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The Stringent Response in Phototrophs

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1. Introduction

Organisms must respond to environmental changes if they are to survive. As a result, species have evolved numerous intracellular and intercellular regulatory systems that often reflect an organism's environment. In bacteria, one of the most important regulatory systems is the stringent response (Cashel et al., 1996). Signaling *via* this response is mediated by guanosine 5′-triphosphate 3′-diphosphate (pppGpp) and guanosine 5′-diphosphate 3′-diphosphate (ppGpp), which function as second messengers. The stringent response was first discovered over 40 years ago in *Escherichia coli*. When *E. coli* cells are grown under nutrient-rich conditions but then transferred to a nutrient-limited environment, intracellular levels of pppGpp and ppGpp ((p)ppGpp) rapidly increase (Cashel et al., 1996). (p)ppGpp controls many vital cellular processes, including transcription and translation. For example, (p)ppGpp directly binds RNA polymerase and alters its promoter-binding affinity (Chatterji et al., 1998; Toulokhonov et al., 2001; Artsimovitch et al., 2004). When nutrient availability changes, therefore, the stringent response simultaneously adjusts the level of transcription for many genes. In *E. coli*, synthesis and degradation of (p)ppGpp are catalyzed by two enzymes RelA and SpoT (Cashel et al., 1996).

Deficiencies in iron, phosphate, nitrogen, or carbon each represent environmental stresses that trigger (p)ppGpp accumulation (Cashel et al., 1996). For photosynthetic bacteria, sunlight is also an important "nutrient". Characterization of a SpoT homolog in the purple photosynthetic bacterium, *Rhodobacter capsulatus*, showed that the stringent response also regulates photosynthesis (Masuda & Bauer, 2004). Genes that encode (p)ppGpp synthases and hydrolases are highly conserved in plants (van der Biezen et al., 2000; Kasai et al., 2002; Yamada et al., 2003; Givens et al., 2004; Tozawa et al., 2007; Masuda et al., 2008a; Kim et al., 2009) and are called RSHs (RelA/SpoT homologs). All known plant RSHs are targeted to chloroplasts, suggesting that they may control chloroplast function. Here we summarize our current understanding of the stringent response in phototrophs. For details concerning the mechanisms of the stringent response itself, several recent reviews are available (Magnusson et al., 2005; Braeken et al., 2006; Jain et al., 2006; Ochi, 2007; Potrykus & Cashel, 2008; Srivatsan & Wang, 2008).

2. (p)ppGpp synthases and hydrolases in bacteria

In *E. coli*, the level of (p)ppGpp is controlled by two enzymes, RelA and SpoT (Cashel et al., 1996). Both enzymes synthesize (p)ppGpp by transferring the pyrophosphate of ATP to the

ribose of GTP or GDP at the 3′ hydroxyl position. (p)ppGpp is hydrolyzed by SpoT, but not RelA, as RelA has only (p)ppGpp synthase activity. Biochemical and crystallographic studies have revealed two distinct domains in SpoT that mediate either synthesis or hydrolysis of (p)ppGpp (Fig. 1) (Cashel et al., 1996). As expected, the (p)ppGpp hydrolysis domain (HD) is not conserved in RelA. The (p)ppGpp HD is found in a superfamily of metal-dependent phosphohydrolases (Aravind & Koonin, 1998; Hogg et al., 2004). In fact, SpoT-like proteins require a divalent cation such a Mn^{2+} for their hydrolase activity (Wendrich et al., 2000).

A large number of bacterial genomes have been sequenced. These data have revealed that SpoT-like proteins, which contain both (p)ppGpp synthase and (p)ppGpp hydrolase domains, are generally conserved among bacterial species (Mittenhuber, 2001a). In contrast, RelA-like proteins, which contain only a (p)ppGpp synthase domain, have only been found in β- and γ- *Proteobacteria*. Phylogenetic analyses suggest that RelA branched off from a SpoT-like protein following the divergence of α- and β- *Proteobacteria* (Mittenhuber, 2001a).

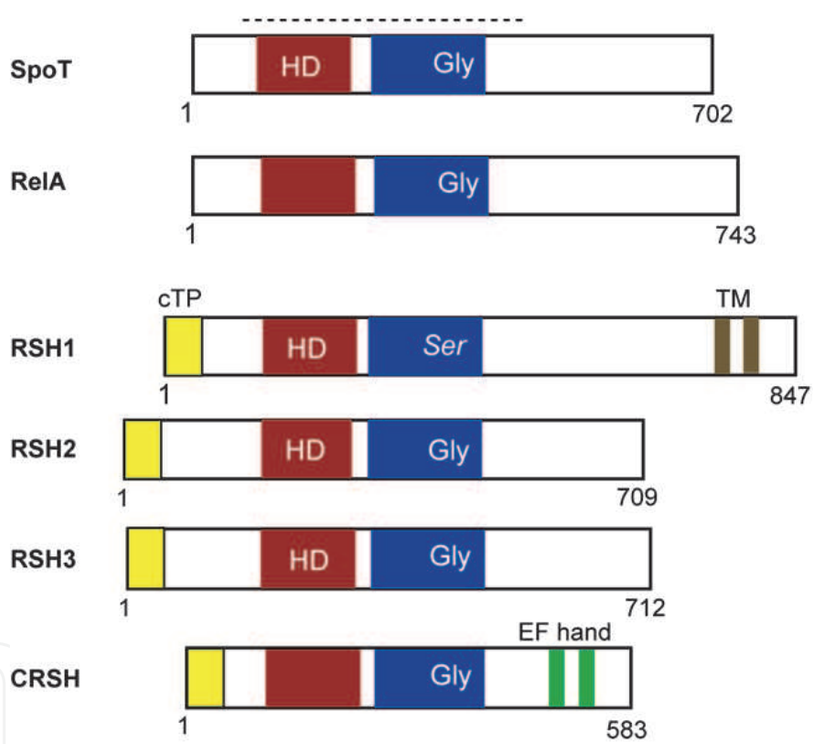

Fig. 1. Schematic depiction of the domain structure of SpoT and RelA from *E. coli* and of Arabidopsis RSHs. The region used to construct the phylogenetic tree (Fig. 3) is indicated by a dashed line. TM: putative transmembrane region; cTP: chloroplast transit peptide; EF-hand: Ca^{2+}-binding domain; HD: HD domain responsible for (p)ppGpp degradation. RelA and SpoT do not conserve several critical amino acids in the HD domain. RSH1 does not conserve the critical Gly residue (changed to Ser) that is necessary for (p)ppGpp synthase activity of RelA. For more detals, see text.

A small (p)ppGpp synthase protein, which lacks an HD domain, has also been found in some *Firmicutes* bacteria (Lemos et al., 2007; Nanakiya et al., 2008). Biochemical analyses indicate that these small enzymes have (p)ppGpp synthase activity.

3. (p)ppGpp synthases and hydrolases in photosynthetic bacteria

To date, six bacterial phyla have been shown to contain species capable of chlorophyll-based photosynthesis: *Cyanobacteria*, *Proteobacteria* (purple bacteria), *Chlorobi* (green sulfur bacteria), *Chloroflexi* (anoxygenic filamentous bacteria), *Firmicutes* (heliobacteria), and *Acidobacteria* (Bryant & Frigaard, 2006; Bryant et al., 2007). All of these photosynthetic bacteria produce SpoT and/or RelA proteins, but a careful analysis of these enzymes has only been done for two species: *Rhodobacter capsulatus* (a purple bacterium) (Masuda & Bauer, 2004), and *Anabaena* sp. PCC7120 (a cyanobacterium) (Ning et al., 2011). Genomic analysis has indicated that both of these bacterial species encode a single SpoT-like protein, but not a RelA-like protein. Based on complementation tests using *E. coli relA* and *relA*/*spoT* mutants, the SpoT-like enzyme of each of these bacteria likely have (p)ppGpp synthase activity. In wild-type strains of both bacteria, cellular levels of (p)ppGpp increase upon amino-acid starvation (caused by addition of serine hydroxamate), suggesting that these SpoT-like proteins induce the stringent response upon amino-acid starvation. In addition, these *spoT*-like genes are essential for cell viability, as loss-of-function mutations are lethal (Masuda & Bauer, 2004; Ning et al., 2011).

R. capsulatus is one of the most extensively studied photosynthetic purple bacteria (Bauer, 2004). It exhibits remarkable bio-energetic versatility and is capable of aerobic respiratory growth, anaerobic respiratory growth (using dimethyl sulfoxide as an electron donor), and photosynthetic growth. As such, this bacterium can adjust its mode of growth in response to environmental conditions (e.g., oxygen concentration, light intensity). In fact, sophisticated regulatory systems that respond to changes in redox and light have been identified (Bauer et al., 2003). Masuda et al. (2004) asked whether the stringent response affects growth-mode control in *R. capsulatus* by functionally characterizing *spoT* of this organism. They found that the lethality associated with a *spoT* mutation could be rescued by loss of *hvrA*, a gene that encodes a nucleoid protein (Masuda & Bauer, 2004). HvrA was originally identified as a *trans*-acting factor that represses transcription of photosynthesis genes under intense light conditions (Buggy et al., 1994). In intense light, *R. capsulatus* down-regulates components of the photosynthetic apparatus (e.g., the light-harvesting complexes, and the photosynthetic reaction center) to avoid photo-damage. *hvrA* mutants, however, cannot reduce photopigment synthesis under intense light. HvrA is a typical bacterial nucleoid protein that resembles *E. coli* H-NS and StpA (Bertin et al., 1999; Masuda & Bauer, 2004). Nucleoid proteins bind curved DNA with low sequence specificity and affect a large number of genes involved in multiple physiological processes (McLeod & Johnson, 2001; Dorman & Deighan, 2003). In fact, *R. capsulatus* HvrA transcriptionally regulates (positively and negatively) genes involved in photosynthesis, nitrogen fixation, and electron transfer, for example (Buggy et al., 1994; Kern et al., 1998; Swem & Bauer, 2002). Although it is not entirely clear why loss of *hvrA* rescues the lethality associated with *spoT*-like loss-of-function, this result suggests a functional link between the (p)ppGpp-dependent stringent response and the nucleoid protein HvrA. A similar phenomenon exists in *E. coli* (Johansson et al., 2000); strains lacking the nucleoid proteins H-NS and StpA have a slow-growth phenotype, which can be partially suppressed by mutations in *spoT* and *relA*. These results suggest that genes regulated by (p)ppGpp are also regulated by the nucleoid structure. Notably, *hvrA* expression itself is controlled by RegA and RegB, a redox-sensitive, two-component system in *R. capsulatus* (Du et al., 1999). Specifically, *hvrA* transcription is repressed or activated under aerobic or anaerobic conditions, respectively. Taken together, these results suggest

that HvrA and SpoT-like proteins are functionally linked in *R. capsulatus* to efficiently utilize energy sources (e.g., oxygen, light, amino acids, nitrogen, and carbon) in response to changing environmental conditions (Fig. 2). In support of this hypothesis, *hvrA* and *spoT*-like double mutants produce significantly lower levels of photopigments (Masuda & Bauer, 2004). Importantly, exogenously added carbon compensates for pigmentation loss in these double mutants (Masuda & Bauer, 2004), suggesting that the stringent response (induced by the SpoT-like protein) promotes photopigment synthesis in *R. capsulatus* specifically during starvation.

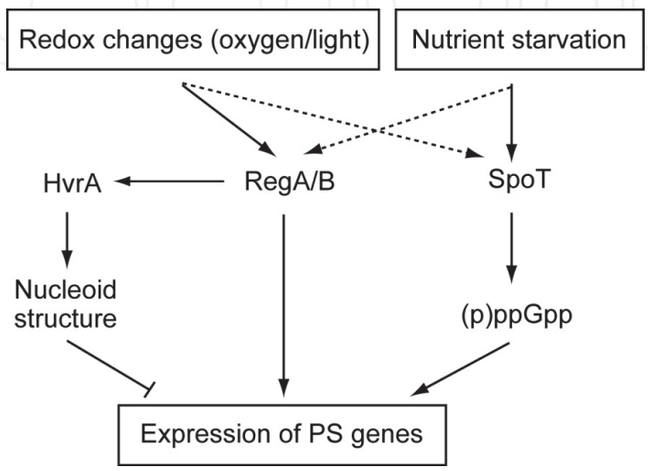

Fig. 2. A model for the coordinated regulation of photosynthesis (PS) gene expression in response to redox, light, and nutrient conditions in the purple bacterium *R. capsulatus*. Putative signaling pathways are indicated by dashed arrows. Solid arrows represent signaling pathways supported by experimental evidence.

Anabaena sp. PCC7120 is a filamentous cyanobacterium that is frequently used as a model organism to characterize nitrogen fixation in heterocysts, which are specialized cells that fix nitrogen. It is well established that nitrogen depletion induces heterocyst formation. In *Anabaena* sp. PCC7120, however, the stringent response is not likely involved in heterocyst formation, as neither SpoT-like proteins nor (p)ppGpp levels increase upon nitrogen depletion (Ning et al., 2011). In these experiments, however, bulk levels of (p)ppGpp were measured, so it is possible that (p)ppGpp levels rise specifically in heterocyst-forming cells. In fact, (p)ppGpp accumulation was previously observed upon nitrogen depletion in other cyanobacteria (Akinyanju & Amith, 1979; Friga et al., 1981). If this is true, then the activity of the SpoT-like protein would be controlled post-translationally (as in *E. coli*) because nitrogen depletion does not affect *spoT*-like expression. To understand the stringent response in cyanobacteria, therefore, genetic analysis of the *spoT*-like gene is necessary.

4. (p)ppGpp synthases and hydrolases in plants and algae

van der Biezen et al. (2000) identified RelA/SpoT-like proteins in the model plant *Arabidopsis thaliana* and designated them as RSHs. Since then, genes encoding RSHs have been found in many plant and algal species (Kasai et al., 2002; Yamada et al., 2003; Givens et al., 2004; Tozawa et al., 2007; Masuda et al., 2008a; Kim et al., 2009). Figure 3 shows a phylogenetic tree that is based on amino-acid sequences of (p)ppGpp synthase and

(p)ppGpp hydrolase domains of SpoT-like and RSH proteins. In this tree, bacterial SpoT-like proteins and plant RSHs are clearly separated, although SpoT-like proteins of the bacterial phyla *Deinococcus-Thermus* form a branch with plant RSH families (RSH1). It has been suggested that plant RSHs were introduced into a proto-plant cell by endosymbiosis of an ancestral cyanobacterium (Givens et al., 2004). Our phylogenetic analysis, however, does not clearly support this hypothesis, although cyanobacterial SpoT-like proteins are relatively similar to plant RSHs. Our results may agree with a previous phylogenetic analysis, which suggested that plant RSHs were introduced into a proto-plant cell by lateral gene transfer from a pathogenic bacterium (van der Biezen, 2000). Additional experiments are necessary to clarify the origin of plant RSHs.

Arabidopsis has four RSHs, RSH1, RSH2, RSH3, and CRSH (Ca^{2+}-activated RSH) (van der Biezen et al., 2000; Masuda et al., 2008a). Primary structures of these RSHs are shown in Fig. 1. Each of these four proteins has a putative chloroplast transit peptide at the N-terminus, suggesting that each functions in plastids. CRSH has two Ca^{2+}-binding domains (EF-hand motifs) at the C-terminus. Sequences similar to the (p)ppGpp synthase and hydrolase domains of *E. coli* SpoT are found in the central region of RSHs. However, the conserved Gly residue, which is necessary for RelA (p)ppGpp synthase activity, is not conserved in RSH1 (changed to Ser). In addition, the HD domain that mediates (p)ppGpp hydrolase activity in SpoT is not conserved in CRSH (Masuda et al., 2008a). Both the Gly residue and the HD domain are conserved in RSH2 and RSH3. These results suggest that RSH1 and CRSH may have only (p)ppGpp hydrolase and synthase activity, respectively, whereas RSH2 and RSH3 may have both activities.

Protein domain structures (Fig. 1) and the phylogenetic tree (Fig. 3) clearly show that Arabidopsis RSHs can be classified into three distinct families: RSH1, RSH2/3, and CRSH. Mining existing databases, all three types of RSHs can be found in another dicotyledon plant (*Nicotiana tabacum*), a monocotyledon plant (*Oryza sativa*), and a moss (*Physcomitrella patens*) (Fig. 3). RSH amino acid sequence alignments reveal that the RSH1, RSH2/3, and CRSH families each have a conserved linear arrangement of domains (Fig. 1). In addition, all RSH1-family members lack the conserved Gly residue that is critical for (p)ppGpp synthesis, all CRSH-family members lack the HD domain required for (p)ppGpp hydrolysis but have two EF-hand motifs instead, and all RSH2/3-family members contain both the Gly residue and the HD domain. These results suggest that the functional roles for these three RSH families may have been conserved between plant species. The green algae *Chlamydomonas reinhardtii* has a single *RSH* gene that does not cluster within any other plant RSH (Fig. 3), suggesting that the three plant RSH families (RSH1, RSH2/3 and CRSH) diverged after the separation of algae and mosses but before the separation of mosses and seed plants. Perhaps these three families were established when plant species adapted to terrestrial growth. Importantly, the *Chlamydomonas* RSH is not similar to bacterial SpoT-like proteins (Fig. 3), suggesting that RSH genes were not introduced into plant cells by endosymbiosis.

Recently, a small family of proteins that contain a domain resembling the SpoT HD domain was identified in metazoa, including *Drosophila melanogaster, Caenorhabditis elegans* and human (Sun et al., 2010). The protein was named Mesh1 (metazoan SpoT homolog 1). Biochemical analyses indicated that *Drosophila* Mesh1 has (p)ppGpp hydrolase activity. Arabidopsis also has a Mesh1 homolog (Sun et al., 2010), found from a cDNA clone submitted to GenBank as part of the Arabidopsis full-length cDNA cloning project (accession no. BAF00616). However, I found that the nucleotide sequence of Arabidopsis *Mesh1* is identical, at least in part, to *RSH2*. Compared to full-length *RSH2*, *Mesh1* lacks ~200

bp from the 5′ end, and it contains the first and third intron sequences. These results indicate that *Mesh1*, if it represents a functional transcript, is likely a splice variant of *RSH2*. Given that no *Mesh1* homologs have been identified in other plant species, and no data concerning Mesh1 function have been reported, the putative (p)ppGpp hydrolase, Mesh1, will not be discussed here.

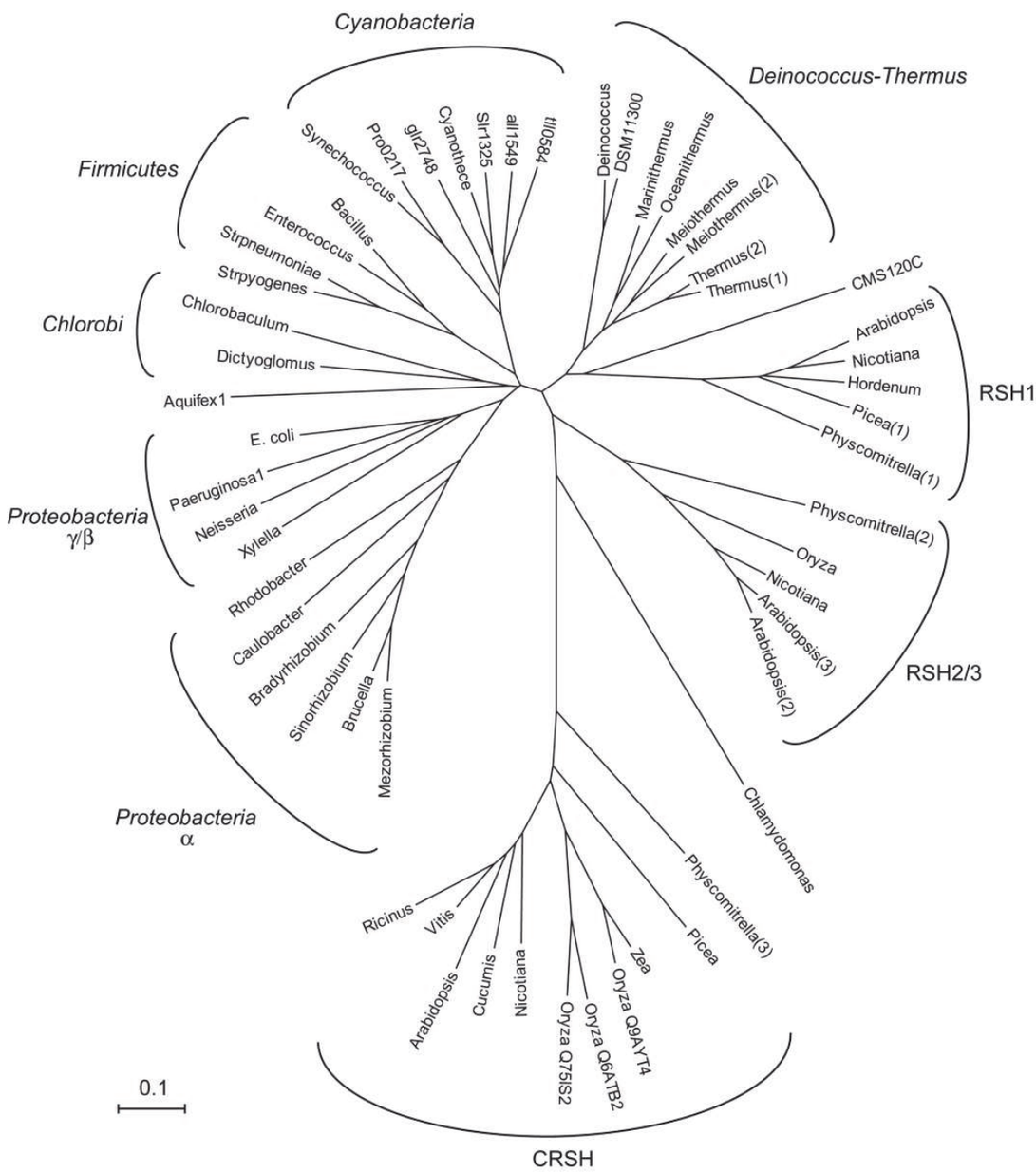

Fig. 3. A phylogenetic tree based on (p)ppGpp synthase and (p)ppGpp hydrolase domains of bacterial SpoT-like proteins and plant RSHs. The region used to construct the phylogenetic tree corresponds to amino acid residues 25–325 of *E. coli* SpoT (indicated by the dashed line in Fig. 1). All gaps in the sequence alignment were omitted, and the tree was constructed using the neighbor-joining method.

Recently, a novel (p)ppGpp degradation system was found in the bacterium *Thermus thermophilus* (Ooga et al., 2009). Specifically, a Nudix (nucleoside diphosphates linked to some moiety X) pyrophosphatase degrades ppGpp (both *in vivo* and *in vitro*) to maintain proper levels of ppGpp. Over 20 genes that encode Nudix pyrophosphatases have been identified in plants (Ogawa et al., 2005; Gunawardana et al., 2009). These proteins have pyrophosphatase activity and degrade a variety of substrates that include (d)NTPs, nucleotide sugars, NADH, and NADPH. Given that some Nudix pyrophosphatases localize to chloroplasts (Ogawa et al., 2008), these proteins could be involved in (p)ppGpp degradation by cooperating with the RSH1 and RSH2/3 families of enzymes. If this is the case, Nudix proteins could degrade (p)ppGpp to ppGp or pGpp or pGp, which are strong inhibitors of purine biosynthesis (Pao et al., 1980; Pao and Dyess, 1981). This suggests that the degradation products of (p)ppGpp may participate in the stringent response in plastids.

5. Physiological functions of RSHs in plants

Complementation analyses using *relA* and *relA/spoT* mutants in *E. coli* indicate that plant RSH2/3 and CRSH family members can functionally replace these bacterial enzymes. This suggests that plant RSHs have (p)ppGpp synthase activity (Givens et al., 2004; Tozawa et al., 2007; Masuda et al., 2008a; Mizusawa et al., 2008). This result was confirmed biochemically, clearly demonstrating that plant RSHs synthesize (p)ppGpp *in vitro* (Givens et al., 2004; Tozawa et al., 2007; Masuda et al., 2008a). These *in vitro* studies also showed that the (p)ppGpp synthase activity of CRSH is activated by Ca^{2+} (Tozawa et al., 2007; Masuda et al., 2008a). As mentioned previously, RSH1 may not have (p)ppGpp synthase activity, as it lacks the critical Gly residue found in bacterial RelA proteins. Mizusawa et al. (2008) showed that expression of Arabidopsis RSH1 does not rescue the *E. coli relA* and *relA/spoT* mutants, supporting this hypothesis. van der Biezen et al. (2000), however, reported the opposite result, concluding that RSH1 can complement the *relA* mutation. In addition, the (p)ppGpp hydrolase activities of RSH1, RSH2/3, and CRSH have not been confirmed. Clearly, these two issues require further investigation.

Protein import experiments indicate that the *Chlamydomonas* RSH is targeted to isolated chloroplasts, suggesting that the protein primarily localizes to plastids (Kasai et al., 2002). In addition, when Arabidopsis RSH1 and CRSH are fused to green fluorescent protein, they also localize to chloroplasts (Masuda et al., 2008a; Mizusawa et al., 2008). Finally, the localization of *N. tabacum* RSH2 has been studied by western blotting, which indicated that the enzyme is highly enriched in the chloroplast fraction (Givens et al., 2004). These results suggest that proteins from each RSH family in plants localize to plastids. For each plant RSH, however, the exact localization within chloroplasts seems to differ. *N. tabacum* RSH2 is in the insoluble fraction of chloroplasts (Givens et al., 2004), whereas Arabidopsis CRSH is in the soluble fraction (Masuda et al., 2008a). These localization differences may reflect different modes of enzymatic regulation. It has been suggested that membrane-associated ribosomes interact with *N. tabacum* RSH2 and regulate its activity, as is the case with bacterial RelA proteins (Givens et al., 2004). There is evidence to suggest that CRSH activity depends on Ca^{2+} levels in the chloroplast stroma (Tozawa et al., 2007; Masuda et al., 2008a). Finally, RSH1 contains two putative transmembrane helices in its C-terminal region (Fig. 1) (van der Biezen et al., 2000), suggesting that it localizes to the thylakoid and/or envelope membrane of plastids. This, however, has not been demonstrated experimentally.

The expression patterns of Arabidopsis *RSH* genes also suggest that there are functional differences between RSHs (Mizusawa et al., 2008). Microarray data indicate that *RSH* genes have diurnal rhythms of expression. Peak levels of expression are reached at noon, evening, and midnight for Arabidopsis *RSH2/3*, *RSH1*, and *CRSH*, respectively (Mizusawa et al., 2008). Given that RSH2/3 enzymes have both synthase and hydrolase activities, they likely maintain plastidial (p)ppGpp levels during daylight hours. At evening, RSH1 may then degrade (p)ppGpp, as it seems to have only (p)ppGpp hydrolase activity. At night, (p)ppGpp levels are likely kept low because keeping plants in the dark reduces cellular (p)ppGpp levels (Takahashi et al., 2004). These data suggest that (p)ppGpp is required to control light-dependent plastidial activities, such as photosynthesis. At night, CRSH likely maintains (p)ppGpp levels based on Ca^{2+} levels. Plastidial Ca^{2+} concentrations change in response to environmental stimuli, such as light conditions (Johnson et al., 1995; Sai & Johnson, 2002), allowing CRSH to translate these environmental changes into (p)ppGpp synthesis.

Expression of *RSH2* genes in Arabidopsis, rice and *N. tabacum* is elevated in response to cold and/or exogenous application of the plant hormone jasmonic acid (or its precursor 12-oxo-phytodienoic acid) (Xiong et al., 2001; Givens et al., 2004; Lee et al., 2005; Mizusawa et al., 2008). (p)ppGpp levels similarly increase in response to jasmonic acid (Takahashi et al., 2004). These observations suggest that the RSH2/3 family synthesizes (p)ppGpp in response to abiotic stresses. The plastidial stringent response may play a role in these types of plant defense responses, although the mechanisms remain unclear.

Histochemical analyses indicate that Arabidopsis *RSH2* and *RSH3* are expressed in all green tissue (Mizusawa et al., 2008), suggesting that the RSH2/3 family controls light-dependent plastidial function. Interestingly, all *RSH* genes are expressed at high levels in flower tissues, such as the pistil and stamen, suggesting an important role for (p)ppGpp in flower development (Mizusawa et al., 2008). Genetic knockdown of *CRSH* disrupts flower morphology, as pistil and stamen development are not coordinated. This defect results in infertility (Masuda et al., 2008a), indicating that RSH-dependent (p)ppGpp production is required for plant reproduction. As discussed below, (p)ppGpp may control the biosynthesis of amino acids, fatty acids, and nucleotides in plastids, as has been observed in bacteria. Because these compounds serve as precursors for plant hormones (e.g., jasmonates, cytokinin, and auxin), the plastidial stringent response may control host plant development by regulating hormone biosynthesis.

6. Which plastidial functions are regulated by (p)ppGpp?

In bacteria, (p)ppGpp regulates the transcription of a large number of genes *via* two distinct mechanisms. First, (p)ppGpp allosterically controls RNA polymerase activity through direct association with the β- or β′- subunit of the polymerase (Chatterji et al., 1998; Toulokhonov et al., 2001; Artsimovitch et al., 2004). Second, RelA- and SpoT-dependent (p)ppGpp synthesis uses ATP and GTP (or GDP) as substrates. This significantly reduces the amount of NTPs available for RNA synthesis, thereby indirectly decreasing RNA polymerase activity (Krasny & Gourse, 2004). In chloroplasts, two RNA polymerases transcribe the plastid genome (Shiina et al., 2005; Liere et al., 2011). One is the bacterial type of RNA polymerase called plastid-encoded plastid RNA polymerase (PEP). The other is the T7-phase type of RNA polymerase called nuclear-encoded plastid RNA polymerase (NEP). As with *E. coli* RNA polymerase, (p)ppGpp directly binds to PEP (Sato et al., 2009) and can

inhibit PEP- mediated transcription when exogenously added *in vitro* (Sato et al., 2009). This suggests that (p)ppGpp directly controls PEP activity. The second "indirect" control of plastidial RNA polymerases needs to be studied in more detail. If this mechanism is real, both PEP and NEP activities should be affected. It has recently been reported that some plant hormones, including jasmonates and auxin, affect transcription of chloroplast genes (Zubo et al., 2011). Because *RSH2* expression is induced by jasmonates (Givens et al., 2004; Mizusawa et al., 2008), it is possible that RSH2-dependent (p)ppGpp synthesis mediates the effects of plant hormones on plastidial gene expression.

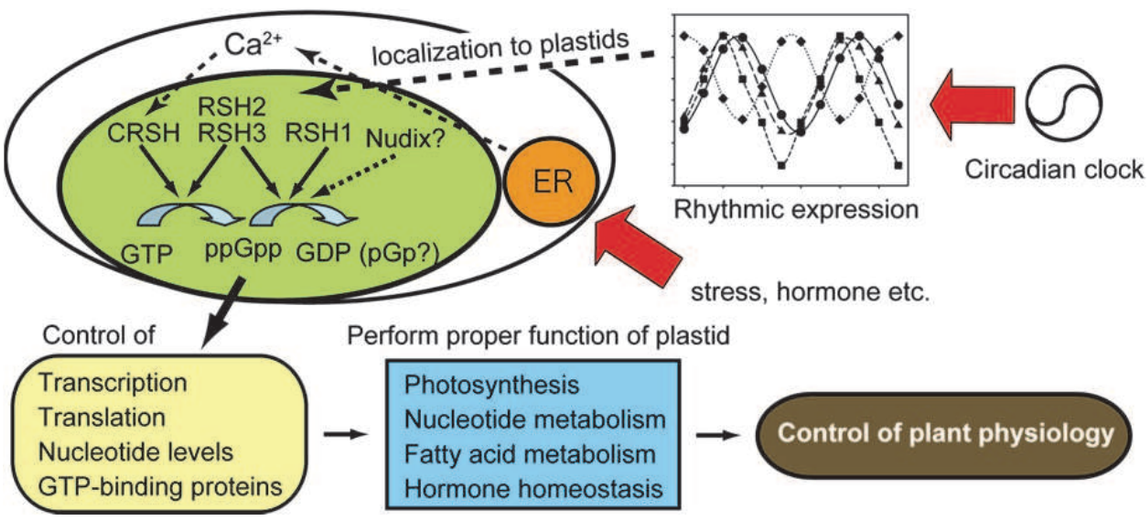

Fig. 4. A model for the stringent response in higher plants.

In addition to regulating transcription, (p)ppGpp also controls translation in bacteria (Milon et al., 2006). The translation initiation factor, IF2, binds and hydrolyzes GTP to initiate translation. (p)ppGpp binds to the GTP-binding pocket of IF2, thereby inhibiting translation initiation (Milon et al., 2006). Given that a bacterial IF2 homolog is found in chloroplasts (Miura et al., 2007), (p)ppGpp may also control translation of plastid genes; the chloroplast genome encodes genes involved in photosynthesis, electron transfer, and fatty-acid biosynthesis, for example. These various plastidial functions, therefore, should be regulated by the (p)ppGpp-dependent stringent response.

Bacteria produce several GTP-binding proteins, some of which are conserved in plants and function in chloroplasts (Mittenhuber, 2001b; Masuda et al., 2008b). Because (p)ppGpp interacts with the GTP-binding pocket of IF2, chloroplast GTP-binding proteins may also be regulated by (p)ppGpp. Several enzymes involved in nucleotide biosynthesis are regulated by (p)ppGpp in an allosteric manner (Gallant et al., 1971; Hou et al., 1999). It is thought that nucleotide biosynthesis can take place in plastids because one of the enzymes that catalyzes phosphoribosyl diphosphate synthesis (the first step in purine and pyrimidine biosynthesis) localizes to plastids (Krath & Hove-Jensen, 1999). As a result, nucleotide biosynthesis in plastids may be directly regulated by (p)ppGpp. Furthermore, consumption of GTP (GDP) and ATP during (p)ppGpp synthesis may also indirectly influence nucleotide metabolism in plastids.

7. Concluding remarks

It has been almost one-half century since (p)ppGpp was first discovered in *E. coli*. Since then, the physiological roles for (p)ppGpp in controlling bacterial cell metabolism have been well documented. The role of the stringent response in photosynthetic bacteria, however, remains unclear, even though photosynthesis is one of the most important anabolic reactions in biology. Future studies are needed if we are to understand how the stringent response controls different types of photosynthesis.

Many plant and algal species produce (p)ppGpp synthases and hydrolases called RSHs. Studies in Arabidopsis indicate that RSHs can be classified into three distinct families, RSH1, RSH2/3 and CRSH, all of which function in plastids. *RSH* gene expression profiles and the domain structures of RSHs suggest that RSH families are functionally diverse. Furthermore, these functional differences are likely necessary to properly regulate plastidial (p)ppGpp levels. Although the specific target proteins of (p)ppGpp remain largely unknown in plastids, the RSH-dependent stringent response regulates many aspects of plastidial function, including transcription, translation, nucleotide metabolism, and biosynthesis of amino acids and fatty acids. As a result, the stringent response may also regulate plant hormone biosynthesis, which is required for host plant development. A model of the stringent response in plastids is shown in Fig. 4. Additional genetic and physiological experiments are needed if we are to understand the precise roles of the (p)ppGpp-mediated stringent response in higher plants.

8. Acknowledgment

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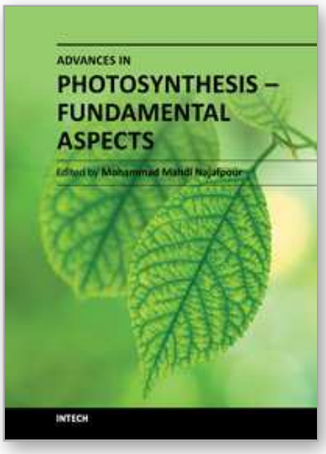

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Photosynthesis is one of the most important reactions on Earth. It is a scientific field that is the topic of many research groups. This book is aimed at providing the fundamental aspects of photosynthesis, and the results collected from different research groups. There are three sections in this book: light and photosynthesis, the path of carbon in photosynthesis, and special topics in photosynthesis. In each section important topics in the subject are discussed and (or) reviewed by experts in each book chapter.

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