Nuclear Export of miRNA precursors

by Jiangsong Jiang
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Nuclear Export of MicroRNA Precursors

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MicroRNAs (miRNAs), which function as regulators of gene expression in eukaryotes, are processed from larger transcripts by sequential action of nuclear and cytoplasmic ribonuclease III–like endonucleases. We show that Exportin-5 (Exp5) mediates efficient nuclear export of short miRNA precursors (pre-miRNAs) and that its depletion by RNA interference results in reduced miRNA levels. Exp5 binds correctly processed pre-miRNAs directly and specifically, in a Ran guanosine triphosphate–dependent manner, but interacts only weakly with extended pre-miRNAs that yield incorrect miRNAs when processed by Dicer in vitro. Thus, Exp5 is key to miRNA biogenesis and may help coordinate nuclear and cytoplasmic processing steps.

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Sue Guo, 1995
Cornell University

Sue Guo's "antisense" experiment

Molecule Injected	Embryo lethality %
ZC22 (par-1)antisense	52
ZC22 (par-1)sense	54
TS antisense	0
Z1 antisense	0
H20	0

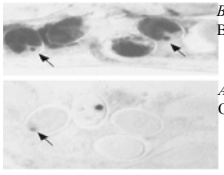
"Surprisingly, injection of sense ZC22 RNA also induced par-1 phenotypes. It is not clear what accounts for this effect… The basis for the sense effect is under investigation and will not be discussed further…"

---- Cell 81(4):611-20 1995

Andy Fire
Carnegie Institution of Washington

Andy Fire's Experiment

Molecules Injected	% twitching
unc-22 "pure" antisense	0
unc-22 "pure" sense	0
unc-22 sense + antisense	100



Before RNAi.
Both cytoplasmic and nuclear RNAs are detected.

After RNAi.
Only nuclear RNAs are detected.

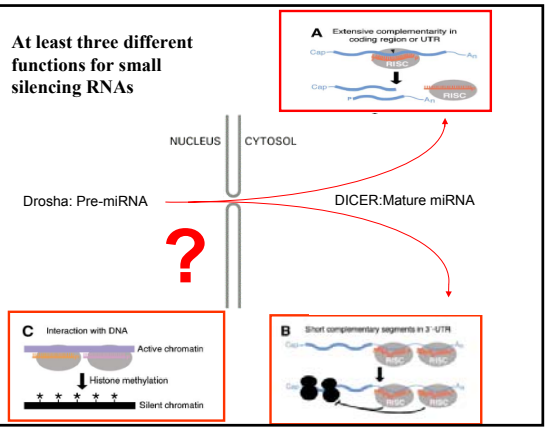
In situ hybridization to *pes-10* transcripts

----- Xu & Fire,PNAS 95, 1998

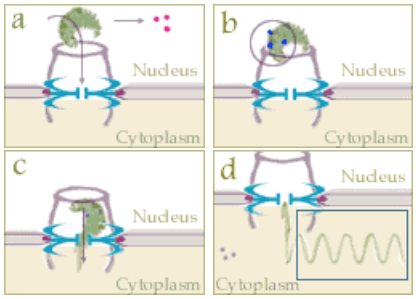
Handy RNAi Terms

- dsRNA: double stranded RNA, precursors, longer than 30 nt
- siRNA: short-interfering RNA, 21-25 nt.
  - Mostly exogenous origin.
  - dsRNA precursors
  - May be target specific
- miRNA: microRNA, 21-25 nt.
  - Encoded by endogenous genes.
  - Hairpin precursors
  - Recognize multiple targets.

At least three different functions for small silencing RNAs



General RNA export through NPC

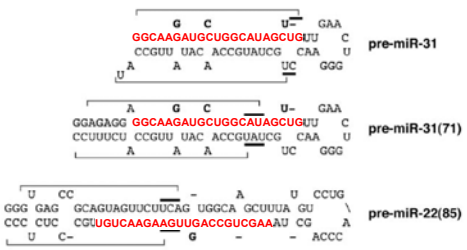


General RNA export through NPC

Do pre-miRNAs use the same way to translocate?

Research subjects

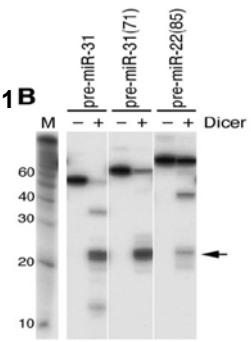
Fig1 A



Q: Are tested pre-miRNA substrates OK and how does DICER cut pre-miRNA ?

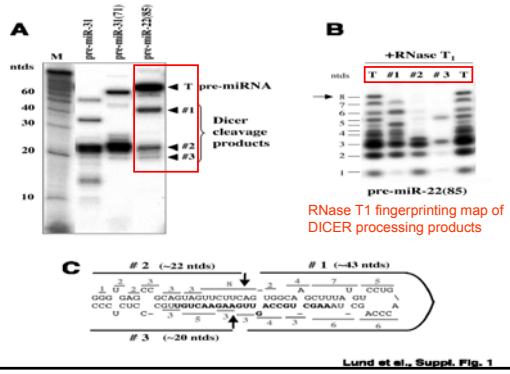
Method: autoradiography of ^{32}P-labeled pre-miRNA substrates cut by DICER .

Fig1 B



Conclusion: DICER cuts pre-miRNA substrates with different efficiencies.

RNase T1 analysis indicates DICER cutting sites.



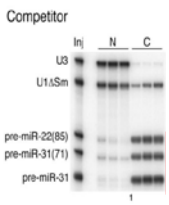
RNase T1 fingerprinting map of DICER processing products

Lund et al., Suppl. Fig. 1

Q: do pre-miRNAs use the same exportin with other RNAs?

Method: inject pre-miRNA and other RNAs into nuclei together and monitor their cometition and aggregation in nuclei and cytoplasm, respectively.

Fig2 A

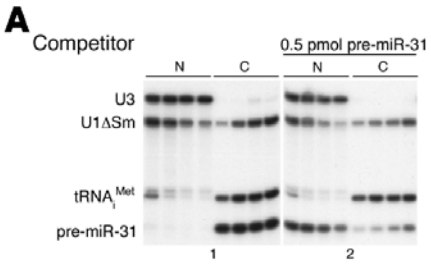


rRNA splicing
mRNA splicing

N: Nucleus
C: Cytoplasm

Conclusion: pre-miRNAs don't compete with U3 and U1△Sm, thus pre-miRNA export is carrier-mediated but unlikely to use the nuclear export receptors CRM1 or Exp-t.

Although pre-miRNA doesn't compete with U3 and U1ΔSm, it does compete with $tRNA_i^{Met}$.

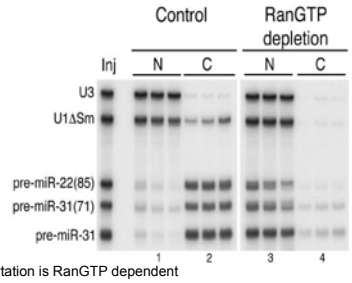


So, pre-miR-31 and **$tRNA_i^{Met}$** may share exportin.

Q: Is RanGTP involved in pre-miRNA export?

Method: Monitoring miRNA transportation in oocytes depleted RanGTP upon preinjection of RanT24N(mutant, which binds weakly to GDP and not to GTP).

Fig2 B



Conclusion: miRNA transportation is RanGTP dependent

Summary

- pre-miRNAs are valid substrates
- pre-miRNAs don't use CRM1 or Exp-t to export
- pre-miRNAs may share exportin with $tRNA_i^{Met}$
- pre-miRNAs transportation is RanGTP dependent

Facts

•Exportin-5-mediated nuclear export of eukaryotic elongation factor 1A and tRNA.

----- The EMBO J. 2002

•"…That exportin-5 preferentially recognizes and transports **minihelix** motif-containing RNAs."
(**minihelix**: a double-stranded stem (>14 nt) with a base-paired 5 end and a 3–8-nt protruding 3 end, pre-miRNA is minihelix)

----JBC 278:5505-5508, 2003

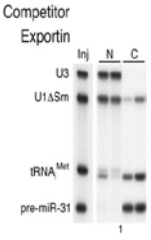
Hypothesis

Exportin-5, a candidate for pre-miRNA export!

Q: Is Exp5 involved in pre-miRNA export?

Method: competition assay

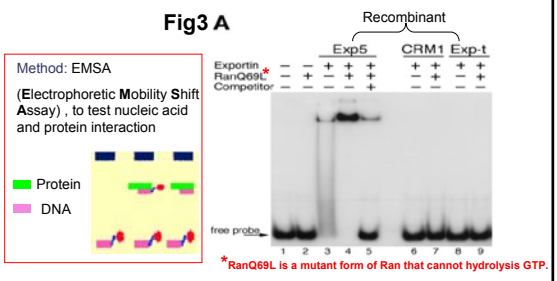
Fig2 C



Conclusion: "…Thus, pre-miRNA export is specifically stimulated by Exp5 ."

"…Can Exp5 bind pri-miRNA directly?..."

Fig3 A

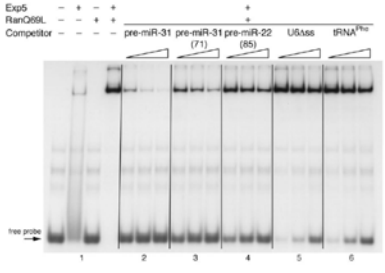


*RanQ69L is a mutant form of Ran that cannot hydrolysis GTP.

Conclusion: Exp5 binds pre-miRNA and RanGTP dramatically enhances this binding, on the contrary, CRM1 and Exp-t don't bind pre-miRNA.

TO further test Exp5's capacity to bind 3 pre-miRNAs & unrelated RNAs

Fig3 B

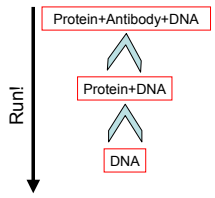


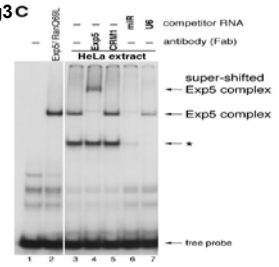
Conclusion: Exp5 shows different binding capacity to 3 pre-miRNAs and unrelated small RNAs poorly compete for Exp5 complex formation.

Using HeLa cell extract to demonstrate the selectivity of the interaction between pre-miRNA and Exp5

Fig3C

Method: super shift EMSA





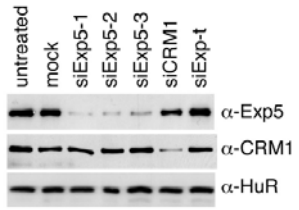
Conclusion: The highly specific interactions between Exp5 and pre-miRNAs

Deleting Exp5 by RNA interference

-----to test Exp5's function in vivo

Method: inject interfering RNAs and controls into cell and total protein extracts were immunoblotted with related antibody to test protein expression.

Fig4A



Conclusion: Treatment of HeLa cells with three different Exp5-specific short interfering RNAs (siRNAs), but not control siRNAs against CRM1 or Exp-t, effectively reduced the levels of Exp5 .

siExp5s destroy Exp5's function in nucleus-cytoplasm transportation

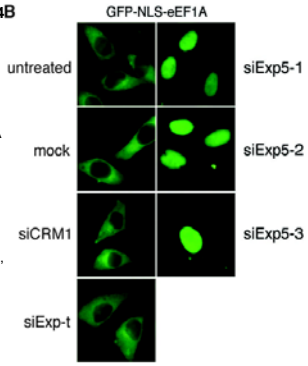
Fig4B

Backgroud: "eEF1A is transported by Exp5 and strictly excluded from nuclei."
----The EMBO J., 2002

Method: construct GFP-NLS-eEF1A HeLa cell line and inject siRNAs against Exp5, CRM1 and Exp-t into cells and monitor GFP signal under fluorescence microscopy

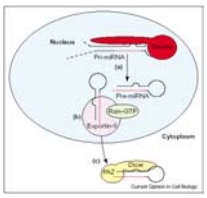
Conclusion: Cells depleted of Exp5 were also impaired for Exp5 function, as indicated by the nuclear accumulation of GFP-NLS-eEF1A

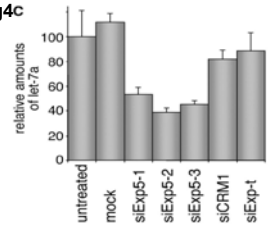
NLS, Nuclear Localization Signal



Depletion of Exp5 by RNAi reveals its role in pre-miRNA processing

Fig4C





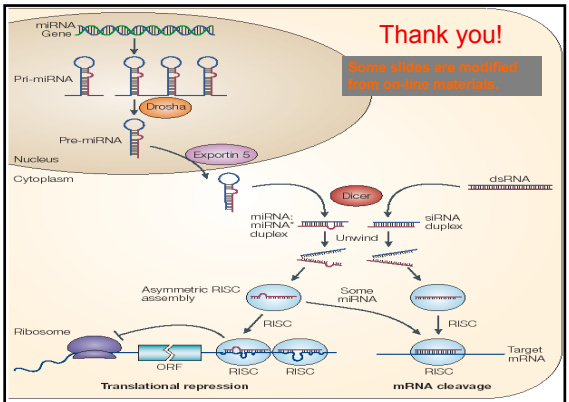
Upon depletion of Exp5 by RNAi for 48 to 72 hours, the levels of let-7a-1 and other mature miRNAs were reduced by 40 to 60%.

HASTY, an ortholog of Exp5 in Arabidopsis, controls several developmental processes

wt / mut	Phenotype
A / B	rosette
C / D	short lateral inflorescences (arrow) unfertilized siliques (asterisk).
E / F	irregularly spaced flowers

"…Thus, the research in science paper demonstrates a direct and central role of Exp5 in miRNA biogenesis and offer a possible explanation for the extensive developmental defects observed in Arabidopsis"

-----Development 130, 1493-1504, 2003



Nature Reviews Genetics **5**, 522–531, 2004