How fly embryos know head from tail

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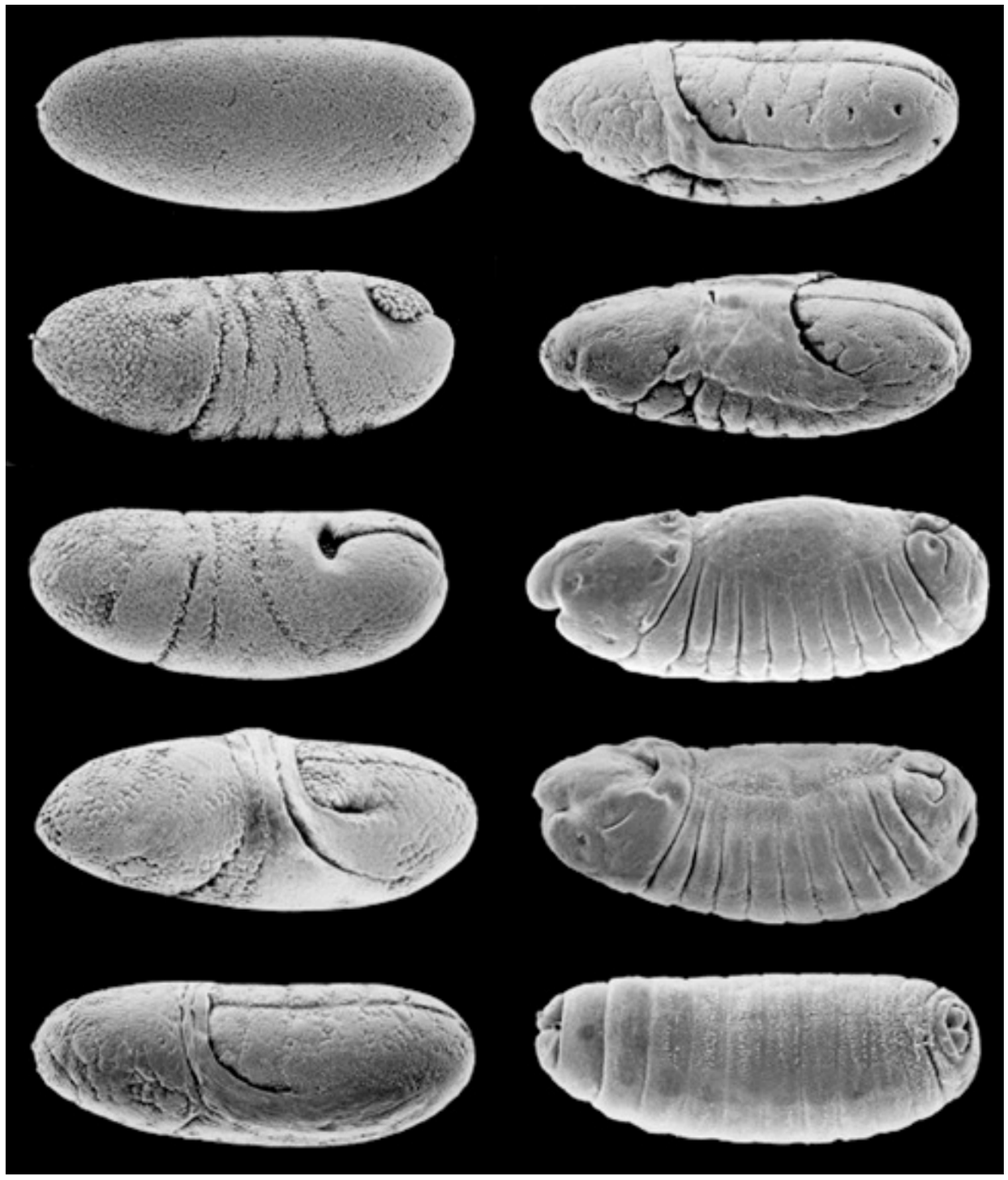
Development



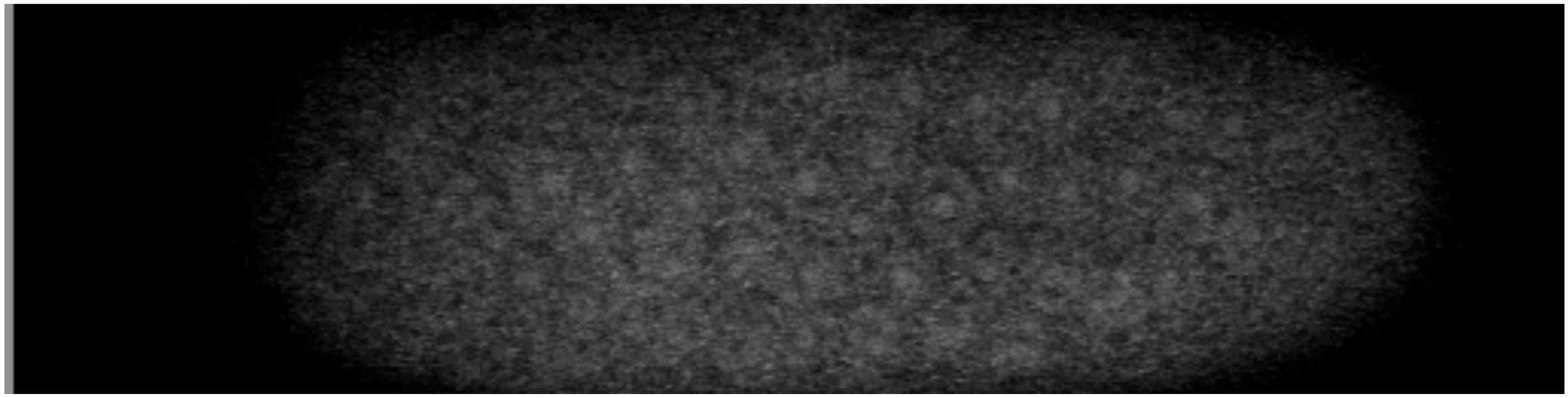
Stages of development

- Development starts with a single fertilized egg
- the genome of every cell in our body remains unchanged

- breaking the symmetry:
  - anterior - posterior (front and back)
  - dorsal - ventral (back and belly)
  - left - right

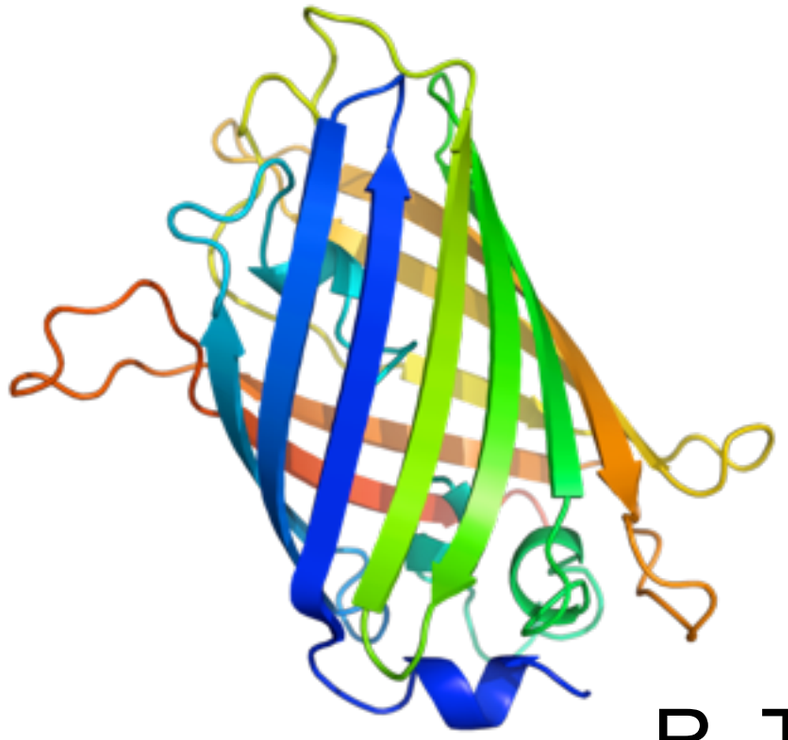
- specification of the different organs, segments....

Drosophila filmfest

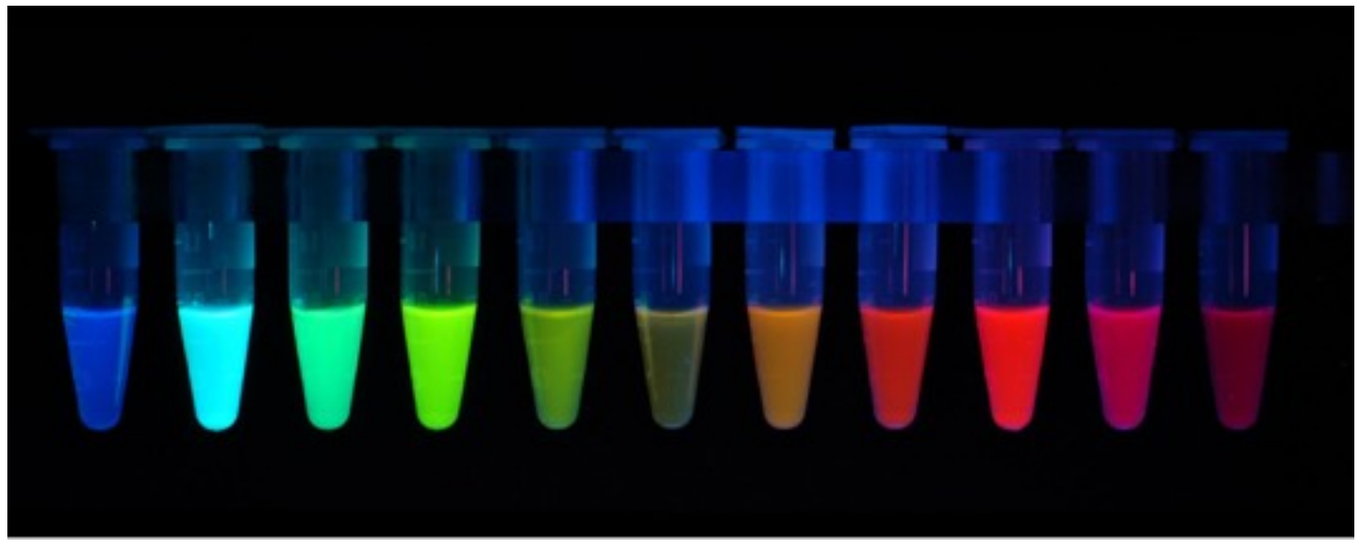


http://www.princeton.edu/~wbialek/rome/Hist04BNT.avi

Green fluorescent protein



R. Tsien



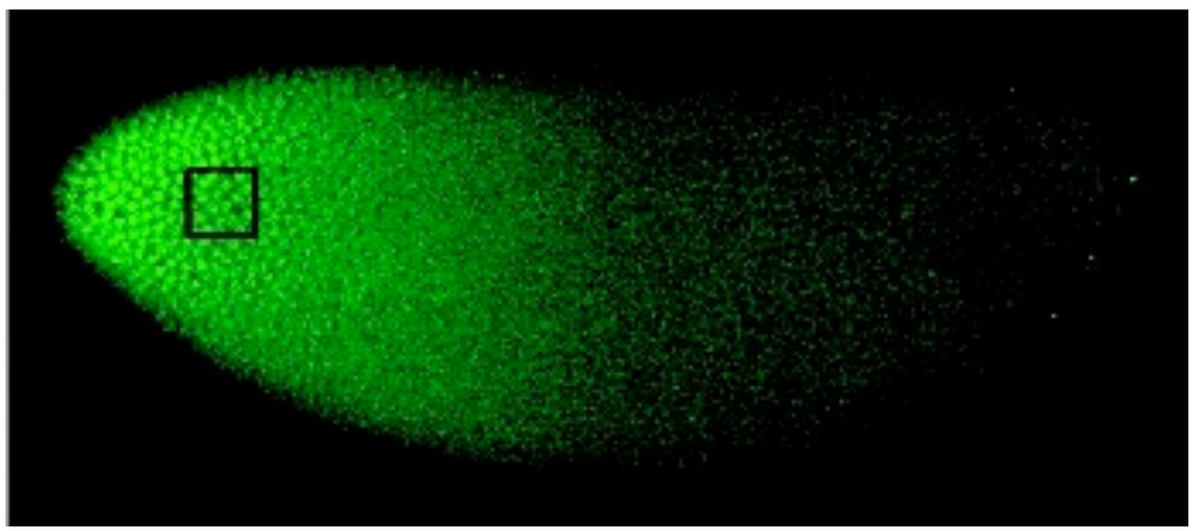
Different colors obtained by modification of the protein

Bicoid mRNA and protein

- bicoid mRNA is localized at the front of the egg, deposited by the mother
- bicoid protein is produced from the mRNA
- Bicoid protein diffuses towards the tail
- Bicoid acts as a transcription factor that turns on cascade of genes that determine head/tail

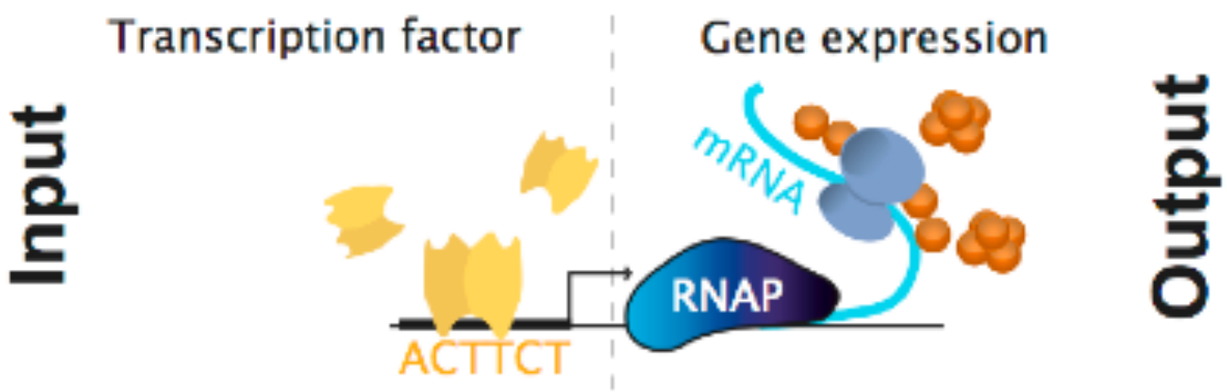
bcd RNA

Bicoid protein



Bill Bialek

Transcriptional regulation



U Irion et al

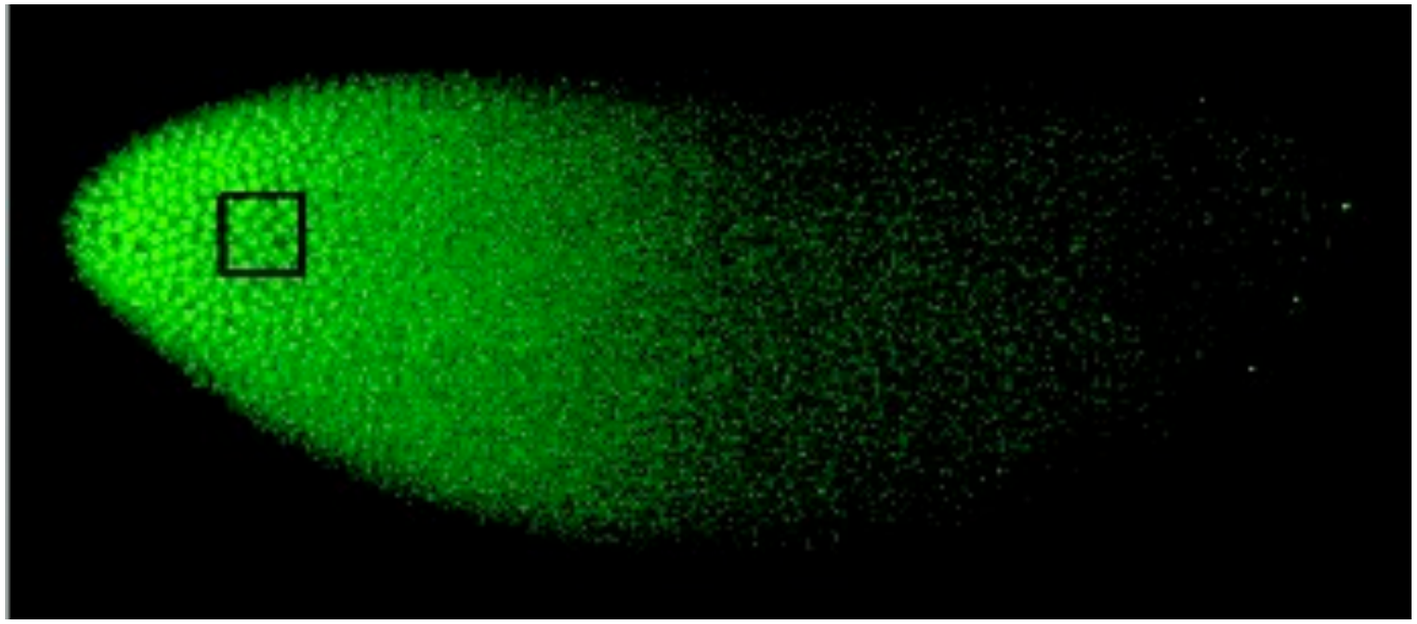
Input – output

- Bicoid regulates downstream genes such as hunchback
- very sharp response: a shallow gradient is transformed into a step like response.
- this sharp response is achieved by a series of amplifications and feedbacks

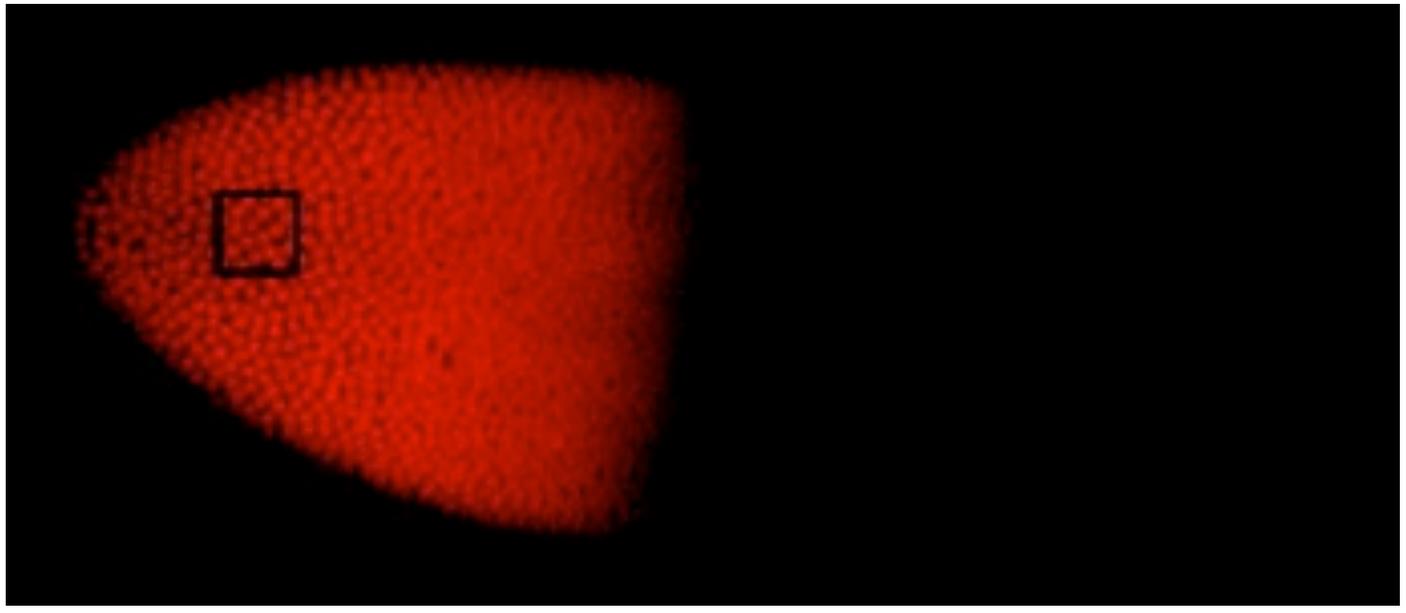
discovered here in Tuebingen by

Driever and Nuesslein-Volhard, 1988

Input: Bicoid protein

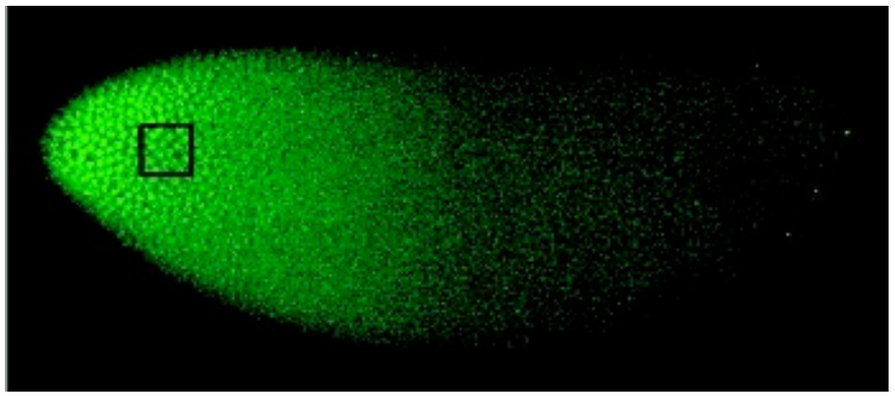


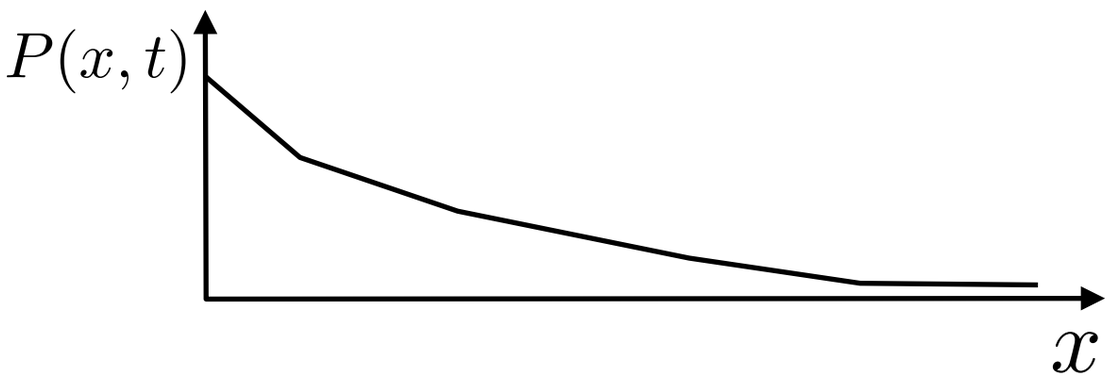
Output: Hunchback protein



(stolen from) Bill Bialek

Models of Bicoid gradient formation





$$\frac{\partial}{\partial t}P(x,t) = D\frac{\partial^2}{\partial x^2}P(x,t) - \frac{P(x,t)}{\tau} + k\delta(x)$$

diffusion degradation production

Steady state solution: $P(x) = \frac{kD}{\tau}e^{-\frac{x}{\sqrt{D\tau}}}$

Estimating the diffusion constant

- The gradient forms in ~90min = 5400s
- The length of the embryo is 500μm
- The typical distance traveled by diffusion in a time t is $\left|\Delta x\right| \sim \sqrt{Dt}$
- Hence for diffusion over 100 μm, we need

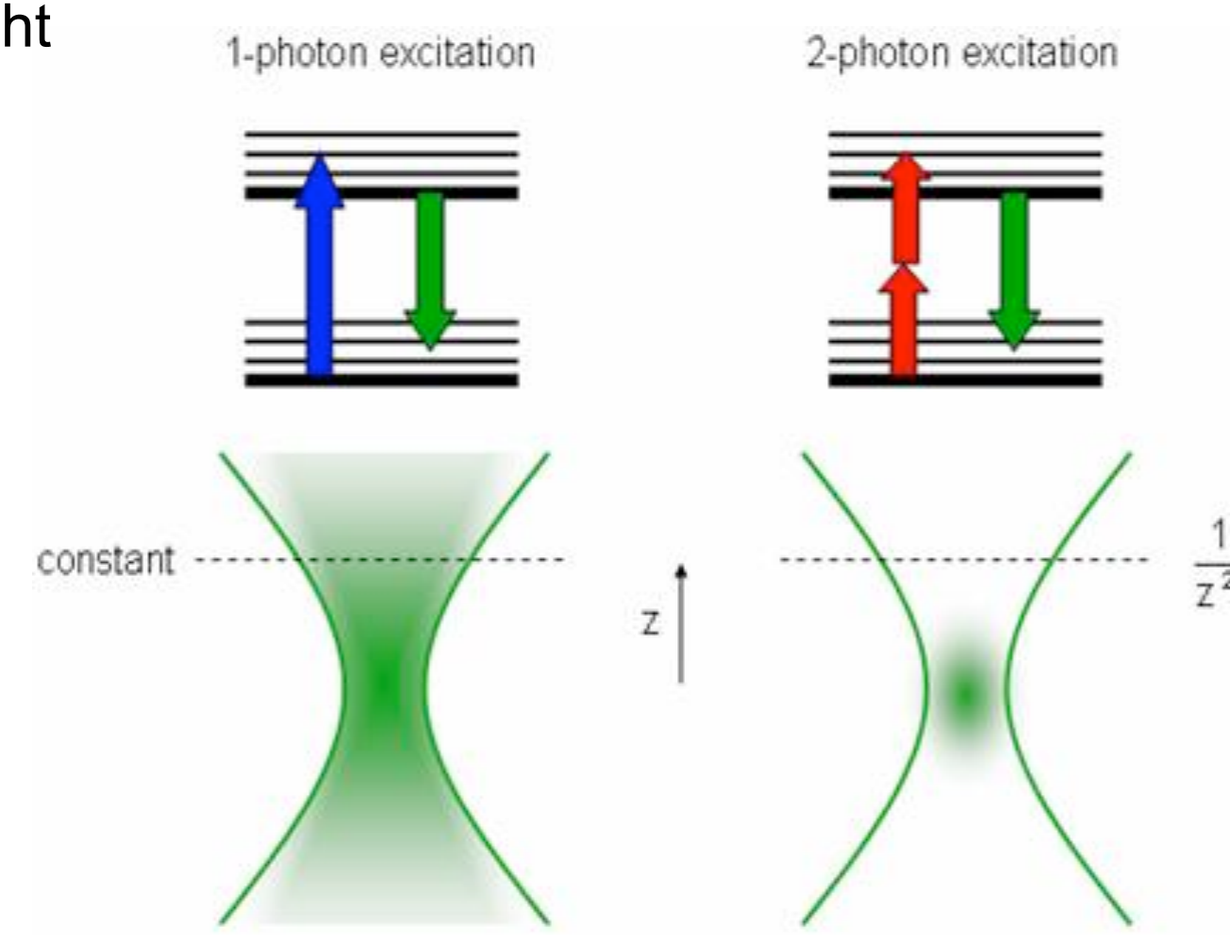
$$D > \frac{10^4}{5000}\frac{\mu m^2}{s} = 2\frac{\mu m^2}{s}$$

$$\tau \approx 1000s$$

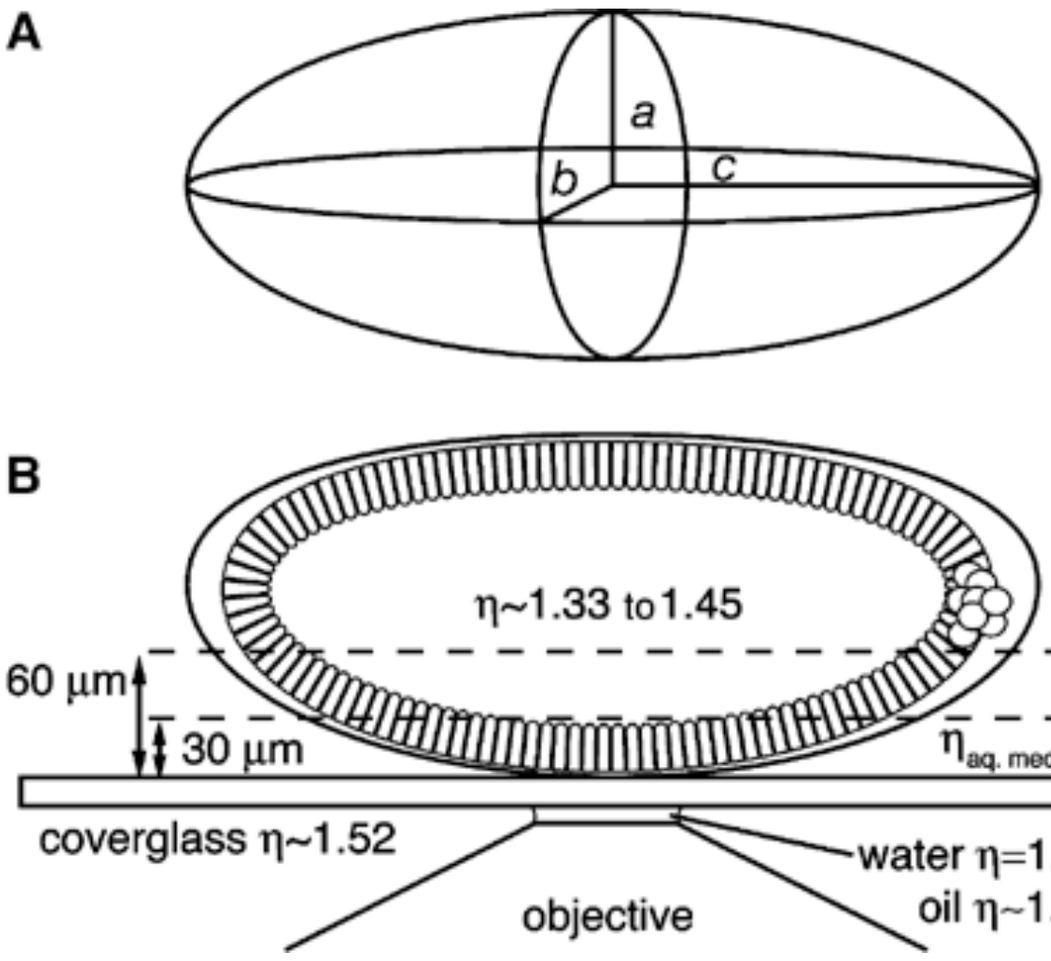
The problem is that measurements suggest a ten-fold smaller D

Two photon microscopy

- A form of fluorescence microscopy: Laser light is used to excite dyes, the emitted fluorescence is recorded
- Normally: one high energy photon per excitation. Excitation is proportional to the intensity.
- In two photon microscopy, simultaneous absorption of two low energy photons. Excitation proportional to intensity squared
- Advantages:
  - low absorption: image deep in tissue
  - good z-resolution



Two-photon microscopy of fly embryos



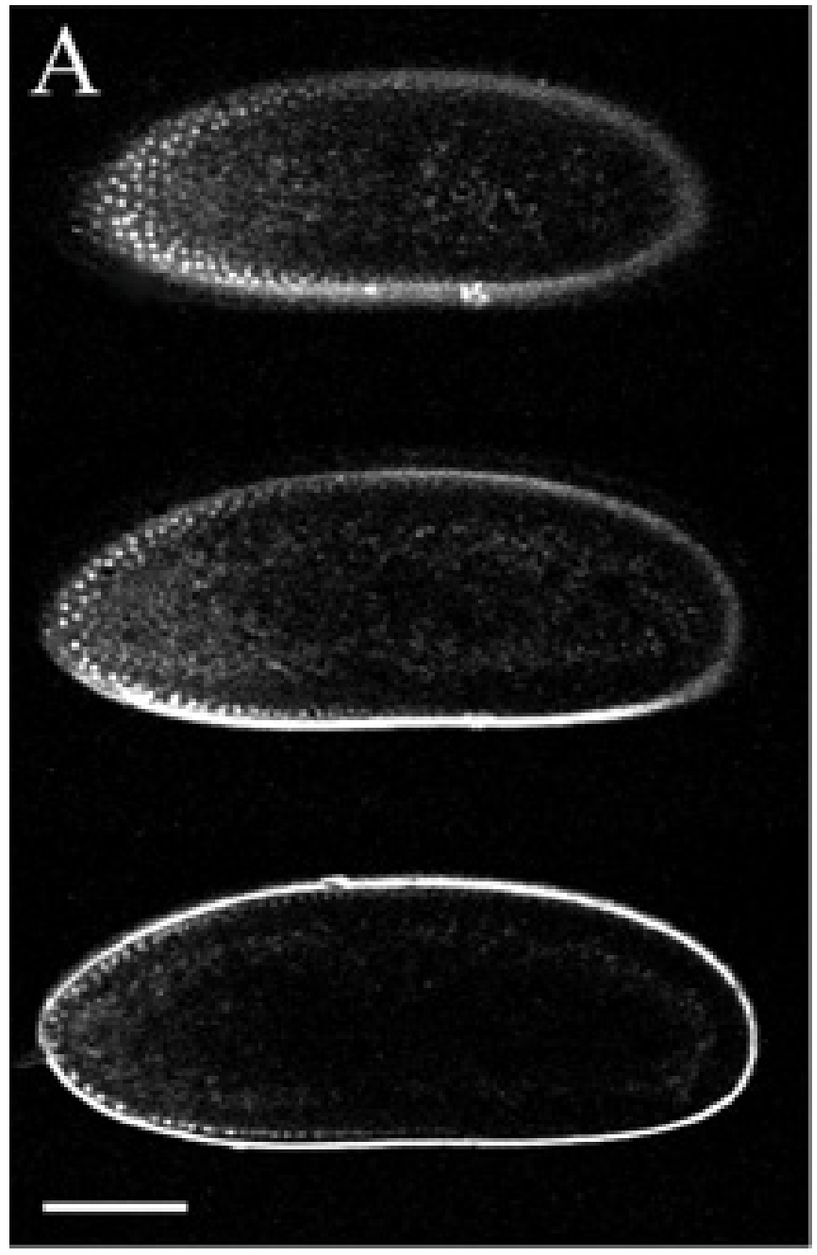
- a=b=100µm
- c=250µm

Mavrakis et al, 2008

30µm

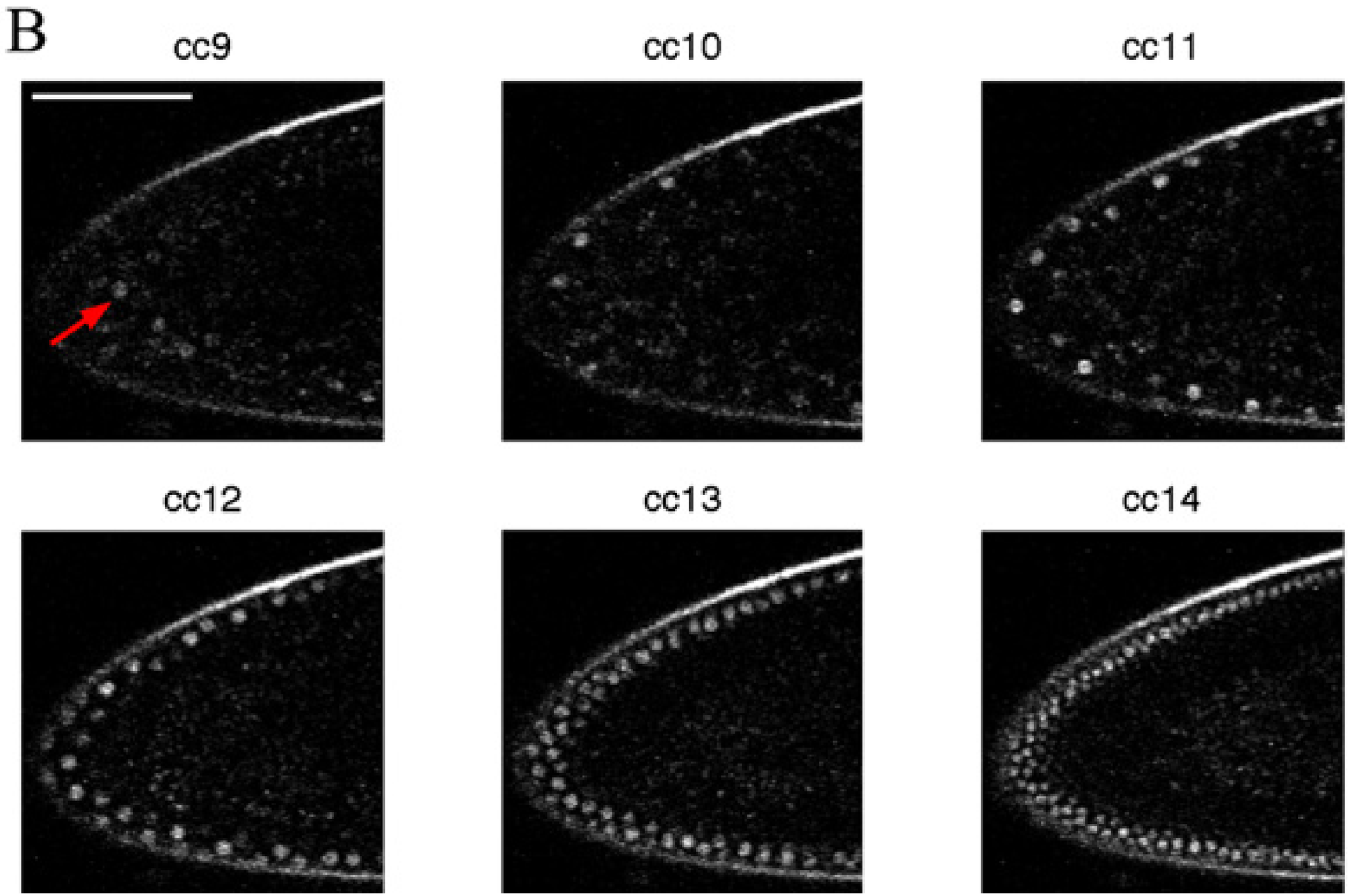
60µm

90µm

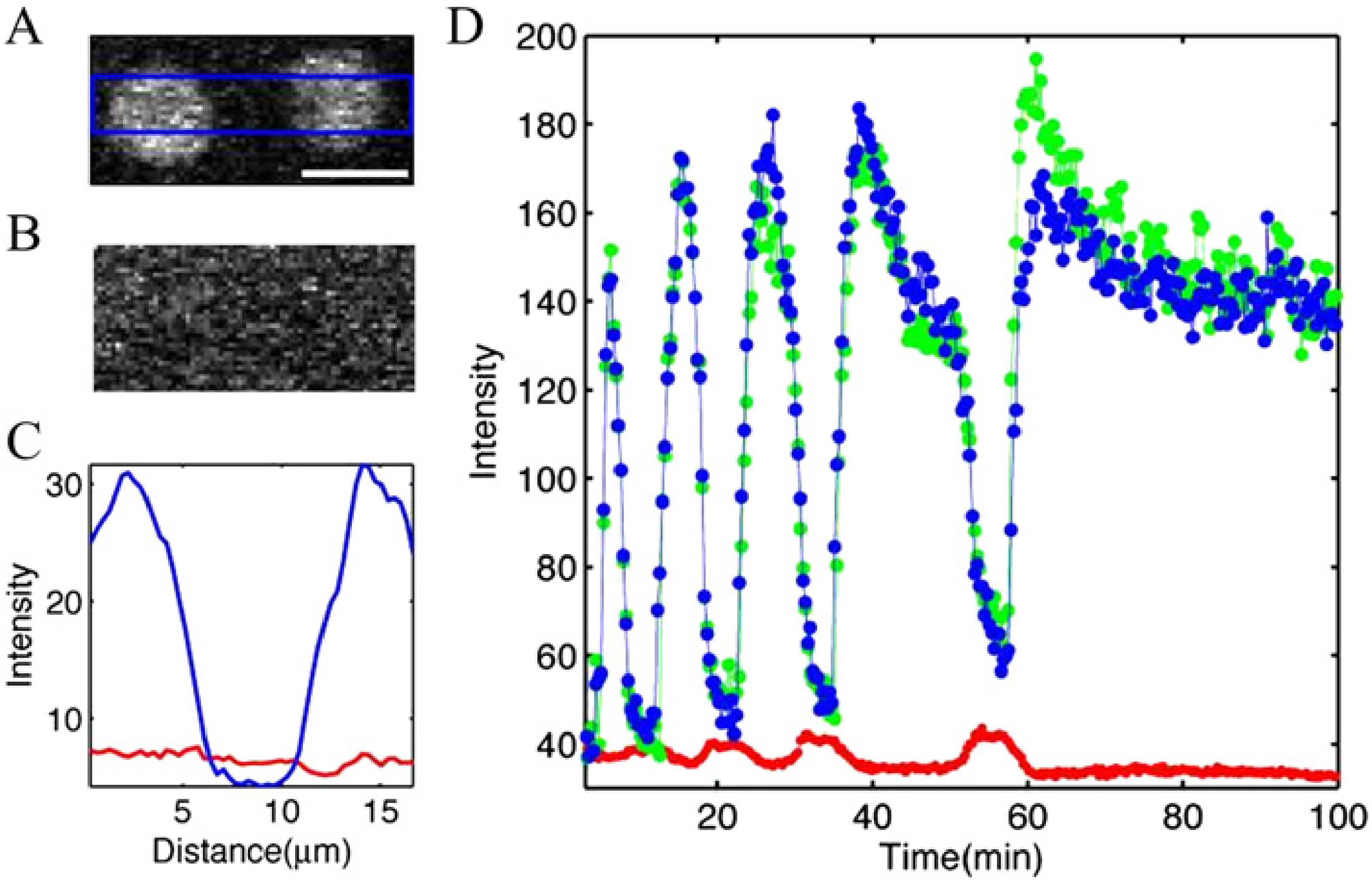


Gregor et al, 2007

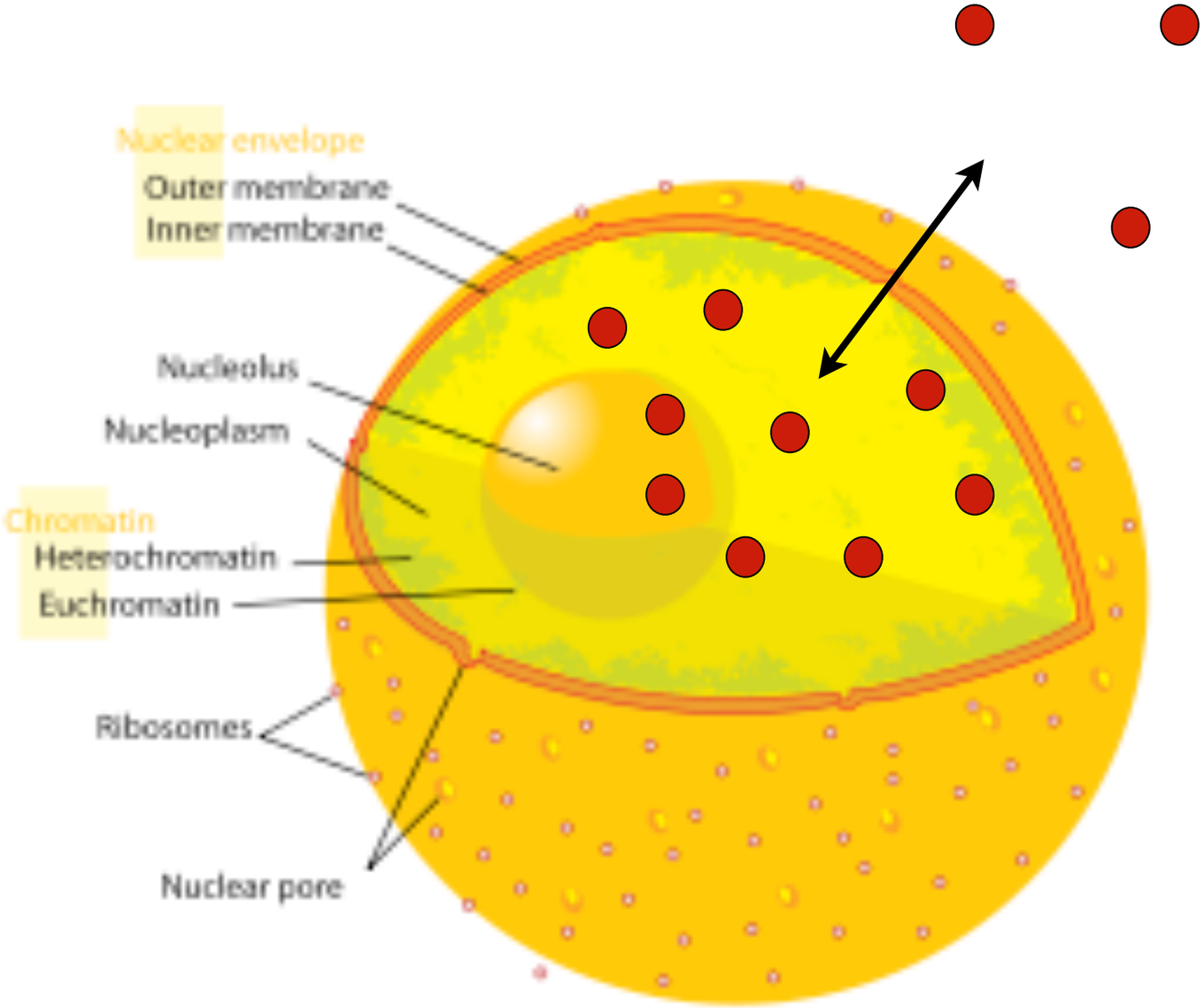
Bicoid protein is localized to nuclei



Nuclei split and reform

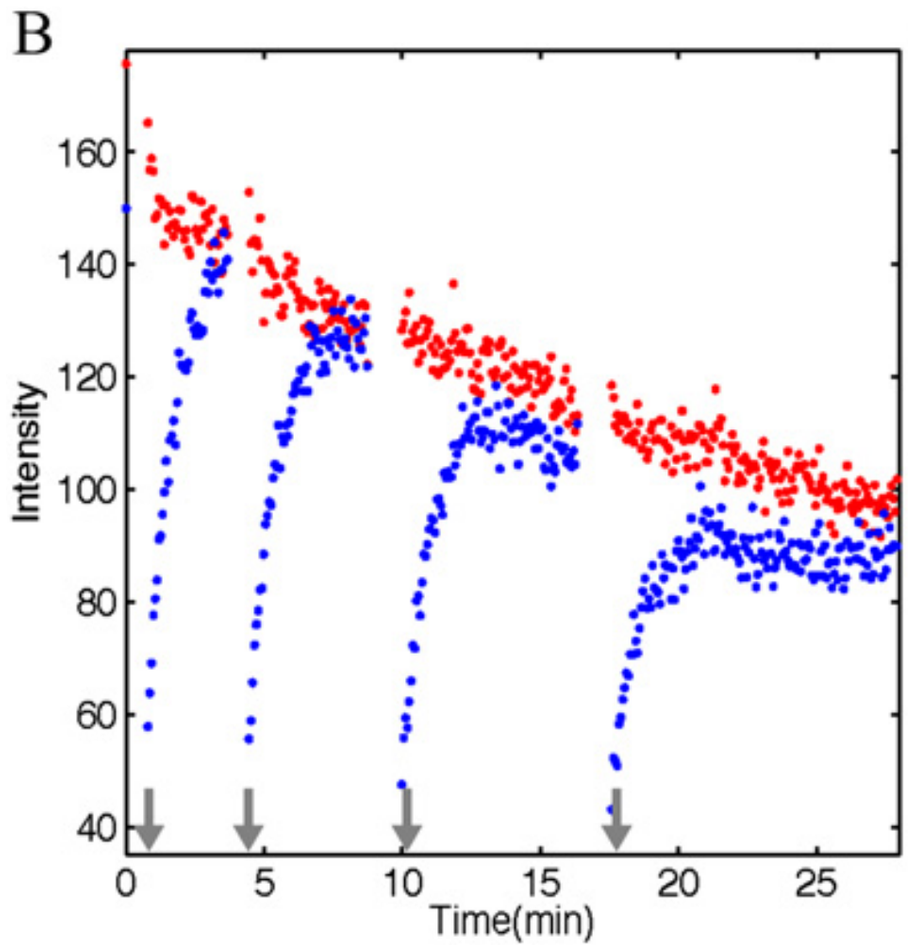


Cell nucleus and nuclear transport

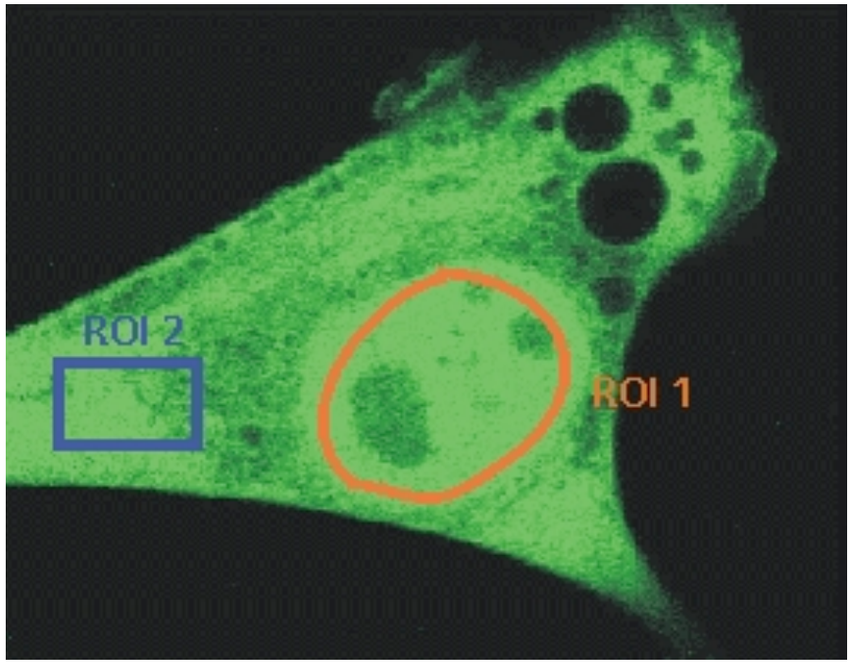


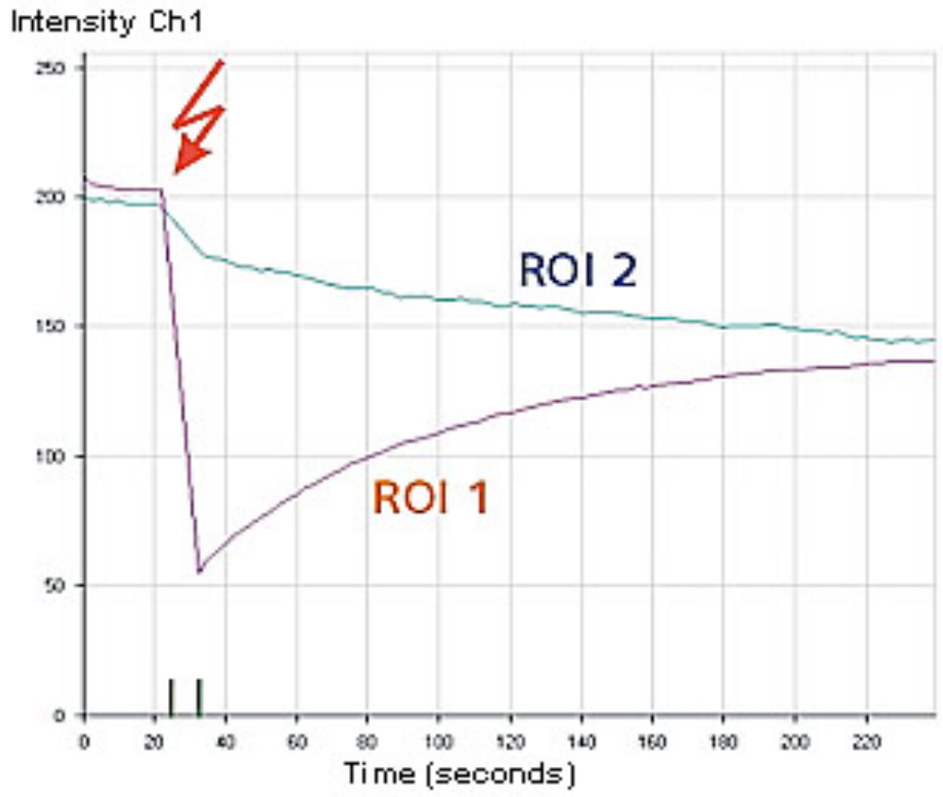
$$\frac{dn(t)}{dt} = k_{in}C_{out} - \frac{n(t)}{\tau_n}$$

$$n(t) = k_{in}C_{out}\tau_n - \Delta n_0 e^{-t/\tau_n}$$



Measuring the diffusion constant





Fluorescence recovery after photo-bleaching (FRAP)

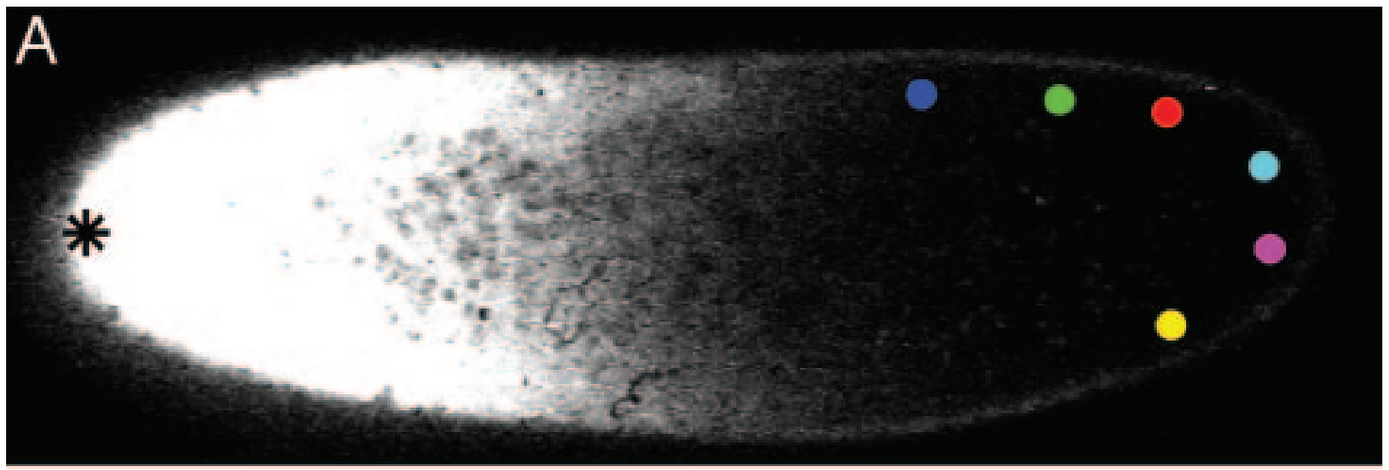
- Locally deplete the dye by photo-bleaching
- Record how it is replenished by diffusion
  (on scales much larger than a nucleus)
- Fit the measurement to the solution of a diffusion equation

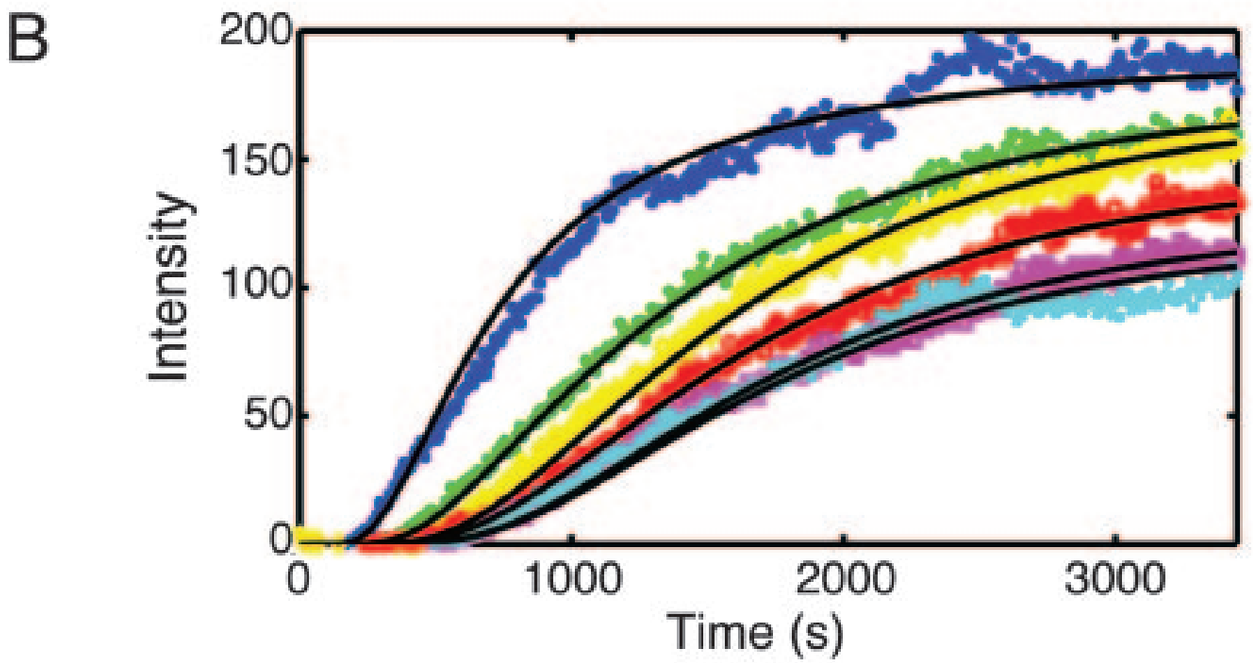
$$D = 0.3 \frac{\mu m^2}{s}$$

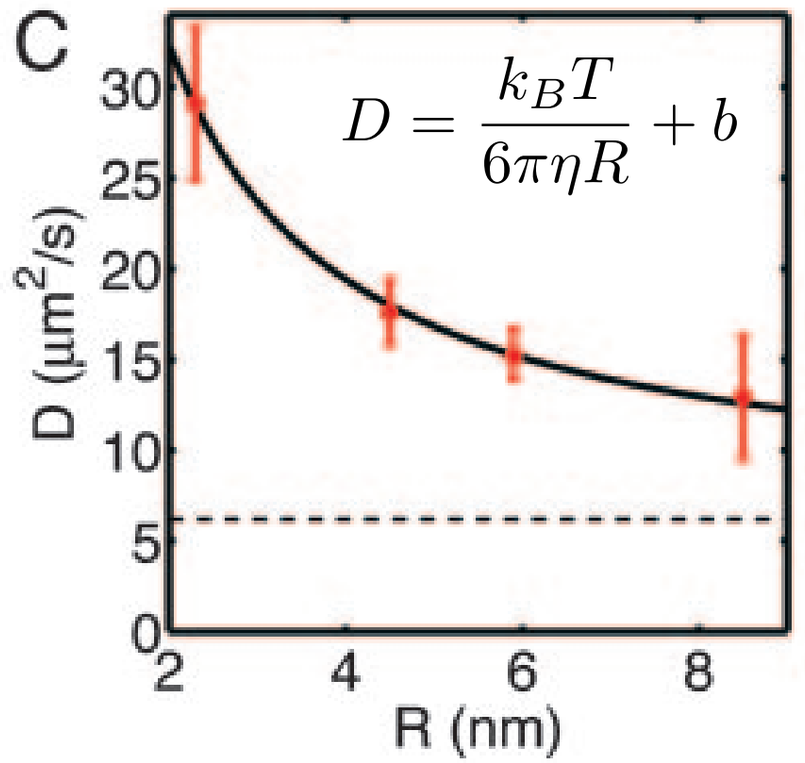
Images by Zeiss.de

Possible resolutions

- Larger diffusion in the center of the embryo (but there is evidence against)
- Active transport (shaking and stirring) on time scales >10 min

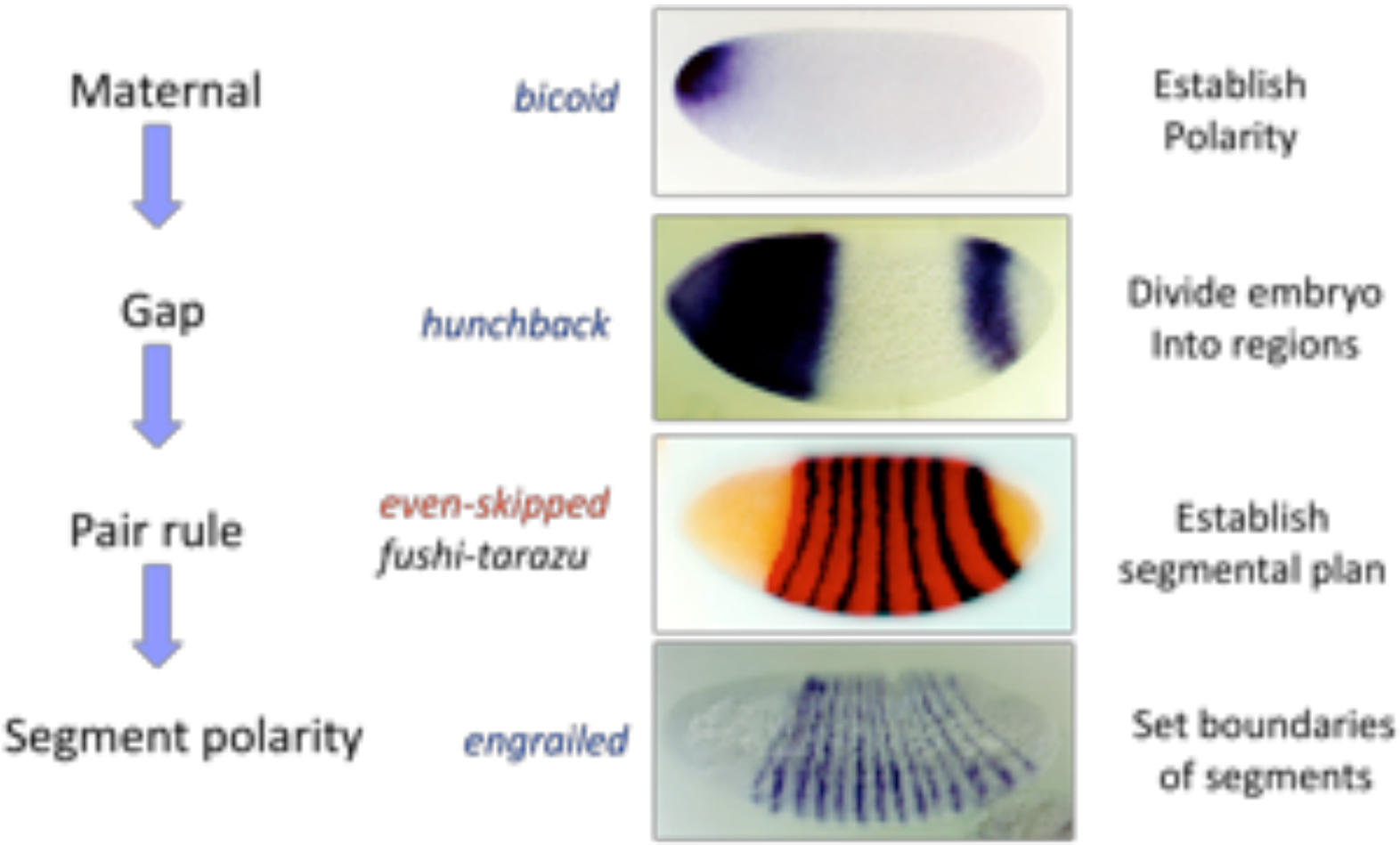






Gregor et al, 2005

Back to biology



Summary

- Development is a fascinating process
- Studying dynamic processes requires dynamics observations
- Fit the measurement to the solution of a diffusion equation