

# Maternal control of embryonic axis formation in *Drosophila*

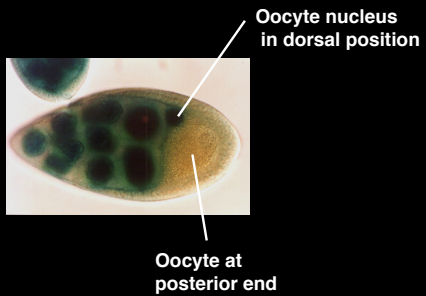
## Part 2 *grk* RNA localization

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HHMI



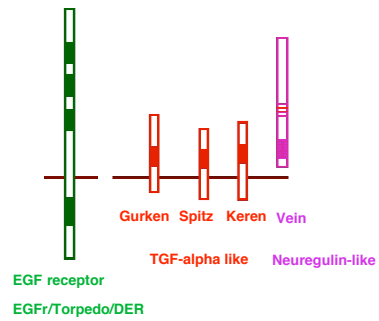
The *Drosophila* ovary

## The future axes of the egg are already visible during oogenesis



**Gurken RNA and protein are localized in the oocyte**

Neuman-Silberberg &amp; Schupbach, 1993, 1996

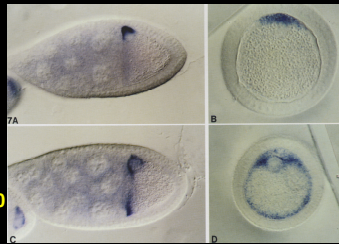


EGF receptor

EGFr/Torpedo/DER

***gurken* RNA is mislocalized in dorsalizing mutants such as *squid*, *fs(1)K10*, *Hrb27c*, *Otu*, *half pint***

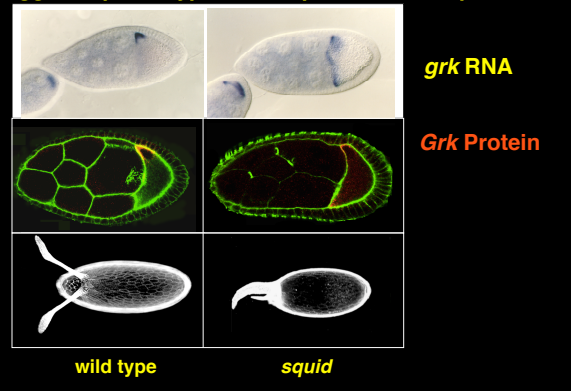
Wt



K10

Roth &amp; Schupbach '94

Eggshell phenotypes correspond to Grk expression



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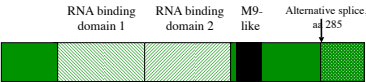
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Squid



Squid encodes a Heterogeneous nuclear ribonuclear protein (hnRNP)  
(Matunis *et al.*, 1992; Kelly,1993)

Squid binds *grk* RNA  
(Norvell *et al.*, 1999)

Squid is required for *grk* RNA localization and translational repression  
(Norvell *et al.*, 1999)

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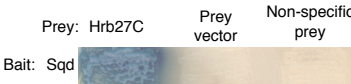
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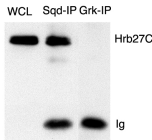
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Finding proteins that interact with Squid

I. Yeast two hybrid



II. Co-immunoprecipitation



Goodrich, Clouse & Schupbach, 2004

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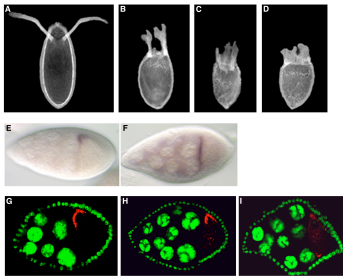
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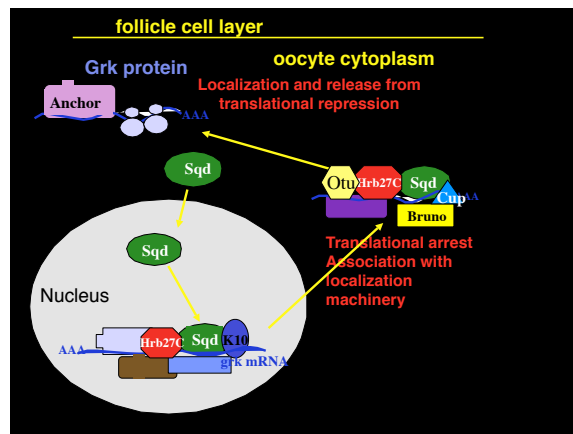
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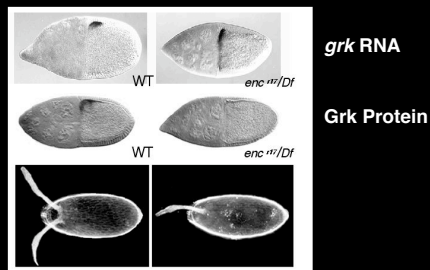
### Germine clones of *hrb27C* mutants produce dorsalized eggs



*Hrb27C/Hrp48* and *Squid* are both required for *grk* RNA localization

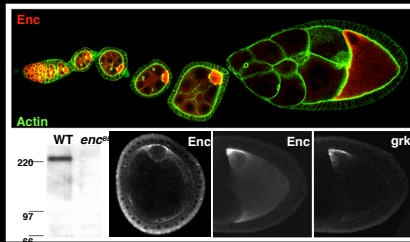


### Encore is required for Gurken protein accumulation



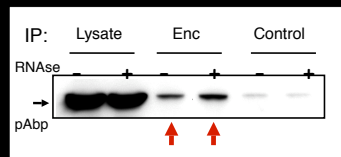


## Enc protein colocalizes with *grk* RNA

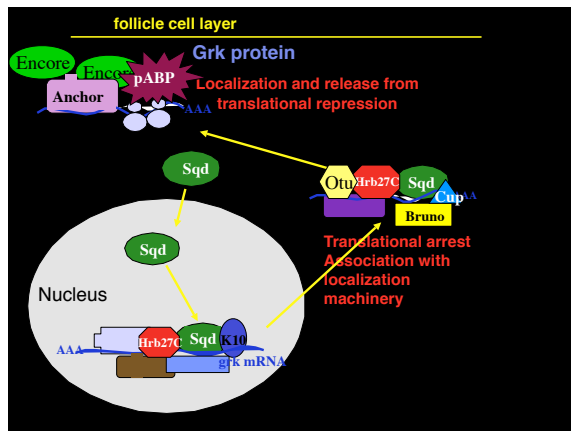


Van Buskirk et al. 2000

## polyA binding protein and *Enc* interact in ovaries



Clouse, Ferguson &amp; Schupbach 2008

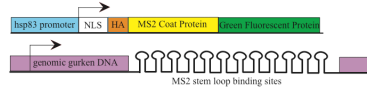


**Dynamics of *grk* mRNA localization***grk* mRNA

A. Jaramillo & T. Schupbach  
In collaboration with E. Gavis

**MS2 MCP-GFP detection system**

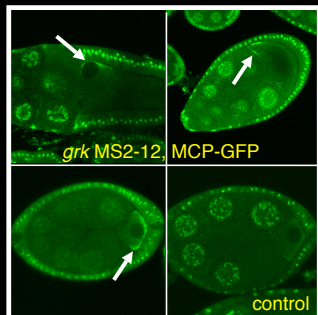
Transgenic Constructs



Detection

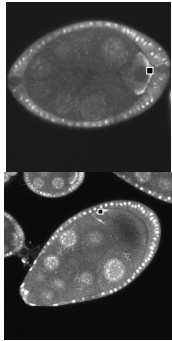


R. Singer and colleagues  
Forrest and Gavis, 2003  
Weil et al., 2006

***grk* MS2-12, MCP-GFP in ovary**

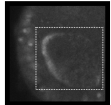
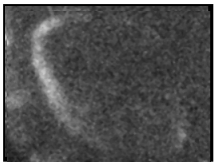
LIVE

### Fluorescence Recovery After Photobleaching (FRAP)

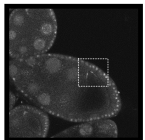
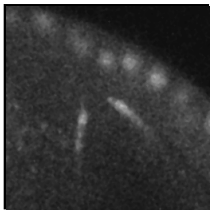


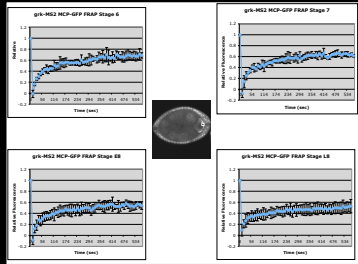
Jaramillo et al., J Cell Sci 2008

### FRAPing *grk* during early stage 8 of oogenesis



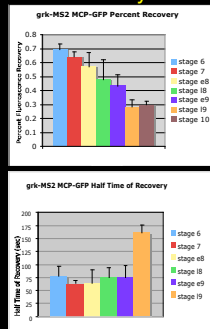
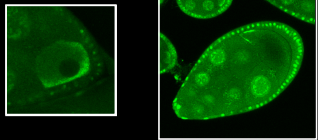
### FRAPing *grk* during stage 9 of oogenesis



FRAP *grk*-MS2 MCP-GFP stages 6-8

There is a steady decrease in recovery from young to older stages

There is a steady decrease in recovery from young to older stages.  
The rate of recovery is initially constant

Is localized *grk* mRNA a static or dynamic molecule?

Early: *grk* mRNA at the posterior pole is dynamic, its localization appears to reflect constant transport.

Late: the *grk* mRNA seems more stably anchored. Squid, Hrb27C required at this stage