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Spatio-temporal gene expression analysis from 3D in situ hybridization images

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Color supplement

Chapter 2

FIG. 1. A panel of marker genes expressed in 24 hpf zebrafish embryos. Row **A** depicts the results of the standard AP detection. Row **B** depicts a characteristic optical section from the 3D image obtained with zebraFISH, using TSA/Cy3-SYTOX Green. Row **C** is a projection of that same image stack into one image. Row **D** depicts 3D reconstructions of the expression pattern, the embryo outline, and some surrounding tissues as obtained from the 3D image.

Column **1** shows *myoD* expression in the somites: (**B1, C1**) *myoD* expression detected in an image stack of 64 slices; (**D1**) 3D reconstruction of *myoD* expression in white, yolk extension in green, and embryo outline in blue. Column 1 is visualized in oblique dorsal orientation. Column **2** shows *krox20* expressed in rhombomeres 3 and 5: (**B2, C2**) *krox20* gene expression in an image stack of 13 slices; (**D2**) 3D reconstruction of *krox20* expression in white, third ventricle in cyan, partial eye outline in salmon, and embryo outline in blue. Column 2 is visualized in oblique lateral orientation. Column **3** shows the *pax2.1* expression pattern at the midbrain-hindbrain boundary and the optic stalk: (**B3, C3**) show gene expression detected in an image stack of 97 slices; (**D3**) 3D reconstruction of *pax2.1* gene expression patterns in white, embryo outline in blue, optic cup in salmon. Column 3 is visualized in oblique lateral orientation. Column **4** shows *otx2* expressed in the diencephalon and mesencephalon: the image stack in (**B4, C4**) is 74 slices; (**D4**) 3D reconstruction of *otx2* gene expression in white, embryo outline in blue, optic cup in salmon. Column 4 is visualized in oblique lateral orientation. For all four genes, the pattern generated with zebraFISH corresponds to the pattern generated with AP detection. The 3D reconstructions give insight into the extension of the pattern within the embryo as well as clear spatial relations with a number of anatomical domains. These domains coincide exactly with the domains annotated in the three-dimensional digital atlas of zebrafish development (bio-imaging.liacs.nl).

FIG. 2. Gene expression patterns of *mpx* in 36 hpf and 48 hpf zebrafish embryos with TSA/Cy3 detection. (**A**) At 36 hpf, an image stack of 70 slices. For this image only TSA detection was applied. The *mpx* expressing cells are clearly visible as dispersed over the yolk and also visible in the head; *mpx* expressing cells also accumulate in the ventral venous plexus (not shown). (**B–D**) At 48 hpf, image stacks of 78 slices using TSA/Cy3-SYTOX Green detection. Expression is visible in single cells scattered over the yolk and in the head. Characteristic slices show single cell imaging (arrow pointing to *mpx* expressing cell) in the brain (**B**) and yolk sac (**C**). (**D**) A projection of the whole stack showing the pattern of the *mpx* gene at 48 hpf.

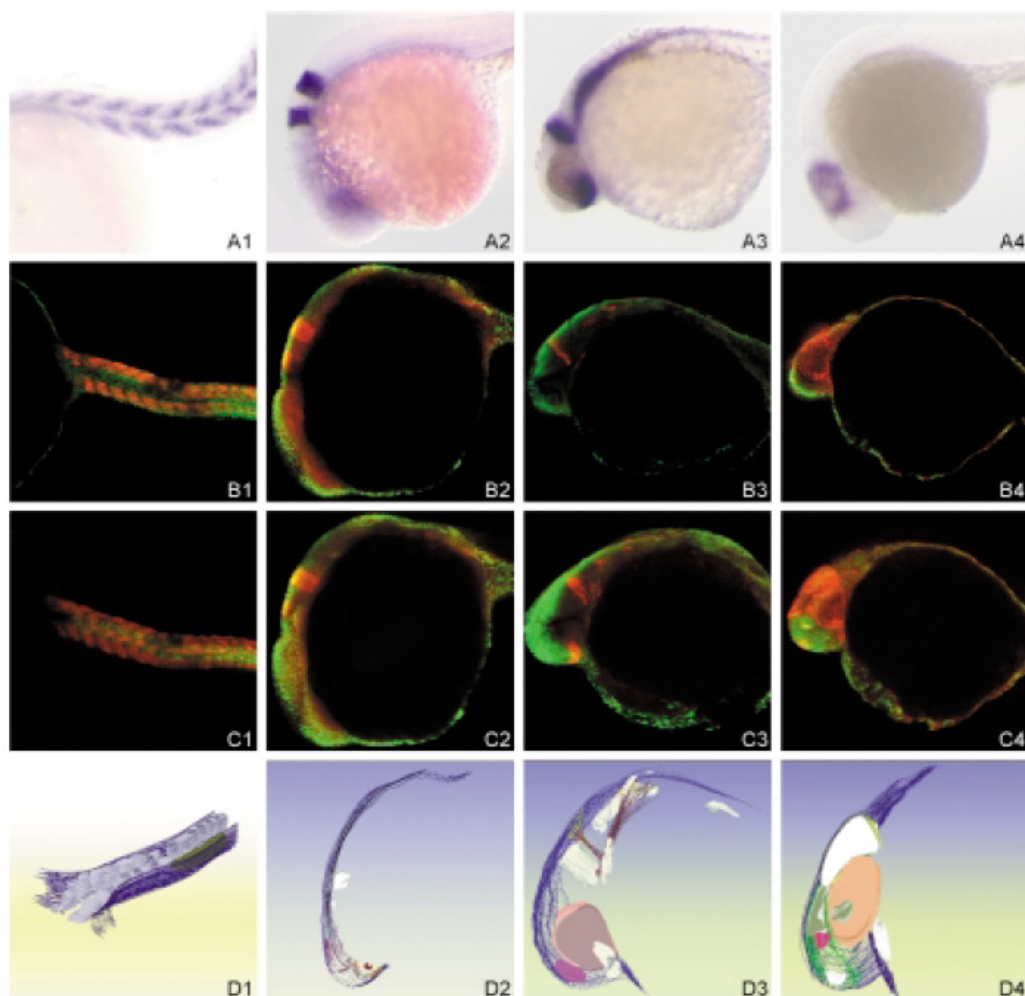


FIG. 1.

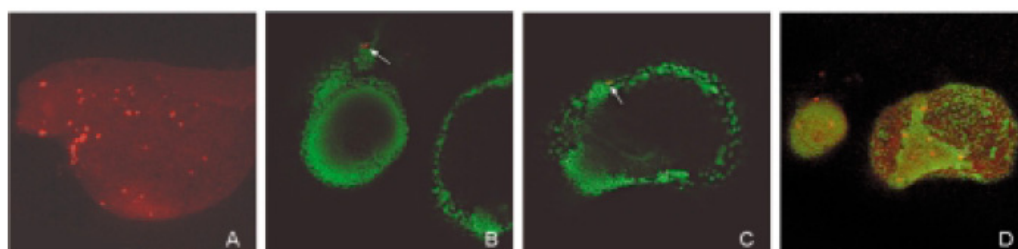


FIG. 2.

Chapter 2 Fig 1 and 2

Chapter 3

Fig.2 A: FISH result for the gene encoding 14-3-3 γ in a 24 hpf embryo. The picture is a confocal image.

C: 14-3-3 γ FISH result in a 48 hpf embryo; a so-called z-projection of the confocal image. Gene expression is in red, i.e. the red channel of the CLSM image. The green depicts a background staining of the cell nuclei with SYTOX Green.

2 B,D: 3D reconstruction of the embryos shown in 2A and 2B respectively. Gene expression for 14-3-3 γ is depicted in white. Both FISH images and 3D reconstruction clearly reveal gene expression patterns in otic vesicle (salmon), optic stalk (orange), cranial ganglia (light yellow), ganglion V (light orange), spinal cord neurons and heart primordium (red) at 24 and 48 hpf.

2 E, F: ISH results for 14-3-3 γ at 24 and 48 hpf. Dorsal view; anterior is to the left.

G: 14-3-3 γ ISH result at 24 hpf. Tissue section after overstaining, revealing gene expression in eye, tectum of the mesencephalon, cranial ganglia and otic vesicle.

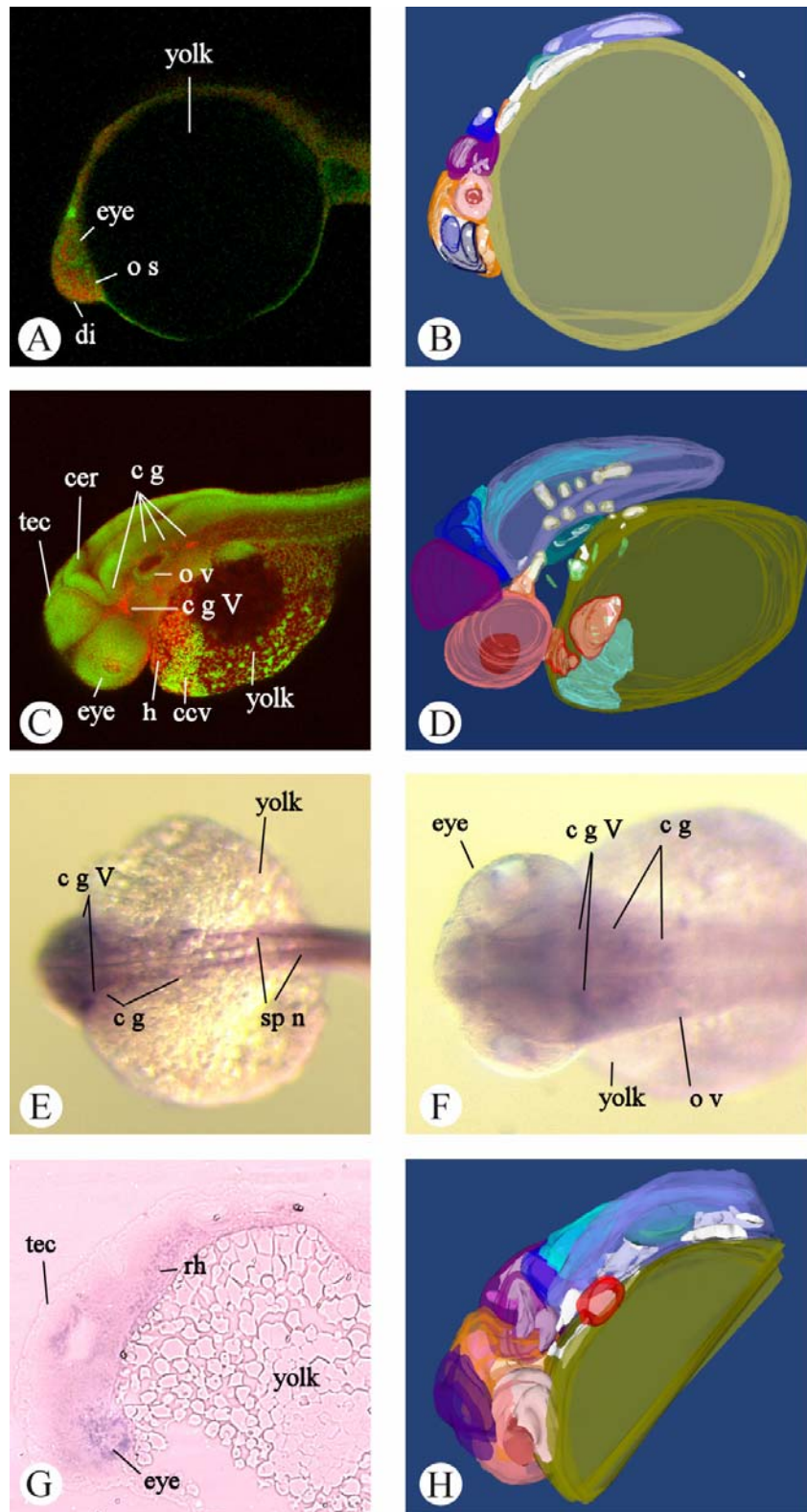
H: 3D reconstruction of the embryo shown in 5A. In 5B, gene expression is displayed in relation to anatomical structures. 14-3-3 γ gene expression domains are depicted in white.

In all images, anterior is to the left, dorsal is to the top.

The findings after overstaining, tissue sectioning and 3D reconstruction of ISH results confirm our findings with FISH.

Abbreviations: ccv, common cardinal vein; cer, cerebellum; c g, cranial ganglia; c g V, fifth cranial ganglion; di, diencephalon; h, heart primordium; o v, otic vesicle; sp n, spinal cord neurons; tec, tectum of the mesencephalon; rh: rhombencephalon.

Color legend: gene expression: white; gill primordia: light green; cerebellum: blue; common cardinal vein: clear blue; diencephalon: orange; eye: salmon; lens: brown; heart primordium: red; otic vesicle: dark green; rhombencephalon: lilac; tectum (mesencephalon): purple; telencephalon: dark blue; 4th ventricle: clear blue.



Chapter 3 Fig.2

Chapter 3

Fig. 3 A: 14-3-3 τ FISH result in a 24 hpf embryo; z-projection of a confocal image.

B: 3D reconstruction of the embryo shown in 3A. 14-3-3 τ gene expression (white) is visible in most brain structures.

C: Gene expression for 14-3-3 τ at 36 hpf, ISH detection.

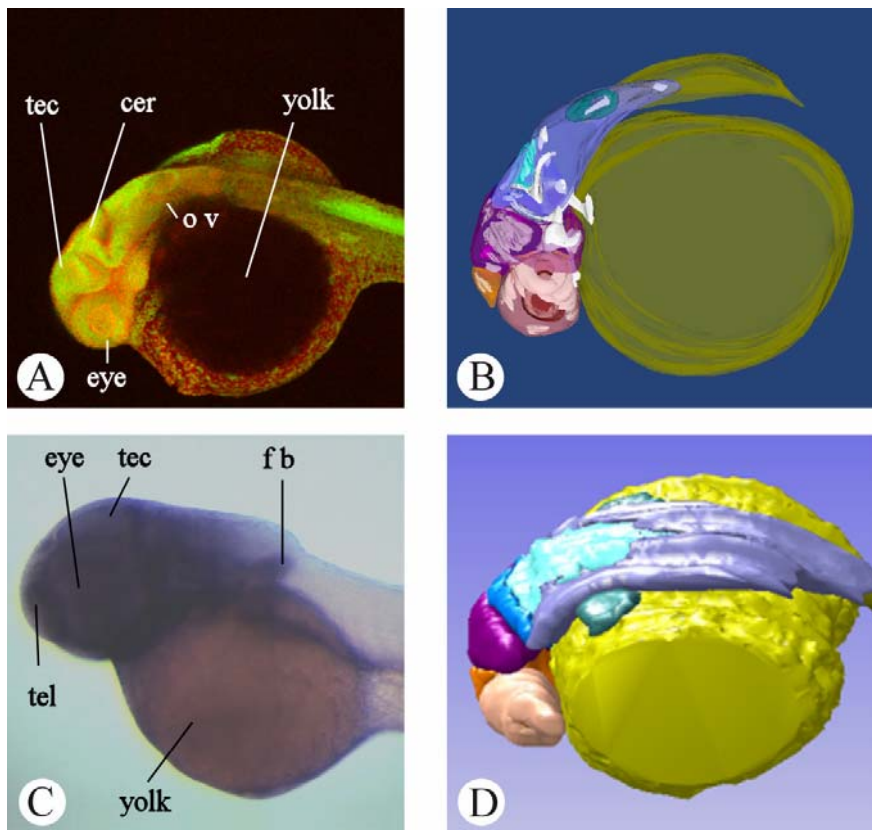
D: Reference image from the 3D atlas, in the same orientation as the embryo in 3A and B.

Abbreviations: cer, cerebellum; f b: fin bud; o v, otic vesicle; tec, tectum of the mesencephalon; tel: telencephalon. In all images, anterior is to the left, dorsal is to the top.

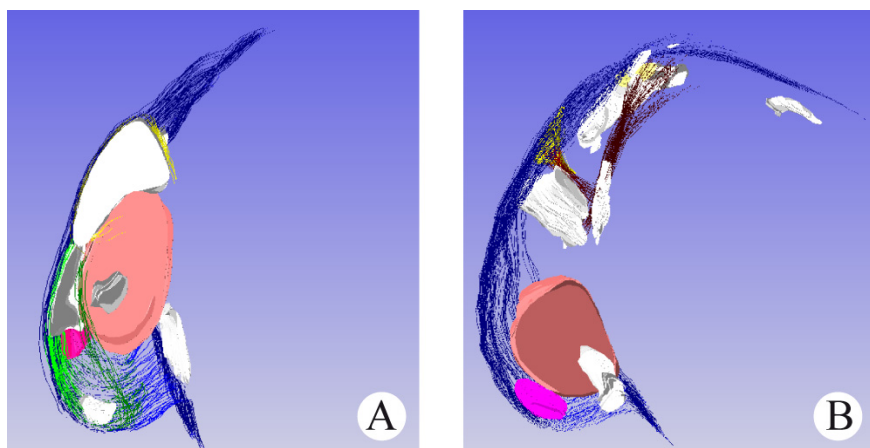
Color legend: gene expression: white; cerebellum: blue; diencephalon: orange; eye: salmon; lens: brown; otic vesicle: dark green; rhombencephalon: lilac; tectum (mesencephalon): purple; 4th ventricle: clear blue.

Fig.4 A, B: 3D reconstructions of *pax2* (4A) and *otx2* (4B) expression domains at 24 hpf, used as a reference for the brain structures.

In all images, anterior is to the left, dorsal is to the top. Color legend: gene expression: white; optic cup: salmon; embryo outline: dark blue



Chapter 3 fig. 3



Chapter 3 fig. 4

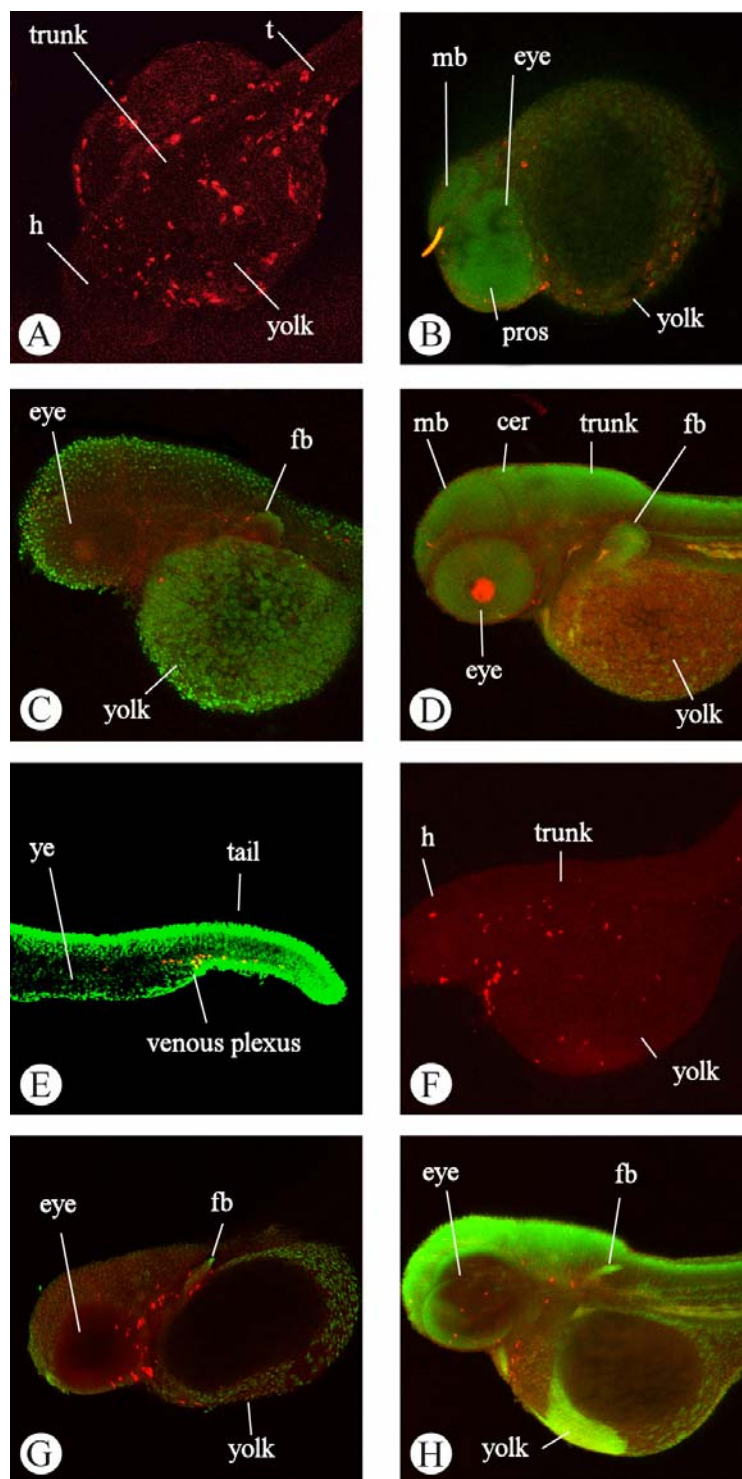
Chapter 4

Fig. 3. Panels A-D: developmental series of *l-plastin* expression in zebrafish embryos at 24, 36, 48 and 60 hpf.

Panels E-H: developmental series of *mpx* expression at 24, 36, 48 and 72 hpf. TSA detection.

A is a dorsal view, anterior is to the left; B-H: Anterior is to the left, dorsal to the top. Images are so-called z-projections of confocal images. Gene expression is in red (TSA signal), counterstaining is in green (SYTOX Green).

Abbreviations: cer: cerebellum; fb: fin bud; h: head; mb: midbrain; pros: prosencephalon; t: tail; ye: yolk extension.



Chapter 4 fig 3

Chapter 4

Fig.6. A-D: *l-plastin* gene expression at 36 hpf (panel A and B) and at 60 hpf (panel C and D), In panel B and D, the 3D reconstructions of the embryos in panel A and C are depicted.

E: *mpx* expression in a 48 hpf embryo. F: 3D reconstruction of the same embryo, depicting *mpx* expressing cells in the common cardinal vein i.e. the light blue structure on the yolk (ochre), visible from the confocal image as well as in the 3D reconstruction.

G: *mpx* expression in the tail of a 72 hpf embryo. H: 3D reconstruction of the same embryo, portraying *mpx* expressing cells in the CHT.

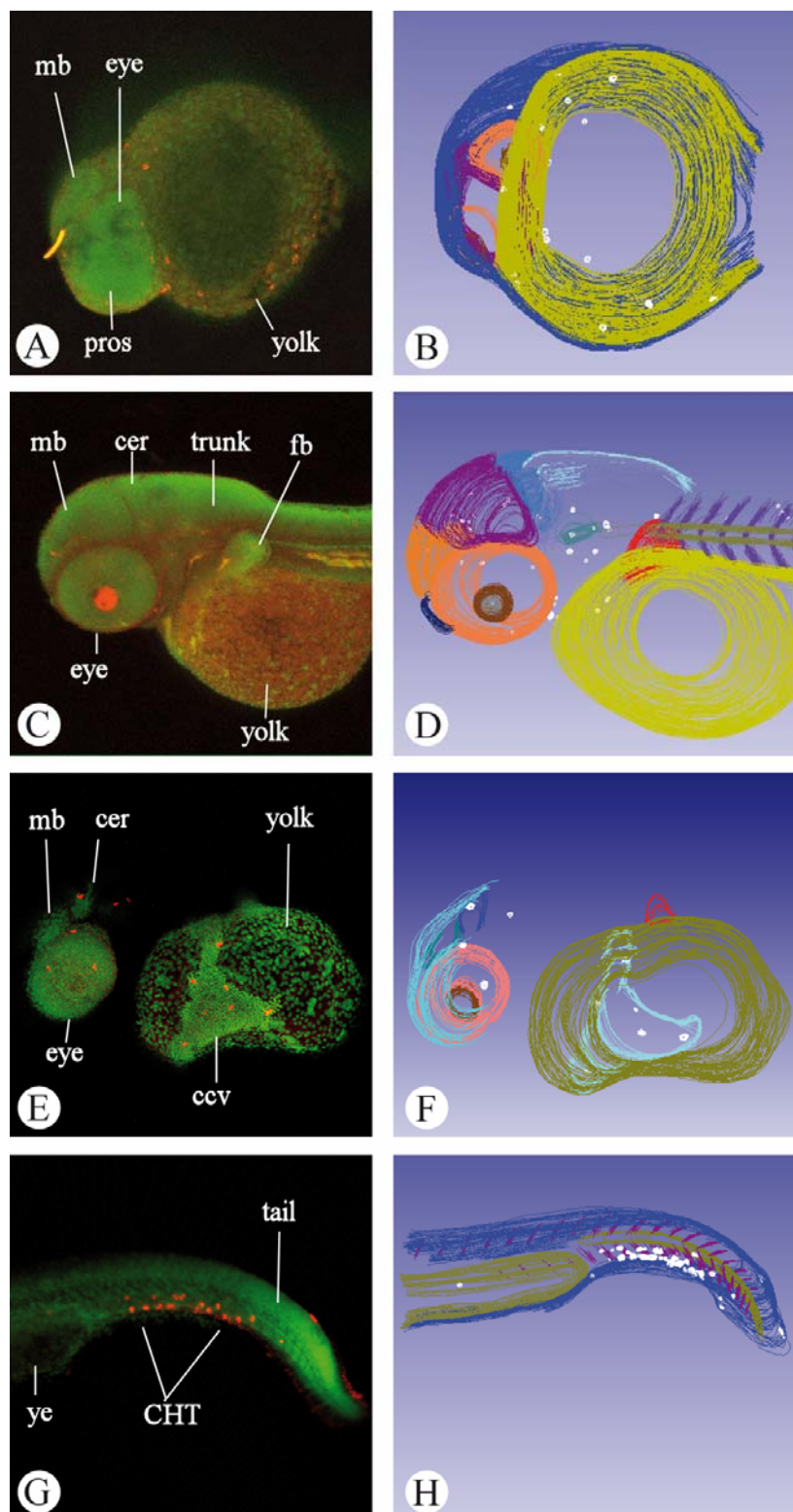
The images of the embryos in panel A, C, E and G are the result of TSA detection; The images are so-called z-projections of a confocal image stack. Gene expression is in red (TSA signal), counter staining is in green (SYTOX Green signal). Anterior is to the left, dorsal to the top.

Color legend for the 3D reconstructions in panel B, D, F and H:

l-plastin or *mpx* positive cells (white). embryo outline (dark blue, diencephalon (orange), yolk and yolk extension (ochre), mesencephalon (purple) optic cup (salmon), pectoral fin (red).

Abbreviations: ccv: common cardinal vein; cer: cerebellum; CHT: caudal haematopoietic tissue; fb: fin bud; h: head; mb: midbrain; pros: prosencephalon; t: tail; ye: yolk extension. **Fig. 5.** A: *l-plastin* expression in the tail of a 96 hpf embryo in the dorsal longitudinal anastomotic vessels. B: *mpx* expression in the tail of a 96 hpf embryo. Notice the clusters of cells in the spaces between the somites, as a part of the CHT (Murayama et al. 2006). ISH detection with AP staining. Anterior is to the left, dorsal to the top.

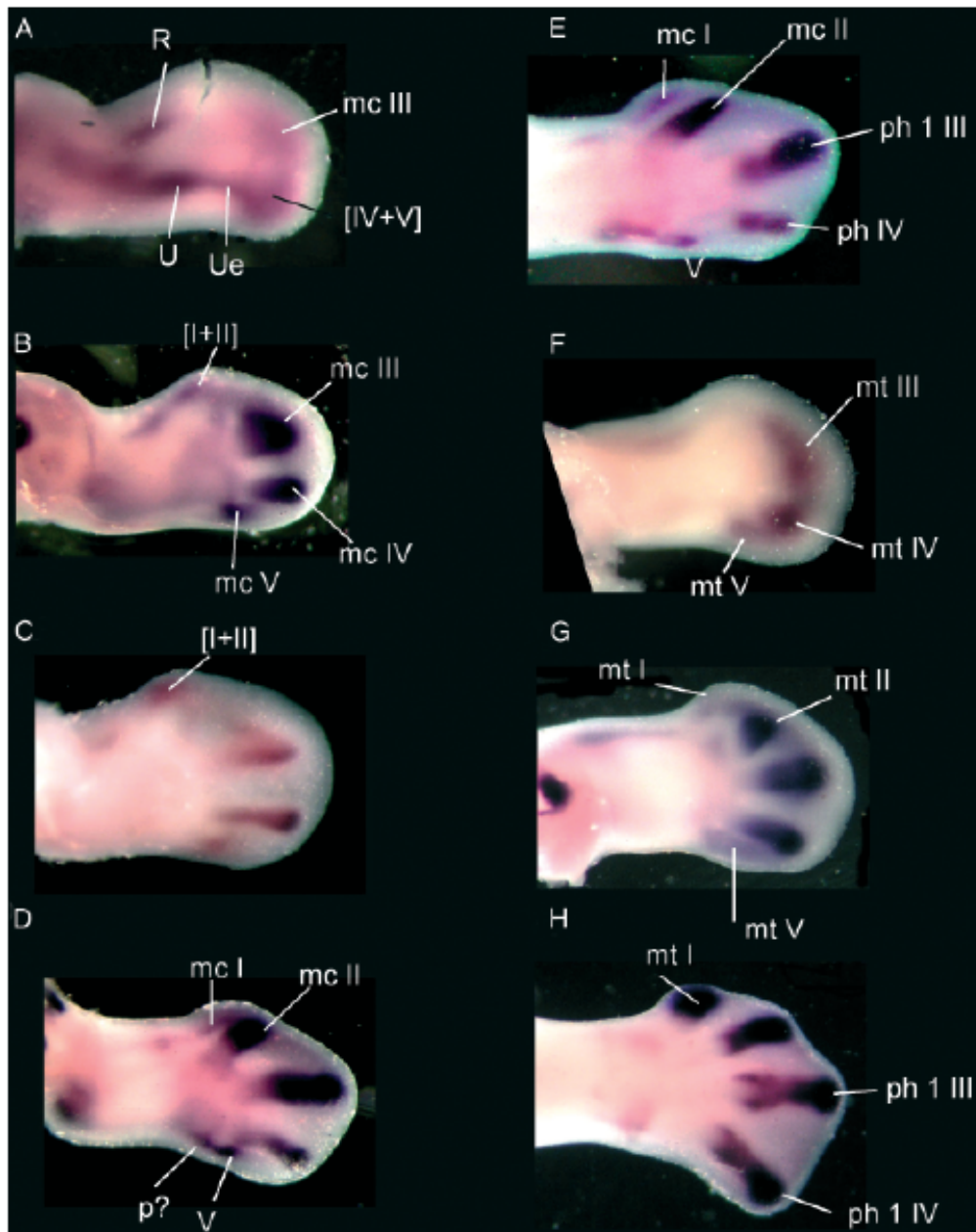
DLAV: dorsal longitudinal anastomotic vessels; CHT: caudal haematopoietic tissue.



Chapter 4 fig 6

Chapter 5

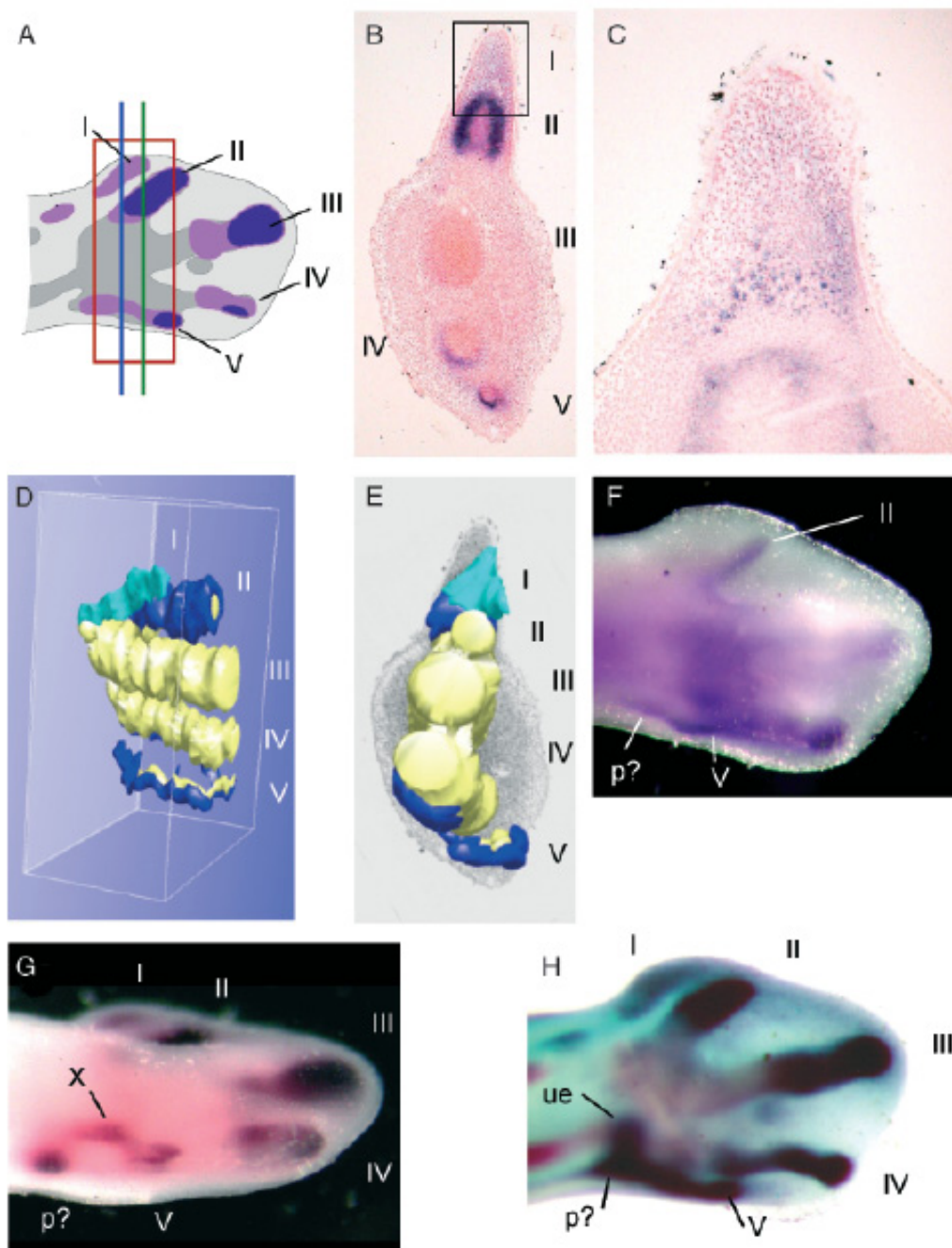
Fig. 3. (A–E) Left chicken wings stage 26–30, after in situ hybridization with *Sox9* probe (anterior is to the top, ventral aspect). (F–H) Left chicken hindlimbs stage 26, 27, and 30, respectively, *Sox9* probe. Anterior is to the top, ventral aspect. Roman numerals, digit or metacarpal number; mc, metacarpal; R, radius; U, ulna; Ue, ulnare; ph, phalanx; p?, pisiform?; [mc IV1V], common expression domain for metacarpals IV and V. Some images were inverted to make the orientation consistent.



Chapter 5 fig. 3

Chapter 5

Fig. 4. (A) Schematic interpretation of the *Sox9* gene expression patterns superimposed on cartilage pattern (alcian blue/ in situ double stains). Roman numerals, digits; vertical green line, plane of section in B; vertical blue line, plane of section in C; dark red box, area reconstructed in D, E. (B and C) Transverse sections of wings (stage 30, hybridized with *Sox9* probe), neutral red counterstain. Sections from the same specimen, C more proximal than B. (C) Detail from boxed area in B, showing expression of *Sox9* in the noncondensed mesenchyme anterior to digit II. (D) Three-dimensional (3D) reconstruction of the same specimen. Yellow, cartilage; dark blue, gene expression digits II–V; light blue, *Sox9* expression, presumptive digit I. Anterior is to the top. Ventral view. (E) Proximal view of the 3D reconstruction. Anterior to the top. The element at the level “V” may consist of mc V1element X. (F) Left chicken wing, stage 30, *Bmpr-1B* probe. Anterior to the top, ventral view. Distinct prechondrogenic domains are seen in digits II–IV, but not anterior to digit II (labeled II). p?, pisiform. (G) Wing, stage 30, oblique posterior–ventral view, *Sox9* probe. X, element “X”; p?, pisiform. (H) Wing, stage 30, overstained in NBT/BCI substrate after *Sox9* hybridization, then counterstained with alcian blue and cleared in methyl salicylate. p?, pisiform; ue, ulnare.

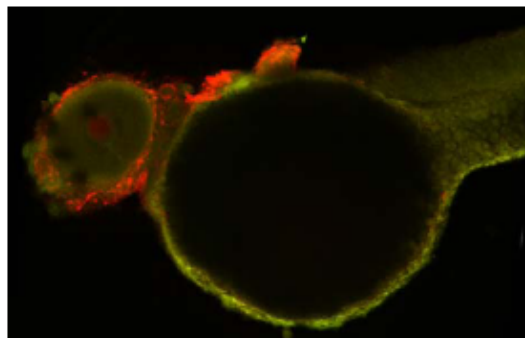


Chapter 5 Fig. 4

Chapter 6

Fig. 2. *bmpr-1b* expression in zebrafish pectoral fin and branchial arches at 48 hpf. Anterior is to the left, dorsal to the top. Left panel : ISH result from AP detection method; Right panel: FISH result. The picture is the result of the projection of a 2 channel 3D confocal image. The expression is in red, i.e. the red channel of the CLSM image, the green depicts a standard staining of the cell nuclei.

Fig. 3. Pattern shift diagram showing episodes, frequently found sequences (Bathoorn et al, in preparation) of skeletal elements plotted against duration of gene expression, using a relative time scale of chicken limb development. The diagram shows a selection from the total analysis of gene expression timing and cartilage formation in chicken wing and hind limb. Onset and duration of gene expression as well as alcian blue staining for cartilage formation are shown in the red bar (wing) and in the green bar (hind limb). Structures are organized in proximal, carpal and digit region. The pattern shift diagram shows that only *sox9* gene expression is found in the presumptive wing metacarpal (mc) I while no subsequent *bmpr1b* expression and cartilage formation are found in the presumptive wing mc I. The onset of gene expression in presumptive wing mc I is relatively late compared to metacarpals and proximal phalanges of digit II and IV. The pattern shift diagram clearly illustrates that the difference in number of digits between wing and hind limb skeleton is the result of a time shift in gene expression



Chapter 6 fig 2

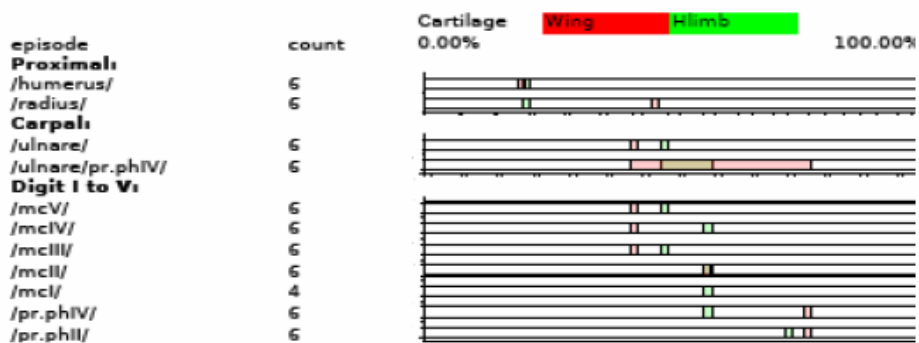
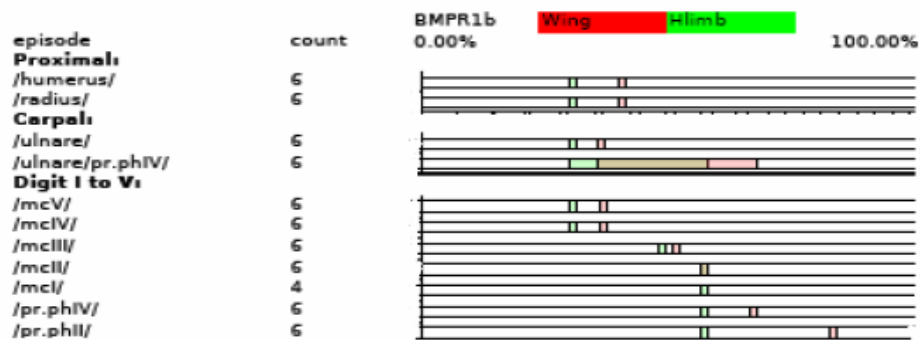
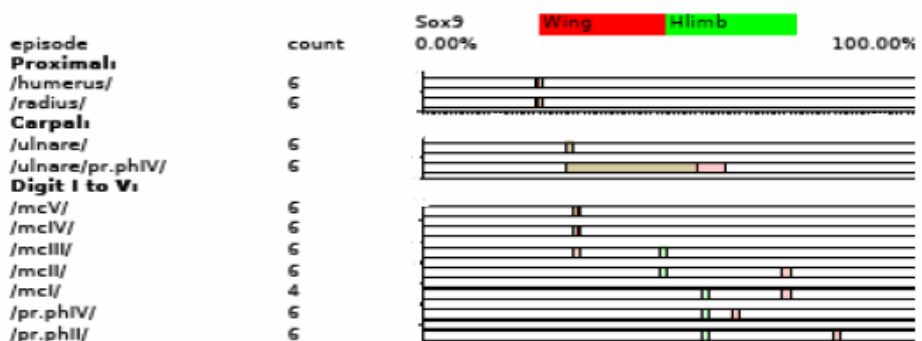


Fig 3