

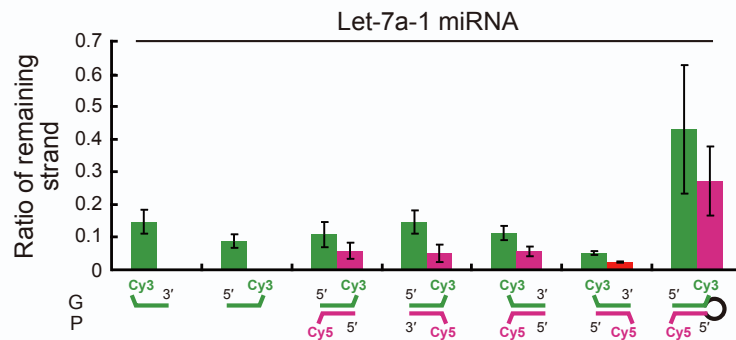
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Supplemental information

**Direct observation of cytoskeleton-dependent
trafficking of miRNA visualized
by the introduction of pre-miRNA**

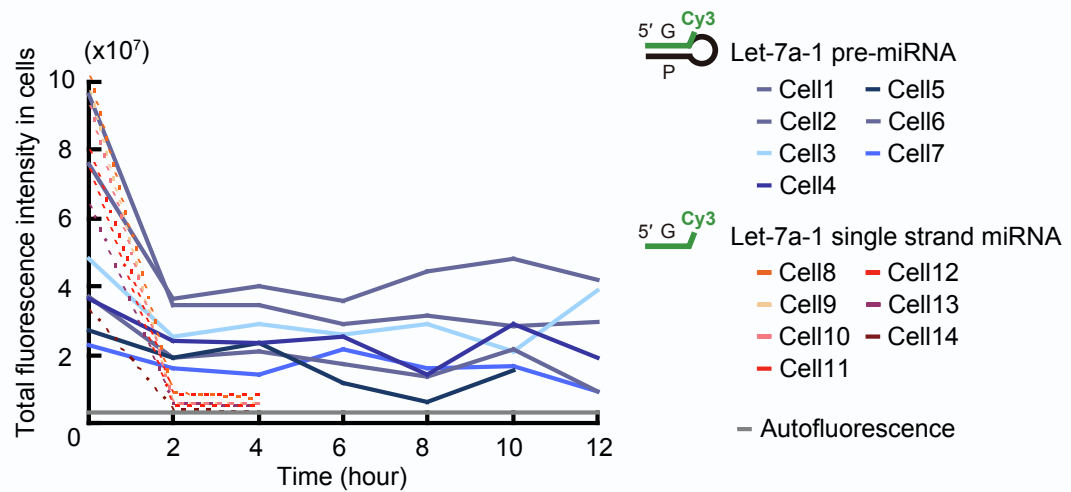
Toshinari Ishikawa, Ko Sugawara, Junwei Zhang, Takashi Funatsu, and Kohki Okabe

Supplementary Figures



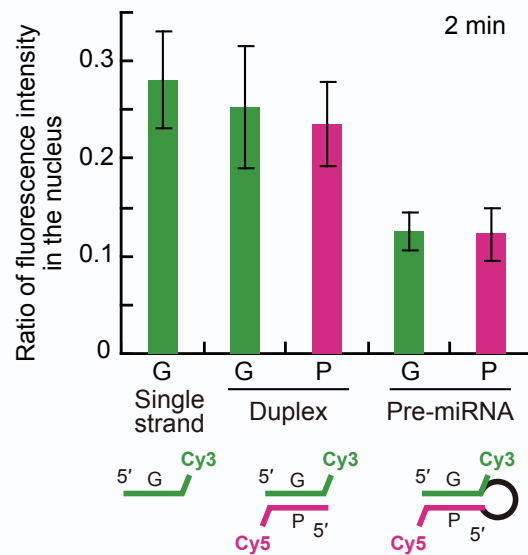
Supplementary Figure S1. Comparison of remaining efficiency upon introduction of various fluorescently labeled miRNA precursors in living HeLa cells, Related to Figure 1

The proportion of remaining strands in the cytoplasm. The results are shown for seven miRNA precursors with guide strands labeled with Cy3 and/or passenger strands labeled with Cy5. The proportion of total fluorescence intensity in the cytoplasm at 60 min after microinjection to that at 2 min after microinjection is shown. Guide (green) and passenger (magenta) strands are shown. Error bars represent SD ($n = 6-12$ cells). G and P stand for guide and passenger sequences of miRNA, respectively.



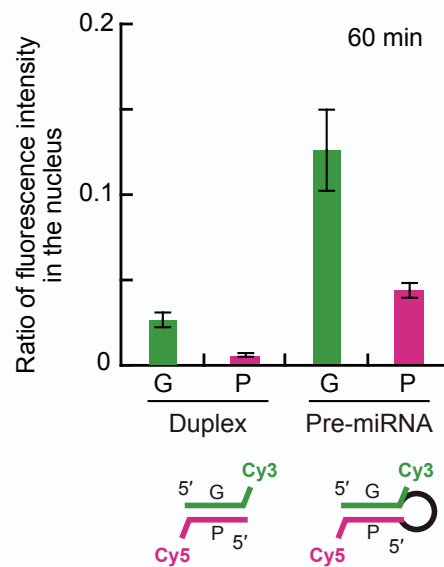
Supplementary Figure S2. Stability of miRNA precursors in living cells, Related to Figure 1

The total fluorescence intensity of let-7a-1 miRNA precursors whose guide strand was labeled with Cy3 microinjected into the cytoplasm of living COS7 cells [cells 1–7 (pre-miRNA) and cells 8–14 (single-strand miRNA)] was tracked using epifluorescence microscopy. The signal of autofluorescence is shown as a green solid line. G and P stand for guide and passenger sequences of miRNA, respectively.



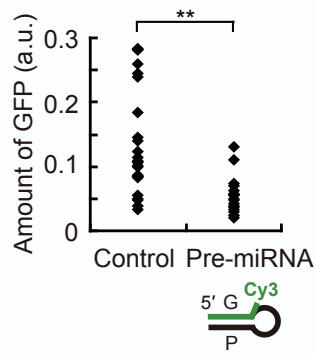
Supplementary Figure S3. Accumulation of miRNA precursors in the nucleus immediately after introduction, Related to Figure 1

The proportion of fluorescence intensity of Cy3 (and Cy5)-labeled let-7a-1 miRNA precursors (single-stranded miRNA, duplex, and pre-miRNA) in the nucleus relative to that of the whole cell at 2 min after introduction into the cytoplasm of living COS7 cells. Green and magenta indicate Cy3-labeled guide strand and Cy5-labeled passenger strand, respectively. Error bars represent SD [$n = 11$ cells (single strand), 12 cells (duplex), 14 cells (pre-miRNA)]. G and P stand for guide and passenger sequences of miRNA, respectively.



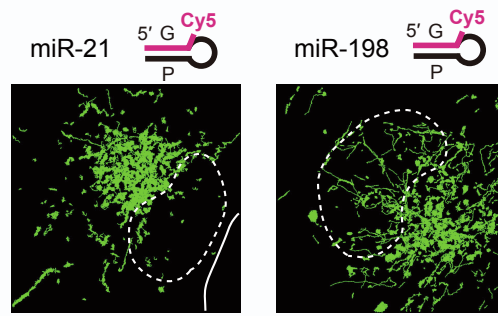
Supplementary Figure S4. Accumulation of miRNA precursors in the nucleus at 60 min after introduction, Related to Figure 2

The proportion of fluorescence intensity of Cy3 (and Cy5)-labeled let-7a-1 miRNA precursors (duplex and pre-miRNA) in the nucleus relative to that of the whole cell at 60 min after introduction into the cytoplasm of living COS7 cells. Green and magenta indicate Cy3-labeled guide strand and Cy5-labeled passenger strand, respectively. Error bars represent SD [$n = 12$ cells (duplex), 14 cells (pre-miRNA)]. G and P stand for guide and passenger sequences of miRNA, respectively.



Supplementary Figure S5. Inhibition of translation by fluorescent miRNA, Related to Figure 2

The level of GFP of the gene containing the target sequence of let-7a-1 miRNA was normalized to that of RFP that did not have an miRNA target. COS7 cells in which Cy3-labeled let-7a-1 pre-miRNA was introduced showed a significantly lower level of GFP. The control represents the results in cells without pre-miRNA introduction. Each rhombus represents a single cell. $**p < 0.01$; Mann-Whitney U test, $n = 25$ cells (Control) and 17 cells (Pre-miRNA). G and P stand for guide and passenger sequences of miRNA, respectively.



Supplementary Figure S6. Tracking of miR-21 and miR-198 particles in living cells, Related to Figure

Cy5-labeled pre-miRNAs were introduced and allowed to mature, followed by single-particle tracking. Dotted and solid lines indicate the nucleus and the cell periphery, respectively. G and P stand for guide and passenger sequences of miRNA, respectively. Scale bar, 10 μm .

Supplementary Tables

Supplementary Table S1 Sequences of miRNA used in this study, Related to Figure 1

Name	Form	Guide/Passenger	Sequence*
Let-7a-1	Single strand	Guide	UGAGGUAGUAGGUUGUAUAGUU-Cy3
	Duplex	Guide	UGAGGUAGUAGGUUGUAUAGUU-Cy3
		Passenger	CUAUACAAUCUACUGUCUUUC - Cy5
	Pre-miRNA	Guide	UGAGGUAGUAGGUUGUAUAGUU(Alexa488)UUAGGGUCACACC†
		Guide	UGAGGUAGUAGGUUGUAUAGUU(Cy3)UUAGGGUCACACC†
		Guide	UGAGGUAGUAGGUUGUAUAGUU(Cy5)UUAGGGUCACACC†
		Guide	UGAGGUAGUAGGUUGUAUAGUU(Amino)UUAGGGUCACACC†
		Passenger	PO ₃ -CACCACUGGGAGAUAAACUAUACAAUCUACUGUCUUU(Cy5)CC†
Passenger		PO ₃ -CACCACUGGGAGAUAAACUAUACAAUCUACUGUCUUUCC	
miR-21	Pre-miRNA	Guide	UGUCGGGUAGCUUAUCAGACUGAUGUU(Amino)GACUGUUGA†
		Passenger	PO ₃ -AUCUCAUGGCAACACCAGUCGAUGGGCUGUCUGACA
miR-198	Pre-miRNA	Guide	UCAUUGGUCCAGAGGGGAGAUAGGUU(Amino)CCUGUGAU†
		Passenger	PO ₃ -UUUCCUUCUUCUCUAUAGAAUAAAUGA

* All sequences are listed from their 5'-end to their 3'-end.

† (Alexa488), (Cy3), and (Cy5) indicate intrachain labelling with corresponding moieties.

Supplementary Table S2 Sequences of antisense 2'-O-methyl RNA oligonucleotides used in this study, Related to Figure 2

Target mRNA	Sequence*
Kras (1)	Amino-AGGCACUCUUGCCUACGCCA-biotin†
Kras (2)	Amino-UCAUGUACUGGUCCUCAUU-biotin†
Kras (3)	Amino-CCUACUAGGACCAUAGGUAC-biotin†
Luciferase‡	Amino-GUUGUUUUGGAGCACGGAAA-biotin†

* All sequences are listed from their 5'-end to their 3'-end.

† Streptavidin was attached to antisense probes via biotin to prevent sequestration into the nucleus.

‡ Control probe that does not have a target in mammalian cells.

Supplementary Movies

Supplementary Movie 1. Tracking of miRNA particles along actin filaments in living cells. Movie of Cy5-labeled let-7a-1 miRNA particles (red, 19 times faster than real time) is merged with averaged image of actin filaments (green).

Supplementary Movie 2. Tracking of miRNA particles along microtubules in living cells. Movie of Cy5-labeled let-7a-1 miRNA particles (red, 19 times faster than real time) is merged with averaged image of microtubules (green).

Supplementary Movie 3. Tracking of miRNA particles in cytoskeleton-disrupted cells. Movie of Cy5-labeled let-7a-1 miRNA particles in living COS7 cells in which both actin filaments and microtubules are destroyed. The movie runs 19 times faster than real time.