth muscle [170]. SRY-box transcription factor 10 (Sox10) is a key transcription factor for studying the ontogeny of NC [171] as Sox10 mutations are associated with developmental defects in PNS neurons, ganglia, myelin sheath, and melanocytes [172–174]. Carney et al. generated a transgenic line to monitor NC-derived cells in vivo using the Tg(sox10:egfp) construct [175]. They cloned a 4.9-kb genomic fragment of the zebrafish *sox10* promoter sequences into the XLT.GFPLT.CS2+ vector containing the egfp gene, and then generated a transgenic line using the linearized construct. The resulting
line marked $sox10^+$ NC-derived cells, including Schwann cells, dorsal root ganglion and cranial ganglia [175], uncovered a role of Sox10 in the development of sensory neurons [175], and revealed mechanism underlying NC migration during craniofacial morphogenesis [176, 177]. As Sox10 is necessary for the differentiation of OLs in the CNS [178], this transgenic line also labels OL lineage.

RG transgenic lines

RG are an early form of glial cells observed during the vertebrate neural development, give rise to intermediate neural progenitors and neurons, such as neural stem cells (NSCs), and act as scaffold cells to help the new neurons migrate towards their target locations in the CNS [180, 181]. Thus, defects in the development and function of RG cause neurodevelopmental diseases such as lissencephaly (which means “smooth brain”) [181], highlighting that RG is critical to CNS development. Bernardos et al. generated transgenic zebrafish, in which GFP expression was driven by the regulatory sequences of zebrafish glial fibrillary acidic protein ($g{fap}$). They cloned $g{fap}$ regulatory sequences into the pEGFP-1 vector (Clontech), which was linearized and then microinjected into zebrafish embryos. The resulting $Tg(g{fap}:GFP)$ line displays RG in the brain, spinal cord, retina and the peripheral spinal nerves [182]. In addition, this line has been used to study the characterization of neural progenitor cells in the adult brain [183], the behavior of NSCs in the normal and injured adult brain [184], and functions of Müller glia in the adult retina [185]. Finally, the $g{fap}$ promoter has been used to generate several other transgenic lines, such as $Tg(g{fap}:nfsb-mcherry)$ that can ablate RG [186] and $Tg(g{fap}:C{re}^{ERT2})$ that can track RG lineages [187].

NSC transgenic lines

NSCs play a crucial role in the production of various types of neurons and glia for the formation of the nervous systems and the maintenance of the stem cell niche, which is a key regulator of stem cells in neurogenesis [188]. The mechanisms underlying generation of various differentiated cell types in the CNS have garnered interest. Nestin, an intermediate filament protein, is a widely employed marker of multipotent NSCs [189]. Lam et al. established a transgenic line expressing GFP under the control of the nestin promoter [183]. They cloned a 3.9-kb nestin regulatory region into the pCS2:GFP-Scel vector, and the resulting $Tg(nestin:gf{p})$ construct was microinjected with 1-Scel meganuclease [50, 183]. The $Tg(nestin:gf{p})$ line labeled the progenitor cells with RG-like morphology and self-renewal capacity located in the ventricular zone of the adult CNS [183]. This line has been also used to study the heterogeneity of neural progenitor cells in the adult brain [190] and the regeneration of the injured brain and spinal cord in adult zebrafish [103, 190]. Finally, Kaslin et al. developed a $TgBAC(nestin:gf{p})$ zebrafish line using BAC recombineering to investigate the stem cell niche in adult cerebellum [191].

Tau transgenic lines

Tau is a microtubule-associated protein, is abundantly expressed in the CNS neurons, and maintain the stability of microtubules in neuronal axons [192]. These proteins undergo biochemical modifications such as phosphorylation for cytoskeletal plasticity [193]. However, abnormally phosphorylated tau proteins form neurofibrillary tangles that lead to cell death and several neurodegenerative diseases, including Alzheimer’s disease and chronic traumatic encephalopathy [194]. To develop therapeutic agents to treat and prevent disease progression, it is important to investigate the underlying mechanism of their pathologies. Paquet et al. generated transgenic zebrafish that recapitulate the pathological features of tauopathy [195]. To generate the tauopathy model, they first employed a bidirectional expression system based on the Gal4/UAS system, which allows for simultaneous expression of transgenes in forward or reverse orientations [195]. They inserted the $E1b$ promoter into the flanking sites of the UAS promoter and then cloned human TAU-P301L and DsRed fluorescent protein gene in both directions of the UAS promoter in the pT2KX1Gdeltasplasmid, resulting in construction of $Tg(UAS-E1B:DsRed,Hsa.MAPT\_P301L)$ plasmid, with which the cognate transgenic line was established using Tol2-mediated integration. This line was used to study a role for BDNF signaling in tauopathy [196], microglial dynamics against tauopathic neurons [197], and the mechanism by which pathological tau proteins aggregate [198, 199].

Dopaminergic neuron transgenic lines

Dopaminergic neurons, which synthesize dopamine (DA) from the amino acid tyrosine, play important roles in the regulation of several physiological and behavioral processes, including voluntary movement, mood, reward, addiction, and memory in the CNS [200]. Deficiency of DA and loss of dopaminergic neurons in the substantia nigra elicit several neurological disorders such as Parkinson’s disease (PD) and impaired motor activity [201]. Therefore, it is necessary to study the pathological mechanisms of PD in association with the development of DA neurons. To label DA neurons in vivo, Xi et al. cloned EGFP gene into dopamine transporter (dat) exon 1 in the
PAC clone containing the 13-kb of regulatory sequences of dat and transferred the resulting PAC-EGFP to pGEM-Tol2 vector [202], thereby generating Tol2-PAC-EGFP construct, with which they created the Tg(dat:EGFP) line using Tol2-mediated transgenesis. This line expresses EGFP under the control of dat regulatory elements and thus labeled DA neurons in the ventral diencephalon, retina, olfactory bulb, pretectum and caudal hypothalamus [202]. In this line, individual DAT-expressing neurons can be tracked before and after treatment with L-DOPA, a precursor of dopamine [203]. Moreover, a Tg(dat:CFP-NTR) line that can ablate DA neurons chemogenetically was developed to investigate PD with locomotor phenotype [204].

The urogenital system

Introduction

Nephron segment patterning and cellular composition are conserved between zebrafish and mammals [205]. Zebrafish embryos develop functional pronephros by 2 dpf and a fully functioning mature pronephric kidney by 4 dpf [206, 207]. Many lines labeling pronephric structures have been established (Table 5), allowing the precise determination of the location, function, and expression profiles of particular cells in the kidney and providing extraordinary tools to model and visualize the biological processes underlying kidney development and disease [208].

While fish generate nephrons throughout their lifespan and regenerate nephrons de novo after injury, mammals can only partly repair their nephrons and cannot form new ones [209, 210]. As such, fish are an appropriate model to study molecular mechanism underlying renal regeneration, which in turn may lead to development of therapeutic activation of mammalian renal regeneration. Zebrabsh renal disease models can be generated with genetic alteration, transient gene knockdown or genome-editing technology. Crossing these zebrafish models to transgenic fluorescent zebrafish lines may yield embryos with abnormal pronephros that can be imaged with in vivo fluorescence microscopy. Zebrafish kidney injury models successfully recapitulate the features of mammalian acute kidney injury (AKI), such as characteristic histological changes, reduced renal function and pericardial edema [211–213].

Development of germline requires the specification and survival of primordial germ cells (PGCs) in the embryo as well as the maintenance of gamete production during

| Table 5 Transgenic reporter zebrafish lines targeting the urogenital system |
| Construct name | Construct ID | Transgenic name | Transgenic ID | Target | References |
|----------------|--------------|-----------------|---------------|--------|------------|
| Tg(wt1b:EGFP) | ZDB-TGCON-STRCT-071127-1 | Tg(-26wt1b:EGFP) | ZDB-ALT-071127-1 | Pronephric kidney | [215–220] |
| Tg(lhx1a:EGFP) | ZDB-TGCON-STRCT-100323-1 | Tg(lhx1a:EGFP) | ZDB-ALT-100323-3 | Marginal cells, shield, notochord and bilateral mesoderm | [220, 222, 223] |
| Tg2(cdh17:EGFP) | ZDB-TGCON-STRCT-140116-2 | Tg2(cdh17:EGFP) | ZDB-ALT-140116-11 | Whole length pronephric tubule | [220] |
| Tg(sox10:EGFP) | ZDB-TGCON-STRCT-150414-3 | Tg(PT:EGFP) | ZDB-ALT-150414-3 | Proximal pronephric tubule | [225, 226] |
| Tg(enpep:EGFP) | ZDB-TGCON-STRCT-101123-2 | Tg(enpep:GFP) | ZDB-ALT-101123-3 | Pronephric tubules, ducts and podocyte-like cells of glomeruli | [227–231] |
| Tg(ddx4:ddx4-EGFP) | ZDB-TGCON-STRCT-070814-1 | Tg(vasa:vasa-EGFP) | ZDB-ALT-070814-1 | Proximal pronephric tubule, pituitary gland and adult pronephric duct epithelium | [243, 252–254] |
| Tg(nphs2:GAL4-VP16) | ZDB-TGCON-STRCT-120613-1 | Tg(podocin:GFP) / Tg(2.5nphs2:EGFP) | ZDB-ALT-120613-1 | Podocytes and glomerular epithelial cells | [234–239] |
| Tg(piwi1:EGFP) | ZDB-TGCON-STRCT-110126-5 | Tg(piwi1:EGFP) | ZDB-ALT-110126-6 | PGCs | [245, 257–260] |
| Tg(piwi1:EGFP-UTRNanos3) | ZDB-TGCON-STRCT-181226-1 | Tg(piwi1:EGFP) / Tg(piwi1:GFP) | ZDB-ALT-181226-1 | PGCs | [261–263] |
| Tg(gsdf:EGFP) | ZDB-TGCON-STRCT-130910-1 | Tg(gsdf:EGFP) | ZDB-ALT-130910-2 | Sertoli and granulosa cells | [264, 265] |
Kidney transgenic lines

The Wilms’ tumor suppressor gene wt1 is essential for kidney development and highly conserved among vertebrates. However, most fish species possess two wt1 paralogs, wt1a and wt1b, while mammals have only one wt1 gene [214]. Human WT1 mutations cause Wilms’ tumor, which is childhood kidney cancer, and the developmental anomalies of the urogenital tract. Transgenic lines echoing wt1 gene expression profile have been generated to examine the structure and development of the zebrafish embryonic kidney, the pronephros, and the roles of the wt1 genes in the pronephros development. Among them, the Tg(wt1be:GFP) line was produced by co-injecting into one-cell stage zebrafish embryos I-SceI meganuclease and Tg(wt1be:GFP) plasmid that drives EGFP expression under a 25.9-kb fragment encompassing the entire region between wt1b and the 5’ neighboring gene ga17 minus translational start site of wt1b [215]. In this line, GFP expression recapitulates the expression pattern of endogenous wt1b in the glomeruli. Also labeled in this line are the pronephric tubules and the proximal regions of the ducts, in which endogenous wt1b gene is not observed, allowing for visualization of the entire pronephric kidney encompassing glomeruli, tubules and ducts [216]. Fluorescence imaging of Tg(wt1be:GFP) zebrafish larvae revealed the segmental organization of each nephron into glomerulus, neck, proximal convoluted and straight tubules, corpuscle of Stannius and pronephric duct, which fuse to the cloaca at 2 dpf [217, 218]. The Tg(wt1be:GFP) line has contributed to the research on the development and regeneration of kidney as well as the drug screening against nephrotoxicity and genetic kidney diseases including hereditary glomerulopathies and cystic kidney diseases [217, 219, 220].

The Lim1 homeobox protein, Lhx1, is essential for establishing the kidney field in embryonic development and in kidney regeneration after injury [221]. To develop a quantitative high-content screening assay for agents that increase the Lhx1a expression during development, the Tg(lhx1a:EGFP) line was generated through I-SceI meganuclease-mediated transgenesis [222]. This line labels marginal cells, shield, notochord and bilateral intermediate mesoderm that give rise to the kidney, in addition to the polster and the diencephalon. At later stages, this line also marks the pronephric proximal and distal tubules and duct as well as the anterior lateral line ganglia, forebrain, hindbrain, midbrain, otic vesicle, posterior lateral line ganglia, spinal cord and tailbud [222]. The renal progenitors isolated from Tg(lhx1a:EGFP) can generate new nephrons when introduced into a damaged host kidneys [223]. A high-content assay using Tg(lhx1a:EGFP) zebrafish embryos was developed employing cognition network technology, an artificial intelligence-based image analysis, that can identify small molecules expanding the kidney field [220]. HDAC inhibitors that facilitate recovery from AKI in zebrafish larvae were screened using this zebrafish system as a regeneration model for drug discovery [220].

The zebrafish homolog of mammalian kidney-specific cadherin, cdh17, is expressed in the epithelium and ducts of the entire tubule during larval development and adult [224]. To establish cdh17 reporter zebrafish, a 5-kb genomic region containing the promoter and the 5’UTR sequences of the cdh17 locus was cloned into the 5’ region of the pI-SceI:EGFP plasmid, which was in turn used to generate the Tg(cdh17:egfp) transgenic line via I-SceI meganuclease-mediated transgenesis [220]. This line at 48 hpf labels the kidney including a pair of tubular structures that converge at the cloaca and the tubular subdomains, excluding the glomeruli. At later points of development, however, the entire embryonic tubular segments are labelled. Easily detected in this line is kidney field expansion, which helps identify novel agents that may have the potential to augment kidney regeneration after injury [220].

To visualize pronephric tubular injury caused by gentamicin in zebrafish larvae, the Tg(PT:EGFP) line was generated by expressing EGFP under the sox10 promoter [225]. This line labels cells in the proximal pronephric tubule and NCCs [225]. In contrast, the Tg(cdh17:EGFP) line labels the whole length of the pronephric tubule [220]. These two lines were combined to identify the spatial expression of molecular markers in the infection-associated AKI [226].

The gene enpep encodes glutamyl aminopeptidase implicated in the regulation of blood pressure, blood vessel formation and tumorigenesis. The Tg(enpep:egfp) line was generated, in which EGFP expression is driven by the 2.3-kb enpep promoter [227]. Tg(enpep:GFP) larvae label both pronephric tubules and ducts as well as podocyte-like cells of the glomeruli in early development [227]. This line has been used to identify and characterize the genes associated with pronephric development, kidney diseases and cellular dynamics during adult zebrafish kidney regeneration [228–231].

To understand a role for gtsfβ in kidney tubule morphogenesis, the Tg(gtsfβ:GFP) line was created, in which
GFP is expressed under the control of the gshβ promoter [232]. In this line, GFP is expressed in proximal pronephric tubules as well as in pituitary gland during embryogenesis and kidney duct epithelium in adult fish, conferring a tool to study the tubular development with genetic or chemical approaches [232]. This line has deepened our understanding of the signaling pathways that trigger renal tubular damage during lethal lipopolysaccharide (LPS)-induced septic shock in an AKI model, suggesting that this transgenic zebrafish is an ideal model to carry out in vivo screen for potential antisepsis therapeutic strategies [233].

The Tg(podocin:GFP) line was generated using the Tg(-2.5nphs2:EGFP) construct via Tol2 transposon-mediated transgenesis. EGFP expression is driven by the 2.5-kb zebrafish podocin promoter [234]. The loss of glomerular GFP expression in the Tg(podocin:GFP) embryos indicates developmental aberrations of and injury to the glomerulus [235–239]. To investigate the podocyte injury, several techniques to induce zebrafish AKI are available. For example, Tg(nphs2:GAL4-VP16) [240] and Tg(pod:NTR-mCherry) [210, 241] lines were established to temporally ablate or injure podocytes, the visceral glomerular epithelial cells, by expressing NTR under the control of the zebrafish nphs2 promoter. To visualize the spatiotemporal pattern of mesonephrogenesis and investigate the development and postinjury regeneration of the mesonephros in adult zebrafish, cytoxin was induced in podocytes of larval or adult transgenic zebrafish that expressed NTR under the control of the podocin promoter, eliciting glomerular injury including podocyte loss, reduced expression of podocyte marker genes and foot process effacement [210, 240, 241].

**Germline and gonadal transgenic lines**

ddx4 [DEAD (Asp-Glu-Ala-Asp) box polypeptide 4], which was previously known as vasa or vas, is a conserved gene belonging to the DEAD box helicase family. Being specifically expressed in the germline across metazoans, it has been extensively used as a germ cell marker [242]. The Tg(vasa:vasa-EGFP) zebrafish was generated using the Tg(vasa:vasa-eGFP) or Tg(ddx4:ddx4-EGFP) construct that includes the vasa regulatory region fused to egfp [243]. This line expresses EGFP in the PGCs from 24 hpf onwards [243]. During early development, however, only maternal GFP signal is detected in the germ line, and zygotic transcription of vasa-eGFP driven by the vasa regulatory region begins after sexual differentiation when the germ cells enter meiosis [244, 245]. This line has been widely used to investigate roles of the genes involved in gonadal development [246–251] as well as the gonadal stem cell features [252, 253]. To visualize the germline in real time and in vivo from 1 dpf through 12 weeks post-fertilization, the Tg(vasa:vasa-EGFP) line was combined with zebrafish mutants mitfΔm2 [melanocyte inducing transcription factor a; ZFIN ID ZDB-ALT-990423-22] or mpv17Δb/b [mitochondrial inner membrane protein 17; ZDB-GENO-141218-8], which renders both larvae and adult zebrafish transparent [254].

ziwi encodes an RNA-binding zinc finger protein and similar to vasa, it is specifically expressed in the zebrafish germline throughout development [255, 256]. Tg(piwil1:EGFP) was generated using the Tg(piwil1:EGFP) construct comprising the promoter elements of ziwi (piwil1), the zebrafish homolog for Droso phila piwi, fused to egfp [245]. In this line, ziwi:EGFP is maternally supplied in embryos and zygotic EGFP is first detected around at 7 dpf [245], implying that this line is less dependent on maternal EGFP to label germ cells early in development than the Tg(vasa:vasa-EGFP) line. The Tg(piwil1:EGFP) line has characterized the roles of the genes involved in gonad development [257–260].

Another transgenic line that can display the PGCs, Tg(piwil1:egfp-UTrnanos3), was generated using the Tg(piwil1:EGFP-UTrnanos3) construct [261]. This line labels PGCs at the shield stage, oogonia and oocytes at early stages in the ovary, and spermatogonia, spermatocyte and spermatid at early stage in the testis, thus illuminating the zebrafish germline throughout the lifespan [261]. This line has been utilized to analyze gene functions in gonadal development [262, 263].

gsdf is expressed in the Sertoli and granulosa cells. To illuminate these cells, the Tg(gsdf:EGFP) line was generated using the Tg(gsdf:EGFP) construct including the 2-kb proximal promoter region of gsdf via Tol2 transposase-mediated transgenesis [264]. EGFP expression is first detected at 16 dpf and progresses from the posteroventral region lining the swim bladder towards the anteroventral region and the urogenital papilla at 19–42 dpf prior to the sexual differentiation of the gonad [264]. This line has been also employed to visualize the 3D architecture of the testis and its cellular content [265].

**Digestive system**

**Introduction**

The zebrafish digestive system can be divided into the gastrointestinal (GI) tract including esophagus, intestinal bulb, mid intestine, posterior intestine and anus, and accessory organs such as liver and pancreas. To investigate developmental process of the digestive system in zebrafish, transgenic fluorescent zebrafish lines have been widely used. Here, we summarize the representative zebrafish transgenic reporter lines that label various cell types in the GI tract, liver and pancreas (Table 6).
The intestinal architecture of the zebrafish closely resembles the mammalian counterpart [266–268]. To understand molecular mechanisms underlying development of the intestinal lumen and enteric nervous system (ENS), various transgenic fluorescent zebrafish lines have been generated.

To establish a gut-specific expression of target genes, the Jen-Leih Wu group at Academia Sinica constructed a plasmid harboring 4.5-kb intestine specific promoter of zebrafish intestine fatty acid-binding protein (I-FABP) fused to RFP. This plasmid was linearized and microinjected into zebrafish embryos to generate the Tg(fabp2:RFP) line, which labeled the zebrafish intestinal bulb and mid intestine [269]. His group also reported that the 192-bp region in the I-FABP promoter sufficed for gut-specific expression [270]. This Tg(fabp2:RFP) line carrying the 4.5-kb promoter has been widely used to study functional organization of zebrafish intestine and human GI diseases such as inflammatory bowel disease and enterocolitis [271–274].

In addition, the Bagnat group at Duke University Medical Center linearized the TgBAC(cldn15la:GFP) construct with AsiSI (New England Biolabs) and microinjected the linearized construct into one-cell stage zebrafish embryos to generate the TgBAC(cldn15la:GFP) line, which exhibits GFP expression in the membranes of epithelial cells spanning from the intestinal bulb to posterior intestine [275]. This line has been used to study the mechanism of the intestinal epithelial formation and patterning as well as intestinal barrier function and inflammation in zebrafish [275–279].

The ENS is the largest part of the vertebrate PNSs. To image the ENS development in real time and in vivo, the Iain Shepherd group at Emory University constructed a plasmid encoding the wavelength-sensitive fluorescent protein Kaede under the control of the enhancer of paired-like homeobox 2b (phox2b), a gene involved in the development of enteric neuron progenitors [280]. Subsequently, they microinjected the resulting construct with Tol2 transposase mRNA into embryos to generate the Tg(-8.3bphox2b:Kaede) line. This line has been used to study de novo enteric neurogenesis in post-embryonic zebrafish, a role of retinoic acid in colonization of the gut by vagal neural crest cells [281, 282], and Hirschsprung disease caused by failure of the ENS [283, 284].

Liver

Cell types and metabolic pathways in the liver are comparable between zebrafish and mammals [285–289]. Hence, zebrafish has emerged as an important animal model to study the development [290, 291] and diseases of the liver [292–296]. Transgenic lines that express fluorescent proteins in hepatocytes, intrahepatic biliary cells and hepatic stellate cells have been developed.

To label zebrafish hepatocytes with EGFP, the Wu group at National Taiwan Ocean University cloned the promoter region of liver-FABP (L-FABP) into the

### Table 6 Transgenic reporter zebrafish lines targeting the digestive system

| Construct name | Construct ID | Transgenic name | Transgenic ID | Target | References |
|----------------|--------------|----------------|---------------|--------|------------|
| Tg(fabp2:RFP) | ZDB-TGCON-STRCT-110208-2 | Tgfabp2:RFP| ZDB-ALT-110208-3 | Intestinal bulb and mid intestine | [269, 271–274] |
| TgBAC(cldn15la:GFP) | ZDB-TGCON-STRCT-140613-1 | TgBAC(cldn15la:GFP)| ZDB-ALT-140613-1 | Epithelial cells (intestinal bulb to posterior intestine) | [275–279] |
| Tgf(-8.3bphox2b:Kaede) | ZDB-TGCON-STRCT-150305-1 | Tgf(-8.3bphox2b:Kaede)| ZDB-ALT-150305-1 | ENS | [280–284] |
| Tgf(-2.8fabp10a:EGFP) | ZDB-TGCON-STRCT-070117-123 | Tgf(-2.8fabp10a:EGFP)| ZDB-ALT-110523-10 | Hepatocytes | [297–302] |
| Tg(EPV/TP1-Mmu.Hbb.EGFP) | ZDB-TGCON-STRCT-090625-1 | Tg(EPV/TP1-Mmu.Hbb.EGFP)| ZDB-ALT-090625-1 | Intrahepatic biliary cells | [304–308] |
| TgBAC(hand2:EGFP) | ZDB-TGCON-STRCT-110128-8 | TgBAC(hand2:EGFP)| ZDB-ALT-110128-40 | Hepatic stellate cells | [309–312, 325] |
| Tgl(ela3:EGFP) | ZDB-TGCON-STRCT-070117-92 | Tgela3:EGFP| ZDB-ALT-060710-10 | Pancreatic exocrine cells | [313] |
| Tg(gcga:GFP) | ZDB-TGCON-STRCT-070215-2 | Gcga:GFP| ZDB-ALT-070215-2 | Pancreatic α-cells | [314, 317, 318] |
| Tgmnx1:GFP | ZDB-TGCON-STRCT-070117-40 | Gmnx1:GFP| ZDB-ALT-051025-4 | Pancreatic β-cells | [315, 316] |
| Tg(ins:DsRed) | ZDB-TGCON-STRCT-080826-1 | Gmns:DsRed| ZDB-ALT-080826-1 | Pancreatic β-cells | [317, 318] |
| TgXla.Eef1a1:GFP | ZDB-TGCON-STRCT-070117-34 | Gmns:GFP| ZDB-ALT-030702-2 | Digestive tract, liver and pancreas | [298, 320–324] |
pEGFP-C1. The resulting construct pLF2.8-EGFP was used to generate the Tg(-2.8fabc10a:EGFP) line [297]. More than 50 studies have taken advantage of this line to investigate liver development. For example, this line was used to determine the role of cellular signaling pathways in liver development [298, 299] as well as drug-induced hepatic injury or genetically-induced hepatic diseases [300–302]. Of note, the Didier Stainier group at University of California, San Francisco established the Tg(fabc10a:DsRed) line to study patterning and differentiation of the hepatopancreatic ductal system [303].

To create a Notch reporter line, the Steven Leach group at Johns Hopkins University fused six copies of the Epstein-Barr Virus terminal protein 1 (EPV.TP1) promoter region to β-globin minimal promoter and EGFP using the Gateway technology. The resulting construct Tg(EPV.TP1-Mmu.Hbb:EGFP) and Tol2 transposase mRNA were coinjected into zebrafish embryos. As a result, the Tg(EPV.TP1-Mmu.Hbb:EGFP) line was generated, which labels intrahepatic biliary cells [304]. This line has been adopted to reveal molecular mechanisms by which endodermal notch signaling, cannabinoid receptor signaling and inhibitor of DNA binding (Id) protein induce bile duct development and liver diseases [305–308].

To generate a transgenic fluorescent zebrafish line that marks hepatic stellate cells, the Stainier group employed the regulatory region of heart and neural crest derivatives expressed 2 (hand2), which encodes a basic helix-loop-helix transcription factor implicated in organ development, determination of intestine left–right asymmetry and nervous system development. A BAC clone harboring the regulatory region of hand2 was used to generate the TgBAC(hand2:EGFP) line by BAC transgenesis. Because activation of hepatic stellate cells plays a key role in regeneration upon hepatic injury, this line has been employed to study liver diseases, to visualize activation of hepatic stellate cells upon ethanol-induced hepatic injury, and to create NTR / MTZ induced hepatic fibrosis models [309–312].

**Endodermal organs**

The Tg(Xla.Eef1a1:GFP) line (more popularly called the gutGFP line) was generated incidentally by the Herwig Baier group at University of California, San Francisco. Microinjection of linearized plasmid pESG that encodes GFP downstream of the Xenopus elongation factor (EF)-1α promoter into zebrafish embryos yielded the line. GFP expression in this line was ubiquitous at early stage, yet was restricted to the endoderm, eye and hatching gland by 22 hpf [320]. The insertion site of the transgene has not been characterized yet. As this line expresses GFP in the developing digestive tract, liver and pancreas, most of early research on these organs had benefited from the gutGFP line. Using this line, the Stainier group published three papers on zebrafish liver, pancreas and intestinal epithelium morphogenesis, respectively [320–322]. These papers laid the foundation for the research on the zebrafish GI tract and its accessory organs. As such, it is not surprising that this line has significantly contributed to uncover molecular mechanisms underlying development of these organs. For example, Wnt signaling was shown to be required for liver development and regeneration [298], pancreas associated transcription factor 1α (Ptf1a) was reported to regulate endocrine versus exocrine fate in pancreas development [323], and the target of rapamycin (TOR) signaling was demonstrated to regulate intestinal epithelium morphogenesis [324].
Intracellular organelles

Introduction

Zebrafish is an excellent animal model to study embryogenesis and human diseases because it allows for visualization and manipulation of cellular organelles and processes [326]. Development of organisms is governed by the spatial and temporal regulations of proliferation, apoptosis, migration, differentiation and morphological specialization. Proteins are synthesized at the endoplasmic reticulum, glycosylated at the Golgi complex, and trafficked to target locations, either intracellular or extracellular [327]. Secretory proteins go through the secretory pathway comprising diverse protein complexes at each step to recruit Rab GTPases and SNARE proteins to tether vesicles to target organelles and facilitate membrane fusion [328]. The understanding of this pathway from the perspective of intracellular organelles is fundamental to study the behavior and biology of cells in organisms. Many human genetic diseases such as mitochon- driopathies [329] and diseases of protein trafficking [330] are linked to abnormal organelle functions. Hence, development of transgenic reporter lines labeling organelles was required.

Nucleus transgenic lines

To visualize overall zebrafish embryonic development, the $Tg(\alpha ctb2: H2B-GFP)$ line was generated (Table 7). Under the regulation of $\beta$-actin promoter, this line expresses fusion proteins of human histone-2 and GFP that localize to chromatin, and has been used to analyze cell cycle length, nuclear architecture and the temporal dynamics of the nuclear compartment [331]. When driven by tissue specific promoters such as islet ($Tg(\text{isl1a:NLS-GFP})$), mylpfa ($Tg(\text{mylpfa:Hsa.HIST1H2BJ-GFP})$) and runx ($Tg(Mmu.Runx1:NLS-mCherry)$), these lines can easily monitor neuronal, muscular and hematopoietic development in real time and in vivo [332–334].

Golgi apparatus transgenic lines

To image the trans-Golgi secretory pathway in the developing embryos in real time and in vivo, the Gerhart group cloned amino acids 1–6 from human B4GALT1 fused to GFP to the region downstream of the zebrafish semi-ubiquitous $\beta$-actin promoter using Gateway technology, thereby creating $Tg(\beta$-act:GalT-GFP). Galactotransferase (GalT) is a Golgi-retained enzyme. Using this construct and Tol2 mediated transgenesis [335], they generated the

Table 7  Transgenic reporter zebrafish lines targeting intracellular organelles

| Construct name                | Construct ID       | Transgenic name                        | Transgenic ID | Target                             | References |
|-------------------------------|--------------------|----------------------------------------|---------------|------------------------------------|------------|
| $Tg(\alpha ctb2: H2B-GFP)$   | ZDB-TGCON-STRCT-170619-1 | $Tg(\alpha ctb2: H2B-GFP)^{12}$ | ZDB-ALT-170619-1 | Chromatin (Nucleus)                | [331]      |
| $Tg(\text{isl1a:NLS-GFP})$   | ZDB-TGCON-STRCT-201203-1 | $Tg(\text{isl1a:NLS-GFP})^{158}$ | ZDB-ALT-201203-5 | Neurons                            | [334]      |
| $Tg(\text{Mmu.Runx1:NLS-mCherry})$ | ZDB-TGCON-STRCT-150512-2 | $Tg(\text{Mmu.Runx1:NLS-mCherry})^{1,200}$ | ZDB-ALT-150512-2 | Hematopoietic cells                | [110]      |
| $Tg(\text{mylpfa:Hsa.HIST1H2BJ-GFP})$ | ZDB-TGCON-STRCT-160728-1 | $Tg(\text{mylpfa:Hsa.HIST1H2BJ-GFP})^{12}$ | ZDB-ALT-160728-1 | Muscular cells                     | [333]      |
| $Tg(\alpha ctb2: H4GALT1-GFP)$ | ZDB-TGCON-STRCT-120419-3 | $Tg(\alpha ctb2: H4GALT1-GFP)^{12}$ | ZDB-ALT-120419-5 | Golgi apparatus                    | [336]      |
| $Tg(\text{h2afx:EGFP-rab5c})$ | ZDB-TGCON-STRCT-111017-3 | $Tg(\text{h2afx:EGFP-rab5c})^{12}$ | ZDB-ALT-111017-5 | Early endosome                     | [341]      |
| $Tg(\text{h2afx:EGFP-rab7a})$ | ZDB-TGCON-STRCT-111017-4 | $Tg(\text{h2afx:EGFP-rab7a})^{12}$ | ZDB-ALT-111017-6 | Late endosome                      | [341]      |
| $Tg(\text{h2afx:EGFP-rab11a})$ | ZDB-TGCON-STRCT-111017-7 | $Tg(\text{h2afx:EGFP-rab11a})^{12}$ | ZDB-ALT-111017-7 | Recycling endosome                 | [341]      |
| $Tg(CMV:EGFP-map1lc3b)$      | ZDB-TGCON-STRCT-091029-1 | $\text{GFP-Lc3, Tg(CMV:EGFP-map1lc3b)}^{12}$ | ZDB-ALT-091029-2 | Lysosomes                          | [349]      |
| $Tg(CMV:EGFP-gabarapa)$      | ZDB-TGCON-STRCT-091029-2 | $\text{GFP-Gabarapa, Tg(CMV:EGFP-gabarapa)}^{12}$ | ZDB-ALT-091029-1 | Lysosomes                          | [349]      |
| $Tg(\text{fabp10a:EGFP-map1lc3b})$ | ZDB-TGCON-STRCT-120710-1 | $Tg(\text{fabp10a:EGFP-map1lc3b})^{12}$ | ZDB-ALT-120710-1 | Liver lysosomes                    | [351]      |
| $Tg(Xla.Eef1a1:mlsEGFP)$     | ZDB-TGCON-STRCT-171018-1 | $Tg(Xla.Eef1a1:mlsEGFP)^{12}$ | ZDB-ALT-171018-4 | Lysosomes                          | [352–354]  |
| $Tg(\text{gna2:GFP-map1lc3b})$ | ZDB-TGCON-STRCT-140416-5 | $Tg(\text{gna2:GFP-map1lc3b})^{12}$ | ZDB-ALT-140416-5 | Photoreceptor cell lysosomes       | [337]      |
| $Tg(\text{Xla.Eef1a1:EGFP-LC3-RFP-LC3ΔG})$ | ZDB-TGCON-STRCT-090309-1 | $Tg(\text{Xla.Eef1a1:EGFP-LC3-RFP-LC3ΔG})^{12}$ | ZDB-ALT-090309-2 | Mitochondria                       | [357]      |
Endosome transgenic lines

Rab proteins mediate endocytosis and vesicle trafficking in development, disease and cellular homeostasis [339]. More than 65 Rab genes have been identified to date and are essential communicators with their effector proteins in formation, motilities and tethering of vesicles in membrane-bounded organelles [340]. To facilitate imaging of endosomes in real time and in vivo, Clark et al. generated transgenic fluorescent zebrafish lines [341]. To label early, recycling and late endosomes, they chose Rab5c (early), Rab11a (recycling), and Rab7 (late) based on localization and functions of these endosome subtypes in zebrafish and other species.

RAB5 has been studied for receptor-mediated signaling pathway in vesicle transport and fusion of membrane between clathrin coated vesicles of the plasma membrane and newly formed endosomes [342]. Among five zebrafish paralogs (rab5a-c, rab5aa and rab5ab) of Drosophila Rab5, rab5c was selected for an early endosomal marker due to its ubiquitous expression in zebrafish embryos [343]. Rab11 protein is localized to the pericentral-recycling compartment, the recycling endosome [342]. Zebrafish rab11 has four paralogs: rab11a, rab11alike, rab11ba and rab11bb. Of which, Rab11a was used as a recycling endosome marker due to its highest protein sequence similarity to Drosophila Rab11a. In addition, Rab7 is localized to late endosomes and implicated in biogenesis of late endosomes and targeting trafficking vesicles to degradation [342]. Unlike Rab5c and Rab11a, the zebrafish Rab7 has no paralog. Rab5c, Rab11a and Rab7 were N-terminally fused to EGFP individually and then cloned downstream of the quasi-ubiquitous h2afx promoter [341]. The resulting constructs were used to generate respective transgenic fluorescent zebrafish lines via Tol2 mediated transgenesis [335]: Tg(h2afx:EGFP-Rab5c), Tg(h2afx:EGFP-rab11a) and Tg(h2afx:EGFP-Rab7). These lines were validated with co-localization between the fluorescent puncta from the EGFP-Rab lines and lipophilic dye FM4-64 at the early, recycling and late endosomes, respectively. Furthermore, these lines have been utilized to study the endocytosis, recycling endosomes of protein secretory pathway and their intracellular dynamics in development [344] and human diseases [231].

Autophagy transgenic line

Autophagy is an essential cellular degradative pathway that delivers cytoplasmic cargo to the lysosome. During autophagy, an autophagosome, a double-membrane vesicle, fuses with a lysosome, hydrolyses, and then degrades the macromolecules, which in turn recycle back into the cytosol for use as cellular nutrients. Autophagy plays an important role in host immune defense, tumor suppression, cardiovascular disease, gastrointestinal disorders, neurodegeneration and longevity [345–348]. The real-time monitoring of autophagy is an essential tool to reveal the process of autophagosome formation and to determine clearance of aggregate-prone proteins in vivo small molecule screens for the autophagy regulators. To this end, He et al. generated two transgenic fluorescent zebrafish lines expressing a GFP fused to Lc3 [Tg(CMV:EGFP-map1lc3b)] or to Gabarap N-terminally [Tg(CMV:EGFP-gabarapa)] under the control of the constitutive CMV promoter [349]. Gabarap is gamma-aminobutyric acid type A receptor-associated protein. When they treated 3 dpf Tg(cmav:GFP-Lc3) embryos with rapamycin and lysosomal inhibitors, they observed that GFP puncta were formed and translocated to lysosomes. These lines have been adopted to monitor autophagic activity during embryogenesis in real time and in vivo [348, 350]. In addition, expression of GFP-Lc3 in a tissue specific manner [liver: Tg(fabp10:EGFP-Lc3); photoreceptor: Tg(TaCP:GFP-Lc3)] has made it feasible to study tissue-specific autophagy [337, 351].

To measure autophagic activity in real time and in vivo, the Mizushima group generated the Tg(Xla.Eef1a1:GFP-LC3-RFP-LC3ΔG) construct. Once translated, this gene product is cleaved by ATG4 protease into GFP-LC3 and RFP-LC3ΔG. The former is conjugated with phosphatidyl-lethanolamine (PE), targeted to the autophagosomes, and then degraded upon fusion of the autophagosomes with the lysosomes. However, RFP-LC3ΔG cannot be conjugated with PE due to lack of C-terminal glycine, which renders RFP-LC3ΔG stable in the cytoplasm. As such, the GFP/RFP signal ratio can act as readout of autophagic activity. Using this construct, they generated a Tg(Xla.Eef1a1:GFP-LC3-RFP-LC3ΔG) line via Tol2 mediated transgenesis [352]. This line has been employed to assess effect of 1-phenyl 2-thiourea (PTU) on autophagic flux in zebrafish embryos [353] and to demonstrate requirement of autophagy for maturation of surfactant-containing lamellar bodies in the zebrafish swim bladder [354].

Mitochondria transgenic line

Mitochondria are double-membrane bound organelles and play an important role in energy production, metabolism, apoptosis, calcium storage and cellular signaling [355]. As such, mitochondria research has been a hot field and thus a need arose to image mitochondria in zebrafish. The Choi group cloned the mitochondrial localization sequence (MLS) of zebrafish cytochrome c
oxidase subunit VIII A (cox8a) (NCBI accession number NM_001303053.1) to the 5′ end of EGFP gene on a mini-Tol2 vector [356, 357]. Its expression was driven by a Xenopus EF-1 α promoter, a ubiquitous promoter. The resulting construct Tg(Xla.Eef1α1:mlsEGFP) was used to generate a zebrafish line expressing mitochondrially targeted GFP via Tol2 transposon-mediated transgenesis [164]. As expected, mitochondria in almost all types of cells in this line were labeled with GFP and mitochondrial fragmentation in chemically induced apoptosis was able to be imaged in real time and in vivo [357]. This line has been employed to visualize mitochondria in motor neurons [358], erythroid cells [359], photoreceptor cells [360] and skeletal muscles [361], and to study degradation of mitochondrial GFP [362] and effect of polycyclic aromatic hydrocarbon and hypoxia on mitochondrial content [363]. In addition, MLS-EGFP derived from the construct Tg(Xla.Eef1α1:mlsEGFP) was used to image dopaminergic neurons [364, 365], Mauthner cells [366], mitophagy [367] and sensory neurons [368].

Conclusion and perspectives

This review summarized the transgenic fluorescent zebrafish lines that have been broadly used in biomedical research. As our understanding of promoters and enhancers widens, CRISPR/Cas9 mediated knock-in technology makes translational fusion easier than ever, and super resolution microscopes become commercially available, transgenic fluorescent zebrafish would make it feasible to image and track each and every protein in real time and in vivo in the near future. In addition, spatiotemporal regulation of fluorescent protein expression could be achieved by combining the transgenic fluorescent zebrafish lines with chemical or physical induction of fluorescent protein expression, which may become an invaluable tool in biomedical research.

Abbreviations

AIM: Acute kidney injury; AP: Anterior posterior; BAC: Bacterial artificial chromosome; BMP: Bone morphogenetic protein; BRP: BEM response element; Cas9: CRISPR-associated protein 9; CF: Cyan fluorescent protein; CHT: Caudal hematopoietic tissue; CMV: Cytomegalovirus; CNC: Cranial neural crest; CNS: Central nervous system; COVID-19: Coronavirus disease-19; CRISPR: Clustered regularly interspaced short palindromic repeats; DA: Dopamine; dat: Dopamine transporter; dizi-fgfl1: Dominant negative Fgf receptor; DNA: Deoxyribonucleic acid; dpg: Days post fertilization; DsRed: Discosoma sp. red; E. coli: Escherichia coli; EF-1 α: Elongation factor-1α; EGFP: Enhanced green fluorescent protein; ENS: Enteric nervous system; EPV: Epstein-Barr virus; FGF: Fibroblast growth factor; FACS: Fluorescence-activated cell sorting; FGF: Fibroblast growth factor; FOXP2: Forkhead box protein terminal protein 1; ER: Endoplasmic reticulum; FACS: Fluorescence-activated cell sorting; FGF: Fibroblast growth factor; FOXP2: Forkhead box protein P2; GaIT: Galacto transferase; GFAP: Glial fibrillary acidic protein; GFP: Green fluorescent protein; GI: Gastrointestinal; HDAC: Histone deacetylase; hpf: Hours post fertilization; HSC: Hematopoietic stem cells; hsp: Heat shock protein; i-FABP: Intestinal fatty acid-binding protein; ICM: Intermediate cell mass; IPN: Interpeduncular nucleus; LEF: Lymphoid enhancer-binding factor; LFBAP: Liver fatty acid binding protein; LPS: Lipopolysaccharide; MBP: Myelin basic protein; MH: Midbrain-hindbrain; MLS: Mitochondrial localization sequence; MN: Motor neurons; MPEG: MacroPhage Expressed Gene 1; MPX: Myeloperoxidase; MS: Multiple Sclerosis; MTZ: Metronidazole; NC: Neural crest; NCBi: National Center for Biotechnology Information; NLS: Nuclear localization sequence or signal; NSC: Neural stem cell; NTR: Nitroreductase; OL: Oligodendrocyte; PAC: P1-derived artificial chromosome; PD: Parkinson’s disease; PGC: Primordial germ cell; PIM: Posterior lateral mesoderm; PNS: Peripheral nervous system; PTU: 1-Phenyl 2-thiourea; RBC: Red blood cell; RG: Radial glia; RNA: Ribonucleic acid; RUNX2: Runt-related transcription factor 2; SARS-CoV-2: Severe acute respiratory syndrome coronavirus 2; SOX9: SRY-Box transcription factor 9; TCF: T cell factor protein; Tg: Transgenic; TGF-β: Transforming growth factor beta; TORA: Target of rapamycin; TSS: Transcription start site; UAS: Upstream Activation Sequence; WISH: Whole mount in situ hybridization; ZFIN: Zebrafish information network.

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References

1. Hamilton F. An account of the fishes found in the river Ganges and its branches. London: Constable & Co.; 1822.
2. Streissinger G, Walker C, Dower N, Krauber D, Singer F. Production of clones of homzygous diploid zebra fish (Brachydanio rerio). Nature. 1981;291(5813):293–6.
3. Stuart GW, McMurray JV, Westerfield M. Replication, integration and stable germ-line transmission of foreign sequences injected into early zebrafish embryos. Development. 1988;103(2):403–12.
4. Komori T, Yagi H, Nomura S, Yamaguchi A, Sasaki K, Deguchi K, et al. Targeted disruption of Cbfa1 results in a complete lack of bone formation owing to maturational arrest of osteoblasts. Cell. 1997;89(5):755–64.
5. Ruzicka L, Howe DG, Ramachandran S, Toro S, Van Slyke CE, Bradford VM, et al. The Zebrafish information network: new support for...
non-coding genes, richer gene ontology annotations and the alliance of genome resources. Nucleic Acids Res. 2019;47(D1):D867–73.
6. Schier AF, Talbot WS. Molecular genetics of axis formation in zebrafish. Annu Rev Genet. 2003;37:561–613.
7. Fuentes R, Tajer WS. Molecular genetics of axis formation in zebrafish. Curr Top Dev Biol. 2020;140:341–89.
8. Sidi S, Goutel C, Peyriéras N, Rosa F. Maternal induction of ventral fate by zebrafish radar. Proc Natl Acad Sci U S A. 2003;100(6):3315–20.
9. Ro H, Dawid IB. Organizer restriction through modulation of Bmp signaling in the zebrafish. FEBS J. 2007;274(12):2960–7.
10. Pyati UJ, Webb AE, Kimelman D. Transgenic zebrafish reveal stage-specific roles for Bmp signaling in ventral and posterior mesoderm development. Development. 2005;132(10):2333–43.
11. Polli BR, Kimmel CB. Dynamic and spatially restricted expression domains of the zinc finger transcription factor sp7 in the cranial neural crest. PLoS Genet. 2012;8(5):e1002710.
12. Dorsky RI, Sheldahl LC, Moon RT. A transgenic Lef1/beta-catenin-dependent reporter is expressed in spatially restricted domains throughout zebrafish development. Dev Biol. 2002;241(2):229–37.
13. Lawson ND, Weinstein BM. In vivo Wnt signaling tracing through a transgenic biosensor fish reveals novel axis activity domains. Dev Biol. 2012;366(2):327–40.
14. Lee HJ, Hou Y, Chen Y, Dailey ZZ, Riddihough A, Jang HS, et al. Response to Nodal morphogen gradient is determined by the kinetics of target gene induction. Elife. 2015;4:e05042.
15. Barolo S. Transgenic Wnt/TCF pathway reporters: all you need is Lef? Methods. 2006;39(3):183–8.
16. Geurtzen K, Knopf F, Cherkura A, Kurth T, Hans S, Weber C, et al. Bone regeneration via dedifferentiation of osteoblasts in the zebrafish fin. Dev Cell. 2011;20(5):713–24.
17. DeLaurier A, Eames BF, Blanco-Sanchez B, Peng G, He X, Swartz ME, et al. Zebrafish sp7EGFP: a transgenic for studying otic vesicle formation, skeletogenesis, and bone regeneration. Genesis. 2010;48(8):505–11.
18. Hammond CL, Schulte-Merker S. Two populations of endochondral osteoblasts with differential sensitivity to Hedgehog signalling. Development. 2009;136(23):3991–4000.
19. Yang Z, Jiang H, Chiahsanchasilak T, Geng Y, Xu W, Heintz N, et al. Modified bacterial artificial chromosomes for zebrafish transgenesis. Methods. 2006;39(3):183–8.
20. Paul S, Schindler S, Giovannone D, de Millo TA, Mariani FV, Crump JG. Ihh induces hybrid cartilage-bone cells during zebrafish jawbone regeneration. Development. 2016;143(2):2066–76.
21. Li N, Selker E, Elks F, Croucher P, Roehl HH. Tracking gene expression during zebrafish osteoblast differentiation. Dev Dyn. 2009;238(2):459–66.
22. Knopf F, Hammond C, Cherkura A, Kurth T, Hans S, Weber C, et al. Bone regeneration via dedifferentiation of osteoblasts in the zebrafish fin. Dev Cell. 2011;20(5):713–24.
23. Geurtzen K, Knopf F, Wehner D, Huitema LF, Schulte-Merker S, Weidinger G. Mature osteoblasts dedifferentiate in response to traumatic bone injury in the zebrafish fin and skull. Development. 2014;141(11):2225–34.
24. Mishra R, Sehring I, Cederlund M, Mulaw M, Weidinger G. NF-kappaB signaling negatively regulates osteoblast dedifferentiation during zebrafish bone regeneration. Dev Cell. 2020;52(2):167-82.e7.
25. Kaga E, Roy P, Asselin G, Hu G, Simonet J, Stanley A, et al. Osterix/Sp7 limits cranial bone initiation sites and is required for formation of sutures. Dev Biol. 2016;413(2):160–72.
26. Grabher C, Joly JS, Wittbrodt J. Highly efficient zebrafish transgenesis mediated by the meganuclease I-SceI. Methods Cell Biol. 2004;77:381–401.
27. prokop DJ, Kivirikko KI. Collagens: molecular biology, diseases, and potentials for therapy. Annu Rev Biochem. 1995;64:403–34.
53. Zhou G, Gorosalo S, Mukhopadhay Y, Kefebvre V, Smith CN, Eberspaecher H, et al. A 182 bp fragment of the mouse pro alpha 1(III) collagen gene is sufficient to direct chondrocyte expression in transgenic mice. J Cell Sci. 1995;108(Pt 12):3677–84.

54. Dale RM, Topczewski J. Identification of an evolutionarily conserved regulatory element of the zebrafish col2a1a gene. Dev Biol. 2011;357(2):518–31.

55. Kawakami K, Shima A. Identification of the Tol2 transposase of the medaka fish Orzayas latipes that catalyzes excision of a nonautonomous Tol2 element in zebrafish Danio rerio. Gene. 1999;240(1):239–44.

56. Kwan KM, Fujimoto E, Graber C, Mangum BD, Hardy ME, Campbell DS, et al. The Tol2kit: a multsite gateway-based construction kit for Tol2 transposon transgenesis constructs. Dev Dyn. 2007;236(1):3088–99.

57. Dougherty M, Kamel G, Grimaldi M, Gfrerer L, Shubinets V, Ethier R, et al. Askary A, Mork L, Paul S, He X, Izuhara AK, Gopalakrishnan S, et al. Distinct requirements for wnt4a and irf6 in extension and integration mechanisms during zebrafish palate morphogenesis. Development. 2013;140(1):76–81.

58. Aikaray A, Mork L, Paul S, He X, Izuhara AK, Gopalakrishnan S, et al. Iroquois proteins promote skeletal joint formation by maintaining chondrocytes in an immature state. Dev Cell. 2015;35(3):358–65.

59. Unlu G, Qi X, Garmazon ER, Melville DB, Patel N, Rushing AR, et al. Phenome-based approach identifies R1C1-linked Mendelian syndrome genes. J Biol. 2020;26(1):e145242.

60. Bonkowski JL, Wang X, Fujimoto E, Lee JE, Chien CB, Dorsky RI. Domain-specific regulation of fox2b CNS expression by left. BMC Dev Biol. 2008;8:103.

61. Eames BF, DeLaurier A, Ullmann B, Huycke TR, Nichols JT, Dowd J, et al. FishFace: interactive atlas of zebrafish craniofacial development at cellular resolution. BMC Dev Biol. 2013;13:23.

62. McGurk PD, Swartz ME, Chen JW, Galloway JL, Eberhart JK. In vivo imaging of the zebrafish heart tube is a dynamic model for cardiogenesis. Proc Natl Acad Sci U S A. 2008;105(19):6919–24.

63. Nichols JT, Blanco-Sanchez B, Brooks EP, Parthasarathy R, Dowd J, et al. Ligament versus bone cell identity in the zebrafish hyoid skeleton is regulated by mef2ca. Development. 2016;143(3):4450–40.

64. Nichols JT, Pan L, Moens CB, Kimmel CB. barx1 represses joints and promotes cartilage in the craniofacial skeleton. Development. 2015;142(23):4430–40.

65. Muyrers JP, Zhang Y, Testa G, Stewart AF. Rapid modification of bacterial transposon transgenesis constructs. Dev Dyn. 2007;236(11):3088–99.

66. Eames BF, DeLaurier A, Ullmann B, Huycke TR, Nichols JT, Dowd J, et al. FishFace: interactive atlas of zebrafish craniofacial development at cellular resolution. BMC Dev Biol. 2013;13:23.
99. Niethammer P, Grabher C, Look AT, Mitchison TJ. A tissue-scale gradient of hydrogen peroxide mediates rapid wound detection in zebrafish. Nature. 2009;459(7249):996–9.

100. Yoo SK, Deng Q, Cavarj PJ, Wu YI, Hahn KM, Huttenlocher A. Differen-
tial regulation of protrusion and polarity by PI3K during neutrophil motility in live zebrafish. Dev Cell. 2010;18(2):226–36.

101. Balla KM, Lugo-Villarino G, Spitsbergen JM, Stachura DL, Hu Y, Banuelos K, et al. Eosinophils in the zebrafish: prospective isolation, characterization, and eosinophilia induction by heminth determin-
ists. Blood. 2010;116(19):3944–54.

102. Pei W, Tanaka K, Huang SC, Xu L, Liu E, Sinclair J, et al. Extracellular HSP60 triggers tissue regeneration and wound healing by regulating inflammatory and cell proliferation. NPJ Regen Med. 2016;1:16013.

103. Goldshmit Y, Ztal TE, Jusuef PR, Hall TE, Nguyen-Chi M, Currie PD. Fgf-dependent glial cell bridges facilitate spinal cord regeneration in zebrafish. J Neurosci. 2012;32(22):7477–92.

104. Evans MA, Smart N, Dube KN, Bollini S, Clark JE, Evans HG, et al. Thy-
mosin beta[4]-sulfoxide attenuates inflammatory cell infiltration and promotes cardiac wound healing. Nat Commun. 2013;4:2081.

105. Misselbeck K, Parolo S, Lorenzini F, Savoca V, Leonardelli L, Bora P, et al. A network-based approach to identify deregulated pathways and drug effects in metabolic syndrome. Nat Comm. 2019;10(1):5215.

106. Ferrero G, Mahony CB, Dupuis E, Yvernogeau L, Di Ruggiero E, Pope HM, Voigt MM. Peripheral glia have a pivotal role in the initial response to axon degeneration of peripheral sensory neurons in zebrafish. PLoS ONE. 2014;9(7):e103283.

107. Li D, Xue W, Li M, Dong M, Wang J, Wang X, et al. VCAM-1(mac) trans-
tion regulated genes in zebrafish. Dev Cell. 2004;7(1):133–44.

108. Orkin SH, Zon LI. Hematopoiesis: an evolving paradigm for stem cell biology. Cell. 2008;132(4):631–44.

109. Mahony CB, Bertrand JY. How HSCs colonize and expand in the fetal niche of the vertebrate embryo: an evolutionary perspective. Front Cell Dev Biol. 2019;7:34.

110. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299(5608):887–90.

111. Chen J, Jette C, Kanki JP, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299(5608):887–90.

112. Kawakami K, Takeda H, Kawakami N, Kobayashi M, Matsuda N, Mishina M. A transposon-mediated gene trap approach identifies developmentally regulated genes in zebrafish. Dev Cell. 2004;7(1):133–44.

113. Distel M, Wullimann MF, Koster RW. Optimized Gal4 genetics for permanent gene expression mapping in zebrafish. Proc Natl Acad Sci U S A. 2009;106(32):13365–70.

114. Bussmann J, Schulte-Merker S. Rapid BAC selection for tol2-mediated transgenesis in zebrafish. Development. 2011;138(19):4327–32.

115. Jessen JR, Distel M, Lin S. Artificial chromosome transgenesis reveals long-distance negative regulation of rag1 in zebrafish. Nat Genet. 1999;23(1):15–6.

116. Jessen JR, Jessen TN, Vogel SS, Lin S. Concurrent expression of recombina-
tion activating genes 1 and 2 in 2 zebrafish olfactory sensory neurons. Genesis. 2001;29(4):156–62.

117. Jessen JR, Meng A, McFarlane RJ, Paw BH, Zon LI, Smith GR, et al. Modification of bacterial artificial chromosomes through chi-stimulated homologous recombination and its application in zebrafish transgen-
esis. Proc Natl Acad Sci U S A. 1998;95(9):5121–6.

118. Langenau DM, Traver D, Fernando AA, Kutok JL, Aster JC, Kanki JP, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299(5608):887–90.

119. Chen J, Jette C, Kanki JP, et al. Myc-induced T cell leukemia in transgenic zebrafish. Science. 2003;299(5608):887–90.

120. Hikosaka O. The habenula: from stress evasion to value-based decision-
-making. Nat Rev Neurosci. 2011;12(7):503–13.

121. Bianco RH, Wilson SW. The habenular nuclei: a conserved asymmetric relay station in the vertebrate brain. Philos Trans R Soc Lond B Biol Sci. 2009;364(1519):1005–20.

122. Arro A, Aizawa H, Takaokoh M, Kobayashi M, Takahashi R, Aoki T, et al. Identification of the zebrafish ventral habenula as a homolog of the mammalian lateral habenula. J Neurosci. 2010;30(4):1566–74.

123. Aizawa H, Bianco RH, Hamaoka T, Miyashita T, Lemura G, Concha ML, et al. Laterotopic representation of left-right information on the dorso-ventral axis of a zebrafish midbrain target nucleus. Curr Biol. 2005;15(3):238–43.

124. Higashijima S, Okamoto H, Ueno N, Hotta Y, Eguchi G. High-frequency generation of transgenic zebrafish which reliably express GFP in whole muscles or the whole body by using promoters of zebrafish origin. Dev Biol. 1997;192(2):289–99.

125. Chou MY, Arro R, Kinoshita M, Cheng BW, Shimazaki H, Agetsuma M, et al. Social conflict resolution regulated by two dorsal habenular subregions in zebrafish. Science. 2016;352(6281):87–90.

126. Kim E, Jeong I, Chung AJ, Kim S, Kwon SH, Seong JY, et al. Distribution and neuronal circuit of spexin 1/2 neurons in the zebrafish CNS. Sci Rep. 2019;9(1):5025.

127. Kimura Y, Okamura Y, Higashijima S. A link between zebrafish homolog of Chx10, marks ipsilateral descending excitatory interneurons that...
participate in the regulation of spinal locomotor circuits. J Neurosci. 2006;26(21):5684–97.

145. Amo R, Fredes F, Kinoshita M, Aoki R, Aizawa H, Agetsuma M, et al. The habenulo-raphe serotonergic circuit encodes an aversive expectation value essential for adaptive active avoidance of danger. Neuron. 2014;84(4):1034–48.

146. Suster ML, Sumiyama K, Kawakami K. Transposon-mediated BAC transgenesis in zebrafish and mice. BMC Genomics. 2009;10:477.

147. Andalman AS, Burns VM, Lovett-Barron M, Broxton M, Poole B, Yang SJ, et al. Neuronal dynamics regulating brain and behavioral state transitions. Cell. 2019;177(4):970–85.e20.

148. Shin J, Park HC, Topczewska JM, Mawdsley DJ, Appel B. Neural cell fate analysis in zebrafish using olig2 BAC transgensics. Methods Cell Biol. 2003;75(1):27–14.

149. Yu D, Ellis HM, Lee EC, Jenkins NA, Copeland NG, Court DL. An efficient recombination system for chromosome engineering in Escherichia coli. Proc Natl Acad Sci U S A. 2000;97(11):5978–83.

150. Shin J, Poling J, Park HC, Appel B. Notch signaling regulates neural precursor allocation and binary neuronal fate decisions in zebrafish. Development. 2007;134(10):1911–20.

151. Park HC, Shin J, Roberts RK, Appel B. An olig2 reporter gene marks oligodendrocyte precursors in the postembryonic spinal cord of zebrafish. Dev Dyn. 2007;236(12):3402–7.

152. Reimer MM, Sorensen I, Kuscha V, Frank RE, Liu C, Becker CG, et al. Motor neurons expressing PKD2L1: evidence for a conserved system from fish to mammal. Dev Dyn. 2010;239(2):592–7.

153. Ohnmacht J, Yang Y, Maurer GW, Barreiro-Iglesias A, Tsarouchas TM, Sidik H, Talbot WS. A zinc finger protein that regulates oligodendrocyte precursor allocation and binary neuronal fate decisions in zebrafish. Dev Dyn. 2007;236(12):3402–7.

154. Sato T, Takahashi M, Okamoto H. HuC/kaede, a useful tool to label neural morphologies in networks in vivo. Genesis. 2006;44(3):136–42.

155. Faucherre A, Lopez-Schier H, Delaying Gal4-driven gene expression in the zebrafish with morpholinos and Gal80. PLoS ONE. 2011;6(1):e16587.

156. Panier T, Romano SA, Olive R, Pietri T, Sumbre G, Candelier R, et al. Fast functional imaging of multiple brain regions in intact zebrafish larvae using selective plane illumination microscopy. Front Neural Circuits. 2013;7:65.

157. Jung SH, Kim S, Chung AI, Kim HT, So JH, Ryu J, et al. Visualization of myelination in GFP-transgenic zebrafish. Dev Dyn. 2010;239(2):592–7.

158. Fisher S, Grice EA, Vinton RM, Bessling SL, Urasaki A, Kawakami K, et al. Evaluating the biological relevance of putative enhancers using To2 transposon-mediated transgenesis in zebrafish. Nat Protoc. 2006;1(3):129–35.

159. Chung AI, Kim S, Kim H, Bae YK, Park HC. Microarray screening for genes involved in oligodendrocyte differentiation in the zebrafish CNS. Exp Neurobiol. 2011;20(2):85–91.

160. Malataesta P, Goto T. Radial glia—from boring cables to stem cell stars. Development. 2013;140(3):483–6.

161. Krieglstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci. 2009;32:149–84.

162. Bernardos RL, Raymond PA. GFAP transgenic zebrafish. Gene Expr Patterns. 2006;6(8):1007–13.

163. Loye A, Lethe FJ, Gotz M, et al. A direct role for Sox10 in specification of neural crest-derived enteric neurons. Proc Natl Acad Sci U S A. 2009;106(18):7614–9.

164. Sheppard A, Szabo A, Trepat X, Mayor R. Supracellular contraction at the rear of neural crest cell groups drives collective chemotaxis. Science. 2018;362(6412):339–43.

165. Chung AI, Kim S, Kim H, Bae YK, Park HC. Microarray screening for genes involved in oligodendrocyte differentiation in the zebrafish CNS. Exp Neurobiol. 2011;20(2):85–91.

166. Malataesta P, Goto T. Radial glia—from boring cables to stem cell stars. Development. 2013;140(3):483–6.

167. Krieglstein A, Alvarez-Buylla A. The glial nature of embryonic and adult neural stem cells. Annu Rev Neurosci. 2009;32:149–84.

168. Bernardos RL, Raymond PA. GFAP transgenic zebrafish. Gene Expr Patterns. 2006;6(8):1007–13.

169. Loye A, Lethe FJ, Gotz M, et al. A direct role for Sox10 in specification of neural crest-derived enteric neurons. Proc Natl Acad Sci U S A. 2009;106(18):7614–9.

170. Jacob C. Transcriptional control of neural crest specification into peripheral lineages. BioEssays. 2006;28(8):788–98.
192. Drechsel DN, Hyman AA, Cobb MH, Kirschenr MW. Modulation of the dynamic instability of tubulin assembly by the microtubule-associated protein tau. Mol Biol Cell. 1992;3(10):1141–54.

193. Noble W, Hanger DP, Miller CC, Lovestone S. The importance of tau phosphorylation for neurodegenerative diseases. Front Neurol. 2013;4:83.

194. Goedert M, Eisenberg DS, Crowther RA. Propagation of tau aggregates and neurodegeneration. Annu Rev Neurosci. 2017;40:189–210.

195. Paquet D, Bhat R, Sydow A, Mandelkov EM, Berg S, Hellberg S, et al. A zebrafish model of tauopathy allows in vivo imaging of neuronal cell death and drug evaluation. J Clin Invest. 2009;119(5):1382–95.

196. Barbereau C, Yehya A, Silhol M, Cubedo N, Verdier JM, Maurice T, et al. Neuroprotective brain-derived neurotrophic factor signaling in the TAU-P301L tauopathy zebrafish model. Pharmacol Res. 2020;158:108465.

197. Hassan-Abdi R, Brenet A, Bennici M, Yanicostas C, Soussi-Yanicostas N. Neurons expressing pathological tau protein trigger dramatic changes in microglial morphology and dynamics. Front Neuropsi. 2019;13:1199.

198. Giustiniani J, Chambraud B, Sardin E, Dounane O, Guillemeau K, Chambraud B, et al. H537T expression is critical for the abnormal phosphorylation of tau in Alzheimer’s disease-related tau pathology. Brain. 2015;138(Pt 5):1339–54.

199. Bjorklund A, Dunnett SB. Dopamine neuron systems in the brain: an update. Trends Neurosci. 2007;30(5):194–202.

200. Jankovic J. Parkinson’s disease: clinical features and diagnosis. J Neurol Neurosurg Psychiatry. 2008;79(6):368–76.

201. Drummond IA, Majumdar A, Hentschel H, Elger M, Solnica-Krezel L, Schier AF, et al. Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. Development. 2010;54(4):731–6.

202. Xiu Y, M, Godoy R, Hatch G, Postrs L, Ebker M. Transgenic zebrafish expressing green fluorescent protein in dopaminergic neurons of the ventral diencephalon. Dev Dyn. 2011;240(11):2539–47.

203. Stednitz SJ, Freshner B, Shelton S, Shen T, Black D, Gahtan E, et al. Selective toxicity of L-DOPA to dopamine transporter-expressing neurons and locomotor behavior in zebrafish larvae. Neurotoxicol Teratol. 2015;52(Pt A):51–6.

204. Godoy R, Noble S, Yoon K, Anisman H, Ekker M. Chemogenetic ablation of dopaminergic neurons leads to transient locomotor impairments in zebrafish larvae. J Neurochem. 2015;139(4):793–804.

205. Grunwald DJ, Eisen JS. Headwaters of the zebrafish—emergence of a new model vertebrate. Nat Rev Genet. 2002;3(9):717–24.

206. Drummond IA, Majumdar A, Hentschel H, Elger M, Solnica-Krezel L, Schier AF, et al. Early development of the zebrafish pronephros and analysis of mutations affecting pronephric function. Development. 1998;125(23):4655–67.

207. Drummond IA. The zebrafish pronephros: a genetic system for studies of kidney development. Pediatr Nephrol. 2000;14(5):428–35.

208. Tobin DM, May RC, Wheeler RT. Zebrafish: a see-through host and a model of infection-associated acute kidney injury. Am J Phys Renal Physiol. 2014;319(F3):1227–31.

209. Oltrabella F, Pietka G, Ramirez IB, Mironov A, Starborg T, Drummond IA, et al. Development of an automated imaging pipeline for the analysis of adult nephron progenitors capable of kidney regeneration in zebrafish. Mech Dev. 2002;115(1–2):15–26.

210. Cianciolo Cosentino C, Skrypnik N, Brill L, Chita B, Novitskaya T, Woods C, et al. Histone deacetylase inhibitor enhances recovery after AKI. J Am Soc Nephrol. 2013;24(6):943–53.

211. Seiler C, Pack M. Transgenic labeling of the zebrafish pronephric duct and tubules using a promoter from the enpep gene. Gene Expr Patterns. 2011;11(1–2):118–21.

212. Bedwell VM, Person AD, Larson JD, McLoon A, Balicunas D, Clark KJ, et al. The lineage-specific gene ponz1 is essential for zebrafish pronephric and pharyngeal arch development. Development. 2012;139(4):793–804.

213. Ko YA, Yi H, Quo C, Huang S, Park J, Ledo N, Kottingen A, et al. Genetic-variation-driven gene-expression changes highlight genes with important functions for kidney disease. Am J Hum Genet. 2017;100(6):940–53.

214. McCampbell KK, Springer KN, Wingert RA. Analysis of nephron segmentation. Kidney Int. 2008;73(10):1120–7.

215. Perner B, Englert C, Lomzer NF, Wingert RA. Laser ablation of the zebrafish pronephric duct. PLoS ONE. 2014;9(6):e97806.

216. Bollig F, Mehringer R, Pemer B, Hartung C, Schafer M, Scharl M, et al. Identification and comparative expression analysis of a second wt1 gene in zebrafish. Dev Dyn. 2006;235(2):554–61.

217. Gehring J, Pandey G, Westhoff JH. Zebrafish as a model for drug screening in genetic kidney diseases. Front Pediatr. 2016;4:183.

218. Wingert RA, Davidson AJ. The zebrafish pronephros: a model to study nephron segmentation. Kidney Int. 2008;73(10):1120–7.

219. Westhoff JH, Giselbrecht S, Schmidt M, Schindler S, Beales PL, Tonshoff B, et al. Development of an automated imaging pipeline for the analysis of the zebrafish larval kidney. PLoS ONE. 2013;8(12):e82137.

220. Sanker S, Cino MC, Vollmer LL, Goldberg ND, McDermott LA, Hukriede NA, et al. Development of high-content assays for kidney progenitor cell expansion in transgenic zebrafish. J Biomol Screen. 2013;18(10):1193–202.

221. Cirio MC, Hui Z, Haldin CE, Cosentino CC, Stuckenholz C, Chen X, et al. Lhx1 is required for specification of the renal progenitor cell field. PLoS ONE. 2011;6(4):e18858.

222. Swanhart LM, Takahashi N, Jackson RL, Gibson GA, Watkins SC, Dawib IV, et al. Characterization of an lhx1a transgenic reporter in zebrafish. Int J Dev Biol. 2010;54(4):731–6.

223. Diep CQ, Ma D, Deo RC, Holm TM, Naylor RW, Arora N, et al. Identification of adult nephron progenitors capable of kidney regeneration in zebrafish. Nature. 2011;470(7332):95–100.

224. Holm JF, Ramachandran A, Reuter K, Wallevåg E, Collins-Racie L, Crocker K, et al. Cadherin-17 is required to maintain pronephric duct integrity during zebrafish development. Mech Dev. 2002;115(1–2):15–26.

225. Cianciolo Cosentino C, Skrypnik N, Brill L, Chita B, Novitskaya T, Woods C, et al. Histone deacetylase inhibitor enhances recovery after AKI. J Am Soc Nephrol. 2013;24(6):943–53.

226. He B, Ebarasi L, Zhao Z, Guo J, Ojala JR, Hultenby K, et al. Lmx1b and Shh expression in zebrafish. Am J Physiol Renal Physiol. 2010;299(5):F1040–7.
240. Wan X, Lee MS, Zhou W. Dosage-dependent role of Rac1 in podocyte injury. Am J Physiol Renal Physiol. 2016;310(8):F777–84.

241. Zhou W, Hildebrandt F. Inducible podocyte injury and proteinuria in transgenic zebrafish. J Am Soc Nephrol. 2012;23(6):1039–47.

242. Gustafson EA, Wessel GM. Vasa genes: emerging roles in the germ line and in multipotent cells. BioEssays. 2010;32(7):626–37.

243. Krovel AV, Olsen LC. Expression of a vas::EGFP transgene in primordial germ cells of the zebrafish. Mech Dev. 2002;116(1–2):141–50.

244. Krovel AV, Olsen LC. Sexual dimorphic expression pattern of a splice variant of zebrafish vasa during gonadal development. Dev Biol. 2004;271(1):190–7.

245. Leu DH, Draper BW. The ziwi promoter drives germline-specific gene expression in zebrafish. Mech Dev. 2002;116(1–2):141–50.

246. Houwing S, Berezikov E, Ketting RF. Zili is required for germ cell differentiation and maturation in zebrafish. EMBO J. 2006;25(20):2702–11.

247. Huang HY, Houwing S, Kaaij LJ, Meppelink A, Redl S, Gauci S, et al. Tdrd1 acts as a molecular scaffold for Pwi proteins and piRNA targets in zebrafish. EMBO J. 2011;30(16):3298–308.

248. Crespo D, Lemos MS, Zhang YT, Sañan D, Norberg B, Bogerd J, et al. Phospho2 inhibits spermatagonia differentiation in zebrafish: interaction with Fsh and an androgen. J Endocrinol. 2020;244(1):163–75.

249. Nobrega RH, Morais RD, Crespo D, de Waal PP, de Franca LR, Schulz RW, et al. Kawasaki T, Saito K, Shinya M, Olsen LC, Sakai N. Production of zebrafish spermatozoa via Igf3. Endocrinology. 2015;156(10):3804–17.

250. Draper BW, McCallum CM, Moens CB. nanos1 is required to maintain spermatogonia and spermatocytes. Biol Reprod. 2014;90(2):45.

251. Kaufman OH, Lee K, Martin M, Rothhamel S, Marlow FL. rbpms2 expression in zebrafish. Genes Dev. 2020;83(4):533–9.

252. Leerberg DM, Sano K, Draper BW. Fibroblast growth factor signaling pathway. Biol Reprod. 2014;90(2):45.

253. Presslauer C, Bizuayehu TT, Razmi K, Fernandes JM, Babiak I. See-GFP transgenic fish. Cell. 2007;129(1):69–82.

254. Dranow DB, Hu K, Bird AM, Lawry ST, Adams MT, Sanchez A, et al. Bmp15 is an oocyte-produced signal required for maintenance of the adult female sexual phenotype in zebrafish. PLoS Genet. 2010;6(9):e1001323.

255. Kaufman OH, Lee K, Martin M, Rothamel S, Marlow FL. bphox2b functions in Balbiani body architecture and ovary fate. PLoS Genet. 2018;14(7):e1007489.

256. Leeb-Lundberg DM, Sano K, Draper BW. Fibroblast growth factor signaling is required for early somatic gonad development in zebrafish. PLoS Genet. 2017;13(9):e1006993.

257. Ye D, Zhu L, Zhang Q, Xiong F, Wang H, Wang X, et al. Abundance of early embryonic primordial germ cells promotes zebrafish female differentiation as revealed by lifetime labeling of germ line. Mar Biotechnol. 2019;21(2):217–28.

258. Feng K, Cui X, Song Y, Tao B, Chen J, Wang J, et al. Gnrh3 regulates PGC proliferation and sex differentiation in developing zebrafish. Endocrinology. 2020;161(1):bqaa048.

259. Gauthier A, Sohm F, Joly JS, Le Gac F, Lareyre JJ. The proximal promoter region of the zebrafish gsdf gene is sufficient to mimic the spatiotemporal expression pattern of the endogenous gene in Sertoli and granulosa cells. Biol Reprod. 2011;85(6):1240–51.

260. Fretaud M, Riviere L, Job E, Gay S, Lareyre JJ, Joly JS, et al. High-resolution 3D imaging of whole organ after clearing: taking a new look at the zebrafish testis. Sci Rep. 2017;7:43012.

261. Lickwar CR, Camp JG, Weiser M, Cocchiara JL, Kingsley DM, Furey TS, et al. Genomic dissection of conserved transcriptional regulation in intestinal epithelial cells. PLoS Biol. 2017;15(8):e2002054.

262. Wallace AS, Burns AJ. Development of the enteric nervous system, smooth muscle and interstitial cells of Cajal in the human gastrointestinal tract. Cell Tissue Res. 2005;319(3):367–82.

263. Kuil LE, Chauhan RK, Cheng WW, Hofstra RMW, Alves MM. Zebrafish: a model organism for studying enteric nervous system development and disease. Front Cell Dev Biol. 2020;8:620073.

264. Her GA, Chiang CC, Wu JL. Zebrafish intestinal fatty acid binding protein (i-FABP) gene promoter drives gut-specific expression in stable transgenic fish. Genesis. 2004;38(1):26–31.

265. Her GM, Yeh YH, Wu JL. Functional conserved elements mediate intestinal-type fatty acid binding protein (i-FABP) expression in the gut epithelia of zebrafish larvae. Dev Dyn. 2004;230(4):734–42.

266. Okuda KS, Misa JP, Oehlers SH, Hall CJ, Eilert F, Alsmain S, et al. A zebrafish model of inflammatory lymphangiogenesis. Biol Open. 2015;4(10):1270–80.

267. Oehlers SH, Flores MV, Hall CJ, Crosier KE, Crosier PS. Retinoic acid suppresses intestinal mucus production and exacerbates experimental enterocolitis. Dis Model Mech. 2012;5(4):457–67.

268. Oehlers SH, Flores MV, Okuda KS, Hall CJ, Crosier KE, Crosier PS. A chemical enterocolitis model in zebrafish larvae that is dependent on microbiota and responsive to pharmacological agents. Dev Dyn. 2011;240(1):288–98.

269. Wang Z, Du J, Lam SH, Mathavan S, Matsuda G, Gong Z. Morphological and molecular evidence for functional organization along the rostrocaudal axis of the adult zebrafish intestine. BMC Genomics. 2010;11:392.

270. Alvers AL, Ryan S, Scherz PJ, Huisken J, Bagnat M. Single continuous lumen formation in the zebrafish gut is mediated by smooth-dependent tissue remodeling. Development. 2014;141(5):1110–9.

271. Rodriguez-Fraticelli AE, Bagwell J, Bosch-Forteza M, Boncompagni M, Reglero-Real N, Garcia-Leon MJ, et al. Developmental regulation of apical endocytosis controls epithelial patterning in vertebrate tubular organs. Nat Cell Biol. 2015;17(3):241–50.

272. Marjoram L, Alvers A, Deerehake M, Bagwell J, Mankiewicz J, Cicchini JL, et al. Epigenetic control of intestinal barrier function and inflammation in zebrafish. Proc Natl Acad Sci U S A. 2015;112(29):2770–7.

273. Solis CJ, Hamilton MK, Carullo M, Garcia-Lopez JP, Navarrete P, Rocino C. Retinoic acid temporally orchestrates colonization of the gut by vagal neural crest cells. Dev Biol. 2014;390(2):392.

274. Uribe RA, Hong SS, Bronner ME. De novo enteric neurogenesis in postembryonic zebrafish from Schwann cell precursors rather than resident cell types. Development. 2020;147(13):dev166619.

275. Guiri K, Schirmer D, Cheng WW, Chauhan RK, Antinolo G, Berrios C, et al. Whole exome sequencing coupled with unbiased functional analysis reveals new Hirschsprung disease genes. Genome Biol. 2017;18(1):148.

276. Sribudiani Y, Chauhan RK, Alves MM, Petrova L, Brosens E, Harrison C, et al. Identification of variants in RET and IHH pathway members in a large family with history of hirschsprung disease. Gastroenterology. 2018;155(1):118–29.e6.
285. Holtta-Vuori M, Salo VT, Nyberg L, Brackmann C, Enejder A, Panula P, et al. Zebrafish: gaining popularity in lipid research. Biochim J. 2010;429(2):235–42.
286. Pasenik MJ, Cinaroglu A, Gao C, Sadler KC. Hepatic steatosis in response to acute alcohol exposure in zebrafish requires sterol regulatory element-binding protein activation. Hepatology. 2009;49(2):443–52.
287. Goldsmith JR, Jobin C. Think small: zebrafish as a model system of organismal biology. J Biomed Biotechnol. 2012;2012:817341.
288. Tran S, Nowicki M, Chatterjee D, Gerlai R. Acute and chronic ethanol exposure differentially alters alcohol dehydrogenase and aldehyde dehydrogenase activity in the zebrafish liver. Prog Neuropsycopharmacol Biol Psychiatry. 2015;56:221–6.
289. Chu J, Sadler KC. New school in liver development: lessons from zebrafish. Hepatology. 2009;50(5):1656–63.
290. Ober EA, Field HA, Stainier DY. From endoderm formation to liver and pancreas development in zebrafish. Mech Dev. 2003;120(1):5–18.
291. Tao T, Peng J. Liver development in zebrafish (Danio rerio). J Genet Genomics. 2009;36(6):325–34.
292. Goessling W, Sadler KC. Zebrafish: an important tool for liver disease research. Gastroenterology. 2015;149(6):1361–77.
293. Vliegenthart AD, Tucker CS, Del Pozo J, Dear JW. Zebrafish as model organisms for studying drug-induced liver injury. Br J Clin Pharmacol. 2014;78(8):1217–27.
294. Kim SH, Wu SY, Baek JI, Choi SY, Su Y, Flynn CR, et al. A post-developmental genetic screen for zebrafish models of inherited liver disease. PLoS ONE. 2015;10(5):e0125980.
295. Park KH, Kim SH. Low dose of chronic ethanol exposure in adult zebrafish induces hepatic steatosis and injury. Biomed Pharmacother.
296. 2019;117:109719.
297. Park KH, Ye W, Zhang J, Kim SH. Palmitic acid-enriched diet induces hepatic steatosis and injury in adult zebrafish. 2019;16(6):497–504.
298. Her GM, Chiang CC, Chen WY, Wu JL. In vivo studies of liver-type fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (Danio rerio). FLEs Lett. 2003;538(1–3):125–33.
299. Goessling W, North TE, Lord AM, Ceol C, Lee S, Weidinger G, et al. APC mutant zebrafish uncover a changing temporal requirement for wnt signaling in liver development. Dev Biol. 2008;320(1):161–74.
300. Li YH, Chen MH, Gong HY, Hu SY, Li YW, Lin GH, et al. Progranulin A-mediated MET signaling is essential for liver morphogenesis in zebrafish. J Biol Chem. 2010;285(52):41001–9.
301. Amali AA, Rekha RD, Lin CJ, Wang WL, Gong HY, Her GM, et al. Thioacetamide induced liver damage in zebrafish embryo as a disease model for steatohepatitis. J Biomed Sci. 2006;13(2):225–32.
302. Zhang Y, Liu K, Hassan HM, Guo H, Ding P, Han L, et al. Liver fatty acid binding protein deficiency provokes oxidative stress, inflammation, and apoptosis-mediated hepatotoxicity induced by pyrazinamide in zebrafish larvae. Antimicrob Agents Chemother. 2016;60(12):3747–56.
303. Amali AA, Rekha RD, Lin CJ, Wang WL, Gong HY, Her GM, et al. Thioacetamide-induced liver damage in zebrafish embryo as a disease model for steatohepatitis. J Biomed Sci. 2006;13(2):225–32.
304. Zhang Y, Liu K, Hassan HM, Guo H, Ding P, Han L, et al. Liver fatty acid binding protein (L-FABP) gene expression in liver of transgenic zebrafish (Danio rerio). FEBS Lett. 2003;538(1–3):125–33.
305. Cox NJ, Unlu G, Bisnett BJ, Meister TR, Condon BM, Luo PM, et al. Trafficking mechanisms of protein trafficking pathway: Biochemistry. 2018;57(1):91–107.
306. De Matteis MA, Luini A. Mendelian disorders of membrane trafficking. N Engl J Med. 2016;375(5):457–61.
307. Cox NJ, Unlu G, Bisnett BJ, Meister TR, Condon BM, Luo PM, et al. Dynamic glycosylation governs the vertebrate COPII protein trafficking pathway. Biochemistry. 2018;57(1):91–107.
308. Unlu G, Levic DS, Melville DB, Knapak EW. Trafficking mechanisms of extracellular matrix macromolecules: insights from vertebrate development and human diseases. Int J Biochem Cell Biol. 2014;47(5):57–67.
309. Tsedensodnom O, Vacaru AM, Howarth DL, Yin C, Sadler KC. Ethanol metabolism and oxidative stress are required for unfolded protein response activation and steatosis in zebrafish with alcoholic liver disease. Dis Model Mech. 2013;6(5):1213–26.
310. Howarth DL, Yin C, Vliegenthart AD, Tucker CS, Del Pozo J, Dear JW. Defining hepatic dysfunction parameters in two models of fatty liver disease in zebrafish larvae. Zebrafish. 2013;10(2):199–210.
311. Huang M, Chang A, Choi M, Zhou D, Anania FA, Shin CH. Antagonistic interaction between Wnt and Notch activity modulates the regenerative capacity of a zebrafish fibrotic liver model. Hepatology.
312. 2014;60(5):1753–66.
313. Zhang C, Ellis JL, Yin C. Inhibition of vascular endothelial growth factor signaling facilitates liver repair from acute ethanol-induced injury in zebrafish. Dis Model Mech. 2016;9(11):1383–96.
314. Han W, Kozh S, Li Z, Mudumana SP, Kozh V, Jiang YJ, et al. Analyses of pancreas development by generation of gfp transgenic zebrafish using an exocrine pancreas-specific elastaseA gene promoter. Exp Cell Res. 2006;312(9):1526–39.
315. Zucchin F, Filippi A, Biemar F, Tiso N, Pauls S, Ellersdottir E, et al. Distinct delta and jagged genes control sequential segregation of pancreatic cell types from precursor pools in zebrafish. Dev Biol. 2007;301(1):192–204.
316. Flanagan-Steeh H, Fox MA, Meyer D, Sande JR. Neuromuscular synapses can form in vivo by incorporation of initially aseptic perisynaptic specializations. Development. 2005;132(20):4471–81.
317. Arkipovva V, Wendik B, Devos N, EK O, Peers B, Meyer D. Characterization and regulation of the hbf2/mnx1 beta-cell progenitor specific enhancer in zebrafish. Dev Biol. 2012;365(1):290–302.
318. Anderson RM, Bosch JA, Goll MG, Hesselson D, Dong PD, Shin D, et al. Loss of Dnmt1 catalytic activity reveals multiple roles for DNA methylation during pancreas development and regeneration. Dev Biol. 2009;324(1):213–23.
319. Andersson O, Adams BA, Yoo D, Ellis GC, Gut P, Anderson RM, et al. Adenosine signaling promotes regeneration of pancreatic beta cells in vivo. Cell Metab. 2012;15(6):885–94.
320. Chuang WS, Andersson O, Row R, Kimmelman D, Stainier DY. Suppression of Aki8-mediated Bmp signaling cell-autonomously induces pancreatic beta-cells in zebrafish. Proc Natl Acad Sci U S A. 2010;107(3):1142–7.
321. Field HA, Ober EA, Roessler T, Stainier DY. Formation of the digestive system in zebrafish I. Liver morphogenesis. Dev Biol. 2003;253(2):279–90.
322. Field HA, Dong PS, Beis D, Stainier DY. Formation of the digestive system in zebrafish II. Pancreas morphogenesis Dev Biol. 2003;261(1):197–208.
323. Ng AN, de Jong-Curtain TA, Mawdsley DJ, White SJ, Shin J, Appel B, et al. Formation of the digestive system in zebrafish III. Intestinal epithelium morphogenesis. Dev Biol. 2005;286(1):114–35.
324. Dong PDS, Provost E, Leach SD, Stainier DY. Graded levels of Ptf1a differentially regulate endocrine and exocrine fates in the developing pancreas. Genes Dev. 2008;22(11):1445–50.
325. Scholpp S. Introduction in vivo cell biology in zebrafish. Histochim Croat Biol. 2010;54(5):457–61.
326. Cox NJ, Unlu G, Bisnett BJ, Meister TR, Condon BM, Luo PM, et al. Dynamic glycosylation governs the vertebrate COPII protein trafficking pathway: Biochemistry. 2018;57(1):91–107.
332. Kobayashi I, Kondo M, Yamamoto S, Kobayashi-Sun J, Taniguchi M, Kanemaru K, et al. Enrichment of hematopoietic stem/progenitor cells in the zebrafish kidney. Sci Rep. 2019;9(1):14205.

333. Zhang W, Roy S. The zebrafish fast myosin light chain mylpfa:H2B-GFP transgene is a useful tool for in vivo imaging of myocyte fusion in the vertebrate embryo. Gene Expr Patterns. 2016;20(2):106–10.

334. Isabella AJ, Grash BR, Stonick JA, Dubrule M, Joens CB. Retinoic acid organizes the Zebrafish Vagus motor topographic map via spatiotemporal coordination of Hgf/met signaling. Dev Cell. 2020;53(3):344-57.e5.

335. Kawakami K. Transposon tools and methods in zebrafish. Dev Dyn. 2005;234(2):244–54.

336. George AA, Hayden S, Adelman VP, Brockerhoff SE. Synaptojanin 1 is required for endolysosomal trafficking of synaptic proteins in cone photoreceptor inner segment membranes. PLoS ONE. 2014;9(1):e84394.

337. Zhang J, Schulze KL, Hiesinger PR, Suyama K, Wang S, Fish M, Clark BS, Winter M, Cohen AR, Link BA. Generation of Rab-based transgenic lines for in vivo studies of endosome biology in zebrafish. Dev Dyn. 2011;240(1):2452–65.

338. Chavrier P, Vingron M, Sander C, Simons K, Zerial M. Molecular cloning of YPT1/SEC4-related cDNAs from an epithelial cell line. Mol Cell Biol. 1990;10(12):6578–85.

339. Thisse B, Heyer V, Lux A, Alunni V, Degrave A, Seiliez I, et al. Spatial and temporal expression of the zebrafish genome by large-scale in situ hybridization screening. Methods Cell Biol. 2004;77:505–19.

340. Martinet W, Knaapen MW, Kockx MM, De Meyer GR. Autophagy in zebrafish larvae in culture. J Neurosci Methods. 2012;205(2):277–82.

341. Crespo C, Knust E. Characterisation of maturation of photoreceptor cell subtypes during zebrafish retinal development. Bio Open. 2018;7(11):bio036632.

342. Sokol AM, Uszczyńska-Ratajczak B, Collins MM, Bazala M, Wopf U, Lunde-gaard PR, et al. Loss of the Mia40a oxidoreductase leads to hepato‑pancreatic insufficiency in zebrafish. PLoS Genet. 2018;14(11):e1007743.

343. Daniel K, Icha J, Horenburg C, Muller D, Norden C, Mansfeld J. Conditional control of fluorescent protein degradation by an auxin-dependent nanobody. Nat Commun. 2018;9(1):3297.

344. Lindberg CD, Di Giulio RT. Polycyclic aromatic hydrocarbon and hypoxia exposures result in mitochondrial dysfunction in zebrafish. Aquat Toxicol. 2019;216:105298.

345. Dukes AA, Bai Q, Van Laar VS, Zhou Y, Ilin V, David CN, et al. Live imaging of mitochondrial transport in single-axon regeneration of Zebrafish Mauthner cells. Front Commun Biol. 2020;3(1):49.

346. Xu Y, Chen M, Hu B, Huang R, Hu B. In vivo imaging of mitochondrial transport in single-axon regeneration of Zebrafish Mauthner cells. Front Cell Neurosci. 2017;11:14.

347. Princey Abudu Y, Pankiv S, Mathai BJ, Hakon Lystad A, Bindesboll C, Brenne HB, et al. NIPSNAP1 and NIPSNAP2 act as ‘eat me’ signals for Schwann cells from chemotoxicity by delaying axon degeneration. Commun Biol. 2020;3(1):49.

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