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Research review paper

The essential role of mRNA degradation in understanding and engineering *E. coli* metabolism

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ABSTRACT

Metabolic engineering strategies are crucial for the development of bacterial cell factories with improved performance. Until now, optimal metabolic networks have been designed based on systems biology approaches integrating large-scale data on the steady-state concentrations of mRNA, protein and metabolites, sometimes with dynamic data on fluxes, but rarely with any information on mRNA degradation.

In this review, we compile growing evidence that mRNA degradation is a key regulatory level in *E. coli* that metabolic engineering strategies should take into account. We first discuss how mRNA degradation interacts with transcription and translation, two other gene expression processes, to balance transcription regulation and remove poorly translated mRNAs. The many reciprocal interactions between mRNA degradation and metabolism are also highlighted: metabolic activity can be controlled by changes in mRNA degradation and in return, the activity of the mRNA degradation machinery is controlled by metabolic factors. The mathematical models of the crosstalk between mRNA degradation dynamics and other cellular processes are presented and discussed with a view towards novel mRNA degradation-based metabolic engineering strategies. We show finally that mRNA degradation-based strategies have already successfully been applied to improve heterologous protein synthesis.

Overall, this review underlines how important mRNA degradation is in regulating *E. coli* metabolism and identifies mRNA degradation as a key target for innovative metabolic engineering strategies in biotechnology.

1. Introduction

Biotechnological processes are directly related to the efficiency of microbial cell factories and extensive efforts have been dedicated to engineer microbes with improved performances. Engineering strains to optimize the production of proteins and metabolites of interest requires molecular tools (plasmids, promoters, sequences) to construct (new) efficient protein synthesis pathways (Rosano et al., 2019). This is also associated with the development of optimized host strains. For example, *E. coli* strains have been engineered with lower proteolytic activity or reduced protein aggregation, and to express toxic proteins or facilitate protein folding (Gopal and Kumar, 2013). Another type of optimization in the field of metabolic engineering involves helping host cells cope with the metabolic burden associated with new enzyme activities, which compete for cellular resources with other processes (Nielsen, 2017; Liu et al., 2020). *E. coli* cells have for instance been modified to express extra tRNA coding genes to compensate for tRNA competition, or to express engineered cofactor pathways to ensure cell redox and energy balance (Gopal and Kumar, 2013; Wang et al., 2017).

The current challenge is to further improve the performance of engineered strains to develop economically viable biotechnological applications. Systems biology, using mathematical models to guide the design of optimal metabolic networks, is one of the best approaches to discover the innovative strategies required. Systems biology approaches have already proven very successful in solving problems of resource allocation and improving microbe efficiency (Becker and Wittmann,

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2018; Weimer et al., 2020; Yoo et al., 2020). Systems biology approaches require a general understanding of metabolism encompassing different levels of metabolic regulation and their interplay (Nielsen, 2017). Different regulatory levels are accessed by large-scale data on mRNA concentrations (the transcriptome), protein concentrations (the proteome), metabolite pools (the metabolome) and dynamic processes such as metabolite fluxes. Very few metabolic models include information on mRNA degradation rates. The fact that mRNA degradation is rarely considered in systems biology impedes rationally designed engineering strategies based on regulating mRNA stability. This is at least partially explained by the scarcity of large-scale mRNA stability datasets in the literature. Rates of mRNA degradation are generally determined by modelling the exponential decay of the mRNA concentration after transcription is arrested by adding rifampicin (Laguerre et al., 2018). Even for *E. coli*, which is one of the best studied model bacteria, large-scale mRNA stability data are only available for a very limited number of growth conditions (Bernstein et al., 2002; Esquerre et al., 2014; Chen et al., 2015; Morin et al., 2020) and mutant strains (Bernstein et al., 2004; Esquerre et al., 2016; Moffitt et al., 2016; Potts et al., 2017; Hadjeras et al., 2019). The fact is however, that since mRNAs are short lived relative to the cell cycle, mRNA degradation affects mRNA concentrations and should therefore also influence bacterial metabolism. This raises the questions of the precise role of mRNA degradation in regulating metabolism and its potential as a target for metabolic engineering.

Significant efforts have been made to elucidate the molecular mechanisms of mRNA degradation in *E. coli*, which has been well described in several recent reviews (Andrade et al., 2009; Carpousis et al., 2009; Hui et al., 2014; Mohanty and Kushner, 2016; Bechhofer and Deutscher, 2019). Briefly, mRNA degradation is initiated most of the time by an endonucleolytic cleavage carried out by RNase E (Regnier and Hajnsdorf, 1991; Bernstein et al., 2004) and also by RNase III (Regnier and Grunberg-Manago, 1990; Altuvia et al., 2018), RNase G (Mohanty and Kushner, 2008), RNase P (Mohanty and Kushner, 2007), RNase LS (Otsuka and Yonesaki, 2005) and RNase Z (Perwez and Kushner, 2006). RNase E cleaves mRNA by either 5′-end independent (direct entry) or by 5′-end dependent mechanisms. 3′-end exoribonucleases (polynucleotide phosphorylase (PNPase), RNase II, RNase R) then convert the endonucleolytic cleavage products to oligonucleotides that are further degraded to mononucleotides by oligoribonuclease. RNase E forms the RNA degradosome in association with PNPase, RNA helicase RhlB and enolase through its C-terminal scaffold region. 5′-end decapping (dephosphorylation by RppH to generate 5′-end monophosphate) (Celesnik et al., 2007) and 3′-end polyadenylation by poly (A)polymerase (PAP I) also favor mRNA degradation (Hajnsdorf et al., 1995; Mohanty and Kushner, 1999). PAP I preferentially polyadenylates mRNAs terminated by a Rho-independent transcription terminator (Haugel-Nielsen et al., 1996; Mohanty and Kushner, 2006). The stability of mRNA depends on the activity of the mRNA degradation machinery itself, but can also be affected by several transcript-specific mechanisms, being strongly dependent on sequence [5'UTR, secondary structure, codon bias (Hui et al., 2014)]; binding of regulatory small RNAs (sRNAs) (Gottesman and Storz, 2011; Lalaouna et al., 2013; Wagner and Romby, 2015) and/or RNA-binding proteins [e.g. ribosomes, Hfq, CsrA and ProQ (Folichon et al., 2003; Holmqvist et al., 2018; Holmqvist and Vogel, 2018; Romeo and Babitzke, 2018)]; and on subcellular localization (Irastortza-Olaziregi and Amster-Choder, 2020). Overall mRNA stability is also very sensitive to bacterial growth conditions (Vargas-Blanco and Shell, 2020). For *E. coli*, significant differences in the stability of the entire or parts of the mRNA population have been observed in cells growing at different rates or under stress (Bernstein et al., 2002; Esquerre et al., 2014; Chen et al., 2015; Morin et al., 2020). How and to what extent the mRNA degradation process is connected to the metabolic state in *E. coli* is not clear. A better understanding of the crosstalk between mRNA degradation, other cellular processes involved in gene expression and cell metabolic activity is essential to develop realistic models of *E. coli* cell function.

The goal of this review is to summarize for the first time the growing evidence that mRNA degradation is a regulatory level of *E. coli* metabolism. We will show that mRNA degradation interacts with transcription and translation, two other processes of gene expression. We will illustrate in greater detail how mRNA degradation regulates and is regulated by carbon, energetic and stress-related metabolism. Mathematical models of mRNA degradation able to account for complex crosstalk with other cellular processes will then be described. These models, which could be used in the future to evaluate and quantify the role of mRNA degradation in regulating global metabolic networks, should help understand the dynamics of mRNA degradation and reveal original mRNA degradation-based strategies for bacterial metabolic engineering. The last part of the review presents the mRNA degradation-based strategies that have already been used to enhance heterologous protein synthesis, highlighting the potential of mRNA degradation as a tool in biotechnology applications.

2. Crosstalk between mRNA degradation, transcription and translation

In this section we analyze the crosstalk between mRNA degradation and two cellular processes involved in gene expression, transcription and translation. In particular, we show that mRNA degradation can be used by cells to balance transcription regulation and eliminate poorly translated mRNAs.

2.1. The concentration dependence of mRNA degradation counterbalances transcription

The crosstalk between mRNA degradation and transcription is studied by analyzing the link between mRNA degradation and mRNA concentrations under various conditions. Several studies have shown that mRNA degradation is enhanced at high mRNA concentrations. A recent study found that for 30% of *E. coli* mRNAs, the frequency of cleavage peaked when the molecules accumulated (Lacoux et al., 2020). In addition, several modelling and experimental studies have shown that mRNA half-lives are inversely related to mRNA concentrations, indicating that concentration is a key determinant of mRNA stability (Bernstein et al., 2002; Mohanty and Kushner, 2003; Esquerre et al., 2015; Dressaire et al., 2018). While this negative correlation between concentration and half-life was not found in a further study (Chen et al., 2015), this negative relationship was unequivocally confirmed by the result that *lacZ* mRNA stability decreases when its transcription is artificially increased (Nouaille et al., 2017).

This crosstalk between mRNA degradation and transcription is orchestrated by the partitioning of RNA polymerase in the nucleoid and the action of the RNA degradosome at the inner membrane impeding any direct physical interaction between the machineries of transcription and degradation. From a dynamic point of view, most mRNA degradation is indeed post-transcriptional. At the mechanistic level, the inverse correlation between mRNA half-life and concentration has been attributed to an increased probability of RNase binding as mRNA concentrations increase (Nouaille et al., 2017) (Fig. 1). This phenomenon was recently modelled as mRNAs competing to bind to the same finite pool of RNase E (Etienne et al., 2020). The fact that mRNAs encoding inner membrane proteins, which co-localize with the RNA degradation machinery in the inner cytoplasmic membrane, are less stable (Moffitt et al., 2016) is experimental evidence that mRNA degradation is regulated by the probability of RNase binding. However, this destabilization was found elsewhere to not be statistically significant (Hadjeras et al., 2019). Further studies are required to decipher the role of RNase binding probability in regulating mRNA degradation, perhaps by modifying the distribution of RNase E between its mRNA targets by artificial titration.

Although mRNA degradation is generally a post-transcriptional process, it has been proposed that long mRNAs undergo co-transcriptional degradation (defined as the 5′-end being degraded before the 3′-end is

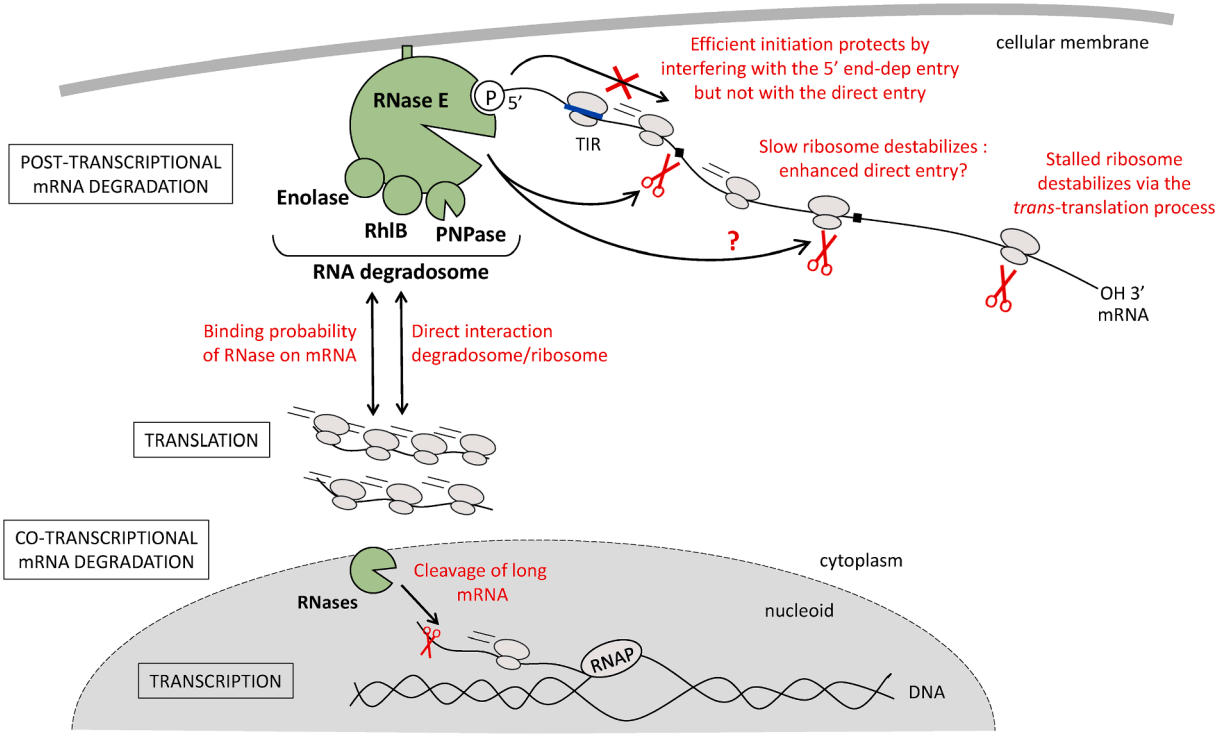

Fig. 1. Schematic representation of coordinated mRNA degradation, transcription and translation in *E. coli*. The small black boxes on the mRNA represent RNase E cleavage sites; the translation initiation region (TIR) is in blue. The two lines to the left of the ribosomes indicate fast translation along the mRNA. Scissors indicate cleavage activity. Arrows point out the actions specified by accompanying red text. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

synthetized) (Chen et al., 2015) (Fig. 1). Chen et al.'s modelling results suggest indeed that a sizeable fraction of mRNAs undergo co-transcriptional degradation (Chen et al., 2015). This finding is inconsistent with the nucleoid localization of RNA polymerase-bound mRNAs unless (i) co-transcriptional degradation is viewed as a stochastic process in which full-length and partially degraded mRNAs coexist and (ii) degradation is performed by free RNases independent of the membrane-anchored RNA degradosome. No effect of co-transcriptional degradation on the relationship between mRNA degradation and concentration has been reported.

The physiological implications of an inverse correlation between mRNA stability and concentration are still unknown. It seems that the concentration dependence of mRNA degradation counterbalances transcription by destabilizing the most abundant mRNAs. It has been speculated that the higher stability of low-concentration mRNAs could be a survival strategy that would favor the translation of mRNAs involved in stress response when present at low concentrations (Vargas-Blanco and Shell, 2020). More generally, the stabilization of low-concentration mRNAs would avoid their being completely degraded. Conserving residual mRNAs could be a cellular strategy to avoid wasting resources on *de novo* mRNA synthesis when growth resumes under better environmental conditions. The reason why highly concentrated mRNAs are destabilized is less clear. The apparent waste in energy involved in first synthesizing and then degrading mRNAs could be a control strategy to allow rapid changes in mRNA concentrations when required. This could confer an adaptive advantage to bacteria in competition with other microorganisms.

2.2. Poorly translated mRNAs are quickly degraded

The crosstalk between mRNA degradation and translation is mediated by the physical interaction between ribosomes and mRNA molecules. Translating ribosomes may impede or accelerate mRNA degradation. On one hand, the fact that mRNAs are generally destabilized when the ribosome density is reduced suggests that ribosomes are protective: i) decreasing the ribosome density by limiting translation initiation (via mutation in the ribosome binding site) or by creating regions without ribosome coverage (by prematurely terminating translation and decoupling transcription and translation) accelerates mRNA degradation (Deana and Belasco, 2005; Dreyfus, 2009), and ii) lowering ribosome coverage by selecting fast translating codons at the beginning of the sequence has been shown to destabilize *lacZ* mRNA (Pedersen et al., 2019). The proposed protective mechanism is that the ribosome impedes 5′-end-dependent degradation by RNase E. Ribosomes are expected to have less of an effect on RNase E degradation through the direct entry pathway, except in the rare cases where ribosomes directly shield a cleavage site (Fig. 1). On the other hand, ribosomes can trigger mRNA decay (Deana and Belasco, 2005; Dreyfus, 2009): i) slowly elongating ribosomes often accelerate bacterial mRNA decay, and ii) ribosomes stalled at the 3′-end because of inefficient translation termination or paused within the coding sequence because of special sequence features or amino acid starvation induce rapid cleavage of the mRNA. The mechanisms involved have only partially been elucidated but could be related to ribosomes acting as antennae to enhance the rates of capture of the RNA degradosome (Tsai et al., 2012) (Fig. 1). Only in the case of stalled ribosomes has the mechanism clearly been identified as *trans*-translation (a quality control mechanism, see ((Dulebohn et al., 2007) for a review).

Recent construction of large-scale sequence libraries and the generation of genome-wide mRNA stability data have extended the study of the role of ribosomes on mRNA degradation to the system level. These studies have also found both protective and destabilizing effects. Ribosomes were found to have a protective effect when translation initiation is increased in two studies of large-scale synthetic sequence libraries of ribosome binding sites and translation initiation regions (Kosuri et al., 2013; Cambray et al., 2018). In contrast, ribosomes were found to have a destabilizing effect on slowly translated mRNAs (encoded by less optimized codons) in two genome-wide studies (Esquerre et al., 2015; Boel

et al., 2016) but not by Lenz et al. probably because they used a different codon bias metric (Lenz et al., 2011). The lower stability of slowly translated mRNA was recently named "slowness-mediated decay" in a review to highlight the general relevance of ribosome-dependent mRNA destabilization (Rak et al., 2018).

In conclusion, ribosomes appear to act as positive effectors of mRNA stability when the rate of translation initiation is high but as negative effectors when the rate of translation elongation is slow or zero (Fig. 1). As a result, mRNAs with inefficient translation initiation or termination, or slow translation elongation, are quickly degraded, ensuring that poorly translated mRNAs that are no longer needed do not impose regulatory constraints, create resource allocation problems or become an energetic burden on the cell (Deana and Belasco, 2005). This apparently contradictory role of ribosomes in the protection and destabilization of mRNAs thus appears to be physiologically coherent.

3. Crosstalk between mRNA degradation and metabolic activity

Here, we focus on the crosstalk between mRNA degradation regulation and metabolic adaptations to growth conditions. The first section describes how the activity of metabolic pathways can be controlled by changes in mRNA degradation and the second section shows that the activity of the mRNA degradation machinery is in turn controlled by metabolic components, altogether demonstrating reciprocal regulatory interactions.

3.1. Metabolic activity can be regulated by changes in mRNA degradation

We show that i) the activity of the central carbon metabolism, ii) metabolic responses to stress (high osmotic pressure, iron or oxygen deprivation and presence of reactive forms of oxygen), and iii) adaptations to temperature, are controlled by mRNA degradation regulation at several levels (blue arrows in Table 1).

3.1.1. Central carbon metabolism

This metabolic pathway is widely affected by degradation regulation (as illustrated in Fig. 2). mRNA degradation regulates the utilization of various carbon sources (glucose, mannose, galactose, fumarate and succinate) in response to cell demand. In general, this involves feedback control at the level of carbon source uptake or the first steps of the metabolic pathway. The *ptsG* gene encoding the main glucose transporter of *E. coli*, EIICB^{Glc}, is destabilized via RNase E-mediated degradation after binding to SgrS sRNA and Hfq (Vanderpool, 2007; Morita et al., 2008). The *manX* mRNA encoding the mannose transporter is similarly destabilized by SgrS sRNA and the RNA degradosome (Rice and Vanderpool, 2011), but also by another molecular mechanism involving the RNA-binding protein CsrA (Esquerre et al., 2016). The *galK* mRNA encoding galactokinase, the first enzyme of the galactose degradation pathway, is destabilized by binding of the sRNA Spot42, leading to discordant expression of the whole *gal* operon and inhibition of galactose utilization (Moller et al., 2002). For the *ptsG*, *galK* and *manX* mRNAs, degradation regulation was the consequence of translation repression (Vanderpool, 2007; Morita et al., 2008; Rice and Vanderpool, 2011). In the cases of fumarate and succinate, although the molecular mechanism has not been elucidated, their utilization is dependent on RNA degradosome formation (Tamura et al., 2013).

As well as regulating the first steps of the central carbon metabolism, mRNA degradation also affects the core of the metabolic pathway. More than 20 mRNAs of the glycolysis and TCA cycle have been identified as degradation-regulated transcripts, meaning that varying their stability in different growing environments directly affects their concentration (Esquerre et al., 2014). For several examples in the glycolysis and the TCA cycle, concentrations of key enzymes or mRNAs have been shown to depend on the expression of particular components of the mRNA degradation machinery. The expression of phosphoenolpyruvate (PEP) carboxylase is drastically reduced in an RNase E and RNA helicase CsdA double mutant, impeding the strain's growth on glucose (Tamura et al., 2013). The mRNA levels of several glycolytic enzymes (*ptsA,* encoding the sugar phosphotransferase system component; *glk,* glucokinase; *pgi,* phosphoglucose isomerase; *tpiA,* triose-phosphate isomerase; and *eno,* enolase*)* are linked to the expression levels of RNase G and/or RNase III (Lee et al., 2002; Sim et al., 2010) and two mRNAs, *aceEF* encoding pyruvate dehydrogenase and *sdhC* encoding succinate dehydrogenase, are specifically destabilized by RNase III-mediated cleavage (Gordon et al., 2017). In addition, various mRNAs involved in glycolysis (*pgi*, encoding phosphoglucose isomerase; *pfkA*, phosphofructokinase*; pgk*, phosphoglycerate kinase; *ldhA*, lactate dehydrogenase; and *pck*, the gluconeogenic protein PEP carboxykinase) and the TCA cycle (*gltA*, encoding citrate synthase; *sucA*, 2-oxoglutarate dehydrogenase; and *sucC*, succinyl-CoA synthetase) are specifically stabilized in the absence of PNPase (Bernstein et al., 2004; Dressaire et al., 2018). The stability of many of these mRNAs (*pgi, pfkA, pgk, ldhA, pck* and *gltA*) also changes when the activity of the glycolytic partner of the degradosome, enolase, is affected (Bernstein et al., 2004). This is also true for *pfkA*, *tpiA*, *pck* and the malic enzyme mRNAs *maeA* and *maeB* when the activity of the post-transcriptional regulator CsrA is modified (Esquerre et al., 2016; Potts et al., 2017)). Altogether, these results show that the activities of the glycolysis pathway and of the TCA cycle are regulated by mRNA degradation.

mRNA degradation is also involved in the regulation of the very last steps of the central carbon metabolism, end-product management, specifically intracellular glycogen accumulation and acetate excretion. CsrA promotes the degradation of several transcripts involved in glycogen metabolism, *glgCAP* (Liu et al., 1995; Baker et al., 2002) and *glgB* (Esquerre et al., 2016). For *glgCAP*, mRNA degradation follows CsrA-mediated translation inhibition (Baker et al., 2002). Acetate metabolism is regulated by the sRNA SdhX, which negatively regulates the *ackA* gene encoding acetate kinase (De Mets et al., 2019). SdhX is cleaved from the 3′-end of the *sdhCDAB-sucABCD* transcript in the TCA cycle by RNase E (and to a lesser extent by RNase III), thereby linking acetate metabolism to the TCA cycle (De Mets et al., 2019; Miyakoshi et al., 2019).

3.1.2. Stress-related metabolic responses

Resistance to osmotic stress has been shown to be related to the stabilization of *betT* mRNA, which encodes a choline transporter (Sim et al., 2014). Under hyper-osmotic stress conditions, the half-life of *betT* mRNA was significantly increased in association with decreased RNase III activity. When iron is scarce, the RyhB sRNA destabilizes mRNAs in the Fur regulon via an RNase E-dependent pathway to down-regulate the expression of genes encoding iron containing proteins (*sodB*, for superoxide dismutase; *sdhCDAB*, succinate dehydrogenase; *acnA*, aconitase; and *fumA*, fumarase) (Masse and Gottesman, 2002; Masse et al., 2007). RyhB stability and function are in turn controlled by polyadenylation via PAP I (Sinha et al., 2018). The expression of genes involved in iron metabolism is also repressed by CsrA upon variations in intracellular iron concentrations, but more likely indirectly (Potts et al., 2017). Protection against oxidative stress involves PNPase, which is able to bind and remove RNAs damaged by H_2O_2, thereby favouring growth in oxidative conditions (Hayakawa et al., 2001; Wu et al., 2009). Under oxidative stress the capped mRNA *trxB*, which encodes thioredoxin reductase, is stabilized by the inactivation of the decapping enzyme ApaH. This may limit disulfide bond formation and oxidative stress damage (Luciano et al., 2019). Finally, under low oxygen conditions, expression of the glycolytic enzyme enolase is promoted by coordinated RNase III and RNase G regulation (Lee et al., 2019). Under these conditions, increased RNase III expression favours RNase III-mediated cleavage of *eno* mRNA, facilitating its translation. At the same time, RNase III destabilizes the *rng* mRNA, which leads to a decrease in RNase G concentrations and thereby to a reduction in RNase G-mediated degradation of *eno* mRNA.

Table 1
Crosstalk between mRNA degradation and metabolism in *E. coli*. This table presents examples of interconnections between mRNA degradation and metabolic pathways or stress responses. The green arrows indicate regulation of an mRNA degradation component by a metabolic factor, and the blue arrows regulation of a metabolic pathway by a component of mRNA degradation. CSP: cold shock protein. *non-specifically targeted mRNAs.

Metabolism		Direction	Components of the degradation	Type of interaction	References
Pathway	Factors (metabolite, mRNA or protein)				
Central carbon metabolism					
Sugar uptake and utilization	*ptsG*	←	RNase E, mediated by SgrS	mRNA destabilization	Vanderpool (2007); Morita et al. (2008)
	manXYZ	←	RNase E, mediated by SgrS and CrsA	mRNA destabilization	Rice and Vanderpool (2011); Esquerre et al. (2016))
	galK	←	mediated by Spot42	mRNA destabilization	Moller et al. (2002)
Glycolysis	Eno	→	RNA degradosome	Normal degradative activity	Bernstein et al. (2004)
	eno	←	RNase G	mRNA destabilization	Lee et al. (2002); Gordon et al. (2017)
	eno	←	RNase III	mRNA processing	Lee et al. (2019)
	Eno	→	RNase E	Enzyme localization	Murashko and Lin-Chao (2017)
	pgi, pgk, ldhA	←	PNPase, enolase	mRNA destabilization	Bernstein et al. (2004); Dressaire et al. (2018)
	tpiA	←	Mediated by CsrA	mRNA stabilization	Esquerre et al. (2016)
	pfkA	←	PNPase, enolase, mediated by CsrA	mRNA stabilization	Bernstein et al. (2004); Esquerre et al. (2016); Dressaire et al. (2018)
Connection glycolysis/ TCA	*pck*	←	PNPase, enolase, mediated by CsrA	mRNA destabilization	Bernstein et al. (2004); Potts et al. (2017); Dressaire et al. (2018)
	aceEF	←	RNase III	mRNA destabilization	Gordon et al. (2017)
	maeA, *maeB*	←	Mediated by CsrA	mRNA destabilization	Potts et al. (2017)
Glycogen biosynthesis	*glgCAP*, *glgB*	←	Mediated by CsrA	mRNA destabilization	Romeo et al. (1993); Liu et al. (1995); Baker et al. (2002); Esquerre et al. (2016)
TCA	Citrate	→	PNPase	Enzyme inhibition	Nurmohamed et al. (2011)
	sucA	←	PNPase	mRNA destabilization	Dressaire et al. (2018)
	sucABCD	←	RNase E or RNase III	mRNA processing	De Mets et al. (2019); Miyakoshi et al. (2019)
	gltA	←	PNPase, enolase	mRNA destabilization	Bernstein et al. (2004); Dressaire et al. (2018)
	sdhCDAB	←	Mediated by RyhB and PAP I	mRNA destabilization	Masse and Gottesman (2002); Sinha et al. (2018)
	sdhC	←	RNase III	mRNA destabilization	Gordon et al. (2017)
	acnA, fumA	←	Mediated by RyhB	mRNA destabilization	Masse and Gottesman (2002)
Acetate metabolism	*ackA*	←	RNase E, mediated by SdhX	mRNA destabilization	De Mets et al. (2019)
Slow growth/stationary phase	Pka	→	RNase R	Protein instability	Liang and Deutscher (2012)
		→	RNase II	Enzyme inhibition	Song et al. (2016)
Nucleotide and energy metabolism					
Nucleotide and energy metabolism	ATP	→	PNPase	Enzyme inhibition	Del Favero et al. (2008)
	Cyclic di-GMP	→	PNPase	Enzyme activation	Tuckerman et al. (2011)
	ATP	→	PAP I, RhlB	Enzyme substrate	Raynal and Carpousis (1999); Worrall et al. (2008)
	Np_nN, NAD	→	ApaH, RppH, NudC	mRNA protection	Cahova et al. (2015); Luciano et al. (2019); Hudecek et al. (2020)
Amino acid metabolism					
Lysine biosynthesis	DapF	→	RppH	Enzyme activation	Lee et al. (2014); Gao et al. (2018)
Glutamine metabolism	glucosamine-6-phosphate	→	RNase E	Enzyme inhibition	Mardle et al. (2020)
Amino acid starvation	(p)ppGpp	→	Endoribonuclease toxins	Indirect activation	Pacios et al. (2020)
Amino acid starvation	ppGpp, DksA	→	PAP I	Transcription inhibition	Nadratowska-Wesolowska et al. (2010)
Iron homeostasis, osmotic and oxidative stress					
ROS detoxification	*sodB*				

(*continued on next page*)

Table 1 (*continued*)

Metabolism		Direction	Components of the degradation	Type of interaction	References
Pathway	Factors (metabolite, mRNA or protein)				
		←	Mediated by RyhB and PAP I	mRNA destabilization	Masse and Gottesman (2002); Sinha et al. (2018)
	oxidized mRNA	←	Free PNPase	mRNA destabilization	Hayakawa et al. (2001), Wu et al. (2009)
Iron metabolism	Fur regulon	←	RNase E, mediated by RyhB and CsrA	mRNA destabilization	Masse and Gottesman (2002); Potts et al. (2017)
Osmotic stress	*betT*	←	RNase III	mRNA destabilization	Sim et al. (2014)
Oxidative stress	*trxB*	←	ApaH	mRNA destabilization	Luciano et al. (2019)
	Cd^{2+}	→	ApaH	Enzyme inactivation	Luciano et al. (2019)
Adaptation to temperature					
Cold temperature	CSPs	←	PNPase, RNase II, RNase III	mRNA destabilization	Zangrossi et al. (2000); Yamanaka and Inouye (2001); Kim et al. (2008)
	bulk mRNAs *	←	RNase R, CsdA	mRNA destabilization	Prud'homme-Genereux et al. (2004); Zhang et al. (2018)
	cold shock induction	→	CsdA	Degradosome composition	Jones et al. (1996); Prud'homme-Genereux et al. (2004)
High temperature	*groEL, dnaK, hslV, htpG*	←	CspC	mRNA stabilization	Shenhar et al. (2009)

3.1.3. Adaptation to temperature shifts

Many components of the mRNA degradation machinery are involved in adaptations to temperature shifts. During acclimation to cold temperatures, RNase R and cold shock proteins ensure appropriate mRNA degradation as part of a two-member mRNA surveillance system (Zhang et al., 2018). PNPase is essential for *E. coli* growth at low temperatures. During acclimation, the PNPase mRNA is stabilized by the down-regulation of RNase III activity by the protein YmdB resulting in a twofold increase in PNPase activity (Zangrossi et al., 2000; Kim et al., 2008). At the end of the acclimation phase, PNPase selectively destabilizes the mRNAs of cold shock proteins to facilitate a return to basal expression levels and reinitiate cell growth (Yamanaka and Inouye, 2001). It is noteworthy that RNase II can compensate the loss of PNPase under cold conditions (Awano et al., 2008), but RNase R cannot (Cairrao et al., 2003). The resistance of *E. coli* to very low temperatures also depends on the helicase activity of cold shock induced CsdA to degrade double-stranded RNAs (Prud'homme-Genereux et al., 2004). The heat shock response involves no changes in stability of bulk mRNA but the specific stabilization of the major heat shock regulator *rpoH* mRNA and destabilization of the four major heat shock mRNAs (*groEL*, *dnaK*, *hslVU* and *htpG*) (Henry et al., 1992; Shenhar et al., 2009). This destabilization is the consequence of the lower concentration of the RNA chaperone CspC upon temperature shift-ups (Shenhar et al., 2009).

3.2. The mRNA degradation machinery is regulated by metabolic factors

The preceding section described how metabolic activities are regulated by changes in mRNA degradation and how widespread these interactions are across the metabolic network. The present section focusses on the ways in which metabolites and metabolic enzymes control the mRNA degradation machinery. These interactions involve i) central carbon metabolism, ii) nucleotide and amino acid metabolism, and iii) stress and temperature shift metabolism (Table 1, with green arrows where the factors are known).

3.2.1. Central carbon metabolism

Citrate, a metabolite of the TCA cycle whose concentration depends strongly on the carbon source (Bennett et al., 2009), inhibits the exoribonuclease activity of PNPase (Nurmohamed et al., 2011). Citrate-mediated inhibition of PNPase is probably widespread in all three domains of life, since citrate has also been predicted to bind mitochondrial and archaeal PNPases (Stone et al., 2017). Normal mRNA turnover also depends *in vivo* on the activity of enolase, a glycolytic enzyme of the central carbon metabolism (Bernstein et al., 2004), which particularly affects the subcellular localization and activity of RNase E under anaerobic conditions (Murashko and Lin-Chao, 2017).

3.2.2. Nucleotide and amino acid metabolism

In nucleotide metabolism, ATP is the direct substrate of two enzymes in the degradation machinery, PAP I and the RhlB RNA helicase, which respectively add adenosine residues at the 3′-end of RNAs and unwind double-stranded RNAs to facilitate their degradation (Raynal and Carpousis, 1999; Worrall et al., 2008). The two nucleotides, cyclic di-GMP (a signalling metabolite synthetized from two GTP molecules) and ATP (with a binding site distinct from that of the substrates), are respectively positive and negative allosteric effector of PNPase activity (Del Favero et al., 2008; Tuckerman et al., 2011). More generally, a new mechanism of mRNA protection by nucleotides is currently emerging ((Cahova et al., 2015; Luciano et al., 2019; Hudecek et al., 2020), see (Vargas-Blanco and Shell, 2020) for a review). This involves the 5′-end capping of RNA by nucleoside polyphosphate (Np_n) or nicotinamide adenine dinucleotide (NAD). Under stress, certain transcripts acquire a 5′ protective Np_n-cap through an increased concentration of its precursor, dinucleoside polyphosphate (Np_nN). The mechanism of NAD-capping and its physiological significance are still unknown. In amino acid metabolism, the enzyme diaminopimelate epimerase (DapF) interacts with the decapping enzyme RppH and enhances its activity in promoting mRNA degradation (Lee et al., 2014; Gao et al., 2018). DapF is involved in lysine biosynthesis and its expression is stimulated in amino acid-rich medium (Lee et al., 2014). In addition, glucosamine-6-phosphate, produced by the aminotransferase GlmS in glutamine metabolism, has recently been shown to inhibit RNase E (Mardle et al., 2020).

3.2.3. Stress metabolism and temperature adaptation

The accumulation of (p)ppGpp in bacteria under nutritional starvation leads to the stringent response (Cashel and Gallant, 1969; Magnusson et al., 2005). Under these conditions, the activity of the major promoter of PAP I is drastically inhibited by ppGpp and DksA (Nadratowska-Wesolowska et al., 2010). This regulation avoids ATP being wasted on polyadenylation in non-growing cells. (p)ppGpp also activates endoribonuclease toxins that cleave specific sequences of mRNAs, inhibit protein synthesis and trigger cell death (Yamaguchi and Inouye, 2011; Yamaguchi et al., 2011). It has been suggested that (p)ppGpp may facilitate the accumulation of inorganic polyphosphate, an activator of

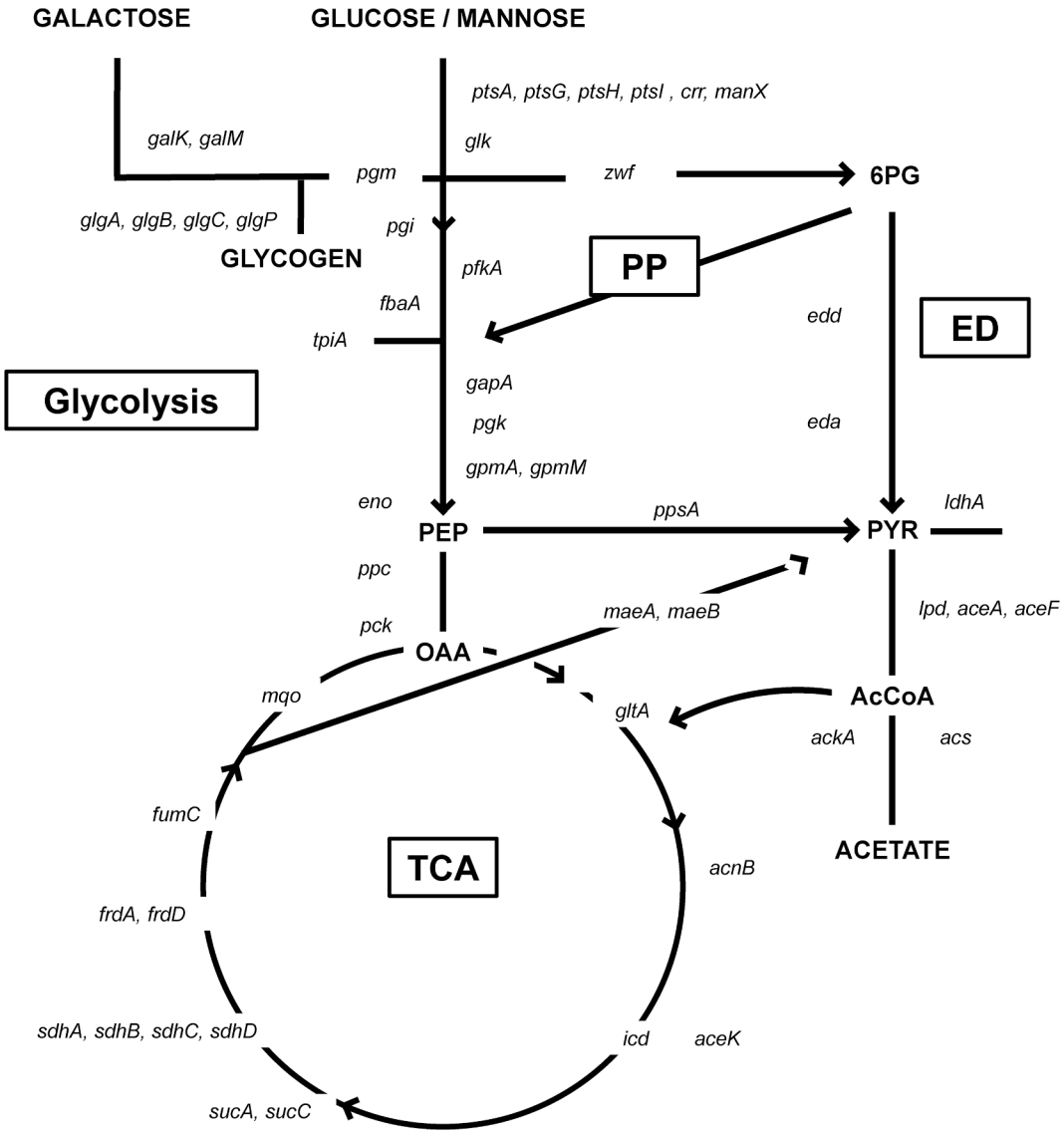

Fig. 2. Genes in the central carbon metabolism whose expression is regulated by mRNA degradation. The 51 genes shown in the figure have their expression regulated by mRNA degradation; they represent around 20% of all the genes involved in carbohydrate transport and metabolism in *E. coli* (according to the database of Clusters of Orthologous Groups of proteins). The functions of the genes are described in the text.

Lon protease implicated in antitoxin degradation and thus endoribonuclease toxin activation.

Under stress, the degradation machinery's activity is also influenced by post-translational modifications controlled by metabolic enzymes. Levels of Pka, which acetylates lysine residues, depend strongly on growth conditions and stress through the positive regulation of its expression by cAMP-CRP (a mediator of catabolite repression) and via the cooperative effect of acetyl-CoA, the donor of the acetyl group, on Pka activity (Castano-Cerezo et al., 2011; de Diego Puente et al., 2015). This enzyme plays a central role in the regulation of 3′ exoribonuclease activity since acetylation stabilizes RNase R and inhibits the catalytic activity of RNase II (Bechhofer and Deutscher, 2019). Another example of a regulatory post-translational modification is the inactivation of the decapping enzyme ApaH through the formation of disulfide bonds under oxidative stress (Luciano et al., 2019).

Several studies have also shown that the composition and/or activity of the degradation machinery depend strongly on growth conditions. Although RNase E, PNPase and RNase III autoregulate their mRNA concentration to achieve activity homeostasis (Hui et al., 2014), mRNA concentrations of enolase, RhlB, RppH and PAP I have been shown to change when the growth rate is decreased (Esquerre et al., 2014). Furthermore, upon entering stationary phase, the induction of RraA expression, an inhibitor of RNase E (Zhao et al., 2006), potentially affects the concentrations of more than 2000 mRNAs (Lee et al., 2003). The composition of the RNA degradosome has also been reported to vary with growth conditions (Tejada-Arranz et al., 2020). Cold temperatures indeed induce the expression of CsdA, which replaces RhlB in the RNA degradosome, facilitating the degradation of structured mRNAs and allowing *E. coli* to grow normally (Jones et al., 1996; Prud'homme-Genereux et al., 2004). In all these examples, the mechanisms involved in coordinating the degradation and metabolic responses to the growth conditions remain to be identified.

4. Integration of mRNA degradation into systems biology approaches

Systems biology is a powerful means to address the complex cross-talk between mRNA decay and other cellular processes. In this context, mathematical modelling has long been a companion to experimental studies of mRNA degradation, whether to extract decay rates from data

or to help integrate and analyze new experimental findings. In this section, we will illustrate with a few examples what has been achieved to date in the mathematical modelling of mRNA degradation and its interplay with other cell processes.

4.1. Simple kinetic models of mRNA degradation

The simplest and most widely used model describes the change in time of the concentration $x(t)$ of a given mRNA as the difference between its synthesis rate, v_s, and its degradation rate, v_d: $\frac{dx(t)}{dt} = v_s - v_d$. In the case of transcription arrest ($v_s = 0$), the model simplifies to: $\frac{dx(t)}{dt} = -v_d$ (Eq. 1). Assimilating the degradation mechanism to a first-order reaction with degradation constant k_{deg}: $v_d = k_{deg} \times x(t)$, leads to the ordinary differential equation (ODE) $\frac{dx(t)}{dt} = -k_{deg} \times x(t)$ and its solution $x(t) = x(0) \times e^{(-k_{deg} \times t)}$. Fitting this exponential function to experimentally measured decay curves yields k_{deg} and the mRNA half-life: $t_{1/2} = \frac{log(2)}{k_{deg}}$. Using alternative expressions for v_d leads to a greater diversity of decay curves. For instance, mRNAs competing for the same limited pool of endoribonucleases leads to non-exponential decay (Etienne et al., 2020), as well as multi-step degradation processes in which mRNA molecules are in different stages of degradation (Deneke et al., 2013a).

4.2. Models coupling mRNA degradation to transcription and translation

The exponential model assumes that mRNA decay takes place independently of other cellular processes. Considering the complex relationships between mRNA degradation and metabolic activity, transcription and translation has led to the development of more complex models. For instance, models coupling transcription to decay in mRNA stability analyses have been shown to agree well with residual levels of transcription elongation by RNA polymerase measured after transcription initiation arrest (Chen et al., 2015; Moffitt et al., 2016; Dar and Sorek, 2018) and with residual mRNA levels at the end of the degradation process (Moffitt et al., 2016). The transcription rate, v_s, is non-zero in this case and compensates for the degradation rate v_d during the time taken by RNA polymerase to complete mRNA elongation. The transcription rate becomes zero thereafter and mRNA concentrations decay exponentially.

Another well-studied interaction is the sensitivity of mRNA stability to ribosome loading and the rate of ribosome translation. Translation-degradation models describe the dynamics of ribosomes along the mRNA using a variety of formalisms (Carrier and Keasling, 1997; Deneke et al., 2013b; Mier-y-Teran-Romero et al., 2013); see (von der Haar, 2012; Zur and Tuller, 2016) for a review). Deneke et al. (2013b) use a stochastic model combining these two processes, whereby translation elongation proceeds in steps with the ribosome either moving to another position on the mRNA or the latter being degraded if it is accessible to endonucleolytic enzymes. The stabilizing effect of translating ribosomes leads to predicted degradation profiles that diverge from exponential decay and agree better with dynamic transcriptomics data from *E. coli*.

Other stochastic models have been used to quantify the regulation of mRNA degradation by sRNAs during stress responses, through the modulation of their concentration, the involvement of protein Hfq or their interplay with ribosomes (Levine et al., 2007; Adamson and Lim, 2011; Lavi-Itzkovitz et al., 2014; Arbel-Goren et al., 2016). In Lavi-Itzkovitz et al. (2014) for instance, quantitative experiments and stochastic modelling revealed that bound ribosomes facilitate the binding of a sRNA to the 5'UTR of a target mRNA. The increased translation rates result in strengthened repression by the sRNAs and destabilization of the target mRNA.

Further models combining mRNA degradation, transcription and translation have notably been used to study the propagation of noise during bacterial protein expression and the occurrence of bursts in protein production (McAdams and Arkin, 1997; Thattai and van Oudenaarden, 2001; Paulsson, 2005; Friedman et al., 2006; Kim and Jacobs-Wagner, 2018). For instance, while the transcriptional origins of bursts have been well characterized, modelling helped demonstrate that the short lifetimes of bacterial mRNAs also contribute to this phenomenon (Kim and Jacobs-Wagner, 2018).

4.3. Models coupling mRNA degradation to metabolism

The tight coupling between metabolic activity and mRNA degradation having only recently come into focus, this is reflected by the relative scarcity of modelling efforts on this topic. The production and degradation of mRNAs are often omitted in metabolic models that include gene expression. The few models that do include mRNA decay describe it with simple first-order kinetics and do not consider metabolism–degradation crosstalk (Usuda et al., 2010). Studies analyzing the impact of mRNA degradation on cell processes with simplified models are notable exceptions. Mitarai et al. (2008) for example, combined quantitative experiments with modelling of the crosstalk between translation, degradation and energetic metabolism (Mitarai et al., 2008). They found that cells appear to minimize the risk of ribosome collision by selecting codon usage and making mRNAs unstable. The energetic cost of wasting mRNAs (producing and recycling them, and maintaining more RNA polymerases to make up for the mRNA loss) was found to be lower in terms of translation time than that of synthesizing ribosomes to compensate for those that collide. Thomas et al. (2018) used a stochastic coarse-grained model of bacterial growth to show that fluctuations in the degradation of mRNAs coding for metabolic enzymes are a major source of growth heterogeneity at slow growth rates (Thomas et al., 2018). Another deterministic model has been used to describe the production of the transcription-translation machinery from amino acids and nucleotides at the genome-scale, under environmental and biological constraints related to nutrient availability in the growth medium and reaction directionality (Thiele et al., 2010). This model suggests that genes involved in transcription, mRNA degradation (notably the *rne* gene coding for RNase E), protein folding and active tRNA availability are key factors for the optimal production of ribosomes.

4.4. Towards genome-scale models of mRNA degradation crosstalk with other cell processes

The mechanistic models cited above describe mRNA degradation and its coupling with cellular processes at various depths of molecular detail. Models focusing on a given mRNA are generally more precise and informative, but difficult to scale up and parameterize when several if not all cell mRNAs need to be considered. This is problematic as most systems biology studies rely nowadays on the acquisition of high throughput data. At the other end of the spectrum, coarse-grained models simplify molecular details by lumping molecular constituents together (Thomas et al., 2018) or by simplifying the kinetics of the reaction (Thiele et al., 2010). They shed light on the grand principles involved in the control of mRNA degradation and interdependent cell processes but lack the level of molecular detail needed to fully understand how cell processes influence the fate of individual mRNAs. Lastly, the models rely on very different formalisms, stochastic, deterministic, or constraint based, which vary in their capacity to describe cellular processes, from the noise of biochemical reactions to the flux of matter in constraint-based models. Efforts have been made to bring all these formalisms together in a single model. For instance, whole-cell models of *Mycoplasma genitalium* and *E. coli* have been developed combining deterministic constraint-based models of the metabolic network, deterministic ODE models of cell division, and stochastic models of transcription, translation, and of mRNA and protein degradation (Karr et al., 2012; Macklin et al., 2020).

An interesting alternative involves extending genome-scale reconstructions to combine metabolic and gene product expression

pathways (Lerman et al., 2012; O'Brien et al., 2013; Lloyd et al., 2018). These so-called ME-Models, already available for *E. coli* (O'Brien et al., 2013; O'Brien and Palsson, 2015; Salvy and Hatzimanikatis, 2020), have increased predictive capacity and accuracy. Although they come at a higher price in terms of simulation difficulty and conceptual understanding (O'Brien and Palsson, 2015), the possibility of integrating omics datasets – including mRNA degradation rate constants [e.g.(Salvy and Hatzimanikatis, 2020)] – should facilitate exploration of the crosstalk between mRNA decay, metabolism and the other gene expression processes.

The tight coupling of mRNA degradation to cell processes thus poses a challenge to modellers in systems biology. There is no ideal model but clearly, it should be tailored to best exploit the available experimental data and provide information on the crosstalk studied. This represents a challenge to experimentalists as well. Experiments need to be carefully designed to produce quantitative and informative multi-omics data reporting on the states of metabolic activity and gene expression. Ultimately, combining these quantitative experiments and mathematical modelling should help identify missing connections between mRNA degradation, cell metabolism and other cell processes, and grasp how these intertwined connections shape the dynamics of mRNA decay. The resulting models could be used as test beds to elaborate biotechnological strategies aimed at modulating mRNA stability.

5. Playing with mRNA degradation to improve heterologous protein synthesis

While metabolic engineering strategies based on systems biology approaches are yet to emerge, new molecular strategies have already been combined with host strain optimization based on mRNA degradation regulation to improve heterologous protein production. These approaches use recent advances in the understanding of the molecular regulation of mRNA degradation and of the role played by degradation in regulating mRNA concentrations and corresponding protein levels.

5.1. Molecular strategies for mRNA stabilization

These approaches involve stabilizing an mRNA molecule, in this case the mRNA of the heterologous protein, by introducing regulatory sequence elements to up-regulate its expression and thereby the expression of the corresponding protein. The regulatory sequence elements are localized at the extremities and in the coding sequence of the mRNA molecule. Different half-lives and thus levels of mRNA can be achieved by changing the 5'UTR sequence of the molecule (Nouaille et al., 2017; Viegas et al., 2018). Native and artificial 5'UTRs have been used to modulate heterologous protein expression by factors of about 4.5 and 8 for native and artificial UTRs, respectively. On a larger scale, thousands of synthetic sequences of the 5'UTRs and the first codons of the reporter gene have be designed to obtained mRNA half-lives ranging from 2 to 11 min (Cambray et al., 2018). From a molecular point of view, it is known that riboswitches (for example, the *lysC* lysine riboswitch) at the 5′-end extremity can directly influence the corresponding protein level by modulating mRNA degradation when RNase E access is impaired (Caron et al., 2012; Richards and Belasco, 2021).

At the 3′-extremity, inserting synthetic repetitive palindromic sequences to prevent 3′-5′ exonuclease degradation improves heterologous protein expression by 23 to 54% depending on the length of the interval between the repeated palindromic sequence and the stop codon (Deng et al., 2019).

Features of the coding sequence also contribute to a *cis*-control of mRNA stability and protein levels. At the omics scale of endogenous *E. coli* genes, optimized codon sequences promote mRNA stability and protein synthesis during exponential growth (Esquerre et al., 2015; Boel et al., 2016). In the case of polycistronic operons, protective RNA structures within the coding sequence ensure differential mRNA decay and thus differential protein expression (Dar and Sorek, 2018). In the *tatABC* operon for instance, the 2.5-fold greater stability of *tatA* mRNA relative to the *tatBC* region is associated with the 25-fold higher expression of TatA protein compared with TatBC. Including stabilizing RNA elements in synthetic operons may be a way to generate non-uniform protein stoichiometries when expressing artificial metabolic pathways in synthetic biology.

5.2. Optimizing host strains by destabilizing specific mRNAs

In biotechnological processes, acetate inhibits *E. coli* growth by altering the internal pH and therefore limits heterologous protein synthesis. Techniques based on destabilizing the mRNAs of proteins involved in acetate accumulation have been investigated to develop hosts better suited for heterologous protein production. In Negrete et al., specific mRNAs are destabilized by sRNAs via antisense base pairing (Negrete et al., 2013). Over-expression of native SgrS sRNA destabilizes *ptsG* mRNA, decreasing glucose uptake and thereby reducing acetate production fivefold (Negrete et al., 2013). Synthetic antisense RNAs targeting the *pta* and *ackA* genes, respectively encoding phosphotransacetylase and acetate kinase, two key enzymes for acetate synthesis, have also been used to decrease mRNA levels (by 10–20%) and thus acetate production by a factor 2.5 (Kim and Cha, 2003). This reduction in acetate production resulted in a twofold increase in heterologous protein synthesis. A high-throughput screening strategy has been proposed to design synthetic *trans*-regulatory antisense RNAs to down-regulate endogenous gene expression (Sharma et al., 2012).

5.3. Host strain optimization by large-scale regulation of mRNA degradation

Two lines of strain optimization via the regulation of mRNA degradation at the cellular level have been explored (i) global stabilization and (ii) selective destabilization/stabilization.

A direct and simple strategy to increase heterologous protein synthesis is to use ribonuclease-deficient host strains to prevent mRNA degradation. The BL21 Star™(DE3) strain, containing the *rne*-131 allele expressing a truncated RNase E (lacking the C-terminal domain), is thus widely used to overproduce heterologous proteins in *E. coli*. However, the effects of this strategy are not so simple. First, in the case of RNase E, different mRNAs are stabilized to different degrees (Lopez et al., 1999) and RNase E autoregulation partially compensates for the loss of activity (Leroy et al., 2002), while for 3′-5′ exoribonucleases (other than oligoribonuclease), overlapping activities compromise efforts to reduce them (Andrade et al., 2009). Second, mRNA stabilization cannot be extended very far without provoking metabolic stress in the host strain because of limited energy resources and the finite number of ribosomes for translation.

An alternative approach for efficient heterologous protein synthesis has been developed that is also based on genome-scale regulation of mRNA degradation but does not lead to competition for cellular resources in the host strain (Mao and Inouye, 2012; Venturelli et al., 2017; Wu et al., 2020). In this approach, the endoribonuclease MazF is used to degrade most mRNAs except for the mRNA of interest, which is protected by the absence of MazF recognition sites in the sequence. The cells remain metabolically active but unable to grow and their resources (energy, ribosomes, etc.) are redirected from biomass formation to the expression of the protein of interest. Heterologous protein levels have thereby been increased by a factor of between 3 and 11 depending on the level of MazF induction (Venturelli et al., 2017; Wu et al., 2020).

6. Conclusions

This review demonstrates that mRNA degradation is a central part of metabolic regulation in *E. coli*. mRNA degradation affects gene expression, transcription and translation and a large variety of metabolic pathways and stress responses, and mRNA degradation is itself affected by cellular metabolic activity.

The evidence discussed here of the many individual metabolic pathways regulated by mRNA degradation raises questions about how metabolism and mRNA degradation are coordinated at the cellular level: how is the metabolic state sensed to regulate mRNA degradation? Are there master regulators that coordinate the control of metabolism and mRNA degradation? The most obvious connection between the metabolic state and mRNA degradation is enolase, which is both a glycolytic enzyme and a component of the RNA degradosome. Its possible role as a sensor connecting mRNA degradation to the energetic demands of the cell has been suggested (Carpousis, 2007), but experimental evidence is lacking. The presence of metabolic enzymes in several bacterial RNA degradosomes (aconitase in *Caulobacter crescentus* (Hardwick et al., 2011) and enolase and phosphofructokinase in *B. subtilis* (Commichau et al., 2009; Newman et al., 2012)) supports the physiological relevance of this connection between mRNA degradation and central carbon metabolism. Master regulators such as CsrA and ppGpp are involved in mRNA degradation regulation. They also coordinate mRNA degradation with other metabolic regulatory networks such as the catabolite repression (through CsrA) and the stringent response (via CsrA and ppGpp) (Romeo and Babitzke, 2018).

Having appreciated the important role of mRNA degradation in metabolic regulation and how it can be modulated to optimize cell performance in biotechnological applications, the role of mRNA degradation process in physiology and adaptation must now be elucidated at the cell system level. Genome-wide studies showed that mRNA half-lives range from a few minutes to almost one hour in *E. coli* (Bernstein et al., 2002; Esquerre et al., 2014; Chen et al., 2015). For the large majority of mRNAs (with stability lower than 10 min), the regulation of gene expression by fast mRNA turnover may constitute a means to rapidly adapt to changing growth conditions and cell demands. For example, mRNA stability can rapidly be controlled by sRNAs: it does not require protein synthesis and can easily be shut off by degradation of the regulatory sRNA. In addition, the activities of the major enzymes in the mRNA degradation machinery are mainly regulated at the post-transcriptional and post-translational levels, which also facilitates rapid response. For the minority of more stable mRNAs, a role in cell survival and regrowth could be postulated by ensuring a basal level of essential mRNAs. At the cellular level, metabolic responses mediated by mRNA degradation are not neutral in terms of resource allocation. On one hand, mRNA degradation recycles ribonucleotides and reduces competition between mRNAs for ribosomes. On the other hand, the cycle of mRNA synthesis and degradation seemingly "wastes" energy: RNA degradation costs energy, just as transcription does, notably through the ATP-dependent degradative pathway (involving PAP I and RhlB) and in the regeneration of triphosphate nucleosides from the mono or di-phosphate nucleosides released by degradation. Only systemic approaches covering all aspects of *E. coli* physiology (gene expression, metabolic activity and resource allocation) can hope to clarify and predict the physiological consequences of modulating mRNA degradation and outline innovative metabolic engineering strategies. Large-scale mRNA stability data will have to be integrated into these mathematical models.

Finally, while this review focusses on the degradation of "unstable" mRNA, the recent finding of a growth condition dependency in the lifetimes of "stable" *E. coli* ribosomal RNAs (Fessler et al., 2020; Hamouche et al., 2021) and transfer RNAs (Svenningsen et al., 2017; Sorensen et al., 2018), extends the need to integrate degradation in physiological studies of *E. coli* to all types of RNA.

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Declaration of Competing Interest

The authors declare that they have no conflicts of interest.

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