

LARVI'09, Ghent, Sept. 9th, '09

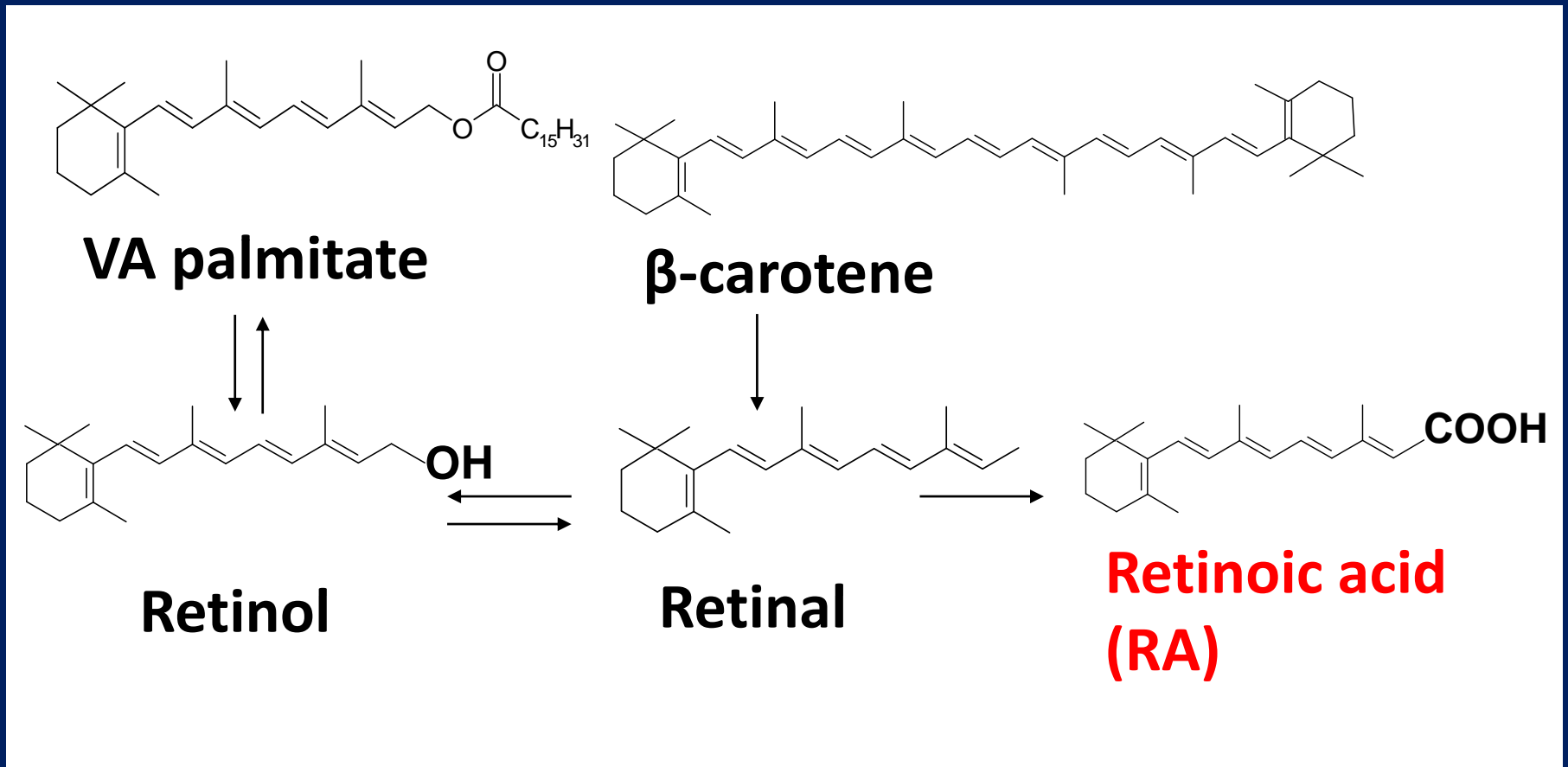
Analysis of skeletal deformity in fish using vitamin A- induced bone deformity model

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Tomonari Kotani (Fukuyama Univ.),
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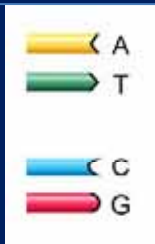
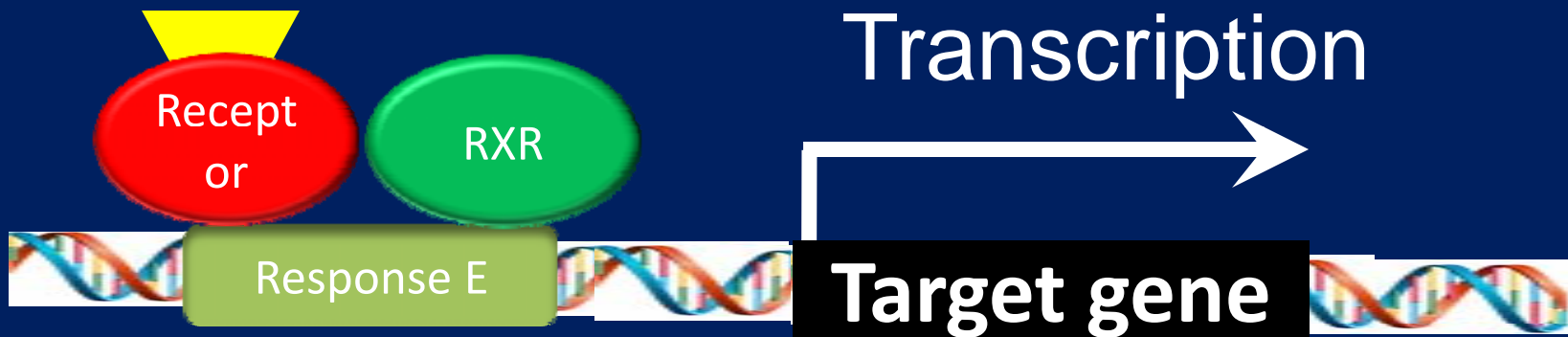
Contents of presentation

- Molecular mechanism of bone deformity induced by retinoid receptor agonist
- Vertebral deformity in fish larvae induced by retinoic acid

Metabolic pathway of vitamin A (VA)



Mode of action of nuclear receptors



Two classes of retinoid receptors



RAR

:Retinoic Acid Receptor

(RAR α , β , and γ)



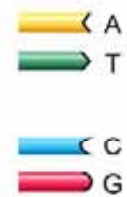
RXR

:Retinoid X Receptor

(RXR α , β , and γ)

Mode of action of RARs/RXRs

Q1. Which of RAR/RXR is important?



Q2. Which kind of gene is important?

QUESTION 1

Which of RAR/RXR
is responsible for
bone deformity?

Binding affinity of retinoids used in the experiment

All-trans RA (atRA)

RAR

9-*cis*-RA (9cRA)

RAR/RXR

Am80

RAR

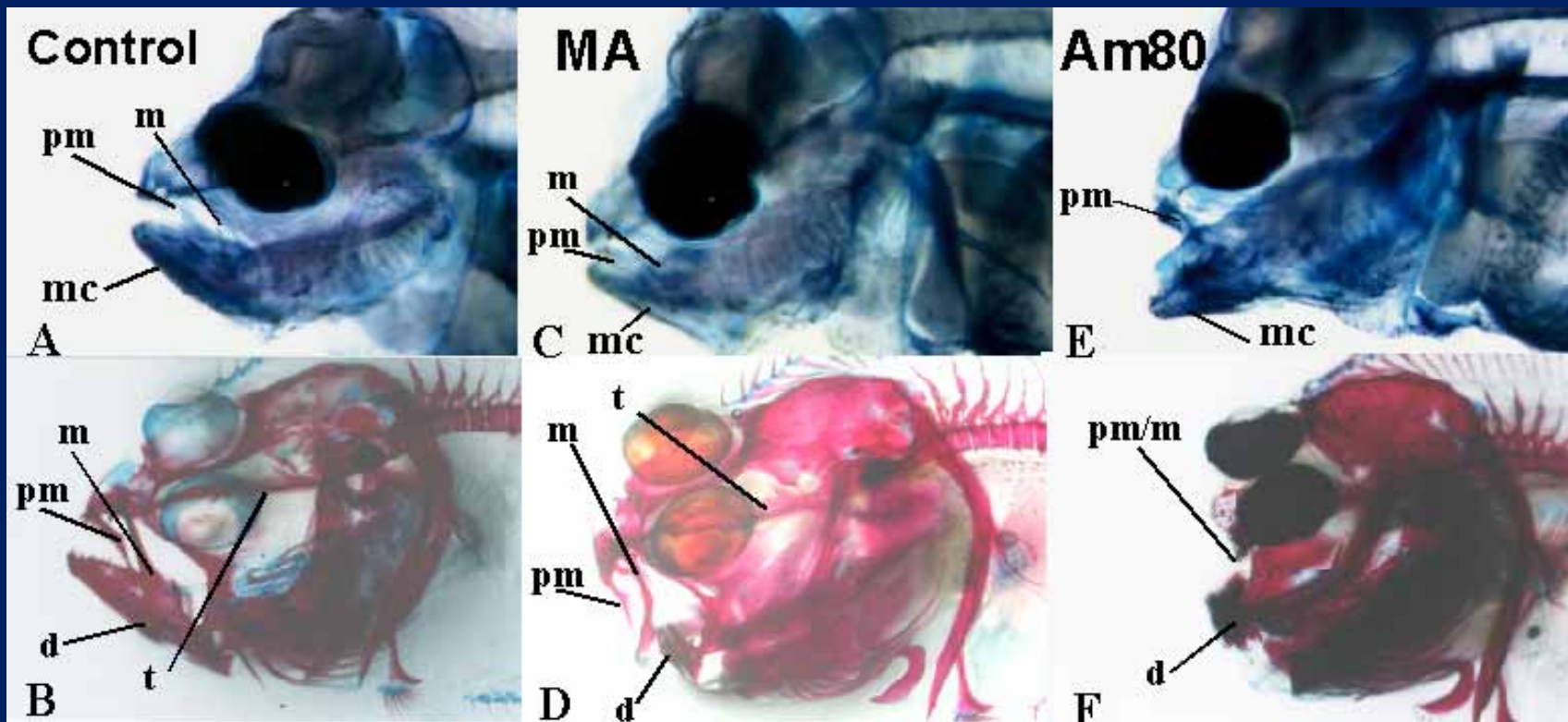
Methoprene acid (MA)

RXR

Lower jaw deformity in flounder at 9 and 58 dph

RXR ligand

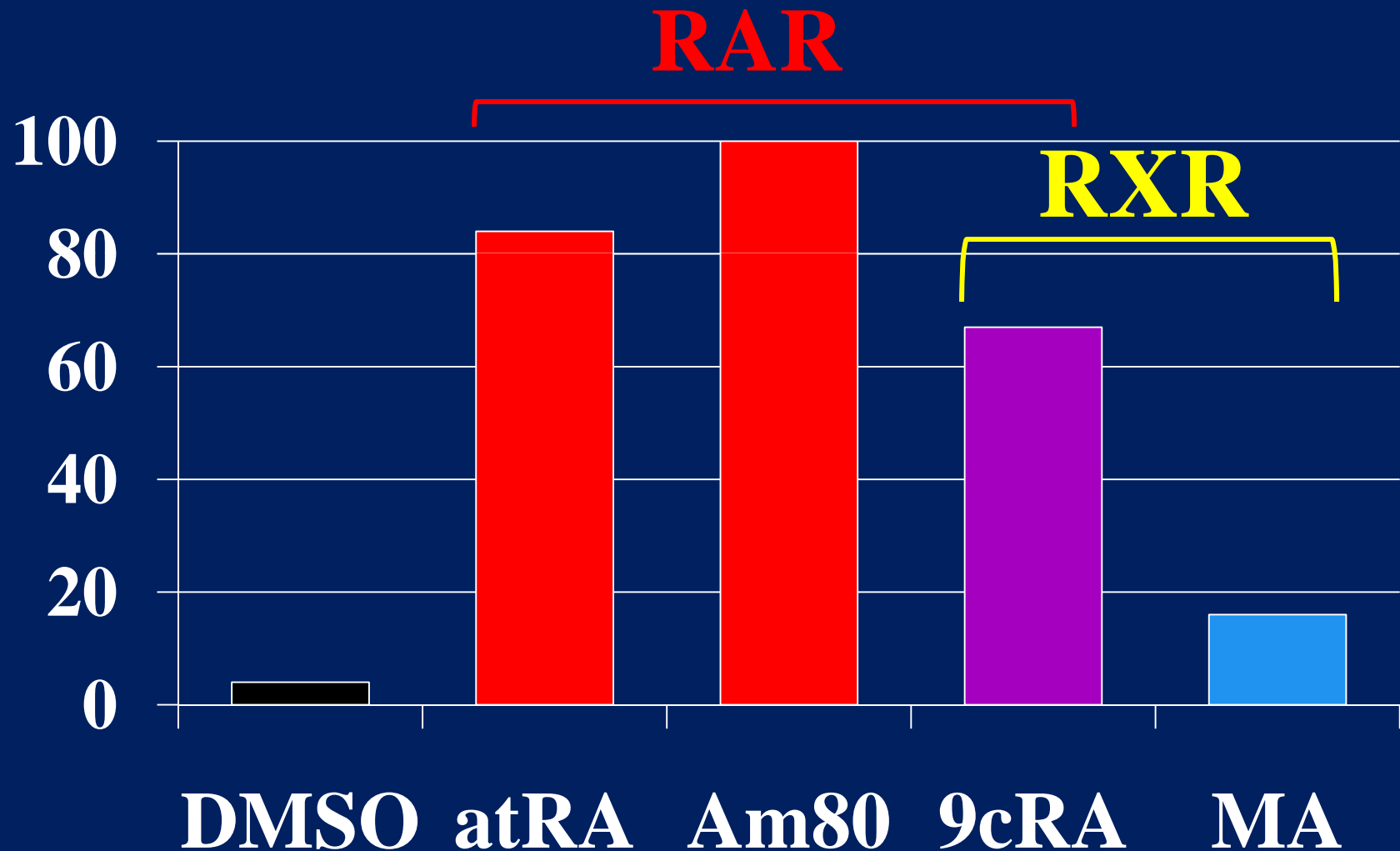
RAR ligand



9 dph (A, C, E) and 58 dph(B, D, F)

Haga *et al.* 2003

Jaw deformity in flounder by RAR/RXR ligands



Modified from Haga et al. 2002. & Haga et al. 2003.

Two classes of retinoid receptors

RAR

*: Retinoic Acid Receptor
(RAR α , β , and γ)*

RXR

*: Retinoid X Receptor
(RXR α , β , and γ)*

QUESTION 2

Which kind of the
genes are
downstream of
RAR/RXR?

Expression of *RAR/RXR* and *patched* in the lower jaw

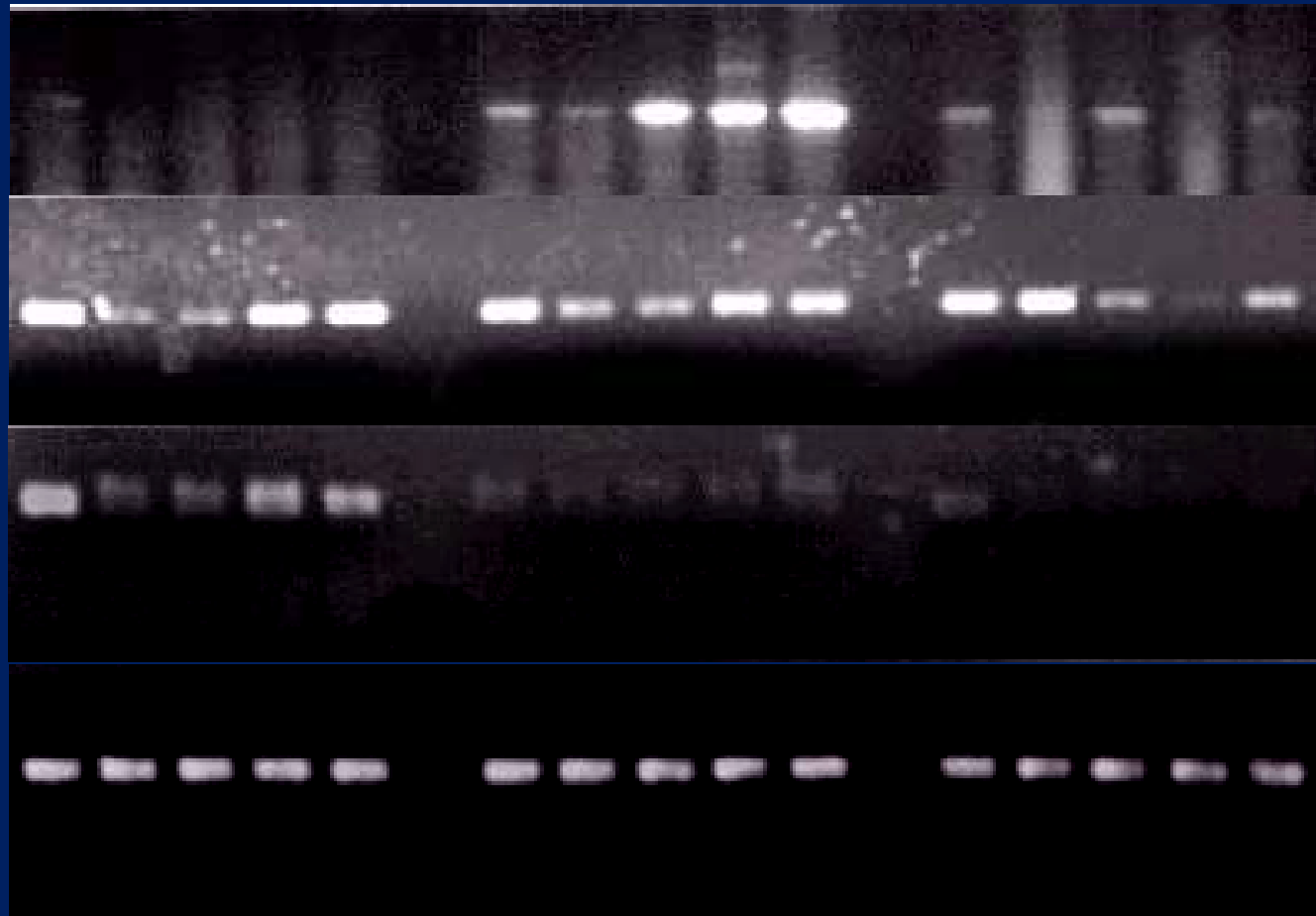
Control RAR ligand RXR ligand
 Am80 MA

RAR

RXR

Patched

EF-1 α

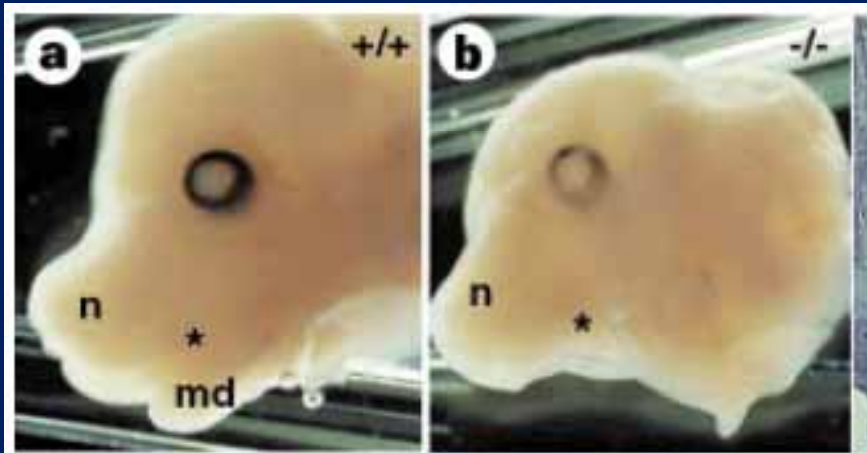
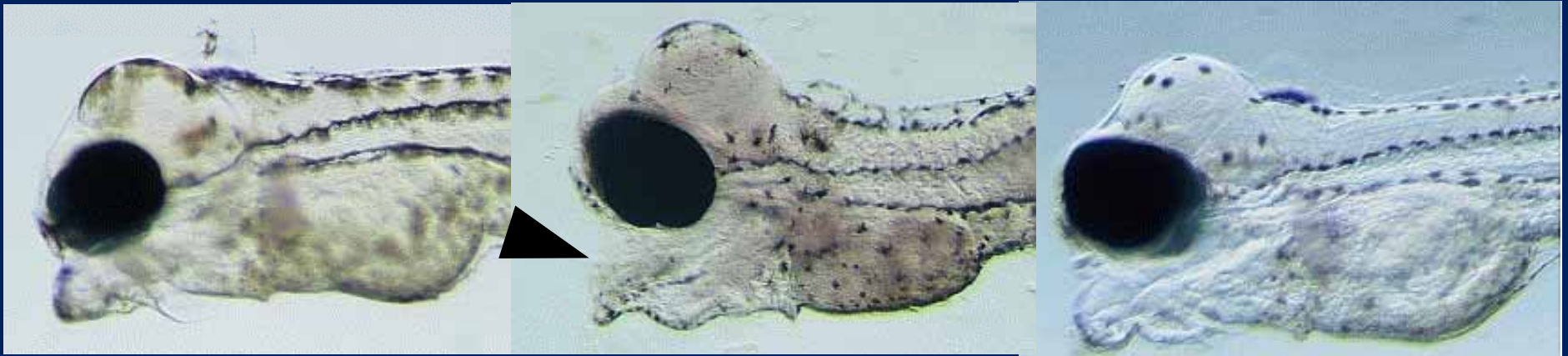


Expression of *pitx-2* in the jaw

Control

RAR ligand
atRA

RAR/RXR ligand
9cRA

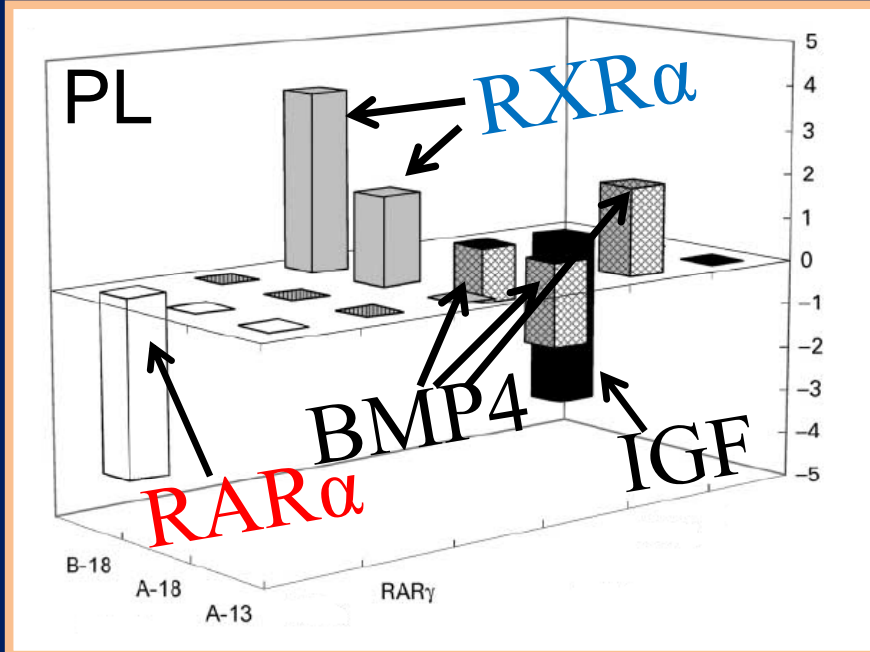
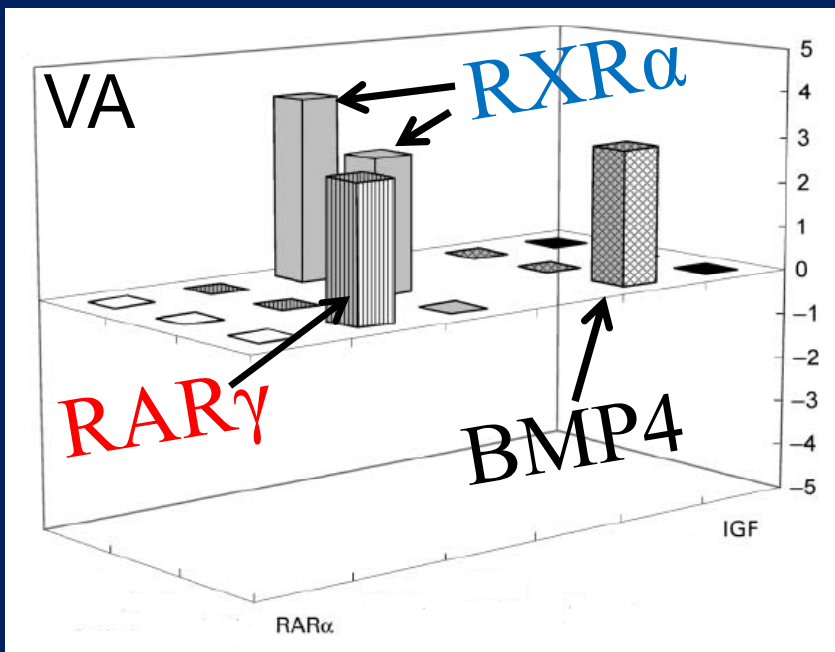
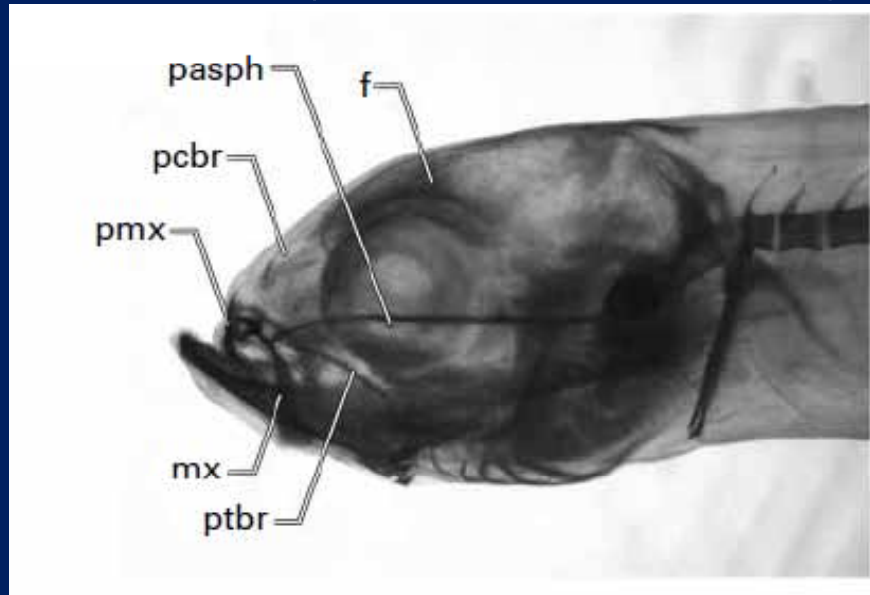
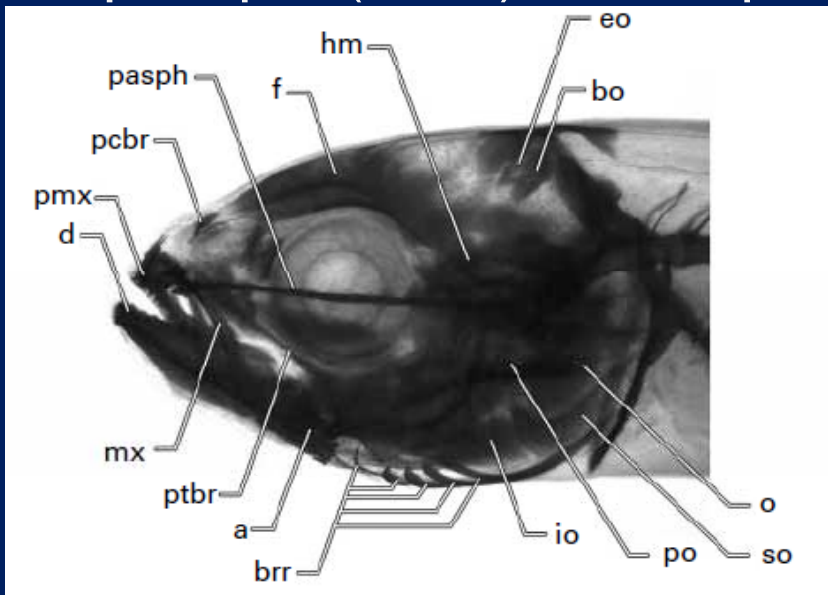


letters to nature

**Function of Rieger syndrome gene
in left-right asymmetry and
craniofacial development**

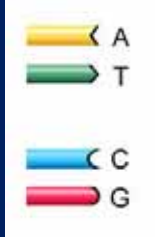
Mei-Fang Lu^{*}, Carolyn Pressman[†], Rex Dyer^{*}, Randy L. Johnson[†]
& James F. Martin^{*}

Deformity and altered gene expression induced by VA and phospholipid (DHA) in European sea bass (Villeneuve et al. 2006)



Mode of action of RARs/RXRs

Q1. Which of RAR/RXR is important?



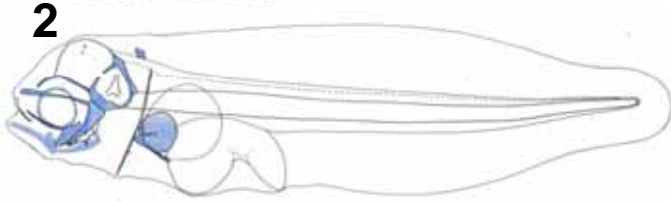
Q2. Which kind of gene is important?

Stages and skeletal development of Japanese flounder (Fushimi)

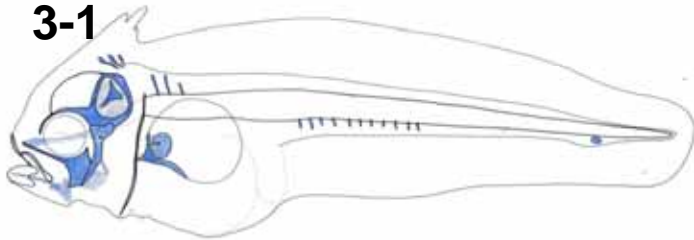
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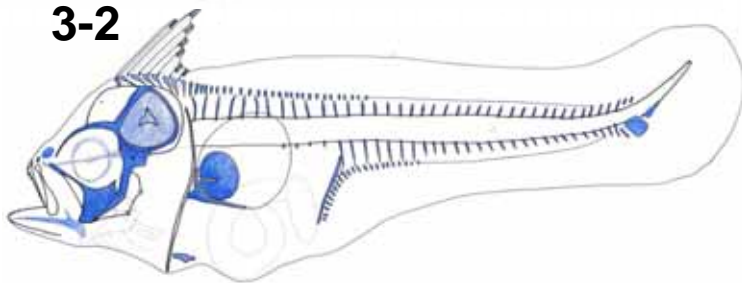
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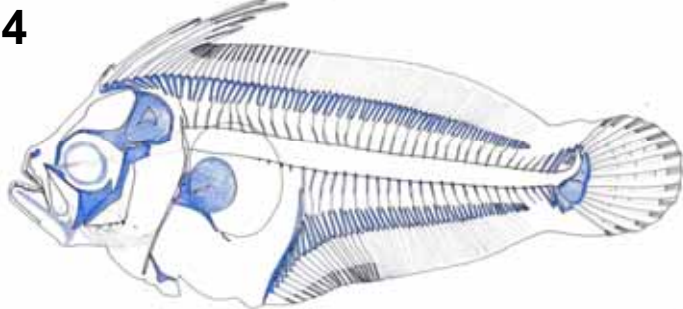
3-1



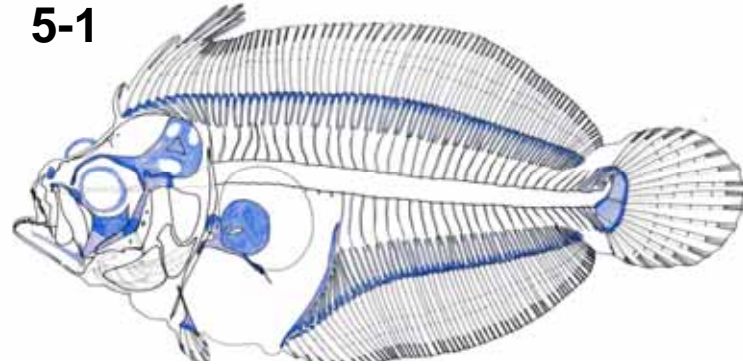
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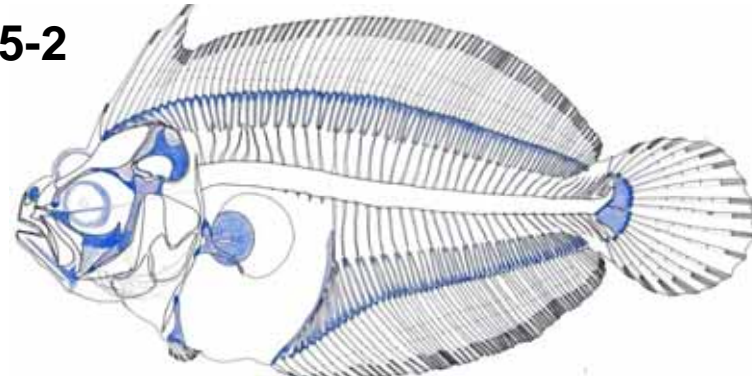
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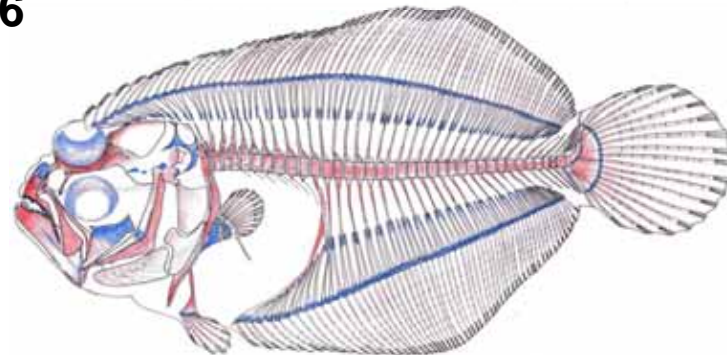
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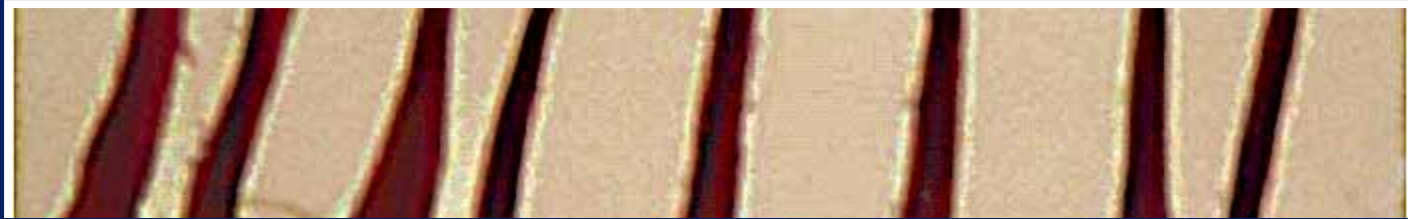
5-2



6



Loss of the intervertebral discs in fused vertebrae in red sea bream



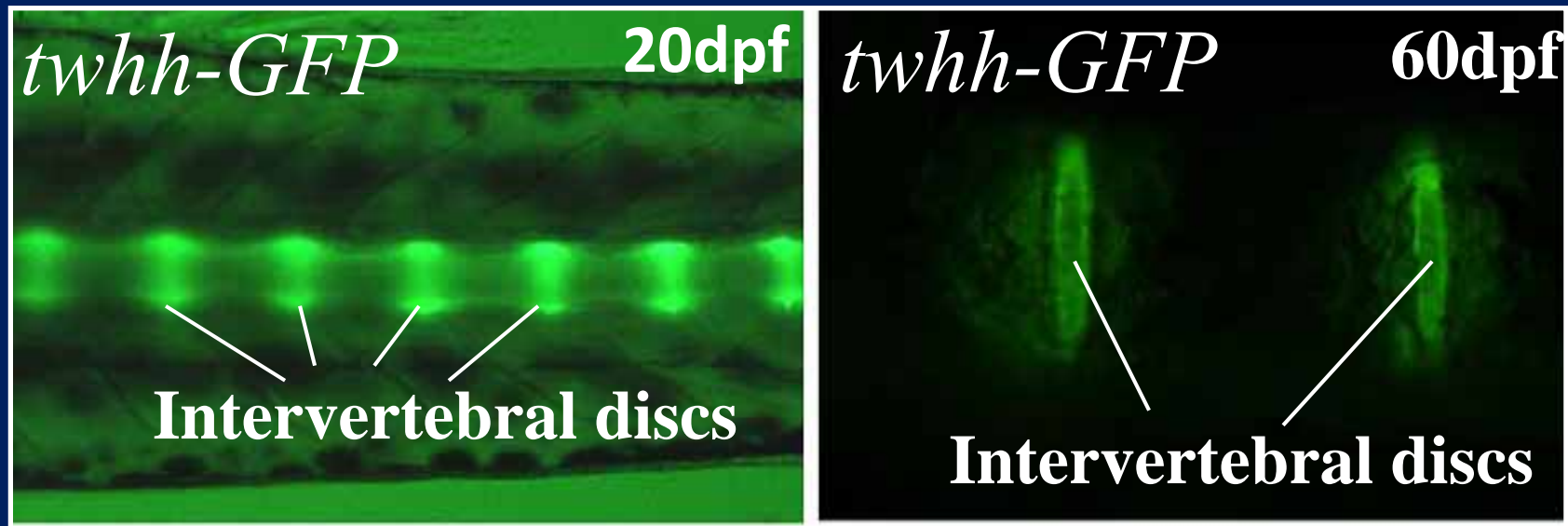
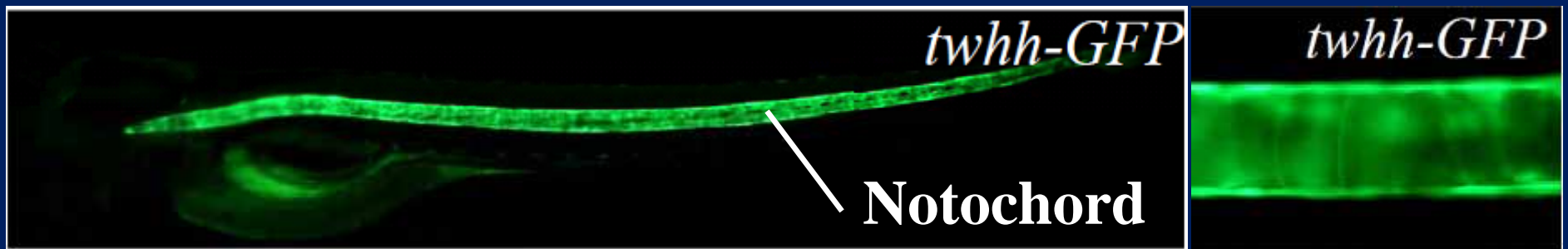
How do intervertebral discs develop?



Production of zebrafish carrying
GFP in intervertebral disc

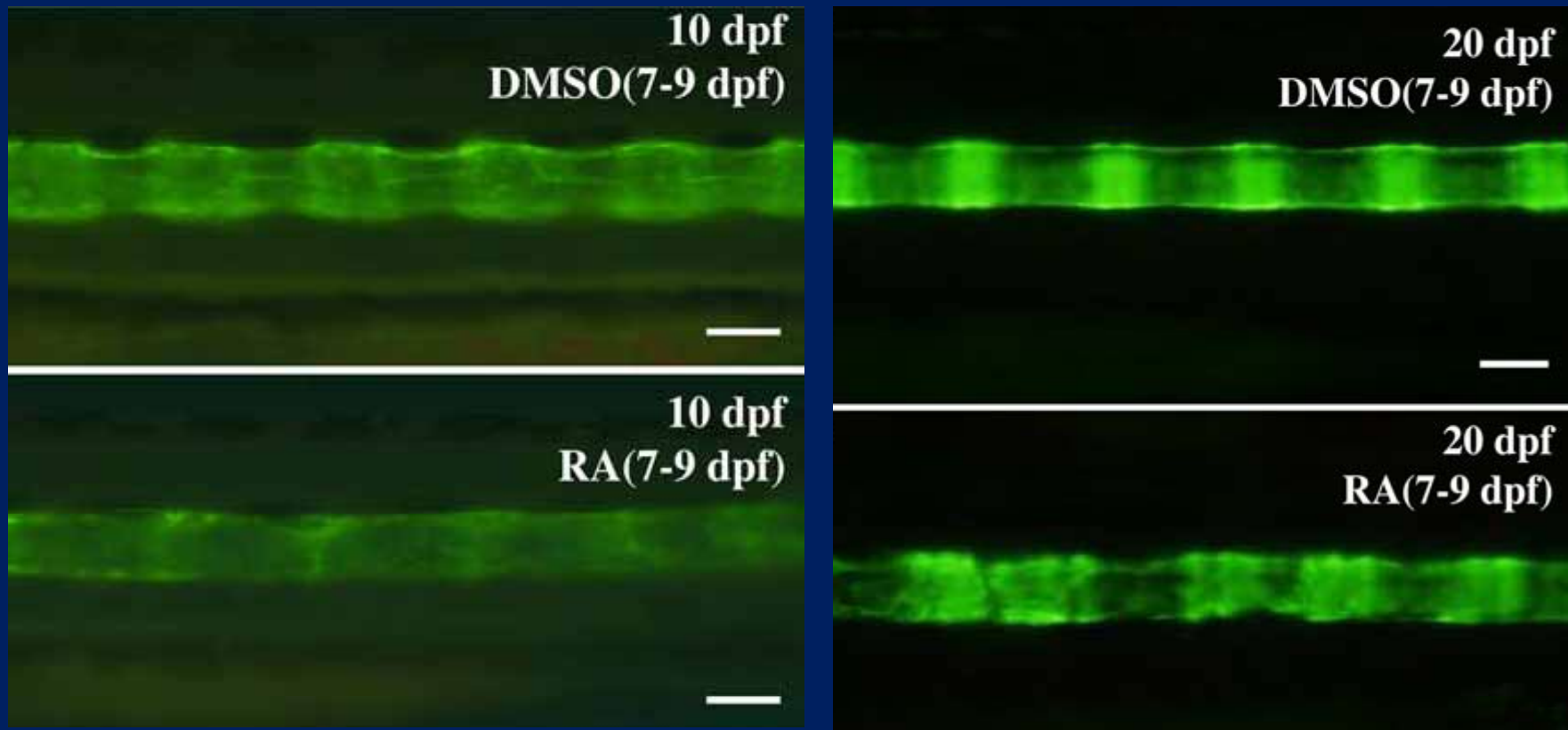


GFP expression in *twhh*-GFP transgenic zebrafish



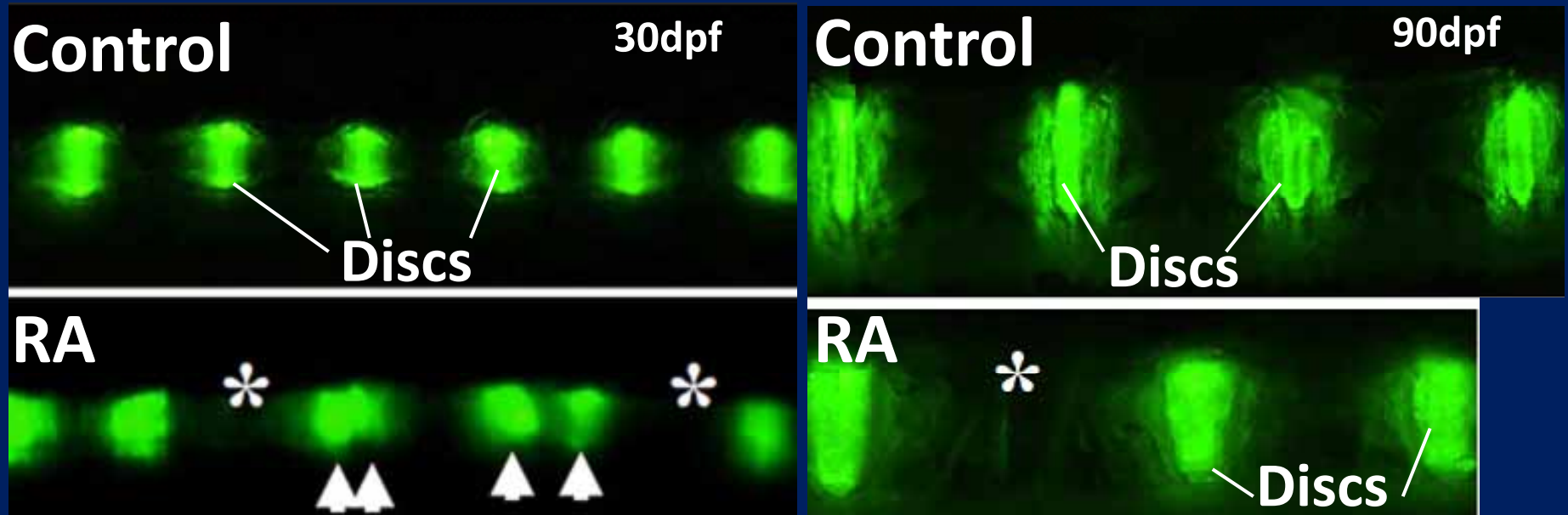
Haga *et al.* 2009. Transgenic Res. in press

RA induced disruption of GFP expression pattern in *twhh*-GFP zebrafish



Haga *et al.* 2009. Transgenic Res. in press

RA induced disruption of GFP expression pattern in *twhh*-GFP zebrafish



Haga *et al.* 2009. Transgenic Res. in press

Summary

- Altered gene expression was demonstrated in larvae showing high percentage of deformity
- *Twhh*-GFP transgenic zebrafish is useful resource to study intervertebral disc development and vertebral fusion in fish

Perspective

Process of production of cultured fish



Early diagnosis of skeletal deformity

Market-size fish