Green magic: regulation of the chloroplast stress response by (p)ppGpp in plants and algae

Ben Field

Green magic: regulation of the chloroplast stress response by (p)ppGpp in plants and algae

Ben Field

Aix Marseille Univ, CEA, CNRS, France
Correspondence: ben.field@univ-amu.fr

Abstract

The hyperphosphorylated nucleotides guanosine pentaphosphate and tetraphosphate [together referred to as (p)ppGpp, or 'magic spot'] orchestrate a signalling cascade in bacteria that controls growth under optimal conditions and in response to environmental stress. (p)ppGpp is also found in the chloroplasts of plants and algae where it has also been shown to accumulate in response to abiotic stress. Recent studies suggest that (p)ppGpp is a potent inhibitor of chloroplast gene expression in vivo, and is a significant regulator of chloroplast function that can influence both the growth and the development of plants. However, little is currently known about how (p)ppGpp is wired into eukaryotic signalling pathways, or how it may act to enhance fitness when plants or algae are exposed to environmental stress. This review discusses our current understanding of (p)ppGpp metabolism and its extent in plants and algae, and how (p)ppGpp signalling may be an important factor that is capable of influencing growth and stress acclimation in this major group of organisms.

Introduction

The chloroplasts of plants and algae arose from the endosymbiosis of a photosynthetic bacterium by a eukaryotic cell that occurred more than one billion years ago. Since that initial symbiosis, the majority of bacterial genes have either been lost or transferred to the nucleus. In parallel, while retaining the photosynthetic machinery and a bacteria-like gene expression system, the chloroplast has become the host for other critical cellular functions, including nitrogen and sulphur assimilation, fatty acid biosynthesis, amino acid and nucleotide biosynthesis, and the production of phytohormones. Perhaps unsurprisingly, the chloroplast has also emerged as a key player in the acclimation of plants to their changing environment (Spetea et al., 2014; Dietz, 2015; Chan et al., 2016; Kmiecik et al., 2016; Serrano et al., 2016; Leister et al., 2017).

There is now increasing evidence that an ancient bacterial stress-signalling pathway mediated by the hyperphosphorylated nucleotides guanosine tetraphosphate and pentaphosphate [referred to as (p)ppGpp hereafter] plays an important role in regulating chloroplast function in response to environmental stress. In this review, I will discuss our current understanding of (p)ppGpp

metabolism in plants and algae using the bacterial system as a reference, and then how (p)ppGpp signalling may be involved in influencing both growth and stress acclimation.

(p)ppGpp plays a major role in bacterial stress responses

Depriving bacteria of amino acids has long been known to cause a general repression of RNA and protein synthesis that is known as the stringent response. In the 1960s, Michael Cashel and colleagues found that two 'magic spots' appeared on thin-layer chromatography plates during amino acid starvation of Escherichia coli labelled with 32P. These magic spots were identified as pppGpp and ppGpp, and were shown to be essential for the stringent response (Cashel and Gallant, 1969; Cashel and Kalbacher, 1970). We now know that in E. coli (p)ppGpp is synthesised from ATP and GTP/GDP by the RelA and SpoT enzymes, and that in many bacteria (p)ppGpp directly and indirectly modulates enzymes involved in proliferative processes such as transcription, translation, and replication (Dalebroux and Swanson, 2012; Hauryliuk et al., 2015) (Fig. 1A). In general, these modifications of cellular metabolism reduce proliferation to conserve resources and allow the activation of acclimatory pathways. Basal levels of (p)ppGpp are also present even in the absence of stress, and are required for optimal, balanced growth (Potrykus et al., 2011; Gaca et al., 2013; Kriel et al., 2014).

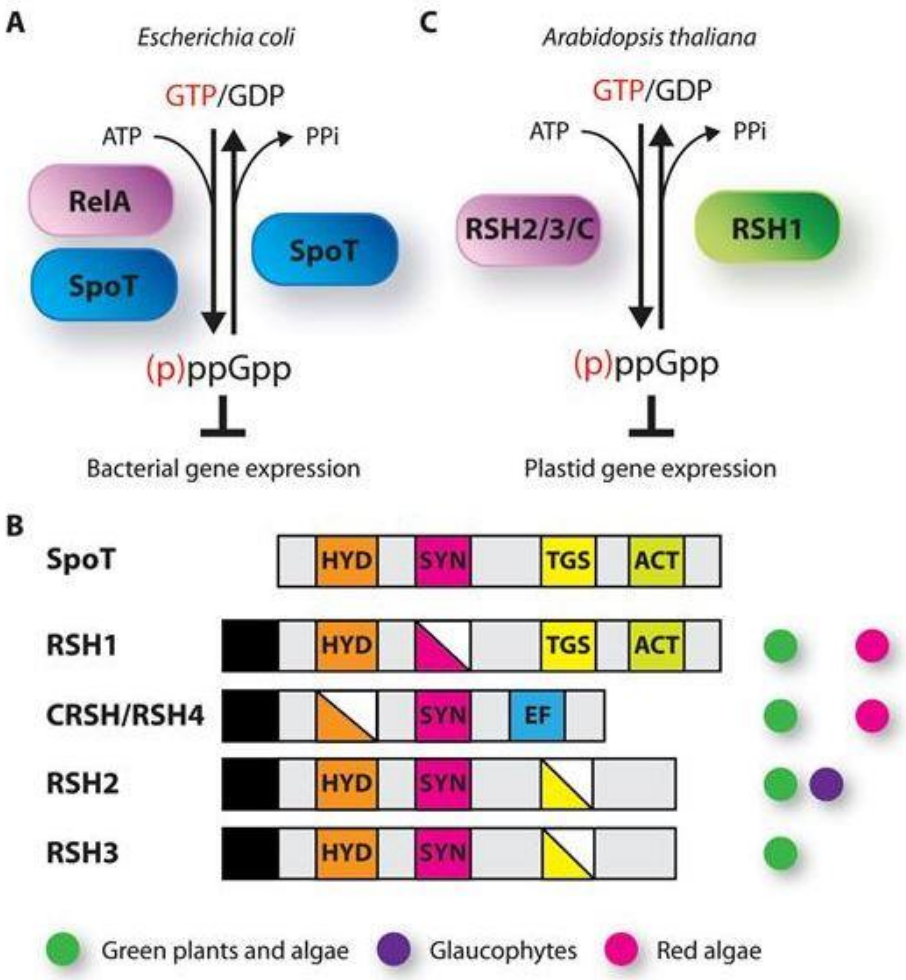

Fig. 1. (p)ppGpp metabolism in bacteria and plants. (A) In the gamma proteobacteria E. coli, (p)ppGpp synthesis and hydrolysis are mediated by the monofunctional RSH RelA and the bifunctional RSH SpoT. (B) The domain organisation of SpoT from E. coli and the four major RSH clades found in plants and algae. These clades are conserved in one or more of the three groups of the Archaeplastida that contain primary chloroplasts (indicated by coloured circles). Black square, chloroplast transit peptide; HYD, (p)ppGpp hydrolase domain; SYN, (p)ppGpp synthase domain; TGS and ACT, conserved interaction domains; EF, EF hand calcium-binding domain. Diagonally filled squares indicate that the corresponding domain is not always present or functional in this clade. (C) Our current state of knowledge of (p)ppGpp metabolism in the model flowering plant Arabidopsis thaliana. The closely related RSH2 and RSH3 participate in (p)ppGpp synthesis with an undetermined contribution from CRSH (represented as RSH/C in the diagram). The monofunctional RSH1 contributes exclusively to (p)ppGpp hydrolysis. *(p)ppGpp metabolism in bacteria and plants. (A) In the gamma proteobacteria E. coli, (p)ppGpp synthesis and hydrolysis are mediated by the monofunctional RSH RelA and the bifunctional RSH SpoT. (B) The domain organisation of SpoT from E. coli and the four major RSH clades found in plants and algae. These clades are conserved in one or more of the three*

groups of the Archaeplastida that contain primary chloroplasts (indicated by coloured circles). Black square, chloroplast transit peptide; HYD, (p)ppGpp hydrolase domain; SYN, (p)ppGpp synthase domain; TGS and ACT, conserved interaction domains; EF, EF hand calcium-binding domain. Diagonally filled squares indicate that the corresponding domain is not always present or functional in this clade. (C) Our current state of knowledge of (p)ppGpp metabolism in the model flowering plant Arabidopsis thaliana. The closely related RSH2 and RSH3 participate in (p)ppGpp synthesis with an undetermined contribution from CRSH (represented as RSH/C in the diagram). The monofunctional RSH1 contributes exclusively to (p)ppGpp hydrolysis.

(p)ppGpp signalling is widespread and diverse among bacteria: RelA and SpoT homologues (RSH) have been found in almost all investigated bacterial groups, (p)ppGpp is involved in the response to a wide range of stresses, and in different bacteria (p)ppGpp has been shown to be required for processes such as pathogenicity, antibiotic resistance, development, and differentiation (Dalebroux and Swanson, 2012; Hauryliuk et al., 2015).

Elements of the stringent response identified in plants and algae

Plant RSHs were first discovered in the model flowering plant Arabidopsis thaliana when RSH1 was identified in a yeast two-hybrid (Y2H) screen for proteins that interact with the nucleotide-binding leucine-rich repeat pathogen-resistance protein RPP5 (van der Biezen et al., 2000). RSH genes have now been identified in land plants and in algae containing primary chloroplasts (red algae, green algae, glaucophytes), as well as in those containing secondary or more complex chloroplasts (stramenophiles, haptophytes) (Atkinson et al., 2011; Ito et al., 2017) (Fig. 1B). My own survey indicates that RSH genes are also present in the other algal groups that possess complex chloroplasts, including the euglenids, rhizaria, and cryptomonads (see Dorrell and Smith, 2011, for an overview of the major algal groups), as well as in the photosynthetic chromatophore of Paulinella chromatophora, which was acquired in a recent and independent primary endosymbiosis event (Nowack et al., 2008). Among the photosynthetic eukaryotes, at least four distinct families of chloroplast-targeted RSH enzymes have been discovered (Atkinson et al., 2011) (Fig. 1C). Surprisingly, different phylogenetic analyses suggest that the RSH enzymes group more closely with the deinococci than with the cyanobacteria (Givens et al., 2004; Atkinson et al., 2011; Ito et al., 2017). Several different explanations have been proposed to explain these uncertain prokaryotic origins of the RSH gene families, including that the grouping with deinococci is an artefact of the phylogenetic inference (Atkinson et al., 2011) or that the different RSH families are the result of multiple horizontal transfer events (Ito et al., 2017). The increasing number of available algal genomes may allow

a more precise picture of RSH and chloroplast evolution to emerge. (p)ppGpp metabolism was also recently linked to photosynthesis in the cyanobacterium Synechococcus elongatus (Hood et al., 2016). The presence of RSH genes in all photosynthetic eukaryotes examined up to now, including those containing chloroplasts derived from secondary or more complex endosymbioses, suggests that there is also a strong link between the capacity for (p)ppGpp metabolism and photosynthesis. Indeed, recent genome sequences from photosynthetic and non-photosynthetic Alveolates (Woo et al., 2015) show that loss of photosynthesis is accompanied by the loss of RSH genes. I have observed that the genomes of two photosynthetic algae in the Chromerida group possess RSH genes, while the genomes of eight species in the Apicomplexa, a sister group of parasitic organisms where photosynthesis was lost and plastids retained, lack RSH genes (BLAST searches of CrytpoDB; Heiges et al., 2006). However, the association between (p)ppGpp and photosynthesis is not absolute across the photosynthetic eukaryotes: the expression of RSH genes can be observed in the transcriptome of the non-photosynthetic and obligate mycoheterotroph flowering plant Monotropa hypopitys (pinesap) (Beletsky et al., 2017). This may suggest that (p)ppGpp signalling has acquired essential new roles outside of photosynthesis in the flowering plants. These new roles are potentially associated with the multicellular lifestyle of these organisms.

While the (p)ppGpp synthesis activity of RSH genes is often tested by complementation in E. coli RelA and SpoT mutants, there are few direct reports of (p)ppGpp measurements in plants. Shortly after the identification of RSH genes, ppGpp and pppGpp were detected in the organs of different flowering plants and in the green algae Chlamydomonas reinhardtii (Takahashi et al., 2004). Levels of (p)pGpp were also shown to vary in response to stress and phytohormone treatments. However, since this initial study, (p)ppGpp levels have rarely been reported in plants and not at all in algae, probably due to the challenging nature of the reported ppGpp extraction procedure. This is now changing thanks to the development of an efficient new method based on ppGpp enrichment followed by high-performance liquid chromatography coupled to mass spectrometry (HPLC-MS/MS) (Ihara et al., 2015). While Takahashi et al. (2004) did detect low levels of pppGpp in their study, a sensitive method for the detection and quantification of the pentaphosphate form of (p)ppGpp has so far not been reported for plants or algae.

The evidence for (p)ppGpp metabolism in chloroplasts is very clear. The mitochondrion is another organelle of prokaryotic origin, whose ancestor may have also possessed a functional (p)ppGpp signalling system. However, no enzymes with conserved (p)ppGpp synthase domains have been reported in non-photosynthetic eukaryotic organisms, suggesting that (p)ppGpp

signalling was not present in the mitochondrial ancestor, or alternatively was lost following endosymbiosis. Furthermore, in photosynthetic eukaryotes there have been no reports of a mitochondrial localisation for RSH enzymes. Surprisingly, metazoans and a few other groups of eukaryotes possess Metazoan SpoT Homologue 1 (MESH1) and MESH1-like enzymes that consist of a single (p)ppGpp hydrolase domain that shows strong conservation with the (p)ppGpp hydrolase domain from bacterial RSHs such as SpoT (Sun et al., 2010; Atkinson et al., 2011). The eukaryotic enzymes group with a clade of α-, β-, and δ-proteobacteria, and are thought to have been acquired by multiple horizontal gene transfer events. Notably, eukaryotic MESH1 enzymes do not possess mitochondrial targeting sequences (Atkinson et al., 2011). Drosophila melanogaster MESH1 can function as a specific (p)ppGpp hydrolase, and Drosophila lacking MESH1 show increased sensitivity to amino acid deprivation (Sun et al., 2010). However, these observations have yet to be reconciled with the facts that (p)ppGpp has not been detected in Drosophila, and that Drosophila does not possess genes encoding known (p)ppGpp synthase domains (Sun et al., 2010).

(p)ppGpp homeostasis in plants and algae

The genome of the model plant Arabidopsis thaliana, where plant (p)ppGpp homeostasis is currently most well understood, encodes four chloroplast-localized RSH enzymes from three families: RSH1 that lacks (p)ppGpp synthase activity and appears to function as the major (p)ppGpp hydrolase (Sugliani et al., 2016), the closely related RSH2 and RSH3 that appear to act as the major (p)ppGpp synthases (Mizusawa et al., 2008; Maekawa et al., 2015; Sugliani et al., 2016), and a calcium-activated RSH (CRSH) that possesses a C-terminal EF-hand domain implicated in calcium binding, and has calcium-dependent (p)ppGpp synthesis activity in vitro (Masuda et al., 2008a). CRSH lacks a functionally conserved (p)ppGpp hydrolase domain and, intriguingly may be involved in Arabidopsis flower development (Masuda et al., 2008a). However, the contribution of CRSH to (p)ppGpp synthesis in planta is currently the least clear out of all the RSHs, perhaps because it acts redundantly with RSH2/RSH3 or only under specific conditions. Altogether, this information can be used to propose a model for (p)ppGpp homeostasis in flowering plants (Fig. 1C). One of the notable differences with the situation in bacteria is the lack of direct experimental evidence for a bi-functional (p)ppGpp synthase such as SpoT. RSH2 and RSH3 both have the potential to be bi-functional because they possess (p)ppGpp hydrolase domains that retain the residues necessary for catalytic activity. However,

(p)ppGpp hydrolase activity has not been demonstrated for either RSH2 or RSH3 when heterologously expressed in E. coli (p)ppGpp mutants, and overexpression in plants results in the over-accumulation of (p)ppGpp (Mizusawa et al., 2008; Maekawa et al., 2015; Sugliani et al., 2016). Experiments on the in vitro activities of purified proteins and protein domains for RSH1, RSH2, and RSH3 have not been yet been reported; however, such experiments may shed more light on the full range of functions of which these enzymes are capable.

In bacteria, (p)ppGpp homeostasis is carried out with assistance from guanosine pentaphosphate phosphatases (GppA) and other GTPases that hydrolyse pppGpp to ppGpp (Hauryliuk et al., 2015). Genes encoding GppA homologues have been identified in plants, but surprisingly are not predicted to encode chloroplast target peptides (Ito et al., 2017). Plants may additionally possess alternative mechanisms for regulating (p)ppGpp levels, such as certain chloroplast-localized moiety X (NUDIX) phosphohydrolases that display (p)ppGpp hydrolysis activity in vitro (Ito et al., 2012; Tanaka et al., 2015).

Despite the wide distribution of RSH genes in algae containing primary and more complex chloroplasts (see above), (p)ppGpp metabolism has so far received little attention and is effectively uncharacterised in these organisms. Indeed, the only current example is in the green algae Chlamydomonas, where one RSH has been studied and shown to have (p)ppGpp synthase activity by complementation of an E.coli relA spoT mutant (Kasai et al., 2002). Interestingly, Chlamydomonas does not possess any members of the RSH clade containing Arabidopsis RSH1, whose members are characterised by the presence of a conserved hydrolase domain and an inactive synthase domain. As Arabidopsis RSH1 is the major (p)ppGpp hydrolase (Sugliani et al., 2016), this suggests that (p)ppGpp metabolism in Chlamydomonas may be significantly different to how it is in land plants and could, for example, include major contributions to (p)ppGpp degradation by other enzyme families.

The molecular mechanisms of (p)ppGpp function in plants

The chloroplasts of plants and algae possess a bacteria-like gene expression system with complex elaborations. In flowering plants, the transcription of polycistronic plastid transcripts is performed by the bacterial-like plastid encoded polymerase (PEP) and two nucleus-encoded polymerases (NEPs), which play a relatively minor role in green tissues (Börner et al., 2015; Pfannschmidt et al., 2015). PEP is encoded on the plastid genome, but for full function it requires a suite of polymerase-associated proteins and sigma factors that are encoded on the nuclear genome (Lerbs-Mache, 2011; Pfalz and Pfannschmidt, 2013; Chi et al., 2015). After transcription, the polycistronic RNAs then undergo extensive editing and splicing (Stern et al.,

2010; Hammani et al., 2014; Schmitz-Linneweber et al., 2015) before translation on bacteria-like 70S ribosomes (Tiller and Bock, 2014; Sun and Zerges, 2015).

In Arabidopsis, (p)ppGpp has been shown to inhibit chloroplast transcription. An assay based on the in planta incorporation of the base analogue 4-thiouridine into nascent chloroplast RNA was used to show that the accumulation of (p)ppGpp inhibits the transcription of PEP and, to a lesser extent, NEP genes in developing seedlings (Sugliani et al., 2016). Quantification of 32P-UTP incorporation into nascent RNA in lysed chloroplast extracts (run-on transcription assays) was also independently used to show that RSH2 and RSH3 are implicated in abscisic acid (ABA)-dependent inhibition of PEP and NEP transcription in both seedlings and in leaves (Yamburenko et al., 2015). However, while around 30 genes were analysed in these studies, the full extent of transcription inhibition by (p)ppGpp has not been ascertained, and the mechanism by which (p)ppGpp inhibits transcription in vivo is currently unknown. In bacteria, two distinct mechanisms have been described (Hauryliuk et al., 2015). In E. coli, (p)ppGpp directly interacts with two sites on RNA polymerase (RNAP), one at the interface between the β′ and ω subunits (Mechold et al., 2013; Ross et al., 2013; Zuo et al., 2013), and another on the β subunit in co-operation with the transcription factor DksA (Ross et al., 2016). RNAP–(p)ppGpp binding results in decreased/increased transcription initiation depending on the kinetic properties of the bound promoter. Transcription from rRNA is inhibited particularly strongly. In contrast, in Bacillus subtilis, and in Gram+ bacteria in general, DksA is absent and RNA polymerase is insensitive to (p)ppGpp (Krásný and Gourse, 2004). (p)ppGpp instead causes a decrease in the GTP pool by the direct inhibition of enzymes in the GTP synthesis pathway, such as guanylate kinase, the enzyme that catalyses the conversions of GMP to GDP (Kriel et al., 2012). A decreased GTP pool in turn leads to the inhibition of transcription initiation for genes where GTP is the initiating NTP; these genes notably include the rRNA genes (Krásný and Gourse, 2004). To date, in vitro studies of plant enzymes have given inconclusive and conflicting results regarding the mechanism by which (p)ppGpp inhibits transcription. Studies on in vitro chloroplast extracts have shown that (p)ppGpp binds and inhibits PEP but not NEP (Takahashi et al., 2004; Sato et al., 2009). However, the physiological consequences of these findings are not clear because the 50% inhibitory concentrations (IC50) for PEP are rather high (~1 mM, Sato et al., 2009; ~2 mM, Takahashi et al., 2004). In addition, no DksA homologues have been identified in either plants or algae, and PEP lacks a homologue of the RNAP ω subunit that is required for the action of (p)ppGpp on E. coli RNAP in the absence of DksA (Vrentas et al., 2005; Börner et al., 2015). More recent work suggests that a Bacillus subtilis-like mechanism might be more likely because recombinant chloroplastic guanylate kinase enzymes from rice

and Arabidopsis are as sensitive to inhibition by (p)ppGpp in vitro as the B. subtilis guanylate kinase with IC50s of around 30 µM (Nomura et al., 2014). In favour of this idea, Sugliani et al. (2016) observed that GTP has been identified as the initiating NTP for the chloroplast rRNA operon containing the 16S and 23S rRNAs in many plant species (Suzuki et al., 2003; Swiatecka-Hagenbruch et al., 2007) and in addition, mutations in the plastidial purine pathway have major effects on plastid gene expression and rRNA accumulation (Kusumi and Iba, 2014). The mechanism of (p)ppGpp function in algae may be significantly different due to large differences in the plastid gene expression machinery. For example, Chlamydomonas has a more bacterial-like transcription machinery than plants, with a single bacteria-like PEP, one sigma factor, and many fewer of the PEP-associated proteins found in plants (Pfalz & Pfannschmidt, 2013).

In addition to its effect on transcription in bacteria, (p)ppGpp also reduces proliferation by binding to and inhibiting several other enzymes, including translation-related GTPases (EF-TU, EF-G, etc.), ribosome biogenesis-related GTPases (Era/Obg GTPases), the DNA primase involved in replication, and others (Steinchen and Bange, 2016). Due to retention of a bacteria-like gene expression system, the orthologues of several of these bacterial (p)ppGpp targets are present in the chloroplasts of plants and algae and may also be involved in (p)ppGpp signalling, as has been previously suggested (Masuda et al., 2008b). There are also hints that (p)ppGpp may have acquired new targets in plants. For example, (p)ppGpp has been shown to influence the rate of Rubisco degradation during dark-induced senescence (Sugliani et al., 2016), an effect that might not be possible to explain simply by reduced rates of chloroplast transcription or translation. It has also been suggested that (p)ppGpp might function within the cytosol following the observation that the cytosolic accumulation of ppGpp caused by overexpression of a bacterial RSH severely affects plant growth (Ihara and Masuda, 2016). However, this is likely to be a non-specific effect. Although (p)ppGpp is not usually synthesised in yeast, the artificial accumulation of (p)ppGpp in yeast can affect gene expression and inhibit growth (Ochi et al., 2012; Hesketh et al., 2017). The effects of (p)ppGpp accumulation in the chloroplast are also different and distinct to those in the cytosol (Maekawa et al., 2015; Sugliani et al., 2016), and can be completely blocked by the expression of the (p)ppGpp hydrolase MESH1 in the chloroplast but not in the cytosol (Sugliani et al., 2016). Finally, there is currently no evidence to suggest that the highly polar (p)ppGpp can leave the chloroplast where it is synthesised. Indeed, GTP, a molecule that is biophysically very similar to (p)ppGpp, is independently synthesised in the cytosolic and chloroplastic compartments, and is not transported across the chloroplast envelope (Olsen and Keegstra, 1992; Kusumi and Iba, 2014).

The effects of (p)ppGpp on chloroplast function and plant growth and development

The roles and effects of (p)ppGpp in planta have only recently been addressed using RSH mutants and lines overexpressing RSH enzymes (Maekawa et al., 2015; Sato et al., 2015; Yamburenko et al., 2015; Sugliani et al., 2016). Taken together, these studies indicate that (p)ppGpp is a potent inhibitor of chloroplast function, and that it is involved in mediating co-operation between the chloroplast and the nucleocytoplasmic compartments during plant growth and development. Arabidopsis lines that over-accumulate (p)ppGpp show a reduction in chloroplast volume per cell due to a reduction in chloroplast size (Maekawa et al., 2015; Sugliani et al., 2016) that is partially compensated for by an increase in chloroplast number per cell (Sugliani et al., 2016). Conversely, greater chloroplast volume per cell is seen in lines with lower (p)ppGpp levels (Sugliani et al., 2016). Photosynthesis is also affected. Over-accumulation of (p)ppGpp due to RSH2/RSH3 overexpression causes a large drop in the maximal yield (Fv/Fm) of photosystem II (PSII) and a striking increase in the ratio of the nucleus-encoded light-harvesting antenna complexes (LHCII) to plastid-encoded PSII core subunits (Maekawa et al., 2015; Sugliani et al., 2016). Together with a recent study showing that (p)ppGpp over-accumulation also has distinct effects on non-photochemical quenching (Honoki et al., 2018), these data indicate that (p)ppGpp metabolism is intimately linked to photosynthetic function. (p)ppGpp over-accumulation also has distinct effects on plant growth, although somewhat contradictory results have been reported. In one case, an Arabidopsis line overexpressing RSH3 showed an enhanced plant growth phenotype (Maekawa et al., 2015), while in another case, RSH3 and RSH2 overexpression lines showed reduced plant growth despite similar levels of (p)ppGpp over-production (Sugliani et al., 2016). These differences may be linked to growth conditions, or the differing genetic backgrounds of the transgenic plants. A small decrease in growth was also noted in the protonema of Physcomitrella patens lines overexpressing PpRSH2a and PpRSH2b, although an increase in (p)ppGpp levels was not directly confirmed (Sato et al., 2015). (p)ppGpp over-accumulation has been a useful tool for understanding (p)ppGpp function, but care must be taken because findings might not reflect the physiological function. RSH mutants that lack (p)ppGpp are therefore a valuable and complementary tool that allow the in planta function of (p)ppGpp to be more accurately ascertained. Experiments using insertion mutants for the different Arabidopsis RSH genes were used to show that the antagonistic activities of RSH enzymes are required for maintaining a basal (p)ppGpp pool in vegetatively growing plants, and that this pool is necessary for optimum growth and the stoichiometry of PSII (Sugliani et al., 2016). Thus, it would appear that, as in bacteria, basal levels of (p)ppGpp play a role in regulating growth and photosynthesis in

unstressed conditions. In addition to plant growth, (p)ppGpp also affects plant development. The Arabidopsis RSH2 and RSH3 genes are highly expressed in older tissues of the plant, and are induced during senescence (Breeze et al., 2011). Altered (p)ppGpp biosynthetic capacity in RSH mutants and overexpression lines was also found to affect the progression of natural senescence (Sugliani et al., 2016). Altered flower development and reduced fertility were also observed in a transgenic line where CRSH was silenced by co-suppression (Masuda et al., 2008a). However, the artificial reduction of (p)ppGpp levels by overexpression of different (p)ppGpp hydrolases does not recapitulate the reduced-fertility phenotype, suggesting that reduced (p)ppGpp levels may not be the explanation (Sugliani et al., 2016).

(p)ppGpp metabolism is wired into general stress responses in plants

While (p)ppGpp metabolism contributes to normal plant growth and development (Masuda et al., 2008a; Maekawa et al., 2015; Sugliani et al., 2016), it is also likely to play an important role in stress acclimation, and to be wired into well-known stress-signalling pathways. In pea shoots, ppGpp has been shown to rapidly increase (within 1–2 h) in response to a wide range of stress conditions (Takahashi et al., 2004). These included wounding, unexpected darkness, heat shock, excess salinity, acidity, drought, UV irradiation, and heavy metal treatment. Unexpected darkness also causes an increase in ppGpp levels in Arabidopsis (Ihara et al., 2015). Interestingly, the only stress tested by Takahashi et al. (2004) that did not cause an increase in ppGpp was cold stress. This is strikingly reminiscent of the situation in bacteria where cold stress causes a decrease in (p)ppGpp, and relA spoT mutants show better acclimation to cold shock than the wild-type (Jones et al., 1992). Takahashi et al. (2004) also showed that the stress-related hormones ABA, jasmonic acid (JA), and ethylene (ET) provoke rapid increases in ppGpp levels in pea shoots that can be completely suppressed by co-treatment with indole-3-acetic acid (IAA). Furthermore, in the case of JA, this response required the synthesis of new proteins because pre-treatment of the pea shoots with the cytosolic translation inhibitor cycloheximide suppressed the ppGpp increase in response to JA. In parallel, run-on transcription assays on chloroplast extracts have been used to show a reduction of transcription rates in chloroplasts isolated from plants pre-treated with ABA (Yamburenko et al., 2013) or methyl jasmonate (MeJA) (Zubo et al., 2011). Furthermore, the ABA-dependent down-regulation of chloroplast transcription in Arabidopsis is partly dependent on the presence of RSH2 and RSH3, suggesting that (p)ppGpp may be responsible (Yamburenko et al., 2015). Conversely, cytokinin (CK) signalling is involved in the up-regulation of chloroplast transcription, although the involvement of (p)ppGp has not yet been tested (Zubo et al., 2008; Danilova et al., 2017). Taken together with the work of Sugliani et al. (2016), which

demonstrated a direct effect of (p)ppGpp on chloroplast transcription, all these data suggest a simple mechanism for abiotic stress-induced (p)ppGpp synthesis in plants (Fig. 2A). In the following sections, I will discuss how stress and hormone perception might lead to an increase in (p)ppGpp levels, and the relevance of (p)ppGpp accumulation for the acclimation of plants and algae to stress.

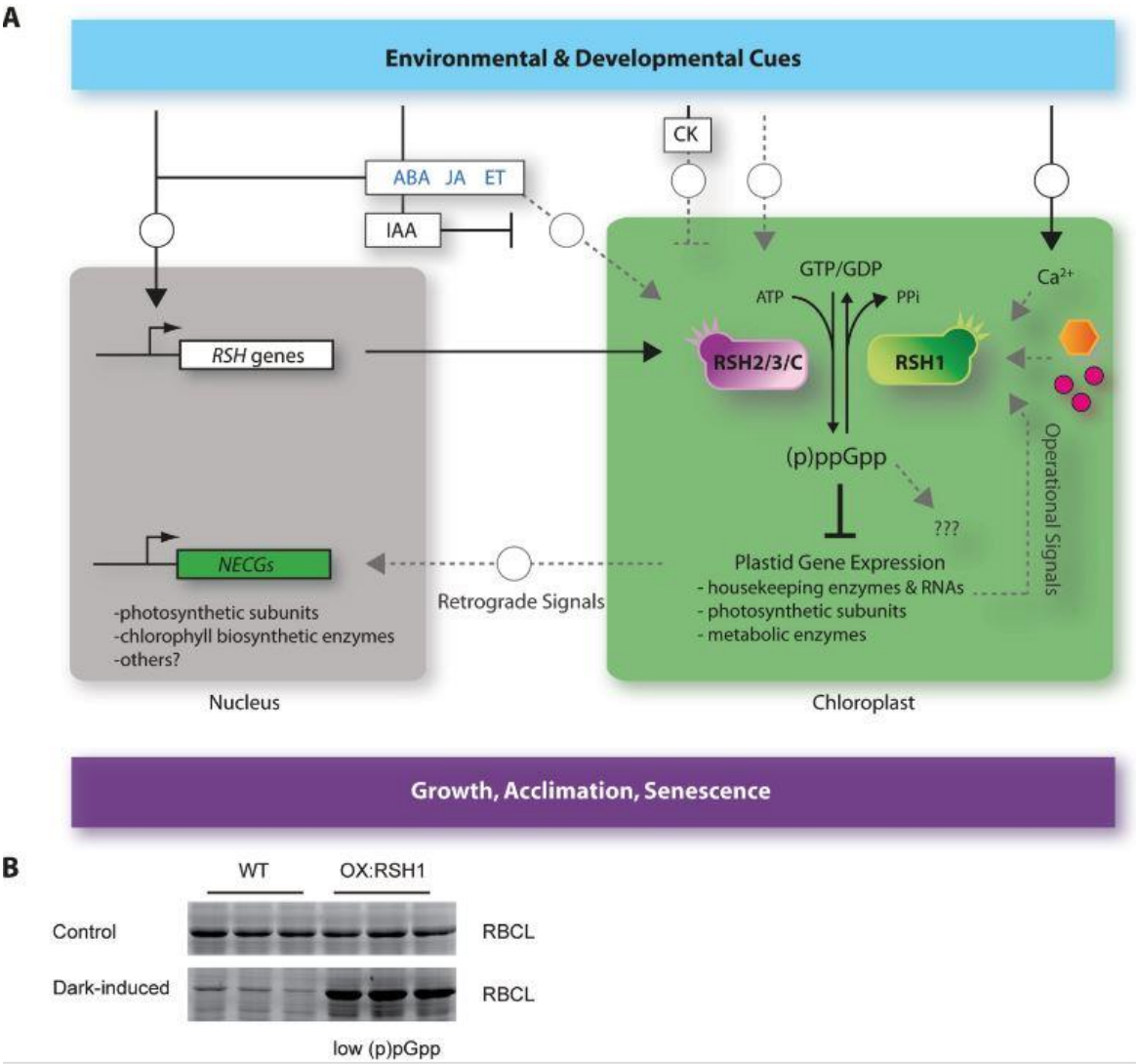

Fig. 2. (A) A model for the regulation of (p)ppGpp metabolism in Arabidopsis in response to developmental and environmental cues. Inputs from internal developmental processes or changes in the external environment can lead to alterations in the transcription of RSH enzymes and/or synthesis of (p)ppGpp in the chloroplast. These signals can be relayed by phytohormones, or via other signalling pathways yet to be identified. Environmental cues such as darkness or pathogen perception can lead to changes in the levels of the secondary messenger Ca2+ in the chloroplast. In turn, Ca2+ has the potential to activate CRSH (represented as RSH/C in the diagram) via binding to the EF hand domain, although this has not been demonstrated in vivo. Within the chloroplast, the activity of the RSH enzymes may be

regulated via their abundance, interactions with other proteins (hexagon), or small molecule interactions (pink circles). RSH enzymes may also be regulated via operational signals generated during chloroplast function, such as redox status. (p)ppGpp accumulation inhibits plastid gene expression, and may also regulate the expression of nucleus encoded chloroplast genes (NECGs) via retrograde signalling pathways. Black arrows denote processes for which in vivo experimental evidence exists, grey dashed lines denote processes that are possible, but for which no direct evidence exists. Circles within lines indicate that multiple steps may be required. (B) (p)ppGpp is required for degradation of Rubisco during dark-induced senescence of detached leaves. Equal quantities of total protein from wild-type (WT) and OX:RSH1 plants were separated by SDS-PAGE and visualised by Coomassie Brilliant Blue after extraction from the leaves after 6 d of darkness (dark-induced) or from non-treated leaves (control). OX:RSH1 plants overexpress the (p)ppGpp hydrolase RSH1, and have lower (p)ppGpp levels than the WT. Adapted from Sugliani et al. (2016), Copyright American Society of Plant Biologists.

Stress-induced regulation of RSH activity

It has not yet been clearly demonstrated at the mechanistic level how the accumulation of a stress-related hormone such as ABA can lead to increased (p)ppGpp levels. The expression of RSH genes themselves is induced by treatment with the jasmonate precursor 2-oxo-phytodienoic acid and ABA, as well as in response to abiotic stresses such as wounding and salt treatment (Mizusawa et al., 2008; Chen et al., 2014; Yamburenko et al., 2015). Given that overexpression of RSH2 or RSH3 is sufficient to cause an increase in (p)ppGpp levels (Maekawa et al., 2015; Sugliani et al., 2016), it is therefore plausible that (p)ppGpp levels increase directly in response to a natural increase in RSH2 or RSH3 expression. A similar mechanism of transcriptional regulation has also been shown to be a route to increased (p)ppGpp levels in bacteria (Geiger et al., 2014). However, in this case the genes that are induced encode short monofunctional (p)ppGpp synthases (small alarmone synthases, SAS), which lack the regulatory C-terminal domain (CTD) found in long RSHs. These enzymes are therefore not equivalent to the plant and algal RSHs, which all have a synthase and hydrolase domain and possess CTD extensions that are conserved within each family and even show conservation with bacteria in the case of the RSH1 family (Fig. 1B). Indeed, in bacteria, post-translational regulatory mechanisms are generally recognised as the main level of control for long RSH enzymes, and thus of (p)ppGpp synthesis, in response to stress. For example, in E. coli, the RelA CTD interacts with the ribosome and permits RelA activation during amino-acid starvation (Hauryliuk et al., 2015; Brown et al., 2016). SpoT also interacts with the acyl carrier protein (ACP) involved in fatty acid synthesis to activate (p)ppGpp synthesis during fatty acid

stress (Battesti and Bouveret, 2006), as well as with the ribosome-associated GTPase Obg (Wout et al., 2004). The primary structure of plant RSH enzymes suggests that they are likely to be controlled in a similar manner. In a remarkable example of evolutionary conservation, it has been shown that the CTD of RSH1 has a conserved TGS domain that is required for the interaction of RSH1 with the plastidial homologue of Obg, ObgC (Bang et al., 2012; Chen et al., 2014). Unfortunately, while the link to ribosomes and translation is intriguing, the physiological significance of the Obg–RSH interaction is still unknown both in plants and bacteria. A second example are the Arabidopsis and rice CRSH enzymes that possess CTD EF hands and Ca2+-dependent (p)ppGpp synthesis activity in vitro (Tozawa et al., 2007; Masuda et al., 2008a). CRSH, S-ADENOSYLMETHIONINE TRANSPORTER1-LIKE, and a type-II NAD(P)H dehydrogenase NDA2 are the only reported chloroplast enzymes to possess EF hand domains (Stael et al., 2012; Hochmal et al., 2015). The link between CRSH and Ca2+ is intriguing because the chloroplast is a major cellular reserve of Ca2+, and Ca2+ has an important signalling role within the chloroplast, the molecular mechanisms of which are not yet fully understood. Elevated Ca2+ concentrations inhibit enzymes of the Calvin–Benson cycle, and via calmodulin and other Ca2+-binding proteins influence photosynthesis, chloroplast protein import, vesicle transport, and other metabolic reactions. Increases in stromal Ca2+ are a well-known response to light–dark transitions, and can also occur following pathogen perception, cold shock, and high salt (Sai and Johnson, 2002; Stael et al., 2012; Hochmal et al., 2015; Loro et al., 2016). (p)ppGpp levels rapidly increase in Arabidopsis and pea plants in response to light–dark transitions as well as to high salt (Takahashi et al., 2004; Ihara et al., 2015). Therefore, it is tempting to speculate that the Ca2+-dependent activation of CRSH may be responsible for the observed (p)ppGpp increases during these responses.

Evidence for (p)ppGpp-mediated stress acclimation

As described above, there is now a considerable body of evidence demonstrating the presence of a (p)ppGpp signalling pathway in chloroplasts that can modulate chloroplast function. (p)ppGpp is also clearly linked to plant stress because (p)ppGpp levels and RSH gene expression are affected by different stresses and by hormone application. Furthermore, chloroplastic RSH enzymes appear to have preserved key domains that are involved in relaying stress signals in their bacterial orthologues. However, despite these findings, there have so far been few reports that show how (p)ppGpp is required to help plants acclimate to stress, and no reports regarding algae.

Under stress conditions, many plants launch a program of accelerated senescence that allows the recycling of nutrients from source tissues to reproductive organs (Sade et al., 2018). Accelerated senescence enhances plant fitness by prioritising the survival of the next generation, but can also cause significant yield loss in agricultural crops. In addition to a potential role in natural senescence, (p)ppGpp has been shown to be involved in dark-induced senescence (Sugliani et al., 2016). The incubation of detached leaves in the dark results in carbon starvation, which induces an accelerated senescence response accompanied by the degradation of chlorophyll and Rubisco. Strikingly, different plant lines with depleted (p)ppGpp levels show reduced chlorophyll degradation and dramatically reduced Rubisco degradation during dark-induced senescence (Fig. 2B) (Sugliani et al., 2016). Plants with higher (p)ppGpp levels show the opposite phenotype, i.e. faster chlorophyll and Rubisco degradation. This stress-related role for (p)ppGpp is not limited to carbon deficiency because a reduced Rubisco degradation phenotype was also observed during nitrogen starvation-induced senescence in a rsh2 rsh3 double-mutant (Honoki et al., 2018). While (p)ppGpp is required for normal chlorophyll and Rubisco degradation, it is not yet clear whether (p)ppGpp itself accumulates during senescence. However, interestingly, an Arabidopsis RSH3 overexpression line that over-accumulates (p)ppGpp shows greater tolerance to nitrogen deprivation, with a higher fresh weight, fewer chlorotic leaves, and lower starch accumulation than the wild-type (Maekawa et al., 2015; Honoki et al., 2018). While the mechanism and physiological relevance of this finding are not yet clear, especially in relation to senescence, it indicates at least that ectopic (p)ppGpp accumulation can influence the response of plants to nitrogen starvation-induced senescence.

The RSH genes and (p)ppGpp have also been shown to be required for adjusting the architecture of photosynthetic complexes under normal growth conditions (Sugliani et al., 2016). Photosynthesis must also be strictly controlled under stress conditions to avoid over-excitation and the generation of damaging reactive oxygen species (ROS). Stress-induced alterations in (p)ppGpp levels could therefore be involved in regulating the architecture of photosynthetic complexes as part of the acclimation process. Interestingly, nitrogen deprivation caused changes in non-photochemical quenching (NPQ) and Fv/Fm in a RSH3 overexpression line (Honoki et al., 2018); these changes were not observed in wild-type plants. Again, this suggests that RSH3 activity, and thus (p)ppGpp levels, are regulated during senescence.

(p)ppGpp is also required for plant responses to biotic stress. In addition to the yeast two-hybrid interaction between RSH1 and RPP5, suggesting a link between (p)ppGpp and biotic stress (van der Biezen et al., 2000), it has been shown that RSH3 family genes are up-regulated in different

plants in response to the bacterial pathogen E. carotovora carotovora (Givens et al., 2004), fungal pathogen elicitors, and the defense-related phytohormone salicylic acid (Kim et al., 2009). And in a more recent study it was shown that RSH3 overexpression lines that over-acumulate (p)ppGpp show reduced defense-gene expression, reduced levels of the defense hormone salicylic acid, and increased susceptibility to Turnip Mosaic Virus (TuMV) (Abdelkefi et al., 2018). In contrast, plants with lower (p)ppGpp levels show reduced susceptibility to TuMV, and this is associated with the precocious up-regulation of defense-related genes and increased SA content. Interestingly, the extracellular perception of pathogen-associated molecular patterns leads to Ca2+ oscillations in the chloroplast and cytosol that require the chloroplast calcium-sensing receptor (CAS) (Nomura et al., 2012). CAS mutants fail to activate salicylic acid biosynthesis and pathogenesis-related gene expression in response to pathogen perception, and are more susceptible to bacterial pathogens. These phenotypes are remarkably similar to those in plants with higher (p)ppGpp levels, although an increase in (p)ppGpp has not yet been demonstrated in response to pathogen infection. In the future it will be fascinating to discover how Ca2+ and (p)ppGpp signalling intersect and interact in plant immunity, as well as in response to other stresses.

Conclusions

The ‘magic spot’, (p)ppGpp, has emerged as a significant regulator of chloroplast function that is required for normal plant growth and development, and which is strongly implicated in plant stress acclimation. Along with the general sigma factors (Chi et al., 2015), and redox control (Pfannschmidt et al., 2001), (p)ppGpp could be considered as one of the few known factors that can exert a general effect on chloroplast gene expression. The transcription, translation, and post-translational regulation of chloroplast gene expression can change dramatically during development and in response to environmental signals and stress (Rochaix, 2013; Dodd et al., 2014; Börner et al., 2015; Pfannschmidt et al., 2015; Sun and Zerges, 2015; Leister et al., 2017; Liebers et al., 2017). However, it is generally thought that, due to the high stability of many chloroplasts mRNAs, the major control of chloroplast gene expression is not at the level of transcription (Klaff and Gruissem, 1991; Kim et al., 1993; Sun and Zerges, 2015). This raises a difficult conundrum about the relevance of (p)ppGpp signalling in the chloroplast, because current in vivo work shows that (p)ppGpp appears to act principally at the level of transcription. Resolving this conundrum will require a better understanding of exactly how (p)ppGpp affects chloroplast function following developmental cues or stress perception, of the time scales involved, and of the tissue and species specificity.

To date, (p)ppGpp signalling has been studied in only a limited part of the photosynthetic eukaryotes. The emergence of new and tractable algal model organisms such as the red alga Cyanidioschyzon merolae and the stramenopile Phaedodactylum tricornutum (Cock and Coelho, 2011) will allow us to determine whether the general features of (p)ppGpp signalling are conserved in these organisms, and to potentially identify new adaptive innovations in (p)ppGpp signalling that are associated with their diverse lifestyles.

(p)ppGpp signalling in plants and algae, for all its similarities to the bacterial system, is also fundamentally different because it occurs within an organelle. Indeed, (p)ppGpp appears to play an important role in promoting co-operation between the plastid and nuclear genomes. However, very little is known about how growth, hormone, and stress-signalling pathways relay their status to the RSH enzymes or vice versa. Chemicals that inhibit plastid gene expression such as lincomycin and norflurazon are well known activators of retrograde signalling pathways. Therefore, it would seem likely that (p)ppGpp accumulation, via the inhibition of plastid gene expression, can itself naturally trigger retrograde signalling to the nucleus. Revealing how this ancient bacterial signalling pathway is wired into the myriad signalling networks of the eukaryotic cell in plants and in algae will be an exciting and rewarding challenge.

Abbreviations:

ABA, abscisic acid;

ACP, acyl carrier protein

CAS, calcium sensor

CK, cytokinin

CRSH, Ca^{2+}-activated RelA/SpoT homolog

CTD, C-terminal domain

ET, ethylene

$\boldsymbol{F_v/F_m}$**,** maximum quantum yield of PSII in a dark-adapted state

HPLC-MS/MS, high-performance liquid chromatography coupled to tandem mass spectrometry

IAA, indole-3-acetic acid

JA, jasmonate

LHCII, light-harvesting complex II

MeJA, methyl jasmonate

MESH1, Metazoan SpoT homologue 1

NECG, nucleus encoded chloroplast gene; NEP, nucleus-encoded polymerase

NPQ, non-photochemical quenching

NUDIX, nucleoside diphosphates linked to X

PAMP, pathogen-associated molecular pattern

PEP, plastid-encoded polymerase

(p)ppGpp, guanosine tetraphosphate and pentaphosphate

PSII, photosystem II

ROS, reactive oxygen species

RNAP, RNA polymerase

RSH, RelA/SpoT homologue

Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase

TuMV, Turnip Mosaic Virus

Acknowledgements

Work on (p)ppGpp in our laboratory is supported by Agence Nationale de la Recherche (ANR) grants SIGNAUX BioNRJ (ANR-15-CE05-0021-03) and G4PLAST (ANR-17-CE13-0005). I

thank Emmanuelle Bouveret, Benoit Menand, and Bérengère Ize for critical reading of the manuscript, and Christophe Robaglia for useful discussions about chloroplast evolution.

References

Abdelkefi H, Sugliani M, Ke H, et al. 2018. Guanosine tetraphosphate modulates salicylic acid signalling and the resistance of Arabidopsis thaliana to Turnip mosaic virus. Molecular Plant Pathology. In press . doi:10.1111/mpp.12548.

Atkinson GC, Tenson T, Hauryliuk V. 2011. The RelA/SpoT homolog (RSH) superfamily: distribution and functional evolution of ppGpp synthetases and hydrolases across the tree of life. PLoS ONE 6, e23479.

Bang WY, Chen J, Jeong IS, et al. 2012. Functional characterization of ObgC in ribosome biogenesis during chloroplast development. The Plant Journal 71, 122–134.

Battesti A, Bouveret E. 2006. Acyl carrier protein/SpoT interaction, the switch linking SpoT-dependent stress response to fatty acid metabolism. Molecular Microbiology 62, 1048–1063.

Beletsky AV, Filyushin MA, Gruzdev EV, Mazur AM, Prokhortchouk EB, Kochieva EZ, Mardanov AV, Ravin NV, Skryabin KG. 2017. De novo transcriptome assembly of the mycoheterotrophic plant Monotropa hypopitys. Genomics Data 11, 60–61.

Börner T, Aleynikova AY, Zubo YO, Kusnetsov VV. 2015. Chloroplast RNA polymerases: role in chloroplast biogenesis. Biochimica Et Biophysica Acta 1847, 761–769.

Breeze E, Harrison E, McHattie S, et al. 2011. High-resolution temporal profiling of transcripts during Arabidopsis leaf senescence reveals a distinct chronology of processes and regulation. The Plant Cell 23, 873–894.

Brown A, Fernández IS, Gordiyenko Y, Ramakrishnan V. 2016. Ribosome-dependent activation of stringent control. Nature 534, 277–280.

Cashel M, Gallant J. 1969. Two compounds implicated in the function of the RC gene of Escherichia coli. Nature 221, 838–841.

Cashel M, Kalbacher B. 1970. The control of ribonucleic acid synthesis in Escherichia coli. V. Characterization of a nucleotide associated with the stringent response. The Journal of biological chemistry 245, 2309–2318.

Chan KX, Phua SY, Crisp P, McQuinn R, Pogson BJ. 2016. Learning the languages of the chloroplast: retrograde signaling and beyond. Annual Review of Plant Biology 67, 25–53.

Chen J, Bang WY, Lee Y, et al. 2014. AtObgC–AtRSH1 interaction may play a vital role in stress response signal transduction in Arabidopsis. Plant Physiology and Biochemistry 74, 176–184.

Chi W, He B, Mao J, Jiang J, Zhang L. 2015. Plastid sigma factors: their individual functions and regulation in transcription. Biochimica et Biophysica Acta 1847, 770–778.

Cock JM, Coelho SM. 2011. Algal models in plant biology. Journal of Experimental Botany 62, 2425–2430.

Dalebroux ZD, Swanson MS. 2012. ppGpp: magic beyond RNA polymerase. Nature Reviews Microbiology 10, 203–212.

Danilova MN, Kudryakova NV, Doroshenko AS, Zabrodin DA, Rakhmankulova ZF, Oelmüller R, Kusnetsov VV. 2017. Opposite roles of the Arabidopsis cytokinin receptors AHK2 and AHK3 in the expression of plastid genes and genes for the plastid transcriptional machinery during senescence. Plant Molecular Biology 93, 533–546.

Dietz KJ. 2015. Efficient high light acclimation involves rapid processes at multiple mechanistic levels. Journal of Experimental Botany 66, 2401–2414.

Dodd AN, Kusakina J, Hall A, Gould PD, Hanaoka M. 2014. The circadian regulation of photosynthesis. Photosynthesis Research 119, 181–190.

Dorrell RG, Smith AG. 2011. Do red and green make brown? Perspectives on plastid acquisitions within chromalveolates. Eukaryotic Cell 10, 856–868.

Gaca AO, Kajfasz JK, Miller JH, Liu K, Wang JD, Abranches J, Lemos JA. 2013. Basal levels of (p)ppGpp in Enterococcus faecalis: the magic beyond the stringent response. MBio 4, e00646-13.

Geiger T, Kästle B, Gratani FL, Goerke C, Wolz C. 2014. Two small (p)ppGpp synthases in Staphylococcus aureus mediate tolerance against cell envelope stress conditions. Journal of Bacteriology 196, 894–902.

Givens RM, Lin MH, Taylor DJ, Mechold U, Berry JO, Hernandez VJ. 2004. Inducible expression, enzymatic activity, and origin of higher plant homologues of bacterial RelA/SpoT stress proteins in Nicotiana tabacum. The Journal of Biological Chemistry 279, 7495–7504.

Hammani K, Bonnard G, Bouchoucha A, Gobert A, Pinker F, Salinas T, Giegé P. 2014. Helical repeats modular proteins are major players for organelle gene expression. Biochimie 100, 141–150.

Hauryliuk V, Atkinson GC, Murakami KS, Tenson T, Gerdes K. 2015. Recent functional insights into the role of (p)ppGpp in bacterial physiology. Nature Reviews Microbiology 13, 298–309.

Heiges M, Wang H, Robinson E, et al. 2006. CryptoDB: a Cryptosporidium bioinformatics resource update. Nucleic Acids Research 34, 419–422.

Hesketh A, Vergnano M, Wan C, Oliver SG. 2017. Bacterial signaling nucleotides inhibit yeast cell growth by impacting mitochondrial and other specifically eukaryotic functions. mBio 8, e01047-17.

Hochmal AK, Schulze S, Trompelt K, Hippler M. 2015. Calcium-dependent regulation of photosynthesis. Biochimica Biophysica Acta 1847, 993–1003.

Honoki R, Ono S, Oikawa A, Saito K, Masuda S. 2018. Significance of accumulation of the alarmone (p)ppGpp in chloroplasts for controlling photosynthesis and metabolite balance during nitrogen starvation in Arabidopsis. Photosynthesis Research. In press . doi:10.1007/s11120-017-0402-y.

Hood RD, Higgins SA, Flamholz A, Nichols RJ, Savage DF. 2016. The stringent response regulates adaptation to darkness in the cyanobacterium Synechococcus elongatus. Proceedings of the National Academy of Sciences, USA 113, 4867–4876.

Ihara Y, Masuda S. 2016. Cytosolic ppGpp accumulation induces retarded plant growth and development. Plant Signaling & Behavior 11, e1132966.

Ihara Y, Ohta H, Masuda S. 2015. A highly sensitive quantification method for the accumulation of alarmone ppGpp in Arabidopsis thaliana using UPLC-ESI-qMS/MS. Journal of Plant Research 128, 511–518.

Ito D, Ihara Y, Nishihara H, Masuda S. 2017. Phylogenetic analysis of proteins involved in the stringent response in plant cells. Journal of Plant Research 130, 625–634.

Ito D, Kato T, Maruta T, Tamoi M, Yoshimura K, Shigeoka S. 2012. Enzymatic and molecular characterization of Arabidopsis ppGpp pyrophosphohydrolase, AtNUDX26. Bioscience, Biotechnology, and Biochemistry 76, 2236–2241.

Jones PG, Cashel M, Glaser G, Neidhardt FC. 1992. Function of a relaxed-like state following temperature downshifts in Escherichia coli. Journal of Bacteriology 174, 3903–3914.

Kasai K, Usami S, Yamada T, Endo Y, Ochi K, Tozawa Y. 2002. A RelA-SpoT homolog (Cr-RSH) identified in Chlamydomonas reinhardtii generates stringent factor in vivo and localizes to chloroplasts in vitro. Nucleic Acids Research 30, 4985–4992.

Kim M, Christopher DA, Mullet JE. 1993. Direct evidence for selective modulation of psbA, rpoA, rbcL and 16S RNA stability during barley chloroplast development. Plant Molecular Biology 22, 447–463.

Kim T-H, Ok SH, Kim D, Suh S-C, Byun MO, Shin JS. 2009. Molecular characterization of a biotic and abiotic stress resistance-related gene RelA/SpoT homologue (PepRSH) from pepper. Plant Science 176, 635–642.

Klaff P, Gruissem W. 1991. Changes in chloroplast mRNA stability during leaf development. The Plant Cell 3, 517–529.

Kmiecik P, Leonardelli M, Teige M. 2016. Novel connections in plant organellar signalling link different stress responses and signalling pathways. Journal of Experimental Botany 67, 3793–3807.

Krásný L, Gourse RL. 2004. An alternative strategy for bacterial ribosome synthesis: Bacillus subtilis rRNA transcription regulation. The EMBO journal 23, 4473–4483.

Kriel A, Bittner AN, Kim SH, et al. 2012. Direct regulation of GTP homeostasis by (p)ppGpp: a critical component of viability and stress resistance. Molecular Cell 48, 231–241.

Kriel A, Brinsmade SR, Tse JL, Tehranchi AK, Bittner AN, Sonenshein AL, Wang JD. 2014. GTP dysregulation in Bacillus subtilis cells lacking (p)ppGpp results in phenotypic amino acid auxotrophy and failure to adapt to nutrient downshift and regulate biosynthesis genes. Journal of Bacteriology 196, 189–201.

Kusumi K, Iba K. 2014. Establishment of the chloroplast genetic system in rice during early leaf development and at low temperatures. Frontiers in Plant Science 5, 386.

Leister D, Wang L, Kleine T. 2017. Organellar gene expression and acclimation of plants to environmental stress. Frontiers in Plant Science 8, 387.

Lerbs-Mache S. 2011. Function of plastid sigma factors in higher plants: regulation of gene expression or just preservation of constitutive transcription?Plant Molecular Biology 76, 235–249.

Liebers M, Grübler B, Chevalier F, Lerbs-Mache S, Merendino L, Blanvillain R, Pfannschmidt T. 2017. Regulatory shifts in plastid transcription play a key role in morphological conversions of plastids during plant development. Frontiers in Plant Science 8, 23.

Loro G, Wagner S, Doccula FG, Behera S, Weinl S, Kudla J, Schwarzländer M, Costa A, Zottini M. 2016. Chloroplast-specific in vivo Ca2+ imaging using yellow cameleon fluorescent protein sensors reveals organelle-autonomous Ca2+ signatures in the stroma. Plant Physiology 171, 2317–2330.

Maekawa M, Honoki R, Ihara Y, Sato R, Oikawa A, Kanno Y, Ohta H, Seo M, Saito K, Masuda S. 2015. Impact of the plastidial stringent response in plant growth and stress responses. Nature Plants 1, 15167.

Masuda S, Mizusawa K, Narisawa T, Tozawa Y, Ohta H, Takamiya K. 2008a. The bacterial stringent response, conserved in chloroplasts, controls plant fertilization. Plant & Cell Physiology 49, 135–141.

Masuda S, Tozawa Y, Ohta H. 2008b. Possible targets of "magic spots" in plant signalling. Plant Signaling & Behavior 3, 1021–1023.

Mechold U, Potrykus K, Murphy H, Murakami KS, Cashel M. 2013. Differential regulation by ppGpp versus pppGpp in Escherichia coli. Nucleic Acids Research 41, 6175–6189.

Mizusawa K, Masuda S, Ohta H. 2008. Expression profiling of four RelA/SpoT-like proteins, homologues of bacterial stringent factors, in Arabidopsis thaliana. Planta 228, 553–562.

Nomura H, Komori T, Uemura S, et al. 2012. Chloroplast-mediated activation of plant immune signalling in Arabidopsis. Nature Communications 3, 926.

Nomura Y, Izumi A, Fukunaga Y, et al. 2014. Diversity in guanosine 3′,5′-bisdiphosphate (ppGpp) sensitivity among guanylate kinases of bacteria and plants. The Journal of Biological Chemistry 289, 15631–15641.

Nowack EC, Melkonian M, Glöckner G. 2008. Chromatophore genome sequence of Paulinella sheds light on acquisition of photosynthesis by eukaryotes. Current Biology 18, 410–418.

Ochi K, Nishizawa T, Inaoka T, Yamada A, Hashimoto K, Hosaka T, Okamoto S, Ozeki Y. 2012. Heterologous expression of a plant RelA-SpoT homologue results in increased stress tolerance in Saccharomyces cerevisiae by accumulation of the bacterial alarmone ppGpp. Microbiology 158, 2213–2224.

Olsen LJ, Keegstra K. 1992. The binding of precursor proteins to chloroplasts requires nucleoside triphosphates in the intermembrane space. The Journal of Biological Chemistry 267, 433–439.

Pfalz J, Pfannschmidt T. 2013. Essential nucleoid proteins in early chloroplast development. Trends in Plant Science 18, 186–194.

Pfannschmidt T, Blanvillain R, Merendino L, Courtois F, Chevalier F, Liebers M, Grübler B, Hommel E, Lerbs-Mache S. 2015. Plastid RNA polymerases: orchestration of enzymes with different evolutionary origins controls chloroplast biogenesis during the plant life cycle. Journal of Experimental Botany 66, 6957–6973.

Pfannschmidt T, Schütze K, Brost M, Oelmüller R. 2001. A novel mechanism of nuclear photosynthesis gene regulation by redox signals from the chloroplast during photosystem stoichiometry adjustment. The Journal of Biological Chemistry 276, 36125–36130.

Potrykus K, Murphy H, Philippe N, Cashel M. 2011. ppGpp is the major source of growth rate control in E. coli. Environmental Microbiology 13, 563–575.

Rochaix JD. 2013. Redox regulation of thylakoid protein kinases and photosynthetic gene expression. Antioxidants & Redox Signaling 18, 2184–2201.

Ross W, Sanchez-Vazquez P, Chen AY, Lee JH, Burgos HL, Gourse RL. 2016. ppGpp binding to a site at the RNAP-DksA interface accounts for its dramatic effects on transcription initiation during the stringent response. Molecular Cell 62, 811–823.

Ross W, Vrentas CE, Sanchez-Vazquez P, Gaal T, Gourse RL. 2013. The magic spot: a ppGpp binding site on E. coli RNA polymerase responsible for regulation of transcription initiation. Molecular Cell 50, 420–429.

Sade N, Rubio-Wilhelmi M, Umnajkitikorn K, Blumwald E. 2018. Stress-induced senescence and plant tolerance to abiotic stress. Journal of Experimental Botany. In press . doi:10.1093/jxb/erx235.

Sai J, Johnson CH. 2002. Dark-stimulated calcium ion fluxes in the chloroplast stroma and cytosol. The Plant Cell 14, 1279–1291.

Sato M, Takahashi K, Ochiai Y, Hosaka T, Ochi K, Nabeta K. 2009. Bacterial alarmone, guanosine 5′-diphosphate 3′-diphosphate (ppGpp), predominantly binds the β′ subunit of plastid-encoded plastid RNA polymerase in chloroplasts. ChemBioChem 10, 1227–1233.

Sato M, Takahashi T, Ochi K, Matsuura H, Nabeta K, Takahashi K. 2015. Overexpression of RelA/SpoT homologs, PpRSH2a and PpRSH2b, induces the growth suppression of the moss Physcomitrella patens. Bioscience, Biotechnology, and Biochemistry 79, 36–44.

Schmitz-Linneweber C, Lampe MK, Sultan LD, Ostersetzer-Biran O. 2015. Organellar maturases: a window into the evolution of the spliceosome. Biochimica et Biophysica Acta 1847, 798–808.

Serrano I, Audran C, Rivas S. 2016. Chloroplasts at work during plant innate immunity. Journal of Experimental Botany 67, 3845–3854.

Spetea C, Rintamäki E, Schoefs B. 2014. Changing the light environment: chloroplast signalling and response mechanisms. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 369, 20130220.

Stael S, Wurzinger B, Mair A, Mehlmer N, Vothknecht UC, Teige M. 2012. Plant organellar calcium signalling: an emerging field. Journal of Experimental Botany 63, 1525–1542.

Steinchen W, Bange G. 2016. The magic dance of the alarmones (p)ppGpp. Molecular Microbiology 101, 531–544.

Stern DB, Goldschmidt-Clermont M, Hanson MR. 2010. Chloroplast RNA metabolism. Annual Review of Plant Biology 61, 125–155.

Sugliani M, Abdelkefi H, Ke H, Bouveret E, Robaglia C, Caffarri S, Field B. 2016. An ancient bacterial signaling pathway regulates chloroplast function to influence growth and development in Arabidopsis. The Plant Cell 28, 661–679.

Sun D, Lee G, Lee JH, et al. 2010A metazoan ortholog of SpoT hydrolyzes ppGpp and functions in starvation responses. Nature Structural and Molecular Biology 17, 1188–1194.

Sun Y, Zerges W. 2015. Translational regulation in chloroplasts for development and homeostasis. Biochimica et Biophysica Acta 1847, 809–820.

Suzuki JY, Sriraman P, Svab Z, Maliga P. 2003. Unique architecture of the plastid ribosomal RNA operon promoter recognized by the multisubunit RNA polymerase in tobacco and other higher plants. The Plant Cell 15, 195–205.

Swiatecka-Hagenbruch M, Liere K, Borner T. 2007. High diversity of plastidial promoters in Arabidopsis thaliana. Molecular Genetics & Genomics 277, 725–734.

Takahashi K, Kasai K, Ochi K. 2004. Identification of the bacterial alarmone guanosine 5′-diphosphate 3′-diphosphate (ppGpp) in plants. Proceedings of the National Academy of Sciences 101, 4320–4324.

Tanaka S, Kihara M, Sugimoto M. 2015. Structure and molecular characterization of barley nudix hydrolase genes. Bioscience, Biotechnology, and Biochemistry 79, 394–401.

Tiller N, Bock R. 2014. The translational apparatus of plastids and its role in plant development. Molecular Plant 7, 1105–1120.

Tozawa Y, Nozawa A, Kanno T, Narisawa T, Masuda S, Kasai K, Nanamiya H. 2007. Calcium-activated (p)ppGpp synthetase in chloroplasts of land plants. The Journal of Biological Chemistry 282, 35536–35545.

van der Biezen EA, Sun J, Coleman MJ, Bibb MJ, Jones JD. 2000. Arabidopsis RelA/SpoT homologs implicate (p)ppGpp in plant signaling. Proceedings of the National Academy of Sciences, USA 97, 3747–3752.

Vrentas CE, Gaal T, Ross W, Ebright RH, Gourse RL. 2005. Response of RNA polymerase to ppGpp: requirement for the omega subunit and relief of this requirement by DksA. Genes & Development 19, 2378–2387.

Woo YH, Ansari H, Otto TD, et al. 2015. Chromerid genomes reveal the evolutionary path from photosynthetic algae to obligate intracellular parasites. eLIFE 4, e06974.

Wout P, Pu K, Sullivan SM, Reese V, Zhou S, Lin B, Maddock JR. 2004. The Escherichia coli GTPase CgtAE cofractionates with the 50S ribosomal subunit and interacts with SpoT, a ppGpp synthetase/hydrolase. Journal of Bacteriology 186, 5249–5257.

Yamburenko MV, Zubo YO, Borner T. 2015. Abscisic acid affects transcription of chloroplast genes via protein phosphatase 2C-dependent activation of nuclear genes: repression by guanosine-3′-5′-bisdiphosphate and activation by sigma factor 5. The Plant Journal 82, 1030–1041.

Yamburenko MV, Zubo YO, Vanková R, Kusnetsov VV, Kulaeva ON, Börner T. 2013. Abscisic acid represses the transcription of chloroplast genes. Journal of Experimental Botany 64, 4491–4502.

Zubo YO, Yamburenko MV, Kusnetsov VV, Börner T. 2011. Methyl jasmonate, gibberellic acid, and auxin affect transcription and transcript accumulation of chloroplast genes in barley. Journal of Plant Physiology 168, 1335–1344.

Zubo YO, Yamburenko MV, Selivankina SY, et al. 2008. Cytokinin stimulates chloroplast transcription in detached barley leaves. Plant Physiology 148, 1082–1093.

Zuo Y, Wang Y, Steitz TA. 2013. The mechanism of E. coli RNA polymerase regulation by ppGpp is suggested by the structure of their complex. Molecular Cell 50, 430–436.