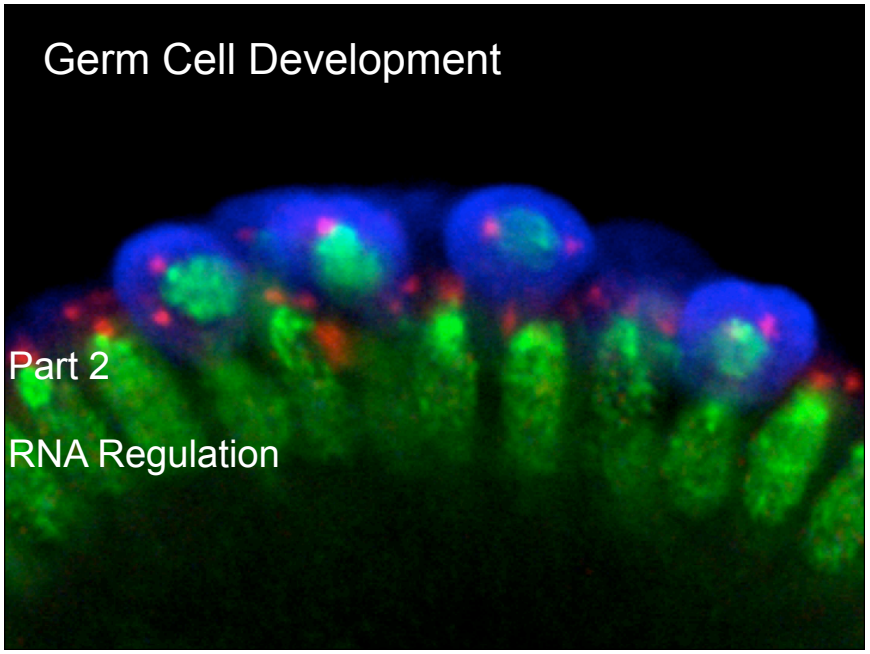
Germ Cell Development

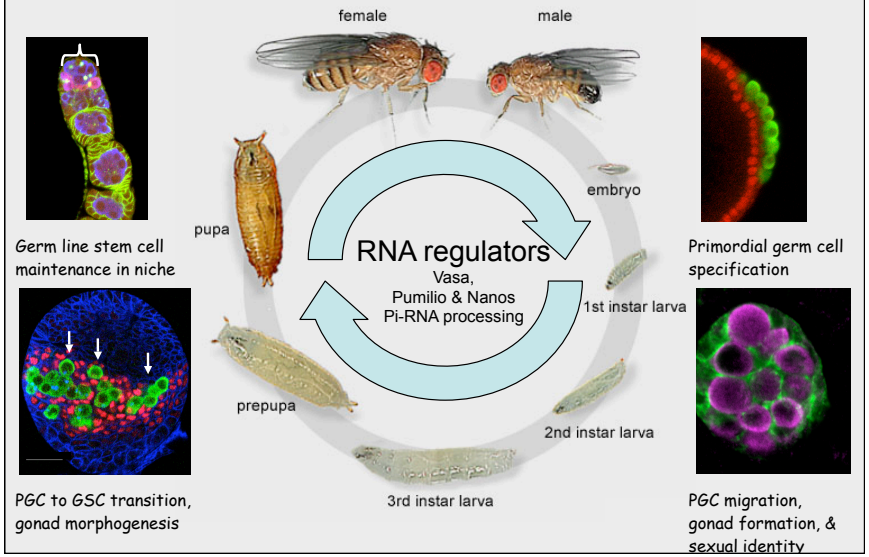


Part 2

RNA Regulation

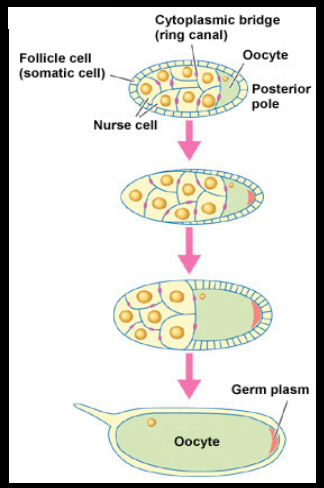
1

The same RNA Regulators are present throughout germ line cycle



2

Germ plasm assembles during oogenesis



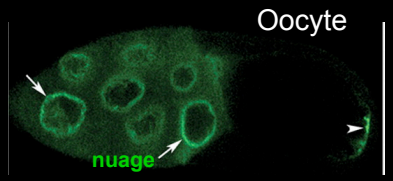
3

The basic unit of Drosophila oogenesis is the egg chamber, which is comprised of an oocyte and 15 nurse cells surrounded by a layer of somatic follicle cell. The oocyte is connected to the nurse cells by a network of cytoplasmic bridges called ring canals. This network allows the nurse cells to synthesize various mRNAs that are required for early embryogenesis and transport them in a microtubule-dependent manner to discrete locations within the oocyte. Nuage is a germ line specific organelle and it is remarkably conserved between species. It appears as a dense, fibrous organelle that is not membrane-bound and is often associated with mitochondrial clusters or is concentrated in the perinuclear cytoplasm. Nuage is though to be the precursor of germ plasm cause they share a lot of common components.

In one model nuage has been suggested to serve as a precursor to polar granules, a view initially based on ultrastructural similarities of the two organelles (Mahowald, 1968 ; Mahowald

Germ plasm assembles during oogenesis

Germ plasm components :
Vasa, Aubergine, Tudor



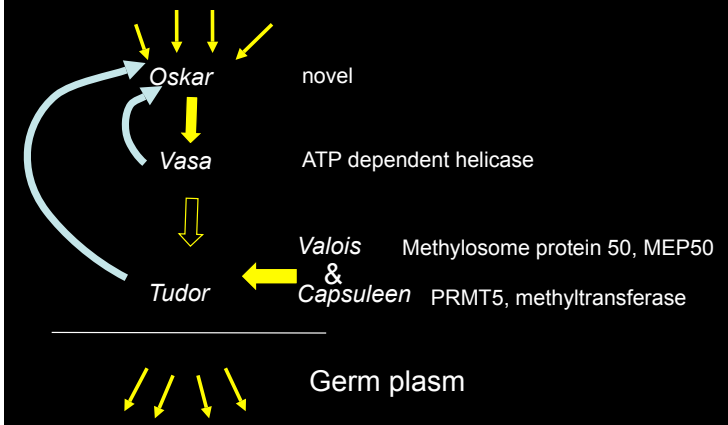
Nuage components :
Vasa, Maelstrom, Spindle-E,
Aubergine, Tudor

4

The basic unit of Drosophila oogenesis is the egg chamber, which is comprised of an oocyte and 15 nurse cells surrounded by a layer of somatic follicle cell. The oocyte is connected to the nurse cells by a network of cytoplasmic bridges called ring canals. This network allows the nurse cells to synthesize various mRNAs that are required for early embryogenesis and transport them in a microtubule-dependent manner to discrete locations within the oocyte. Nuage is a germ line specific organelle and it is remarkably conserved between species. It appears as a dense, fibrous organelle that is not membrane-bound and is often associated with mitochondrial clusters or is concentrated in the perinuclear cytoplasm. Nuage is though to be the precursor of germ plasm cause they share a lot of common components.

In one model nuage has been suggested to serve as a precursor to polar granules, a view initially based on ultrastructural similarities of the two organelles (Mahowald, 1968 ; Mahowald

Germ plasm assembly



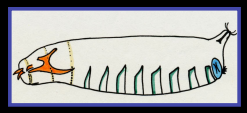
Germ plasm

localized effectors

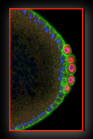
5

Germ plasm effectors

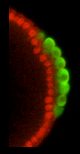
Abdomen formation
nanos (nos), pumilio (pum)



Germ cell formation
germ cells less (gcl)



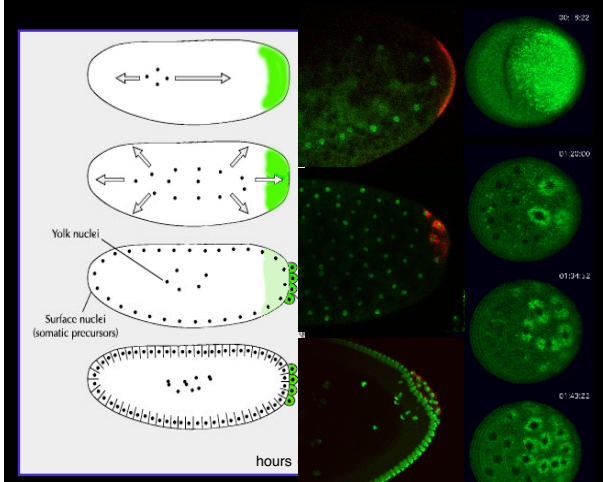
Germ cell transcription
Polar granule component pgc)



Germ cell migration
nanos, pumilio, tre1, wunen

6

Germ plasm RNA segregation into germ cells

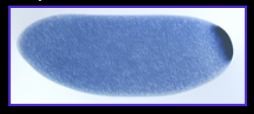


Leslie Ciruna

7

Regulated Translation and stability of Germ plasm RNAs

- Germ-line specific localization of maternal mRNA



nos RNA

- Germ line-specific stability of maternal RNA

nos RNA

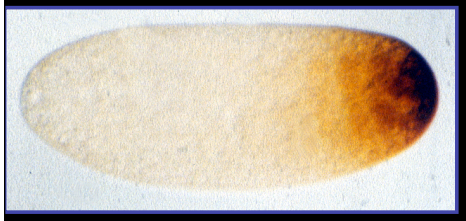
- Germ-line specific translation of maternal mRNA

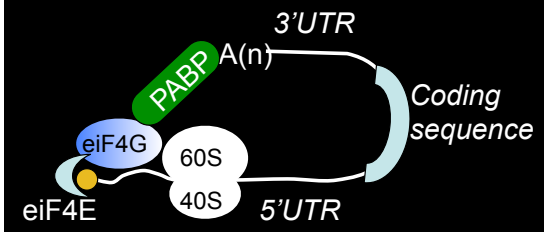
Nos protein

Wang and Lehmann R. 1991
Gavis and Lehmann R. 1994

8

Spatial translational regulation of *nanos* RNA



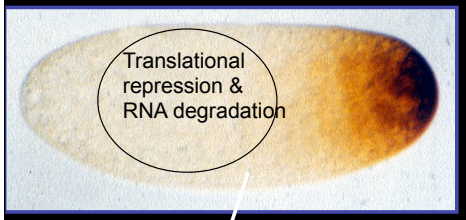


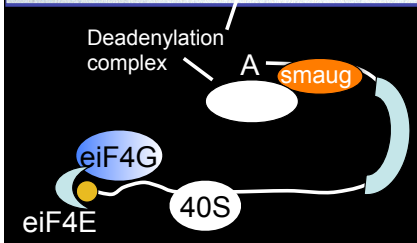
after Besse and Ephrussi,
Nature Reviews MCB, Vol9, 2008 971-980

Nature Reviews | Molecular Cell Biology Figure 1 | Spatial translational activation of ASH1 mrNa in budding yeast. Trans-acting factors, such as She2, first associate with ASH1 mRNA in the nucleus (step 1), and are subsequently exported together with the mRNA to the cytoplasm. A mature transport ribonucleoprotein particle (RNP) is then assembled (step 2) by further recruitment of motor proteins and translational repressors (Khd1 (also known as Hek2) and pumilio-homology domain family-6 (Puf6)). Note that Puf6 strongly accumulates in the nucleus but has not been shown to associate with the mRNA in this compartment. During transport along actin filaments (step 3), ASH1 mRNA translation initiation is blocked by two complementary mechanisms (inset) that prevent assembly of the eukaryotic translation initiation factor-4F (eIF4F) complex and recruitment of the 40S ribosomal subunit (Khd1-mediated mechanism; left), and prevent recruitment of the 60S ribosomal subunit (Puf6-mediated mechanism; right). After reaching the bud tip, ASH1 RNP contacts the membrane-

9

Spatial translational regulation of *nanos* RNA





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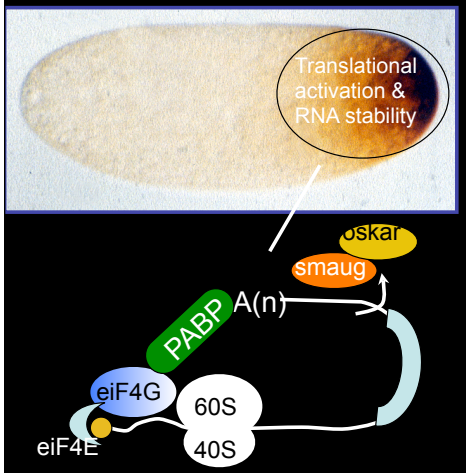
10

Spatial translational regulation of *nanos* RNA



after Besse and Ephrussi,
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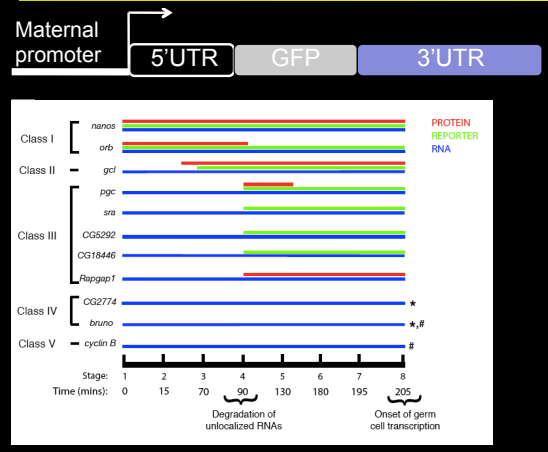
11

Nature Reviews | Molecular Cell Biology Figure 1 | Spatial translational activation of ASH1 mrNa in budding yeast. Trans-acting factors, such as She2, first associate with ASH1 mRNA in the nucleus (step 1), and are subsequently exported together with the mRNA to the cytoplasm. A mature transport ribonucleoprotein particle (RNP) is then assembled (step 2) by further recruitment of motor proteins and translational repressors (Khd1 (also known as Hek2) and pumilio-homology domain family-6 (Puf6)). Note that Puf6 strongly accumulates in the nucleus but has not been shown to associate with the mRNA in this compartment. During transport along actin filaments (step 3), ASH1 mRNA translation initiation is blocked by two complementary mechanisms (inset) that prevent assembly of the eukaryotic translation initiation factor-4F (eIF4F) complex and recruitment of the 40S ribosomal subunit (Khd1-mediated mechanism; left), and prevent recruitment of the 60S ribosomal subunit (Puf6-mediated mechanism; right). After reaching the bud tip, ASH1 RNP contacts the membrane-

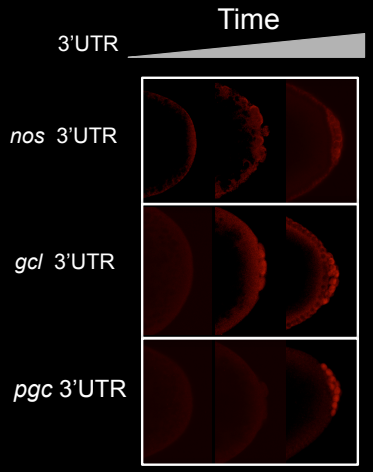
3'UTRs control temporal fine-tuning of translation



Rangan et al. (2009) Curr. Biol. 19(1):72-7

12

Temporal regulation of translational activation of localized RNA



Rangan et al. (2009) Curr. Biol. 19(1):72-7

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Distinct Timing of Translational Activation

Stage	Gene	Function
Germ plasm	*Nanos (nanos)*	RNA translational repressor *Germ cell specification and survival*
Germ buds	*Germcell-less (gcl)*	Nuclear envelop protein *Germ cell formation*
Germ cells	*Polar granule component (pgc)*	P-TEFb kinase inhibitor *Germ cell transcriptional silencing*

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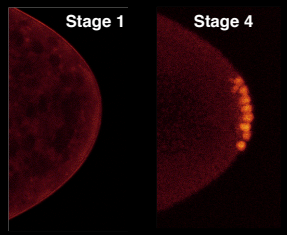
pgc 3'UTR controls localization and translation

***gfp-pgc* 3'UTR**

GFP RNA

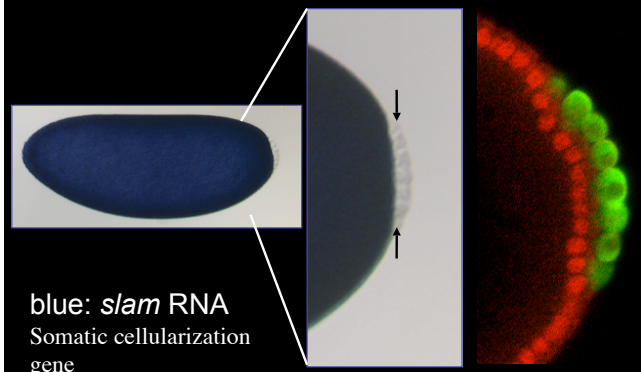
GFP Protein



Prash Rangan

15

Transcription is repressed in early germ cells



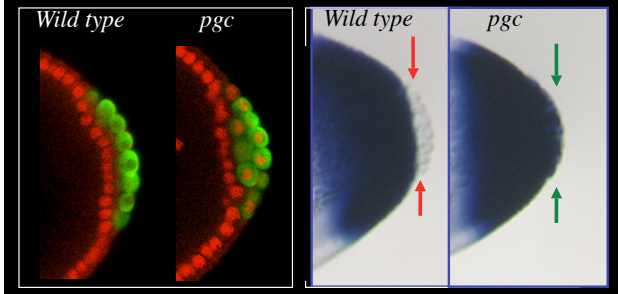
blue: *slam* RNA
Somatic cellularization gene

red: pSer2-CTD
(active PolII)

green: Vasa
Germ cells

16

The *polar granule component* gene represses transcription in early germ cells



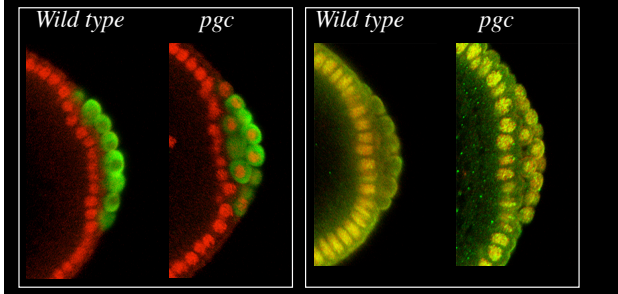
red: pSer2 CTD
green: Vasa

tailless mRNA
(somatic patterning gene)

Martinho et al. 2004
Hanyu-Nakamura et al. 2008

17

The *polar granule component* gene affects chromatin state in early germ cells



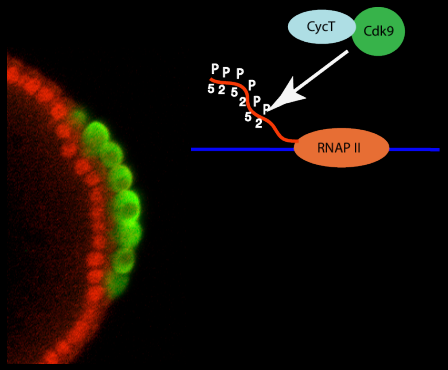
red: pSer2 CTD
green: Vasa

pSer2-CTD
Me-K4 His3
Active chromatin

Martinho et al. 2004

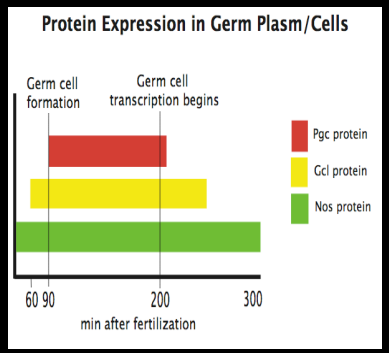
18

PGC blocks Ser 2 Phosphorylation by Blocking P-TEFb Recruitment in the Germ Line



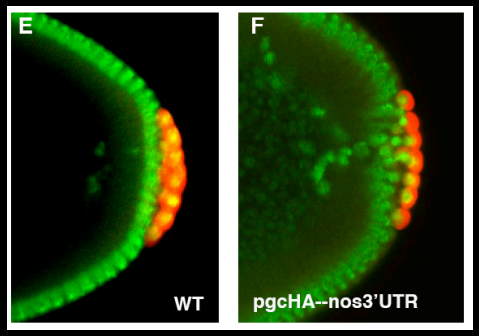
Hanyu-Nakamura et al 2008

Does temporal regulation of localized RNA matter?



Rangan et al. (2009) Curr. Biol. 19(1):72-7

Timing of translational activation is critical for normal development

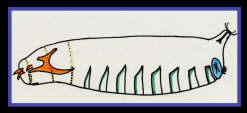


Rangan et al. 2009

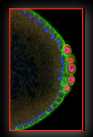
21

Regulation of Germ plasm effectors

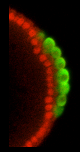
Abdomen formation
nanos (nos), pumilio (pum)



Germ cell formation
germ cells less (gcl)

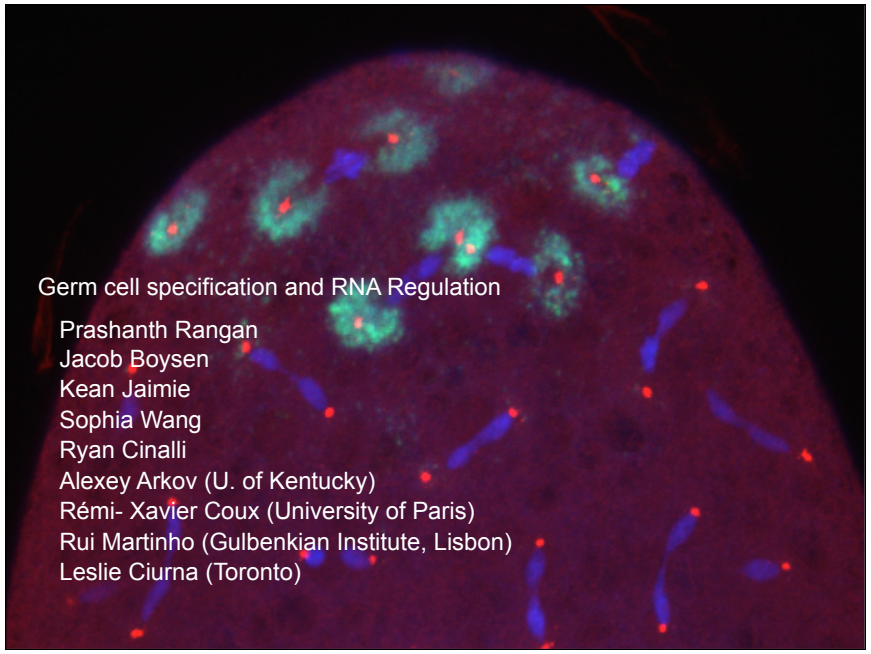


Germ cell transcription
Polar granule component pgc)



Germ cell migration
nanos, pumilio, tre1, wunen

22


Germ cell specification and RNA Regulation
Prashanth Rangan
Jacob Boysen
Kean Jaimie
Sophia Wang
Ryan Cinalli
Alexey Arkov (U. of Kentucky)
Rémi- Xavier Coux (University of Paris)
Rui Martinho (Gulbenkian Institute, Lisbon)
Leslie Ciurna (Toronto)