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Enhanced gene regulation by cooperation between mRNA decay and gene transcription☆

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Abstract

It has become increasingly clear in the last few years that gene expression in eukaryotes is not a linear process from mRNA synthesis in the nucleus to translation and degradation in the cytoplasm, but works as a circular one where the mRNA level is controlled by crosstalk between nuclear transcription and cytoplasmic decay pathways. One of the consequences of this crosstalk is the approximately constant level of mRNA. This is called mRNA buffering and happens when transcription and mRNA degradation act at compensatory rates. However, if transcription and mRNA degradation act additively, enhanced gene expression regulation occurs. In this work, we analyzed new and previously published genomic datasets obtained for several yeast mutants related to either transcription or mRNA decay that are not known to play any role in the other process. We show that some, which were presumed only transcription factors (Sfp1) or only decay factors (Puf3, Upf2/3), may represent examples of RNA-binding proteins (RBPs) that make specific crosstalk to enhance the control of the mRNA levels of their target genes by combining additive effects on transcription and mRNA stability. These results were mathematically modeled to see the effects of RBPs when they have positive or negative effects on mRNA synthesis and decay rates. We found that RBPs can be an efficient way to buffer or enhance gene expression responses depending on their respective effects on transcription and mRNA stability.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Keywords

mRNA buffering; Crosstalk; Transcription; mRNA decay; Yeast; Gene regulation

1. Introduction

It has been postulated that crosstalk between transcription and degradation machineries acts to control the mRNA concentration ([mRNA], [1-3]. This crosstalk has two branches, transcription to degradation (direct: from the nucleus to the cytoplasm), and degradation to transcription (reverse: from the cytoplasm to the nucleus; see Fig. 1), which is an example how gene expression works in a circular rather than in a linear fashion [4,5]. The mechanisms and functions of both directions can differ [6]. For the nucleus-cytoplasm direction, the mechanism has been demonstrated as the co-transcriptional imprinting [7] of mRNAs with RNA-binding proteins (RBPs). mRNA imprinting has been shown in the yeast *Saccharomyces cerevisiae* for: RNA polymerase II subunits Rpb4/7 [8-11]; basal transcription machinery factor Taf7 [12]; Hst3 histone deacetylase [13]; the Ccr4-NOT complex [14,15]; specific decay factors Cth2 [16]; Puf3 [17]. Imprinting RBPs should return to the nucleus later. This return to the nucleus can be used as a reverse direction mechanism (Fig. 1, see below). Note that most of these RBPs have been originally described as part of either transcriptional machinery (Rpb4/7, Taf7) or degradation pathways (Cth2, Puf3), or of both (Xrn1, Ccr4-Not). mRNA imprinting can have compensatory effects by increasing or decreasing synthesis and decay rates) and, at the same time, causing mRNA buffering. Buffering can be global by acting on the whole mRNA concentration ($[mRNA]_t$) or being restricted to a subset of genes depending on the fraction of the transcriptome bound by RBPs.

Global $[mRNA]_t$ buffering has been demonstrated to occur when subunits of transcriptional or decay machineries are depleted. In the yeast mutants that lack some decay subunits, increased mRNA stabilities (decreased decay rates) are balanced with lower global mRNA synthesis rates, which maintain the steady-state levels of most mRNAs stable [2,4,13]. Similarly, in other yeast mutants that lack subunits of transcription complexes, lowered synthesis rates are balanced by increasing mRNA stabilities [18]. The molecular mechanisms underlying this balancing process are not well understood. As previously explained, direct crosstalk may be based on mRNA imprinting with the RBPs that modulate mRNA stability later in the cytoplasm [7]. Reverse crosstalk needs a mechanism to not only send information from the cytoplasm to the nucleus (see below), but to also convert this information into a transcriptional effect. One possible mechanism for this is the regulation of transcription elongation by RBPs. Slobodin et al. [19] found that regulation at this level impacts the length of poly(A) tails and the expression of decay machinery (Ccr4-NOT) as a mechanism for $[mRNA]_t$ buffering. The effect of the Ccr4-NOT complex as a stimulator of transcription elongation has also been demonstrated in yeast [15,20]. We also found that the effect of Xrn1 on synthesis rates in yeast is executed, at least in part, by favoring the elongation rates of RNA polymerases [4] in parallel to Ccr4-NOT [21]. This occurs by preventing backtracking [22], especially near the poly(A) site [23,24].

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The reverse crosstalk direction also needs a mechanism to send information from the cytoplasm to the nucleus. This can be achieved by the nuclear import of the cytoplasmic proteins coupled with the direct crosstalk direction to bring them back to the cytoplasm. This idea has been developed in the models proposed by B. Glaunsinger and T. Jensen [25,26]. These models propose poly(A)-binding proteins as sensors of the level of cytoplasmic poly(A) mRNAs. Interestingly, the reverse crosstalk in the study of Gilbertson et al. [25] in mammalian cells does not act by activating transcription as a buffering system to compensate for an increase in mRNA decay but, on the contrary, to enhance the drop in most mRNA levels. This example shows that transcription/degradation crosstalk may be not only compensatory for mRNA buffering, but also additive to reinforce a primary regulatory stimulus [27].

The consequence of crosstalk can also differ when acting on a limited number of mRNA targets. The crosstalk direction from the nucleus to the cytoplasm may have the effect of determining the future cytoplasmic life of some mRNAs according to which environmental circumstances they were synthesized in. For instance, the mRNAs transcribed during stress responses can be imprinted to become more stable to more quickly increase cytoplasmic levels [19] or, conversely, less stable to provoke a sharper response peak that reduces the cost of the response [10,28]. Sending information from the cytoplasm to the nucleus for a limited set of mRNAs has been shown in the phenomenon called genetic compensation in higher eukaryotes. During this process, the mRNAs bearing premature stop codons (PTCs) are degraded by the usual non sense-mediated decay (NMD) route for PTCs mRNAs, but at a higher SR for other sequence-related paralogous genes, which partially compensates their mRNA [29,30]. This can partly compensate the phenotypic defect caused by the primary mutation. This mechanism is not, however, universal because it does not apparently exist in *S. cerevisiae* [3].

We reasoned that in order to investigate the existence of both crosstalk directions in a limited set of mRNAs, it is necessary to study the factors that have been described to control a group of genes at either the transcriptional or the mRNA stability level. In this study, we used some new and previously published genomic datasets of several yeast mutant strains of the model eukaryote *S. cerevisiae*. These strains are depleted of proteins, previously known to be related to either transcription or mRNA decay, but not known to possess activity in the other process. Here we show that some RBPs, which are probably exported as being bound to mRNAs from the nucleus, seem to increase the synthesis and decay rates of their target mRNAs. This occurs when the protein is both a presumed transcription factor (Sfp1) or a presumed decay factor (Puf3, Upf2/3), and suggests that these RBPs make specific crosstalk to control the mRNA levels of their targets by combining transcriptional and mRNA stability effects. We also mathematically modeled the different possibilities for transcription-mRNA decay crosstalk by the RBPs bound co-transcriptionally in the nucleus. We corroborated that mRNA imprinting by RBPs is a very efficient way to buffer [mRNA] when RBPs have parallel (stimulating or inhibitory) effects on synthesis and decay rates. We also demonstrated that upon having an additive effect, the activity of RBPs at the transcription and post-transcriptional levels speeds up and enhances the response, which suggests that they could be useful as a regulatory mechanism for eukaryotic cells.

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2. Materials and methods

2.1. Yeast strains and culture media

The *sfp1Δ* yeast (the Y05352 strain from the Euroscarf collection: BY4741 *sfp1Δ*::KanMX4) and wild-type (BY4741: *Mat a leu2Δ*, *his3Δ*, *met15Δ*, *ura3Δ*) cells were grown in YPD medium (1 % yeast extract, 2 % peptone, 2 % glucose) at 30 °C. Pre-cultures were grown overnight. The next day, pre-cultures were diluted to OD_{600} = 0.05 to be grown in 250 mL flasks with agitation at 190 rpm until an OD_{600} of ~0.5 was reached. Cells were recovered by centrifugation and flash-frozen in liquid nitrogen. The yeast mutants analyzed in Fig. 2 are also from BY4741 background and were grown in similar conditions as described in the original publication by Sun et al. [2].

2.2. Genomic methods

Genomic run-on (GRO) in the *sfp1Δ* mutant was performed as described in [31]. Briefly, GRO detects, by macroarray hybridization, genome-wide active elongating RNA pol II, whose density per gene is taken as a measurement of its synthesis rate (SR). At the same time, the protocol allows the mRNA amounts (RAs) for all the genes to be measured. mRNA half-lives (HLs) are calculated as RAs/SRs by assuming steady-state conditions for the transcriptome.

2.3. Modeling

We start by first describing a simple model of gene expression that is later expanded to include an RBP as the crosstalk factor (CF). The model can be represented by the following reactions

$$Promoter \rightarrow RNAP \text{ (Binding of polymerase to the promoter)}$$

$$RNAP \rightarrow Promoter \text{ (Dropping off of polymerase from promoter)}$$

$$RNAP \rightarrow mRNA \text{ (Polymerase completing transcription elongation to make mRNA)}$$

$$mRNA \rightarrow \otimes \text{ (mRNA decay)}$$

The model assumes that an infinite pool of RNA polymerases initiate transcription at promoters with rate k_r, and R_b is the level of promoter-bound polymerase (see Fig. 4A left panel). This promoter-bound polymerase either drops off with rate k_{off}, or elongates with rate k_e to form a cytoplasmic mRNA whose level we denote by m. The drop-off parameter k_{off} will be important later on, as the binding of CF to promoter-bound polymerase can stabilize (decrease k_{off}) or destabilize it (increase k_{off}). This gene expression model can be described by the following system of ordinary differential equation systems

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$$\frac{dR_b}{dt} = k_r(M - R_b) - k_e R_b - k_{off} R_b$$

$$\frac{dm}{dt} = k_e R_b - \gamma_m m$$

where γ_m is the mRNA decay rate constant. Here M denotes the total number of promoters and $M - R$ is the number of free promoters where transcription can be initiated.

We now introduce the CF in the above gene expression model that binds to bound polymerase making it less/more likely to drop off (see Fig. 4A right). This leads to additional reactions:

$$RNAP + Nuclear\ Factor \rightarrow Factor\ bound\ RNAP$$

$$Factor\ bound\ RNAP \rightarrow Promoter$$

$$Factor\ bound\ RNAP \rightarrow Factor\ bound\ mRNA$$

$$Factor\ bound\ mRNA \rightarrow \otimes$$

These reactions represent CF in the nucleus binding to promoter-bound polymerase with rate k_m and we denote the level of this CF-bound polymerase as R_{bf}.CF-bound polymerases are assumed to elongate at the same rate k_e, but drop-off at a different rate $\hat{k}_{off}$. We further assume that CF imprints the nascent RNA complex and is exported with it to the cytoplasm to create CF-bound mRNAs with level m_b. Once this CF-bound mRNA is degraded in the cytoplasm with rate $\hat{\gamma}_m$, the factor is immediately imported back to the nucleus. The transcription and mRNA stability impacts of the CF are modeled through parameters $\hat{k}_{off}$ and $\hat{\gamma}_m$. For example, an activator-only CF reduces the $\hat{k}_{off}$ of the CF-bound polymerase, which now makes them more likely to complete transcription elongation to synthesize cytoplasmic mRNA. An activator that destabilizes mRNA can be modeled by a reduced $\hat{k}_{off}$ (compared to k_{off}) and an increased $\hat{\gamma}_m$ (compared to γ_m),and similarly, a stabilizer reduces $\hat{\gamma}_m$.

In the model, the CF can be present in three different forms: a free factor in the nucleus that binds to promoter-bound polymerases; CF bound to the elongating RNA polymerase complex; CF bound to mRNA in the cytoplasm. If T is the total intracellular level of the CF, then the concentration of the free CF in the nucleus is $T - R_{bf} - m_b$. Overall, this leads to the following modified model with CF regulation

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$$\frac{dR_b}{dt} = k_r(M - R_b - R_{bf}) - k_e R_b - k_{off} R_b - k_m(T - R_{bf} - m_b)R_b$$

$$\frac{dR_{bf}}{dt} = k_m(T - R_{bf} - m_b)R_b - k_e R_{bf} - \hat{k}_{off} R_{bf}$$

$$\frac{dm}{dt} = k_e R_b - \gamma_m m$$

$$\frac{dm_b}{dt} = k_e R_{bf} - \hat{\gamma}_m m_b$$

Note that in this modeling the terms of the type (k_r) are equivalent to the synthesis rate (SR) we use along the manuscript and the terms of the type (γ_m) are equivalent to decay rates (DR). DR is related to mRNA half-life with the simple equation

$$HL = ln2 \,/\, DR$$

To generate Fig. 4B, we plot the net mRNA level ($m_b + m$) as a function of the initiation rate k_r for an activator that stabilizes mRNA ($k_{off} = 1\ min^{-1}$, $\hat{k}_{off} = 0.2\ min^{-1}$, $\gamma_m = 1\ min^{-1}$, $\hat{\gamma}_m = 0.3\ min^{-1}$), only the activator ($k_{off} = 1\ min^{-1}$, $\hat{k}_{off} = 0.2\ min^{-1}$, $\gamma_m = \hat{\gamma}_m = 1\ min^{-1}$), and an activator that destabilizes mRNA ($k_{off} = 1\ min^{-1}$, $\hat{k}_{off} = 0.2\ min^{-1}$, $\gamma_m = 1\ min^{-1}$, $\hat{\gamma}_m = 2\ min^{-1}$). Other parameters can be taken, such as $T = 3000$, $M = 1000$, $k_e = 1\ min^{-1}$, $k_m = 100\ min^{-1}$.

In Fig. 4C, we plot the net mRNA level as a function of time by solving the ODE model for turning an activator ON. For the transient solution, we make further model simplification by approximating the term $k_r(M - R_b - R_{bf})$ as $k_r M$. We use the parameters in Fig. 4B to consider synergistic regulation (activator that stabilizes mRNA), no crosstalk (only the activator) and buffering (the activator that destabilizes mRNA). In addition to the parameters in Fig. 4B, we also consider $k_r M = 300\ min^{-1}$ and the initial conditions of the ODE are taken as:

$$R_b = \frac{k_r M}{k_e + k_{off} + k_r}, m = \frac{k_e k_r M}{\gamma_m(k_e + k_{off} + k_r)}, R_{bf} = 0, m_f = 0$$

that correspond to the steady-state value of the ODE model in the absence of the activator. The net mRNA levels are normalized to their value at time $t = 0$.

In Fig. 4D, we consider that a repressor is turned ON with four different cases: a repressor that destabilizes mRNA ($k_{off} = 1\ min^{-1}$, $\hat{k}_{off} = 5\ min^{-1}$, $\gamma_m = 1\ min^{-1}$, $\hat{\gamma}_m = 3\ min^{-1}$),

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a repressor that only impacts transcription ($k_{off} = 1\,min^{-1}$, $\hat{k}_{off} = 5\,min^{-1}$, $\gamma_m = \hat{\gamma}_m = 1\,min^{-1}$), a repressor that only destabilizes mRNA ($k_{off} = \hat{k}_{off} = 1\,min^{-1}$, $\gamma_m = 1\,min^{-1}$, $\hat{\gamma}_m = 3\,min^{-1}$) and a repressor that stabilizes mRNA ($k_{off} = 1\,min^{-1}$, $\hat{k}_{off} = 5\,min^{-1}$, $\gamma_m = 1\,min^{-1}$, $\hat{\gamma}_m = 0.5\,min^{-1}$). All the other parameters and the plotting procedure is as depicted in Fig. 4C.

3. Results

3.1. Additive crosstalk in post-transcriptional regulons

In order to check the existence of different crosstalk modes based on RBPs, we ran a meta-analysis of a previously published set of yeast deletion mutants, where each one lacked a factor related to a pathway involved in mRNA decay [2]. In that study, the existence of global $[mRNA]_t$ buffering was established for almost all the analyzed mutants [2].

This dataset included some examples of RBPs with specific sets of targets, e.g. the Puf and Upf proteins that have been related to mitochondria (Puf3), ribosome biogenesis (Puf4) or cell periphery (Puf1/2) [32], or the preferred mRNAs of the NMD pathway (Upf2/3) [33]. We analyzed the relative behavior of the targets in mRNA, half-lives (HLs) and SRs. As expected, the relative HLs of their targets clearly increased for mutants *puf3*, *upf2* and *upf3*, along with relative increases in SRs, which resulted in an additive effect upon which the mRNA levels of their targets increased by lowering decay and raising transcription (Fig. 2A-C). *puf4*, however, showed a significant effect on the HLs of its targets, but not at the transcription level (Fig. 2D). This indicates that it is not a crosstalk factor. Finally, *puf1* and *puf2* had no significant effects on the mRNA stability of their targets in spite of their presumed activity (Fig. 2E-F), and only a weak effect on *puf1* SR.

These results reveal that some RBPs, which have been initially presumed to affect only the mRNA stability of their targets (e.g. Puf3, Upf2, Upf3), also act in transcriptional repression for those genes demonstrating the existence of specific crosstalk, which seems to act by summing up effects on synthesis and decay rates as a way to increase the levels of a group of RBP target mRNAs.

3.2. Crosstalk mRNA transcription/decay in transcriptional regulons: studying the *sfp1Δ* mutant

We decided to extend our analysis to transcriptional activators. To this end, we used the case of the yeast Sfp1 factor. Previously published works reveal that steady-state mRNA measurements in *sfp1Δ* strains show the up- or down-regulation of a relatively large numbers of genes [34,35], which makes it a good candidate to explore the existence of specific crosstalk. Sfp1 is a transcription factor that primarily binds the promoter of ribosomal proteins (RPs) and affects ribosome biogenesis [36].

We performed a Genomic Run-On (GRO) analysis [37] to elucidate the effect of Sfp1 depletions on SR, RA and HL parameters. The comparison of Sfp1 targets against the effect on the rest of the transcriptome (Fig. 3) showed in *sfp1Δ* that, as expected, there was a strong effect on the RNA pol II SR of their RP targets as regards the global average.

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There was, however, a stronger effect on their mRNA levels. This was caused by additional specific destabilization in RP mRNAs (Fig. 3). These results indicate that Sfp1 activates all its RP targets at the transcription level, but it also has an additive effect by increasing their mRNAs' stability, which contributes to the final mRNA levels.

3.3. Mathematical modeling of mRNA levels based on transcription/decay crosstalk pathways

After the meta-analysis of some new and published datasets, we decided to mathematically model the behavior of RBPs with either mRNA stabilizing or destabilizing activity, and to also show the ability to influence transcription either positively (activator) or negatively (repressor). For this purpose, we used an ordinary differential equation-based mathematical model to capture the biomolecular crosstalk circuit dynamics, which allowed us to predict the mRNA level outcome according to the SR (Fig. 4B). We assumed that RBP binds mRNA co-transcriptionally, and can either enhance or diminish the overall transcriptional efficiency of RNA pol II based on our previous results with the Xrn1 and Ccr4 factors [21]. We also assumed that the bound RBP factor travels with mRNA to the cytoplasm and remains bound to it until mRNA is degraded [38].

We modeled the cases of the factors that activate transcription and enhance mRNA degradation (i.e., Rpb4/7; Xrn1, Ccr4-NOT, etc.) compared to simple activators, which do not influence mRNA stability (see Materials and Methods for a detailed description). Fig. 4B depicts not only how such an activator (orange line) is less efficient in increasing mRNA levels at any SR, but also how the maximum mRNA level possible is lower than that obtained with an activator without mRNA binding activity (red line). If the activator possesses mRNA stabilizing activity (blue line), it serves to obtain higher mRNA levels for any SR. As far as we know, such transcriptional activators have not yet been described until the case of Sfp1 we describe above. The case of transcriptional repressors possessing mRNA-binding activity and effects on stability is similar to that of activators, but it considers that gene activating occurs when the repressor is turned off and when gene repression is turned on. The obtained lines are similar to those that appear in Fig. 4B (not shown).

Having seen the enhancement caused by RBPs, which act additively on mRNA levels upon transcription and mRNA stability, we wondered what would happen during the kinetics of the activation or repression of a gene (or a group of co-regulated genes) regulated by this kind of RBPs. In Fig. 4C-D, we compare these results (blue lines) to the case of no effect mRNA stability (red and black lines), and also to the cases of antagonist effects (orange lines). During activation, the RBPs that play dual activities have strong effects on both the kinetics and final levels of mRNAs (Fig. 4C). If the transcription activator is also an mRNA stabilizing factor, mRNA levels more quickly increase and reach higher levels than when it acts only at the transcription level (compare the blue and the red lines in Fig. 4C). Similar results are obtained if a factor that acts as a transcriptional repressor and destabilizes mRNA is switched off. In contrast, we obtain buffering behavior (orange line) when the transcription activator induces mRNA degradation.

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We also modeled the action of the same series of factors during the repression kinetics (Fig. 4D). When a repressor with mRNA destabilizing activity is switched on (or an activator is switched off), repression is faster and stronger if the repressor has additive activities on mRNA stability (blue line) than when it acts only at the transcription level (red line). Similar results are obtained when an RBP possesses only mRNA destabilizing activity and not a transcriptional repressor (black line). Buffering is achieved (orange line) when the activator possesses mRNA destabilizing activity after being switched off.

All the possible kinds of such RBPs can be called crosstalk factors (CFs) because they serve to send information from the nucleus to the cytoplasm by means of mRNA imprinting. Depending on their activities on chromatin and in the cytoplasm, they can serve as buffering factors (BFs) or, alternatively, to speed up transcriptional responses as regulatory factors (RFs). For [mRNA] buffering, we previously showed that it is mostly global in the yeast *S. cerevisiae* by controlling total [mRNA], and not the concentration of particular sets of mRNA. With RFs however, it seems logical that they can be devoted to specific groups of genes.

4. Discussion

mRNA transcription/degradation crosstalk is a process that, in spite of having been discovered a few years ago (see ref. [39] for a review), is attracting plenty of attention because it probably influences all eukaryotic gene expression aspects. The features and mechanisms of this crosstalk are, however, much less known. In this study we wondered whether mRNA buffering is the only outcome of these crosstalk mechanisms or if they can also contribute to regulate changes in gene expression.

We analyzed the genomic datasets of several yeast mutants in the transcription or decay factors known to have a set of specific targets; that is to say, transcriptional or post-transcriptional regulons. The expected result for a regulon would be the SR of the genes activated by the depleted transcription factor to lower and, because of this, so would their mRNA levels. The expected result for a post-transcriptional regulon is that the HLs of the mRNAs bound by an RBP, which induces their degradation, would increase and, consequently, so would their mRNA levels. If specific crosstalk exists for these mRNAs, the changes observed in their levels would differ by either raising or lowering from global mRNA changes. The relative change in the average mRNA levels of the genes that behave with the regulon, compared to the change in the global mRNA levels, would inform us if there was i) specific compensatory crosstalk to buffer regulon mRNA levels, ii) additive crosstalk that exacerbates the effect on those mRNAs or iii) no specific crosstalk.

We first investigated seven previously described post-transcriptional regulons driven by RBPs (Upf1/2/3 and Puf1/2/3/4). In those mutants, a previous study showed that global buffering was not affected [2]. In four of them, we confirmed that the HLs of their target mRNAs had increased (*puf3*, *puf4*, *upf2*, *upf3*). When we analyzed the transcriptional effect on their target genes, we found no effect on *puf4* (Fig. 2D), which indicates that it is a simple regulator that operates only at the post-transcriptional level. This case is similar to the previously studied one of *upf1* (see [3]). Nevertheless, and quite unexpectedly, we

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noted additive behavior for three other studied cases: *puf3* (Fig. 2A), *upf2* (Fig. 2B) and *upf3* (Fig. 2C). In all three cases, we concluded that these RBPs not only stimulate the decay of a selected group of mRNAs, but also play a negative role in their transcription. Therefore, the relative increase in the mRNA levels observed in their target mRNAs is due to not only the disappearance of a specific decay factor, but also to lack of specific transcriptional repression. We do not know what mechanism of transcription repression these RBPs follow, and if it is direct or indirect, but we recently found that Puf3 is also a nuclear protein that binds chromatin and imprints its mRNA targets [17]). For the Upf2/3 proteins, nuclear location has been demonstrated in human cells, where hUpf3 shuttles between the cytoplasm and the nucleus, hUpf2 is perinuclear and hUpf1 is mainly cytoplasmic [40]). The results are less clear in yeast, but Upf3 has an NLS (nuclear location signal; see SGD: https://www.yeastgenome.org/). The results herein obtained for *upf2 / 3* contrast with those obtained for *upf1* [3]. This result is, at first glance, surprising given the presumed function of all three proteins in the NMD pathway that forms a protein complex [41,42]. The functions of these three proteins can, however, be multiple and differ from one another. Indeed, it has been demonstrated that hUpf1 can be imported to the nucleus where it plays a role in nonsense-mediated altered splicing (NAS), a pathway that probably does not exist in yeast, which indicates that the putative nuclear functions associated with Upf1 emerged recently during evolution and might be a unique feature of the mammalian Upf11 protein [43]. Thus if yeast Upf1 is only involved in cytoplasmic NMD, but Upf2/3 perform additional nuclear functions, only the last two Upf proteins may affect transcription and imprint mRNAs similarly to Puf3. In fact, it has been demonstrated that Upf machinery interacts at the nucleus with epigenetic machinery in higher eukaryotes, which provides a link between the cytoplasm and the nucleus for this response [29,30].

For Puf3 (Fig. 2A), whose targets are mRNAs that encode mitochondrial proteins [44], additive action on synthesis and degradation rates could contribute to a faster down-regulation of the mRNA levels of targets (as modeled in Fig. 4C: blue vs. black lines) when cells undergo fermentation conditions, and act repressively upon both mRNA synthesis and decay.

We also studied an example of a well-known activator that drives a transcriptional regulon. We analyzed by Genomic Run-On the *sfp1Δ* mutant strain following the same principle previously described for post-transcriptional regulons. The results we obtained with the Sfp1 regulon indicate the existence of an additive effect on the RP mRNAs mediated by their co-transcriptional imprinting (Fig. 3). The influence of a transcription factor (TF) on the mRNA HL that it activates has been demonstrated in yeast ([45-47]. In such cases, specific *cis* elements of the promoter, which are bound by specific TFs, direct the imprinting of transcribed mRNAs that are either more quickly exported (Hsf1-dependent heat-shock mRNAs, [47]) or accelerate decay (Rap1-dependent mRNAs, [45]; and cell cycle-regulated mRNAs during mitosis, [46]). Imprinting can be done by the same proteins that bind the promoter. One such case is Dbf2 for cell cycle mRNAs [46] or, alternatively, the TF can direct mRNA imprinting by a more general factor like the Mex67 export protein in heat-shock mRNAs [47]. However, the positive effect of Sfp1 on the mRNA stability of their targets is a new finding. Our modeling shows that these kinds of factors will enhance and

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speed up the transcriptional response (Fig. 4). By doing so, they would contrast with the crosstalk factors involved in buffering, which display opposite effects in transcription and mRNA decay.

Based on the results of this study and those in a previous paper [3], we proposed a model based on the imprinting mechanism by RBPs to explain the direct crosstalk direction. Imprinting might vary from very specific to global depending on the nature of RBPs, which can bind a variable number of mRNAs. RBPs affect the stability of their targets in the cytoplasm and should then return to the nucleus (Fig. 1). The nuclear import of free RBPs would be the mechanism for the reverse crosstalk direction [38]. We demonstrate here that this would be an efficient way to buffer $[mRNA]_t$ if RBPs possess transcriptional activation activity, but it can also be a mechanism for the additive effect if RBPs negatively affect transcription and mRNA decay, such as Puf3 and Upf2/3. Possible models for the mechanism of reverse global crosstalk have been published. They are based on the nucleo-cytoplasmic shuttling of a kind of wide-binding spectrum RBPs: polyA binding proteins. Glaunsinger's group [25] proposed an antibuffering mechanism mediated by the cytoplasmic poly(A) binding protein (PABPC), whereas Jensen's group posited a buffering mechanism based on the Nab2 function in nuclear mRNA decay [26]. RBPs are the best candidates to shuttle information forward and backward from the nucleus to the cytoplasm. They can sense the $[mRNA]_t$ in the cytoplasm and be imported to the nucleus whenever they are free, where they can have positive or negative effects on transcription [27]. The existence of proteins that are simultaneously gene transcription factors, bind DNA, play roles in mRNA metabolism and bind mRNA is becoming a frequent finding [48,49].

Additive crosstalk promotes regulatory changes whereas compensatory crosstalk ensures mRNA buffering (Fig. 5). These two mechanisms operate in opposite directions. As buffering operates globally ([3], it should act as a general obstacle for gene regulation, particularly when many genes have to coordinately change their expression significantly. Under these conditions, slow regulation might be insufficient to counteract robust global buffering mechanisms. According to our mathematical modeling, additive changes by simultaneous actions upon mRNA synthesis and decay would involve a kinetic advantage to overturn global buffering via a mechanism of signal amplification. From this point of view, crosstalk mechanisms would contribute to global [mRNA] homeostasis by diminishing stochastic noise (compensatory action, buffering) and, at the same time, favoring fast regulations in specific regulons in response to meaningful regulatory signals (amplified regulation) (Fig. 5). Hence, the fact that we herein show how Sfp1 regulates RPs genes, which are highly active under fermentative growth conditions, whereas Puf3 regulates mitochondria-related genes, which are expressed under respiratory conditions, is extremely interesting. In both cases, the activation or repression of the regulon should compensate for the general buffering mechanism that occurs when the carbon metabolism changes [37]. Interestingly, we observed how these two regulons feature among the few that escape from global [mRNA] buffering during the growth rate variation caused by transcription-mRNA decay crosstalk [50].

Finally, we recall that changes in mRNA levels do not necessarily correspond quantitatively with changes in protein levels. mRNAs imprinted with RBPs can alter their translational

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efficiency apart from their mRNA stability [7]. This can be caused either by the direct effect of the RBP on translation [6,7,51] or by the effect of the RBP on the formation of mRNA granules. In fact, this has been shown for Puf3 in yeast [52] and for Upf1 and 3 factor in mammals and yeast cells respectively [53].

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Data availability

The GRO data for the *sfp1* mutant are stored in the GEO repository (accession number GSE57467). The rest of the analyzed mutants datasets are from the ArrayExpress database (accession number E-MTAB-1525).

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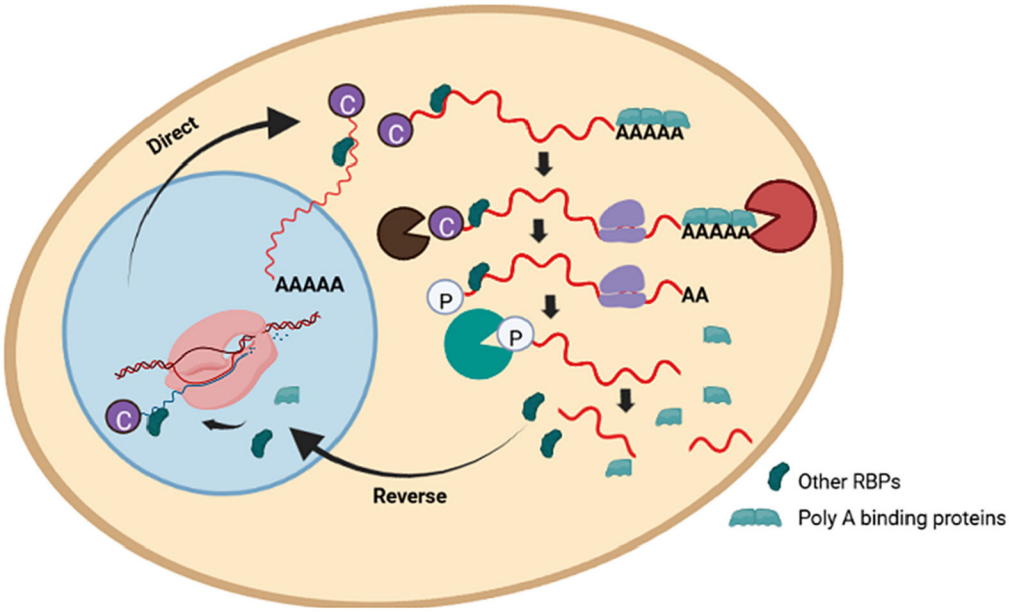

Fig. 1.

Transcription-degradation crosstalk pathways. Crosstalk has two branches: direct from the synthesis (transcriptional) machinery for the mRNAs in the nucleus to the decay machineries in the cytoplasm; reverse from the cytoplasm to the nucleus. RNA-binding proteins (RBP, either polyA-binding or others) can co-transcriptionally imprint mRNAs and be transported with them to the cytoplasm where they affect the mRNA life, especially mRNA stability. Once mRNA is degraded, they can be imported back to the nucleus where they affect transcription. The potential effect of the RBPs on the mRNA depends on their effect as activators or repressors of transcription and mRNA decay. This is discussed later in the main text. Circles with C or P denote 5′ capped or 5′ phosphate mRNA molecules, respectively. Different colored pacmans represent enzymes or complexes involved in mRNA decay. See the main text for further explanations. Figure drawn using BioRender.

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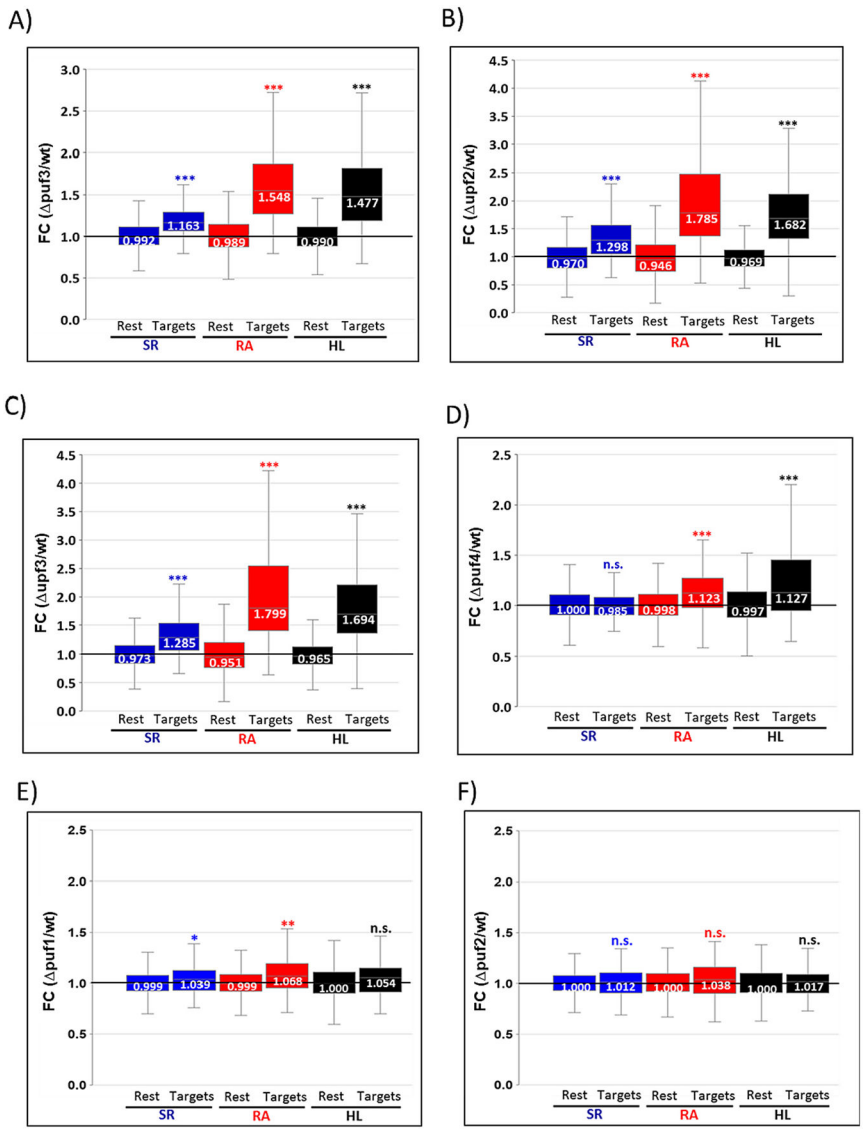

Fig. 2.

Transcriptomic study of several yeast strain mutants in mRNA decay factors. Fold changes (FC) for the synthesis rates (SR), mRNA half-lives (HL) and mRNA levels (RA) in the mutants vs. the wild type strains are represented in the box-plots. We compared the median of the FC of the targets for Puf1 and Puf2 (70 genes), Puf3 (210 genes), Puf4 (145 genes), Upf2 and Upf3 (479 genes) to the rest of the transcriptome. The results show the additive effect of the Puf3 (A), Upf2 (B) and Upf3 (C) factors. However, Puf4 (D) only affects the HLs of its targets, whereas Puf1 (E) and Puf2 (F) have very slight effects. The numbers in boxes show the median FC value. The statistical significance for the pair-wise comparisons (targets vs. the rest) was estimated by the Kolmogorov-Smirnov test of the differences between distributions (*** is *p*-value $<10^{-5}$; ** p-value <0.005; * p-value <0.05; n.s. not significant). All analyzed SR, RA and HL data are from Sun et al. [2].

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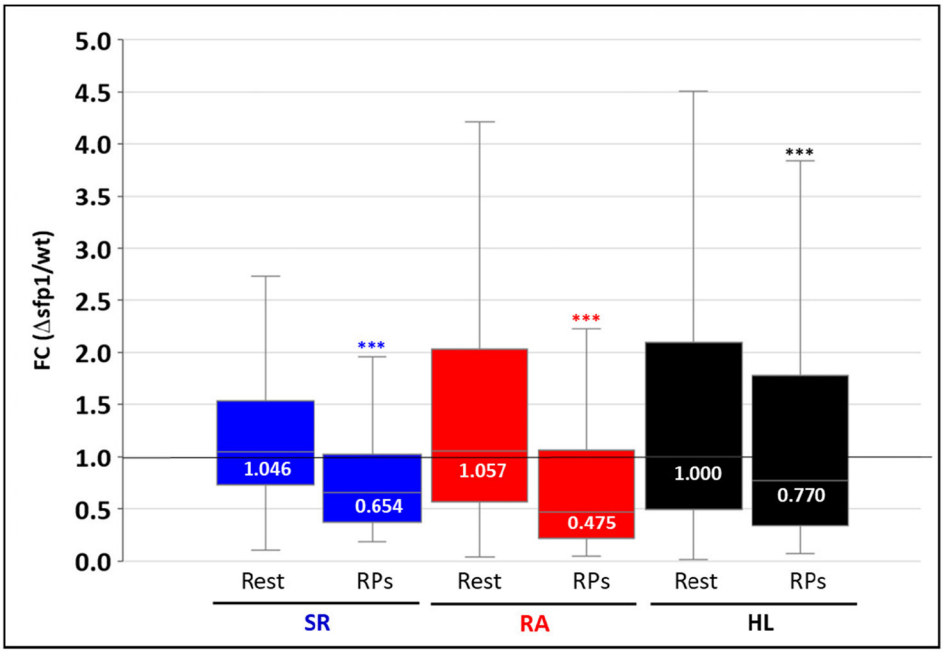

Fig. 3.

Transcriptomic analysis of the *sfp1* mutant. We performed a GRO analysis of the *sfp1* mutant compared to its wild-type strain (BY4741). Then we compared the fold changes of the group of 106 RP Sfp1 targets to the rest of the transcriptome as explained in Fig. 2. The experiment was run in three biological replicates. The numbers in boxes show the median value of the ratio (FC). The statistical significance for the pair-wise comparisons (targets vs. the rest) was estimated by the Kolmogorov-Smirnov test of the differences between distributions. *** is *p*-value $<10^{-5}$.

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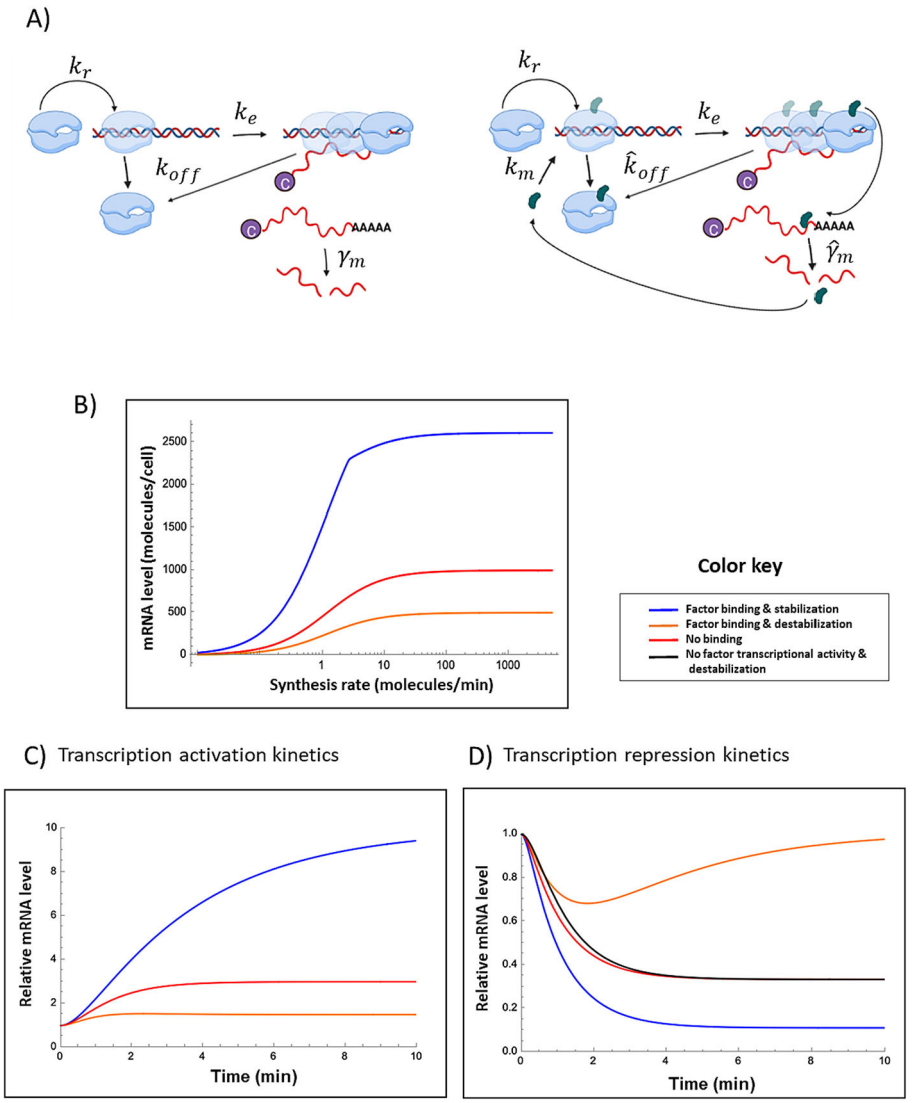

Fig. 4.

Mathematical modeling of the transcriptional responses conducted by crosstalk factors. A) The schematic on the left shows the model of gene expression in the absence of the crosstalk factor. Here RNA pol II (represented as a blue molecule) binds to gene promoters with rate k_r. Bound polymerase either abort transcription by dropping off with rate k_{off}, or finish the transcriptional elongation/export process with rate k_e to produce an mRNA transcript that decays with rate γ_m. The schematic on the right shows the process with the crosstalk factor (green symbol) that binds to the RNA pol II with rate k_m. The factor-bound polymerase has a different drop-off rate $\hat{k}_{off}$, and the corresponding factor-imprinted mRNA has an altered degradation rate $\hat{\gamma}_m$. Please see the Materials and Methods for a detailed model description. Figure drawn using BioRender. B) mRNA response as a function of the synthesis rates for the case of a transcriptional activator with stabilizing (blue line) or destabilizing (orange line) activity compared to the case in which the factor has no mRNA binding capacity (red line). C, D) Modeling different response curves during transcription

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activation (C) or repression (D) for the cases of the RBPs that possess parallel or antagonist activities at the synthesis and decay rates. Transcriptional activation means turning on a transcriptional activator or turning off a transcriptional repressor. Repression means the opposite in both cases. Blue curves are for the cases with a transcription activation factor that possesses stabilizing activity. A transcriptional repressor with destabilizing activity would show similar plots. Orange curves are for the RBP transcription factors with opposite effects on mRNA stability and provoke buffering. There is no crosstalk for a TF with no mRNA binding properties (red curves) or for a destabilizing factor with no transcriptional activity (black curve). The time scale shown is the case of an mRNA with an HL of 1 min as a reference.

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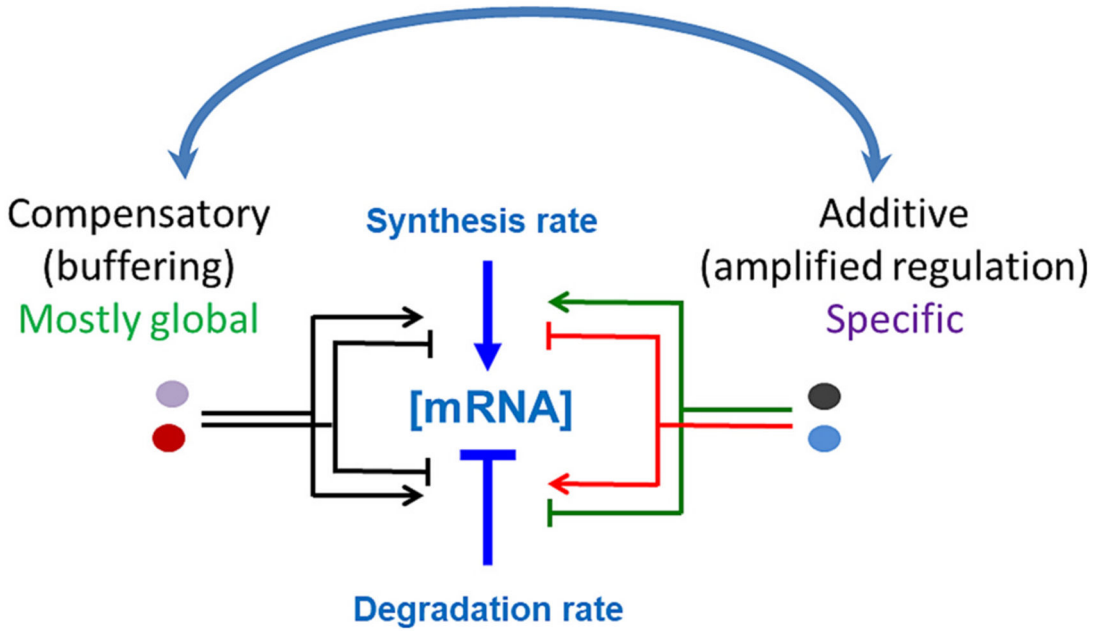

Fig. 5.

Summary of compensatory or additive effects on the transcription-mRNA decay crosstalk mediated by RNA binding proteins. Crosstalk can affect the respective synthesis or degradation rates by increasing them, which sums up the effect of changing (raising or lowering) the mRNA level [mRNA] (right). Alternatively, crosstalk can act by compensating the effects of rates changes in a way that buffers [mRNA] (left). mRNA buffering normally affects global [mRNA] [2,3], whereas additive crosstalk acts on specific subsets or co-regulated genes by provoking an amplification of their regulation (see Fig. 4). The existence of global [mRNA] buffering and specific additive regulation of a subset of mRNAs should involve a mutual influence (upper blue arrow).