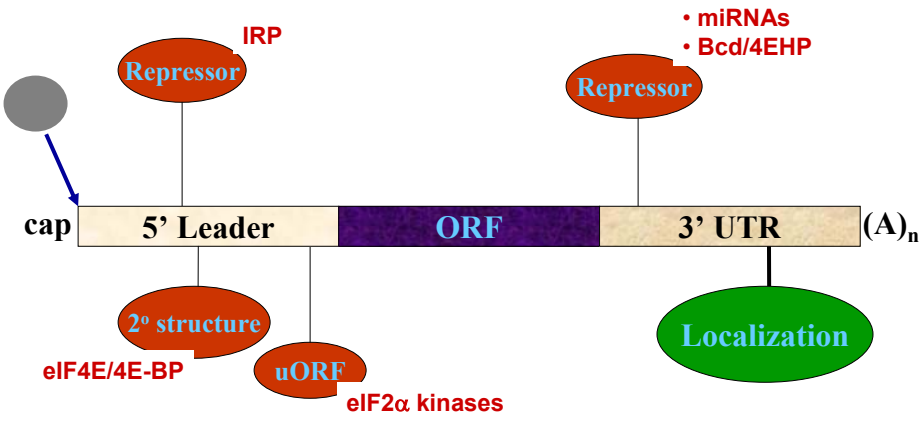
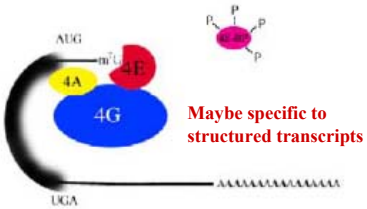
# mRNA *cis* Elements Located in Non-Protein-Coding Regions

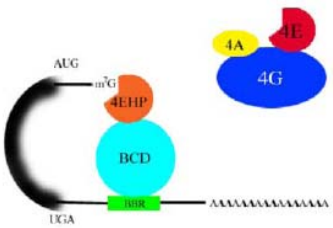



# Transcript-independent versus specific competition at the 5' cap

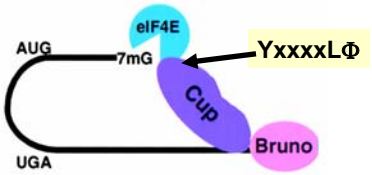
**Transcript-independent inhibition by 4E-BPs**




**Transcript-specific inhibition by mRNA-bound bicoid**



## Other 3'-tethered repressors are 4E-BPs that complete with eIF-4G




| Target mRNA | 4E-BP | RNA-BP |
|---|---|---|
| *oskar* | Cup | Bruno |
| *nanos* | Cup | Smaug |
| cyclin B1* | Maskin | CPEB |

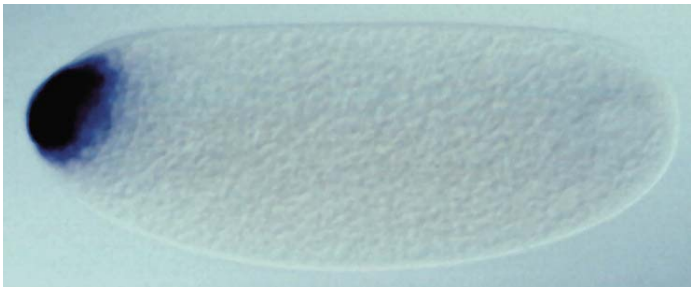
*Xenopus

## Mechanisms of transcript-specific translational control

- **Translational repressors (proteins, microRNAs) that bind to specific *cis* elements**
- **Interplay between structural characteristics of an mRNA (uORFs, secondary structure) and the activity of a general initiation factor (e.g. eIF2, eIF4E) can convert global to gene-specific regulation**

# Lecture 3

## *Mechanisms of Localized mRNA Translation in Cells*



## Reasons for mRNA Localization

- Targets protein to appropriate region of cell.
- Prevents expression elsewhere.
- Response to local requirements.
- Independent control in different regions.
- Localized synthesis necessary for assembly.
- More efficient transport
  *one mRNA vs. many proteins*

## Mechanisms of mRNA Localization

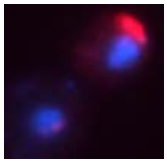
- Diffusion and localized entrapment.
- Localized degradation.
- Active transport on cytoskeletal motors.

## Steps in mRNA localization

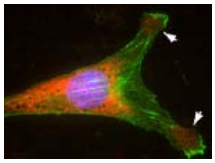
- Nuclear “priming” of transcripts.
- Translational silencing in cytosol.
- Specific association with motor proteins.
- Transport.
- Tethering at destination.
- Translational derepression.

# Examples of mRNAs Actively Transported and Localized

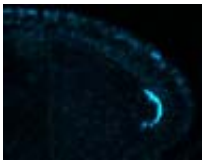
***ASH1***
**Bud tip in dividing**
***S. cerevisiae***

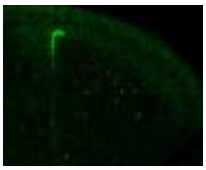


***β-actin***
**Leading edge of migrating fibroblast**



***oskar***
**Posterior of *drosophila* oocyte**





***bicoid***

## *ASH1*

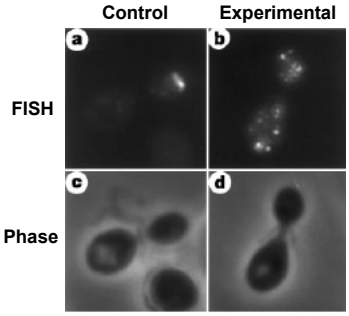
- **Transcription factor**
- **Represses mating type switching in daughter cells.**
- **One of ~2 dozen mRNAs that localized to daughter cells**

  ***all seem to use the same mechanism***

## *ASH1* mRNA travels along the actin cytoskeleton

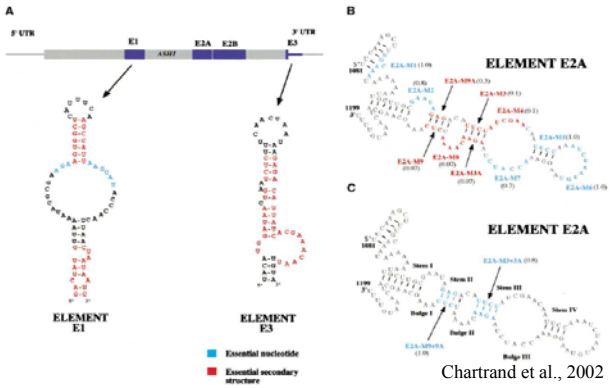
**Delocalized by:**

- *ACT1* mutants
- Actin assembly proteins (*SHE5*)
- *MYO4* mutants
- Actin depolymerization drugs




Takizawa et al., 1997

## *ASH1 cis*-Elements



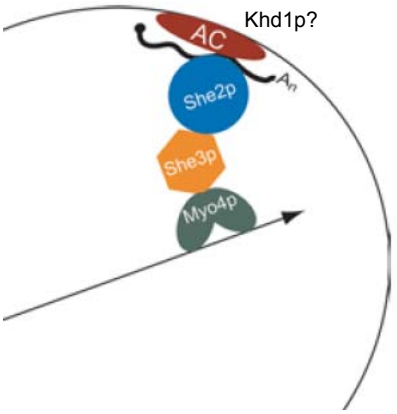

Chartrand et al., 2002

- Located in both the ORF and the 3'UTR
- Structure, not sequence, is important
- Inhibit translation as well as direct localization
- Each acts independently
  - Additive influence on *ASH1* localization
  - Each will localize a reporter gene individually

# Model for *ASH1* mRNA localization

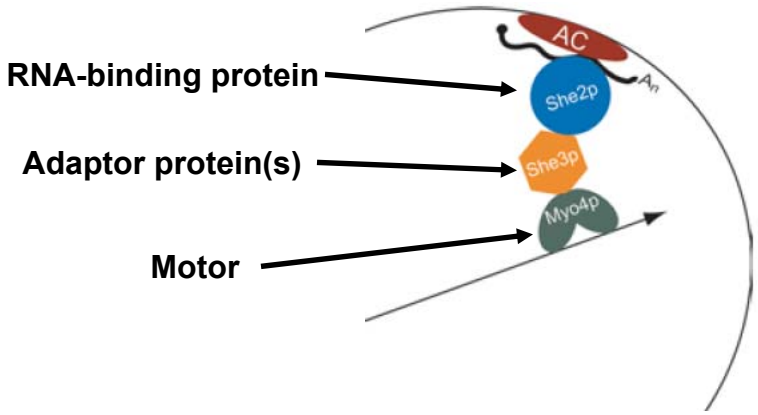
- **She2p**
  **RNA-binding protein**
  **present in nucleus**
- **Myo4p**
  **myosin motor protein**
- **She3p**
  **interacts with She2p & Myo4p**
  **required for Myo4p—She2p**

- Multiple motors per transcript?
- Mechanism of anchoring?
- Activation of translation?




Gonsalvez et al., 2005

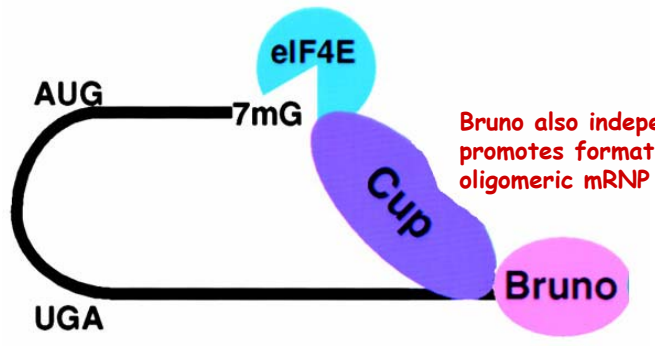
# General Picture



## *oskar*

- Localized to posterior.
- Localization requires factors acquired in the nucleus.
- Cotransports with kinesin-HC (plus-end-directed MT motor).
- Not transported in khc$^{-}$ mutants.
- Staufen
  - highly conserved dsRNA binding protein
  - required and colocalizes

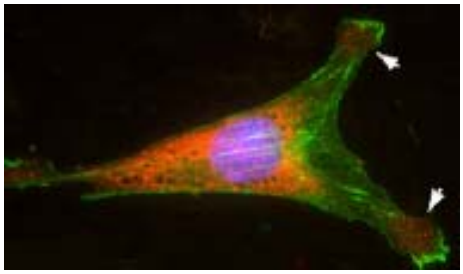
## Inappropriate *oskar* translation is inhibited by tethering eIF4E




Bruno also independently promotes formation of oligomeric mRNP particles

How is it regulated once the posterior site is reached?

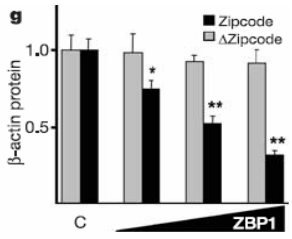
# *β-Actin* mRNA in Fibroblasts



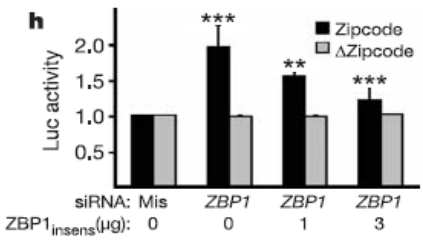
- Localizes to the leading edge of migrating cells.
- Mediated by two 54-nt elements in 3'UTR ("zipcodes").
- ZBP1 binds to the zipcodes and is required for transport.
- ZBP1 assembles onto the 3'UTR and travels with the *β-actin* mRNA.
- Active myosin and intact actin filaments are required.
- ZBP1 also inhibits translation of *β-actin* mRNA.

# ZBP1 inhibits translation in a zipcode dependent manner

***In vitro***

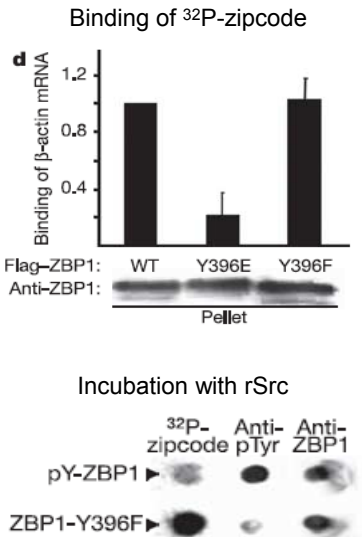



***In vivo***

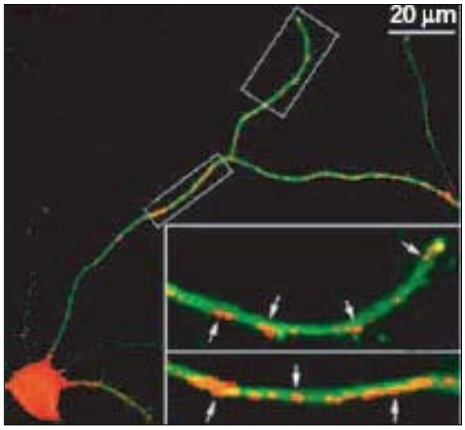



Huttelmaier et al., 2005

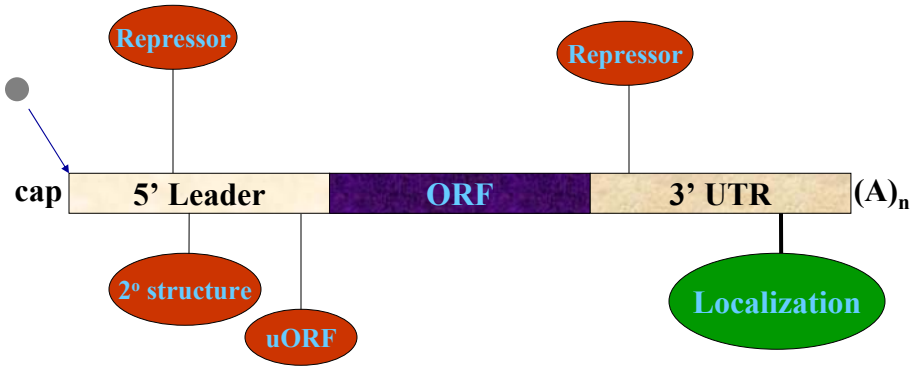
**Phosphorylation of Tyr396 by Src kinase blocks interaction of ZBP1 with zipcode RNA**




**ZBP1 is also involved in β-actin mRNA movement in dendrites, but in contrast to fibroblasts, transport is on microtubules using kinesin motors.**



# mRNA *cis* Elements Located in Non-Protein-Coding Regions




**Most of these regulatory systems do not involve simple one-protein/one-RNA interactions**