

Small Molecules Screen in Embryonic Zebrafish Using Molecular Variations to Target Segmentations

Sandra Richter, Ulrike Schulze, Pavel Tomančák, Andrew C. Oates

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What are Vertebral Malformation Disorders?



1/1000 in human population

Bones of the axial skeleton and muscle are produced during embryonic segmentation

Fraction of genetic causes are known

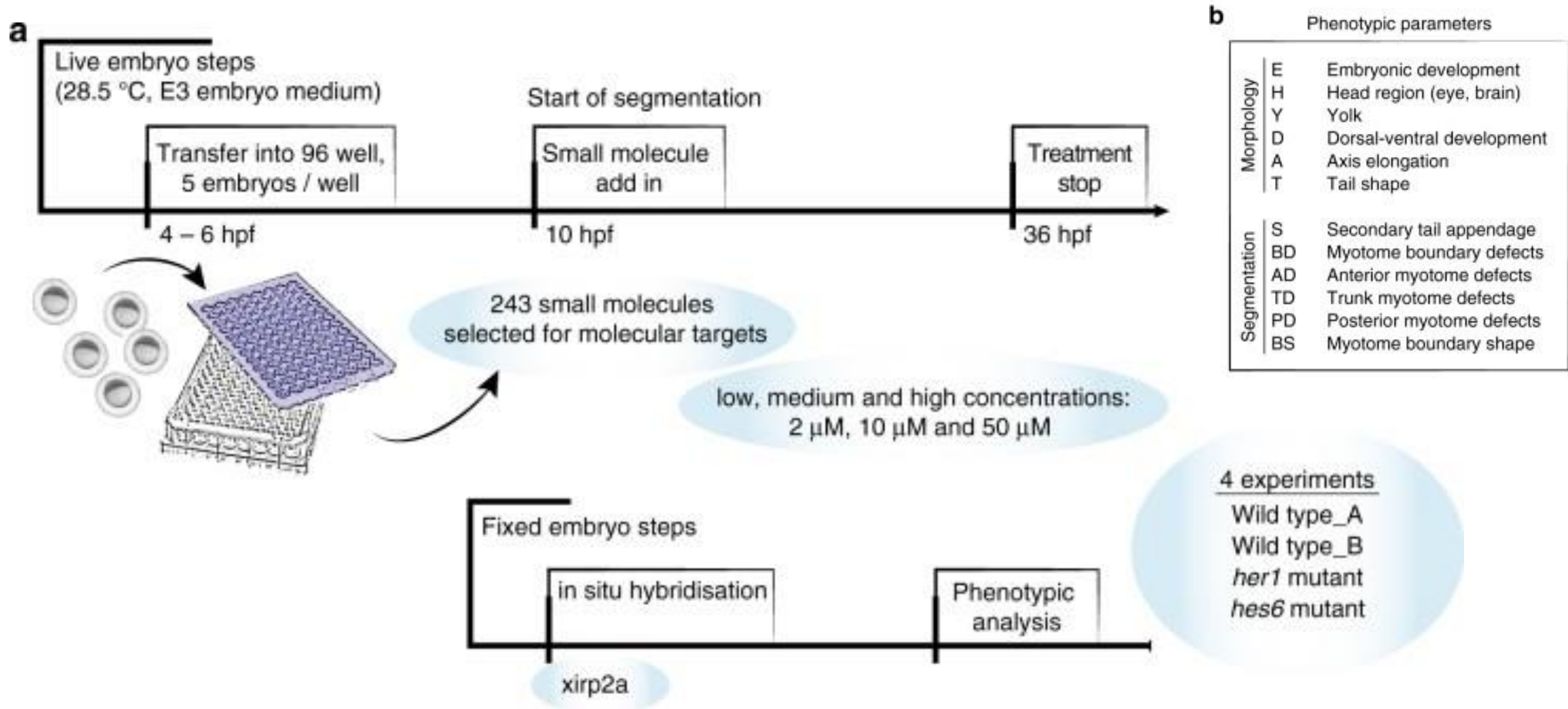
What organism was chosen to model bone malformation disorders?



How was chemical genomics used to identify segmentation defects?

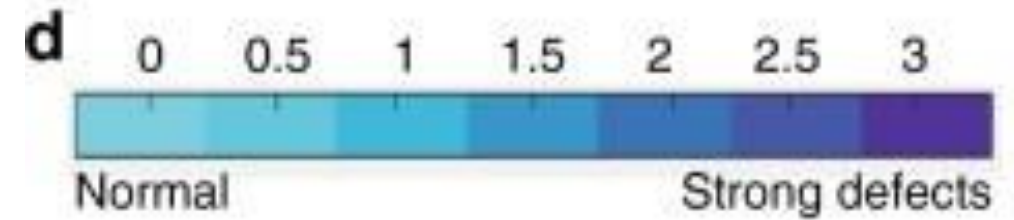
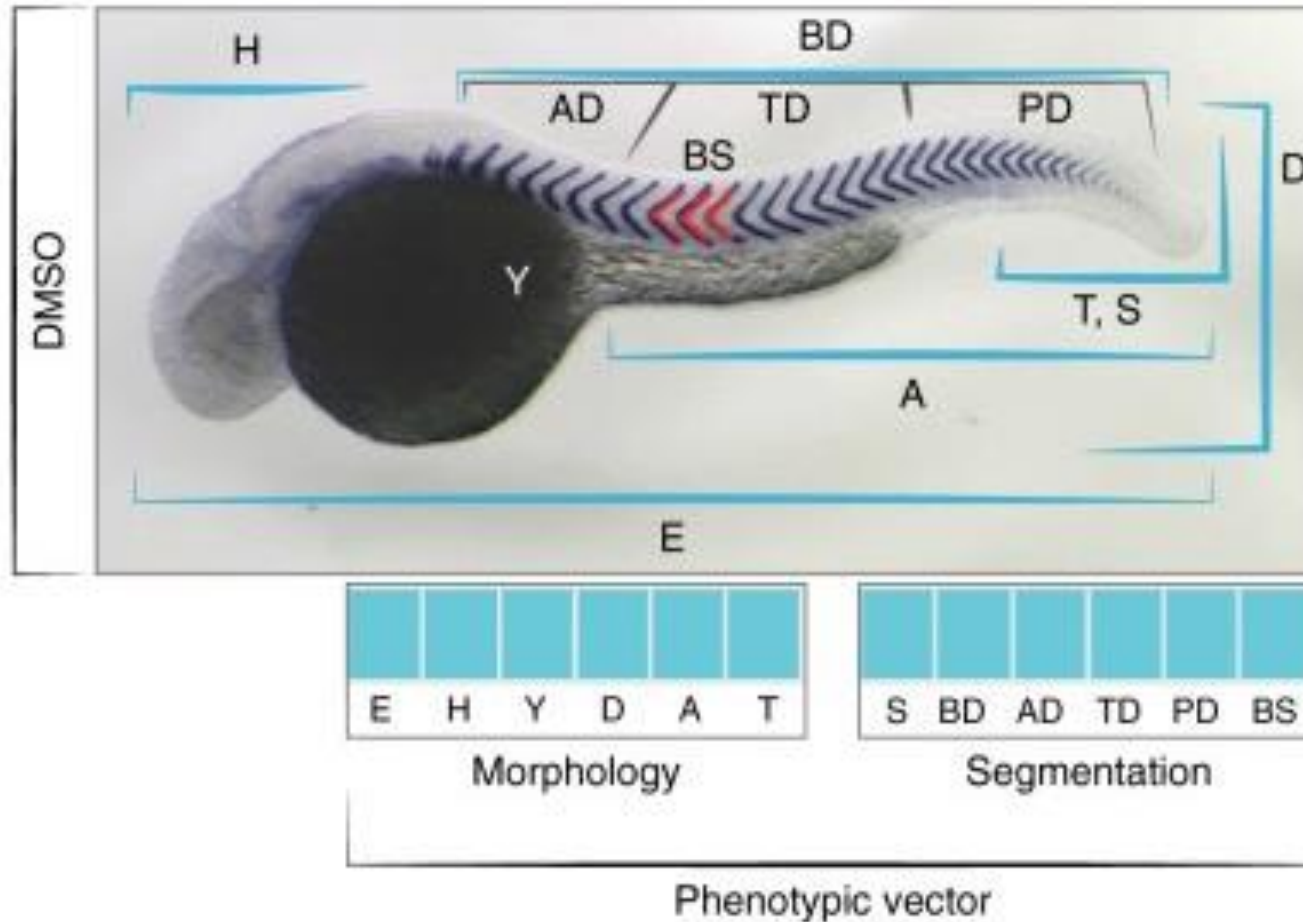


What was the screening and scoring strategy?



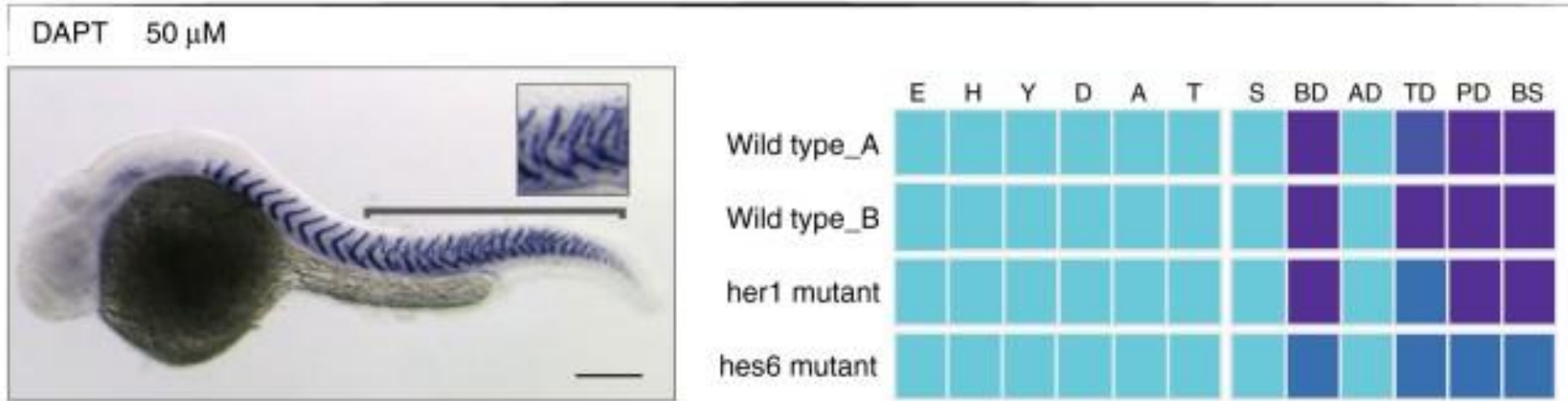
Insitu hybridization to detect xirp2a mRNA was used to get an analysis of segment boundary defects

How did they set up their unique **color coded** parameters to show how the skeletal segmentation is normally aligned?

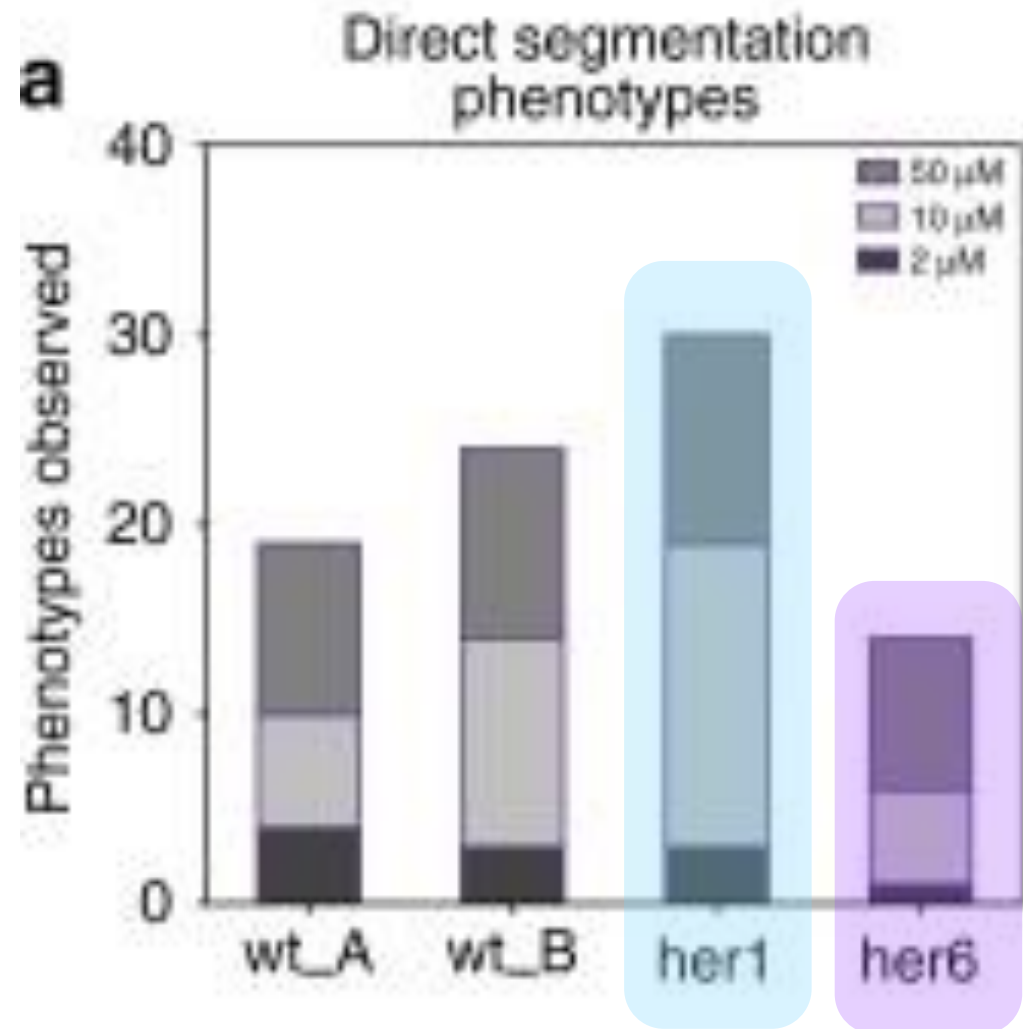


What were some positive control compounds and their parameters in their system?

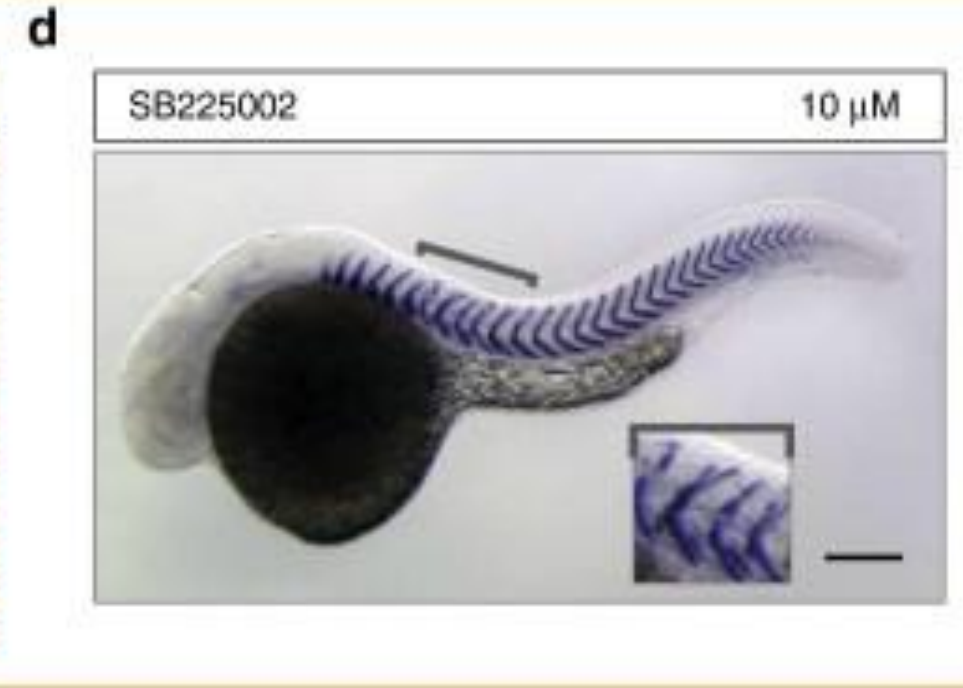
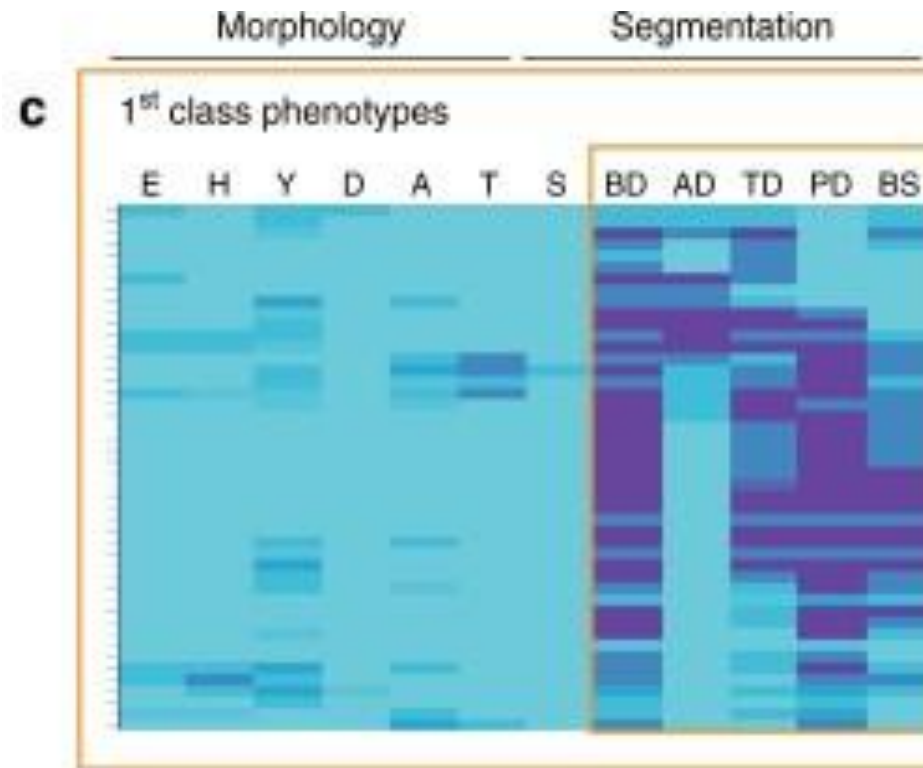
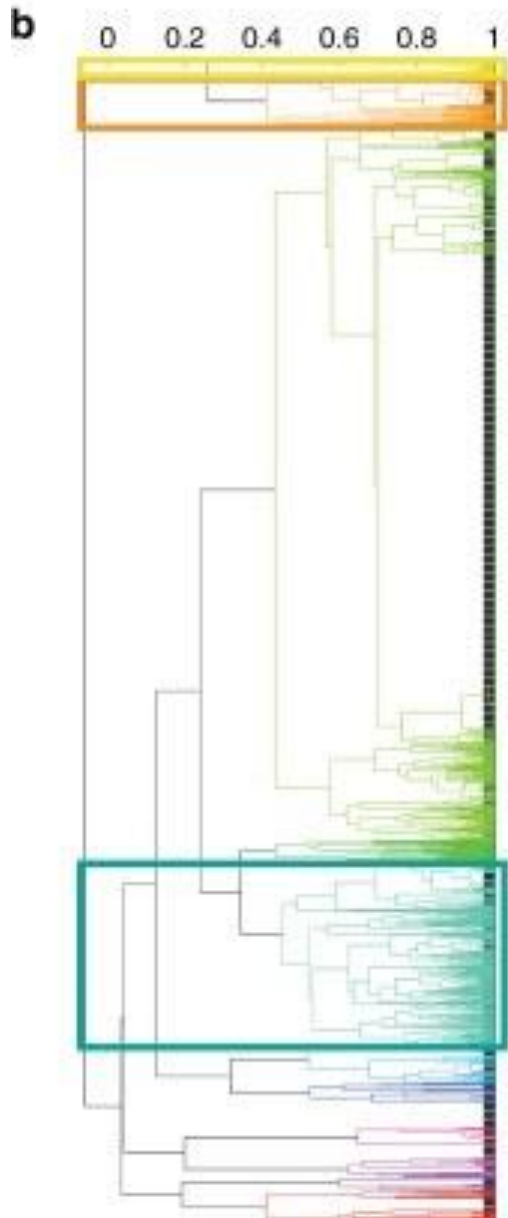
e



What were the phenotypes that resulted from direct segmentation?

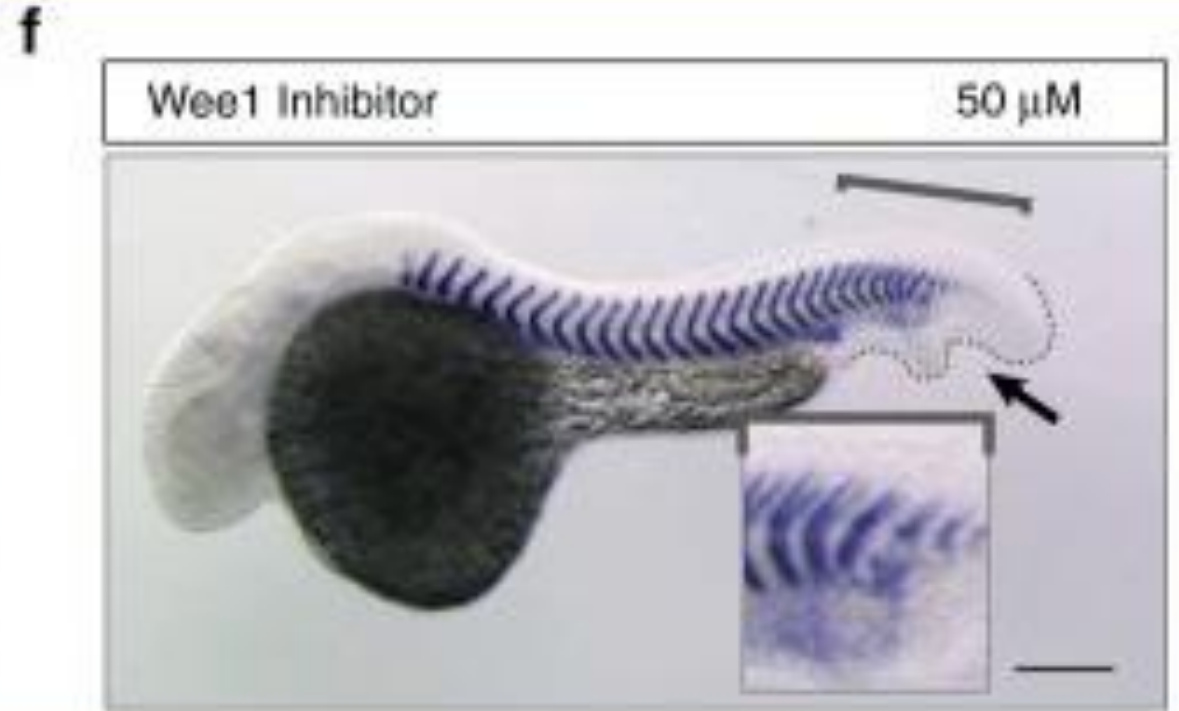
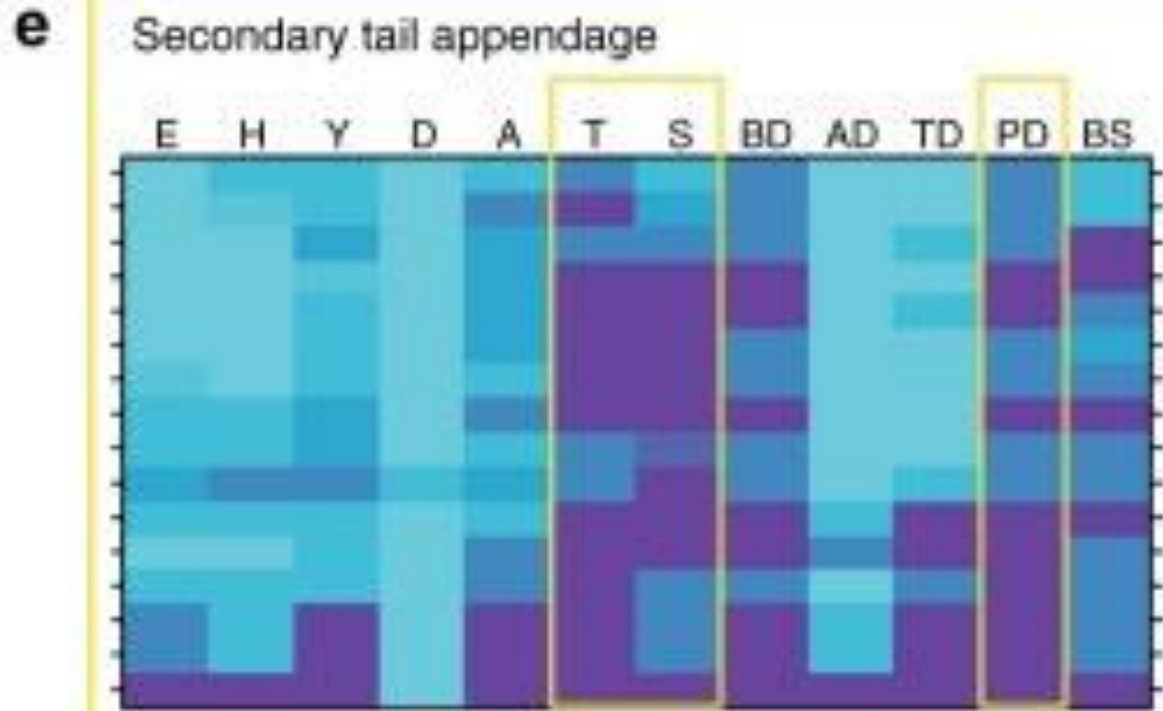


What was the 1st cluster of the hierarchical clustering of phenotypic vectors?



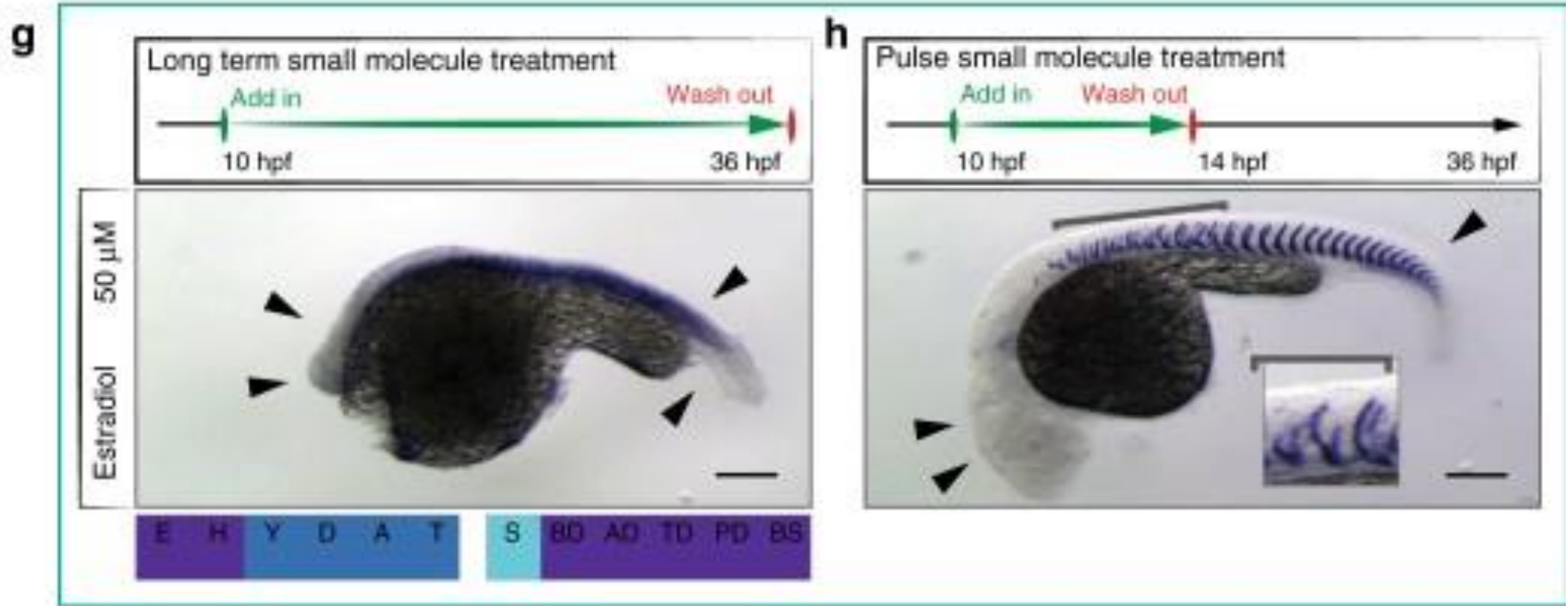
Phenotypes show normal axis elongation but showed specific mid-trunk defects of myotome boundaries

What was the 2nd cluster of the hierarchical clustering of phenotypic vectors?



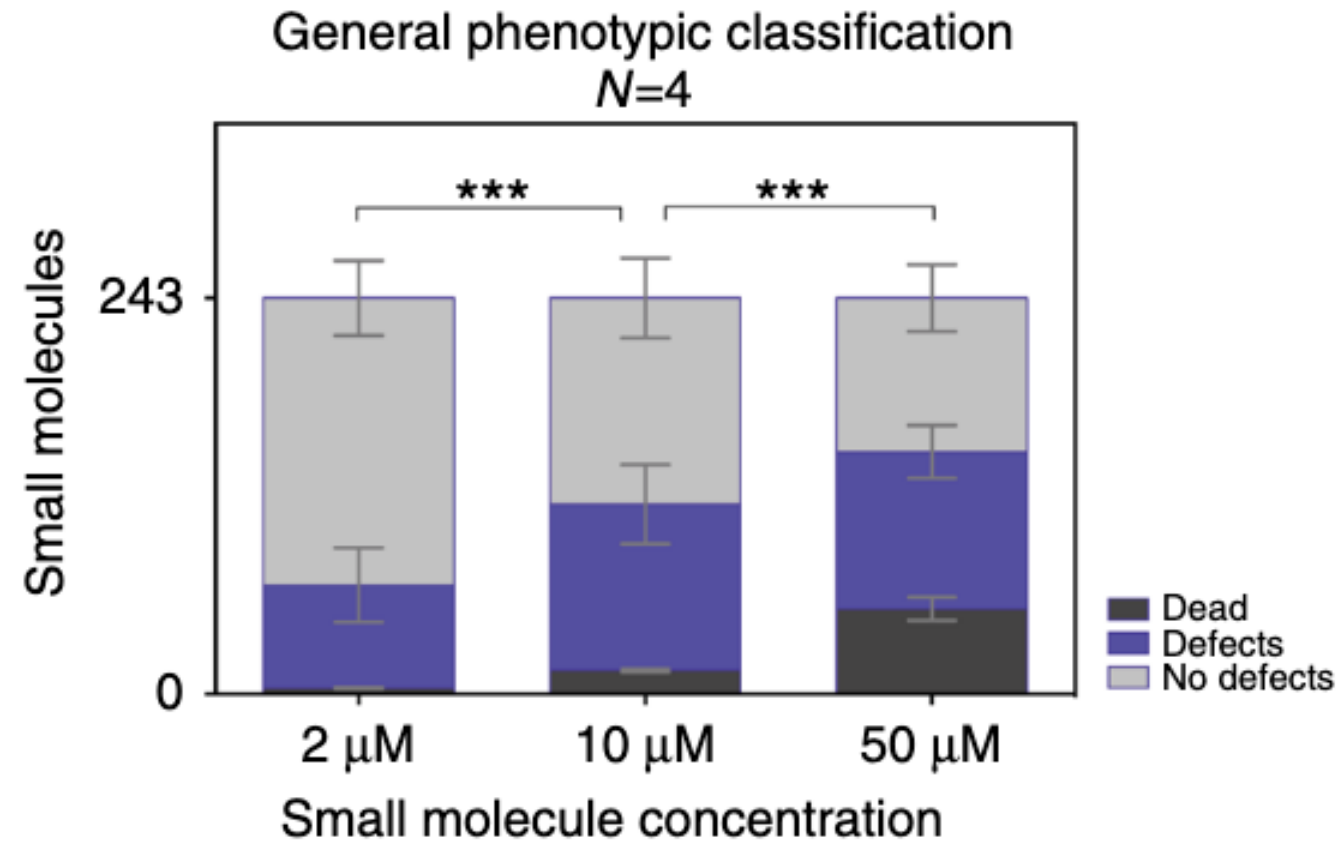
Key feature was its high scores of tail shape and secondary tail and development of a secondary tail appendage

Were there masked direct segmentation defects in the third cluster?

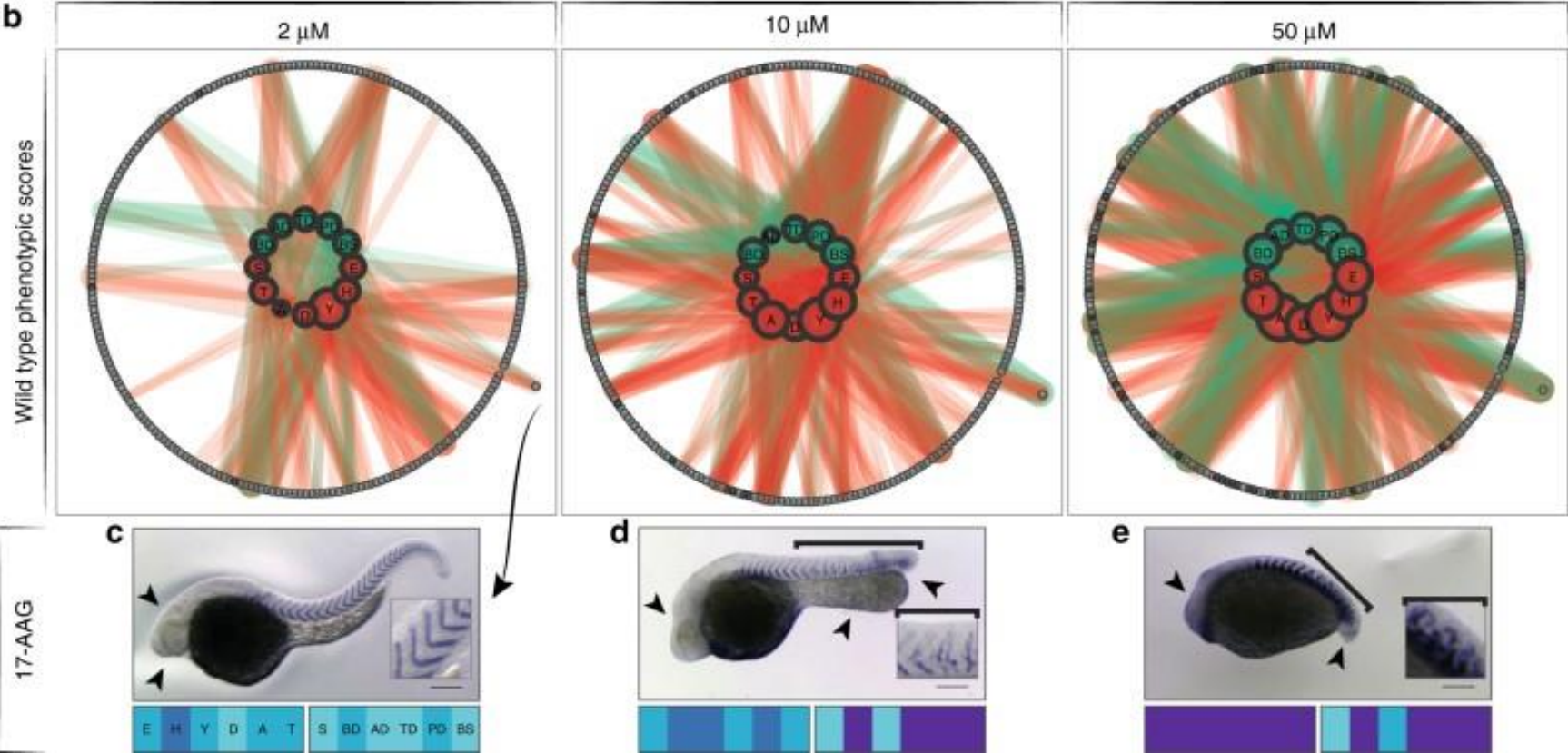


Used a shorter treatment to find masked direct segmentation defects

What were the phenotypes as concentrations increased?



What is the relationship between the CONCENTRATION of small molecules and the skeletal phenotypes?

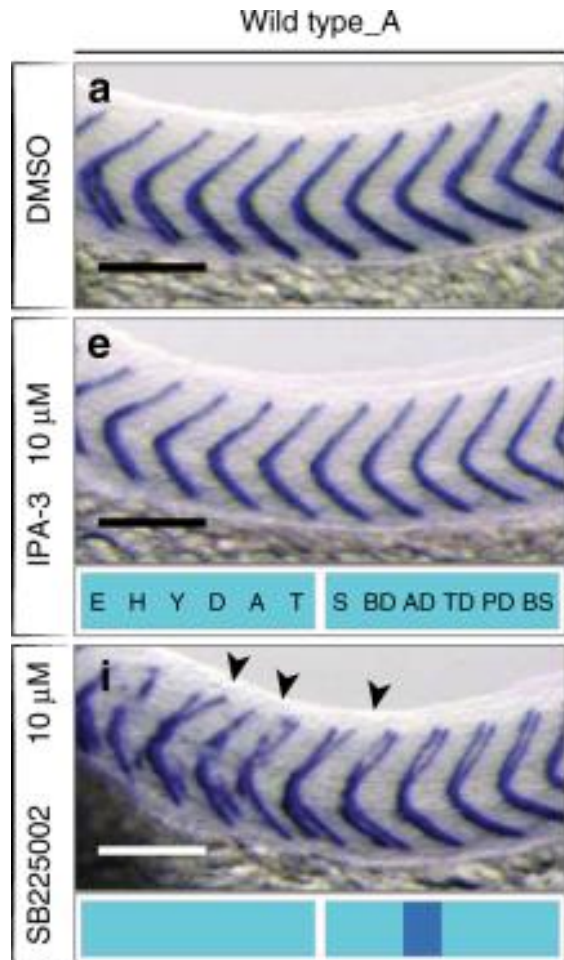


morphology
segmentation

Inside circle: Phenotypes

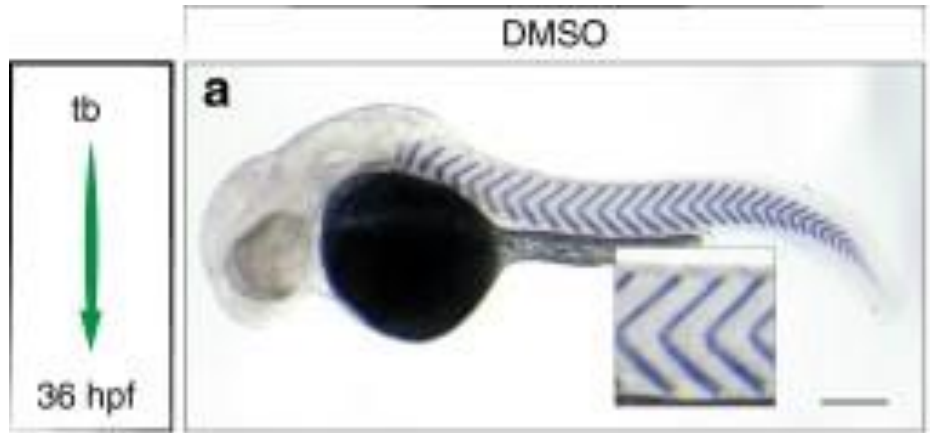
Interactions to outside circle is link of phenotypic parameter to small molecule

How do Her1 and Hes6 affect direct segmentation phenotypes?



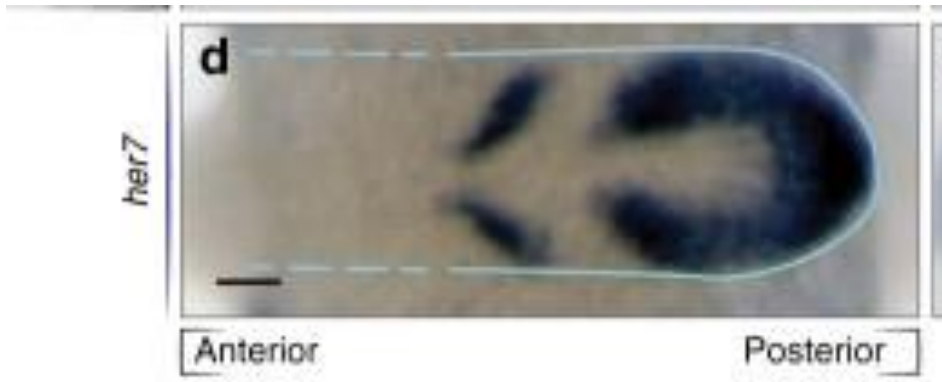
Her1 was found to be an enhancer for small molecule effects
Hes6 gave no advantage to small molecules

What direct segmentation phenotypes did inhibitors from the 1st cluster produce?



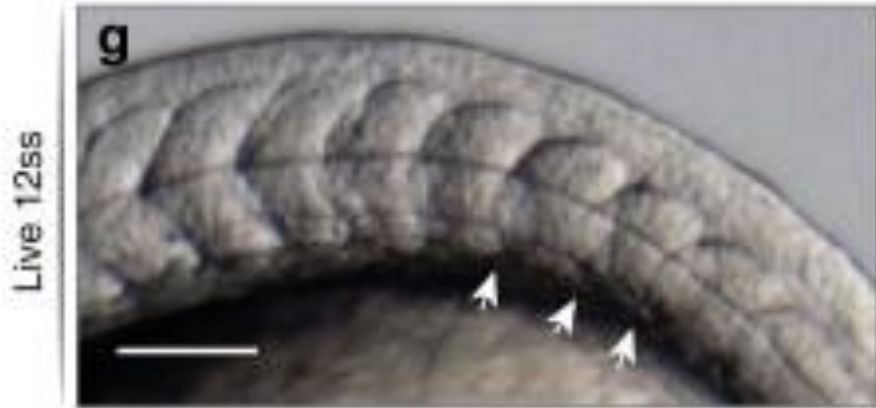
SB225002 caused boundary disruptions only in midtrunk segments
XRP44X showed mild segment defects evenly across the axis

When was the segmentation clock disturbed by SB225002 and XRP44X?



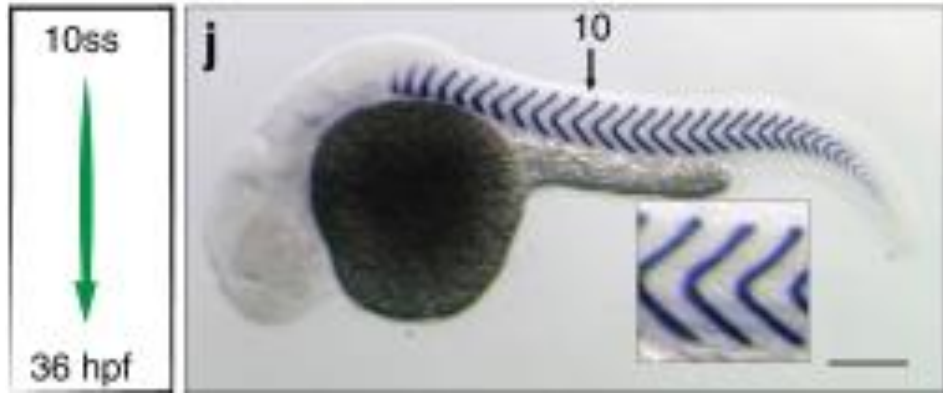
Segmentation clock found undisturbed by SB225002 and XRP44X

Did SB225002 and XRP44X affect the morphology of newly formed somites?



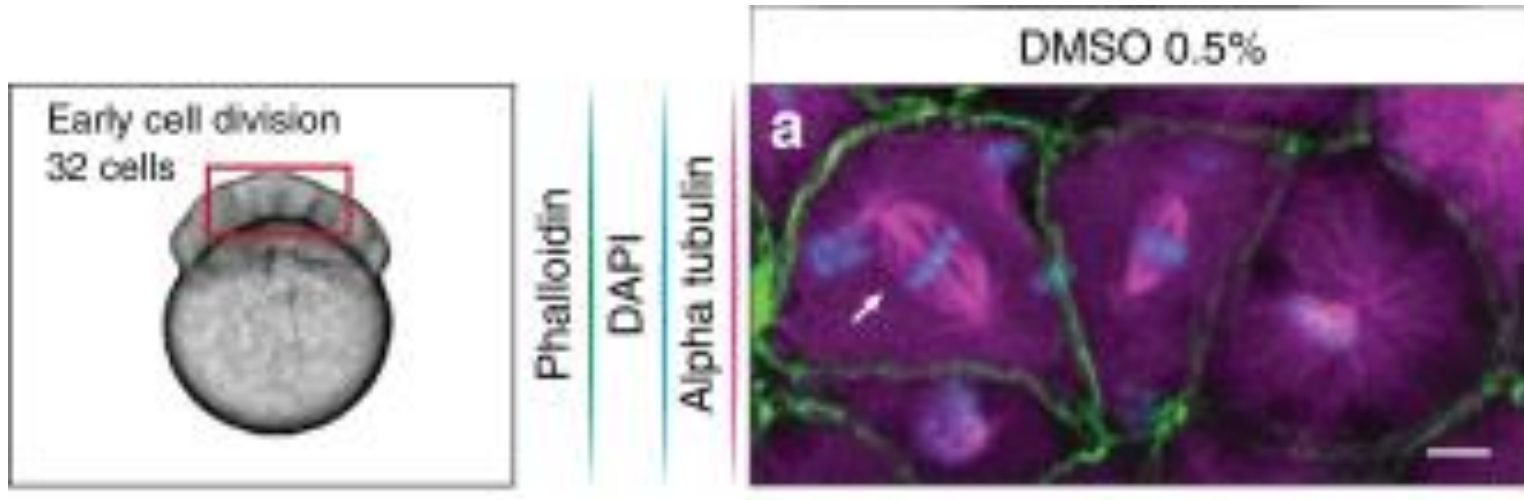
SB225002 affects somites in initial somite boundary formation
XRP44X doesn't affect initially formed somites

Did changing the TREATMENT TIME of SB225002 and XRP44X affect what phenotypes were observed?



SB225002 treatment resulted in normal segmentation.
XRP44X treatment caused defects to the myotomes in the anterior trunk

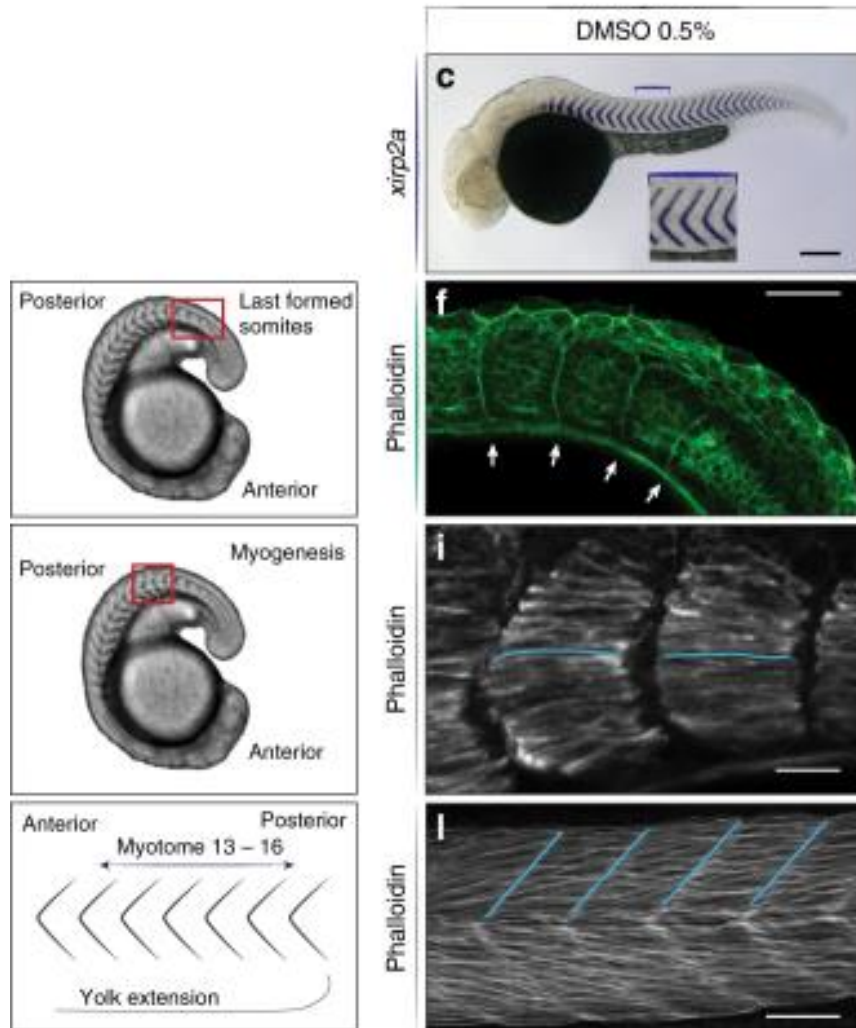
What was the effect of XRP44X on zebrafish embryos during mitotic cleavage?



XRP44X caused mitotic arrest and affected microtubules

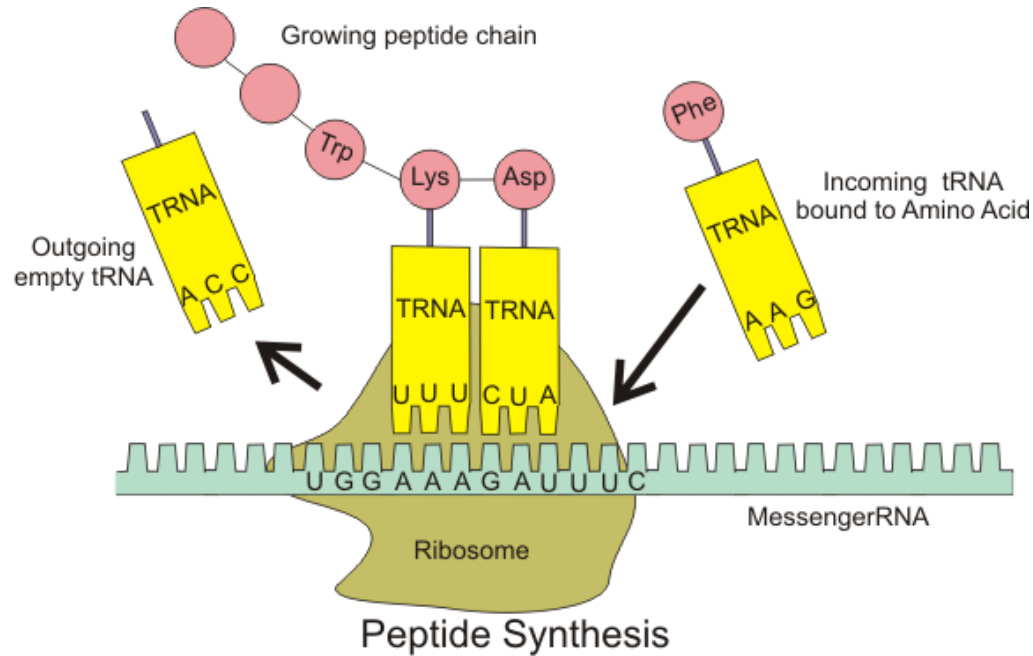
F actin
alpha tubulin

What were the phenotypes of myotomes when treated with XRP44X?

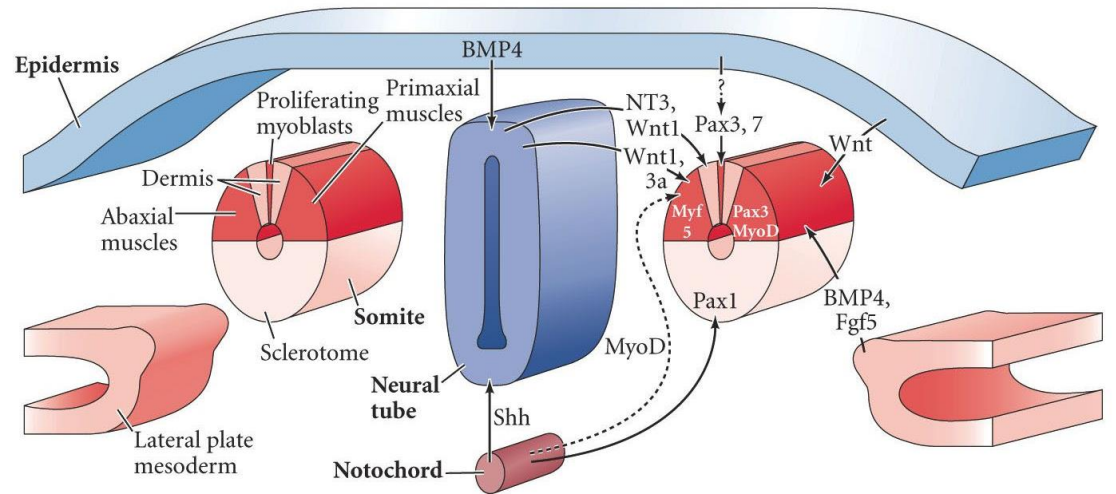


XRP44X affects segmentation through its activity on microtubule stability during somite maintenance and muscle differentiation

What could be concluded about the two compounds **SB225002** and **XRP44X**?

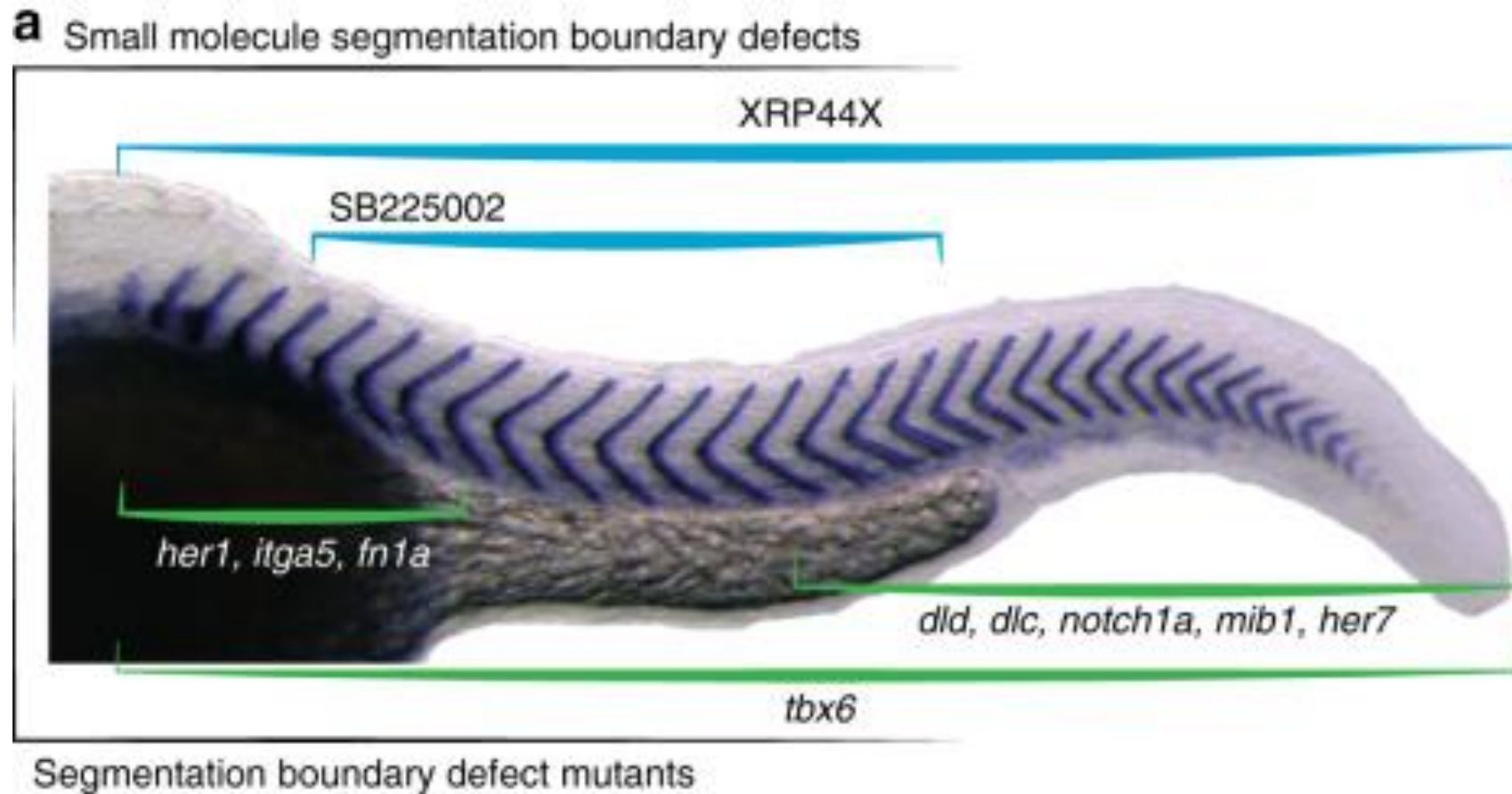


SB225002 interrupts translation

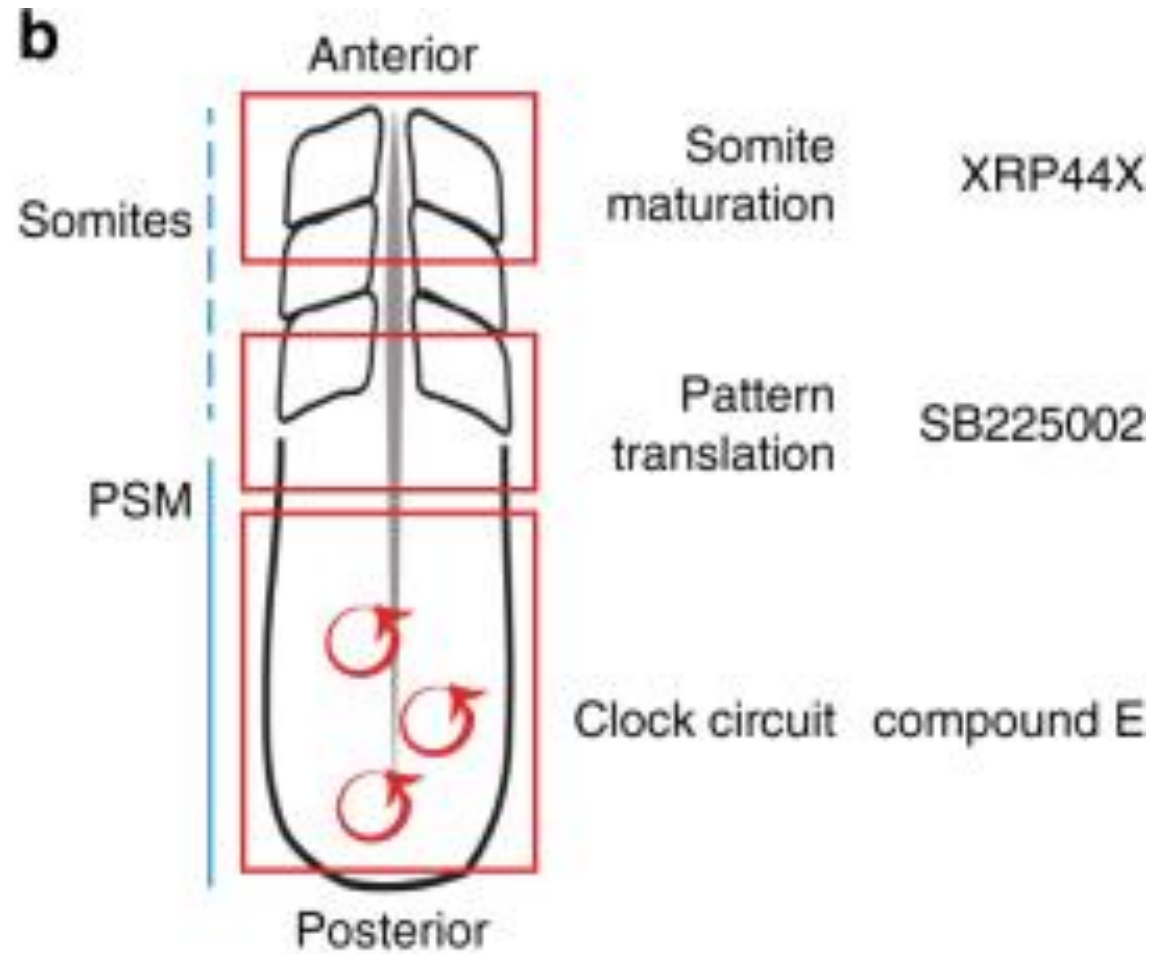


XRP44X affects somite maturation

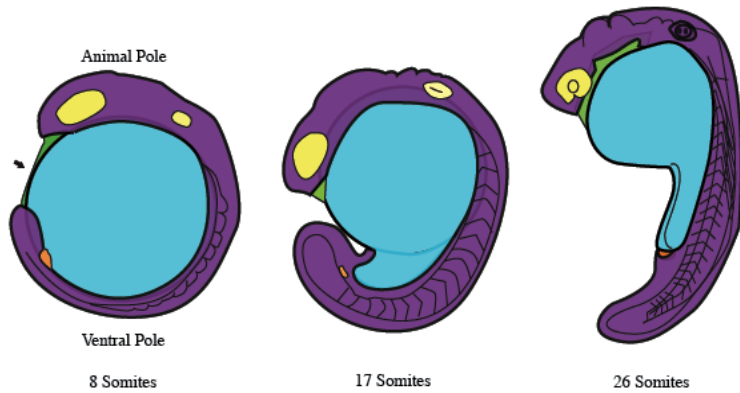
Overview: What are the selected direct segmentation phenotypes they identified?



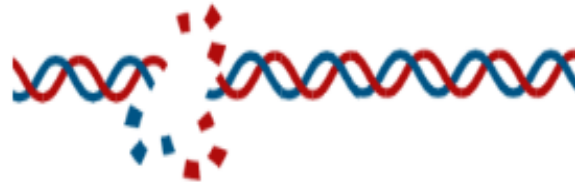
Overview: What were known small molecules to affect the steps of segmentation



What was concluded by scientists in this research?



29 small molecules



Her1 mutant



**Advantage of using a small,
flexible molecule screen**

How does their research translate to human disease?



Miller Syndrome

Resources

- https://embryology.med.unsw.edu.au/embryology/index.php/Zebrafish_-_Segmentation_Period
- <https://orthoinfo.aaos.org/en/diseases--conditions/spinal-deformity-in-children-with-myelomeningocele>
- <https://www.genengnews.com/news/bacterial-protein-found-to-reduce-inflammation-in-the-gut/>
- <https://alchetron.com/Chemical-library>
- https://embryology.med.unsw.edu.au/embryology/index.php/Zebrafish_-_Segmentation_Period
- <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5711842/>