

Regulatory mechanisms and networks couple the different phases of gene expression

Orna Dahan, Hila Gingold and Yitzhak Pilpel

Department of Molecular Genetics, The Weizmann Institute of Science, Rehovot, 76100, Israel

Gene expression comprises multiple stages, from transcription to protein degradation. Although much is known about the regulation of each stage separately, an understanding of the regulatory coupling between the different stages is only beginning to emerge. For example, there is a clear crosstalk between translation and transcription, and the localization and stability of an mRNA in the cytoplasm could already be determined during transcription in the nucleus. We review a diversity of mechanisms discovered in recent years that couple the different stages of gene expression. We then speculate on the functional and evolutionary significance of this coupling and suggest certain systems-level functionalities that might be optimized via the various coupling modes. In particular, we hypothesize that coupling is often an economic strategy that allows biological systems to respond robustly and precisely to genetic and environmental perturbations.

The different stages of gene expression regulation are often coupled

Gene expression is a highly complex process that comprises many steps, each with elaborate regulation. Proper control of gene expression is crucial for the correct implementation of the programs embedded in the genome. The first stage of gene expression is transcription, a process subject to several levels of regulation. In eukaryotes, the pre-mRNA is processed following transcription and exported from the nucleus to the cytoplasm where additional steps of mRNA maturation take place. The mature mRNA is then either translated, stored, or degraded. Until recently the process of gene expression was predominantly considered to be a series of isolated steps, each controlled by separate regulatory mechanisms and networks. However, it is becoming increasingly clear that the different phases of the process are coupled. In our definition, two phases of gene expression are coupled if the control of one affects the other. The two phases are 'bidirectionally coupled' if such effects between them are mutual. For instance, transcription and translation are coupled if the control of transcription also affects translation. Thus, whereas the central dogma is largely unidirectional, the entire gene-expression process can be viewed as a more complex network with feedback between coupled regulatory mechanisms.

Regulatory coupling is likely to be an economic strategy that endows the cells with improved regulatory capabilities, but can also involve trade-offs. We discuss recent findings demonstrating diverse coupling mechanisms between the different levels of gene expression regulation and speculate on their potential biological roles.

Coupling between transcription and RNA processing

Whereas traditional views consider transcriptional and post-transcriptional regulation to be separate processes, it is becoming increasingly clear that the regulation of the two phases is often coupled (Figure 1). In particular, we now understand that control over many post-transcriptional modifications can take place during transcription. Such 'cotranscriptionality' permits crosstalk between the different steps of mRNA biogenesis and provides a platform to control the order and timing of events [1–3]. Coupling between transcription and downstream RNA-processing events (Figure 1, arrow 1) is predominantly achieved by the recruitment of different processing factors to the nascent RNA during transcription, and this takes place via an interaction with RNA polymerase II (RNAPII). RNAPII can adopt different phosphorylation states during transcription, forming a pattern that determines the recruitment of alternative RNA-processing molecules [4]. In this respect the polymerase serves as dynamic docking platform that orchestrates different RNA-processing events during transcription [5]. An interesting example is the connection between transcription and splicing. The phosphorylation status of RNAPII appears to be related to the rate of transcription, which in turn affects (and is affected by) splicing events along transcripts [3,6–9].

Coupling between transcription and RNA localization

Many mRNAs have specific localizations within cells, both in prokaryotes [10,11] and eukaryotes [12], indicating that a major regulatory process is responsible for mRNA localization. It is now becoming clear that information required for localization is embedded onto mRNAs during transcription (Figure 1, arrow 2). Further, mRNA localization can, in turn, affect protein localization. A strong demonstration of this concept has been obtained in *Saccharomyces cerevisiae* where the localization of many transcripts to the bud tip is determined during transcription through recruitment of the mRNA-localization factor to the newly transcribed RNA [13], and this takes place via a protein

Corresponding authors: Dahan, O. (orna.dahan@weizmann.ac.il); Pilpel, Y. (Pilpel@weizmann.ac.il)

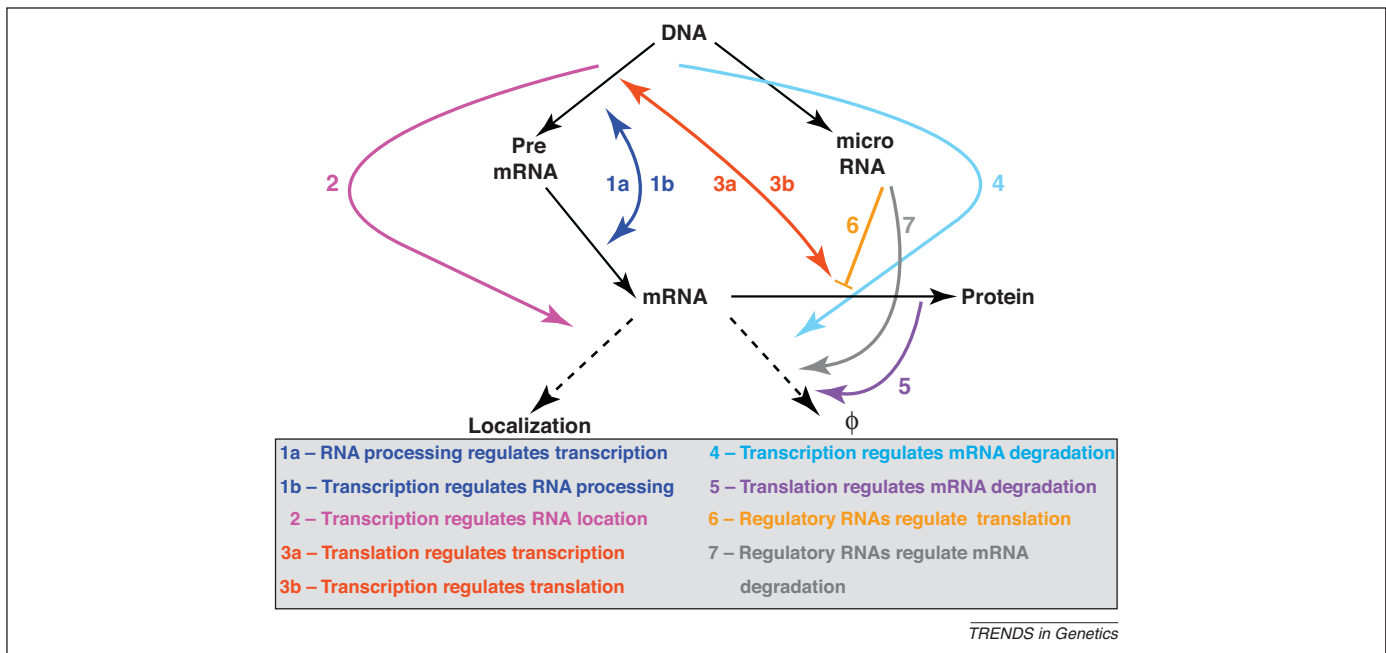


Figure 1. Coupling in gene expression. Schematic representation of the main coupling modes between the different stages of gene expression, including transcription, RNA processing, RNA localization, translation, RNA degradation and regulation by microRNAs. As shown here, the different processes are often coupled and can mutually regulate each other, thus creating an intricate regulatory coupling network. Black solid arrows denote the flow of genetic information in gene expression, in other words, transcription, RNA processing, and translation; black dotted arrows denote the flow of material in gene expression, in other words, degradation (represented here as ‘ ϕ ’) and localization; numbered colored arrows denote regulatory coupling modes.

complex named the ‘locosome’ [14]. Similar examples are found in higher eukaryotes. In one such case, mRNAs were shown to be localized to the leading edge of fibroblasts via shuttling proteins that associate with the nascent mRNA at their site of transcription in the nucleus [15,16].

Bacteria also show interesting relationships between transcription and mRNA and protein localization. Recent data have shown that transcripts tend to remain near their site of transcription for their entire lifespan, demonstrating a type of transcription–localization coupling that appears to be unique to prokaryotes [10]. Further, RNA localization in bacteria can coincide with protein localization [11]. This might suggest an intriguing three-way coupling between DNA, RNA, and protein. According to this hypothesis, the bacterial chromosome is folded such that particular genes reside in proximity to their mRNA and protein products [17].

Coupled regulation of transcription and translation

Transcription and translation are the two major steps in gene expression and both are subject to complex regulation. In bacteria, in contrast to eukaryotes, these two principal events can be coupled in time and space owing to the lack of a membrane-enclosed nucleus. In fact, one of the classical modes of gene expression regulation in bacteria, the ‘attenuation mechanism’, manifests coupling between transcription and translation. In this mode of coupling the availability of specific amino acids determines the translation rate of the enzymes that synthesize these amino acids, and this in turn governs the transcription rate of the corresponding mRNAs [18].

The attenuation mechanism couples translation and transcription but, inherent to its logic, it can only be applied to the expression of amino acid biosynthetic

enzymes. A more general mechanism is needed for proper coordination of the transcription and translation of other genes. One such coupling mechanism was recently described in bacteria where the proximity between RNAP and the ribosome on transcripts is used to coordinate the speed of their respective polymerization reactions. Measuring the *in vivo* rates of transcription and translation in *Escherichia coli* demonstrated that the speed of ribosome movement on the transcript directly affects the rate of transcription elongation [19]. In this case the moving ribosome pushes forward the RNAP, thereby providing mechanical adjustment between the two processes. This model was further supported by the observation of a physical link between the transcription and translation machinery in *E. coli* [20].

In eukaryotic cells, coupling between transcription and translation presents a greater challenge because transcription takes place in the nucleus, whereas translation is cytoplasmic. Can coupling between these two processes still take place across subcellular compartments? A recently emerging view suggests that coupling can take place through an association between nascent RNA and proteins that later regulate the translation of that mRNA. In particular, it was shown that two subunits of the yeast RNA-Pol II (Rpb4 and Rpb7) associate with mRNAs during their transcription – and once in the cytoplasm they target the transcript to the translation apparatus [21]. Such nucleocytoplasmic shuttling molecules could thereby coordinate transcription and translation across subcellular boundaries [21] (Figure 1, arrow 3).

Coupling between transcription and mRNA degradation

Another cytoplasmic process that is crucial to the life of an mRNA is its degradation. Could this process also be cou-

pled to transcription? In yeast, at least, this was found to be the case (Figure 1, arrow 4). A direct coupling mechanism between mRNA production and degradation is mediated by the same RNAPII subunits, Rpb4 and Rpb7, that coordinate transcription with translation. These two subunits, which associated with the mRNA during transcription, and which then escort the transcript to the cytoplasm, can affect mRNA degradation in addition to modulating translation [22–24].

A further example of a direct regulatory link between RNA transcription and degradation is the CCR4–NOT complex (the major yeast mRNA deadenylase) that controls the initial step of mRNA degradation [25–27]. In addition to its role in controlling mRNA decay, the complex is also part of a larger multicomponent assembly that contains diverse transcription initiation proteins, such as members of the SAGA complex [28], several subunits of RNAPII [29], and subunits of the transcription initiation factor TFIID [30–32].

Coupling between translation and mRNA degradation

Both translation and mRNA decay are extensively regulated, but is there a crosstalk between these two processes? One might expect that, during translation, mRNA might be protected from degradation – perhaps by being covered with ribosomes. Technological advances in measuring the genome-wide density of ribosomes on mRNAs [33–36], as well as mRNA degradation rates [37,38], now allow this issue to be examined and the potential modes of coupling between the control of translation and mRNA stability to be explored (Figure 1 arrow 5). One such study on the yeast *Schizosaccharomyces pombe* found that ribosome occupancy and density (i.e. fraction of mRNA molecules associated with ribosomes, and number of ribosomes per transcript, respectively) correlate positively with mRNA stability [34]. Specifically, mRNAs with higher ribosome occupancies and densities, which are thus translated with higher efficiency, were generally found to be more stable. However, such a correlation does not necessarily indicate a causal effect. Translational control, for example by modulating ribosome density on transcripts, is likely to affect mRNA degradation. However, mRNA decay rates would also be expected to affect the efficiency of translation. To resolve this issue and reveal the direction of the causal effect, specific intervention in one of the processes should be made.

More surprising was the recent observation that degradation of mRNAs can take place concomitantly with the last round of translation, presumably alleviating the need to dissociate the ribosomes from the mRNA before its decay [39]. Clearly, regulatory coupling between translation and mRNA decay is far from being understood; studies in both eukaryotes and prokaryotes point toward diverse types of interaction [40–45] and extensive work will be needed to clarify the picture.

A systems view of gene expression coupling

A systems-biology approach, which often considers entire gene modules and genomes, could complement biochemical investigations into the structure, function and evolution of coupling mechanisms. For example, several studies have explored the relationship between mRNA production and

stability at the whole-genome level. Such studies require, in addition to measuring mRNA levels, that the decay rates [37,46–50] and transcription rates [51–54] of all genes in the genome are measured.

In one such study yeast cells were exposed to environmental stimuli, and genome-wide changes in both mRNA levels and stability were measured [48]. This work uncovered an intriguing relationship between RNA production and degradation. For some of the genes a ‘counter-action’ mode of coupling was observed. In this mode, mRNAs of genes induced in response to a specific signal are destabilized, whereas mRNAs of genes repressed at the transcriptional level are stabilized. Other genes, can show the opposite (and perhaps more intuitive) correlation: in other words, induction of these genes is associated with stabilization of their transcript, whereas their repression is accompanied by destabilization (hundreds of genes are either destabilized or stabilized by up to fourfold in response to stress). Interestingly, the choice between the two alternative modes of coupling governed the profile and kinetics of mRNA levels. Transcripts that show ‘counter-action’ coupling display ‘spiked’ dynamics, with fast and transient responses, whereas transcripts that show positive correlation exhibit slow and sustained responses [48]. A very similar ‘counter-action’ coupling was also observed in yeast under different growth conditions and with a different genome-wide measurement approach [51]. More recently, a similar relationship was also demonstrated in mammals. By analyzing a multitude of gene-expression data, combined with genome-wide half-life measurements, a negative correlation was found between the rapidity of changes in transcript abundance and mRNA stability, akin to the yeast situation. This study thus demonstrated, on a global scale in mammals, that unstable transcripts respond to changes in their environment faster than stable ones [55]. Furthermore, it was recently shown that changes in degradation rates are responsible for shaping the spiked dynamic of transcripts in mouse dendritic cells exposed to lipopolysaccharide [54].

Although the mRNA degradation mechanism differs considerably between eukaryotic and prokaryotic cells (reviewed in [56]), similar observations have also been made in *E. coli* – where inverse relationships between mRNA abundance and transcript stability were found under particular growth conditions [46]. A further study revealed more complex dynamics, where the degradation rate was found to change dynamically during the response to stress [49]; future investigations will need to meet the challenge of measuring changes in transcription and decay at fine temporal resolution. All these findings indicate that cells have evolved to exploit coupling between transcription and mRNA degradation to permit dynamic changes in their transcriptome in response to different environmental cues.

‘Soft’ and ‘hard-wired’ coupling mechanisms

The coupling between phases of gene expression can be hard-wired, such as in the cases of shuttling molecules that are associated with a target mRNA at one stage (e.g. transcription), but that also affect its control at a different stage (e.g. mRNA degradation) [22–24]. Another possibility is soft coupling at the level of regulatory networks. In such

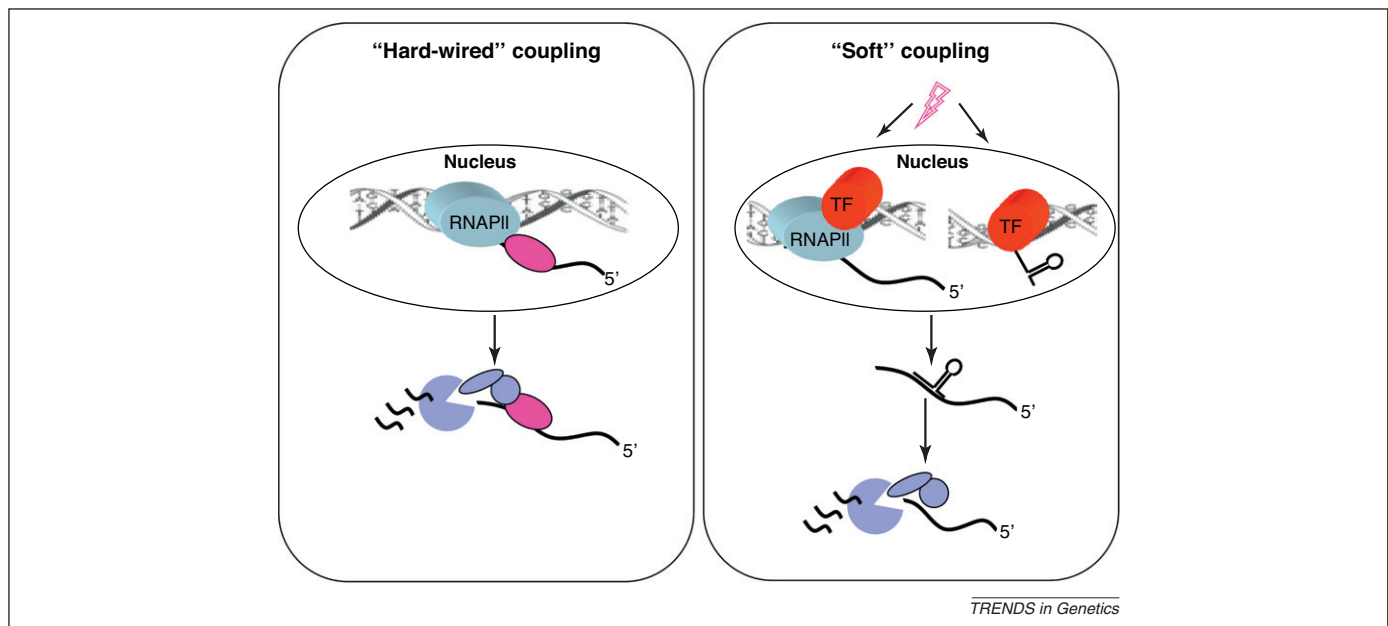


Figure 2. Hard-wired versus soft coupling. Two coupling strategies are presented. Hard-wired coupling refers to cases in which two processes are directly coupled via a molecule that associates them. As illustrated, transcription and mRNA degradation feature such coupling. In this case coupling is achieved by cotranscriptional recruitment of specific proteins (represented as a pink ellipse) to the nascent transcript. When in the cytoplasm these proteins regulate transcript stability by directing the mRNA degradation machinery to the mRNA. Soft coupling refers to cases in which coupling between the two processes is obtained by concerted network wiring. An example for such coupling is the coordination of transcription by a transcription factor (TF) and by a miRNA that serves as a post-transcriptional regulator. In the illustrated example the transcription factor also regulates the transcription of the miRNA with which it coregulates the target mRNA. Thus the same signal that triggers the transcription factor can also ensure proper control of the target genes at the post-transcriptional level (here the miRNA is depicted as a black stem and loop). This is a soft link because the effect of the transcription factor at the post-transcriptional level is not hard-wired into the transcription event and the network could be re-wired, for example by eliminating the miRNA binding site within the target mRNA.

a design two processes are coordinated without any physical interaction between the corresponding molecules. For example, a module of genes can be regulated at the transcriptional level by a common factor, and at the post-transcriptional level by another shared regulator, if the two factors are coregulated then the two processes become coupled (Figure 2).

An interesting example of such soft coupling between transcription and post-transcriptional regulation has been observed in mammals. Many genes in mammals are subject to transcriptional regulation by DNA-binding proteins, and are also regulated at the post-transcriptional level by microRNAs (miRNAs) that either destabilize their transcripts or inhibit their translation [36,57,58]. Computational analyses identified a recurring network motif consisting of pairs of regulators – a transcription factor and a miRNA that both regulate large sets of common targets. Interestingly, in many of these regulator pairs the transcription factor and the miRNA appear to regulate one another. The transcription factor, the miRNA, and the target gene can therefore form a feed-forward loop [59,60] that couple transcriptional and post-transcriptional regulation (Figure 1, arrows 6 and 7; Figure 2).

Another example of soft coupling, identified in *S. pombe*, employs a very similar logic but uses proteins instead of a regulatory RNA. The regulatory circuit here consists of the transcription factor Mei4 and the mRNA stabilization protein Meu5. The two regulators share a significant proportion of their targets and, in addition (as in feed-forward loops), the transcription factor regulates the expression of the mRNA stability regulator. Interestingly, this dual regulation of transcription and RNA stability appears to

dictate the kinetics of the target genes: genes that are regulated only by the transcription regulator Mei4 show spiked dynamics (i.e. fast induction and fast relaxation back to the basal level), but genes that are also regulated by the stabilizing factor show a more sustained dynamics with slower relaxation [50], reminiscent of the situation in *S. cerevisiae* [48].

Coupling, what is it for?

As discussed above, biology employs a rich repertoire of mechanisms to couple the different stages of gene expression. An intriguing question is – what is the function of such coordination? Or, why have such coupling mechanisms repeatedly evolved in different pathways and species? One can also wonder about the potential costs and trade-offs associated with coupling of the different phases of gene expression. In the following section we speculate on the potential functions that coupling might serve in biology.

Coupling as an economic strategy for gene expression regulation

An obvious advantage of coupling consecutive stages of gene expression is increased efficiency and economy. In an economically-efficient chain of events the rate of one process is tuned according to the capacity of a subsequent one. For instance, if the capacity of a cell to translate a given set of genes is compromised under specific conditions it would be logical and economic to downregulate the transcription of those genes so as to avoid wasteful mRNA production. In bacteria this logic appears to be implemented in a coupling mode that mechanically adjusts the rate of transcription elongation according to the speed of translation [19]. One

can further speculate on a generalization of this principle, for example to a situation where the functional level of the general translation apparatus in the cell (the level of ribosomes, tRNAs and other translation factors) modulates the transcription machinery to ensure that genome-wide levels of mRNA do not exceed the mRNA translation capacity of the cell.

Coupling to enhance responsiveness to the environment

Although the economic role of coupling in decreasing the costs and increasing the efficiency of gene expression can be understood intuitively, other coupling regimes could be less economic and their advantage must be found elsewhere. Examples include cases in which a higher production rate of a particular transcript is coupled to its high degradation rate [48], a seemingly wasteful strategy. In these cases cells consume energy to produce and turn-over abundant transcripts rapidly. An appealing explanation for such counter-action coupling is that the system acquires the capacity to respond dynamically to stimuli. This type of relationship has been demonstrated in yeast [48], and might also be present in mammals – where transcription factors and regulatory RNAs appear to cooperate in analogous ways to modulate the kinetic profiles of shared targets. For example, if a transcription factor activates a gene target, and with an appropriate delay the transcription factor then activates the miRNA or an anti-sense transcript that also regulates the same target, then a transient change in target mRNA levels can be obtained, as recently demonstrated [61].

Coupling and the control of stochasticity

Coupling could also serve to determine cell-to-cell variation in protein levels. Given the stochastic nature of molecular recognition, a particular protein can be expressed at widely different levels in different but genetically identical cells, a phenomenon known as ‘noise’. It is therefore conceivable that organisms have evolved mechanisms to control gene noise levels; in other words, to minimize noise when a precise level of expression is required, but to permit stochastic effects when these might be advantageous – for example to create cell-to-cell variation under stress conditions [62]. Because noise can be affected by all stages of gene regulation [62–72], determination of the desired amount of noise could require appropriate coupling between the different stages, exactly as the mean expression level of a protein requires such coupling.

We discuss below two different mechanisms by which coupling can fine-tune noise levels. The first concerns the balance between transcription and translation of a given gene (as previously suggested [73]). If a specific mean expression level of a protein is required, the cell can achieve this precise level by different combinations of transcription and translation rates. The two extremes are a strategy of high transcription combined with low translation and, conversely, a low transcription rate but with efficient translation. The first involves high energy-expenditure, but could ensure reduced noise at the protein level by creating a robust and large pool of mRNA [73,74]. The converse strategy, a low rate of transcription with efficient translation, could be exploited to enhance noise.

A second mechanism to tune the noise level is to coordinate the expression level of a gene with that of its regulatory RNA [58,60]. In such circuits a transcription factor might first activate the regulatory RNA, and only later the target mRNA. The regulatory RNA could thus form a ‘buffer’ that damps fluctuations in the mRNA when the later is expressed at only low levels [75–77].

Coupling and the facilitation of evolvability

Finally, we speculate here on a potential role of coupling in facilitating evolvability – the ability of organisms to evolve. The capacity to evolve can be limited by the need to acquire several mutations simultaneously. Evolvability could therefore be enhanced by a mechanism that reduces the number of mutations needed to produce a novel trait such as a new regulatory program [78]. Clearly, coupling between the different stages of gene expression regulation can increase evolvability. For example, if a new gene is added to the genome, such as by gene duplication or by horizontal gene transfer, the same mutation (for example in the promoter of the gene) that facilitates the integration of the gene into an existing transcription-regulation network could also ensure proper control of the decay of the transcribed mRNA. In the absence of coupling, each stage would require a separate set of mutations, resulting in a slower fixation rate.

Concluding remarks

A classical view in traditional fields of biology such as physiology considers animal and plant bodies as holistic entities in which organs, tissues and other constituents constantly affect each another. The alternative reductionist approach has led biology onto a different track in which molecules and pathways are understood in great detail, but not the regulatory interactions between them. The advent of systems biology is now reintroducing the classical holistic approach to biology by examining the interactions between the parts. We have summarized here a rich set of mechanisms that can be exploited by organisms to couple the different stages of gene expression regulation.

Organisms have evolved analogous coupling solutions in multiple systems. However, the differences between prokaryotes and eukaryotes are notable. In prokaryotes many coupling mechanisms take advantage of the lack of subcellular compartments to couple directly the different steps of gene expression. Eukaryotes have overcome subcellular barriers through ‘cotranscriptionality’ [3] – in other words, the use of nucleocytoplasmic shuttling molecules, or ‘mRNA coordinators’, that are associated with mRNAs during transcription in the nucleus and that control a range of post-transcriptional events in the cytoplasm [21,22,79,80]. Another interesting difference in coupling solutions is between the soft and hard-wired alternatives. For instance, whereas yeast display hard-wired coupling in the binding of molecules to mRNA during transcription, higher eukaryotes with a miRNA machinery also appear to use soft-coupling in the wiring of their regulatory networks (not excluding the possibility that hard-wired coupling also occurs in higher eukaryotes). In both cases these can fulfill a similar regulatory task; for example, both strategies can counteract an increase in transcription rate by an inhibitory signal, thereby generating spiked expression dynamics.

Future challenges are substantial. We need to determine if there are additional modes of coupling. For example, although the production and degradation of mRNA are often coupled, is this true also at the protein level? In particular, do cells coordinate translation and protein degradation, for example by already tagging proteins for degradation during translation to determine their subsequent lifespan? This is exploited as a quality control mechanism to rapidly eliminate mistranslated proteins [81–83], but is it used also for properly-translated proteins? There could well be additional modes of coupling – for instance, protein degradation could signal to the transcription machinery to modulate its activity. Analysis of possible correlations between changes taking place in the different phases of gene expression would be an important first step, to be followed by the challenging task of deciphering the underlying molecular mechanisms that might serve as coordinators.

Although diverse mechanisms are clearly exploited to couple the different phases of gene expression, a major conceptual challenge for the coming years will be to understand the selective advantages of such coupling. As hypothesized above, coupling could be used to reduce the energetic cost of gene expression or to regulate attributes of gene expression such as noise and dynamics in response to the environment. Were these features part in the evolutionary driving forces that led to coupling between the different levels of gene expression? Would the performance of regulatory networks change significantly without particular types of coupling? Does coupling entail a cost to the cells, and do cells face more complicated trade-offs when coupling is implemented? One potential cost of coupling is compromised flexibility – for instance, if the mRNA decay rate is determined during transcription, the cell could then be committed early on to a particular kinetics of mRNA degradation, thereby losing the ability to control transcript dynamics later on.

Essential for answering these fundamental questions will be to experimentally uncouple naturally coupled processes and examine the effects on the system. For example, a single mutation in yeast can alone uncouple transcription from mRNA degradation and translation [22]. Potentially, such mutations will allow the effects of coupling to be studied with minimum additional perturbing effects. Such a genetic tool could be used to determine the effects of uncoupling on mRNA and protein kinetics, and on cell fitness. In addition, such uncoupling mutations could be used to abolish the feed-forward loop that connects transcription factors and miRNAs – for example by a mutation that disconnects a miRNA from the coregulatory transcription factor. How would such a modified system, in which the two regulators no longer regulate one another, function compared to the wild-type system?

Finally, we anticipate that with the rapid progress of technologies to measure the transcriptome and proteome, and with the advent of synthetic biology, we will gain a much deeper understanding of gene expression networks and establish the entire process as an integrated system with diverse mutual effects that function in harmony as a result of multiple coupling modes.

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