

## Patterning Development in the Early Embryo

### Part 2

#### Stability of Morphogen Gradients Movement of Molecules

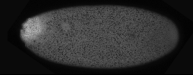
Eric Wieschaus

HHMI, Princeton University

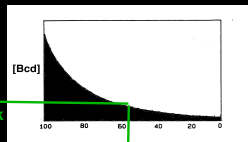
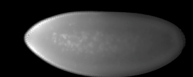
Thomas Gregor

Bill Bialek, David Tank

Bicoid  
RNA

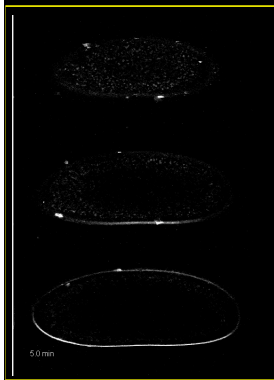


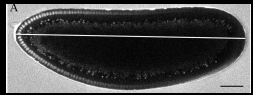
Bicoid  
protein



Hunchback

#### EGFP Bicoid in living embryos (Thomas Gregor)



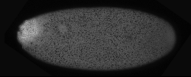


**Nuclear Bicoid**  
Concentration stable from  
Cycle 10

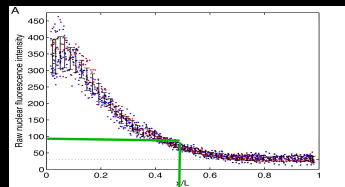
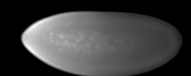
Little variation from  
embryo to embryo

Nuclear concentration provides  
sufficient positional information to  
distinguish each nucleus from its  
anterior and posterior neighbor

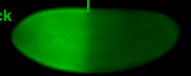
Bicoid  
RNA



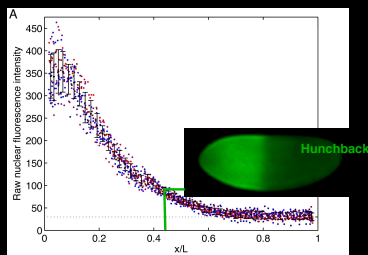
Bicoid  
protein



Hunchback

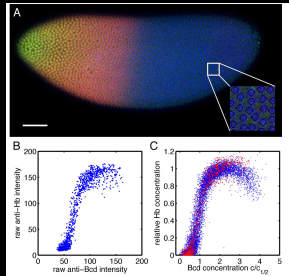


**Exponential decline consistent with  
diffusion from anterior source**



Hunchback transcription activated at 48%EL  
(cells discriminate 10% drop in Bcd level)

### Hb response to Bcd highly precise and highly nonlinear

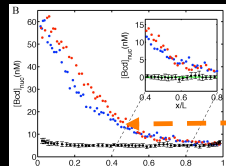
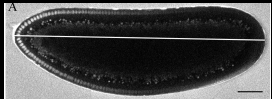


$$Hb = Hb_{max} \frac{Bcd^n}{Bcd^n + Bcd_{1/2}^n}$$

Best fit for Hill coefficient ~ 5

### Measuring Absolute Concentrations of Bcd

EGFP Bicoid embryo bathed in 36 nM GFP



Bcd Concentration at Hb Boundary 8 nM ( $\pm 2$ )

690 Bicoid molecules per nucleus

How does the Hb control region in adjacent nuclei distinguish 700 Bicoid molecules from 630 or 770?

(given that it has only five to seven sites that can bind Bcd?)

Things we don't know  
about Bicoid's  
transcriptional activation

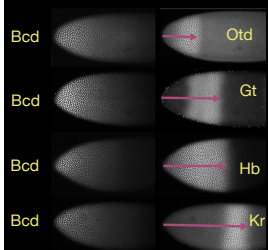
Time required for sampling

Bicoid binding & occupancy

Molecular basis of time  
averaging memory

Do we really know  
the answers to  
these questions for  
any eukaryotic  
transcription  
factors?

**Is Hunchback privileged?**  
**Are other Bicoid responses**  
**equally precise?**

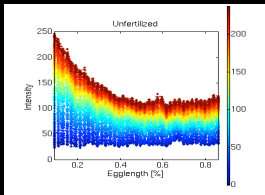


Other gap genes respond to higher or  
lower concentrations of Bicoid

Even more things we  
don't know about  
Bicoid

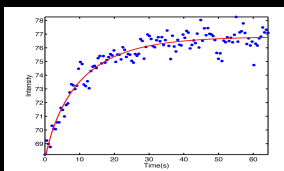
How does Bcd protein  
actually move?

Do any molecules  
move in the egg by  
pure diffusion?



Diffusion constants needed  
for stable proteins modeled  
in whole embryos over  
hours

4-8  $\mu\text{m}^2/\text{sec}$



Diffusion constant modeled  
by photobleaching small  
cytoplasmic volumes and  
short time frames

0.3  $\mu\text{m}^2/\text{sec}$

### A diffusion constant of $0.3 \text{ microns}^2 / \text{sec}$

This value would require several hours to produce a gradient that would span the *Drosophila* egg.

In the simplest model, if the diffusion constant is small, the half life of the protein would have to be long to establish a stable gradient.

### Localized synthesis can produce stable long term gradients if movement from source is balanced by degradation

$$C(x) = C_0 e^{-x/\lambda}$$

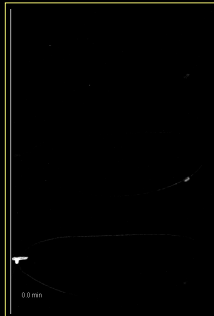
where the length constant  $\lambda$  reflects the point at which the concentration has fallen to 1/e of the peak level at source.

#### At steady state

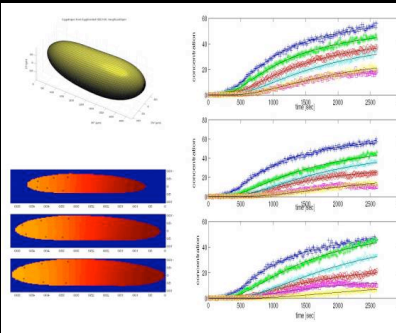
$$\lambda = \sqrt{D \cdot \tau}$$

$D$  = diffusion constant  
 $\tau$  = life time of protein

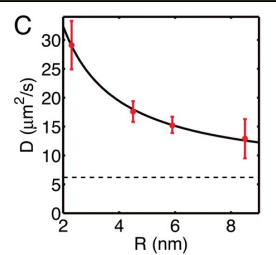
### Measuring effective diffusion constants by injecting labeled Dextran



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What fraction of Dextran's movement is due to diffusion?  
What fraction follows Stokes/Einstein relationship?



Diffusion constants for different dextrans

10kD 29μm²/sec

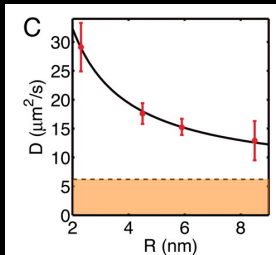
40kD 18μm²/sec

70kD 15μm²/sec

150kD 13μm²/sec

Size of EGFP Bicoid

70% of diffusive movement of biologically inert  
Dextrans follows the Stokes/Einstein relationship



Size independent component of  
dextran motility = 6 μm²/sec

What accounts for size independent movement of molecules in the egg?

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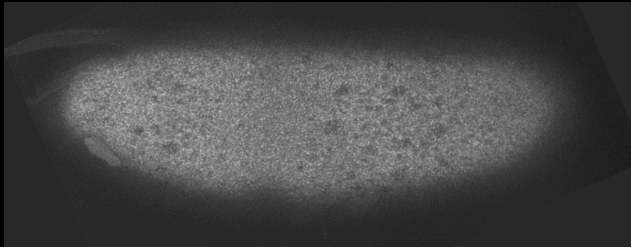
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Will random cytoplasmic streaming during cleavage mimic diffusion over the larger times scales available during cleavage?



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If Bicoid movement in the egg has a biological basis, is it regulated?

Is it a target of natural selection?

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