Simulation of RNA Silencing Pathway for Time-dependent Transgene Transcription Rates

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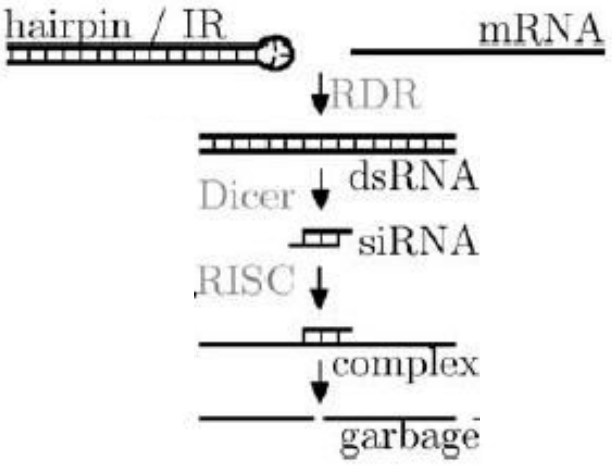
Introduction

- Invading nucleic acids, such as transgenes and viruses, are silenced through the action of small homologous RNA molecules. This process is an outcome of the cellular defense mechanism against transposons and viruses.

(T. A. Volpe *et al.*, *Science*, 297, 1833-1837, Sept 2002;

Pal-Bhadra et al., *Mol. Cell*, 9, 315-327, Feb 2002)

- Usually known as RNA silencing, RNA Interferencing (RNAi) or Post-Transcriptional Gene Silencing (PTGS). It protects the eukaryotic cells against viruses and transposons.



Hutbágner et al., Curr. Opin. Genet. Dev. 12, 225-232 (2002)

Possible Input-Output Scenario of a Silencing Pathway

- Viruses produce double-stranded RNA (dsRNA) during reproduction, which can trigger silencing of virial RNA

- RNAi can be triggered by a sufficiently high expression of transgenes, a mechanism known as co-suppression or transgene induced silencing

- The activation of transgene induced RNAi is directly linked to the function of RNA directed RNA polymerase (RDR). Overexpression of RDR significantly reduces the number of transgene needed to trigger RNAi

- RNAi deficient mutants show enhanced expression of transposons

  $\Rightarrow$ Transposons could trigger RNAi when

  (1) the transposons have multiple inverted repeats (IRs) that form dsRNA transcripts

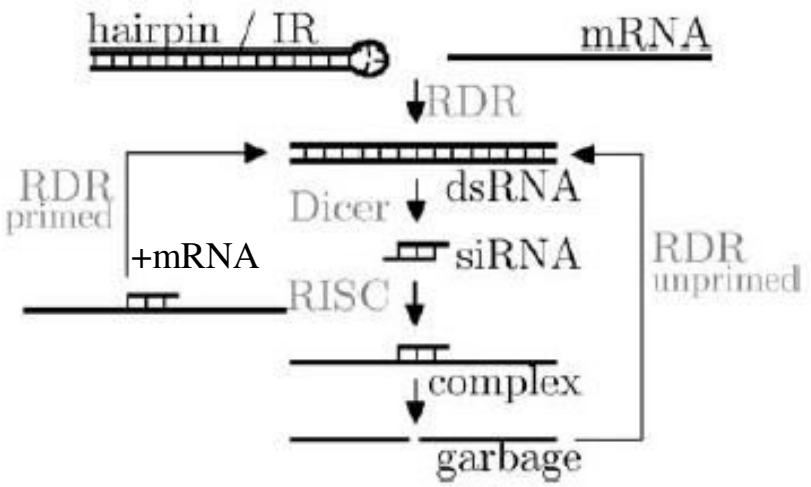
  (2) the copy number of transposon is sufficiently high

Understanding the Silencing Pathway

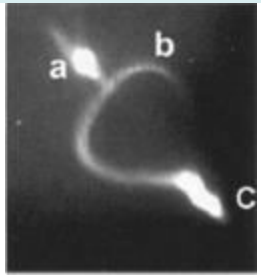
- **Structure and function dominated processes.**

  This involves various subsystem dynamics

  - Hairpin/IR interaction with mRNA
  - Thermodynamic energy landscape in favour of dicing (by Rnase III class enzyme) to form small interfering RNA (siRNA, 21-25 nucleotides long) from dsRNA.
  - Antisense base pairing which leads to the cleavage of the target mRNA near the center of the siRNA
  - Expression of RDR (primed and unprimed loops) which can form additional dsRNA in some species (e.g., Caenorhabditis elegans)



Understanding the Silencing Pathway

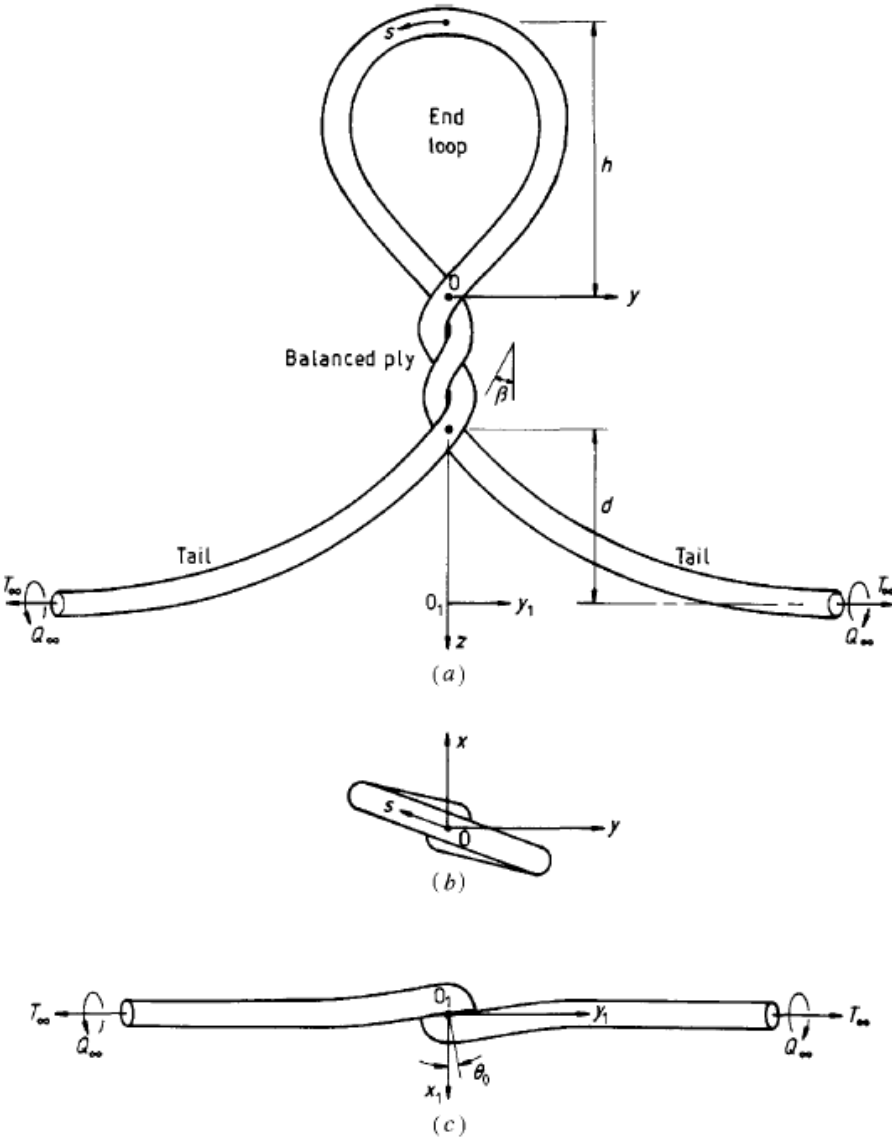


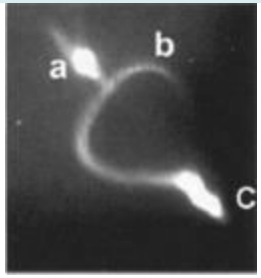
- Mechanics dominated processes
  - Hairpin/IR and dicer transport, force field and end-loop formation
  - Gene-gene interaction (genetic network – Novak-Tyson model)
  - Transcription coupled DNA supercoiling (e.g., Escherichia coli). There exists extensive experimental support for a twin-domain model in which positive DNA supercoils are generated ahead of a translocating RNA polymerase complex and negative supercoils are formed behind it.

- Leng and McMacken (PNAS, 99, 9139, 2002) have suggested that among the RISCs, those nucleoprotien complexes having sharply bent DNA, can form barriers that impede the diffusion and merger of independent chromosomal supercoil domains.
  In vitro experiments using plasmid DNA templates require precise amount of RDR and long RNA chains for transcription coupled DNA supercoiling.

- Suitable computational models are required to aid *in vitro* experiments.

Understanding the Silencing Pathway





RNA chain

$$\left.\begin{aligned} T = p = \frac{\sin^4\beta}{\cos 2\beta}, \quad Q = -\frac{2\cos\beta\sin^3\beta}{\cos 2\beta}, \\ \boldsymbol{V} = -\frac{\cos\beta\sin^3\beta}{\cos 2\beta}\boldsymbol{b}, \quad \boldsymbol{M} = \sin^2\beta\boldsymbol{b}, \end{aligned}\right\}$$

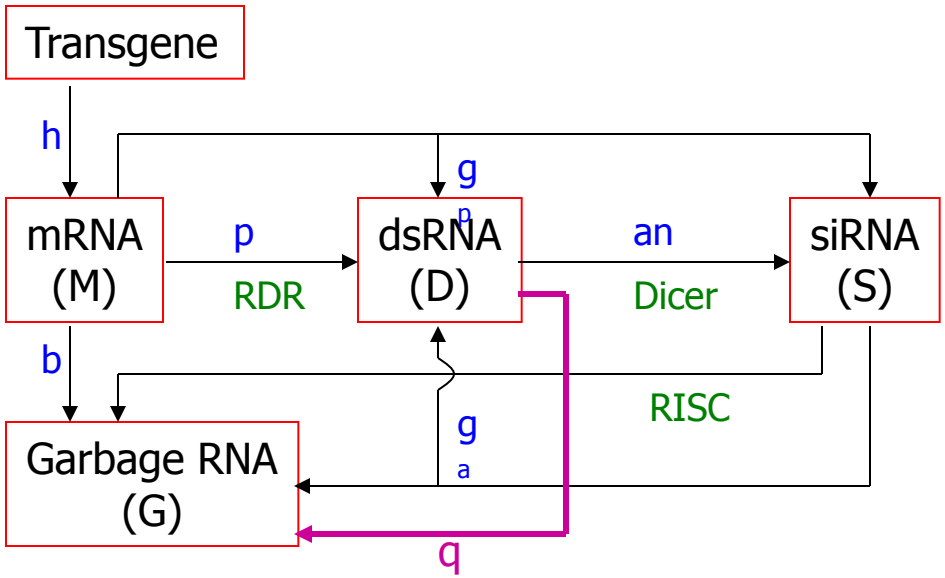
$$U' = \frac{1}{2\kappa}\sin^4\beta(\tan^2 2\beta + \kappa).$$

Mechanics of the loop and the tail regions can be solved numerically

Results of MD calculations are remarkably similar to the results Of continuum mechanics calculations

MD simulation review: Olson, Curr. Opin. Struct. Biol., 6, 242-256 (1996)

Systems Approach for Simulating RNAi



- Time-varying transcription rate (h)
  - realistic virial invasion, trans-membrane protien transport
  - controlled dosage

- Radiation or shock wave induced damage

Systems Approach for Simulating RNAi

- RNAi experimental data for system level modeling
  - P. Brodersen and O. Voinnet, "The diversity of RNA silencing pathways in plants", *TRENDS in Genetics*, 22, 268-280, May 2006.
  - Similar data and others, such as #mRNA versus gene copy number variation (required to validate a pathway model for a specific type of cell)

$$\frac{dM}{dt} = h - d_m M - pM - bSM$$

$$\frac{dD}{dt} = pM - aD$$

$$\frac{dS}{dt} = anD - d_s S - bSM$$

$$\frac{dG}{dt} = bSM - d_g G$$

- d_m, d_s and d_g are degradation rates.
- dsRNA is synthesized from mRNA by RDR with small rate p, and cleaved into n siRNAs with rate a.
- The term bSM is due to binding of mRNA with RISC.
- Transcription rate h
- No transgene induced silencing in this basic model

•**Basic Model**: Bergstrom *et al.*, "Mathematical models of RNA silencing: Unidirectional amplification limits accidental self-directed reactions," PNAS, 100, 11511, 2003.

Systems Approach for Simulating RNAi

- Extended Amplification Model
  - A small amount of siRNA can activate the transformation of mRNA into dsRNA
  - primed and unprimed amplifications of mRNA by garbage RNA

$$\frac{dM}{dt} = h - d_m M - pM - bSM - g_p SM$$

$$\frac{dD}{dt} = pM - aD + g_p SM + g_a SG$$

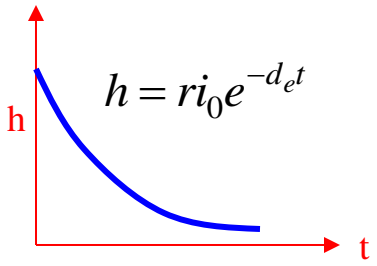
$$\frac{dS}{dt} = anD - d_s S - bSM - g_p SM - g_a SG$$

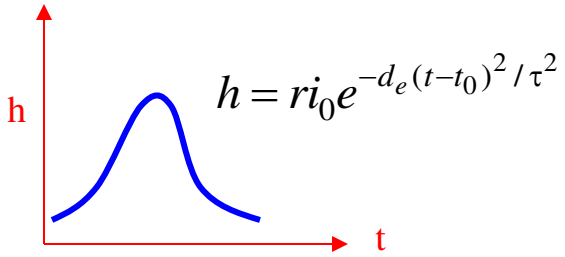
$$\frac{dG}{dt} = bSM - d_g G - g_a SG$$

Systems Approach for Simulating RNAi

- Time-dependent transcription rate

$h = ri(t)$



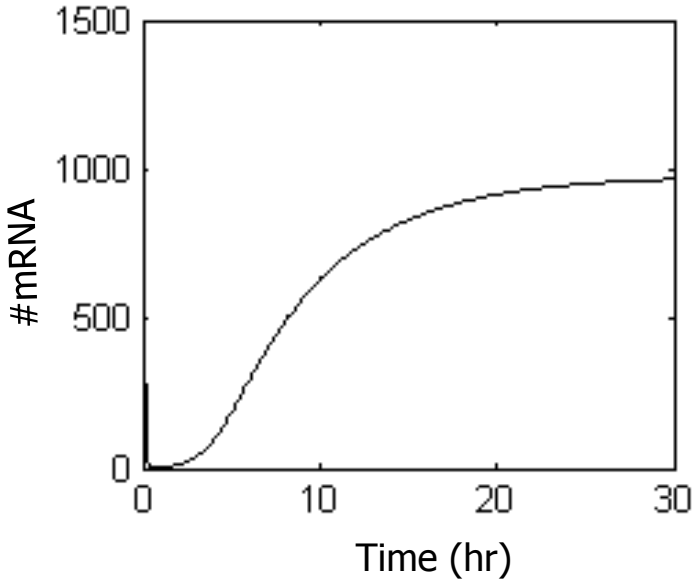


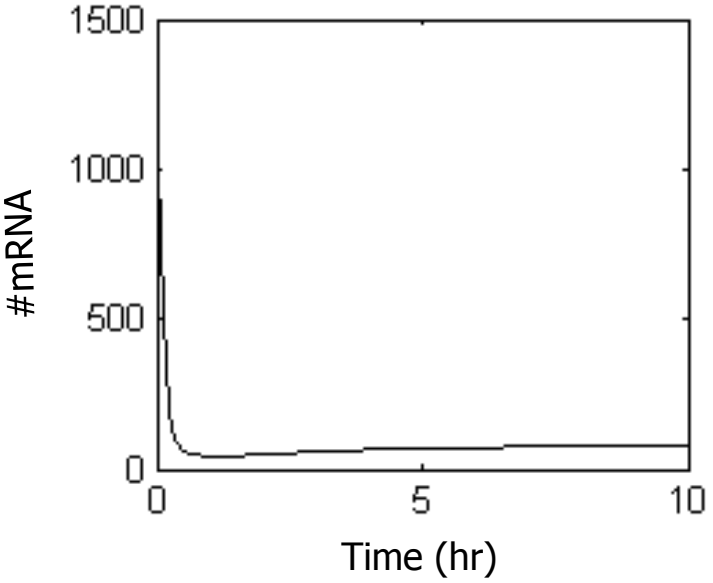
Parameters	Values	Parameters	Values
d_m	0.14 hr^{-1}	d_s	2 hr^{-1}
d_g	2.8 hr^{-1}	r	160 $hr^{-1}cell^{-1}$
p	0.002 hr^{-1}	a	2 hr^{-1}
n	10	b	0.008 cell mol^{-1} hr^{-1}
g_a	0.0008cell mol^{-1} hr^{-1}	g_p	0.0008 hr^{-1}

Groenenboom et al., *PLoS Computational Biology*, 1, e21, 2005

RNAi due to Constant Transcription Rate

- Transgene induced RNAi in human cell





mRNA half-lives may vary greatly between different species
(e.g., Yeast – 1.5hr; human – 1hr to several days)

Analyzing the Model Behaviour

- Equilibrium point solution for constant or low transcription rate: d(.)/dt = 0

$$\begin{cases} h - d_m M - pM - bSM - g_p SM = 0, \\ pM - aD + g_p SM + g_a SG = 0, \\ anD - d_s S - bSM - g_p SM - g_a SG = 0, \\ bSM - d_g G - g_a SG = 0. \end{cases}$$

By rearranging in S,

$$d_s(b+g_2)g_3 S^3 + \left[(2bh - nbh + hg_2 - nhg_2 + d_s d_m + d_s p)g_3 + d_s(b+g_2)(d_g+g_1)\right] S^2 + \left[(bh + g_2 h - ng_2 h + d_s d_m + d_s p)(d_g+g_1) + -pnhg_3 - nbhg_1\right] S - pnh(d_g+g_1) = 0.$$

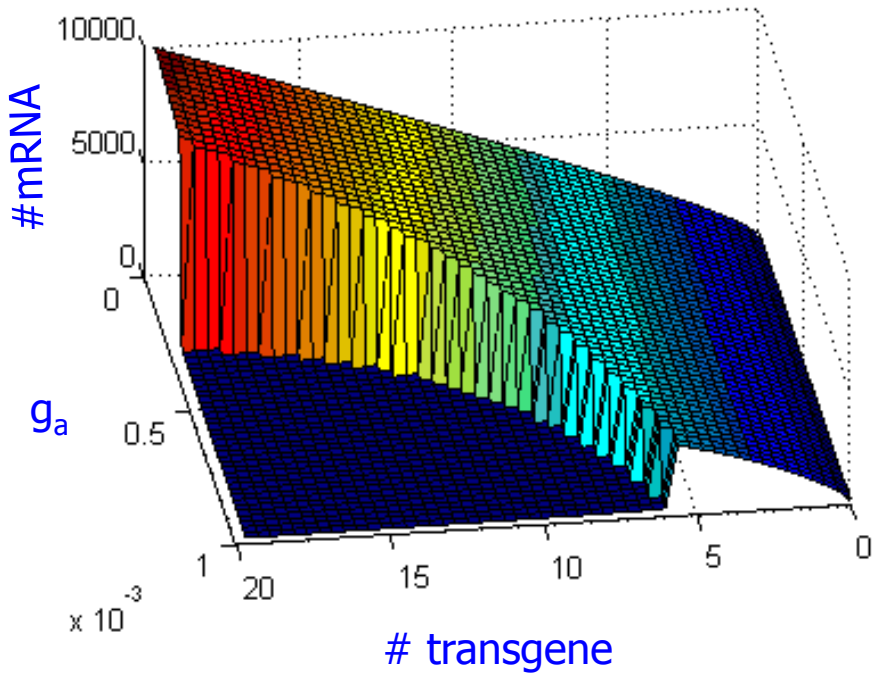
After solving in S,

$$M = \frac{h}{d_m + p + (b+g_2)S} \qquad G = \frac{bSM}{d_g + g_1 + g_3 S} \qquad D = \frac{pM + g_2 SM + g_s SG + g_1 G}{a}$$

- Now select the parameter for a desired #mRNA (M) – a dosage design problem

Analyzing the Model Behaviour

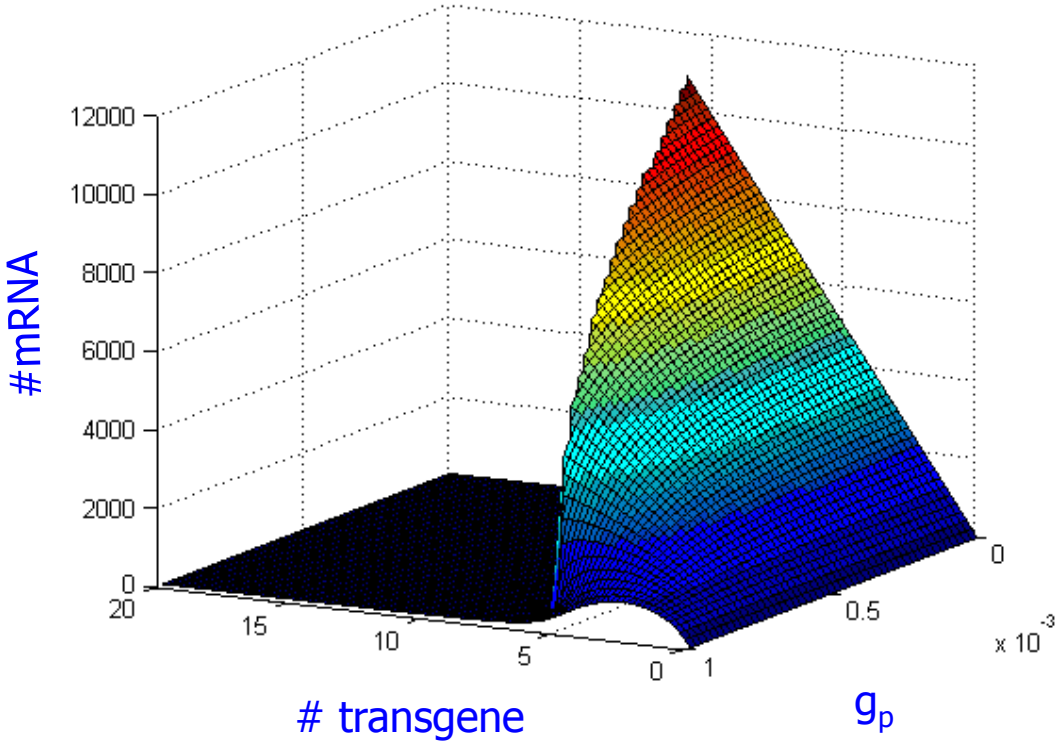
- Equilibrium point solution



- When g_a=0, RNAi does not occur for any initial number of transgenes
  ⇒ Garbage RNA induced amplification of siRNA is indeed a resonable description to simulate transgene induced RNAi

Analyzing the Model Behaviour

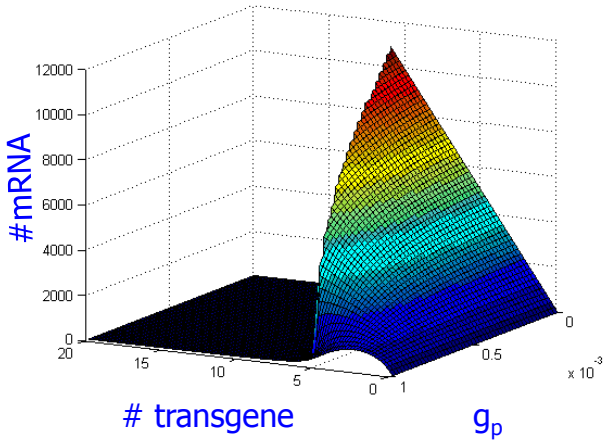
- Time domain finite differencing with RK4

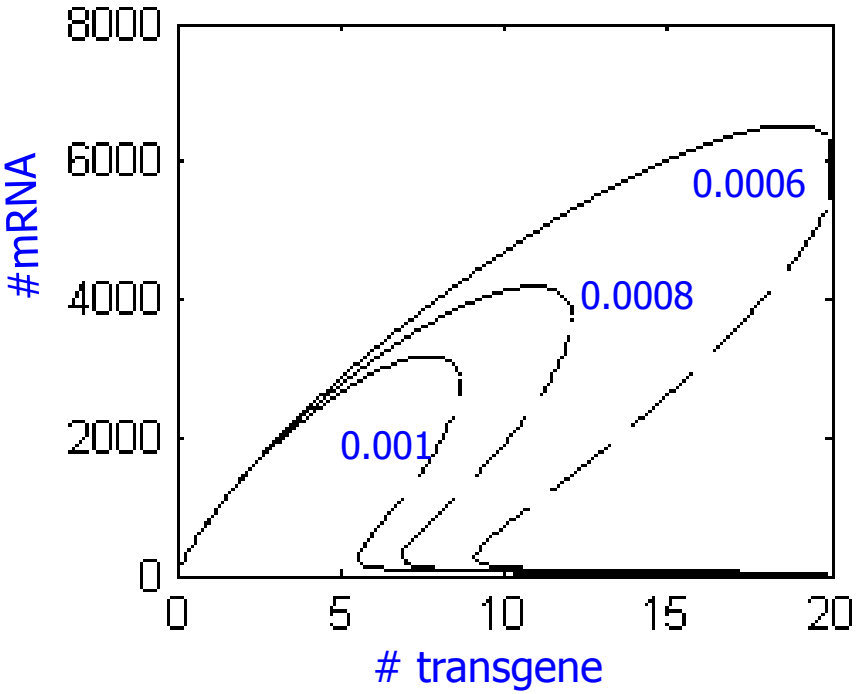


- After 400hr of evolution. The flat region represents RNAi for the chosen initial values of # transgene and siRNA induced activation rate (g_p).

Analyzing the Model Behaviour

- Time domain finite differencing with RK4



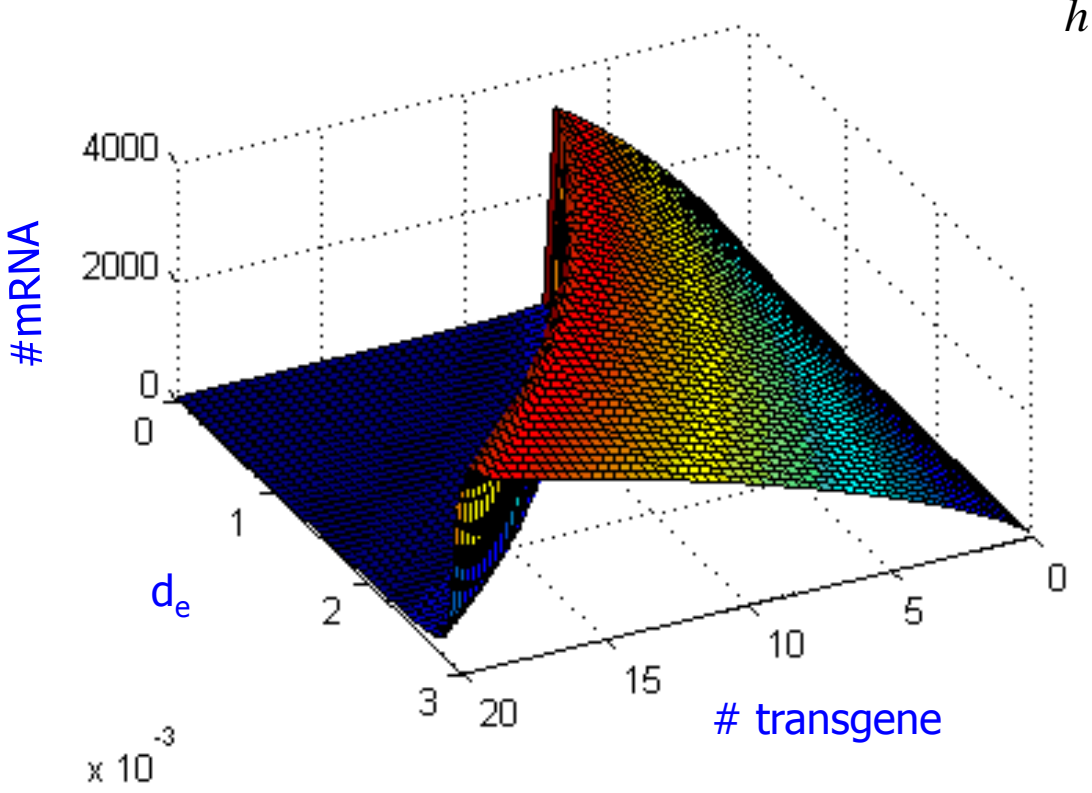


- Increasing g_p leads to decrease in the critical transgene copy number

Analyzing the Model Behaviour

- Time-dependent (exponentially decaying) transcription

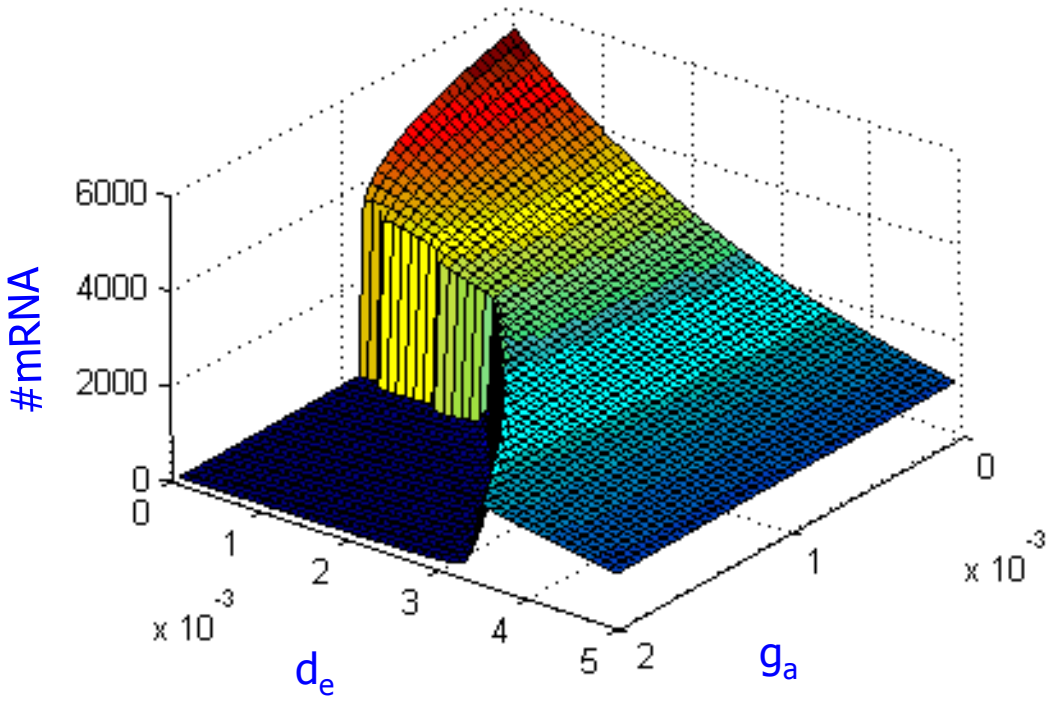
$$h = ri_0 e^{-d_e t}$$



- For higher initial value of # transgene, a fast decaying transcription (higher d_e) can lead to RNAi.

Analyzing the Model Behaviour

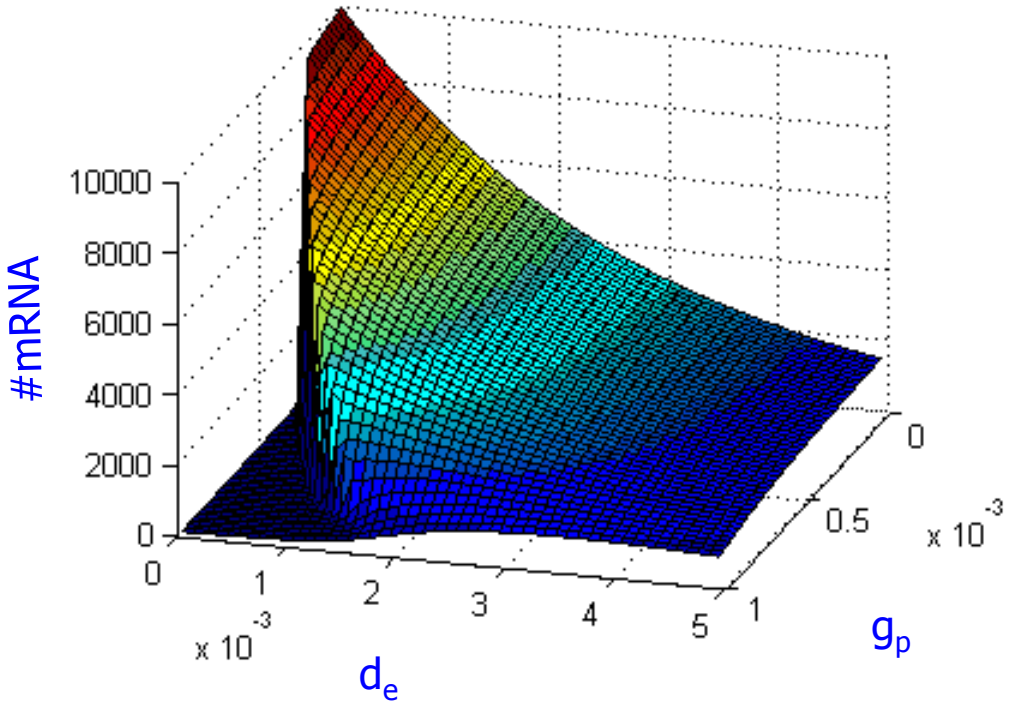
- Time-dependent (exponentially decaying) transcription



- Combined effect of fast decaying transcription and increasing garbage RNA induced amplification may not produce RNAi

Analyzing the Model Behaviour

- Time-dependent (exponentially decaying) transcription

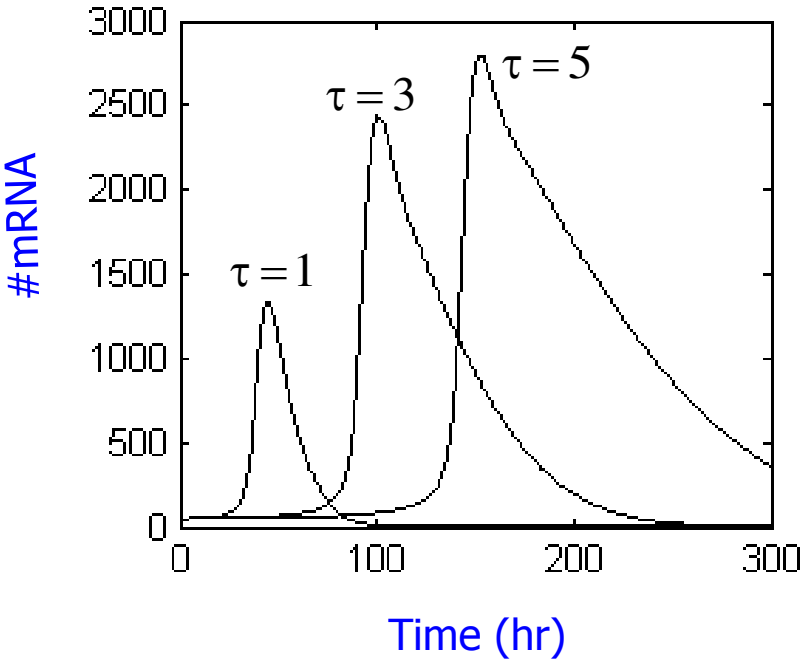


- High siRNA induced activation (high g_p) with small decay in transcription (small d_e) can lead to RNAi

Analyzing the Model Behaviour

- Time-dependent (Gaussian) transcription

$$h = ri_0 e^{-d_e (t-t_0)^2 / \tau^2}$$



- Time to reach complete RNAi varies almost linearly with the time constant τ

Conclusion

- An extended amplification model with time-dependent transcription rate has been analyzed. Experimentally reported data has been employed. The simulated RNAi behaviour for constant transcription rate agrees well with *in vitro* experimental studies reported in published literature.

- The results show interesting nonlinear non-autonomous properties of the model pathway. The results also indicate that a time-dependent transcription at high rate can help in faster RNAi using higher gene copy number.

- Other complicated and non-local effects (e.g., mechanics of loop and bent formation, energy landscape in genetic network and transport of hpRNA-IR-dicer) need to be accounted as a subsystem model within the pathway. Sequence specific effect may also have role in RNAi.