Sustainability of RNA-interference in Rule Based Modelling

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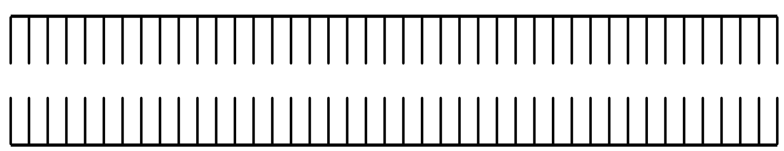
RNA interference

- RNAi (also known as RNA silencing) is a mechanism in which short interfering RNA's (siRNA's) (21～26 nt's) directly control gene expression.
- RNAi consists of three fundamental biochemical processes:

Step 1 RNAi

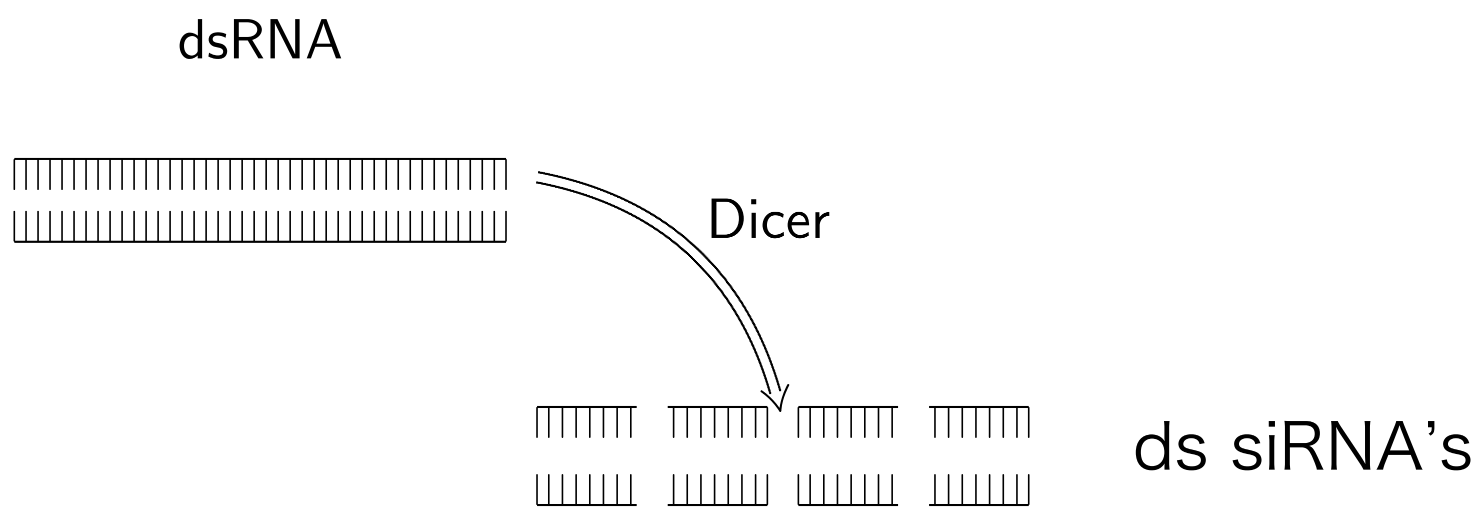
Formation of double stranded RNA (dsRNA)

dsRNA



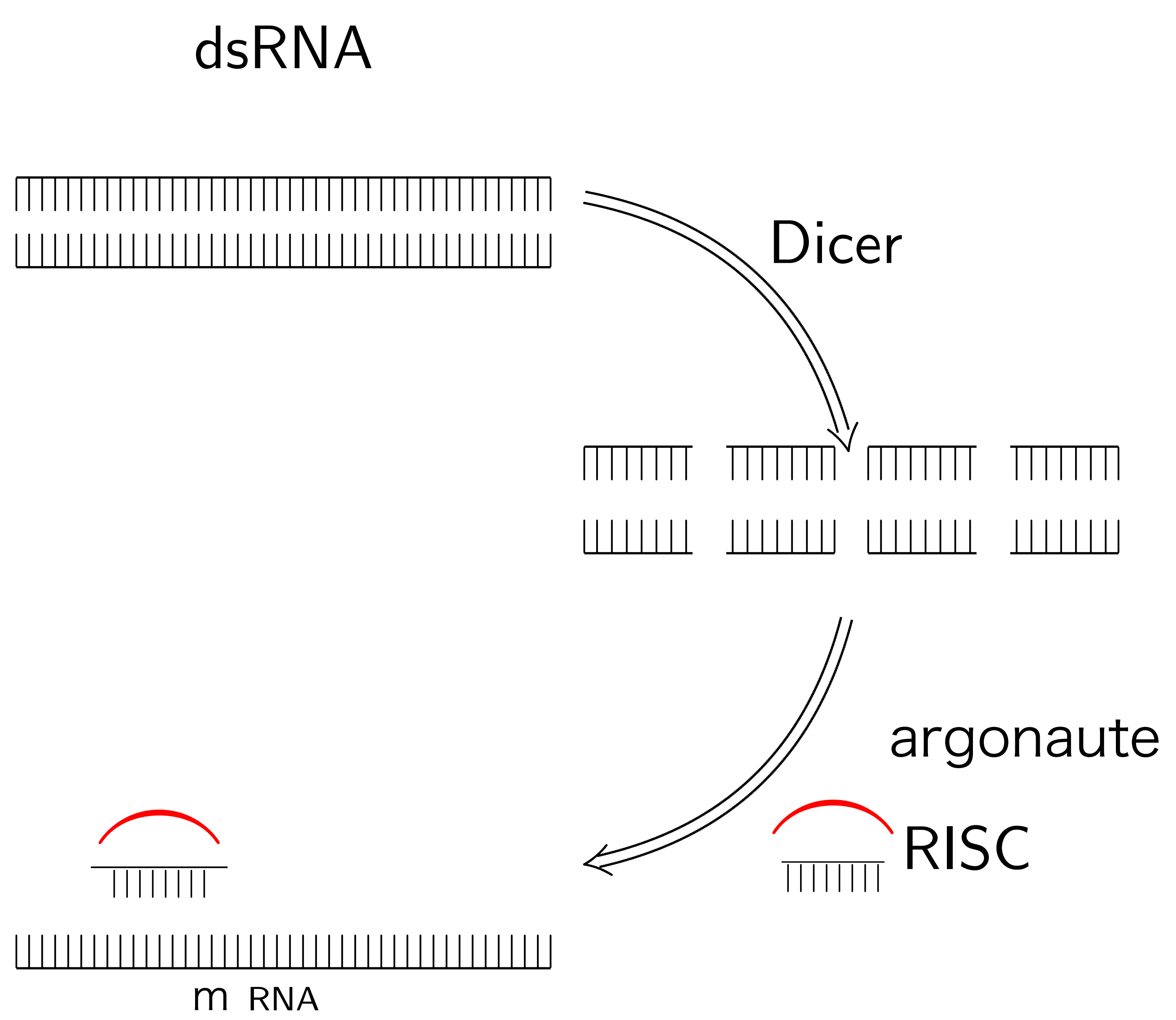
Step 2 RNAi

Dicer enzyme cleaves dsRNA into siRNA’s:

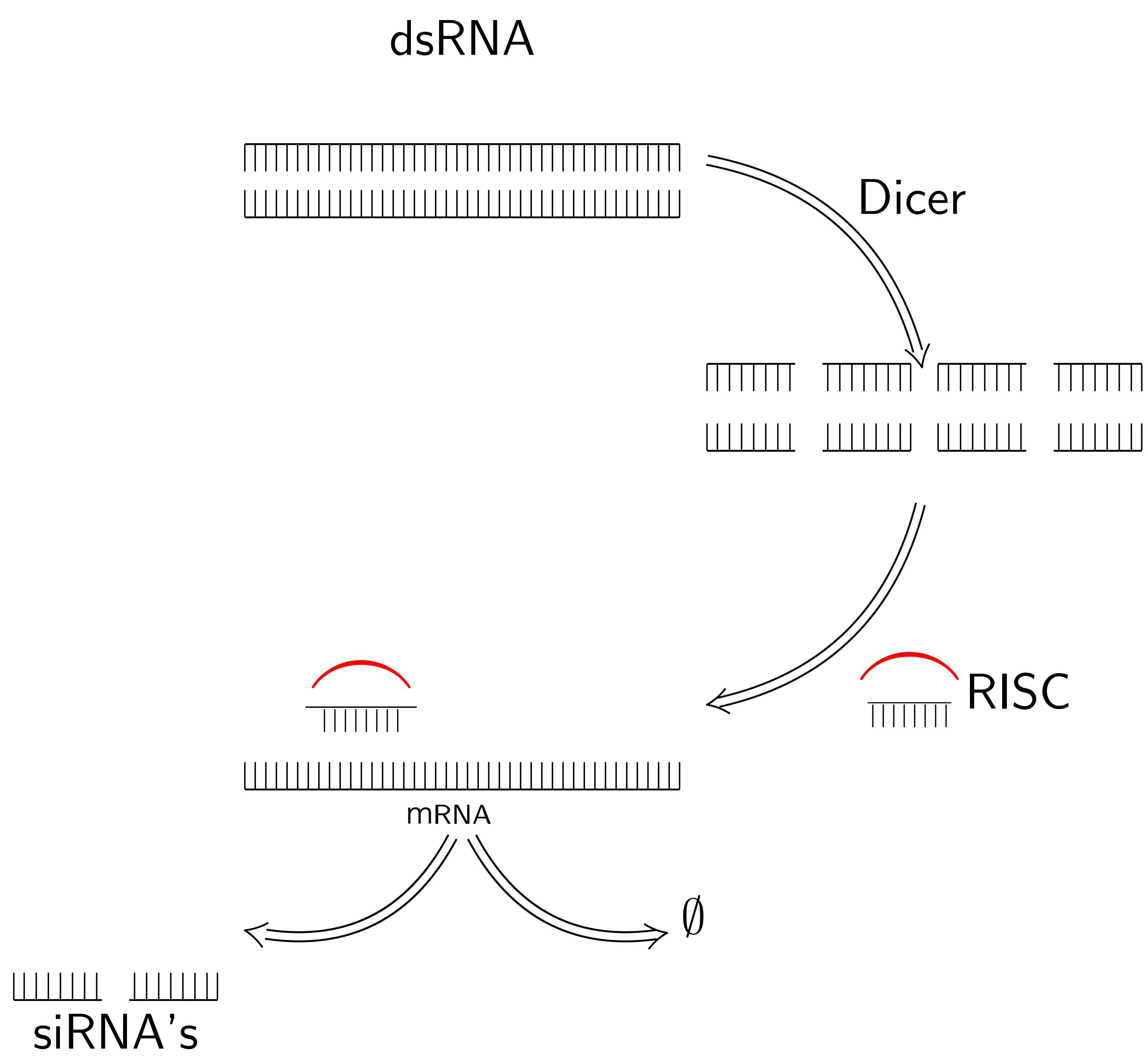


Step 3 RNAi

Incorporation of siRNA into RNA-induced silencing complex (RISC), targeting a long single-stranded mRNA by complementarity.

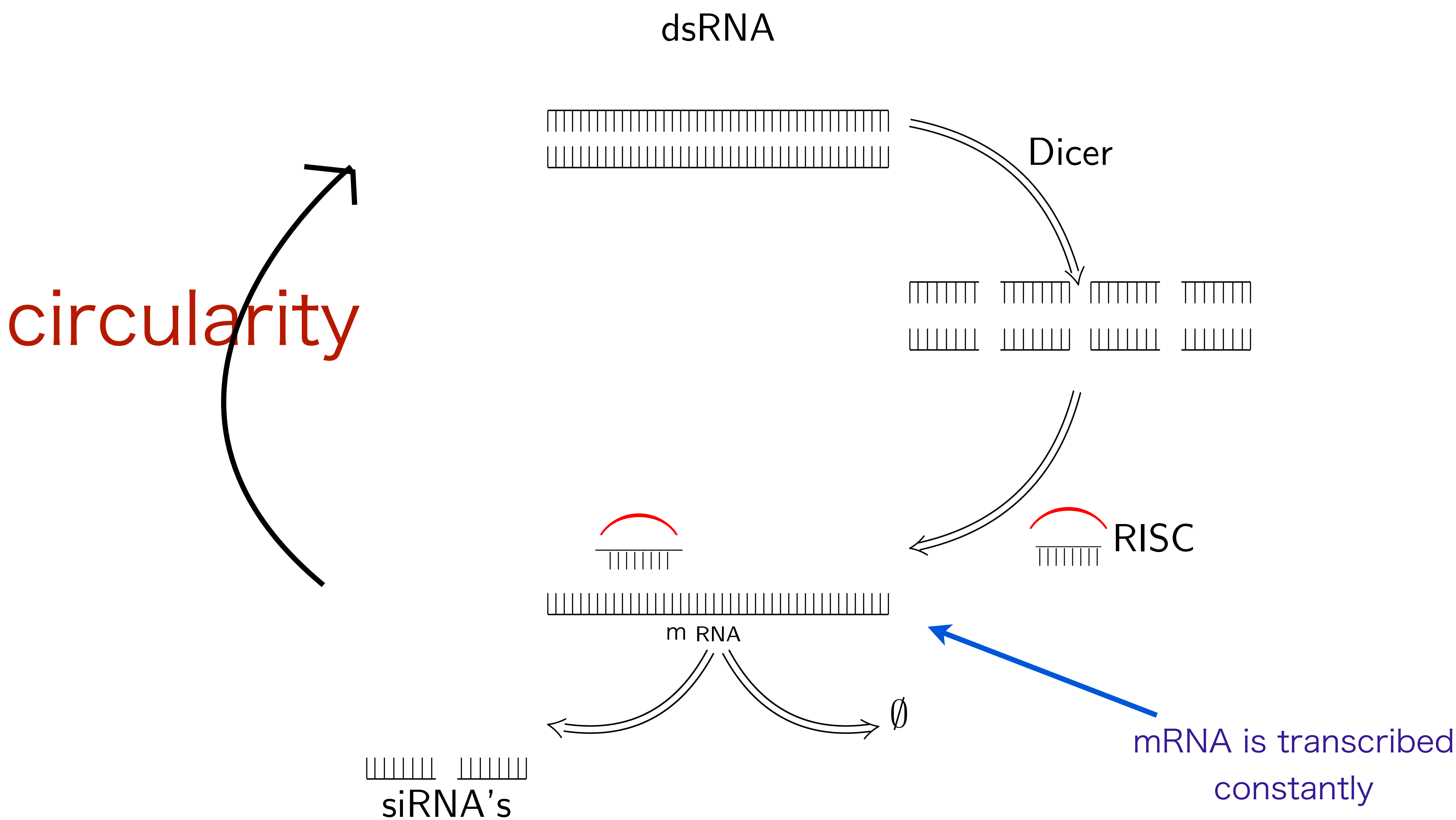


Finally, RISC degrades mRNA or cleaves it into siRNA's.



Motivation

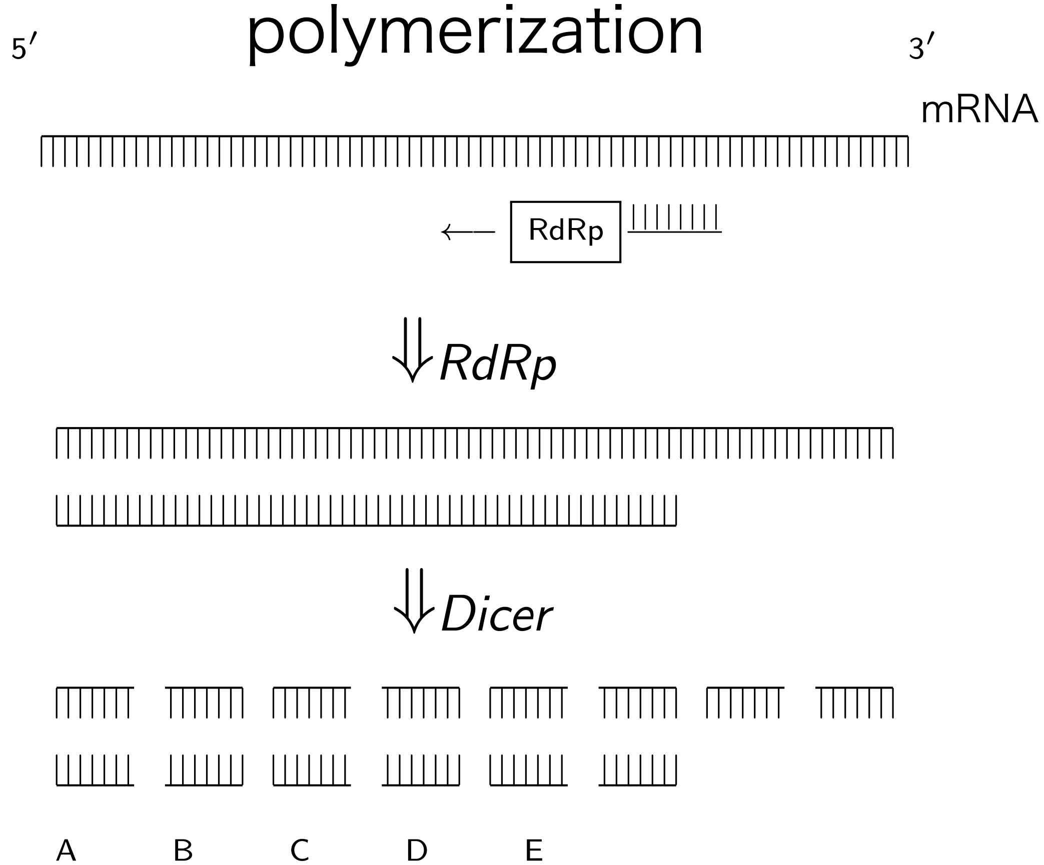
Analyze circularity of RNAi, explaining how RNAi is sustained!



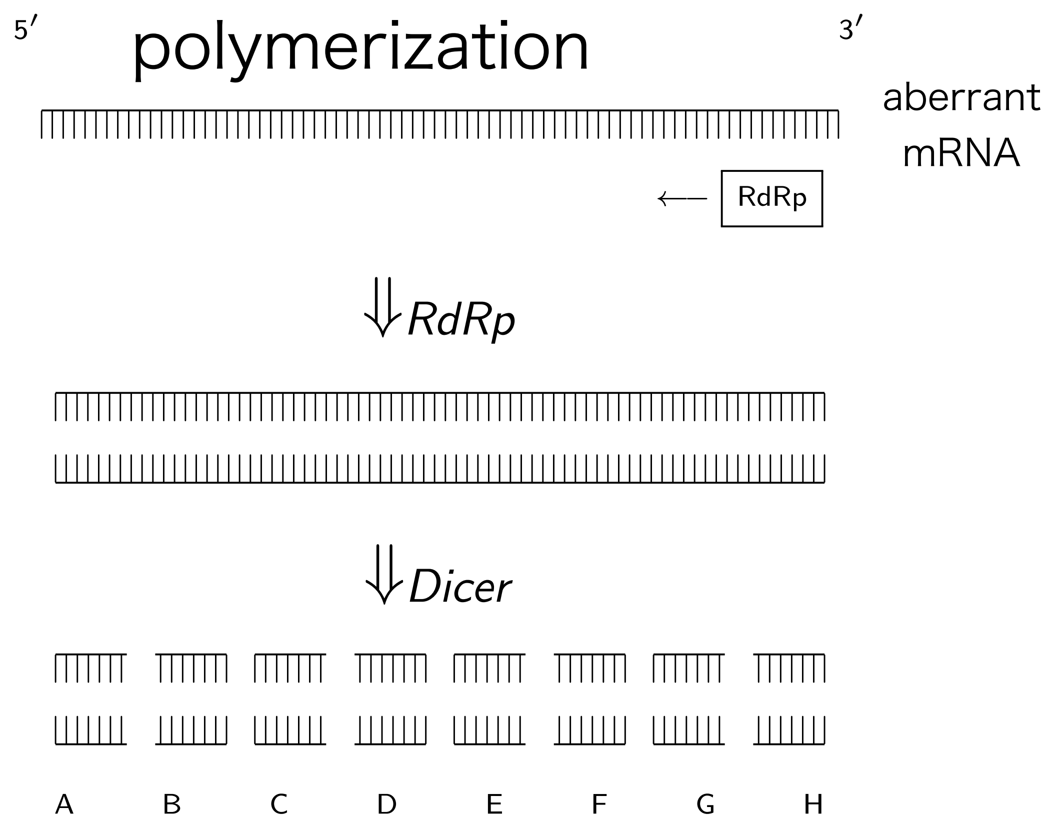
Synthesis of dsRNA by RdRp mediation

Two circulatory paths for the synthesis:

(i) primer dependent polymerization



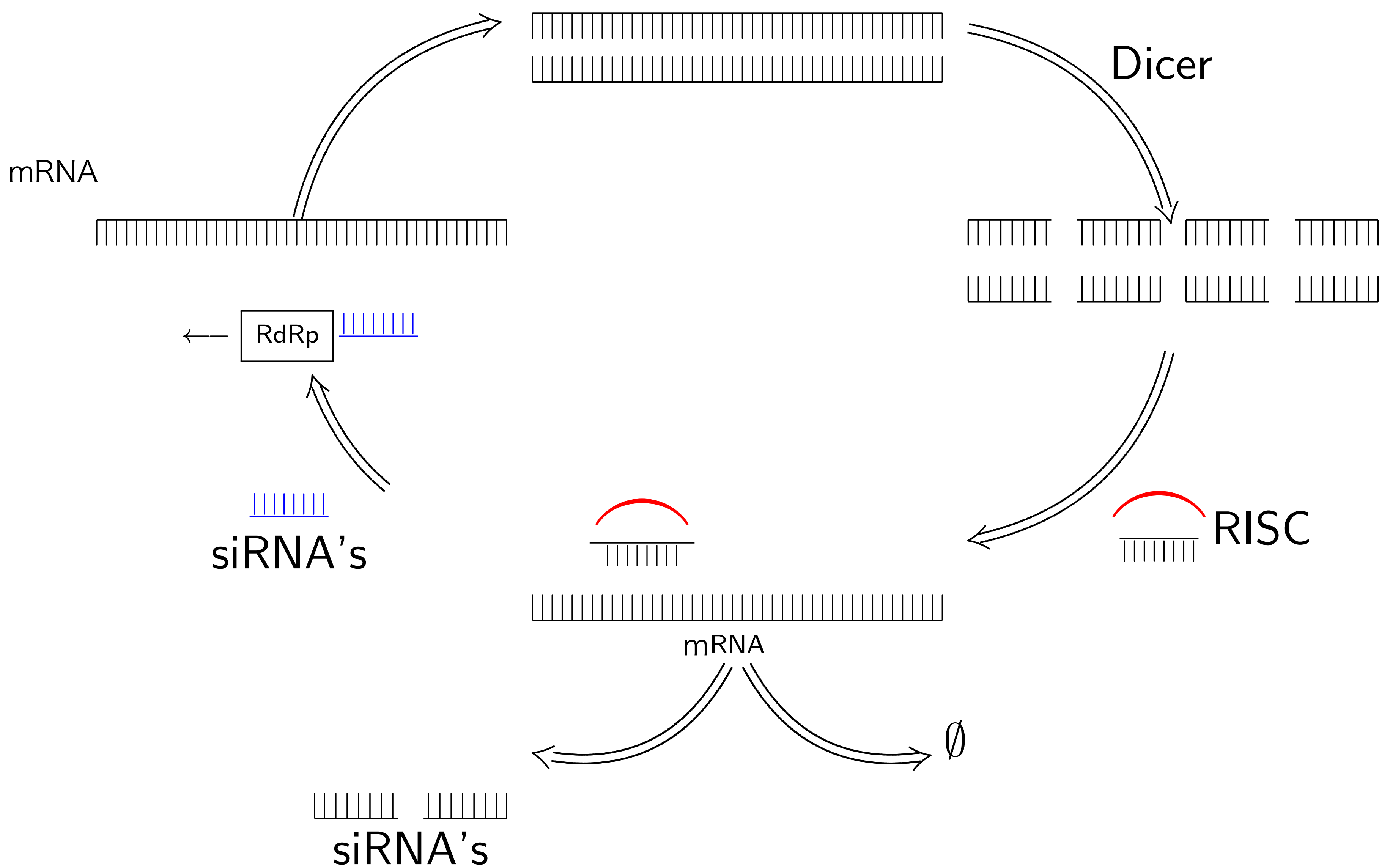
(ii) primer independent polymerization



difference of RNAi between plant and animal

- David Baulcombe, RNA silencing in plants, Nature. (2004)
- Julia Pak and Andrew Fire, Distinct Populations of Primary and Secondary Effectors During RNAi in C. elegans, Science. (2007)

Circularity of RNAi (with primer dependent polymerization)



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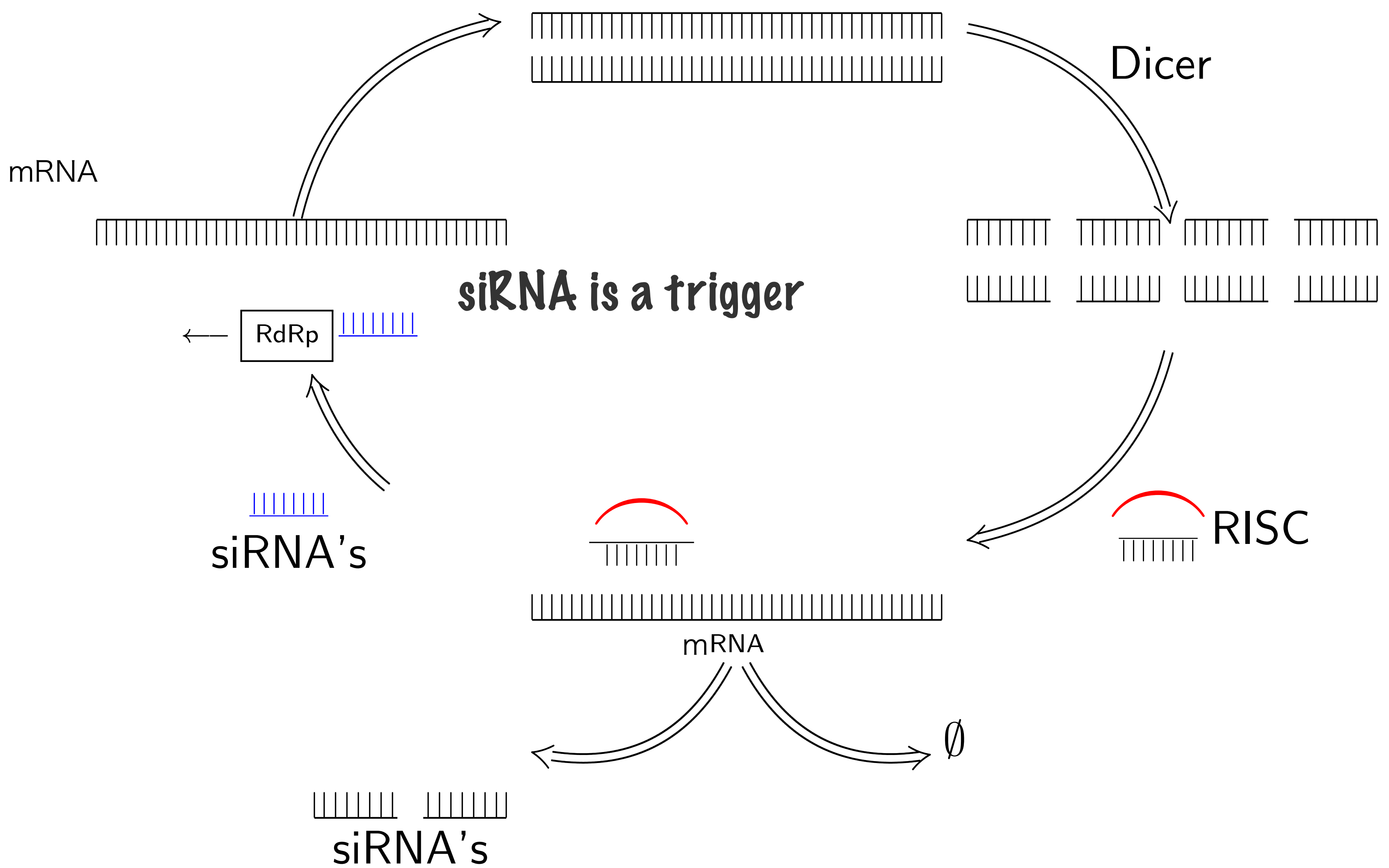
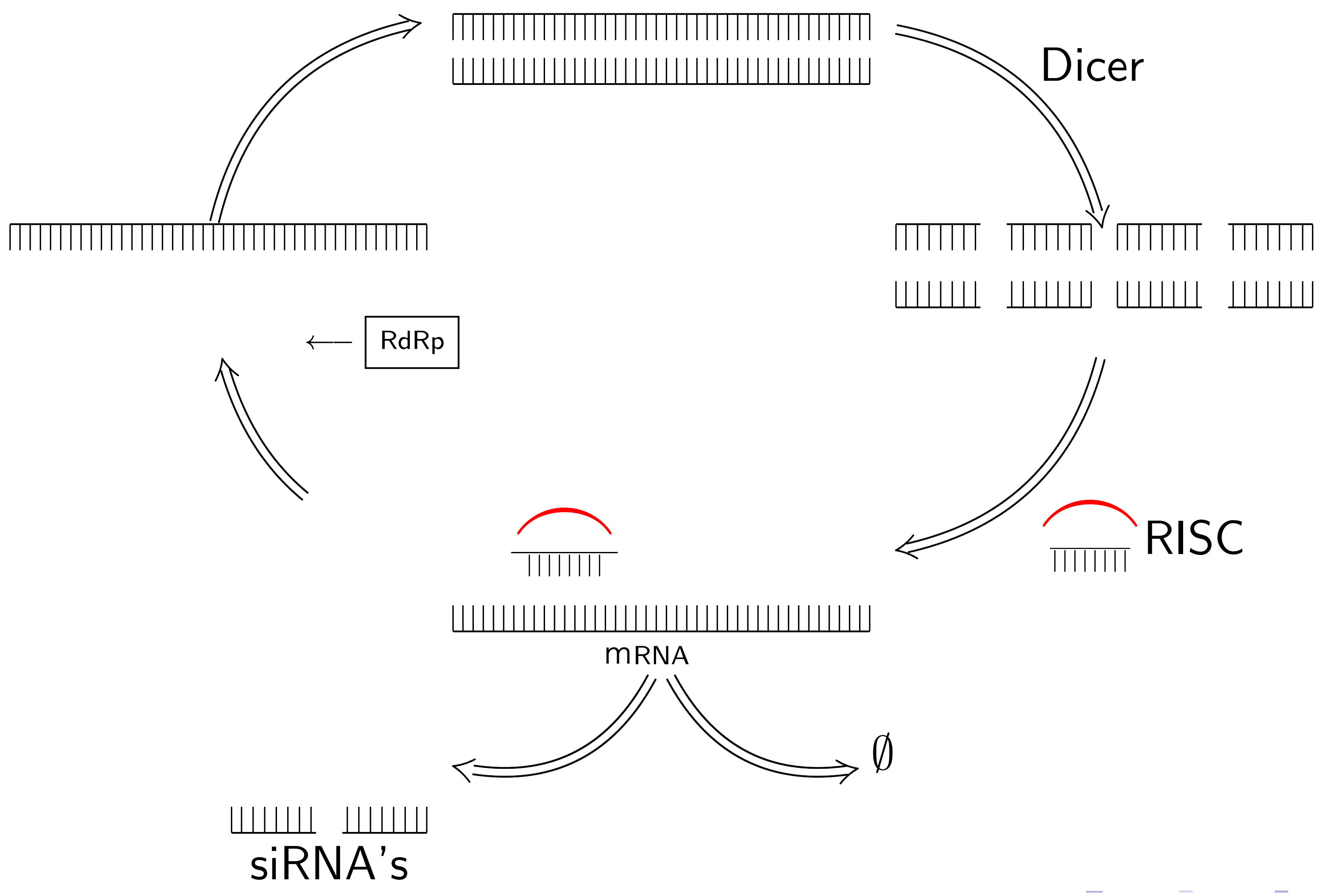
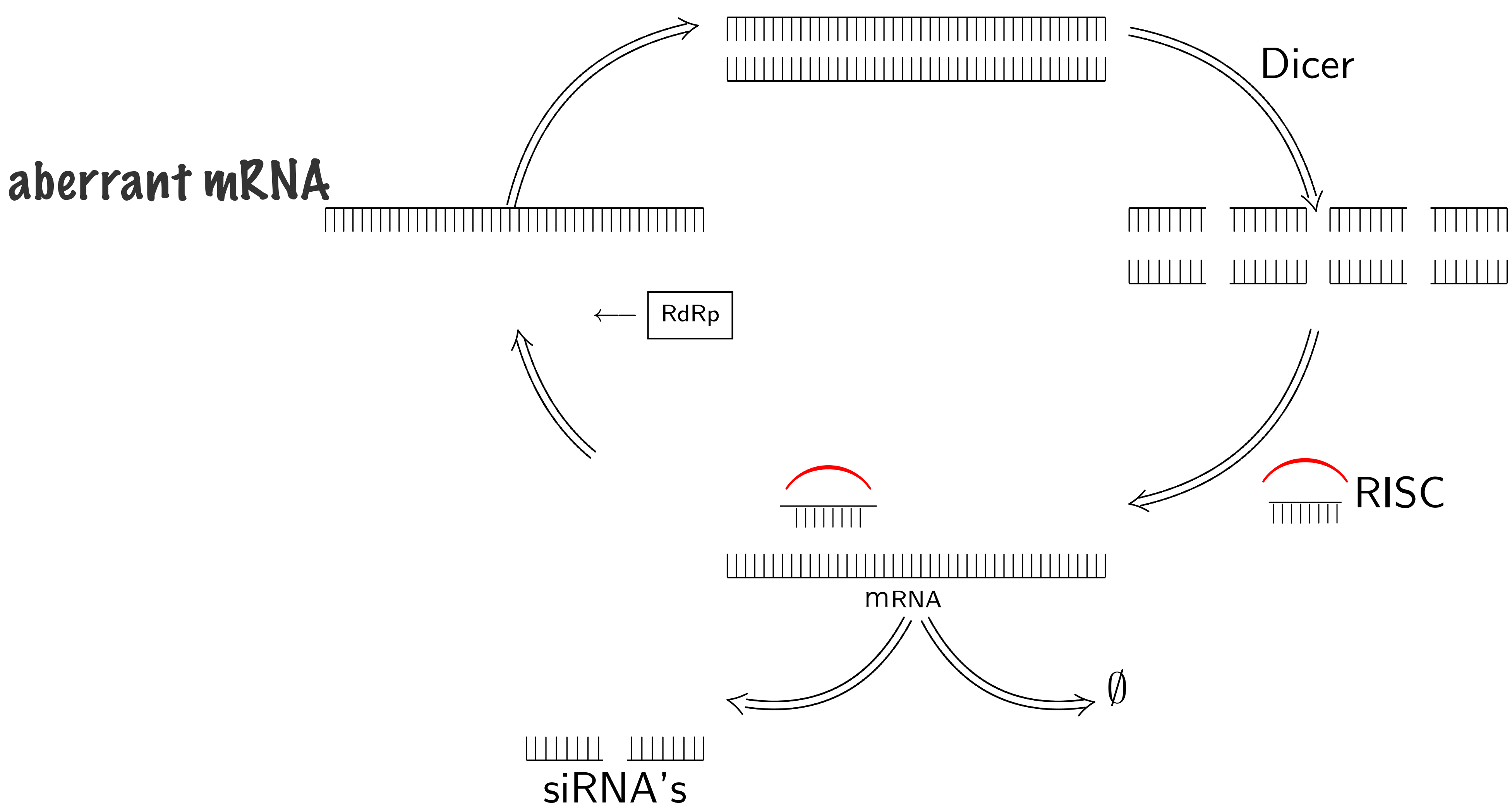


Circularity of RNAi (with primer **in**dependent polymerization)



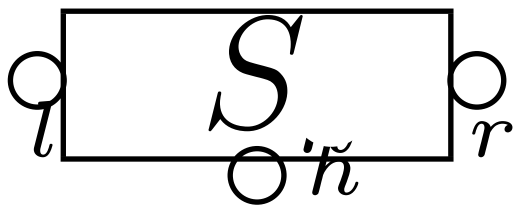
Circularity of RNAi (with primer **in**dependent polymerization)



siRNA as Primitive Agent

siRNA= $S\ (l, h, r)$

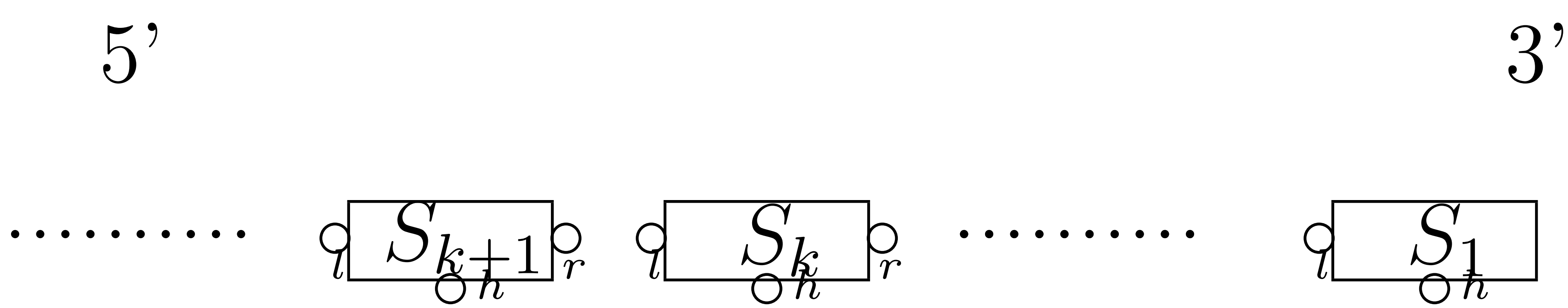
- l and r for phosphate bonds.
- h for a segment of hydrogen bond



Moreover, siRNA Has a Type.

$$\text{siRNA} = S_k(l, h, r)$$

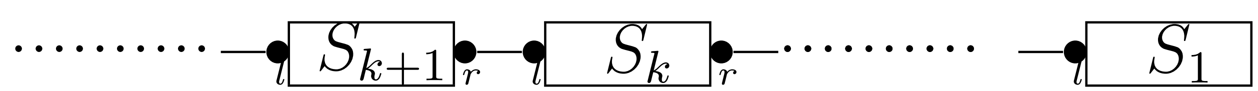
- The type k ∈ {1,2, ..., M } designates its position inside dsRNA, from which siRNA is cleaved.



RNA and dsRNA as Complexes of siRNAs

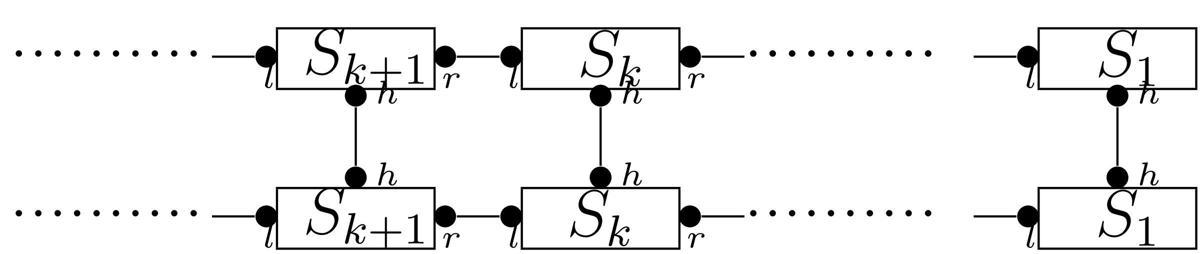
RNA=

$$\ldots, S_{n+1}(l^{n+2}, r^{n+1}), S_n(l^{n+1}, r^n), \ldots, S_2(l^3, r^2), S_1(l^2, r)$$



dsRNA=

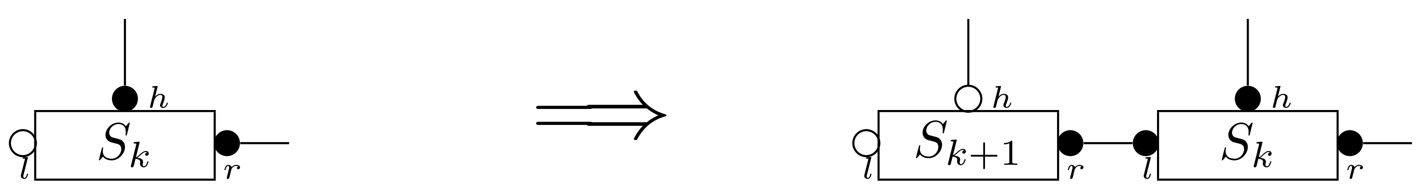
$$\ldots, S_{n+1}(l^{n+2}, h^{1_{n+1}}, r^{n+1}), S_n(l^{n+1}, h^{1_n}, r^n), \ldots, S_2(l^3, h^{1_2}, r^2), S_1(l^2, h^{1_1}, r)$$
$$\ldots, S_{n+1}(l^{\overline{n+2}}, h^{1_{n+1}}, r^{\overline{n+1}}), S_n(l^{\overline{n+1}}, h^{1_n}, r^{\overline{n}}), \ldots, S_2(l^{\overline{3}}, h^{1_2}, r^{\overline{2}}), S_1(l^{\overline{2}}, h^{1_1}, r)$$



Reactions of RNAi as Rules

(i) Polymerization

$$S_k(l, h^{1_k}, r^k) \longrightarrow S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k)$$

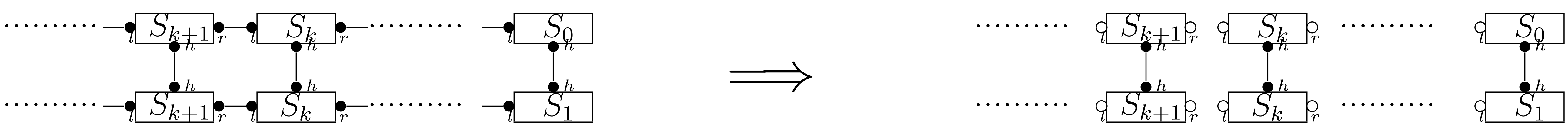


compact description !

Reactions of RNAi as Rules

(ii) cleavage

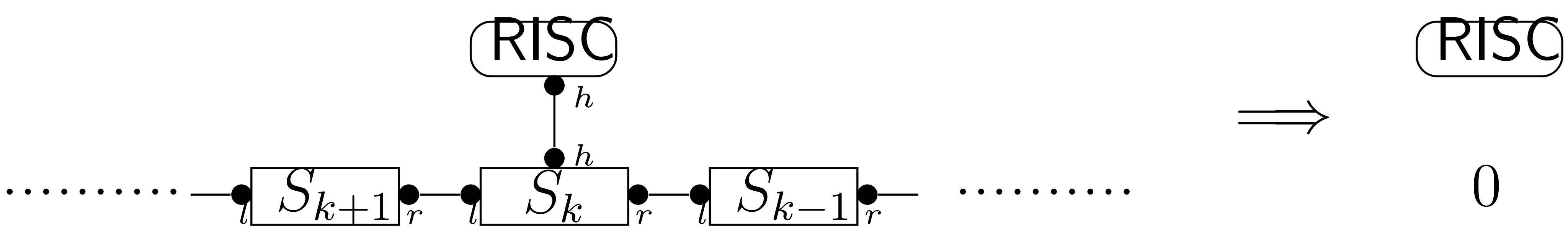
$$\prod_{i\in T}(S_i(l^{i+1},h^{1_i},r^i) \mid S_i(l^{i+1},h^{1_i},r^i)) \longrightarrow \prod_{i\in T}(S_i(l,h^{1_i},r) \mid S_i(l,h^{1_i},r))$$



Reactions of RNAi as Rules

(iii) degradation

$$\mathsf{RISC}(h^{1_k}),\quad S_k(l^{k+1},h^{1_k},r^k)\mid \prod_{i\in T\setminus\{k\}} S_i(l^{n+1},r^n)\longrightarrow \mathsf{RISC}(h),\ 0$$



Purpose of this work

Show:

- the difference between the two synthesis paths of dsRNA in terms of their effectiveness for sustainability (by κ's semantics of Markov branching processes).
- validity of the compact description of polymerization-rule (by κ's rule refinement).

Multytype Branching Process
(Galton-Watson)

Multitype Branching Process

- random variables for the n-th generation of each type

$$\mathbf{Z}(n) = (Z_1(n), \ldots, Z_m(n))$$

- The mean matrix $M = (m_{ij})$ describes the evolution of the process.

$$m_{ij} = E[Z_j(1) \mid \mathbf{Z}(0) = \mathbf{e}_j]$$

$$u(n) = E[\mathbf{Z}(n)] = (E[Z_1(n)], \ldots, E[Z_m(n)])$$

$$u(n) = u(0)M^n$$

Irreducible Branching Process

Each type i of individual eventually may have progeny of any other type j

∀ (i, j) ∃n ≥ 1

$$P[Z_j(n) > 1 \mid \mathbf{Z}(0) = \mathbf{e}_i] > 0.$$

Any initial configuration cam lead to any composition !

Irreducibility is a criterion discriminating the two kind of synthesis of dsRNA

- RNAi with primer dep. synthesis is reducible.
- RNAi with primer indep. synthesis is irreducible.

Our Slogan

To capture sustainability of RNAi
in terms of
(non-)extinction of siRNA population

Extinction of siRNA

The probability q_i of eventual extinctions of SIRNA of type i (initiated with a single particle)

$$q_i = \lim_{n \to \infty} q_i(n)$$

with $q_i(n) = P[\mathbf{Z}(n) = \mathbf{0} \mid \mathbf{Z}(0) = \mathbf{e}_i]$

The growth/extinctions of irred. B.P is characterized by Perron-Frobenius root ρ

A mean matrix M of irreducible B.P. has a maximal eigenvalue ρ so that

$$M^n = \rho^n M_1 + o(\rho^n)$$

(M_1 is) determined by right/left eigenvectors of M

The growth/extinction of irred. B.P is characterized by Perron-Frobenius root ρ

Thm (irreducible branching process)

For qi extinction probability of type i ,

- If $\rho \leq 1$, then qi=1 for all types i=1, ..., M.
- If $\rho > 1$, then qi < 1 for all types i=1, ..., M.

Although intrinsically heterogeneous, uniform extinction for red. B.P

Thm (reducible branching process)

If $\rho \leq 1$, then the extinction probability $q_i = 1$ for all types $i = 1, \ldots, M$.

The mean matrix for primer dep. polymerization

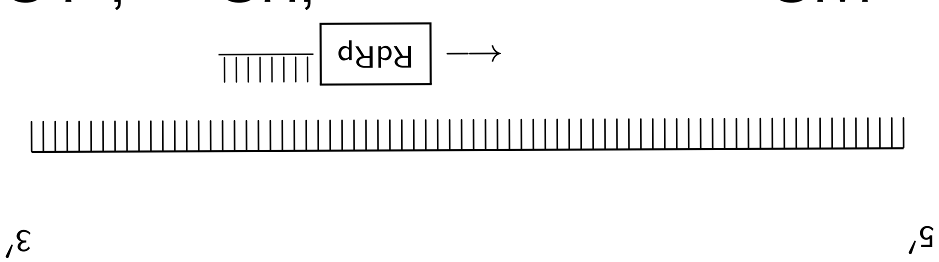
The mean matrix M_{dep} is triangular

$$M_{dep} = \begin{pmatrix} s_1 & & & * \\ & s_2 & & \\ & & \ddots & \\ 0 & & & s_m \end{pmatrix}$$

The n-th row u_n describes the birth-probabilities of children Si of types i (i=1,･･･ , M):

$$u_n = (0, \ldots 0, s_n, m_{n,n+1}, \ldots, m_{nm})$$

S1 ,･･･Sn, ･･･ SM



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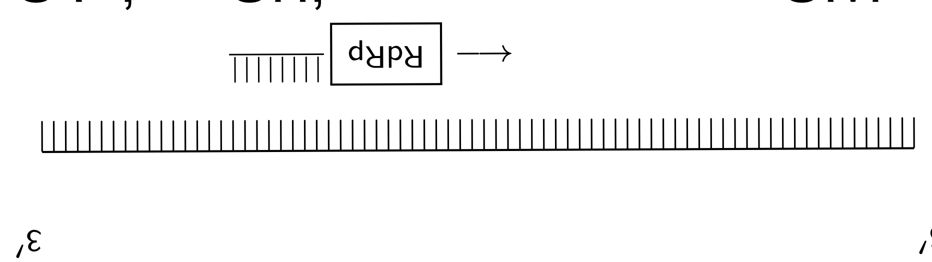

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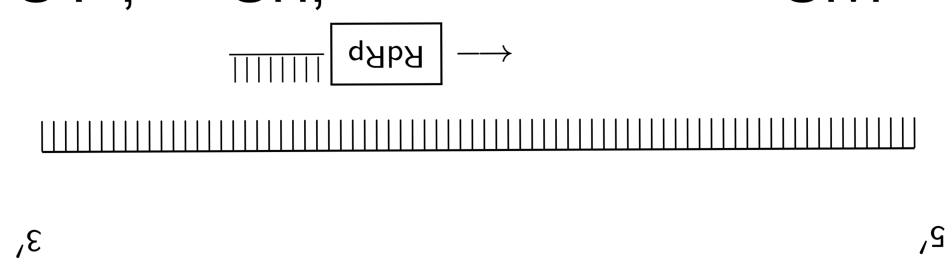

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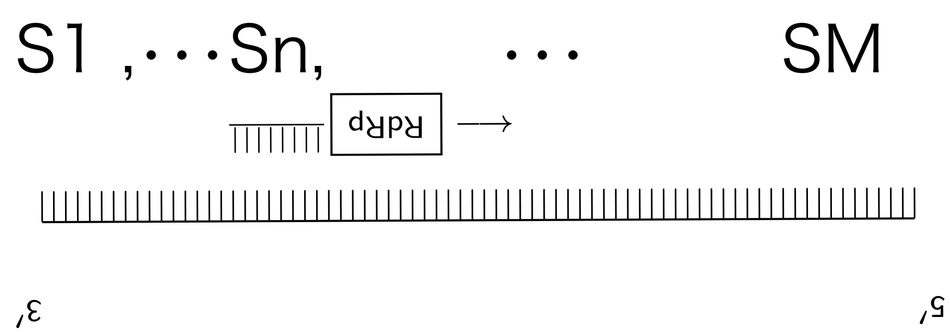
no children of these types produced !



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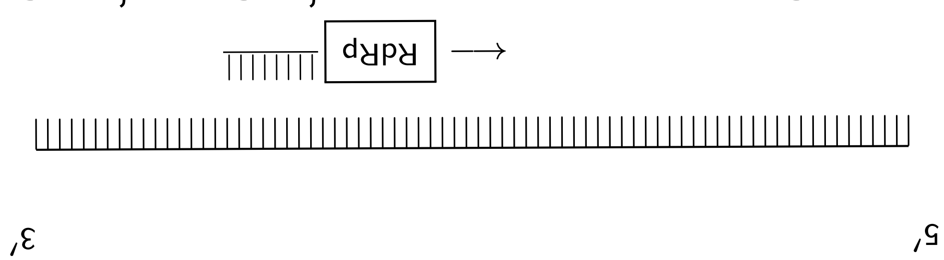

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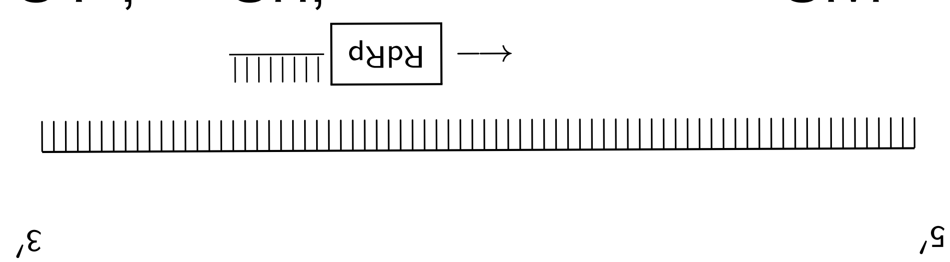

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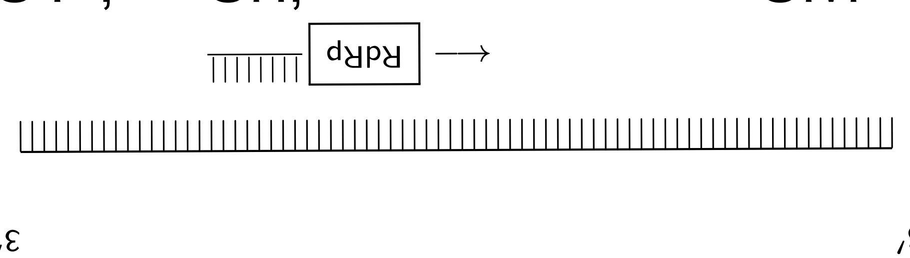

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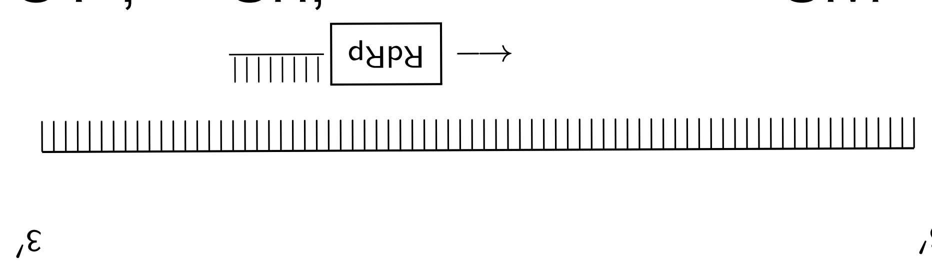

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compact polymerization

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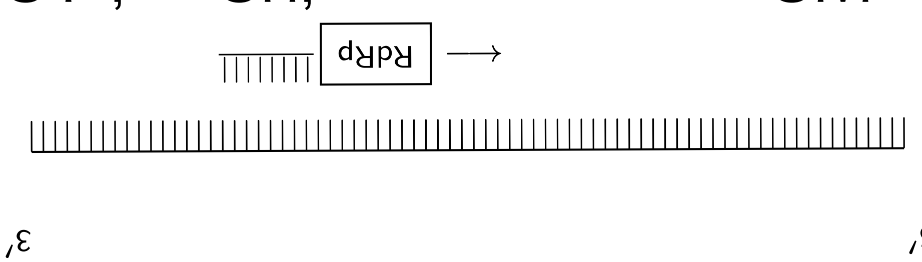

no children of these types produced !

compact polymerization

- s_n determined by sites of Sn
- $m_{n,i}$ determined by sites of S_{i-1} and S_i

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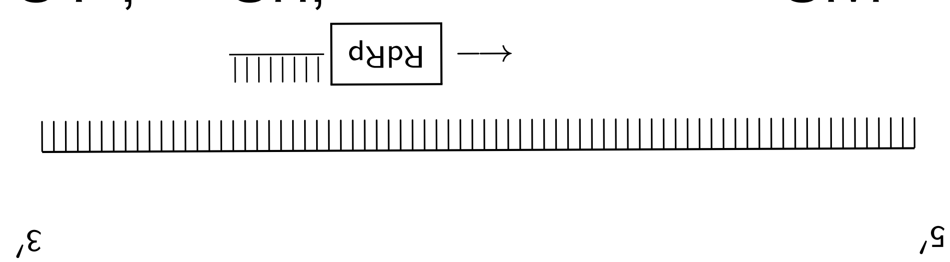


no children of these types produced !

compact polymerization

- s_n determined by sites of Sn
- $m_{n,i}$ determined by sites of S_{i-1} and S_i

E.g., • s_n probability of non-decay of Sn of type n

- $m_{n,i} = s_n\, q\, (1-r)^i\, (1-h)^{i-1}$

q the probability of Sn's binding to mRNA

h the probability of denaturation of hydrogen bonds

r the probability of breaking ligation bond

Prop.

The populations of siRNAs Si's of all types i eventually extinct with primer dep. synthesis only.

Proof.

Since the eigenvalues of the triangular M_{dep} (whose maximal is Perron-Frobenius) are given by the diagonal elements ≤ 1.

The mean matrix for primer indep. polymerization

$$M_{indep} = \sum_{j=1}^{m} \mathbf{u} \otimes {}^t\mathbf{e}_j$$

$$\mathbf{u} = (q,\ qc,\ qc^2, \ldots\ , qc^{m-1})$$

q the probability of RdRp mediation

c a certain constant, e.g., c = (1-h)(1-r)

Prop.

RNAi may sustain with primer indep. synthesis.

The probability q_i of extinction < 1

for every type i.

Proof.

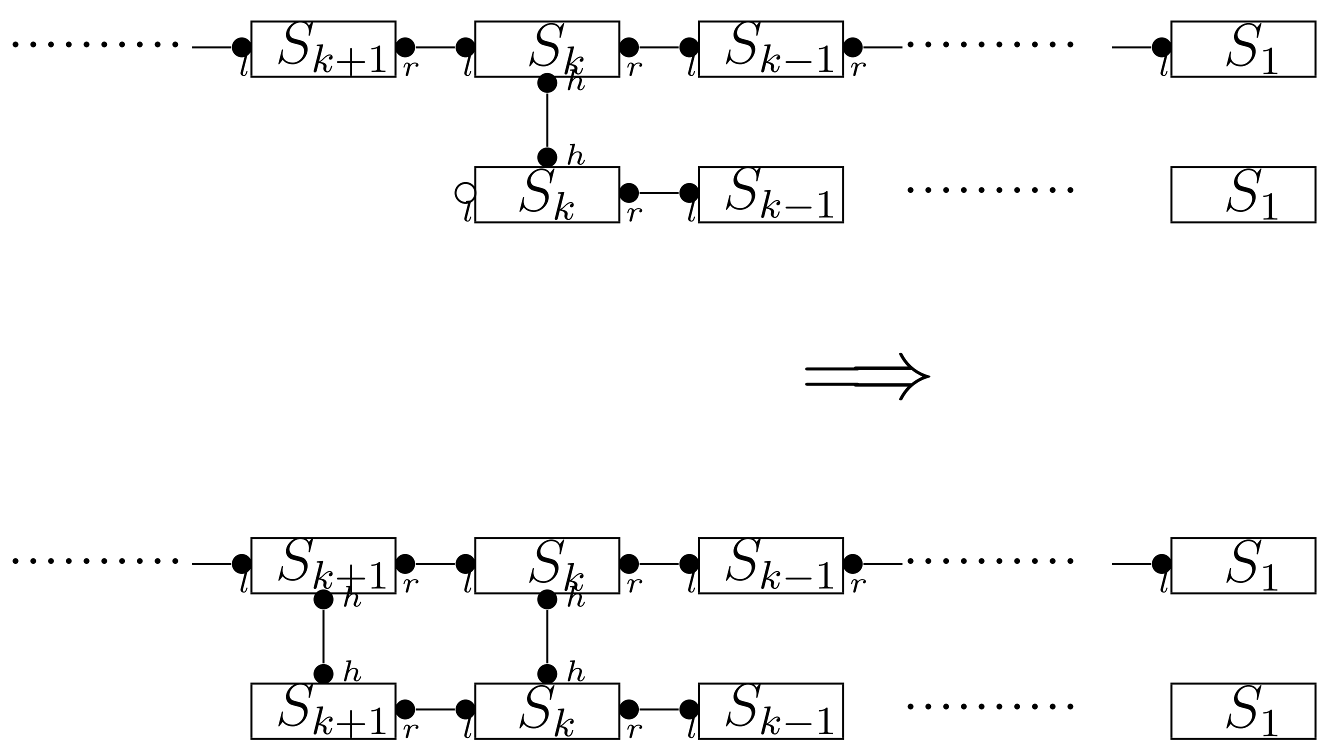
Perron-Frobenius root of M_{indep} is given by

$$\rho = \sum_{i=1}^{m} u_i = q\frac{1-c^m}{1-c}$$

, which is made > 1 with appropriate q and c.

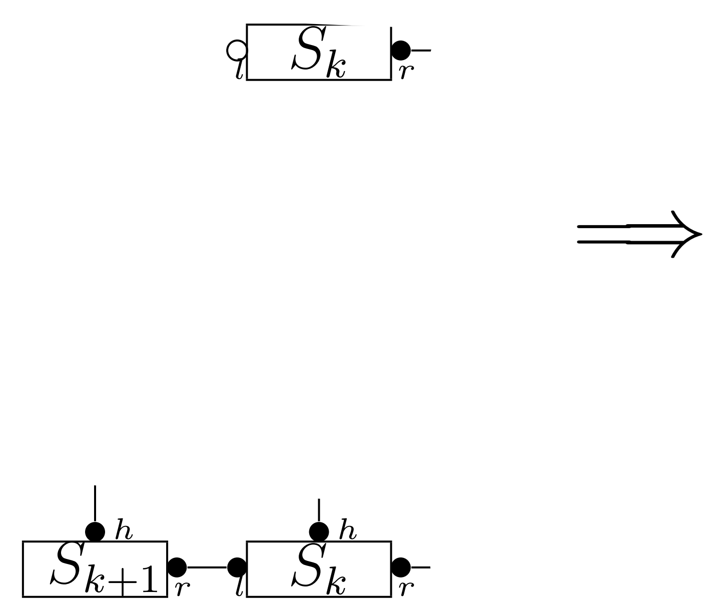
Model Refinement for Polymerization

$$\begin{array}{c} S_k(l, h^{1_k}, r^k), \\ \prod_{j<k} S_j,\ \text{mRNA}(h^{1_k}, h) \end{array} \longrightarrow \begin{array}{c} S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k), \\ \prod_{j<k} S_j,\ \text{mRNA}(h^{1_k}, h^{1_{k+1}}) \end{array}$$



The original rule of polymerization

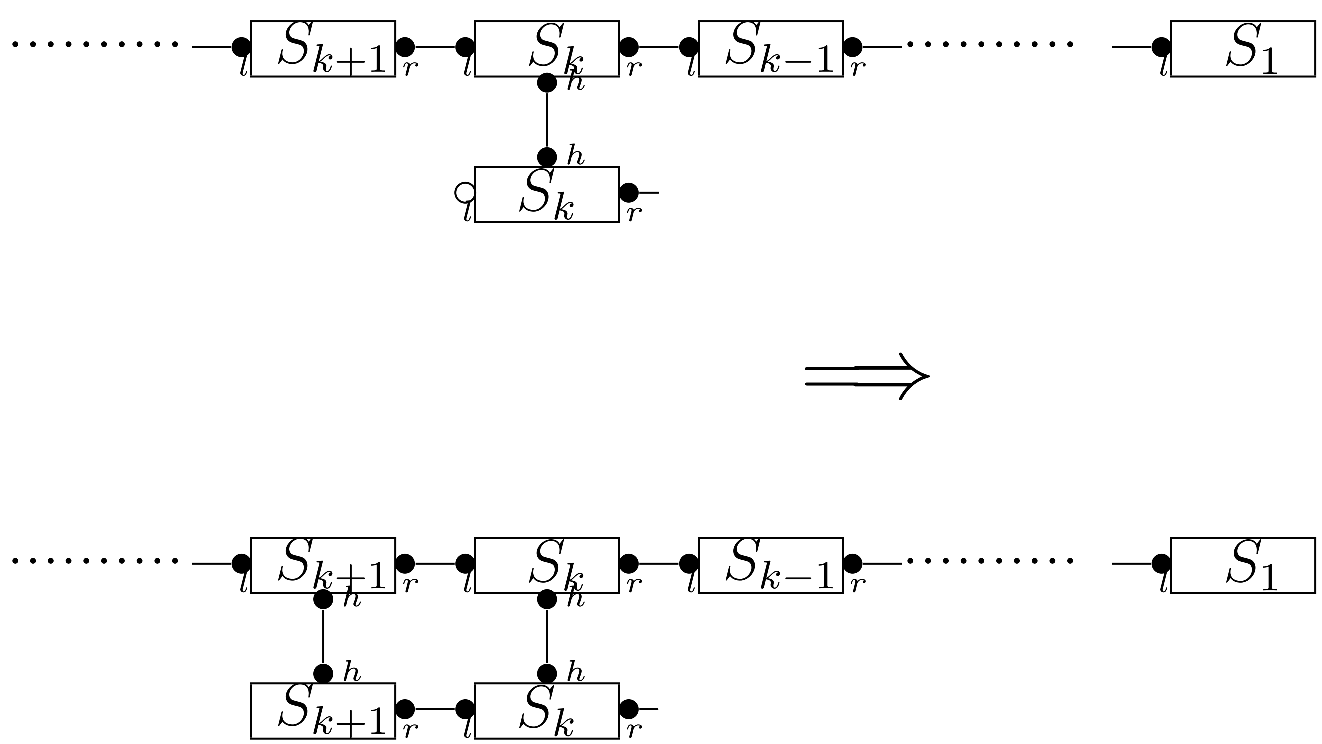
$$S_k(l, h^{1_k}, r^k), \longrightarrow S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k),$$



The original compact rule is globalized !

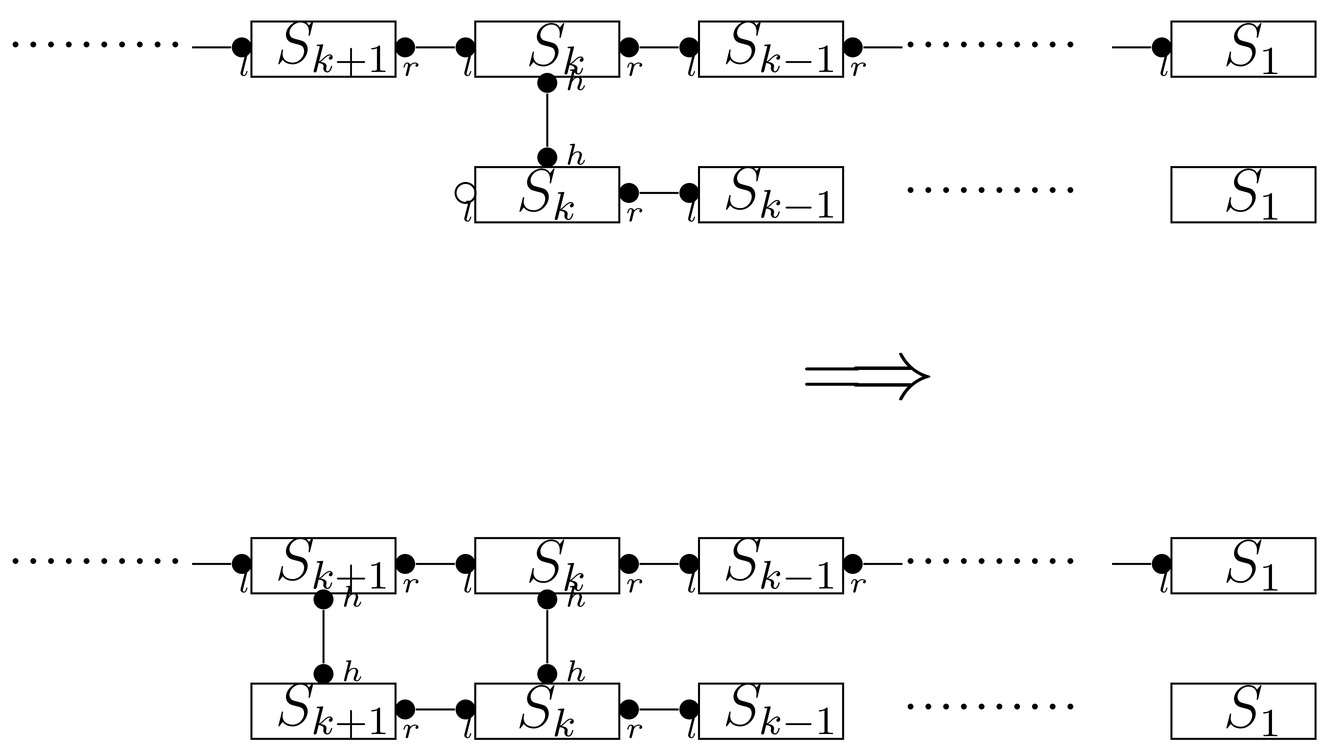
Adding Context 1 : mRNA

$$S_k(l, h^{1_k}, r^k), \quad \text{mRNA}(h^{1_k}, h) \longrightarrow S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k), \quad \text{mRNA}(h^{1_k}, h^{1_{k+1}})$$



Adding Context 2: Sj's

$$\begin{array}{c} S_k(l, h^{1_k}, r^k), \\ \prod_{j<k} S_j,\ \text{mRNA}(h^{1_k}, h) \end{array} \longrightarrow \begin{array}{c} S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k), \\ \prod_{j<k} S_j,\ \text{mRNA}(h^{1_k}, h^{1_{k+1}}) \end{array}$$



The mean matrix for the refined rule is again triangular, but whose n-th row

$$u_n = (0, \dots 0, s_n, m_{n,n+1}, \dots, m_{nm})$$

is given by

$$s_n = \mathsf{site}_n(S_n, \mathsf{mRNA})$$

$$m_{n,i} = \mathsf{site}_{n,i}(S_n, S_{n+1}, \dots, S_{i-1}, S_i, \mathsf{mRNA})$$

invariance

Thm.

The extinction property is invariant under the rule refinement of polymerization.

Proof.

Throughout the refinement, Perron-Frobenius root does not increase.

Conclusion

- [Sustainability of RNAi]
  (Primer dep. synthesis)
  siRNAs eventually become extinct (with the probability 1) hence RNAi cannot sustain.
  (Primer indep. synthesis)
  RNAi may sustain since the probability of siRNA-extinction is less than 1.

- [Invariance under refinement]
  ・Rule refinement for polymerization preserves extinction of siRNAs.
  ・Compact description of κ is valid for capturing the sustainability of RNAI.

Future Works

- Heterogeneity, peculiar to reducible branching process ?
  E.g., distribution of spreading of concentrations of each typed siRNA in primer dep. polymerization.
- Model abstraction as a dual notion of model refinement ?