Mathematical modeling of dynamic regulation of poly(A) tail in gene expression control

Project Description

1. Overview

Nearly all mRNAs in eukaryotic cells acquire a poly(A) tail, which plays a crucial role in post-transcriptional control of the mRNA, such as splicing, nuclear export, translation, silencing and decay (*1-4*). The poly(A) tail is highly dynamic, regulated at various stages of its life by a number of molecular mechanisms that physically interact with other mechanisms of gene expression, such as transcription and mRNA decay (*5-7*). In essence, *poly(A) regulation organizes a highly interconnected system of gene expression control*. Although much is known about the molecular composition of the machineries regulating poly(A) tails, yet missing is a mechanistic understanding of the dynamics of poly(A) regulation and its functional coupling with other mechanisms of gene expression control. Filling this gap of knowledge will improve our arsenal to control gene expression, which is crucial for biological research and applications.

As a powerful tool to study complex dynamic controls, mathematical modeling integrates existing knowledge and data, provides mechanistic insights, and generates nonintuitive predictions about complex systems. The field of poly(A) regulation has accumulated sufficient basic knowledge, data, as well as puzzles, for modeling studies, thanks to the increasingly detailed picture of the molecular mechanisms for poly(A) regulation (*3, 5-12*), and genome-wide characterization of poly(A) dynamics and its relationship with gene expression dynamics (*13-19*). ***The overarching goal of this proposal is to build a mathematical modeling framework to investigate the dynamic control of the poly(A) tail from its birth to death, and its interplay with the dynamics of mRNA transcription, translation and decay***. The proposed study will focus on the following aspects of poly(A) regulation, which have been sufficiently studied by experiments and are ready for modeling studies.

Aim 1: Investigate dynamic regulation of nuclear cleavage and polyadenylation. mRNAs first acquire the poly(A) tail in the nucleus through the cleavage and polyadenylation (CPA) process as transcription progresses. For many genes, CPA can occur at alternative sites, generating different mRNA isoforms (*7, 20*). The outcome of alternative polyadenylation (APA) is controlled by dynamic coupling between transcription and CPA (*7*). We will ***combine modeling with live-cell RNA imaging*** (to be performed at Prof. Robert Singer's lab at Albert Einstein College of Medicine) to investigate how transcriptional dynamics and CPA dynamics interplay and how the coupled dynamics affects the outcome of APA.

Aim 2: Investigate poly(A) and mRNA regulation by poly(A)-binding proteins (PABP). The poly(A) tail is bound by PABPs throughout its life and poly(A)-mediated regulation of gene expression is essentially carried out by the PABPs. The most well-studied and ubiquitous PABP, Pab1, interacts with and regulates the translation and deadenylation machineries (*3*). We will build models to test existing hypotheses about how Pab1 dynamically binds with poly(A) (*21*), and investigate how mRNA deadenylation, decay and translation are centrally regulated by Pab1.

Starting off with the above poly(A) regulatory mechanisms will launch the long-term trajectory of my research in gene expression dynamics. In the long run, as more data become available in the field, I will expand and fill gaps in the modeling framework, with the ultimate goal of coherently integrating the complex interconnections between poly(A) regulation and other mechanisms of gene expression, and bringing a holistic understanding of how gene expression is dynamically regulated.

2. Intellectual merit

The proposed modeling research on poly(A) regulation will advance fundamental, quantitative understanding of gene expression control, as the poly(A) tail is involved in every stage of the mRNA's life, including transcription, translation and decay. Mathematical modeling will bring novel insights into the complex dynamic coupling of these gene expression mechanisms, for example, how the cell coordinates

transcription, translation and mRNA decay (*10, 22-32*), which apparently promotes economic utilization of the cell's resources. Furthermore, the predicted gene expression dynamics from the research will allow re-examination of the simple assumptions for gene expression kinetics that are widely used in modeling of gene regulation, and suggest possible improvements. This will strengthen the basis of predictive modeling in systems biology and synthetic biology, as gene expression lies at the root of gene regulatory networks and cell signaling.

4. Background

Poly(A) as a universal and central regulator of mRNA

3'-poly(A) tail is a universal feature in nearly all eukaryotic mRNAs, except for the histone mRNA (*1, 4*). The poly(A) tail is first synthesized in the nucleus, and is further shortened (deadenylation) or elongated (polyadenylation) in the cytoplasm (*1, 2, 5, 44*). The poly(A) tail plays a crucial role in post-transcriptional regulation (*3, 4*). In the nucleus, the poly(A) tail is involved in RNA quality control and nuclear export (*11, 45, 46*). In the cytoplasm, the poly(A) tail controls mRNA stability and translatability (*2, 13*). Deadenylation is the essential leading step of major mRNA decay pathways (*47-52*).

Dynamic regulation of the poly(A) tail is mediated by a number of molecular mechanisms, which often involve formation of macromolecular complexes that physically interact with other mechanisms of gene expression and regulation (*5-7*). To name perhaps the most striking example, among others, the Ccr4/NOT complex, a major deadenylation machinery, interacts with nearly all mechanisms of gene expression and regulation, from transcription initiation and elongation to translation and mRNA decay steps downstream of deadenylation (*11*). ***Essentially, poly(A) regulation organizes a highly interconnected system of gene expression control***. Such interconnection can mediate dynamic coupling between different gene expression processes. Indeed, genome-wide correlation was observed between translation and mRNA decay (*25-32*) and between transcription and mRNA decay (*22-24, 53-56*). However, many puzzles remain, such as the controversial relationship between poly(A) tail length and translation (*13-18*). Overall, it is poorly understood how poly(A) regulation and the interconnected gene expression mechanisms coordinately shape the dynamics of gene expression.

Mathematical modeling of poly(A) regulation

Mathematical modeling has proven a powerful tool for studying complex dynamic controls. For example, a great number of mathematical models have enabled deep understanding of the dynamics and function of gene regulatory mechanisms and networks (*57, 58*). Mathematical models have been built and combined with temporal data on poly(A) tail lengths to investigate the connection between deadenylation and mRNA decay (*48, 49, 59*). However, dynamic coupling between poly(A) regulation and other gene expression processes has not been a focus of modeling studies. These dynamic couplings likely play a critical role in gene expression control. They also stand at the perfect point for modeling investigation, as experimental studies are generating increasingly detailed pictures for the poly(A) regulatory mechanisms (*3, 5-12*), and genome-wide data on poly(A) dynamics and its correlation with gene expression dynamics (*13-18*). Modeling will provide novel mechanistic insights into how poly(A) regulation coordinates gene expression dynamics, predict key factors controlling the dynamics, and guide downstream experimental studies.

Out of the vast complexity of poly(A) regulation, this proposal chooses to concentrate on the following intensively studied mechanisms of poly(A) regulation (Fig. 1) and their dynamic interplay with transcription, translation and decay of mRNA.

Nuclear cleavage and polyadenylation (CPA) mechanism

Aim 1 will address the CPA mechanism that gives birth to the poly(A) tail. The CPA machinery is assembled at specific polyadenylation sites (PAS) in a newly transcribed RNA (Fig. 1A). Activity of the CPA machinery cleaves the RNA and adds adenosines at its 3' end. An important gene regulatory function

of CPA lies with alternative polyadenylation (APA): a large number of genes contain multiple PASs (*60-63*) and CPA at different PASs generates mRNA isoforms with different stability and translatability (*20*).

CPA is functionally coupled with transcription. The CPA machinery physically interacts with the transcription machinery (Fig. 1A) (*4, 7, 64-69*). The dynamics and outcome of CPA is intertwined with the dynamics of transcription, especially transcriptional pause. Specifically, CPA factors induce pausing of the RNA polymerase II complex (Pol II) downstream of the PAS (*70-75*). In turn, slow transcription promotes selection of the proximal PASs, and vice versa (*76-79*). However, the real-time dynamics of the coupling between CPA and transcription is yet unknown, hindering our understanding of the principles behind regulation of APA. In this aim, we will model the coupled dynamics of CPA and transcription (Fig. 1B), and parametrize the model using live-cell RNA imaging. We will use the model to investigate how the positioning of PASs in a gene affects CPA dynamics and PAS selection, and distill the design principle of PAS placement in APA (Fig. 1B).

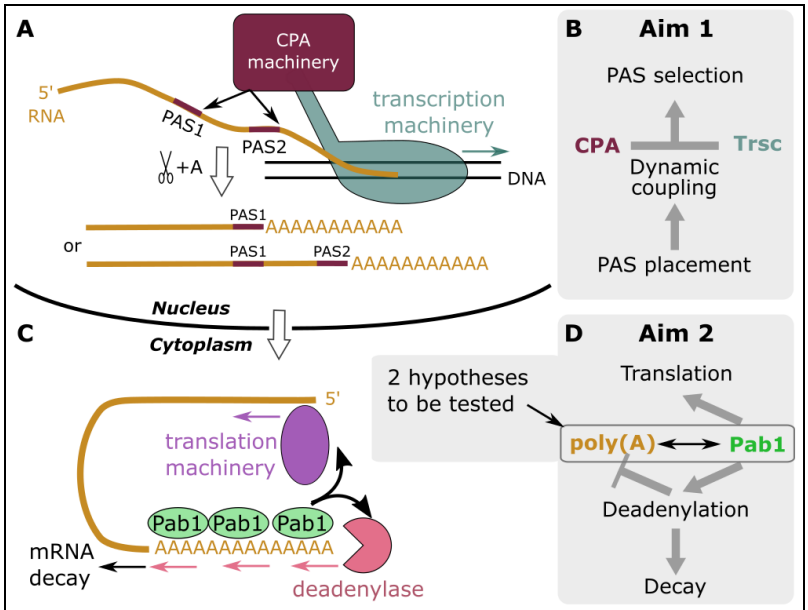

Fig. 1: Molecular mechanisms to be modeled. (**A**) CPA process with alternative PASs. CPA machinery is physically and dynamically coupled with the transcription machinery. (**B**) Summary of Aim 1. (**C**) Pab1-mediated regulation of deadenylation and translation couple mRNA decay and translation. (**D**) Summary of Aim 2. Details of the two hypotheses about poly(A)-Pab1 binding dynamics will be described in the Research Plan.

Poly(A)-binding proteins (PABP)

Aim 2 will address the role of poly(A)-binding proteins (PABP) in gene expression. The poly(A) tail is bound by PABPs throughout its life (*3, 80*). Through their interactions with multiple RNAs and proteins, PABPs serve as central mediators for metabolism and functions of mRNAs (*3, 80, 81*). Among the number of PABPs, Pab1 is the major cytoplasmic PABP and is ubiquitously expressed in all eukaryotes (*3*). Pab1 regulates deadenylation (which eventually lead to mRNA decay) by recruiting deadenylases to the poly(A) tail (Fig. 1C) (*21, 82-84*). Pab1 also regulates translation by interacting with translation initiation factors (Fig. 1C) (*85-89*). Taken together, Pab1 potentially contributes to coordination between mRNA decay and translation (*25-32*).

Although the structure of Pab1-poly(A) complex has been characterized (*90, 91*), a clear picture of how Pab1 dynamically interact with poly(A) remains lacking, and even less is known about how Pab1-poly(A) binding dynamically regulates deadenylation and translation. In this aim (Fig. 1D), we will first build biophysics models based on the structure (*90, 91*) and binding measurements (*21, 91-98*) to test two existing hypotheses about how Pab1 dynamically interact with poly(A) as deadenylation chews away the poly(A) (*21*) (see Research Plan for detail). Then we will expand the model to investigate how Pab1-poly(A) orchestrate the dynamics of mRNA deadenylation, decay and translation.

5. General modeling strategies and example previous work

Poly(A) tail regulation is undoubtedly complicated and experimental data are scarce in many aspects, such as the details of the dynamic interplay between poly(A) regulation and other gene expression mechanisms.

When facing uncertainty in model construction and parametrization, we will adopt the following general strategies to cater the type of model, level of complexity, and questions to be addressed by the models to the limitation of available information.

- ***Start simple*** and focus first on the most general and salient features;
- ***Coarse-grain*** the model if necessary and possible;
- ***Explore global parameter space*** to identify key factors underlying major qualitative behaviors.

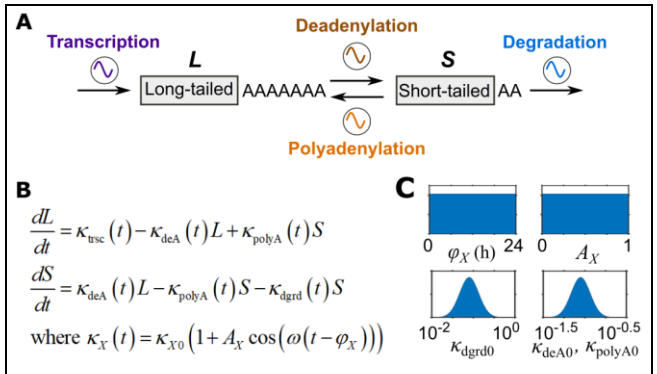

Fig. 2: Model for rhythmic control of poly(A). Adapted from (*33*). (A) Schematic of the model. (B) Equations of the model. (C) Global parameter space explored. Shaded areas show distributions from which parameter values were drawn. Distributions for mean rates were chosen based on transcriptomic data in (*99, 100*).

Our recent publication on circadian regulation of poly(A) tail (*33*) showcases the above modeling strategies, as well as the type of conclusions that can be drawn from such models. Rhythmic control of gene expression in single cells lies at the root of the biological circadian rhythm (*101, 102*), and happens at both transcriptional and post-transcriptional levels (*103-107*). Recent studies revealed hundreds of mRNAs whose poly(A) tail lengths exhibit robust circadian rhythmicity (*108*). To determine the extent to which rhythmic poly(A) regulation influences rhythmicity in gene expression, we ***started simple*** by constructing a parsimonious model for poly(A) length regulation (Fig. 2). Since the experimental study (*108*) quantified the rhythmicity of poly(A) tail length based on the ratio between the long-tailed and short-tailed fractions of mRNAs, in the model we ***coarse-grained*** the poly(A) regulation as reversible conversion between the long and short states, too (Fig. 2A, B) (*33*).

Because the four processes in the model can happen with widely diverse mean rates, phases and amplitudes, we explored the behaviors of the model on the ***global parameter space*** (Fig. 2C). Our ***global parameter sensitivity analysis*** via the Sobol's variance-based method (*109, 110*) revealed the rhythmicity of deadenylation as the most important factor determining the rhythmicity of poly(A) tail length (represented by the long/short ratio) and long-tailed mRNA abundance (*33*). The latter quantity can be considered as a rough proxy for mRNA translatability, in light of the role of poly(A) tail in regulating translation (*9, 111-113*). In fact, the influence of deadenylation rhythm is so strong that distinct phases in deadenylation, but not the other processes, can robustly cluster the phases of L/S and L into distinct

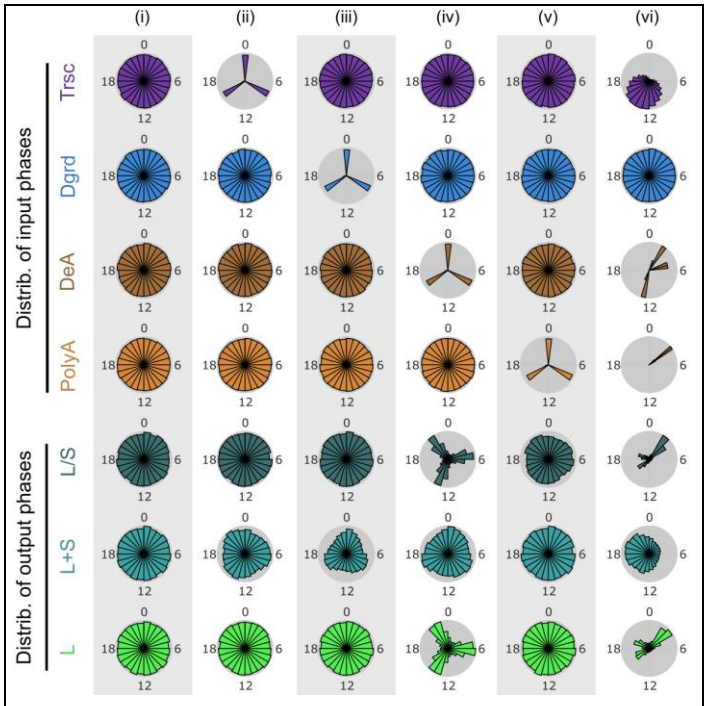

Fig. 3: Rhythmicity of deadenylation is key determinant of the rhythmicity of long/short ratio and long-tailed mRNA abundance. (i) All four phases evenly distributed around the clock. (ii-v) Transcription (i), degradation (ii), deadenylation (iii), or polyadenylation (iv) rate peaks in 3 narrow time windows, while phases of the other three rates are evenly distributed around the clock. (vi) Distribution of phases suggested by experimental observations in (*108, 114*). In all cases, amplitudes and means assume random values following distributions in Fig. 2C. Adapted from (*33*).

narrow windows (Fig. 3, ii-v), while all the other parameters are randomly chosen from the global parameter space. This striking clustering effect suggests that the experimentally observed distinct phases in the expression of deadenylases (*108*) could potentially cluster mRNAs into distinct groups with coordinated rhythms (Fig. 3, vi).

Although our model above is very simple, global parameter analysis allowed us to draw a strong conclusion that calls rhythmic deadenylation to the attention of the field of circadian gene expression. It is worth noting that the strong qualitative behaviors often rely on the most general and basic features of the system (e.g., topology of a network), but not additional details. In the proposed work, we will follow similar strategies to deal with unknowns and draw conclusions from findings that truly stand out in the global parameter space.

6. Research Plan

This proposal aims to devise mathematical models to ***investigate dynamic control of the poly(A) tail and its interplay with the dynamics of mRNA transcription, translation and decay***, with a focus on (1) nuclear polyadenylation and (2) Pab1-mediated mRNA regulation.

Aim 1: Investigate dynamic regulation of nuclear cleavage and polyadenylation

Dynamic coupling between transcription and the cleavage and polyadenylation (CPA) mechanism controls which polyadenylation site (PAS) is selected for RNA cleavage and the subsequent synthesis of poly(A) tail (*7, 69*). In Aim 1.1, we will ***combine mathematical modeling and live-cell RNA imaging experiments*** to devise a mechanistic model for CPA. In Aim 1.2, we will use the model to predict how the placement of PASs in a gene affects its CPA dynamics and PAS selection, and ***distill the design principles of alternative polyadenylation (APA)***.

Preliminary study:

The CPA process entails physical interactions between the CPA factors and RNA polymerase II (Pol II) (*7, 8, 115*). CPA and transcriptional pause are particularly intertwined. CPA factors induce pausing of Pol II downstream of the PAS (*70-75*). During alternative polyadenylation (APA), slow transcription promotes

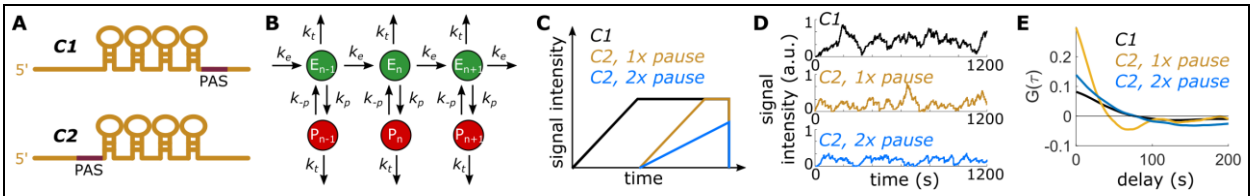

Fig. 5: Proof-of-principle test by model. (**A**) Prototype reporter constructs. Stem loops represent tag sequences. The upper construct serves as a control to separate the effect of the tags on transcriptional pause. (**B**) Mathematical model for transcriptional dynamics. Pol II randomly switches between the elongating (green) and pausing (red) states, and CPA terminates transcription. The rate of termination, k_t, is set to zero before the position of the PAS. The signal level is defined as the number of completely transcribed tag sequences at the current Pol II position, *n*. Rates of transcriptional elongation, pausing and pause release are based on literature data (*117-120*). (**C**) Illustrative time trajectories of the signal generated by a single RNA. (**D**) Model simulated trajectories of signal when multiple transcription events on the tagged gene overlap. (**E**) Autocorrelation curve of the simulated signal trajectory. Averaged over 20 simulated trajectories.

selection of proximal PASs, and vice versa (*76-79*). Hence, transcriptional pause plays an important role in the CPA process.

Transcriptional pause has been widely observed via Pol II occupancy profiling (*70-75*). However, Pol II occupancy only provides a static, bulk-averaged profile, from which the kinetics of transcriptional pause cannot be uniquely determined (*73*). Live-cell RNA imaging measures real-time dynamics of single RNA molecules (*116*) (Fig. 4). When the RNA-binding protein is localized to the nucleus, the experiment can be used to probe transcriptional dynamics (*117, 118*).

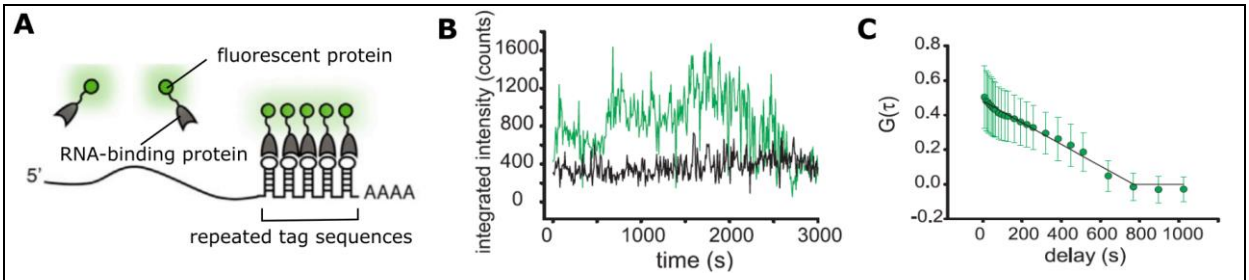

Fig. 4: Live-cell RNA imaging. (**A**) Illustration of live-cell single-RNA imaging technology. The RNA of interest is inserted with multiple repeats of a tag sequence which binds tightly with its cognate RNA-binding protein (e.g., MS2, MCP) fused to a fluorescent protein. Multimerized tags allow single RNAs to be detectable. Adapted from (*122*). (**B**) Example time trajectory of the RNA signal at a single transcription locus. Green line: RNA signal at the transcription locus. Black line: background signal. (**C**) Example autocorrelation curve of the signal trajectory. (B), (C) adapted from (*118*).

To make a proof-of-principle test whether live-cell RNA imaging data can detect changes in transcriptional pause dynamics, we built a model to simulate the fluorescent signal generated by a pair of prototype reporter constructs, with tags inserted before and after the PAS, respectively (Fig. 5A). The model incorporated dynamics of transcription elongation, pause and termination (by CPA) (Fig. 5B). In an ideal world, the constructs would generate clean signal trajectories that clearly display the transcriptional dynamics (Fig. 5C) and inform the rate parameters in each case. But when randomness in the processes and overlapping transcription events on the tagged gene are considered, the predicted RNA signal becomes very noisy (Fig. 5D), making direct comparison of the time trajectories impossible. Additional sources of noise in the experiment will only make the actual data noisier (Fig. 4B). To analyze the data, autocorrelation function averaged over multiple signal trajectories is often used (Fig. 4C) (*118*, *121*). We hence computed the autocorrelation function of the simulated trajectories, and found the curves distinguishable between cases with different transcriptional pause rates (Fig. 5E, yellow vs. blue curves). Through fitting the autocorrelation function from the model to that from the experiment, one can infer the model parameters.

Aim 1.1. Integrate modeling and live-cell RNA imaging to construct model for CPA

We will construct a mechanistic model for CPA dynamics based on the macromolecular architecture of the CPA machinery (*7*, *8*, *64*, *115*). The most salient feature of the CPA machinery is that multiple CPA factors are assembled at the PAS and simultaneously bind with the carboxyl-terminal domain (CTD) of Pol II (Fig. 6A). In light of this salient feature, we will start with a parsimonious model (Fig. 6B), which coarse-grains the CPA factors and their interactions with PAS and Pol II, and focuses on the dynamic feedback between transcriptional pause and assembly of the CPA machinery.

Model parametrization

The model will be parametrized by both literature data and the live-cell RNA imaging data. For example, the basal elongation rate of Pol II is available from the literature (*117-120*). The assembly dynamics of the CPA machinery can be inferred from binding affinity measurements and structural information for CPA factors (*123-130*). Importantly, the kinetics of transcriptional pause and CPA will be quantified in live-cell RNA imaging described below.

Quantifying rates of transcriptional pause and CPA

To quantify the rates of transcriptional pause and CPA, we will combine live-cell RNA imaging with modeling.

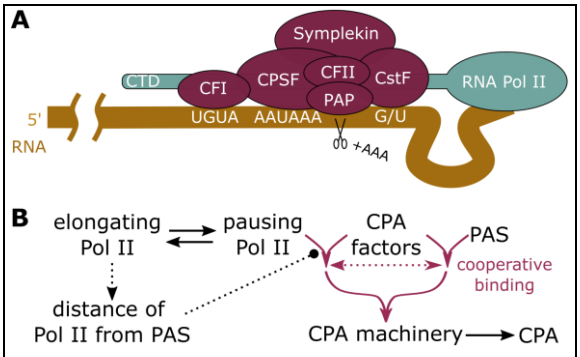

Fig. 6: Modeling the CPA mechanism. (**A**) Molecular architecture of the CPA machinery. (**B**) Starting model for CPA. Solid black arrows: state conversion. Solid maroon arrows: complex formation. Dotted arrow with pointed head: positive impact. Dotted arrow with circle head: binding between Pol II and CPA factors depends on the distance of Poll II from the PAS.

We will first use modeling to guide the design of the reporter. Specifically, we will predict the RNA signal dynamics under different reporter constructs (e.g., different inter-tag spacing, different positioning of the tags relative to the PAS, hybrids of different types of tags), and identify effective construct to answer the following questions.

- How much is transcriptional pause affected by the PAS in a nascent RNA?
- Over what distance (along the transcribed RNA) does a PAS induce transcriptional pause? Pol II occupancy indicates that Pol II could pause several kilobase pairs downstream of the PAS (*70-75*). But Pol II occupancy is affected by both pausing and termination, so the exact range of pausing regulation is not known.
- How does the strength of a PAS affect its regulation of transcriptional pause? The strength of a PAS depends on its nucleotide sequence and is associated with the likelihood that the PAS is selected in APA (*7, 61-63, 131*). The relative strengths of PAS sequences are highly conserved across different organisms (*61, 62*).
- How soon does CPA occur after the PAS is transcribed and how does this depend on the strength of the PAS?
- How do multiple PASs with varying distances affect the kinetics of transcriptional pause and CPA?

Similar to our preliminary study (Fig. 5), we will predict the RNA signal in the presence of intrinsic (e.g., molecular) and extrinsic (e.g., measurement) noise, and ask which designs can distinguish different possible answers to the above questions. In addition, we will derive *approximate analytic formulas* for the autocorrelation functions. Chain reactions as that depicted in Fig. 5B are mathematically expressed as homogeneous linear differential equations; this type of equation is analytically solvable, but deriving the formula for the autocorrelation function usually requires appropriate approximation. These formulas will potentially make straightforward suggestions for designing the reporter construct. More importantly, the formulas will be fitted to the autocorrelation curve from the experimental data to infer the parameters for transcription and CPA.

The live-cell RNA imaging will be conducted using the budding yeast system. The PI will send her student to receive training and conduct the experiments at **Prof. Robert Singer**'s lab at Albert Einstein College of Medicine (see letter). Prof. Singer is the world's leading pioneer in live-cell RNA imaging techniques and RNA dynamics (*117, 118, 132-134*). During model-aided design of reporter constructs, the PI and her student will consult with Prof. Singer and his lab members to ensure that the modeling is seamlessly integrated with experiments, with caveats in the imaging technology and RNA biology carefully considered. The experiments will be conducted over three to four 2~3-week visits to the Singer lab. Ultimately, the experimental data will be used to parametrize transcriptional pause and CPA dynamics in the model, and suggest necessary revision to the model.

Aim 1.2. Investigate regulation of APA by PAS placement

APA generates alternative mRNA isoforms, which can differ in stability, translatability, and even the coding sequence (*20, 115*). APA plays an important role in cellular control. The global APA profile in a cell is dynamically regulated during cell proliferation, differentiation, development, etc. (*20, 135-144*).

The placement of PASs in a gene provides the genomic code that controls APA. Because CPA cannot occur to a PAS before it is transcribed, the positioning of a PAS relative to other PASs would affect its probability of being selected during APA. Intriguingly, although PAS placement is highly diverse across different genes, the most distal PAS or the single PAS of a gene in mammals and fission yeast tend to be the strongest, so-called canonical, PAS (*61-63, 131, 145*). Yet, budding yeast, surprisingly, displays exactly the opposite trend (*145*). Although these observations corroborate the importance of PAS placement, mechanistic understanding of how PAS placement regulates APA is lacking.

We will investigate how PAS placement affects APA with a top-down approach. That is, we will leverage the mechanistic model for CPA constructed in Aim 1.1, and ***predict the APA dynamics and outcomes***

under different PAS placements. Note that the model will focus on the interdependence between CPA and transcription dynamics, without detailing on individual molecules. Therefore, the predictions will center on generic characteristics of the processes represented by model parameters. For example, the strength of a PAS will be represented by its binding affinity with CPA factors, or its regulatory effect on transcriptional pause; the change in CPA or transcription components will be represented by the change in the rates of CPA, transcriptional elongation or transcriptional pause. We aim to answer the following questions.

- How does the distance between PASs affect the dynamics and outcome of APA?
- How does the relative strength of PASs affect the dynamics and outcome of APA?
- It is important to interrogate the role of PAS placement in the context of cellular control. Since the PAS placement does not change with external signal or cell state, APA regulation in cellular controls must rely on changes in factors mediating CPA or transcription. In this regard, how much can up- or down-regulation of CPA and/or transcription activities alter the dynamics and outcome of APA, given a PAS placement?
- What are the design principles of PAS placement to optimize the tunability of APA by up- or down-regulation of CPA and/or transcription?

To cope with unknown information, we will again adopt the strategy of exploring the ***global parameter space***. For example, the realistic levels of up- or down-regulation of CPA and/or transcription activities are likely unknown and diverse across different cellular contexts. We will combine different levels of changes in the corresponding rate parameters, and explore their effects. We will also use ***phase diagrams*** and ***global parameter sensitivity analysis*** to identify the most significant determinant of the probability distribution of PAS usage. As demonstrated in our work on circadian poly(A) regulation (Figs. 2, 3) (*33*), we will draw conclusions from the most outstanding model behaviors in the global parameter space.

Predictions related to the first three questions above will be ***experimentally tested*** by northern blot quantification of the abundance of different mRNA isoforms when PAS placement is altered in the gene construct or when a selected component of the CPA or transcription machineries is inhibited. The experiments will be performed in Prof. Singer's lab. The results will be used to validate the model and provide information for model revision.

Expected outcomes

The research in this aim will generate a novel mechanistic model for the CPA mechanism, which will highlight the dynamic coupling between CPA and transcription. The model will be further used to explore how the PAS placement affects the dynamics and outcome of APA, and distill the design principles of PAS placement in terms of controllability of APA. It is worth noting that we will highlight general, qualitative conclusions. By doing so, although the model will be parametrized by data from budding yeast, many qualitative conclusions will be generalizable to other organisms. Ultimately, this research will generate new knowledge about the mechanism and regulation of CPA and its role in gene expression control.

Pitfalls and alternatives

In Aim 1.1, analytic formulas for the autocorrelation functions are planned to be derived and used for parameter inference. Derivation of the autocorrelation functions will likely require approximation. In case we cannot find a good approximation (to be evaluated via comparison to simulation results), we will directly fit the simulation results to the experimental data to infer parameter values. Furthermore, for simplicity we have assumed that CPA is equivalent to transcriptional termination in our preliminary modeling. In reality, transcription could continue long after CPA and even cause transcriptional readthrough. How long transcription tends to continue after CPA in our reporter RNA can be estimated by inserting different tags before the PAS and at a few varied distances after the PAS, respectively. Disappearance of the pre-PAS signal will indicate CPA and appearance of the post-PAS signal will indicate continued transcription. If prolonged transcription after CPA turns out common, we will take this into account when designing and

modeling reporter construct. One possibility is to insert a Nrd1-Nab3-Sen1 dependent termination site downstream of the distal PAS to force termination (*146*).

Aim 2: Investigate poly(A) and mRNA regulation by poly(A)-binding proteins (PABP)

This aim focuses on the major, ubiquitous cytoplasmic PABP, Pab1 (*3*). Pab1 binds to mRNA poly(A) tails, and interacts with deadenylases (*21, 82-84*) and the translation machinery (*85-89*). Deadenylation is furthermore the leading step of major mRNA decay pathways (*47-52*). Taken together, Pab1 likely contributes to the genome-wide correlation between activation of translation and inhibition of mRNA decay (*21, 28-32*). To gain mechanistic understanding of Pab1's role in gene expression, in Aim 2.1, we will model the dynamic interactions between Pab1 and poly(A) and its feedback with deadenylation. In Aim 2.2, we will model the dynamic interactions between Pab1 and the translation machinery to study how Pab1 regulates translation and coordinates translation with mRNA decay.

Aim 2.1 Modeling the dynamics of Pab1-poly(A) binding and its feedback with deadenylation

Pab1 recruits deadenylases to poly(A) and promotes deadenylation (*21, 82-84*). In turn, deadenylation shortens the poly(A) tract, preventing Pab1 binding. These two processes therefore constitute a feedback.

Modeling in this sub-aim will be based on the structural and biophysical data about Pab1. Pab1 has four RNA recognition motifs (RRM) separated by short linkers (*90, 95*) (Fig. 7). The RRMs bind to poly(A) with different affinities (*92, 147, 148*). The carboxy-terminal domain (CTD) mediates the interaction of Pab1 with multiple proteins, including deadenylases (*92*). Webster et al. hypothesized two alternative mechanisms for deadenylation-driven release of Pab1 (Fig. 7A) (*21*). Both mechanisms start with transient unbinding ("breathing") of the 3'-most RRM from the poly(A). This allows the deadenylase to remove the exposed poly(A) segment and prevent rebinding of the RRM. Afterwards, two possible events may happen: (1) the subsequent RRMs are removed from the poly(A) in the same manner, resulting in "peeling" of the entire Pab1 off the poly(A), or (2) the RRMs slide along the RNA and maintain high-affinity interactions with poly(A). Our modeling will address the following key questions.

- Which of the two mechanisms for deadenylation-driven release of Pab1, i.e., "peeling" or "sliding", is likely or more likely to happen on a physiologically compatible time scale?
- How does the feedback between Pab1-poly(A) binding and deadenylation control the kinetics of mRNA decay?

First, we will build biophysical models to test the "peeling" vs. "sliding" hypotheses of Pab1 release (Fig. 7B). Our model will delineate thermal fluctuations in the binding/unbinding or sliding between the RRM domains and poly(A), where deadenylation serves as a Brownian ratchet (*149*). The interactive energies between Pab1 RRMs and poly(A) will be estimated from the crystal structures (*90, 91*), and measured binding affinities and binding kinetics (*21, 91-98*). The model will predict which hypothesized mechanism is energetically more favorable and hence more likely to happen in reality.

Second, we will extend the model to incorporate the feedback between deadenylation-driven Pab1 release (model selected by Step 1) and recruitment of the deadenylase by Pab1 throughout the full length of the poly(A) tail (Fig. 7C). Note that deadenylase recruitment and activity are likely highly variable depending on the genes and cells, because deadenylation is regulated by many factors, such as AU-rich elements, GU-rich elements and miRNA (*47, 150*) and functioning of these factors can further vary in response to regulatory signals. Instead of pinpointing any specific mRNA, we will vary the parameters for deadenylation in a broad range (e.g., over several orders of magnitude as suggested by (*48*)) to predict the corresponding dynamics in poly(A) length distribution (Fig. 7C, right). Since deadenylation is the rate-limiting step of decay for most mRNAs (*47-52*), this model will also allow us to investigate the mRNA decay kinetics (Fig. 7C, right). Using *global parameter exploration*, we seek to discover the *general principles and constraints* for dynamic regulation of mRNA deadenylation and decay.

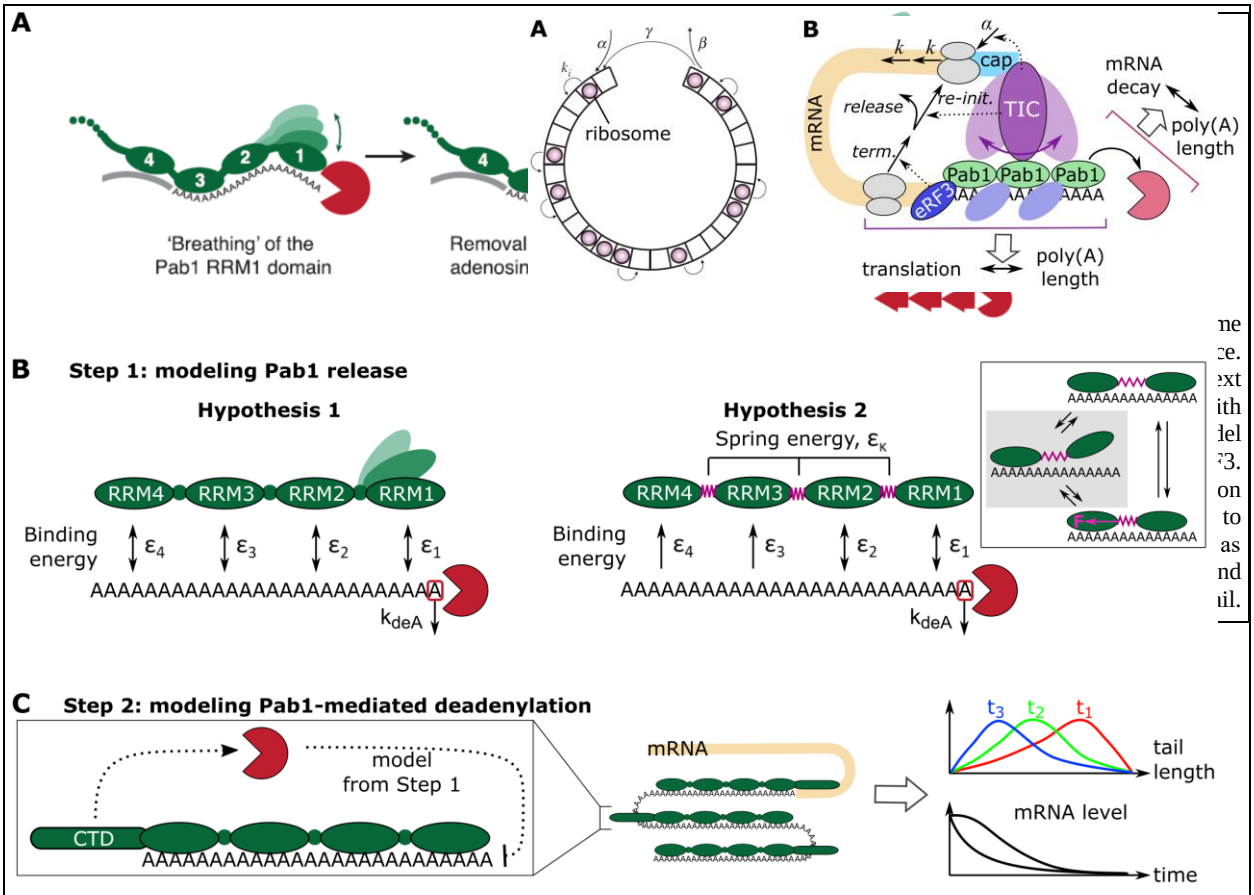

Fig. 7: Aim 2.1. (**A**) Two hypothetical mechanisms proposed for deadenylation-driven release of Pab1 from poly(A). Adapted from (*21*). (**B**) Biophysical models to test the hypotheses. Model for Hypothesis 1 will depict thermally driven binding/unbinding between Pab1 and poly(A). Model for Hypothesis 2 will feature thermally driven sliding allowed by flexible linkers between RRMs. Inset: Sliding may happen directly, or via transient dissociation of the RRMs (grey box). Sliding of one RRM deforms the linker domain (magenta zigzags), and exerts force on the neighboring RRM (magenta arrow). In both models, thermal fluctuations in Pab1-poly(A) interaction are ratcheted by removal of the exposed adenosine by the deadenylase (red pac-man). (**C**) Extended model. Left: in each poly(A) segment covered by one Pab1, activation of deadenylase by Pab1 and deadenylation-driven release of Pab1 forms a feedback loop. Middle: full-length mRNA and its poly(A) tail with multiple Pab1 associated. Right: model will predict length distributions of the poly(A) tail and the corresponding mRNA decay kinetics.

Aim 2.2 Modeling Pab1-mediated regulation of translation and translation-decay coordination

Pab1 is a critical regulator of translation. Pab1 binds with the translation initiation complex (TIC), and the latter binds the 5' cap of the mRNA (*85-89*). These interactions stabilize the TIC and facilitate translation initiation (*93, 151, 152*). They also circularize the mRNA by bringing its two ends into proximity (*111*) (Fig. 8). Furthermore, Pab1 facilitates translation termination by binding with the peptide release factor, eRF3 (*153-155*). After termination, the close proximity between the two ends of the mRNA could facilitate quick recycling of the ribosomes back to 5' end and translation re-initiation (*156*).

To understand ***the role of Pab1 in regulating translation***, we will build upon *a recent model* for translation with ribosome recycling for translation re-initiation (Fig. 8A) (*157*). This model belongs to an extensive class of mRNA translation models termed the totally asymmetric simple exclusion process (TASEP) models (reviewed in (*158, 159*)), which represent translation as uni-directional movement of ribosomes on a 1D lattice, and can predict the translation flux and ribosome density profile on the mRNA. The work in (*157*) used dependence of the overall translation flux on the *de novo* translation initiation rate (α in Fig. 8A) to characterize how generic ribosome recycling with constant rates impacts translation. To interrogate the role

of Pab1, we will add to the model binding between Pab1 and TIC/eRF3 and computationally test their impact on translation (again using dependence of translation flux on α). The interactions will be parametrized by binding measurements (*91, 93, 152, 155, 160-162*). The Pab1-TIC complex will promote translation initiation and re-initiation, and the Pab1-eRF3 complex will promote translation termination (Fig. 8B, dotted arrows). Particularly, including multiple Pab1 molecules bound to the poly(A) tail (Fig. 8B) will also allow us to predict the relationship between poly(A) tail length (~ number of Pab1 loaded) and translation flux. Genes with vastly different translation kinetics may not be influenced by Pab1 in the same way; to understand such differential impacts, we will explore the model behaviors on the ***global parameter space***. To make ***biologically relevant exploration***, we will leverage the parameter sets given in a recent work (*163*), which fit a TASEP model to genome-wide ribosomal density profiling data to infer parameters of translation kinetics for individual genes.

Finally, as the model in Aim 2.1 is established, we will link the translation model with the model from Aim 2.1 to ***explore the role of Pab1/poly(A) in orchestrating mRNA translation and decay*** (Fig. 8B). To address this question in contexts relevant to the previous experimental reports (*21, 28-32*), we will examine how varying deadenylase activity affects the translation flux, and how varying transcription elongation rate (related to codon optimality) or translation initiation rate affects the deadenylation and decay kinetics. We will explore these effects over the ***global parameter space***, using parameter combinations representing fast/slow deadenylation and fast/slow translation. The model will be assessed and revised via comparing its results to genome-wide data on poly(A) tail length distribution (*13, 14, 32, 164*), decay-translation coupling (*21, 28, 29*), decay kinetics (*165-167*) and translation kinetics parameters (*163*) in yeast.

Expected outcomes

The study in this aim will create a mechanistic model framework for Pab1-mediated regulation of mRNA, based on known architectures of the relevant molecular machineries and biophysical/biochemical measurements. The study will take a modular approach, starting from testing hypotheses about the dynamic interactions between poly(A) and Pab1, and eventually growing towards the multiplex coupling between Pab1-poly(A) dynamics, deadenylation, decay and translation. Exploration of the model behaviors on the global parameter space will distill the general principles and constraints in Pab1-mediated mRNA regulation, which can be tested by future experimental studies. As the work progresses, the PI will actively seek experimental collaboration to test the model predictions. Overall, the research will provide mechanistic insights into the central mediator role of Pab1-poly(A) in controlling mRNA stability and translatability.

Pitfalls and alternatives

Study in Aim 2.1 may conclude that both hypotheses work with comparable efficacy. This would indicate a hybrid mode combining the two hypothetical mechanisms. In this case, we will combine the two models into one for Pab1-poly(A) binding dynamics in the hybrid mode, and use the new model in the remaining study of Aim 2. TASEP models are stochastic by nature, and can be computationally costly (*168*). If the computation time is too long in our large-scale parameter sweeping, we will use a deterministic substitute model for the translation part (*168*). In cases where stochastic properties are of interest, we will run the model on a small number of representative parameter sets that span over the range of genome-wide datasets.

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