States of decay: The systems biology of mRNA stability

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States of decay: The systems biology of mRNA stability

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Abstract

An appropriate equilibrium between transcription and mRNA decay is vital for the function of the cell. The RNA-binding complexes regulating mRNA degradation, such as carbon catabolite repression 4-negative on TATA-less, may also control several other stages of the mRNA life cycle, from transcription to translation. This pleiotropic control complicates the analysis of mRNA stability. Computational models have analysed the mechanisms underlying mRNA turnover and have been used to extract mRNA decay rates from high-throughput data sets. Multiomics studies have clarified the actions of RNA-binding complexes, and such studies allow the evolution of more accurate and complex computational models. This review discusses two complementary aspects of systems biology in the study of mRNA decay—computational modelling of mRNA turnover and recent '-omics' studies of the function of RNA-binding proteins controlling mRNA stability.

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mRNA decay, Deadenylation, Computational modelling, Multiomics, RNA binding proteins, CCR4-NOT, PUF3.

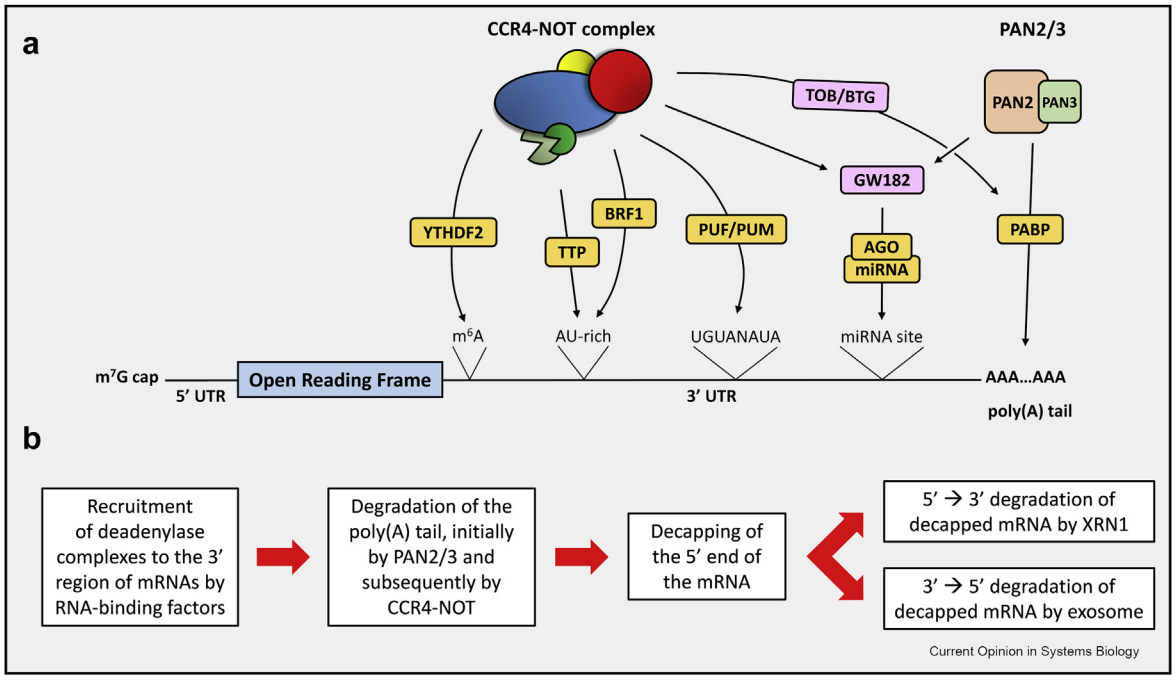
Introduction

Maintaining correct levels of mRNA expression, achieved by the balance between mRNA synthesis and decay, is crucial to the correct function of the cell. While transcription is undoubtedly important for mRNA synthesis, tightly regulated degradation facilitates rapid or localised changes in mRNA availability in response to external stimuli. In humans, dysregulation of mRNA stability may underlie aspects of diseases such as obesity [1], Alzheimer's disease [2], and autism spectrum disorders [3].

Exonuclease-mediated mRNA degradation begins with shortening of the poly(A) tail, and deadenylation is considered the rate-limiting step in mRNA degradation. Two main deadenylase complexes mediate deadenylation, the poly(A)-nuclease deadenylation complex (PAN2/3) and the carbon catabolite repression 4-negative on TATA-less (CCR4-NOT) complex. The deadenylase complexes are recruited to their targets by a variety of RNA-binding factors (Figure 1A). Pan2/3 is recruited by poly(A)-binding protein (PABP) [4], while CCR4-NOT is recruited via a number of proteins binding to elements mainly located in the 3′ untranslated region (3′ UTR) of the mRNA, including Pumilio (PUF/PUM) [5], the AU-rich element-binding proteins tristetraprolin (TTP/Zfs1) [6] and butyrate response factor 1 [7], and the 6-methyl-adenosine (m^6A)—binding protein YTHDF2 [8]. Transducer of ErbB2/B-cell translocation gene family proteins may recruit CCR4-NOT to target mRNAs indirectly via interactions with PABP [9]. Both Pan2/3 and CCR4-NOT are also recruited via miRNAs and Argonaute proteins and GW182 [10,11]. PAN2/3 is thought to act on longer poly(A) tails and catalyses an initial deadenylation step, before further poly(A) shortening by the CCR4-NOT complex (Figure 1B) [12,13]. Degradation of the poly(A) tail triggers the removal of the 5′ 7-methylguanosine cap from the 5′ end of the mRNA by decapping enzymes such as DCP2. The decapped mRNA is then degraded from the 5′ end by 5′-3′ exoribonuclease 1 (XRN1) or from the 3′ end by the exosome. For a more detailed overview of mRNA degradation, see the study by Schoenberg and Maquat [14].

Systems biology provides us with powerful tools with which to reveal the complex mechanisms underlying mRNA stability. This review discusses two related aspects of systems biology—computational modelling of mRNA decay and recent '-omics' studies of the RNA-binding proteins (RBPs) regulating this process. Computational modelling has been used for two major purposes in the study of mRNA decay. Firstly, mechanistic modelling of mRNA deadenylation and decay pathways has allowed analysis of the underlying system dynamics. Secondly, mRNA decay rates have been extracted from time-series gene expression data through

Figure 1



The mechanism of mRNA decay. (a) The deadenylase complexes PAN2/3 and CCR4-NOT are recruited to their mRNAs by a number of RNA-binding factors. Factors directly binding to mRNA are shown in orange; factors interacting indirectly are in pink. Pan2/3 is recruited by poly(A)-binding protein (PABP), while CCR4-NOT is recruited via a number of proteins recognising elements mainly located in the 3′ untranslated region (3′ UTR), including Pumilio (PUF/PUM), tristetraprolin (TTP/Zfs1), butyrate response factor 1 (BRF1) and the 6-methyl-adenosine (m6A)–binding protein YTHDF2. Transducer of ErbB2/B-cell translocation gene (TOB/BTG) family proteins may recruit CCR4-NOT to mRNAs via interactions with PABP. Both Pan2/3 and CCR4-NOT are also recruited via miRNAs and Argonaute (AGO) proteins and GW182. For clarity, not all interactions of CCR4-NOT and PAN2/3 are shown. **(b)** A schematic representation of the process of mRNA decay. The poly(A) tail is initially degraded by PAN2/3 and subsequently by CCR4-NOT. Degradation of the poly(A) tail triggers the removal of the 5′ 7-methyl-guanosine cap from the 5′ end of the mRNA by decapping enzymes such as DCP2. The decapped mRNA is then degraded from the 5′ end by 5′-3′ exoribonuclease 1 (XRN1) or from the 3′ end by the exosome. CCR4-NOT, carbon catabolite repression 4-negative on TATA-less.

sophisticated modelling techniques. Examples of these two applications are first reviewed.

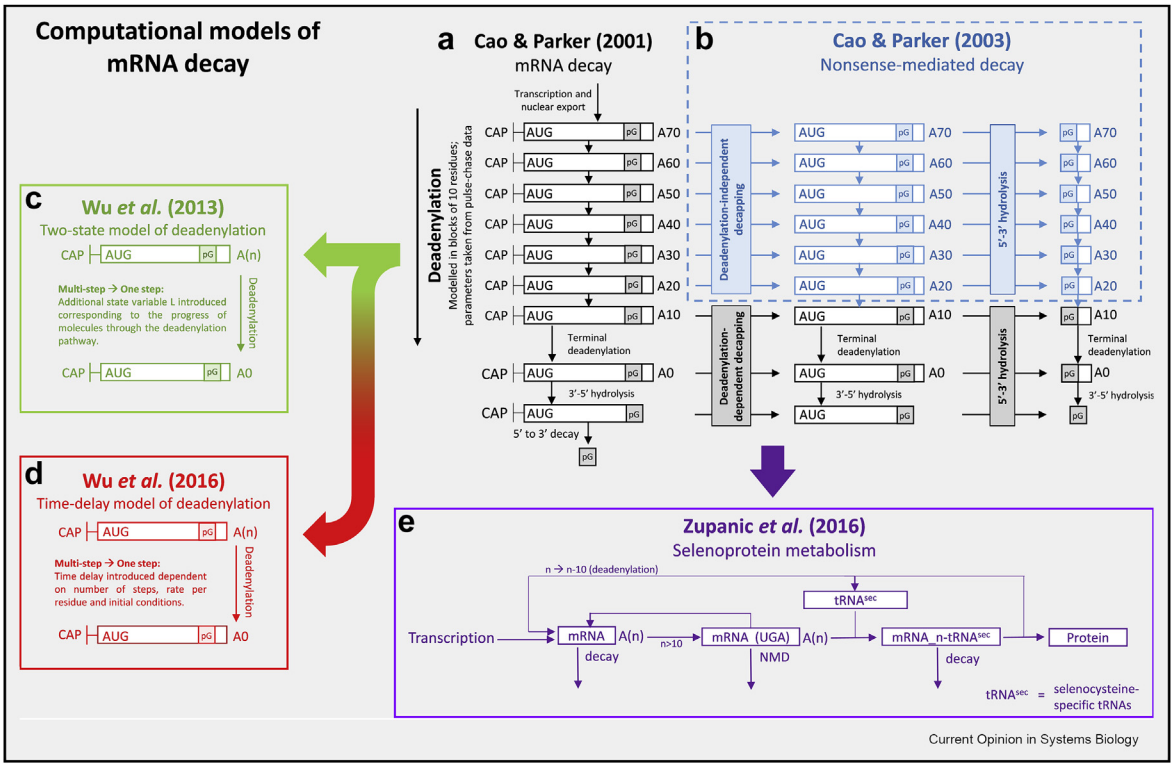
Mechanistic computational modelling of RNA decay and deadenylation

A set of related computational models have explored the dynamics of mRNA decay. Cao and Parker [15] produced a detailed mathematical model of cytoplasmic mRNA turnover with a focus on deadenylation-dependent decay through both decapping and terminal deadenylation (Figure 2A). Simple representations of transcription, nuclear export, and deadenylation-independent decapping are also included. The 21 parameters in the model were rigorously set using experimental data for both a stable mRNA (MFA2pG) and an unstable mRNA (PGK1pG), and simulations were validated against experimental data for the half-life and the relative ratios of full-length mRNAs and 3′ and 5′ fragments. Sensitivity analysis on the model demonstrated, firstly, that deadenylation is the rate-limiting step in mRNA decay. Secondly, perturbations in the rates of different steps in the decay process had variable effects for stable and unstable mRNAs in the model.

In a second study, Cao and Parker [16] extended the model to include pathways mediating decay of nonsense codon–containing mRNAs (Figure 2B). Nonsense-mediated decay is the mechanism that eliminates mRNAs carrying premature termination codons, which arise from errors in transcription [14]. Experimental observations indicate that deadenylation and decapping may be decoupled in nonsense-mediated decay [17,18]. Therefore, deadenylation-independent decapping was included in the model for nonsense-mediated decay in addition to accelerated rates of deadenylation and decapping. In this case, *PGK1* and *HIS4* mRNAs were used for parameter fitting. The simulations suggested that the proximity of the nonsense codon to the poly(A) tail determines the steady-state distribution of poly(A) tail lengths.

Tian et al. [19–21] investigated different methods of simplifying the multistep deadenylation reaction in Cao

Figure 2



Simplified representations of published computational models of mRNA deadenylation and decay. Cao and Parker [15] produced a model of mRNA deadenylation followed by either deadenylation-dependent decapping and decay or terminal deadenylation (**a**; black). The model additionally incorporates equations representing transcription, nuclear decay and nuclear export not shown in the figure. Cao and Parker [16] subsequently expanded this model to include deadenylation-independent decapping in nonsense-mediated decay (NMD; **b**; blue). A two-state model of deadenylation developed by Wu et al. [19] allowed Cao and Parker's multistep reaction to be reduced to a one-step reaction with comparable predictive capacity (**c**; green). The state variable corresponding to the number of molecules in the reaction is supplemented by a second variable corresponding to the progress of each molecule through the multistep reaction. More recently, Wu et al. [20] presented an alternative simplification of Cao and Parker's model which also reduces the multistep deadenylation reaction to a one-step reaction, this time through the inclusion of a time delay (**d**; red). A model of selenoprotein metabolism developed by Zupanic et al. [22] includes an adapted version of Cao and Parker's model of deadenylation (**e**; purple). In this model, NMD is in competition with binding of selenocysteine-specific tRNAs to mRNAs containing a premature stop codon (UGA), followed by translation via selenocysteine-specific mechanisms.

and Parker's model. Firstly, rather than simulating intermediate stages of deadenylation, an additional state variable was introduced corresponding to the progress of molecules through the multistep reactions [19] (two-state model; Figure 2C). A stochastic two-state equation is then used, which is dependent on the total number of molecules in the system, the progress of molecules through the multistep process and the number of steps. Secondly, Wu and Tian reduced the multistep deadenylation reaction to an alternative single reaction through use of a state-dependent time delay [20] (time-delay model; Figure 2D). A previous study by Tian [21] had demonstrated that a range of state-independent time-delay equations was ineffective at simulating mRNA decay. To improve on these, a stochastic formula for calculation of the state-dependent time delay was derived on the basis of the number of intermediate steps in the system, the degradation rate, and the initial state of the system. The accuracy of the models was assessed through comparison with experimental data for the decay of two constructs of *RPL30* after addition of transcription inhibitor 1,10-phenanthroline. Both models were more accurate than a first-order degradation model over the first 45 min after transcription inhibition, as assessed by the absolute error, and gave simulated decay rates comparable to those generated by a stochastic multistep model based on Cao and Parker's deterministic model. However, only the time-delay model was able to accurately reproduce the experimental data at longer times, where the two-state model simulations began to deviate.

Zupanic et al. [22] integrated various mRNA decay submodels into a detailed model of selenoprotein synthesis. Simulations were compared with experimental data for selenoprotein RNAs measured in Caco-2 cells grown in a range of sodium selenite concentrations. A Bayesian information criterion was used to assess model

fit to account for the number of parameters. Compared with a range of simplified models, a detailed representation of deadenylation-dependent mRNA decay based on Cao and Parker's model gave the best fit to the experimental data, highlighting the importance of deadenylation in regulation of selenocysteine metabolism (Figure 2E).

The models of RNA decay developed by Cao and Parker remain the most detailed published to date and are an important resource for studying the underlying mechanisms of deadenylation and decay. As discussed in the following sections, recent and ongoing studies are elucidating the molecular mechanisms underlying mRNA decay, allowing for further expansion and refining of these models in the future. The study by Zupanic et al. highlights the importance of a detailed representation of mRNA decay and deadenylation for studying selenocysteine metabolism. In contrast, reduced models such as those developed by Wu et al. are vital for researchers looking to simulate specific outputs with a similar level of accuracy to the complete models but without requiring information regarding intermediate steps. This allows the simulations to be run with less computational power and requires fitting of fewer parameters.

Deriving mRNA decay rates from transcriptomic data

Computational modelling has been used to infer mRNA decay rates from gene expression data. As discussed by Palumbo et al. [23], a zeroth-order dependence of transcription and a first-order dependence of decay on mRNA level give a good approximation of RNA turnover. For an RNA with concentration $x(t)$, time-dependent transcription rate T and decay rate constant k_d:

$$\frac{dx(t)}{dt} = T(t) - k_d x(t)$$

Historically, decay rates have been calculated either through inhibition of transcription or through mRNA labelling (reviewed in Ref. [24]). In the case of transcription inhibition ($T = 0$), the equation simplifies such that mRNA half-lives and decay constants can be extracted from the experimentally measured exponential decay curves:

$$\frac{dx(t)}{dt} = -k_d x(t); \quad x(t) = x(0)e^{-k_d t}$$

However, when Cacace et al. [25] performed correlation analysis between experimental studies of mRNA degradation rates performed by different groups after inhibition of transcription, they found no correlation between data sets using different methods of transcription inhibition and only moderate correlation between studies using the same method of inhibition. This is at least partly due to sensitivity of decay rates to changes in cell physiology [26] and coupling between transcription and decay [27,28].

A number of studies have combined sophisticated computational and experimental techniques to determine transcription and decay rates without the requirement for major manipulation of cellular physiology (recently reviewed in Ref. [24]). Among these, three major computational pipelines exist [29–31], each based on time-series RNA-seq measurement of RNA levels and 4sU-seq measurement of newly transcribed RNAs (see Box 1 for definitions of -omics terms). Firstly, 'dynamic transcriptome analysis' (DTA) calculates decay constants for each mRNA by assuming constant rates of transcription and decay over short measurement times after labelling (3, 6, 12 and 24 min) [29]. 'Dynamic RNA life cycle' (DRiLL) [30] and 'Inference of Synthesis, Processing and Degradation Rates in Time-Course Analysis' (INSPEcT) [31], meanwhile, assume negligible degradation of 4sU-labelled RNA over short measurement times, such that the transcription rate is directly taken from the 4sU-seq data. DRiLL and INSPEcT both use similar modelling strategies. DRiLL firstly uses a binomial model to estimate the abundances of precursor and mature mRNAs from the RNA-seq data [30]. Next, a kinetic model infers transcription, pre-mRNA processing and mature RNA degradation rates from the time-series measurements. Parameters are determined by gradient descent optimisation. INSPEcT follows a similar framework, additionally testing different models (by default sigmoid and impulse) and parameter sets to identify the best fit to the RNA-seq data, determined by minimisation of residual sum of squares [31].

Rather than using 4sU labelling, Wang et al. [32] demonstrated that mRNA stability can be predicted from measurements of histone modifications together with gene expression data using a computational regression model. mRNA levels were fitted as a linear combination of three different histone modifications. The residuals between the model predictions and experimental RNA-seq data strongly correlated with experimentally determined mRNA half-lives, allowing estimation of decay rates without 4sU labelling.

Computational studies have also included coupling between transcription and decay in analysis of gene expression data. Transcription-decay coupling occurs both as a buffering system, where gene induction promotes RNA degradation [33], and as a cooperative system, where gene induction promotes RNA stability causing rapid change [34].

Box 1. Glossary of abbreviations of computational/-omics terms

4sU-seq: Labelling of newly transcribed RNA with a uridine analogue, 4-thiouracil (4sU/4-SU). Labelled RNA may then be biotinylated and isolated for high-throughput sequencing (HTS).

BioID: Proximity-dependent **bio**tin **id**entification. A bait protein is fused to a biotin ligase to label any protein within a 10-nm radius. Biotinylated proteins are then be purified for identification by mass spectrometry.

DRiLL: Dynamic RNA life cycle. A computational method for deriving mRNA transcription and decay rates from time-series RNA-seq and 4sU-seq data. See also cDTA and INSPEcT.

DTA: Dynamic **t**ranscriptome **a**nalysis. A computational method for deriving mRNA transcription and decay rates from RNA-seq and 4sU-seq data. A modified version, comparative DTA (**cDTA**), allows absolute quantification of mRNA synthesis and decay rates, and therefore more accurate comparison between samples. See also DRiLL and INSPEcT.

GRO-chip: Global **r**un-**o**n. Nuclei are isolated and then nascent transcripts are extended *in vitro* using labelled nucleotides. Analysis of labelled transcripts allows global quantification of transcription. May also be analysed by HTS (GRO-seq).

HITS-CLIP: High-**t**hroughput **s**equencing of RNA isolated by **c**ross**l**inking **i**mmuno**p**recipitation (aka CLIP-seq). A more stringent version of RIP-seq; see also PAR-CLIP.

INSPEcT: Inference of Synthesis, Processing and Degradation Rates in Time-Course Analysis. A computational method for derivation of mRNA transcription and decay rates from transcriptomics data. See also cDTA and DRiLL.

OOPS: Orthogonal **o**rganic **p**hase **s**eparation. A method for isolating RNA-binding proteins and their targets. Crosslinked RNA-protein adducts are isolated from the aqueous–organic interface of acidic guanidinium-thiocyanate-phenol-chloroform (AGPC). May be particularly useful for nonadenylated RNAs.

PAR-CLIP: Photo**a**ctivatable **r**ibonucleoside-enhanced **c**ross**l**inking and **i**mmuno**p**recipitation. A method using photosensitive nucleosides (e.g. 4sU) to improve RBP-RNA crosslinking efficiency before immunoprecipitation. Similar to HITS-CLIP.

Ribo-seq: (Also ribosome footprinting, ribosome profiling). The mRNA is digested, leaving just the fragments protected by bound ribosomes. Sequencing these fragments by HTS and mapping the ribosome locations to the transcriptome allows analysis of various aspects of translation.

RIP-chip: RNA immunoprecipitation (RIP). Immunoprecipitation of an RNA-binding protein of interest in association with its target RNAs, followed by identification of the bound RNAs by microarray.

RIP-seq: RNA immunoprecipitation (RIP) followed by RNA-seq. Similar to HITS-CLIP/PAR-CLIP, the absence of a crosslinking step simplifies the protocol, but the probability of false positives/negatives may be increased.

RNA-seq: Transcriptomic analysis of total RNA using HTS.

RNA-tagging: A protein of interest is fused to the *C. elegans* poly(U) polymerase, pup2. RNAs bound by the fusion protein are tagged at the 3′ end with a string of uridines, allowing isolation and analysis by HTS.

TAIL-seq: Transcriptome-wide sequencing of mRNA 3′ ends, allowing global measurement of poly(A) tail length. A biotinylated adapter is ligated to the 3′ end of the mRNA, the mRNA is fragmented and a second adapter is ligated to the 5′ end. Fragments corresponding to the 3′ end of mRNAs are then isolated using the biotin-tagged 3′ sequence for analysis by HTS.

Farina et al. [35] applied methods developed to study lead–lag relationships in systems and control engineering to gene expression data to identify common regulatory signals between genes taking account of both transcriptional and post-transcriptional regulation. Cacace et al. [25] subsequently developed a stochastic model of coordinated transcription and RNA decay during the reproductive and metabolic cycle of budding yeast. The modelling is based on comparison of all gene pairs to determine common promoter activity and gene-specific activity. Starting from the zeroth-order transcription/first-order decay equation described previously, the authors modelled the transcription rate of all pairs of genes as the sum of a common term between those genes and a stochastic independent term. The linear equations were then converted to discretised

stochastic equations for comparison with experimental measurements at discrete time points.

Sun et al. [36] modified the DTA modelling framework to account for changes in decay and translation rates. Gene-specific transcription and decay rates were multiplied by functions representing global modulation of transcription and decay. Global stabilisation of RNA levels upon changes in transcription or degradation was demonstrated through measurement of global mRNA synthesis and expression under steady-state conditions in wild-type, Pol II mutant (low transcription) and Δ*Ccr4* and Δ*Caf1* (low degradation) yeast and analysis in their modelling framework. Subsequently, by performing a large-scale DTA screen on a wider range of genes involved in transcription and decay, Sun et al. [37] identified Xrn1 as a key regulator of RNA buffering, additionally confirmed by Haimovich et al. [38]. However, owing to inconsistencies in the effects of Xrn1 knockdown in the two studies, a comprehensive model of the molecular mechanisms involved has yet to be developed [39].

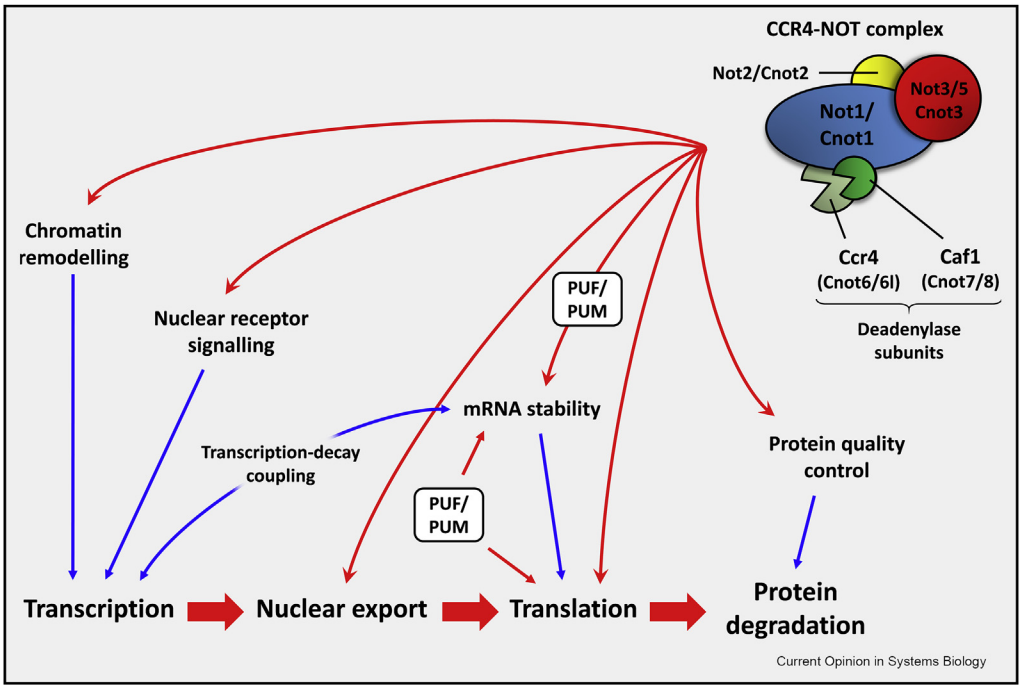
A complete picture of the mechanisms of mRNA homoeostasis requires understanding the function of the highly conserved RBP complexes regulating mRNA stability and decay. In the second part of this review, we discuss recent studies that have used '-omics' approaches to probe the functions of RBPs in regulating mRNA stability and translation.

Multiomics analyses of the deadenylase subunits of the CCR4-NOT complex

The CCR4-NOT complex is a major regulator of mRNA stability in eukaryotes via deadenylation, but the complex is also involved in other cellular processes, including transcription, mRNA nuclear export, translation and protein quality control (Figure 3; [40,41]). CCR4-NOT's pleiotropic control of mRNA from transcription to decay makes it an ideal candidate for integrated multiomics studies.

A recent study used formaldehyde cross-linking to preserve RBP–RNA interactions followed by RIP-seq to identify RNAs bound to three interacting RBPs in *Saccharomyces cerevisiae*: the CCR4-NOT deadenylase subunit Ccr4 (Cnot6/6L in vertebrates), Dhh1 and Puf5 [42]. Unsurprisingly, the targets of the three proteins overlapped considerably (68% of Ccr4-bound mRNAs also interacted with Dhh1 or Puf5; 28% of mRNAs interact with all three proteins), indicating that they

Figure 3



The CCR4-NOT complex regulates the mRNA life cycle at multiple levels. The CCR4-NOT complex is a major deadenylase in eukaryote cells, stimulating mRNA decay via removal of the poly(A) tail. In addition to deadenylation, evidence suggests that CCR4-NOT exerts control of mRNA at several other levels, including transcription, nuclear export, translation and protein quality control. Other RNA-binding proteins also regulate mRNA function at multiple levels. For example, Pumilio/Puf family proteins (PUF/PUM) regulate mRNA stability via CCR4-NOT–dependent and CCR4-NOT–independent pathways and may also affect translation without altering mRNA stability. Red arrows show potential direct effects of CCR4-NOT and PUF/PUM, while blue arrows indicate indirect effects. Not all subunits of CCR4-NOT are shown. CCR4-NOT, carbon catabolite repression 4-negative on TATA-less.

coregulate many of the same mRNAs, predominantly low-abundance transcripts. Mapping the regions of each mRNA bound by the RBPs revealed that Ccr4 bound to 45% of target mRNAs via the coding region or 5′ UTR, rather than the 3′ UTR. This could merely reflect interactions between proteins bound to the 5′ and 3′ ends of the mRNA, but Dhh1 and particularly Puf5 showed a greater affinity for the 3′ UTR. CCR4-NOT is recruited to mRNAs by RBPs such as tristetraprolin and butyrate response factor 1 [40] (Figure 1), and Ccr4-associated mRNAs were enriched for Puf binding sites, suggesting that Puf5 may recruit CCR4-NOT. In agreement with other studies [43], Ccr4/Dhh1 bound to mRNAs involved in responses to nutrient deficiency and metabolism.

There is some evidence that CCR4-NOT regulates transcription [44]. However, integration of RIP-seq data with mRNA synthesis and decay rates from cDTA and GRO-chip found a correlation between Ccr4 binding and degradation, but not transcription [42]. Furthermore, in genes with introns, very few intronic sequences were associated with Ccr4. Together, these data suggest that Ccr4 mainly influences mRNA levels in the cytoplasm at the level of mRNA decay. Ribosome footprinting (Ribo-seq) found that mRNA decay factor recruitment to mRNA was inversely correlated with ribosome occupancy, indicating that RBP binding suppresses translation [42].

Using a different approach, Webster et al. [45] used a temperature-sensitive Pol II allele in yeast to halt transcription globally, followed by time-series RNA-seq measurements to calculate transcriptome-wide mRNA stability. Knockout of the CCR4-NOT deadenylase subunit Caf1 (Pop2; Cnot7/8) stabilised mRNAs containing less optimal codons, which are translated more slowly. *In vitro* assays showed that nonoptimal mRNAs were deadenylated more rapidly by Caf1 than optimal mRNAs. Earlier studies had also indicated that codon optimality and translation rate are important factors in mRNA stability [46, 47], although this view has been challenged more recently [48]. In contrast, Ccr4 regulated mRNA stability more generally. This agrees with previous work suggesting that Ccr4 is the dominant deadenylase CCR4-NOT subunit in yeast [49], with Caf1 fulfilling a more specialised role. However, this may not be the case in higher eukaryotes, for example, Caf1 is thought to be the dominant deadenylase in *Drosophila* [50]. The respective roles of the CCR4-NOT deadenylase subunits have also been investigated in human HeLa cells [51]. Transcriptome-wide poly(A) tail length measurements using TAIL-seq showed that *CNOT7/8* knockdown increased poly(A) length and stabilised mRNA globally, whereas knockdown of the other major deadenylase, *PAN2/3*, had little effect, suggesting that CCR4-NOT is the dominant regulator of mRNA deadenylation and stability. In yeast too, Ccr4-bound mRNAs were not generally affected by Pan2/3, suggesting that the two complexes may target separate subsets of mRNAs [42]. In HeLa cells, PABPC1 stimulated deadenylation by CNOT6/6L, but inhibited CNOT7/8. Conversely, the absence of PABPC1 suppressed CNOT6/6L deadenylase activity, while CNOT7/8 activity increased. Despite this difference, CNOT6/6L and CNOT7/8 were able to compensate for deadenylase-deficient mutants of the other subunit.

Probing the interactome of mRNA-processing protein assemblies

Many factors involved in mRNA processing, translation and degradation colocalize in stress granules (SGs) and processing bodies (PBs). Youn et al. [52] used proximity-dependent biotin identification (BioID [53]) to investigate protein associations within SGs and PBs in human HEK293 cells. The interactions of 119 SG-/PB-associated bait proteins were analysed, identifying 144 core components of RNA granules and over 7400 unique interactions. In PBs, CCR4-NOT bait proteins identified 72 high-confidence interactions with many known RBPs, but also proteins with unknown roles in RNA metabolism, including the E3 ubiquitin ligase RNF219, the centrosomal protein CEP85 and the uncharacterised KIAA0355. These proteins also showed strong associations with the RBPs Argonaute 2 and GW182. In addition, BioID data showed strong evidence for interactions between the RNA-binding E3 ubiquitin ligase CNOT4 and CCR4-NOT in human cells, challenging the current view that CNOT4 does not associate with the complex in vertebrates, although its homologue in yeast, Not4, is considered a CCR4-NOT subunit [54]. This study indicated a modular structure within mRNA granules, with proteins predominantly associating with others of similar function.

Multiomics clarifies the role of Puf3 in mitochondrial function

Similar to CCR4-NOT, the PUF (Pumilio) family of RBPs regulates both mRNA stability and translation [55]. RIP-seq identified over 1000 Puf3-bound mRNAs in *S. cerevisiae* [56], and comparison of these data with previous RIP-chip [57] and PAR-CLIP [58] data sets identified a common ‘core’ set of Puf3 targets. Comparison of steady-state mRNA levels by RNA-seq found few differences in Puf3-bound mRNAs between WT and Δ*Puf3* cells, although mRNA decay and synthesis were not assessed. Almost all the mRNAs upregulated in Δ*Puf3* cells encoded mitochondrial proteins. Mass spectrometry detected 662 proteins encoded by Puf3-bound mRNAs, but surprisingly, only 26 proteins were differentially expressed in Δ*Puf3* cells. Around half of the affected proteins had not shown altered mRNA levels, supporting previous observations that Puf3 may regulate translation independently of mRNA stability

[55]. RNA-seq analysis of ribosome-associated mRNA suggested that Puf3 deletion alters mRNA association with ribosomes, further indicating effects on translation.

More recently, a second study has investigated the role of Puf3 in yeast. RNA-seq data from two different methods, HITS-CLIP [59] and RNA-tagging [60], were integrated to produce a shortlist of 269 high-confidence Puf3 targets [61]. Motif quality appears important for determining stability of Puf3 target mRNAs [62] and sequence analysis of this shortlist showed an enrichment of high-affinity PUF family binding sites, increasing confidence that it represents direct Puf3 targets. As in the earlier study [56], Lapointe et al. [61] found only 24 proteins differentially expressed in Δ*Puf3* cells. However, Puf3 deletion affected many more proteins (160) in cells under fermentation conditions. Of these, 91 were encoded by Puf3-bound mRNAs and were enriched for mitochondrial functions and translation. Proteins whose abundance was Puf3 dependent, but whose mRNA was not associated with Puf3, were also predominantly mitochondrial, with roles in oxidative phosphorylation and the electron transport chain. One specific target was Coq5, overexpression of which in Δ*Puf3* cells suppressed coenzyme Q (CoQ) synthesis. Lipidomic analysis by mass spectrometry confirmed that *Puf3* deletion and *Coq5* overexpression similarly altered levels of CoQ intermediates. Data from a prior MS-based multiomics screen of mutant yeast strains established that Puf3-deficient cells are CoQ deficient in fermentation conditions [63] and the derepression of Coq5 by loss of Puf3 provided the mechanism. The multiomics approach in this study identified with high confidence a fundamental control over mitochondrial function by the direct repressive action on target mRNAs by Puf3.

Concluding remarks

The multilevel regulation of the mRNA life cycle [64] complicates the analysis of RBPs. In addition, novel techniques such as orthogonal organic phase separation have identified many previously unknown RNA–protein interactions [65]. The studies described previously show that a multiomics approach can clarify at which level RBPs are exerting their control. Computational modelling has been used both to extract mRNA decay rates from high-throughput data sets and to analyse the underlying mechanisms involved in deadenylation and decay. Owing to a previous paucity of data, existing computational studies of mRNA turnover have modelled at the level of overall processes rather than the specific molecular mechanisms. Furthermore, there have been no attempts to introduce coupling between transcription and decay into these models. Data from the ongoing multiomics studies and sophisticated analysis of high-throughput data will allow more detailed computational models of mRNA turnover and, in turn, a better understanding of regulation of gene expression.

Conflict of interest statement

Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest

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