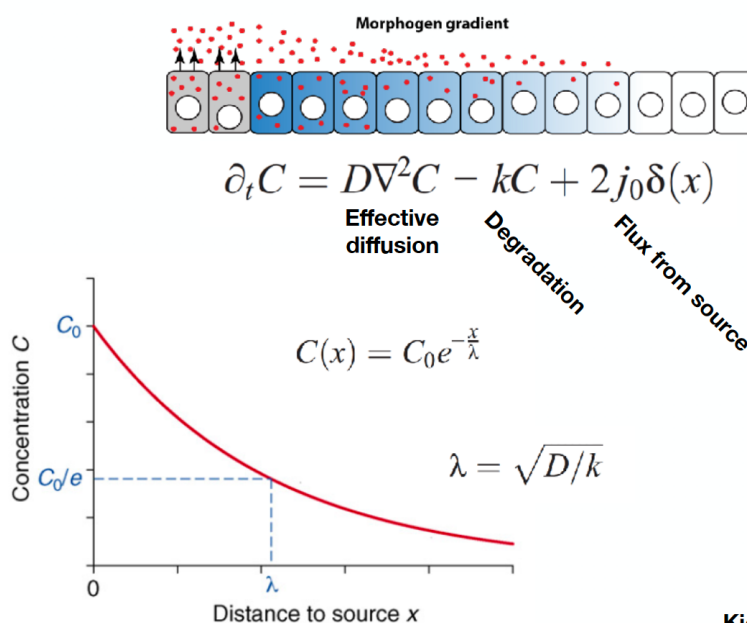


Part 1 ~30 min

1. Grafting and explant experiments have been instrumental in our understanding of *Xenopus* development. What would you predict as the result from the following experiments:
 - a. The 4-cell embryo is separated into two 2-cell half-embryos by cutting vertically between the cells along a line oriented between the sperm entry point and the middle of the grey crescent. What happens to each of the two 2-cell half embryos?
 - b. An animal cap is cultured with cells from the dorsal vegetal part of a different embryo that was previously incubated in colchicine at the time of fertilization. What tissue, if any, is induced?
 - c. Transplanting the dorsal organizer region from an early gastrula into the ventral side of a host embryo induces a second axis with a head and trunk. What effect follows from transplanting the equivalent region of the embryo during the second half of gastrulation? What effect follows from transplanting the equivalent region of the embryo after gastrulation is finished?
2. 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 proteins in the signaling pathway to change the gradient's:
 - a. *shape* - how steeply it declines from the source
 - b. *range* - where in the field of cells it reaches a particular concentration

Morphogen distribution



Part 2 ~1 hr

The zebrafish organizer – fact or fiction?

Explants and grafting experiments have been performed in many different species to identify where the organizer is and the molecules involved as well as to understand general principles underlying body axis establishment. You learnt about organizers in *Xenopus* on Monday. Your task here is to use zebrafish as a model system and *propose experiments* to identify

- (a) where an organizer might be in early embryos, and/or
- (b) what molecules might be involved in the ability of any potential organizer to induce cell fates and embryonic structures.

You should organize this in the following manner:

1. Propose an experiment for addressing the above-mentioned problem(s) in concrete terms.
2. Predict the likely outcome(s) of the experiment. **Send this to Andy andrew.oates@epfl.ch and Emanuel emanuel.vasquez@epfl.ch by Friday evening 11.10.21!**
3. Interpret the experiment (this task will be undertaken on Oct 17).

Cool thing: We will test some of your suggestions and perform real experiments in zebrafish embryos. The experiments will be performed by the Oates lab members and the results will be shared with you next Thursday (Oct 17).

Today: We will first give you a short introduction to basic zebrafish embryology focusing on the start of gastrulation. Key concepts that will be introduced: blastoderm, margin, epiboly, cell involution and convergence.

Techniques that may help you:

1. Microinjection - A technique where DNA, mRNA or morpholino (synthetic oligonucleotides for reducing gene function) is injected into early embryos. This technique allows for increasing or decreasing expression of 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 one embryo (usually called the donor) are moved to a specific region in another embryo (usually called the host).
3. Tissue grafting - A technique where an intact piece of tissue is cut from one embryo and moved into another embryo.

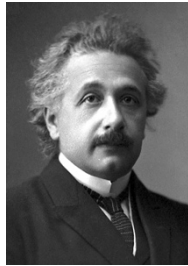
You will be given several clues to help with this exercise. Here are the first clues:

1. *One of the first things you want to do is to know which embryo stage will be of interest to answer the question. Think of the stage that was used in *Xenopus* to do the Spemann-Mangold experiment.*
2. *If you want to propose a transplantation experiment, think about the choice of your donor and host embryos.*

Part 3 ~ 30 min

***Gedanken* experiment – morphogenesis in embryonic tissues**

A *Gedanken* experiment is done away from the laboratory, usually lying down, on a train, or under an apple tree. The goal is to explore a range of possibilities in abstract terms, to gain some insight onto the problem and to see what kind of experiment might be worth doing and what sort of results might be surprising.



Imagination is more important than knowledge.

We have seen movies of embryonic tissues changing shape during elongation, gastrulation, neurulation etc. Consider an epithelium in which cells are joined to their neighbours in a roughly 2D arrangement, forming a single-layer cellular sheet.

- What changes of shape or properties would the cells in a cell sheet need to have to cause a large-scale deformation in the sheet?
- What shapes are possible?
- Given the cell biology we have covered already, what *molecular* mechanisms could be used to drive such changes?