AKT signalling pathway during embryonic development in the tunicate *Oikopleura dioica*

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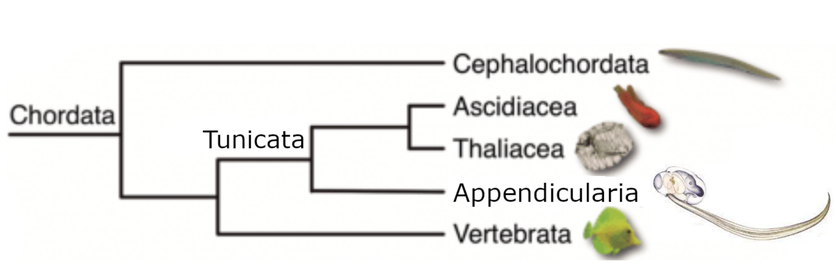
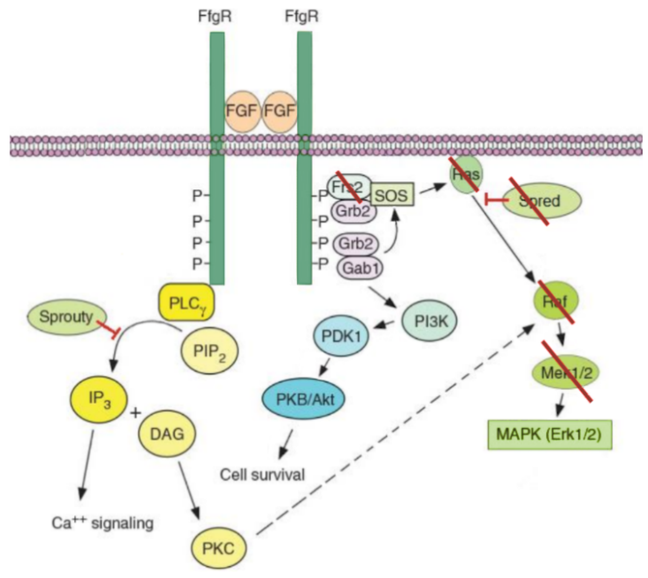
O. dioica, a model for gene loss

O. dioica is an emergent EvoDevo model that occupies a key phylogenetic position within the subphylum of tunicates. It has a short life cycle (around 6 days) and an extremely rapid embryonic development (around 4 h). Its body is divided into a trunk and a tail with a notochord and two bands of 10 muscle cells.

O. dioica's genome has undergone an extreme compaction process that has led to the dismantling of its Retinoic Acid signalling pathway (RA). The RA and Fibroblast Growth Factors pathway (FGF) antagonism is crucial for embryonic development in many animals. This suggests that *O. dioica*'s FGFs pathway has had a significant impact on its evolution and function.





Previous research in the group has shown that *O. dioica* has suffered a dismantling of the Ras/MAPK route. Continuing the lab's research on the FGF signalling pathway, this work is focused on the AKT pathway, which is a key regulator in various cellular functions, including cellular migration, mitogenesis, differentiation and cell survival.

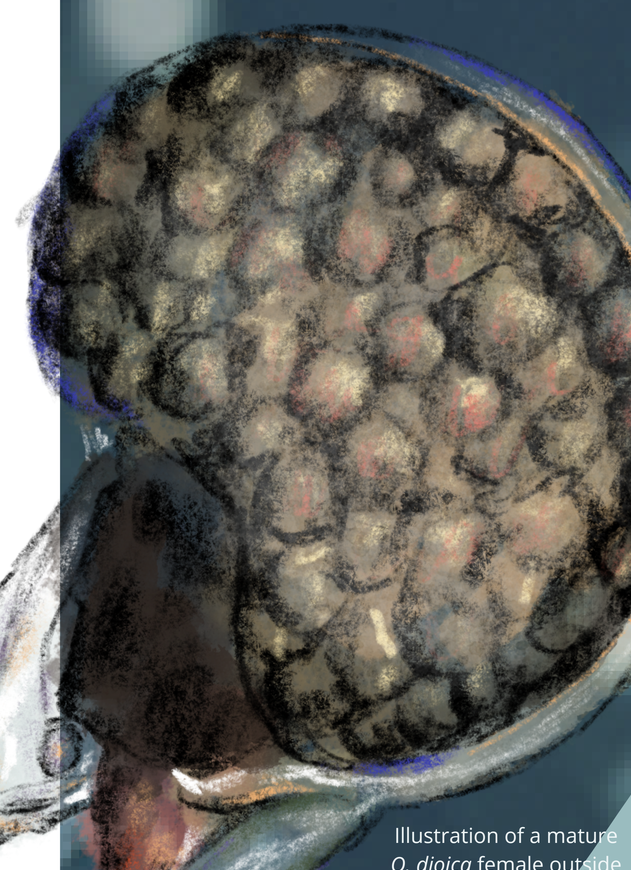


Illustration of a mature *O. dioica* female outside of her house

Objectives

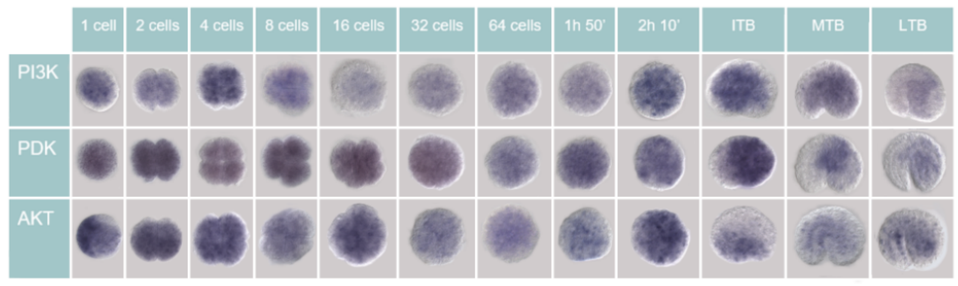
01. To analyze expression patterns of genes in *O. dioica*'s AKT pathway

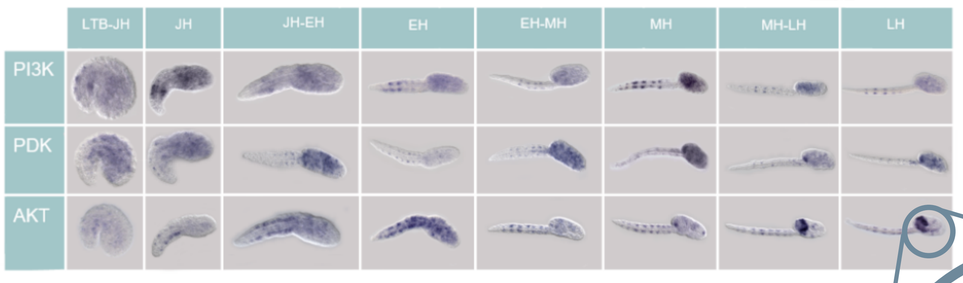
1. To amplify and clone the AKT, PDK and PI3K genes to synthesize their antisense probes
2. To characterize these genes' expression patterns throughout *O. dioica*'s development by whole-mount in situ hybridization

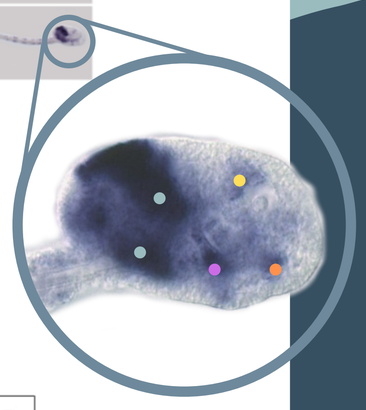
02. To perform inhibitory treatments of the AKT pathway in *O. dioica*

1. To determine Wortmanin's optimal application method to study its effects in *O. dioica*'s embryonic development
2. To characterize developmental abnormalities caused by inhibition of this pathway
3. To study changes in the expression patterns of *O. dioica*'s actin and troponins 3, 4 and 5 through whole-mount in situ hybridization

Expression analysis



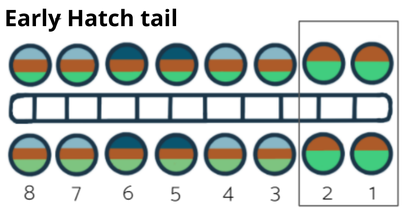




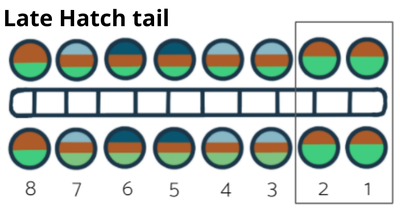
Whole-mount hybridization results of the PI3K, PDK and AKT genes that form the pathway during *O. dioica*'s development. The genes were expressed with a ubiquitous and maternal distribution until the tailbud stage, where the pathway acquired a more tissue-specific expression pattern in the tail and trunk.

From the EH-MH stage until the LH stage, stronger staining was observed in the ● stomach lobes, the ● posterior pharynx floor, the ● migrating oral glands and the ● brain.

Early Hatch tail



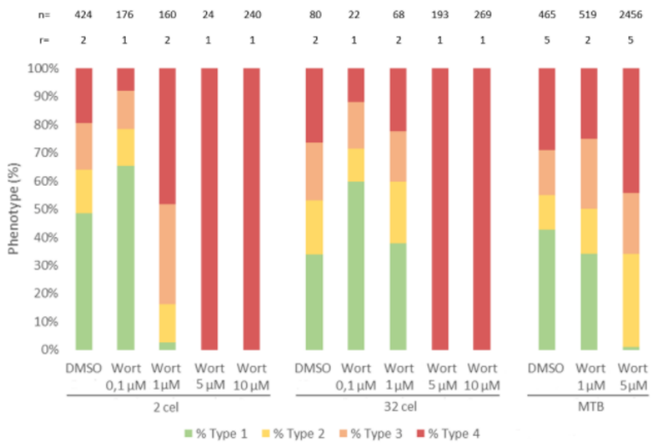
Late Hatch tail

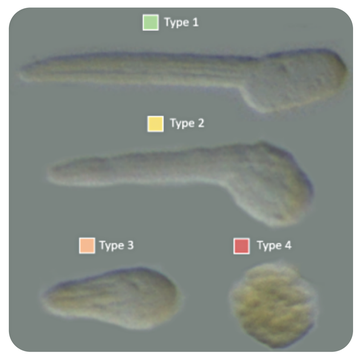


PI3K
PDK
AKT

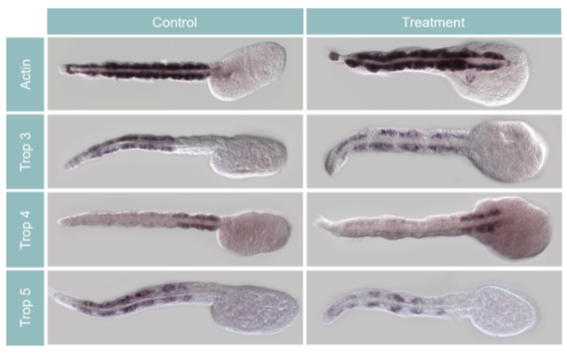
AKT and PDK genes were expressed in all the tail muscle cells at all hatching stages. In the EH stage, the PI3K gene was expressed in all muscle cells, with a higher intensity in the 5th and 6th pairs, with the exception of the 1st and 2nd pairs. In the LH stage the 8th and the first two most anterior muscle pairs showed no PI3K expression.

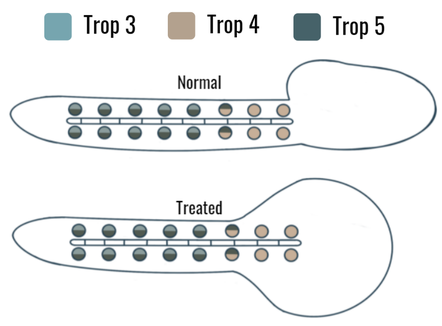
AKT pathway inhibition





To study the pathway's function, we treated embryos with Wortmannin, a PI3K inhibitor. Embryos were classified into 4 types according to the degree of affectation. The table collects the proportions of these phenotypes in the embryos treated with different Wortmannin concentrations or DMSO, at the 2-cells, 32-cells or MTB stages. The most optimal treatment to study the inhibitor's effects in tail development was at a **5 µM** concentration in the **MTB** stage, where a majority of embryos arrived at the hatching stage, but with obvious malformations (type 2) worth studying in more detail.





To study these abnormalities in the tail more detaily, another whole-mount in situ hybridization was carried out using these treated embryos, and the actin and the troponin 3, 4 and 5 genes. No differences in expression were observed between control and treated embryos. However, the first two muscle cell pairs did not complete the separation from the trunk.

Conclusions of the project

The AKT pathway shows a conserved ubiquitous and maternal pattern until the tailbud stage when its expression starts to be tissue-specific. Its basic functions in cell growth during early embryogenesis could be conserved in chordates

In later embryonic stages, the AKT pathway appears to be only active in the stomach lobes, the posterior pharynx floor, the migrating buccal glands, the brain, and in all the tail's muscle cells with the exception of the first two most anterior pairs

The severity of embryonic abnormalities caused by Wortmannin is correlated with the concentration and time window of the treatment

To study the effects of AKT pathway inhibition in *O. dioica*'s tail muscle cell development, the 5 µM MTB Wortmannin treatment is the optimal application

AKT pathway inhibition causes developmental abnormalities and a failure to complete the typical trunk-tail separation in hatched embryos