Spatial organization of bacterial transcription and translation

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Seminar Outline

Introduction – Motivation, Background and Structure of Talk // 3min

Hi. I'm Laura and I will talk about the work of Cast-ell-ana et.al on the spatial organization of bacterial transcription and translation.

Biological Background

To give some motivation why you should care about the complicated sounding topic of this talk, let's start with some underlying biology:

- This is a protein. Proteins are found in all living cells.
- They are complex molecules responsible for nearly all essential task within a cell like
    - structure
    - signaling
    - functions such as catalyzing chemical reactions
    - etc.
- The instructions to synthesize proteins are contained in a molecule called "DNA".
- The procedure of accessing the information coded in the DNA and synthesizing it into a protein can be broken down into two steps.
    - First, "transcription": DNA is used as a template to produce messenger RNA (mRNA).
    - Second, "translation": ribosomes attach to the mRNA, read the sequence and synthesize the corresponding proteins.
- Experiments have shown that there is a spatial localization mechanism for ribosomes and mRNA within cells.

Scientific Questions

- Observing this spatial organization raises two fundamental questions (see ref 1,2,3,4,5):
    1. **What physical processes are responsible for subcellular organization?**
    2. **How does this internal structure influence the basic processes of mRNA transcription and protein translation in the cell?**

Goal of Research

- The reason people are interested in the spatial organization of ribosomes and mRNA is because experiments suggest that these mechanisms may be responsible for specific biological functions in all domains of life.
    - Whereas in *Eukaryotes* mRNA localization is a well-established mechanism for performing different functions (see ref)
    - mRNA localization in *Prokaryotes* is not as well studied. However, there is experimental evidence backing up the notion that there are functional roles in bacteria too (see ref 6,7,8,9).

Outline of approaching the scientific question

- In this talk I will talk you through the strategy of approaching these scientific questions by the authors of this paper.
- First, to answer the question which physical processes drive the macroscopic patterns observed in experiments, a mathematical model is proposed and motivated.
- Then we will have a look numerical solution that result from the model and what conclusions can be drawn from their analysis.

Paper // 17min

Model Setup // 6min

Motivation for RD // 2min

- This paper focuses on prokaryotic cells.
- In rod shaped bacteria such as E. coli, DNA forms a condensed structure called "nucleoid" in the center of the cell. This is where mRNA is made. However, ribosomes and mRNA have been observed away from the nucleoid. Graph
- The goal is to keep this model simple but not oversimplified.
- First let's look at the geometry at hand:
    - Implementing a (1D) line with the x-coordinate running along the major axis of rod-shaped cell makes the simplest of geometries by pretending everything that happen in the 3D cell in projected onto this line.
    - Because of the left-right symmetry of a the rod-shaped cell, considering only one half simplifies the system a little further.
- Next, let's look at the two components of interest:
    - Concentration of free ribosomes (FR) in space and time
    - Concentration of mRNA in space and time
- Finally let's look at the interaction of these components. There may be many interactions but let's begin by consider two which are based on experimental observations:
    - First, we observe ***reactions*** of components with each other. FR can attachment and detachment from mRNA.
        - FR can turn into translating ribosomes (TR) by binding to mRNA and thus synthesizing proteins. Similarly, FR can also turn into transiently bound ribosomes (BR) by binding to mRNA which means they are bound but don't really do any protein synthesis.
        - TR and BR can also detach and turn back into FR
        - Degradation of mRNA by enzymes for example also frees up BR and TR into FR
    - Second, experiments show how both FR and mRNA are moving in the system by a process called ***diffusion***.

Dynamics of System(equation + animation) // 4min

- To describe the dynamics of this model in space and time, according to our observations it seems appropriate to use a reaction/diffusion equation to do so:
    - spatiotemporal dynamics = diffusion term + reaction term (equation)
- Let's look at the dynamics of the FR concentration in a bit of detail as the procedure for deriving the equation of the mRNA concentration is identical
- We start by look at the ***diffusion term***
    - Contrary to other hypothesis for mRNA segregation (29), a specific prediction of the model presented in this paper is that mRNA segregation is due *to excluded volume effects* resulting from the condensed nucleoid DNA.
    - The intuition behind this assumption is that as the size/volume of mRNA increases with each binding ribosome. This means occupy the same space results in a decrease of available volume and incentivizes movement to areas with more available volume.
    - Let's derive a mathematical description of how this effect influences diffusion:
        - We start by slicing the cell into bin of width Δx. Each bin is projected onto the1D x-axis where x_i denotes the position of the bin *i*.
        - The question is: what is the concentration of FR in bin x_i if ribosomes can jump between adjacent bins?
        - After one jump, the amount leaving bin x_i obviously depends on concentration of FR $c_F(x_i)$ inside the bin x_i. The diffusion rate d_F with which FR can jump out of bin is dependent on the available space to the left $v_F(x_{i-1})$ and the right $v_F(x_{i+1})$. V_F is the fractional volume available to a FR within the DNA mash at position x_i. Basically, v_F is a factor influencing d_F. In other words, if there is more available space the rate of diffusion in that direction increases and vice versa.
        - After one jump what enters the bin x_i depends on the concentration of FR on both the left $c_F(x_{i-1})$ and the right $c_F(x_{i+1})$ bin as well as on the available volume inside the bin $v_F(x_i)$.
        - This 'master equation' can be solved by tailoring around $x=x_i$.
        - By taking the continuum limit we get an expression that looks like this. D_F is the diffusion coefficient.
        - This is the diffusion term accounting for the excluded volume.
        - With a keen eye one might notice a striking similarity to the diffusion equation of fick's first law. By comparing the two equations we can easily read of the 1D FR flux at position x. This flux will be important a little bit later
- For the ***reaction term***. Let's look at our reaction diagram from before. The signs are convention if you keep consistent
    - For BR attachment and detachment
        - The amount FR attaching to all available mRNA and becoming BR is dependent on the F->B transition rate, the concentration of FR and the total density of available mRNA
        - The amount of BR unbinding from an mRNA depend on the unbinding rate and the multiplicity factor m in the total density of mRNA accounts for how many BR are bound to all the mRNA.
    - Attachment and detachment of TR is identical to the behavior of FR
    - There is also degradation term of mRNA based on experiments (ref 6). It depends on the degradation rate. The factor m+n in front of the total density of mRNA accounts for how many BR and TR are bound to all the mRNA.

Results // 11min

Goal // 1min

- Now that we have the equations set up, lets solve them!
- Disclaimer: This work produced a lot of results. For the scope of this talk I want to focus on three results that I found insightful
- Before numerically solving the RD for FR and mRNA concentrations we need to add just a little more information about the system:
    - First, since time scales for protein synthesis are so much faster compared to cell doubling times the RD are solved at steady state.
    - Second mass conservation in enforced by constraining the total number of ribosomes as constant in the system.
    - Third, boundary conditions for FR and mRNA concentrations are integral to solving RD equations. Both at the cell pole as well as the cell centre there are no-flux boundary conditions imposed for both FR and mRNA.

Total mRNA profile // 1min

- Result 1:
    - *Solving the RD for mRNA concentration at steady state yields a mRNA concentration profile along the right cell half.*
- Explanation:
    - mRNA is generated at the nucleoid. As the majority of mRNA is being loaded with ribosomes the size/volume of the mRNA increases such that that the excluded volume biases their diffusion away from the nucleoid.
    - The heat map shows most mRNA is loaded with ~10TR and ~2BR (graph). Because each ribosome has a linear size of 20 nm, the effective size of an mRNA molecule with 10 bound ribosomes is significantly larger than the pore size of the DNA mesh in the nucleoid, which is estimated to be 50nm.
    - Because of this, mRNAs experience strong excluded-volume effects which push them out of the nucleoid region.
    - As the number of ribosomes bound to mRNA increases, so does the segregation of the mRNA from the nucleoid. (See density profiles graph)
- Conclusion:
    - The model makes a prediction for strong, genome-wide mRNA localization away from the nucleoid, indicating that ~ 90% of mRNAs are typically located outside the nucleoid (in line with experiments see ref 10)

Ribosome concentration // 3min

- Result 2:
  - *Solving the RD for FR concentration at steady state yields a FR concentration profile along the right cell half. Additionally, the fluxes are calculated from the RD as well.*
- Explanation:
  - As mRNA segregate away from the nucleoid towards the cell poles, it is being loaded with ribosomes implying that the poleward flux of mRNA carries with it a poleward flux of ribosomes.
    - First, multiple FR bind to mRNAs made in the cell nucleoid. Each mRNA is thus loaded with ~10 TR, and ~2 BR.
    - Second, the effects of excluded volume in the nucleoid result in a net flow of these loaded mRNA to the cell poles. Once the loaded mRNA reaches the poles, they ultimately decay and free their ribosomes.
  - Due to conserved ribosome concentration in this model there must be a compensating flux of FR from the poles toward the nucleoid, which is exactly the behavior observed by the model.
  - This "pumping" of TR and BR from the nucleoid to the poles results in lots of FR at the poles. The consequence of particle conservation is a diffusive return flux of FR to the nucleoid.
  - Overall, these results illustrate/quantify a "circular" flux of TR, BR, and FR.
  - The existence of a steady ribosome circulation implies that there must be an external source of energy driving these circular fluxes. There are two possibilities in the model:
    - Process (A): fluxes are caused by nonequilibrium creation and degradation of mRNAs. This process should be strictly dependent on new mRNA production.
    - Process (B): fluxes are caused by mRNA and FR binding in the nucleoid and subsequent expulsion from the nucleoid by excluded-volume effects. This process should persist even in the limit where the mRNA production and degradation rates are both low, with the total number of mRNAs fixed and equal to N_{mRNA}.
  - By varying the mRNA rates together, keeping the total mRNA number constant, the circulation vanished as the mRNA rates slowed, thus identifying process (A), the flux of new mRNAs from nucleoid to pole, as the driver of ribosome circulation (graph)
- Conclusion:
  - mRNA-bound ribosomes flow from the nucleoid to the cell poles, where they unbind from mRNAs and then diffuse back to the nucleoid to bind newly synthesized mRNA
  - the flux of newly synthesized mRNAs from nucleoid to the pole drives ribosome circulation.

Nucleoid size // 5min

- To determine the nucleoid size there is a neat derivation using force balance between an inward force F_{in} exerted by the FR and mRNA on the nucleoid and an outward force F_{out}, exerted by the nucleoid on the FR and mRNA.
    - Let's start with the inward force F_{in}:
        - Since FR are so much smaller than polysomes the inward force is characterized mainly by the force of mRNA. Difference = 20*e-2
        - mRNAs, like particles in a gas, exert an entropic force (pressure) on the nucleoid directed toward the cell-centre - this force can be computed directly from the reaction–diffusion equations.
        - There is a rigorous derivation of the F_{in} which if anyone is interested, we can talk about later but for all intends and purposed this is the result.
            - For me the hand wavy intuition behind this term is that we treat the mRNA distribution outside of the nucleoid region as 1D ideal gas where we cannot distinguish between force and pressure. So pV = NkT in 1D reads pL = NkT and also F = p = N/L kT, N/L is this total mRNA concentration outside the nucleoid.
        - This force exerted by the mRNA results in the compaction of the nucleoid see ref 8, 26.
        - In other words, mRNA segregation to the poles causes nucleoid compaction at mid-cell (F_{IN}).
    - Lets now look at the force exerted by the nucleoid onto the mRNA
        - the natural tendency of the compressed DNA polymer to increase its configurational entropy results in an effective "spring" force pushing outward on the mRNAs (F_{OUT}).
        - The spring force of the nucleoid can be estimated by modeling the nucleoid as a lattice with DNA living on its vertices. The confinement dependent entropy of this self-avoiding DNA is given by ___ intuition????
        - We can estimate the volume by pretending the nucleoid occupies a cylinder with radius R and length 2. x_o :_____
        - The outward entropic force exerted by the nucleoid on the mRNA is simply given by :
- For mechanical equilibrium there must be an opposite equal force between F_{in} /F_{out}.
- Numerically solving this equation yields a profile which match the experimentally observed nucleoid size very well
- The larger the number of mRNAs, the more the nucleoid shrinks toward mid-cell due to the entropic force exerted by the polysomes (graph).
- Using the calculated ribosome and mRNA densities, it is confirmed that mRNA segregation to the poles quantitatively accounts for nucleoid compaction.
- Conclusion:
    - *Physically*, the observed nucleoid size reflects the balance of two competing entropic forces—the compressive force that mRNAs at the poles exert on the DNA (F_{IN}), and the expansive force exerted by the DNA on these mRNAs (F_{OUT}).
    - *Biologically*, the compaction of the nucleoid by mRNAs creates a potential global feedback circuit: Gene expression drives mRNA levels, which, by compacting the nucleoid, impact transcription-factor access and hence gene expression (30).

Extending the model // 1min

- Finding:
    - The minimal model was carefully modified/extended to account for many biological influences:
        - Comparing co-transcriptional translation and post-transcriptional translation
        - Including 30S and 50S ribosomal subunits
        - Including more realistic mRNA degradation by RNase enzymes
        - Conditions of late phase of cell division cycle **(more exp?)**
        - Different growth rates **(more exp?)**
        - Model without BR (since their existence is still an open question)
- Conclusion:
    - By verifying that none of these additional factors influence the behavior of mRNA segregation, this makes a strong case for the *excluded volume effect* being the main driving force behind of the spatial organization

Conclusion // 1min

Strength and Weaknesses of the Paper

- Strengths:
    - What I really like about this approach is that a simple 1D model of RD with relatively few assumptions can still produce robust results agreeing with experimental data.
    - In this paper the author's arguments for certain simplifications are well explained and based on solid biological/chemical reasoning.
- Weaknesses:
    - To reiterate, even though some of the model's predictions agree with experimental data, the fact that no genome-wide mRNA localization away from the nucleoid has been observed in experiments makes the work very theoretical.
    - The claim of this model is that genome-wide mRNA segregation can arise entirely from excluded-volume effects, is a pretty big assumption. As a sanity check I would have been curious to see how calculations with simple diffusion and no excluded volume effect differed. But maybe that just way to obvious?

Outlook

- One could go on and extending the model. For example, in a more realistic geometry like a 2D box. The extra dimension might influence the concentration profiles. These effects are unobtainable in the 1D model and might reveal more aspects of the dynamics.
- I've also been thinking about other ways to approach the question of what drives mRNA segregation and maybe instead of RD other processes like active transport might be interesting too based on the research of____?
- How would you be able to discriminate between different theories? Eg active transport vs reaction diffusion due to excluded colume?