N7. COUPLING BETWEEN MESSENGER RNA SYNTHESIS AND DEGRADATION DURING GENOME EXPRESSION

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Brief: Genome expression involves the synthesis of messenger RNA (mRNA), a short-lived molecule whose translation directs the synthesis of cellular proteins and that is subject to degradation after the translation process. Up to the date, mRNA synthesis and degradation has been considered as a linear phenomenon in which the above steps occur subsequently without a regulatory interconnection. However, gene expression can be studied as a global system in which all their stages are coupled and interconnected by regulatory mechanisms. We have contributed to demonstrate that the machineries of mRNA synthesis and degradation physically and functionally interact in the cell nucleus. We have proposed that it allows the cross-regulation of transcription with mRNA degradation and vice versa, giving rise to a circular system, which would explain the large robustness observed in gene expression (Haimovich et al, 2013).

Transcription is performed by RNA polymerase in cooperation with a set of auxiliary factors, and is divided into three phases: initiation, elongation and termination. During elongation RNA polymerase often undergoes a curious phenomenon of arrest where it translocates backwards with respect to both the DNA template and the RNA transcript, without shortening this one (Gómez-Herreros et al, 2012). This backtracking phenomenon can be reverted through the auxiliary factor TFIIS, allowing the resumption of mRNA synthesis. Experimental evidence obtained in our laboratory suggests that the backtracking process could imprint the synthesized mRNA, conditioning its half-life. Moreover, backtracked polymerases would be the regulatory targets of the mRNA degradation machinery when acting on the transcriptional process.

By using the NetLogo software, we have constructed a computational multiagent-based model of this system, using the main elements of the machinery of synthesis and degradation, as well as some of the auxiliary factors.

The minimal transcription/degradation system that we have modelled is composed of the following elements:

- RNA polymerase II; there are other RNA polymerases in eukaryotic cells but we focus in this because it transcribes protein-encoding genes. When RNA polymerase II is elongating mRNA can be either active or inactive (backtracked).
- TFIIS. This is the factor that promotes the reactivation of backtracked RNA polymerase II
- Ccr4 and Xrn1; these are the two main components of the mRNA degradation machinery. Xrn1 is a 5'-3' RNA exonuclease. Ccr4 is the main mRNA 3' deadenylase. In addition, Ccr4 is a transcription elongation factor that opposes RNA polymerase II backtracking.

The continuous variables of the system are the following:

- Total amount of RNA polymerases engaged in transcription
- Proportion of transcribing RNA plymerases that are active (non backtracked)

- mRNA molecules present in the cell
- Half-life of the mRNA

These continuous variables can be experimentally measured in a rather accurate manner. We have chosen GAL1, one of the best characterized genes of the yeast Saccharomyces cerevisiae, which is our favourite model organism.

The discrete variables of the system are the following:

- Presence of the mRNA degradation factors (Yes/No)
- Presence of the RNA polymerase II reactivation factors (Yes/No)
- Capability of the RNA polymerase II to become backtracked (Yes/No)

Xrn1, Ccr4 and TFIIS are non-essential proteins in yeast. The mutant strains lacking these factors are viable in standard growth conditions. When compared with the wild type, mRNA concentrations are surprisingly similar in these strains. The modelling of the system should allow explaining the robustness of the mRNA synthesis/degradation system.

This work is part of the project entitled "Crosstalk between transcription and mRNA degradation: influence of chromatin and RNA polymerase II backtracking", financed by The Spanish Ministry of Economy and Competitiveness (MINECO) (grant BFU2013-48643-C3-1-P). Although carried out in a yeast experimental system, the high degree of evolutionary conservation of gene expression mechanisms makes this work of general interest for Biology and Biomedicine.

Key question: Is there a cross-regulation between mRNA synthesis and degradation during genome expression?

ODD Protocol:
1. Overview

Goal: Modelling gene transcription and its coupling to mRNA degradation, to increase our knowledge on the molecular behaviour of this system and to test several alternative hypothesis based on previous experimental work.

Entities, process overview and scheduling: There are four kind of agents in the model: Gene, Polymerase, mRNA, TFIIS, Ccr4, Xrn1

- Gene promoter:
  - It does not move
  - Entry site of the polymerase into the gene
  - The promoter may have different levels of activity. This is modulated by Xrn1
  - It blocks its activity when a polymerase is transcribing the gene
- Gene body:
  - It does not move
  - It is downstream of the promoter
  - It is the informative part of the gen. It is copied into mRNA by the polymerase
- Gene terminator:
  - It does not move
  - It is downstream of the gene body
  - Exit site of the polymerase. In this the polyA tail is added to the mRNA and then the mRNA is released from the gene
- Polymerase:
  - Nanomachine that reads the information encoded in the gene body producing the mRNA molecule
  - It moves randomly in the cell
  - Enters the gene through the promoter
  - It may backtrack and arrest
  - Backtracked polymerases can reactivate by its interaction with TFIIS
  - It terminates transcription and mRNA synthesis at the terminator

- mRNA:

a. information (5')

- It is the body of the mRNA and contains the information encoded by the body of the gene
- It is polar: its two ends are different (3' end and 5' end)
- It is produced by the polymerase by reading the gene body
- It also contains a polyA tail in the 3' end
- It moves randomly in the cell together with the polyA tail
- Its polyA tail is degraded by Ccr4
- Its information (5' end part) is degraded by Xrn1, provided that the polyA tail is fully degraded
- It is characterized by its half-life (how fast is it degraded)

b. polyA (3')

- It is the tail composed by a high numbers of A nucleotides that is added to the mRNA 3' end immediately before termination
- It is fused to the informative part of the mRNA in its 3' end
- It moves randomly in the cell together with the informative part
- It is degraded by Ccr4
- Its degradation is a requirement for the degradation of the information part of the mRNA
- During transcription termination, it may be imprinted for degradation by the transfer of Ccr4 from the polymerase to the polyA

- TFIIS
  - It reactivates backtracked polymerases
  - It moves randomly in the cell
- Ccr4
  - It moves randomly in the cell
  - It degrades the polyA
  - After degrading the polyA it stays bound to the mRNA until this is completely degraded by Xrn1
  - When mRNA degradation is complete, Ccr4 acquires de capacity of interacting with the transcribing polymerase, keeping its degradation capacity
  - After interacting with the polymerase, it follows the polymerase until the terminator. Then it is being released and loses its transcriptional capacity.
  - In the terminator it switches back to the degration mode, losing its transcriptional capacity, and binds the polyA of the mRNA that is just transcribed
- Xrn1
  - It moves randomly in the cell
  - It degrades the information part of the mRNA (5' end) after the complete degradation of the polyA by Ccr4
  - After degrading the mRNA it switches to a transcriptional mode: it can stimulate the activity of the promoter, keeping its degradation capacity.
  - After stimulating the promoter, it loses its transcriptional capacity

The variables are absolute numbers, only the promoter activity and frequency of polymerase backtracking ranks between 0 and 1.

The spatial environment of model is the cell (3D) but It is not necessary a very high spatial resolution and we simplify to a 2D space. Your time interval is from t=0 mRNA concentration reaches a dynamic balance (steady state).

2. Design concepts

The model is based on our knowledge on the mRNA transcription and degradation phenomena. Two elements are especially relevant: The mRNA degradation machinery (Ccr4, Xrn1) can moves into the nucleus and influence gene transcription after mRNA

degradation. And the Polymerase backtracking is important for the coupling between gene transcription and mRNA degradation. Many specific molecular details of the degradation/transcription coupling are unknown. The purpose of the study is a first comparison of the global consequences of different possible options for some of these details.

Emergence: The steady state (dynamic balance) of mRNA concentration is an expected emerging result of the model.

Flow chart:

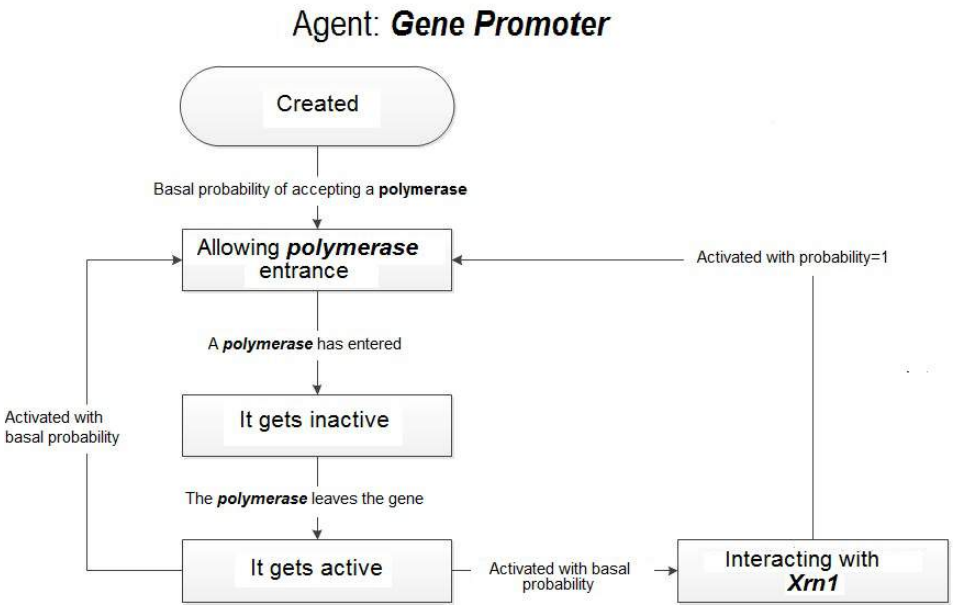

Diagram 1. Agent: Gene promoter

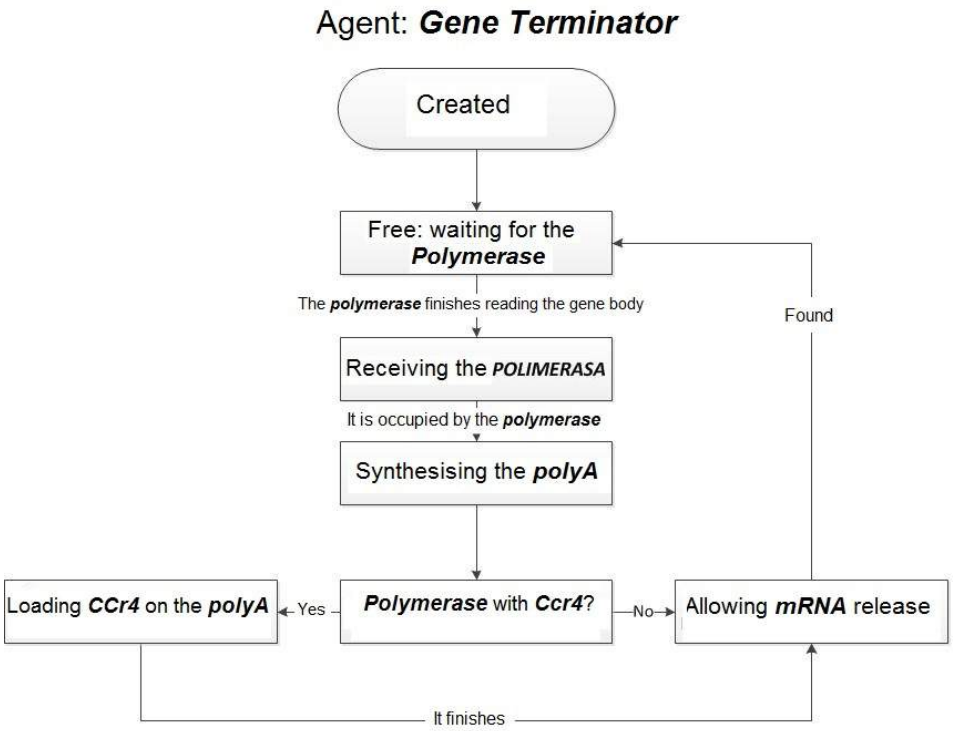

Diagram 2. Agent: Gene terminator

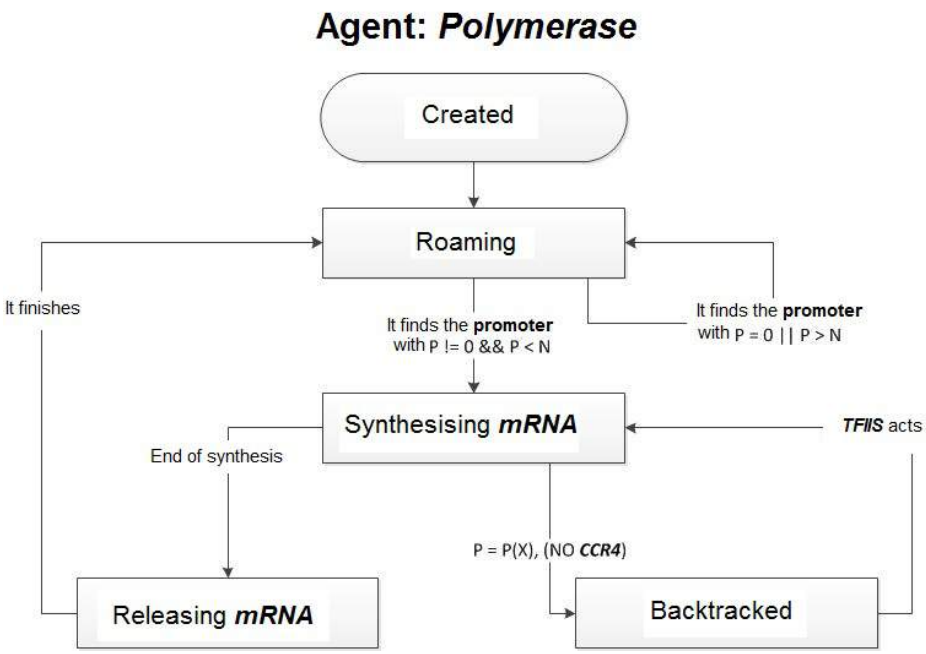

Diagram 3. Agent: Polymerase

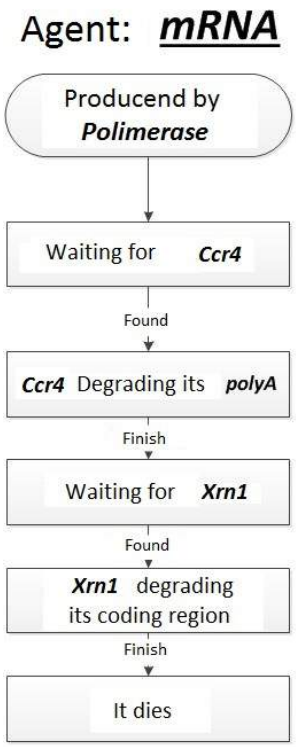

Diagram 4. Agent: mRNA

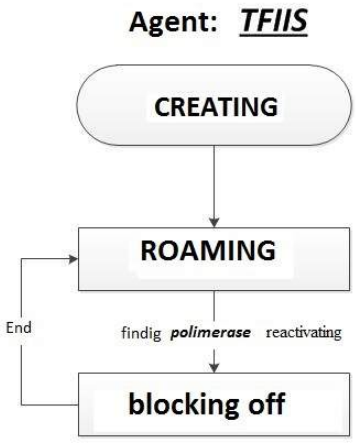

Diagram 5. Agent: TFIIS

Code: N7_code.nlogo
View it on Github: https://github.com/culturadigital/forma14/blob/master/N7.nlogo

Model snapshots:

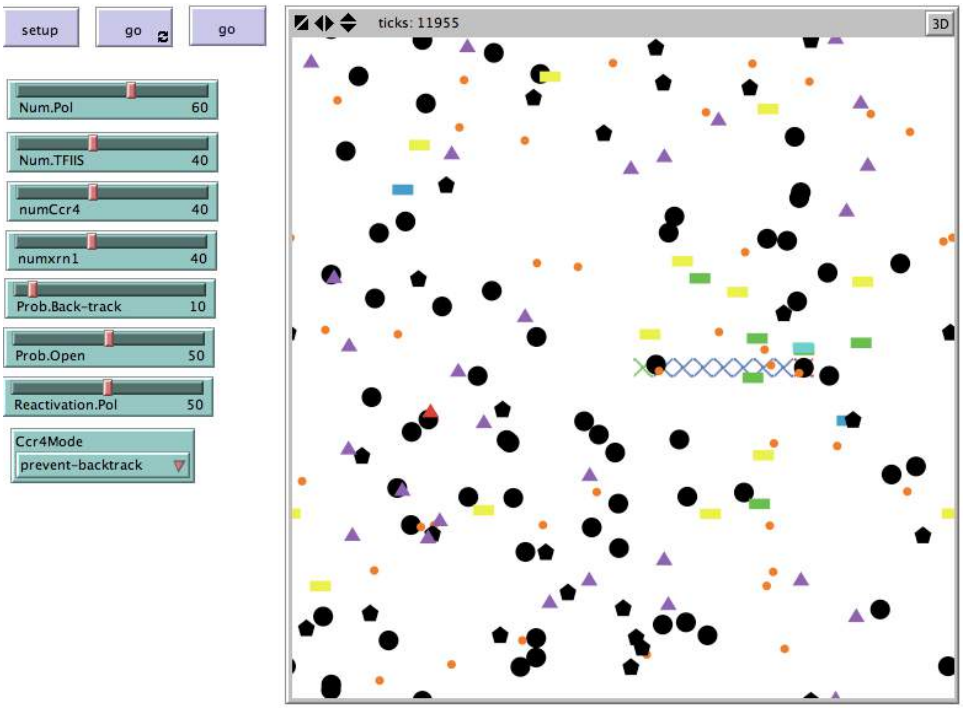

Figure 1. Symbols: X, gene; black circle, RNA polymerase; green rectangle, mRNA; blue rectangle, mRNA being degraded by Ccr4; yellow rectangle, polyA-less mRNA waiting for Xrn1; orange dot, TFIIS; black pentagone; Ccr4; purple triangle, Xrn1.

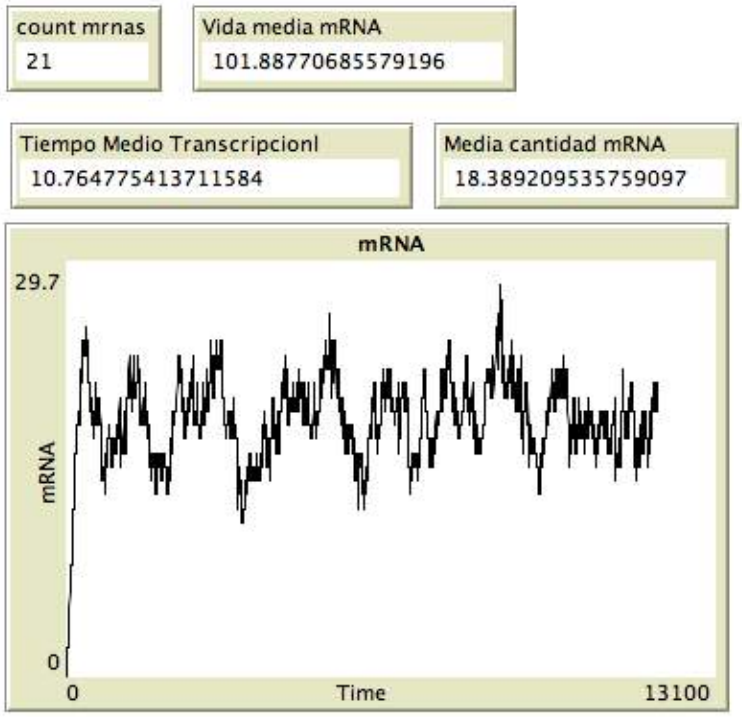

Figure 2. Graphic detail of the steady state (dynamic balance) of mRNA concentration.

Conclusions: By using the NetLogo software, we have developed a computational multiagent-based model of the mRNA synthesis/ degradation system. The results obtained support the expected behaviour for this system in the cellular context. These results, combined with experimental measurement of different variables of the system in mutant strains lacking the key genes of the process, should allow us better defining the synthesis / degradation coupling of mRNA and, in particular, confirming the importance of the backtracking phenomenon in this circular process.

References:

- Haimovich, G; Medina, D; Causs, S; Garber, M; Millán-Zambrano, G; Barkai, O; Chávez, S; Pérez-Ortín, JE; Darzacq, X; Choder, M. (2013) Gene expresión is circular: factors for mRNA degradation also foster mRNA synthesis. Cell. 153: 1000 - 1011.
- Gómez-Herreros, F; de Miguel-Jiménez, L; Millán-Zambrano, G; Peñate, X; Delgado-Ramos, L; Muñoz-Centeno, M.C; Chávez, S. (2012) One step back before moving forward: regulation of transcription elongation by arrest ans backtracking. FEBS Letters 586: 2820-2825.