ΠΑΝΕΠΙΣΤΗΜΙΟ ΘΕΣΣΑΛΙΑΣ
ΤΜΗΜΑ ΒΙΟΧΗΜΕΙΑΣ ΚΑΙ ΒΙΟΤΕΧΝΟΛΟΓΙΑΣ

Η ΡΥΘΜΙΣΗ ΤΗΣ ΓΟΝΙΔΙΑΚΗΣ ΕΚΦΡΑΣΗΣ στους ευκαρυωτικούς οργανισμούς Μέρος Β΄

Η παρεμβολή RNA, RNAi

Νικόλαος Μπαλατσός

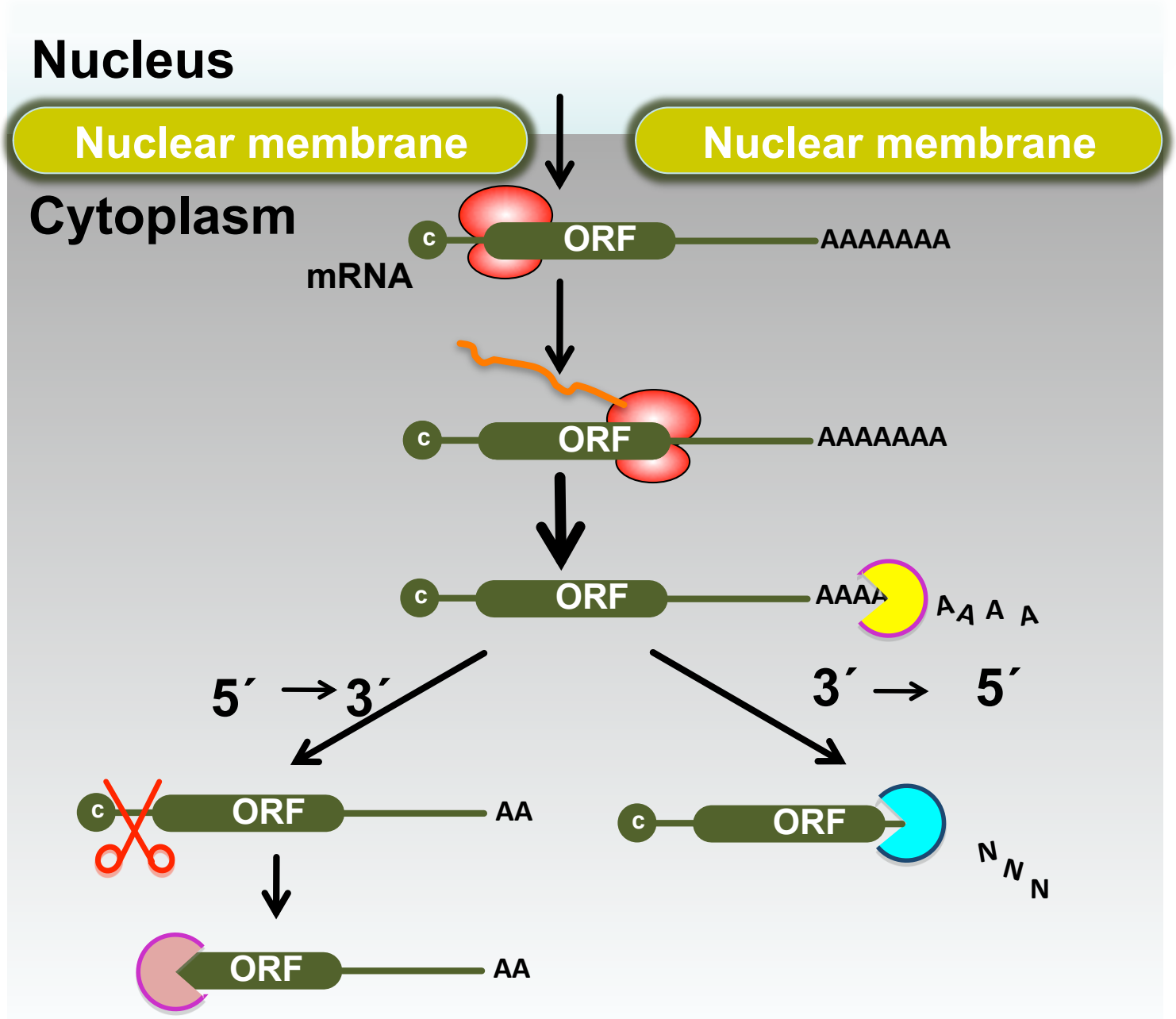
RNA interference, RNAi:

Specific inhibition of gene expression mediated by a short double-stranded RNA, called short interfering RNA or siRNA

Παρεμβολή RNA (RNA interference, RNAi):

Εξειδικευμένη αναστολή γονιδιακής έκφρασης μέσω ενός μικρού δίκλωνου RNA, γνωστού ως siRNA.

Eukaryotic mRNA turnover



Insight and discovery are functionally separable.
The one precedes the other.
Insight can happen every day.
Discovery does not.
Insight takes more intelligence, but it is discovery that is rewarded...

Francis HC Crick

Introduction of a Chimeric Chalcone Synthase Gene into Petunia Results in Reversible Co-Suppression of Homologous Genes *in trans*

Carolyn Napoli,[1] Christine Lemieux, and Richard Jorgensen[2]

DNA Plant Technology Corporation, 6701 San Pablo Avenue, Oakland, California 94608

The Plant Cell, Vol. 2, 279–289, April 1990

We attempted to overexpress chalcone synthase (CHS) in pigmented petunia petals by introducing a chimeric petunia CHS gene. Unexpectedly, the introduced gene created a block in anthocyanin biosynthesis. Forty-two percent of plants with the introduced CHS gene produced totally white flowers and/or patterned flowers with white or pale nonclonal sectors on a wild-type pigmented background; none of hundreds of transgenic control plants exhibited such phenotypes. Progeny testing of one plant demonstrated that the novel color phenotype co-segregated with the introduced CHS gene; progeny without this gene were phenotypically wild type. The somatic and germinal stability of the novel color patterns was variable. RNase protection analysis of petal RNAs isolated from white flowers showed that, although the developmental timing of mRNA expression of the endogenous CHS gene was not altered, the level of the mRNA produced by this gene was reduced 50-fold from wild-type levels. Somatic reversion of plants with white flowers to phenotypically parental violet flowers was associated with a coordinate rise in the steady-state levels of the mRNAs produced by both the endogenous and the introduced CHS genes. Thus, in the altered white flowers, the expression of both genes was coordinately suppressed, indicating that expression of the introduced CHS gene was not alone sufficient for suppression of endogenous CHS transcript levels. The mechanism responsible for the reversible co-suppression of homologous genes *in trans* is unclear, but the erratic and reversible nature of this phenomenon suggests the possible involvement of methylation.

Potent and specific genetic interference by double-stranded RNA in *Caenorhabditis elegans*

Andrew Fire*, SiQun Xu*, Mary K. Montgomery*, Steven A. Kostas*†, Samuel E. Driver‡ & Craig C. Mello‡

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† *Biology Graduate Program, Johns Hopkins University, 3400 North Charles Street, Baltimore, Maryland 21218, USA*
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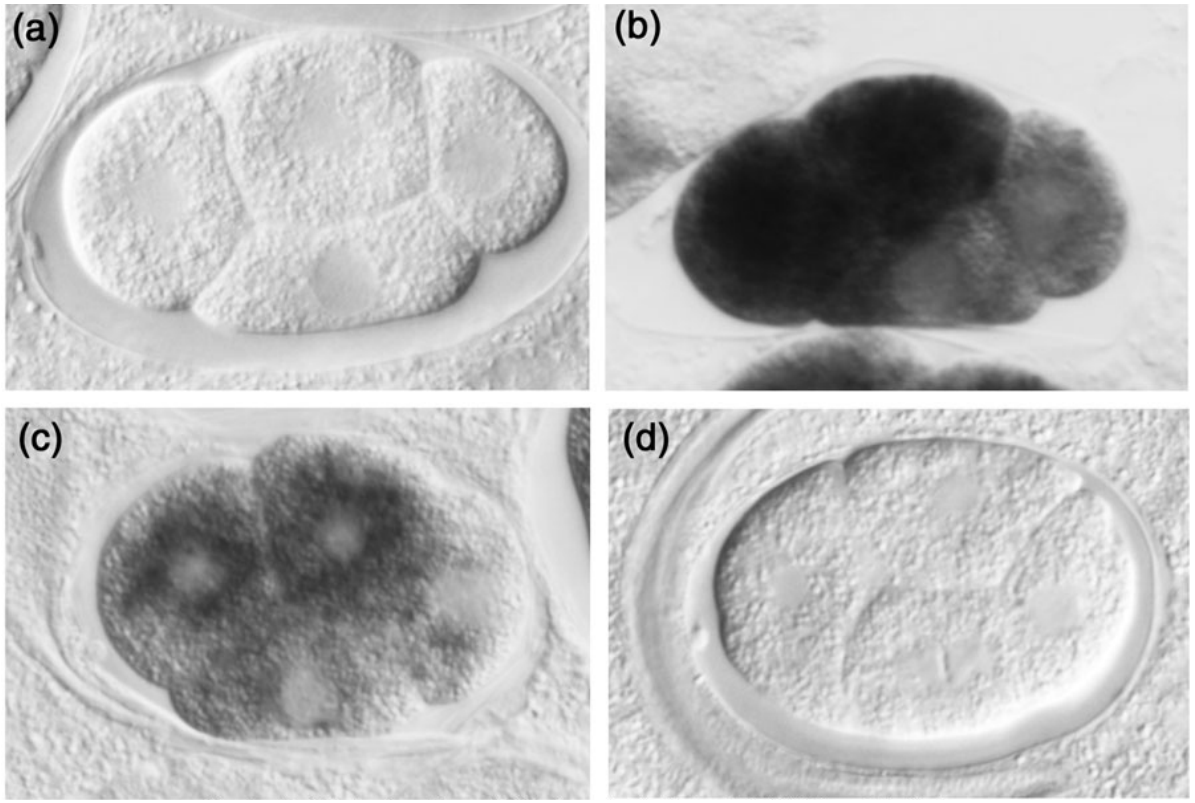
NATURE | VOL 391 | 19 FEBRUARY 1998

Double-stranded RNA-induced RNA interference causes destruction of a specific mRNA in *C. elegans*



Guo, S. and Kemphues, K. J. *Cell* 81, 611-620 (1995)
Fire, A. et al. *Nature* 391, 809 (1998)

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Fire, A. et al. *Nature* 391, 809 (1998)

Duplexes of 21–nucleotide RNAs mediate RNA interference in cultured mammalian cells

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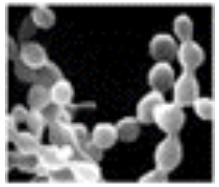
** Department of Cellular Biochemistry, and † Department of Biochemistry and Cell Biology, Max-Planck-Institute for Biophysical Chemistry, Am Fassberg 11, D-37077 Göttingen, Germany*

NATURE | VOL 411 | 24 MAY 2001 |

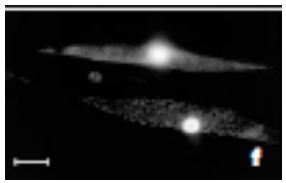
RNAi Timeline

1990
Post transcriptional gene silencing (PTGS)

1992 quelling in neurospora

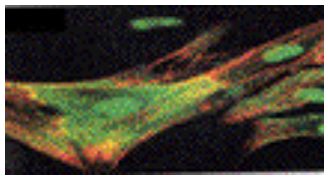


1998
«RNAi» in *C. elegans*
Fire et al. Nature 1998; 391: 806



1998-2001
RNAi in yeast  *Drosophila,* plants, trypanosomes

2001
Successful siRNA experiments in mammalian cells
Elbashir et al Nature 2001; 411: 49



2002
Recombinant Dicer generated siRNAs

2002
«Breakthrough of the year»

2003
Fortune Magazine:
Biotech's Billion Dollar Breakthrough

1990 | 1992 | 1998 | 2001 | 2002 | 2003 | 2006

modified from www.invitrogen.com

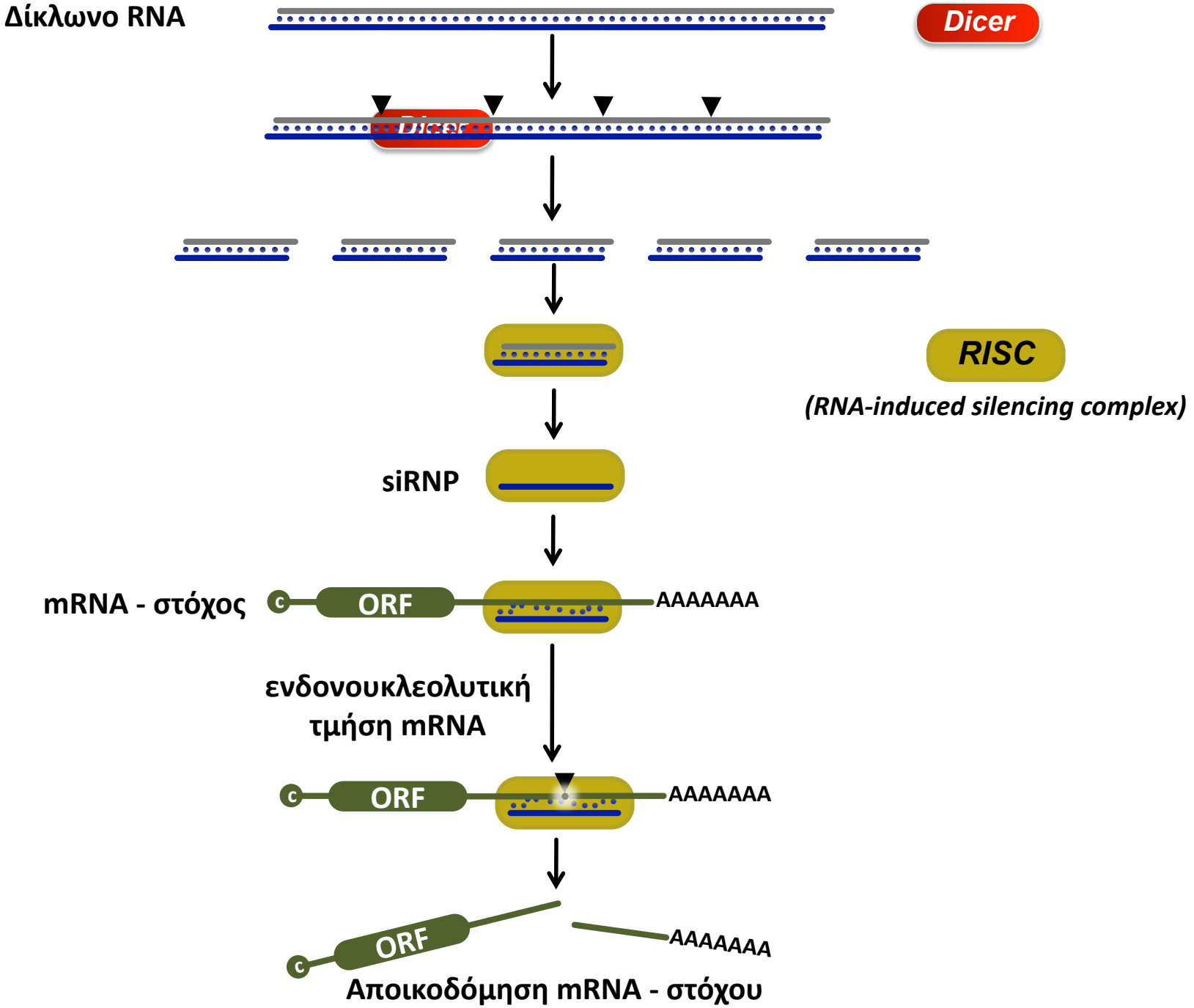
The Nobel Prize in Physiology or Medicine 2006

"for their discovery of RNA interference -
gene silencing by double-stranded RNA"

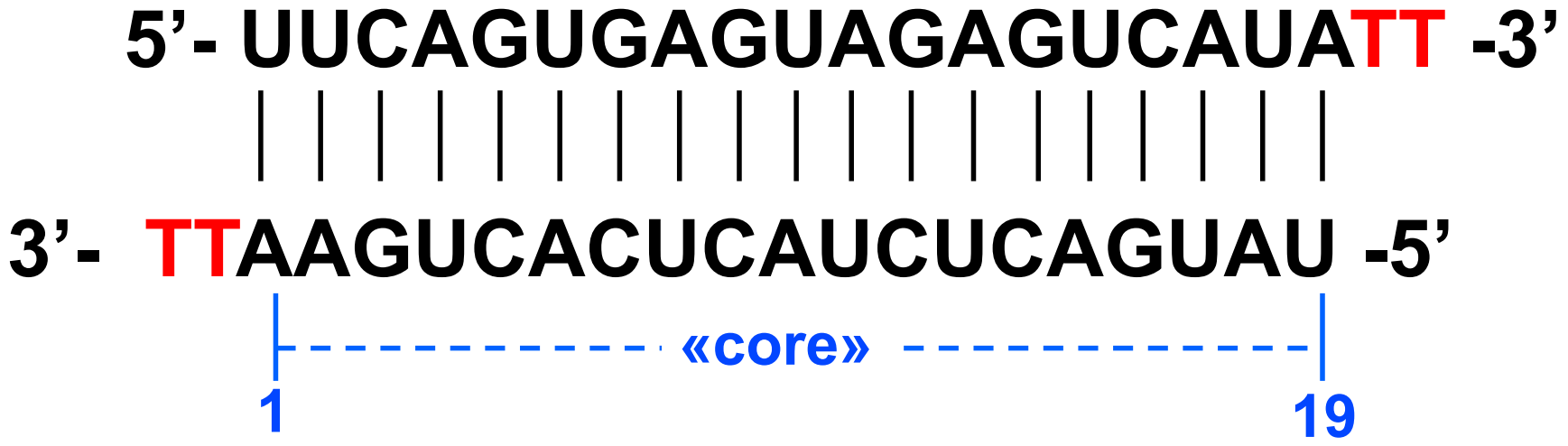
Andrew Z. Fire
1/2 of the prize
USA
Stanford University School of Medicine
Stanford, CA, USA
b. 1959

Craig C. Mello
1/2 of the prize
USA
University of Massachusetts Medical School
Worcester, MA, USA
b. 1960

RNAi: Γενικός μηχανισμός δράσης



The short interfering RNA molecule, siRNA



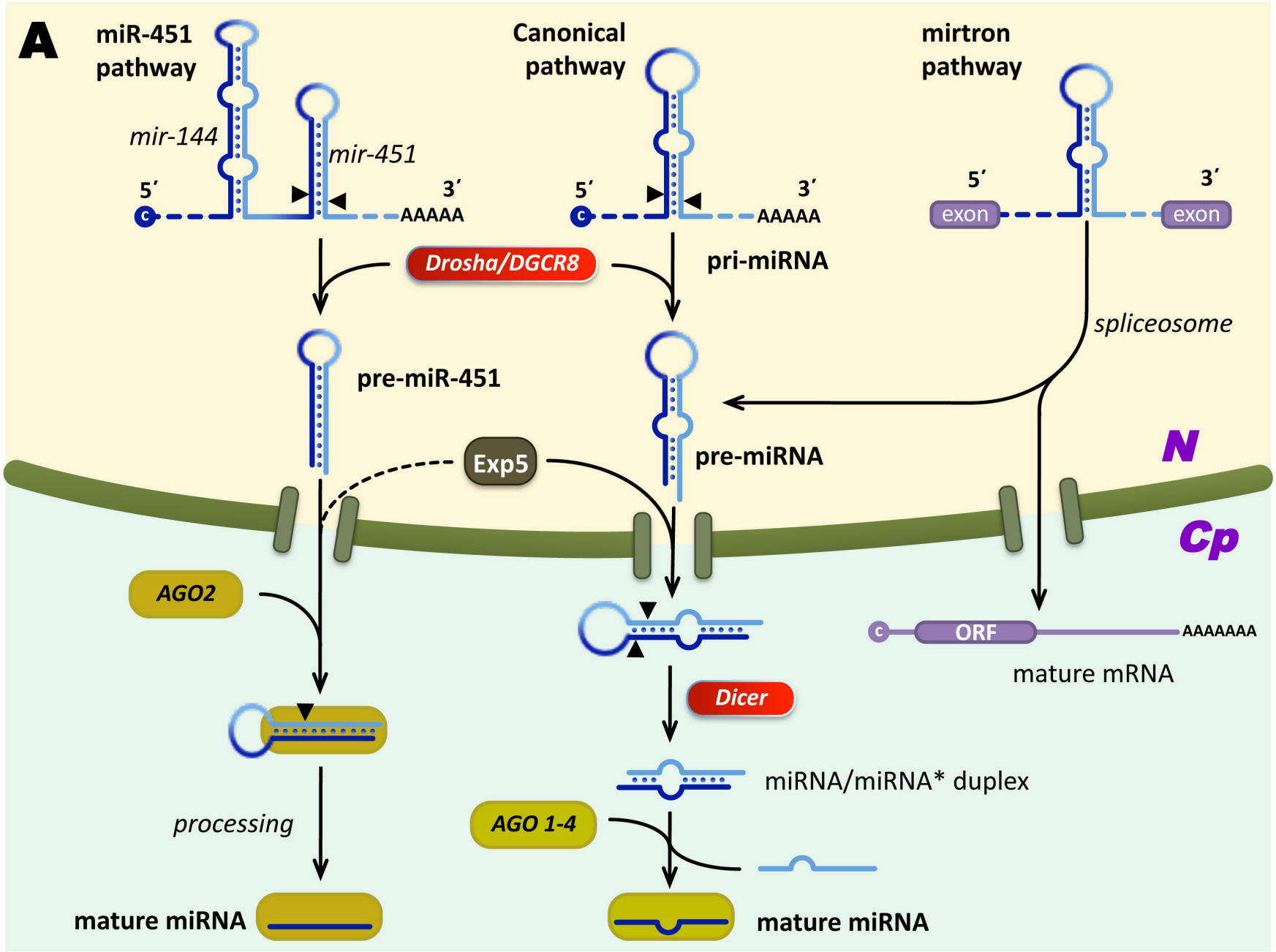
microRNA, miRNA

- 1993: Victor Ambros (*lin-4*)

  (Cell 1993; 75: 843).
- 2000: *Let-7* (Frank Slack, Ruvkun lab)

  (Mol Cell 2000; 5:659. Nature 2000;403:901)
- Είναι αλληλουχίες στο DNA που μεταγράφονται, αλλά δεν μεταφράζονται σε πρωτεΐνη
- Προκύπτει από καλυμμένα (5') - πολυ(Α) πρόδρομα μετάγραφα (pri-miRNA)
- Πρόδρομη θηλιά ~70 nt (pre-miRNA)
- Ώριμο miRNA ~22 nt (miRNA)

Βιογένεση των miRNAs

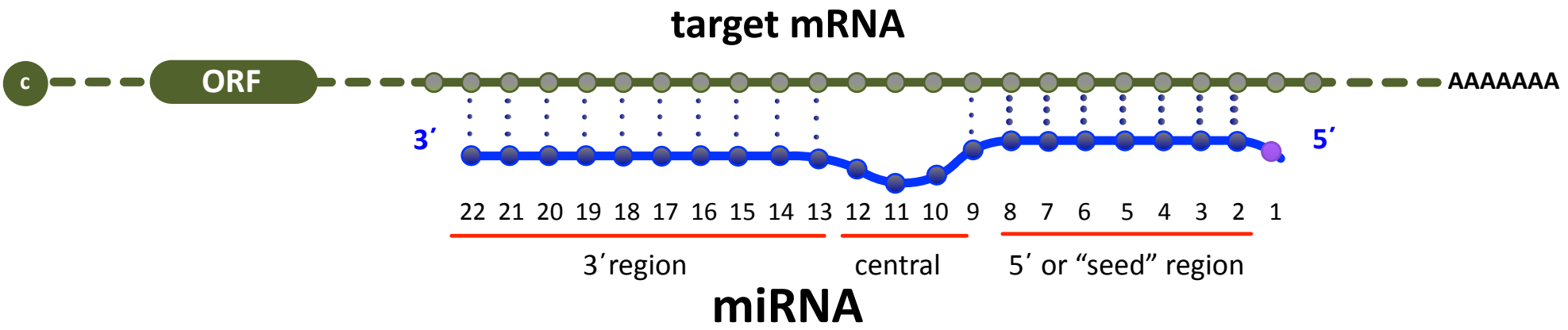


Giannouli et al. 2012, In: *Cancer Biomarkers*

Βιογένεση και λειτουργία των miRNAs: Οι συντελεστές

- Drosha και Pasha: μέλη του “Microprocessor” συμπλόκου (~600-650kDa)
- Drosha και Dicer: RNάσες III
- Pasha: dsRNA προσδενόμενη πρωτεΐνη
- Exportin 5: μέλος πυρηνοκυτταροπλασματικών παραγόντων μεταφοράς καρυοφερίνης (karyopherin nucleocytoplasmic transport factors) Απαιτεί Ran και GTP
- Argonautes: ένζυμα με δράση RNase H

Αλληλεπίδραση mRNA - miRNA



miRNA + intron = mirtron

Intronic microRNA precursors that bypass Drosha processing

J. Graham Ruby[1,2*], Calvin H. Jan[1,2*] & David P. Bartel[1,2]

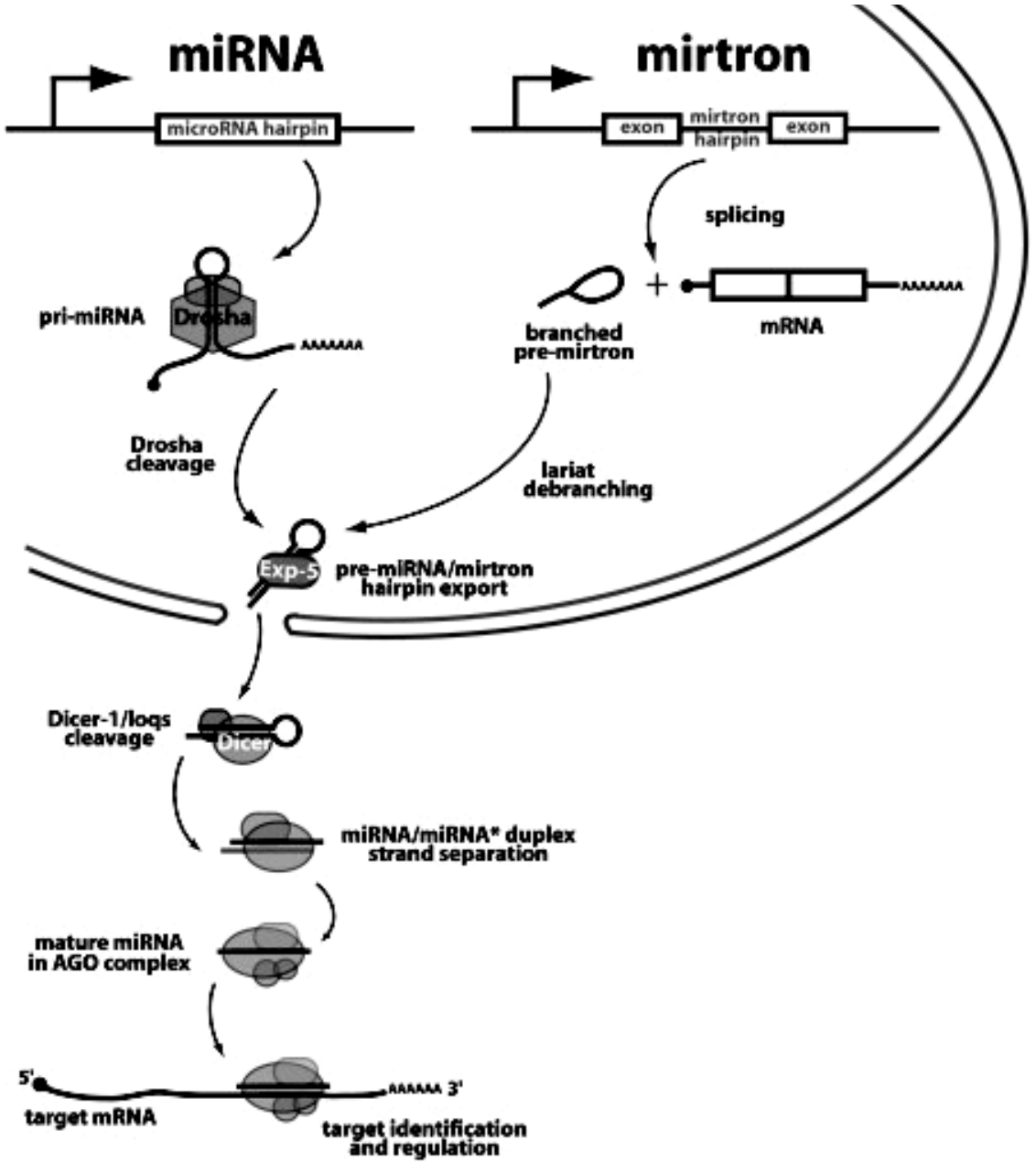
nature Vol 448|5 July 2007|doi:10.1038/nature05983

The Mirtron Pathway Generates microRNA-Class Regulatory RNAs in *Drosophila*

Katsutomo Okamura,[1] Joshua W. Hagen,[1] Hong Duan,[1] David M. Tyler,[1] and Eric C. Lai[1,*]
[1] Memorial Sloan-Kettering Cancer Center, Department of Developmental Biology, 1275 York Ave, Box 252, New York, NY 10021, USA

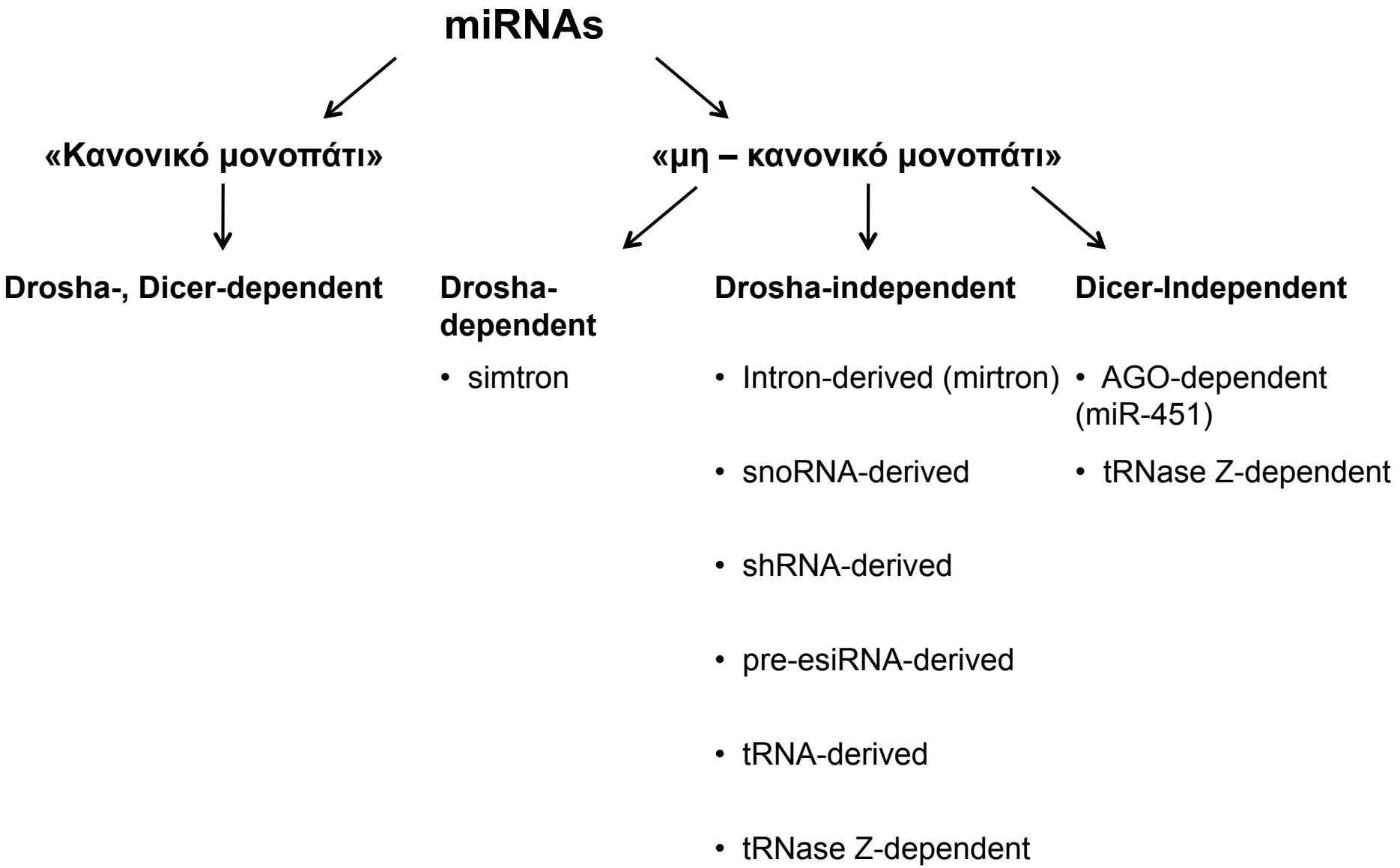
Cell *130*, 89–100, July 13, 2007

Μονοπάτια των mirtron και miRNA

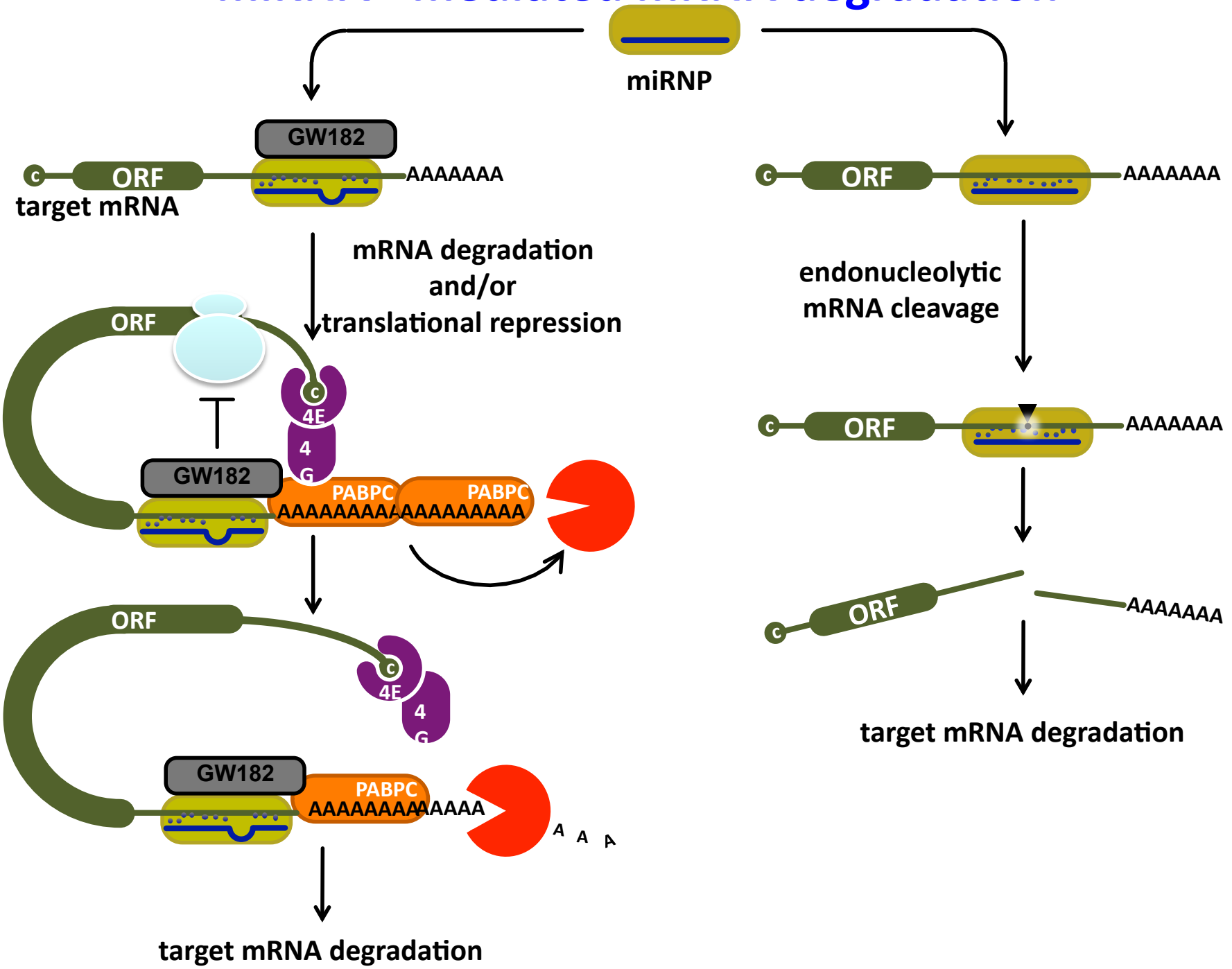


Okamura K et al. Cell 2003; 130: 89

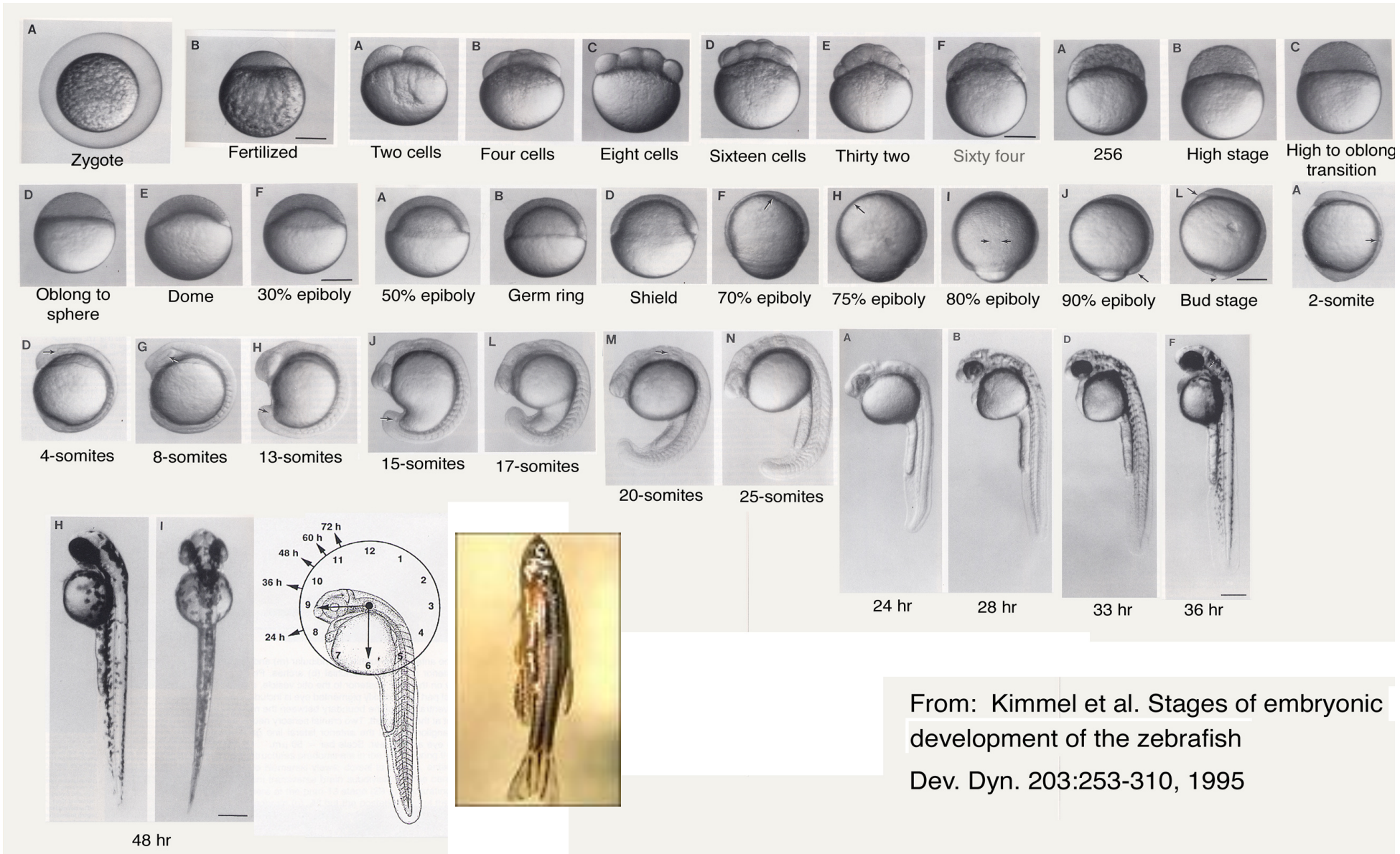
Προέλευση miRNA



miRNA - mediated mRNA degradation



Zebrafish Development

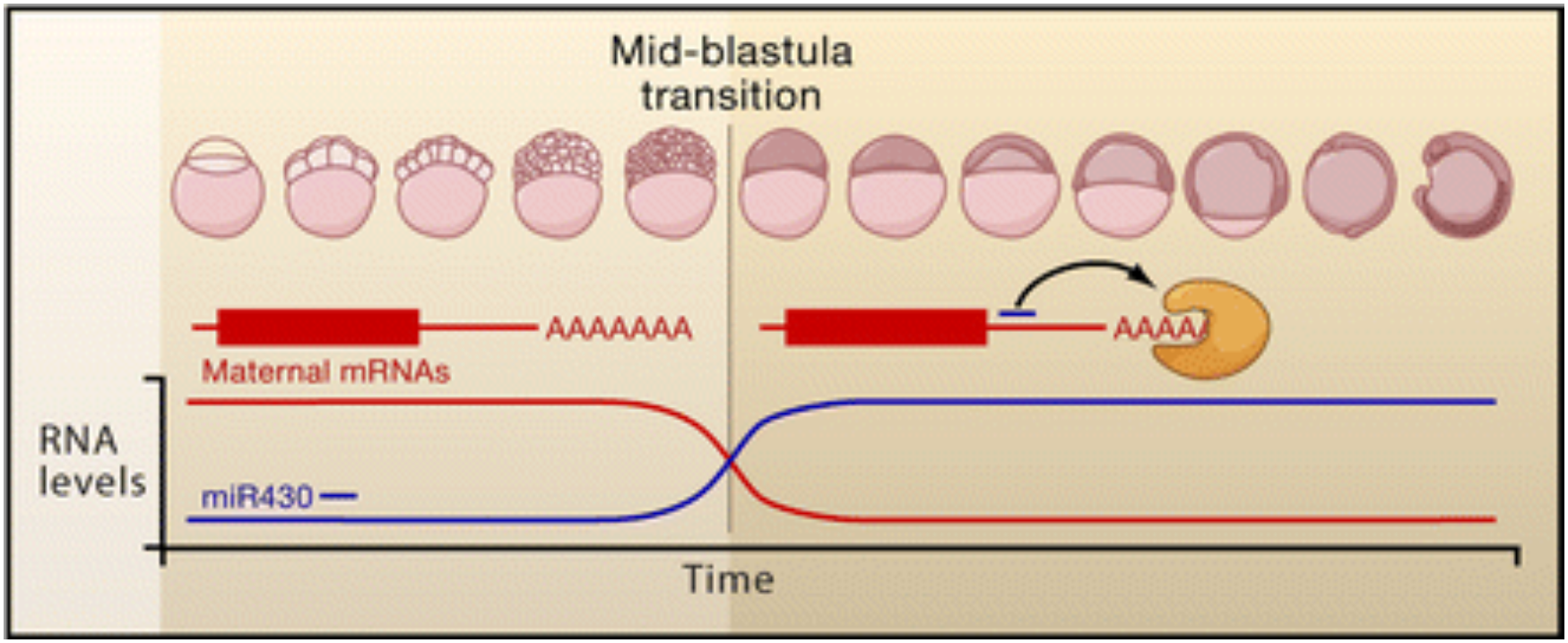


From: Kimmel et al. Stages of embryonic development of the zebrafish

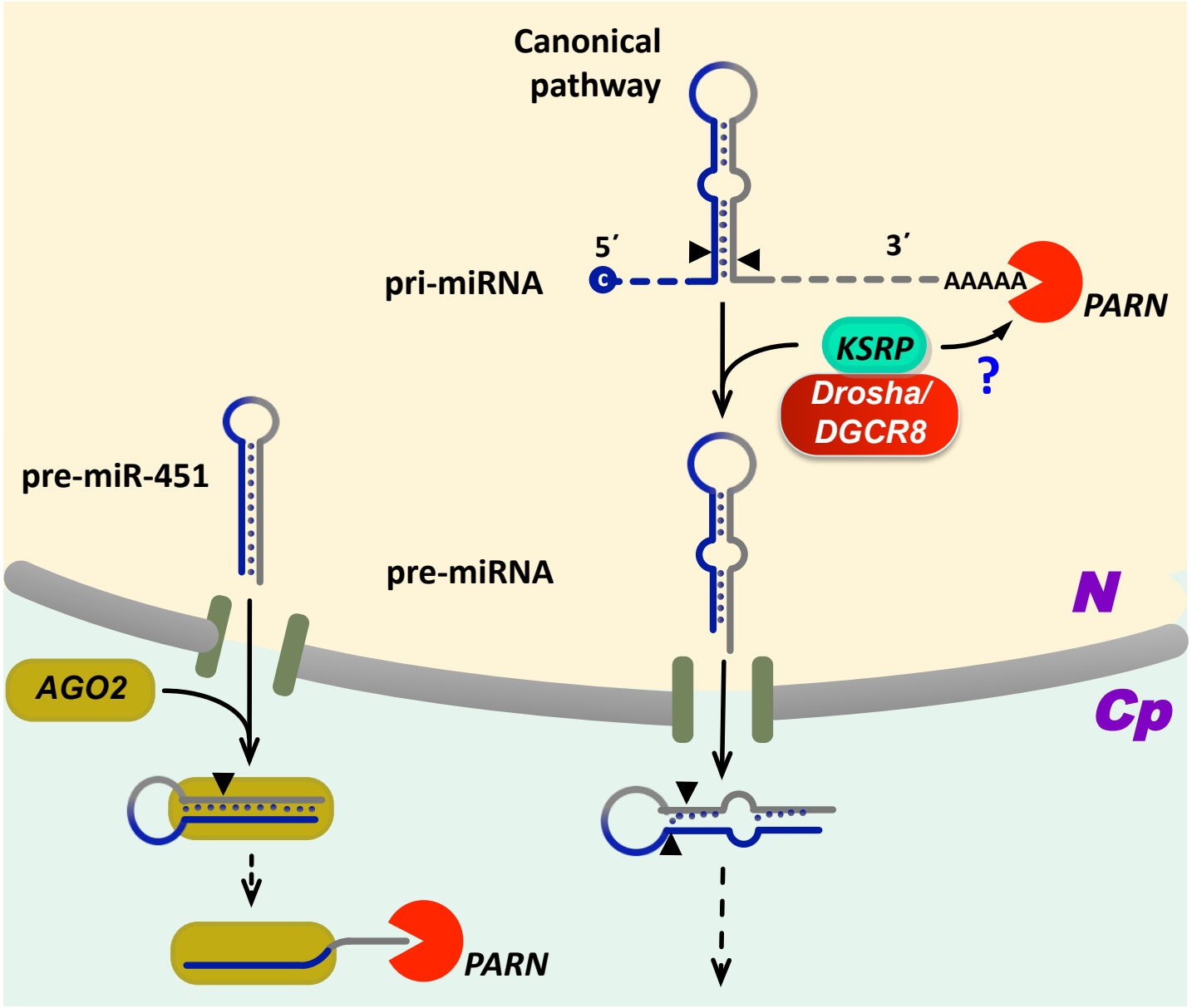
Dev. Dyn. 203:253-310, 1995

http://kirbylab.duhs.duke.edu/gifjpg/zebrafish.jpg

miR430 Promotes Decay of Maternal mRNAs during Zebrafish Development



More roles for PARN in miRNA biogenesis ?



Based on, Yoda et al. 2013, *Cell*; Giannouli et al. 2012, In: *Cancer Biomarkers*

miRNA - siRNA

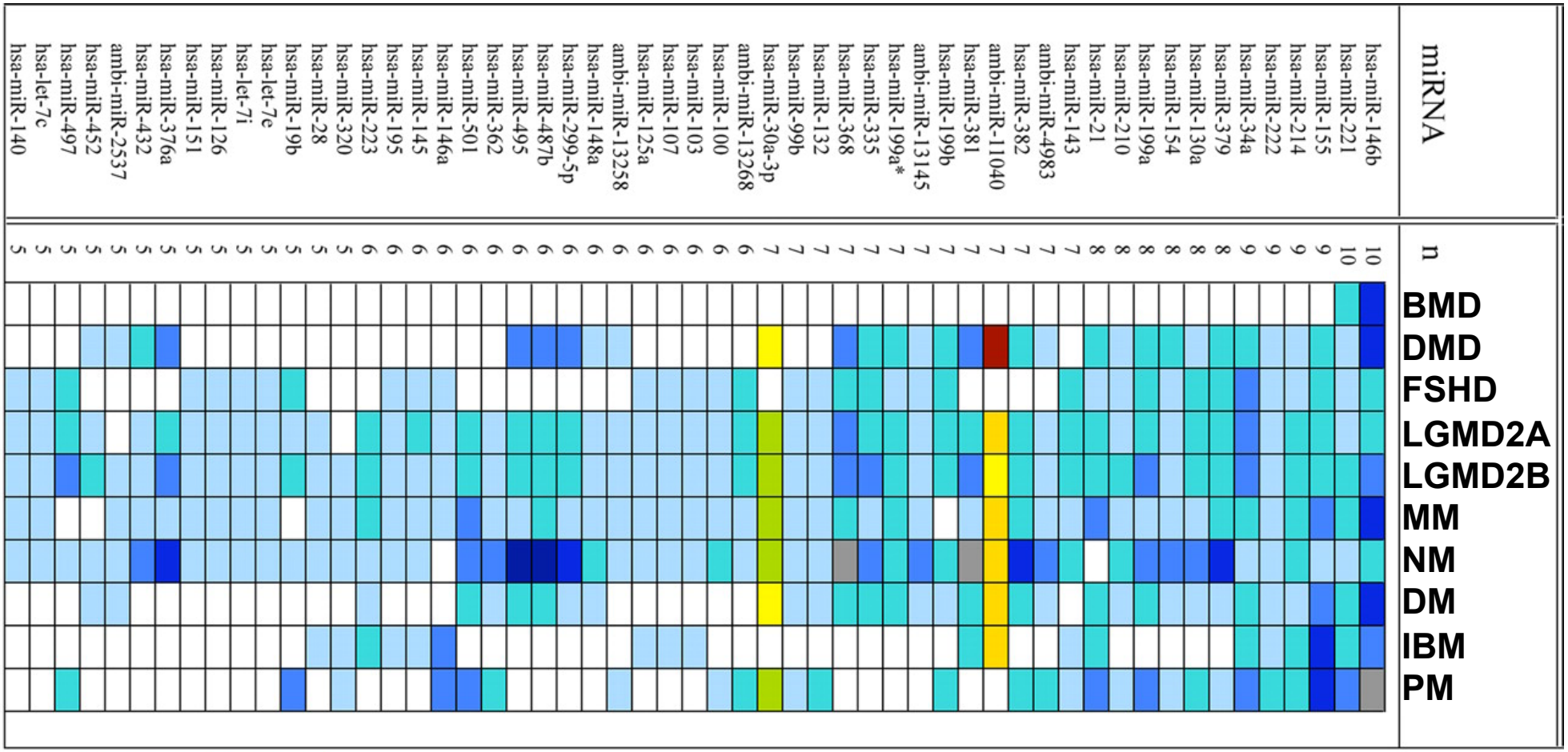
Ρυθμίζουν έκφραση γονιδίων

Διαφορές στην προέλευση:

- siRNA
  1. Εξωγενές
  2. προκύπτει από **δίκλωνο RNA (dsRNA)**
  3. κυρίως η απόκριση σε ξένο RNA (συνήθως ιϊκό)
  4. συνήθως 100% συμπληρωματικότητα με το mRNA-στόχο

- miRNA
  1. Παράγεται από τον ίδιο οργανισμό
  2. Είναι **μονόκλωνο** και προκύπτει από μονόκλωνο **ssRNA** μέσω 2γους δομής θηλιάς
  3. ρυθμίζει μετα-μεταγραφική έκφραση γονιδίων
  4. συνήθως δεν έχει 100% συμπληρωματικότητα με το mRNA-στόχο

miRNAs common to various muscular disorders

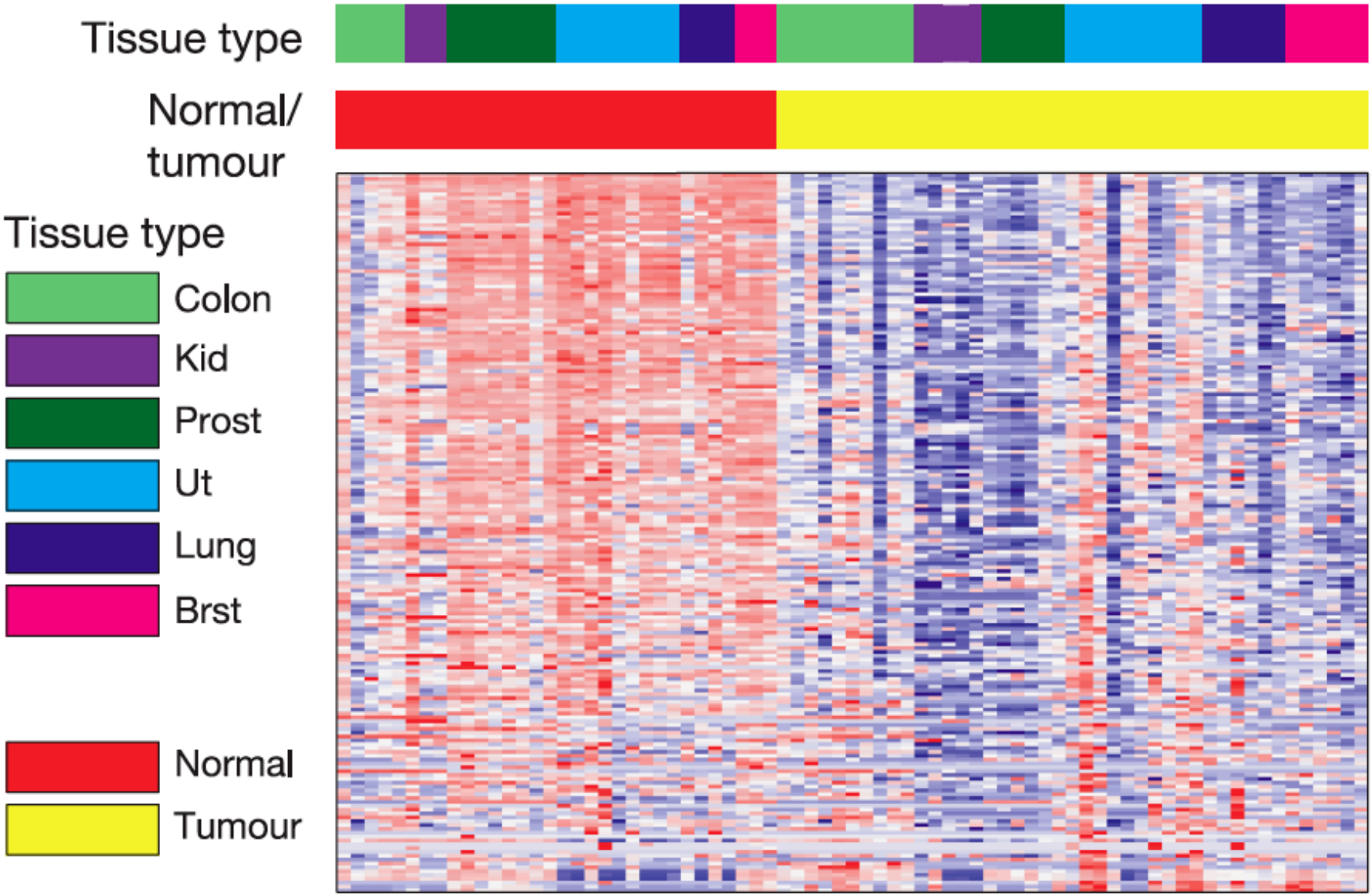


+ 20 - 30
+ 15 - 20
+ 10 - 15
+ 5 - 10
+ 3 - 5
+ 1.5 - 3
- 1.5 - 3
- 3 - 5
- 5 - 10
- 10 - 15
- 15 - 20

DMD, Duchenne muscular dystrophy
BMD, Becker muscular dystrophy
LGMD, Limb girdle muscular dystrophies; LGMD2A, LGMD type 2A; LGMD2B, LGMD type 2B
MM, Miyoshi myopathy
FSHD, Facioscapulohumeral muscular dystrophy
NM, nemaline myopathy
PM, Polymyositis
IBM, Inclusion body myositis
DM, Dermatomyositis

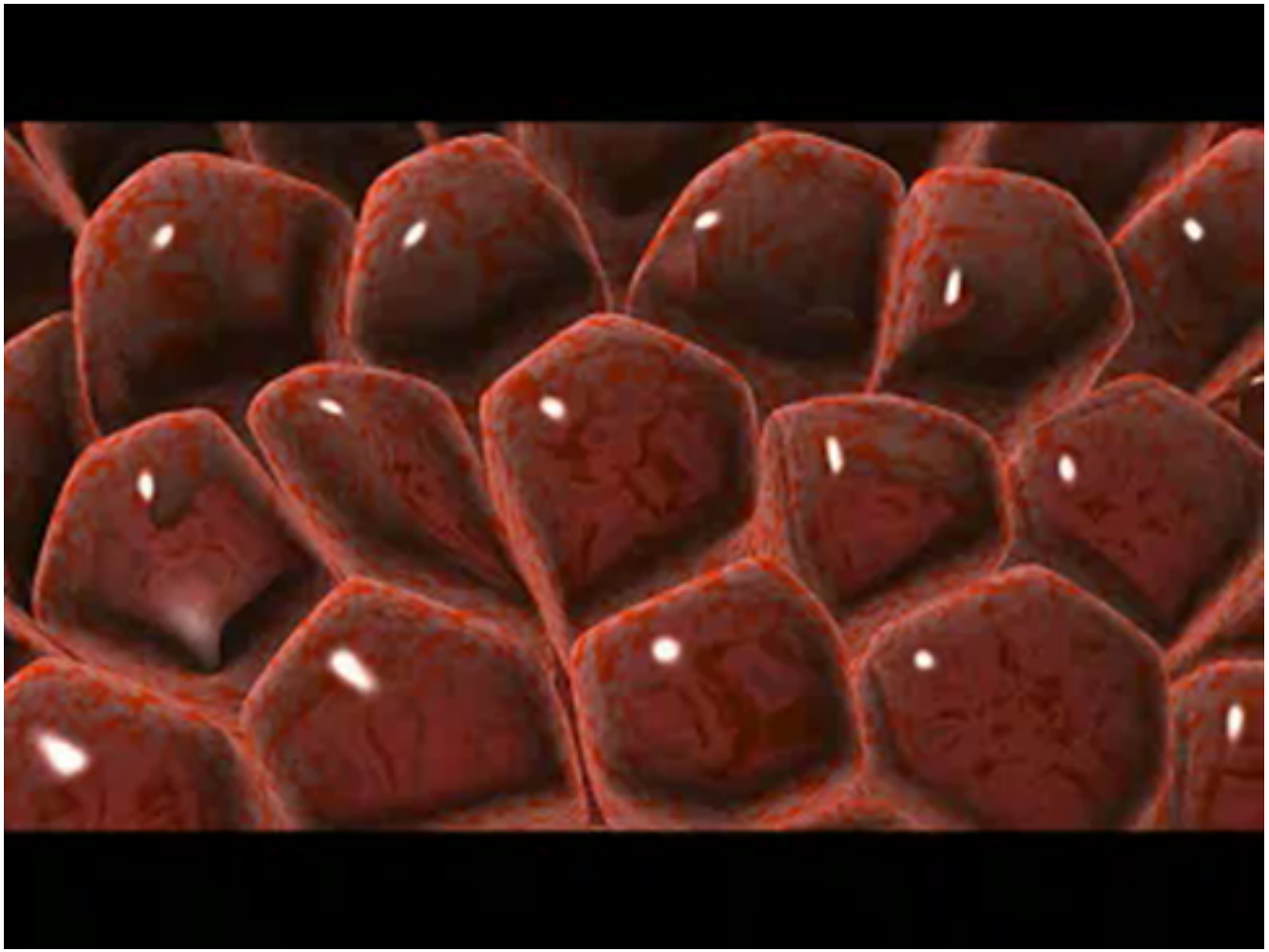
Eisenberg I. et al. (2007) PNAS 104, 17016-17021

miRNA expression profile classify human cancers



Nature 435:834-838, 2005

miRNA biogenesis and function



http://www.nature.com/focus/rnai/animations/med_res.mov

miRNA biogenesis and function

http://www.youtube.com/watch?v=_-9pROnSD-A