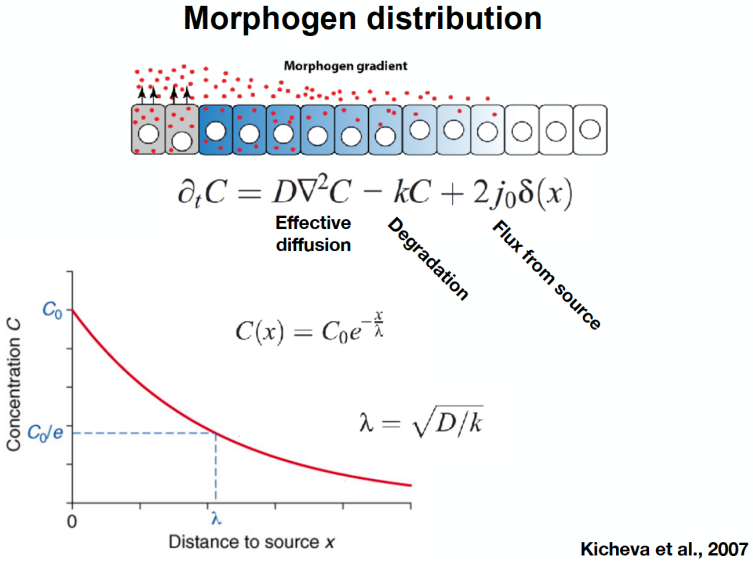
BIO-221 Exercises Week 5 – Week 6

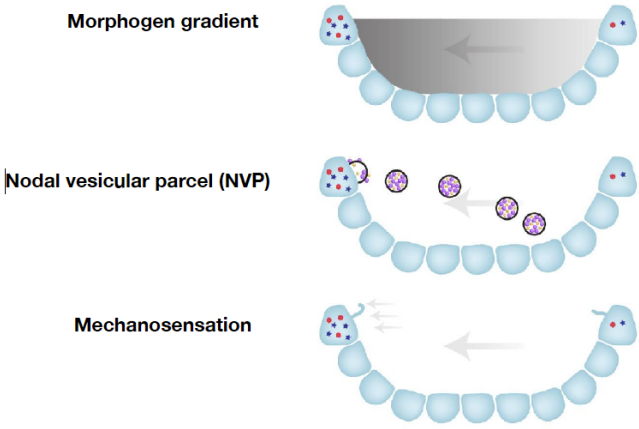
14.10.25 17:00 – 19:00

Part 1 ~30 min

1. Gradients of signals are found throughout development. Using the equations in the morphogen distribution slide, below, and what we discussed about TGF-beta signals, describe what changes you could make to the **biochemical or biophysical properties of one or more of the proteins** in the BMP signaling pathway that could lead to changes in the following features of the BMP gradient in the early zebrafish embryo:
   a. *shape* - how steeply it declines from the source
   b. *range* - where in the field of cells it reaches a particular concentration



2. The left-right laterality of the early mouse embryo is controlled by the activity of the node, a small pit at the end of the developing primitive streak/notochord. We discussed the lateralized flow of fluid across the node induced by aligned, tilted rotating cilia, but the nature of the signal that is generated by or with this flow is still not clear. Design an experiment to distinguish between any **two** of the following **three** hypotheses that we mentioned in the lecture:



Part 2 ~1 hr

The molecular functions of the zebrafish organizer

In the lecture on A-P and D-V axes in the zebrafish, we explored a model of how multiple interacting gradients set up in the blastoderm just before the onset of gastrulation. The signalling gradients of Nodal-related proteins Squint and Cyclops are thought to be of critical importance. As revealed by loss of function experiment with mutations in the *squint* and *cyclops* genes, or with loss of function in the receptor system caused by mutation to *one-eyed pinhead*, nodal signaling is **required** for the induction of all head and trunk mesoderm and endoderm, as well as playing a role in the A-P axis.

In thinking about the organizer, however, the operational definition is a transplantable unit of tissue that is **sufficient** to induce an ectopic axis, just as was seen in the original Spemann-Mangold experiments in 1924. Design an experiment using zebrafish to test whether nodal-related signalling is sufficient to cause organizer activity. Your general experimental approach should be to use injected mRNA encoding the Cyclops / Ndr2 protein, and the transplantation of cells between donor and host embryos at developmental stages up to the onset of gastrulation.

1. Give details of this experiment, including timing and location of manipulations and the time point when the result should be evaluated.
2. Predict the likely outcome(s) of the experiment.
3. You can work in small groups.
4. ***Send this to Emanuel*** emanuel.vasquez@epfl.ch and ***Andy*** andrew.oates@epfl.ch ***by Wednesday October 15th @ 10:00.***

Cool thing: We will perform real experiments in zebrafish embryos and the results will be shared with you during the Wednesday session (October 15).

Remember:

1. Microinjection - A technique whereby mRNA (or DNA, dyes, morpholinos (synthetic oligonucleotides for reducing gene function)) is injected into early embryos. This technique allows for variable expression of a protein of interest. Injection can be done early to get wide-spread distribution, or late, to confine the molecule to a small region.
2. Transplantation - A technique where cells isolated from a location in one embryo (the donor) are moved to a specific region in another embryo (the host).

15.10.25 12:00 – 14:00

1. Evaluate the experimental design, anticipated outcome and real results with Emanuel.