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)
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.

dsRNA

Dicer

Argonaute

RISC

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

Analyze **circularity** of RNAi, explaining how RNAi is sustained!

dsRNA

Dicer

RISC

mRNA is transcribed constantly

siRNA’s

mRNA

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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

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

siRNA is a trigger
Circularity of RNAi (with primer independent polymerization)
Circularity of RNAi (with primer independent polymerization)

aberrant mRNA → Dicer ↓ ⌢ RdRp → RISC ⌢ mRNA → ∅ siRNA’s
siRNA as Primitive Agent

\[
\text{siRNA}= S \ (l, h, r)
\]

- \(l\) 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 \( k \in \{1, 2, \ldots, M\} \) designates its position inside dsRNA, from which siRNA is cleaved.

\[
\begin{array}{c}
5' \\
\ldots\ldots \quad \text{\( \otimes S_{k+1} \)} \quad \text{\( \otimes S_k \)} \quad \ldots\ldots \\
3'
\end{array}
\]
RNA and dsRNA as Complexes of siRNAs

**RNA=**

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

\[ \begin{array}{c}
\ldots & S_{k+1} \rightarrow S_k \rightarrow \ldots \rightarrow S_1
\end{array} \]

**dsRNA=**

\[ \ldots, S_{n+1}(l^{n+2}, h_1 l^{n+1}, r^{n+1}), S_n(l^{n+1}, h_1 l^n, r^n), \ldots, S_2(l^3, h_1 l^2, r^2), S_1(l^2, h_1 l, r) \]

\[ \begin{array}{c}
\ldots & S_{k+1} \rightarrow S_k \rightarrow \ldots \rightarrow S_1
\end{array} \]
Reactions of RNAi as Rules

(i) Polymerization

\[ S_k(l, h_1^k, r^k) \rightarrow S_{k+1}(l, h_1^{k+1}, r^{k+1}), S_k(l^{k+1}, h_1^k, r^k) \]

compact description!
Reactions of RNAi as Rules

(ii) cleavage

\[ \prod_{i \in T} (S_i(l^{i+1}, h^{1i}, r^i) \mid S_i(l^{i+1}, h^{1i}, r^i)) \rightarrow \prod_{i \in T} (S_i(l, h^{1i}, r) \mid S_i(l, h^{1i}, r)) \]
Reactions of RNAi as Rules

(iii) degradation

\[ \text{RISC}(h^{1_k}), \quad S_k(l^{k+1}, h^{1_k}, r^k) \mid \prod_{i \in T \setminus \{k\}} S_i(l^{m+1}, r^n) \rightarrow \text{RISC}(h), 0 \]
Purpose of this work

Show:

- the difference between the two synthesis paths of dsRNA in terms of their effectiveness for sustainability (by $\kappa$'s semantics of Markov branching processes).

- validity of the compact description of polymerization-rule (by $\kappa$'s rule refinement).
Multitype Branching Process (Galton-Watson)
Multitype Branching Process

- random variables for the n-th generation of each type
  \[ Z(n) = (Z_1(n), \ldots, Z_m(n)) \]

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

\[
m_{ij} = E[Z_j(1) \mid Z(0) = e_j]
\]

\[
u(n) = E[Z(n)] = (E[Z_1(n)], \ldots, E[Z_m(n)])
\]

\[
u(n) = u(0)M^n
\]
Irreducible Branching Process

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

$$\forall \ (i, j) \ \exists n \geq 1$$

$$P[Z_j(n) > 1 \mid Z(0) = e_i] > 0.$$ 

Any initial configuration can 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 $q_i(n) = P[Z(n) = 0 \mid Z(0) = e_i]$
The growth/extinctions of irreducible B.P is characterized by Perron-Frobenius root $\rho$

A mean matrix $M$ of irreducible B.P. has a maximal eigenvalue $\rho$ so that

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

determined by right/left eigenvectors of $M$
The *growth/extinction* of irred. B.P is characterized by Perron-Frobenius root \( \rho \)

Thm (irreducible branching process)

For qi extinction probability of type i ,

- If \( \rho \leq 1 \), then \( q_i = 1 \) for all types \( i = 1, \ldots, M \).
- If \( \rho > 1 \), then \( q_i < 1 \) for all types \( i = 1, \ldots, M \).
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

\[ M_{dep} = \begin{pmatrix} s_1 & * \\ & s_2 \\ & \vdots \\ 0 & s_m \end{pmatrix} \]

The n-th row $u_n$ describes the birth-probabilities of children $S_i$ of types $i$ ($i=1, \cdots, M$):

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

Then each element of $S_{j<n}$ is determined by the immediate predecessor $S_n$.

Example 3.4

Each different types may grow at different rates. Nevertheless uniform extinction of other groups can be distinguished. Reducible systems in general can display great heterogeneity. One type may become extinct whereas another thrives.

If $\lambda_i > \lambda_j$, then $q_i < q_j$, and the probability that a type is not extinct at time $t$ is (from left, respectively) given by the maximal eigenvalue $\lambda_i - \lambda_j$. Then with probability $q_i$, which is triangular with lower left elements being 0, thus we have $s_{ij}$. The front 0s indicate that to trigger polymerization triggered by the agent $S_i$, for all the sites of agents inside the arguments.

Let $\lambda = \max \{ \lambda_i \}$, which is the rate of the birth of the individual polymerization triggered by the agent $S_i$. RNA interference: Register Machine and Termination

Amplification is NOT Bi-Directional
The mean matrix for primer dep. polymerization

The mean matrix $M_{dep}$ is triangular

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

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

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$S_1, \ldots, S_n, \quad \ldots \quad SM$

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    s_1 & * \\
    0 & s_2 \\
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no children of these types produced!
The mean matrix for primer dep. polymerization

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  s_1 & * \\
  s_2 & \\
  \vdots & \\
  0 & s_m
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The mean matrix for primer dep. polymerization

The mean matrix $M_{dep}$ is triangular

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

The n-th row $u_n$ describes the birth-probabilities of children $S_i$ of types $i$ ($i=1, \cdots, M$):

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

no children of these types produced!

compact polymerization
The mean matrix for primer dep. polymerization

The mean matrix $M_{\text{dep}}$ is triangular

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

The $n$-th row $u_n$ describes the birth-probabilities of children $S_i$ of types $i$ ($i=1,\cdots,M$):

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

no children of these types produced!

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

![Diagram](image-url)
The mean matrix for primer dep. polymerization

The mean matrix $M_{dep}$ is triangular

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

The n-th row $u_n$ describes the birth-probabilities of children $S_i$ of types $i$ ($i=1, \cdots, M$):

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

no children of these types produced!

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

E.g.,
- $s_n$ probability of non-decay of $S_n$ of type $n$
- $m_{n,i} = s_n q (1-r)^i (1-h)^{i-1}$

$q$ the probability of $S_n$'s binding to mRNA
$h$ the probability of denaturation of hydrogen bonds
$r$ the probability of breaking ligation bond

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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 $\leq 1$. 
The mean matrix for primer indep. polymerization

\[
M_{\text{indep}} = \sum_{j=1}^{m} u \otimes t e_j
\]

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

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 $M_{\text{indep}}$ 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

\[
S_k(l, h^{1_k}, r^k), \quad \Pi_{j<k} S_j, \text{ mRNA}(h^{1_k}, h) \quad \rightarrow \quad S_{k+1}(l, h^{1_{k+1}}, r^{k+1}), S_k(l^{k+1}, h^{1_k}, r^k), \quad \Pi_{j<k} S_j, \text{ mRNA}(h^{1_k}, h^{1_{k+1}})
\]
The original rule of polymerization

\[ S_k(l, h^1, r^k), \quad S_{k+1}(l, h^{1,k+1}, r^{k+1}), S_k(l^{k+1}, h^1, r^k), \]

\[ \begin{array}{c}
\begin{array}{c}
S_k \quad \text{r} \quad h
\end{array}
\end{array} \]

\[ \begin{array}{c}
\begin{array}{c}
S_{k+1} \quad \text{r} \quad h
\end{array}
\end{array} \]

\[ \begin{array}{c}
\begin{array}{c}
S_k \quad \text{r} \quad h
\end{array}
\end{array} \]
The original compact rule is globalized!
Adding Context 1: mRNA

\[ S_k(l, h^{1k}, r^k), \text{ mRNA}(h^{1k}, h) \rightarrow S_{k+1}(l, h^{1k+1}, r^{k+1}), S_k(l^{k+1}, h^{1k}, r^k), \text{ mRNA}(h^{1k}, h^{1k+1}) \]
Adding Context 2: Sj's

\[ S_k(l, h^1_k, r^k), \prod_{j<k} S_j, \text{mRNA}(h^1_k, h) \rightarrow S_{k+1}(l, h^{1_k+1}, r^{k+1}), S_k(l^{k+1}, h^1_k, r^k), \prod_{j<k} S_j, \text{mRNA}(h^1_k, h^{1_k+1}) \]
The mean matrix for the refined rule is again triangular, but whose n-th row

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

is given by

\[ s_n = \text{site}_n(S_n, \text{mRNA}) \]

\[ m_{n,i} = \text{site}_{n,i}(S_n, S_{n+1}, \ldots, S_{i-1}, S_i, \text{mRNA}) \]
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 $\kappa$ 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?