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Review

Participation of non-coding RNAs in plant organelle biogenesis

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The biogenesis of plant mitochondria and plastids is a multistep process that depends on the expression of both, organellar and nuclear genes. A growing body of evidence suggests that the indispensable coordination of different steps in this process may be gained by participation of the non-coding RNAs. A plethora of non-coding RNAs of diverse length, both intraorganellar ones, as well as encoded by the nuclear genome (including microRNAs and short interfering RNAs), were also suggested to play a role in the stress response by regulating the expression levels of targeted genes important for organelle biogenesis. Selected points of current interest regarding the regulation of plant mitochondrial and plastid gene expression by diverse non-coding RNAs, also discussed in the aspect of abiotic stress conditions, are highlighted here.

Key words: non-coding RNAs, chloroplast DNA, mitochondrial DNA, organelle gene expression, stress response

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INTRODUCTION

Despite their structural variability, plant mitochondrial and plastid genomes (further referred to as 'mitogenomes' and 'plastomes', respectively) encode a limited number of proteins. A number of organellar genes were transferred to the nuclear genome in the course of evolution (Alverson *et al*., 2011). Complex and multistep biogenesis of plant organelles must therefore depend on the concerted expression of organellar and nuclear genes. Among various molecular factors, the role of ncRNAs in this process is widely accepted. Regulation of plant gene expression is largely influenced by the participation of various classes of non-coding RNAs (ncRNAs), including microRNAs (miRNAs), small interfering RNAs (siRNAs), natural antisense RNAs (NAT RNAs), intermediate-sized ncRNAs (im-ncRNAs), as well as long non-coding RNAs (lncRNAs) whose length exceeds 200 nt. Many ncRNAs were proved to regulate expression of nuclear genes encoding diverse organellar proteins. In addition, intraorganellar ncRNAs also regulate expression of mitochondrial and plastid genes.

Recently, an excellent review on the intraorganellar ncRNAs focused mainly on the animal and protist data and potential therapeutic applications has been published (Dietrich *et al*., 2015). Some reports on the participation of miRNAs, siRNAs and other ncRNAs in plant stress responses were also published, though without detailed discussion of their potential organellar functions (Chiou, 2007; Sunkar *et al*., 2012; Matsui *et al*., 2013; Zhang *et al*., 2013). Our knowledge regarding status of the plant organelle-encoded ncRNAs as well as nuclear-encoded ncRNAs in the organelle biogenesis has been substantially expanded in the past years. The aim of this review is to provide a comprehensive and timely view of the influence of ncRNAs on the selected aspects of functioning of the plant mitochondria and plastids.

REGULATION OF PLASTID AND MITOCHONDRIAL GENE EXPRESSION BY INTRAORGANELLAR ncRNAs

One of the most intriguing findings in plant organelle biology was the discovery of various ncRNAs which are derived either from *de novo* transcription of plant mitogenome and plastome, or from degradation products of the organelle transcriptomes, as it was shown for some PPR (pentatricopeptide repeat) protein targets (Marker *et al*., 2002; Lung *et al*., 2006; Morin *et al*., 2008; Hotto *et al*., 2011; Ruwe & Schmitz-Linneweber, 2012).

The organellar expression, which is especially complex within plant mitochondria, is regulated in various steps (Dietrich *et al*., 2015), henceforth participation of ncRNA in this process is warranted. However, in interpretation of such results caution must be taken as it is known that the vast parts of mitogenomes and plastomes were inserted into the *Arabidopsis thaliana* nuclear genome and in some approaches it is very difficult to distinguish between such insertions containing ncRNAs and true intraorganellar ncRNAs (Lung *et al*., 2006; Mor-

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Abbreviations: AGO, Argonaute; APS, ATP sulfurylase; asRNA, antisense RNA; atp, ATP synthase; CI-CIV, mitochondrial complex I-IV; ccm, cytochrome *c* maturation; CcmE, mitochondrial heme chaperone; C/D box, C (RUGAUGA) and D (CUGA) sequence motifs; clp, caseinolytic protease; cox, cytochrome *c* oxidase; CMS, cytoplasmic male sterility; CSD, Cu/Zn superoxide dismutase; cyt., cytochrome; DCL, Dicer-like; H/ACA box, H box (ANANNA consensus) and the ACA motif; HY, long hypocotyl; im-ncRNA, intermediate-sized non-coding RNA; LHC, light-harvesting complex; lncRNA, long non-coding RNA; Lon, ATP-dependent protease; miRNA, microRNA; miRTarBase, miRNA-target interactions database; mTERF, mitochondrial transcription termination factor; nad, mitochondrial NADH dehydrogenase; NAT, natural antisense; ncRNA, non-coding RNA; ndh, plastid NAD(P)H dehydrogenase; NRPD, nuclear RNA polymerase D; ORF, open reading frame; OXA, organellar transporter protein; OXPHOS, oxidative phosphorylation; pet, cytochrome b_6f complex; P5CDH, D-pyrroline-5-carboxylate dehydrogenase; PDH, pyruvate dehydrogenase; PNRD, Plant Non-coding RNA Database; PPR, pentatricopeptide repeat; PS, photosystem; psa, photosystem I; psb, photosystem II; rbc, Rubisco; RDR, RNA-dependent RNA polymerase; rrn, rRNA; rpl, ribosomal large subunit; rpo, RNA polymerase; rps, ribosomal small subunit; SAUR, small auxin up RNA; SGS, suppressor of gene silencing; siRNA, short interfering RNA; SnRK, serine/threonine-protein kinase; sprA, small plastid-encoded RNA; sRNA, small RNA; SRO5, similar to RCD one 5; TAS, *trans*-acting small; tasi-RNA, *trans*-acting small interfering RNA; TPR, tetratricopeptide repeat; trn, tRNA; tsRNA, tRNA-derived small RNA; UTR, untranslated region; ycf, hypothetical chloroplast open reading frame

in *et al*., 2008). In addition, some nuclear insertions in the mitogenomes were postulated to be transcribed, for instance in *Brassicaceae*, which makes the evaluation of the ncRNAs' origin challenging (Qiu *et al*., 2014; Wang *et al*., 2014). Due to the fact that the organelle transcription efficiency is counterbalanced by the control of transcript stability (Holec *et al*., 2006), the enrichment of deep-sequencing data with reads for non-coding regions is not always satisfactory. Thus the development of optimal experimental and *in silico* methods for the confirmation of ncRNAs provenance and the use of secondary structure and structure motifs for ncRNA prediction in advanced algorithms seems to be particularly important for the future (Grimes *et al*., 2014; Wu *et al*., 2016).

In Supplementary Table 1 (at www.actabp.pl) the most representative data on plant intraorganellar ncRNAs and their expression are presented and will be discussed below.

Mitogenome-derived ncRNAs

Extensive reports on plant intramitochondrial ncRNAs are still limited. In the initial approach of Marker and coworkers (2002), a few low abundant ncRNA candidates of 62–300 nt were mapped to intergenic regions of the Arabidopsis mitogenome that were flanked by various ORFs (open reading frames), including *trn* genes. In this study, two RNAs generated from the genomic repeats were also identified. Additional ncRNA was proposed to be generated from *nad1* exon 5/3'UTR. All those ncRNAs belonged to the class II of small RNAs (sRNAs) lacking sequence motifs, for instance C/D or H/ACA box of small nucleolar RNA and tRNA sequence motifs known among members of class I. The extremely low abundant antisense RNAs (asRNAs), generated either from the repeat regions of the Arabidopsis mitogenome or from *atp9* and additional 3 *nad* genes, were found by Holec *et al*. (2006) in the poly(A)$^+$ fraction of the mtRNA. Interestingly, some asRNAs were complementary to the transcripts edited in plants containing downregulated mitochondrial polynucleotide phosphorylase gene; thus, they were suggested to originate by transcription from the opposite DNA strand.

Various distinct ncRNAs, including putative im-ncRNAs (found by deep-sequencing of the cDNA library) were mapped to the upper and lower strands of the rice (*Oryza sativa*) mitogenome (Liu *et al*., 2013). In the distinct approach of Wang and coworkers (2014), ncRNAs were sequenced on 454 Life Sciences platform after a chromatography-based selection. Numerous im-ncRNAs were identified to be generated from the Arabidopsis mitogenome, preferably from *trn*, *rrn26* and some protein genes (*atp*, *rpl*, *nad*, *cox3,* as well as the mitochondrial *ccm* genes) and their flanking regions. Their size ranged from 65 to 189 nt. However, some of those ncRNAs were also mapped at high density to the Arabidopsis chromosome 2, and therefore their organellar origin remains disputable.

Recently, Baev and coworkers (2014) identified 74 candidates for sRNAs derived from Arabidopsis mitochondrial transcriptome, including messengers encoding some Ccm proteins (involved in maturation of *c*-type cytochromes), subunits of mitochondrial complexes (CI, CIV) and ATP synthase, which were in majority. In some cases, mitochondrial sRNAs appeared to be generated by a longer transcript degradation (Wu *et al*., 2015). Using Illumina mRNA-seq, as well as sRNA-seq data, the authors analyzed the leaves' transcriptome of the night-flowering catchfly (*Silene noctiflora*). This plant species contains the largest known mitogenome, displaying an extremely bizarre multichromosomal organization. Notably, intergenic sequences encompass almost 99% of this genome. sRNAs (17–25 nt) were mapped to the various intergenic regions of the night-flowering catchfly mitogenome; no asRNAs were found. However, the general pool of sRNAs reads that were mapped to the reference mitogenome was smaller than the one obtained by the mRNA-seq analysis.

Plastome-derived ncRNAs

Compared to the mitochondrial data, our knowledge regarding plastid ncRNAs is far more advanced (Suppl. Table 1 at www.actabp.pl), as some results came from the initial studies over 25 years ago. In the early report of Goldschmidt-Clermont and coworkers (1991), a specific function for a sRNA (ca. 430 nt) in the initial events of *trans*-splicing of *psaA* transcripts in *Chlamydomonas reinhardtii* plastids was suggested. Later on, Vera & Sugiura (1994) studied plastome-encoded RNAs (218 nt) proposed to be involved in 16S rRNA maturation and chloroplast ribosome biogenesis. However, removal by targeted gene deletion of *sprA* gene encoding this sRNA did not affected 16S rRNA maturation. Thus, this gene appeared not to be essential for plastid biogenesis, at least under standard growth conditions (Sugita *et al*., 1997).

Between the years of 2002 and 2008, numerous plastid ncRNAs were detected in various plant species, including Arabidopsis (Marker *et al*., 2002), tobacco (*Nicotiana tabacum*; Billoud *et al*., 2005; Lung *et al*., 2006) and tomato (*Solanum lycopersicum*; Itaya *et al*., 2008). Marker and coworkers (2002) characterized 7 candidates for ncRNA (up to 290 nt) deriving mostly from intergenic regions. Notably, some of them appeared to be tissue-specific. Other ncRNAs of 21–100 nt were analyzed by Billoud and coworkers (2005) who had shown that they also derived from the intergenic regions of the plastome (*ycf1-trnN*), as well as from *trnV* and the 5' coding region of *ndhB* gene. They differed in size depending on the plant material. Itaya *et al*. (2008) studied ncRNAs from young and mature fruits, and from leaves and buds of the tomato plastome. They were also mapped to diverse intergenic regions (*cplP-rpl20*), as well as to some coding regions (*psbA*, *psbB* and *ycf2* genes).

Furthermore, Lung and coworkers (2006) reported 12 short or im-ncRNAs (18–53 nt) that generally mapped to the regions identified earlier by the Marker and coworkers (2002) approach, however, the most abundant ncRNAs derived rather from *rrn16* gene; their functions were not investigated. More im-ncRNAs (60–157 nt) were identified in the Arabidopsis plastome in the recent study of Wang and coworkers (2014), and mapped to the 5' and 3' flanking regions of the known genes, as well as to the intergenic and repeat regions. Hotto and coworkers (2011), in contrast, reported on longer ncRNAs (up to 1300 nt); at least some of them were expressed from dedicated promoters. They were mapped both, to some intergenic regions and *rps*, but not to *rrn* genes. Their abundance correlated with their targets level, implying engagement of ncRNAs in the control of plastid transcriptome stability.

Among diverse ncRNAs, plastid asRNAs were investigated by diverse research groups. Long RNAs (400–650 nt) with antisense orientation to *ndhB* transcripts were characterized by Georg and coworkers (2010) in plastids of 3 plant species. They accumulated predominantly in young leaves and were shown to be involved in

maturation and stability control of plastid mRNA. The level of plastid ncRNAs, including asRNAs (to *atpB-atpE*, *rbcL* and *clpP* mRNAs), can be controlled by RNAse J; nevertheless asRNAs' population is highly complex, with diverse 5' and 3' termini (Sharwood *et al*., 2011*a*). In some cases, asRNAs protect other plastid messengers, for instance *atpB*, from exonucleotic degradation (Nishimura *et al*., 2004). AsRNAs were also involved in inactivation of *psbT* and *psbB* transcripts (Zghidi-Abouzid *et al*., 2011; Chevalier *et al*., 2015). Overexpressed asRNA (AS5) may block 5S rRNA precursor processing and decrease 5S rRNA stability and its association with plastid ribosomes. AS5 forms a duplex that is degraded under standard growth conditions (Hotto *et al*., 2010; Sharwood *et al*., 2011*b*). AS5 also prevented accumulation of misprocessed rRNA. Chevalier and coworkers (2015) investigated the impact of *psbN* gene expression (encoding asRNA to *psbT-psbH-petB-petD* polycistronic messengers) on the *psbT-psbH* transcript processing. One of the processed *psbH* RNAs was generated by a site-specific RNA cleavage (governed by the relevant endoribonuclease) of *psbT-psbH* intergenic region duplexed with such asRNA. Plastid transcriptome from the mature leaves of barley (*Hordeum vulgare*) of the wild type and *albostrians* mutant was assayed by Zhelyazkova and coworkers (2012). Plastids in white leaves and white parts of the striped leaves in the *albostriant* line were ribosome deficient and lacked all plastid-encoded proteins. A number of promoters allowed synthesis of asRNAs to 40 and 45 genes in plastids from green and *albostrians* lines of barley, respectively. Overall, at least 60 diverse ncRNAs were predicted to be transcribed from their own promoters, with different abundance in the two lines tested and in different tissues. However, even more detailed results came with the recent study of Chen and coworkers (2014), who investigated the Chinese sage (*Salvia miltiorrhiza*) transcriptome. A large number of asRNAs (longer than 100 nt) to protein-coding genes and ncRNAs mapped to intergenic regions was revealed. Multiple ncRNAs were mapped to the same genes, including *atpB*, *ndhA*, *psaB*, *rpoC2*, *rrn16* and *ycf2*.

Numerous plastid sRNAs were also found. Using sRNA pyrosequencing, Morin *et al*. (2008) mapped them to *Pinus contorta* and rice plastid *ndh*, *rps*, *psb* and *pet* genes, and speculated about their role in processing of the plastid transcripts by specifying the endonuclease cleavage sites. Other RNAs (up to 36 nt) were mapped to Arabidopsis and Chinese cabbage (*Brassica rapa*) plastomes (intergenic regions, *rrn*, *trn* and protein genes) by Wang and coworkers (2011), who speculated that silencing-like mechanisms may be responsible for the regulation of abundance of the selected targets. Mohorianu and coworkers (2011) revealed a set of tomato sRNAs (21–24 nt) which were upregulated in mature fruits, when post-transcriptional control of the plastome expression is very important. They were mapped to the previously known regions of *rpo, rrn* and *trn* genes, as well as genes encoding the photosynthesis proteins. Later on, Ruwe & Schmitz-Linneweber (2012) mined deep-sequencing data of Rajagopalan and coworkers (2006) and found 50 diverse sRNAs (16–28 nt) of high abundance. Interestingly, plastid mRNA ends co-localized with the sRNA termini, which led to the conclusion that at least some sRNAs were remnants of the PPR protein target messengers. Few sRNAs were also mapped to the intergenic regions. Numerous plastid sRNAs (including putatively novel ones) were also found in moso bamboo (*Phyllostachy heterocycla*) leaves and roots by Xu and coworkers (2014). Interestingly, their distribution was strongly organ-spe-cific. In the leaf (but not root) library used for the deep-sequencing, almost 25% reads mapped to the plastome (mostly *rrn* and *trn* genes). 23% reads were mapped to the intergenic regions and only a portion to the protein genes. Some sRNA loci in particular were in close proximity to *atp*, *psa*, *psb*, *rpo* and *rps* genes. Also, Baev and coworkers (2014) analyzed 87 sRNA-producing transcripts in Arabidopsis plastids, especially the ones encoding various proteins related to photosynthesis.

RELEVANCE OF miRNA AND siRNA IN THE POSTTRANSCRIPTIONAL REGULATION OF THE EXPRESSION OF PLANT NUCLEAR GENES ENCODING ORGANELLAR PROTEINS

Mitochondrial and plastid proteins as important targets of miRNAs

In Supplementary Table 2a (at www.actabp.pl) the predicted organelle targets of Arabidopsis miRNAs from Plant Non-coding RNA Database (PNRD; Yi *et al*., 2015), further verified by psRNATarget (Dai & Zhao, 2011), are shown. A vast number of organellar proteins can be putatively targeted by at least 141 of 377 experimentally confirmed Arabidopsis miRNAs from the current PNRD release. This highlights the relevance of ncRNAs in regulation of the expression level of the nuclear-encoded mitochondrial and plastid proteins. In general, 290 miRNA-organelle target interactions were predicted *in silico*. Interestingly, plastid target interactions prevailed the mitochondrial ones (160 and 130 pairs, respectively).

Numerous messengers encoding plastid proteins participating in various metabolic routes were predicted to be regulated by miRNAs, including enzymes for sulfate, amino acid and carbohydrate metabolism, protein kinases, various transporters and translocases, zinc finger proteins, proteins involved in cytokinin and chlorophyll biogenesis, thylakoid membrane proteins and proteases, as main plastid targets (Suppl. Table 2a at www.actabp.pl). There was a bias between the functional distribution of the putative plastid targets *vs*. all (plastid and mitochondrial) targets. As far as the plastid targets are concerned, enrichment in the small molecule binding (30% *vs*. 21%) and nucleotide binding (20% *vs*. 29%) activities was observed.

On the other hand, predictions for targeted messengers encoding mitochondrial proteins were also high in number. Besides the PPR protein superfamily, they included proteins involved in the DNA replication machinery, RNA processing and editing factors, enzymes for nucleotide and amino acid metabolism, import proteins, Krebs cycle enzymes and, strikingly, some OXPHOS proteins (mostly CII and CIV subunits) and factors necessary for their assembly (Suppl. Table 2a at www.actabp.pl). Overall, transcripts for respiratory proteins accounted for about 7% of all non-redundant messengers related to mitochondrial biogenesis. Those results are in line with Sunkar & Zhu (2004) findings, who pointed out that nuclear transcripts encoding CIV subunits and organellar PPR proteins may be putatively targeted by certain miRNAs.

However among that relatively high number of miRNAs with potential organellar targets, still only a limited pool of ncRNAs was experimentally validated. According to the miRNA-target interactions database (miRTarBase; Chou *et al*., 2016), only 7 of the 33 deposited Arabidopsis miRNAs with experimentally verified targets, regulate the expression

level of 3 plastid and 6 mitochondrial proteins. This resulted in 18 experimentally verified miRNA-organellar target interactions (Suppl. Table 2b at www.actabp.pl). It seems that most of the predicted ncRNAs involved in organelle biogenesis have not been experimentally verified so far.

Using degradome sequencing, Addo-Quaye and coworkers (2008) were able to directly detect cleaved miRNA targets. This approach allowed for the confirmation of some predicted interactions, as well as to find new ones. For instance, plastid Cu/Zn superoxide dismutase isoform 2 (*CSD2*) mRNA was validated as the target of miR398a, previously investigated by Dugas & Bartel (2008). Bouché (2010) found that transcripts for a chaperone protein, essential for the activity of plastid CSD isoform, were novel targets of miR398. Analyses of German and coworkers (2008) allowed for the identification of targets (miR161 and miR400) for messengers encoding 4 mitochondrial PPR and TPR proteins. The authors had sequenced the 5' terminal signatures of polyadenylated products from miRNA-mediated mRNA decay in Arabidopsis inflorescences. Zhang and coworkers (2011) predicted the TPR protein to be a target for miR1888, and a peptide chain release factor 1 to be a target for miR408; expression level of both targets was significantly increased when Arabidopsis *HY5* gene encoding a basic transcription factor was mutated. In addition, mRNA for lipoic acid synthase family protein (putatively plastid-targeted) was found as a novel target for miR837-3p (Addo-Quaye *et al*., 2008).

Other interesting organellar targets (not listed in Suppl Table 2b at www.actabp.pl) for selected miRNAs related to organellar functions were also found in recent years. They include, for instance, new plastid targets (TPR protein, LHCB1.5 subunit of PSII, as well as OXA1 transporter of thylakoid membrane) for three diverse miRNAs of broccoli (*Brassica oleracea* var. *italica*; Chen *et al*., 2015). Shuai and coworkers (2013), using degradome sequencing strategy, found multiple organellar targets for miRNAs of *Populus trichocarpa*. They included the PSII, PSI and PPR proteins, plastocyanin, plastid ascorbate peroxidase, *CSD2* dismutase, as well as the mitochondrial transcription termination factor (mTERF). This finding was important owing to the differential expression of some miRNAs between the two genotypes, varying with sensitivity to the heat treatment. In some cases, the interplay between the level of miRNA and the respective messengers encoding organellar proteins is not direct, for instance when miRNA regulates the level of transcription factors (Wei *et al*., 2015). For instance, miRNA167d in rice regulates the level of an mRNA encoding transcriptional activator responsible for the regulation of root elongation. The downregulation of this factor resulted in alterations of two proteins essential for organellar Fe metabolism (Qi *et al*., 2012). Wei and coworkers (2015) also proposed *CCME* transcripts, encoding a specific heme chaperone located in mitochondria, to be targeted by a novel miRNA-180.

Certain miRNAs were also proposed to be associated with in the cytoplasmic male sterility (CMS) origins, which depends on the large-scale gene rearrangements in the mitogenome resulting in aberrations in the mitochondrial- nuclear signalization. Ding and coworkers (2016) analyzed miRNA targets by degradome sequencing in soybean (*Glycine max*) CMS and maintainer fertile lines. These include various mitochondrial proteins, for instance isocitrate dehydrogenase and the nuclear-encoded 24 kDa subunit of CI (each of them targeted by two diverse miRNAs). In addition, dihydrolipoyl dehydrogenase, a crucial part of pyruvate dehydrogenase (PDH) complex, as well as subunit β (ATP2) of mitochondrial ATP synthase and mTERF family protein were targeted by other miRNAs. Since organellar PPR-like proteins were involved in floral and pollen development, all of these results comply with the general view on the regulation of fertility restoration in plant CMS lines by such proteins (Jiang *et al*., 2014; Ding *et al*., 2016). Recently, by degradome sequencing, Wei and coworkers (2015) identified novel miRNAs specifically expressed in the CMS *Ogura* type of Chinese cabbage (*B. rapa* ssp. *pekinensis*) buds, as well as their targets. Interestingly, transcripts encoding some PPR proteins were also targeted by several miRNAs (miR158, miR161, miR400, miR5654) and tasi-RNAs in male gametophyte. This highlights the relevance of crucial alterations in organelle biogenesis and the transmission of plastids and mitochondria at this developmental stage in the CMS context as well (Grant-Downton *et al*., 2009; Jiang *et al*., 2014; Wei *et al*., 2015). It should be underlined that PPR proteins are often the expression products of fertility restorer genes (Desloire *et al*., 2003). Yang and coworkers (2013) identified transcripts for plastid ATP sulfurylase isoform 1 (APS1), PSI subunit I and the mitochondrial Lon protease isoform 2 as targets of miR395, PC-5p-56 and PC-5p-74, respectively. Overall, miRNAs may regulate the phenotypical expression of CMS by fine-tuning the gene expression in sterile plant lines; in turn, CMS influences the miRNA biogenesis.

Endogenous siRNAs in the regulation of organelle biogenesis

Few papers have reported on regulation of organelle biogenesis by siRNAs. In the initial study of Borsani and coworkers (2005), endogenous siRNAs generated from a pair of *cis*-NATs were described, including a messenger encoding protein involved in the Pro catabolism in plant mitochondria (also see section 4.6). Furthermore, it appeared that the transcripts for functional organellar proteins are overrepresented among Arabidopsis *cis*-NATs. Almost 20% and over 25% of *cis*-NATs belong to the mitochondrial and plastid ones, respectively (Jin *et al*., 2008).

Numerous organellar targets derived from *trans*-acting small (*TAS*) messengers, which produce very abundant secondary siRNAs, including ta-siRNAs, were characterized by Addo-Quaye and coworkers (2008). They had shown ta-siRNA interactions with transcripts encoding 7 mitochondrial PPR proteins. Notably, *TAS* transcripts also targeted PPR protein mRNAs, cleaved by miR173 to the heterogeneous population of phased ta-siRNAs. They were RT-PCR detectable in mature Arabidopsis pollen, contrary to *TAS* transcripts (Grant-Downton *et al*., 2009). Interestingly, this study was the first indication of ta-siRNAs participation in organelle biogenesis in the pollen; however, the precise functions of those ncRNAs remain elusive.

Recently, Yuan and coworkers (2015) in their exhaustive approach analyzed 4080 *cis*- and 2491 *trans*-NATs pairs in Arabidopsis. Interestingly, protein non-coding genes were in majority among *trans*-NATs, whereas protein-coding genes predominated for *cis*-NATs. In addition, a bias for siRNA length derived from *cis*-NATs (21 nt), as well as *trans*-NATs (24 nt), was noted. Among NATs generated from the protein-coding messengers (giving 1280 nat-siRNAs in total) studied by Yuan and coworkers (2015), some transcripts encoded for plastid proteins, which participated in terpene synthesis and fatty acid metabolism. Messengers encoding mitochondrial proteins (F-box protein, SAUR-like auxin-responsive protein family, PPR superfamily member, β-galactosidase related pro-

tein, as well as some uncharacterized proteins) were also involved in the NATs biogenesis. In general, mRNAs for mitochondrial proteins were present in 361 nat-siRNAs, and the ones for plastid proteins only among 45 nat-siRNAs, which gives about 28.2 and 3.5% of all nat-siRNAs from protein-coding transcripts, respectively.

ncRNAs IN ORGANELLAR STRESS RESPONSE

Alterations in plant organellar genomes, transcriptomes, proteomes and metabolomes in response to adverse conditions have been elucidated in various aspects in the past several years (Taylor *et al*., 2009; Jacoby *et al*., 2012; Rurek, 2014). The current data also broadens our view on the participation of various ncRNAs, including intraorganellar ncRNAs, nuclear-encoded miRNAs and siRNAs in the relevant organellar responses (Matsui *et al*., 2013; Zhang *et al*., 2013).

The evidence on miRNAs' role in the plant stress response came from the discovery that certain miRNAs participate in targeting of messengers for stress responsive genes encoding organellar proteins and from the fact that the abundancies of the miRNAs active in plant growth and development were often affected under stress (Sunkar *et al*., 2012). Based on microarray analyses, Gläßer and coworkers (2014) postulated recently that some Arabidopsis miRNAs were generally associated with certain routes of plastid- nucleus retrograde signaling, altered under stress conditions. Some reports on alterations within organellar genome-wide sRNA profiles in adverse treatments are also known. For instance, Baev and coworkers (2014) showed that heat stress (contrary to cold treatment) resulted in alterations in the biogenesis of sRNAs derived from mitochondrial and plastid transcripts (Fig. 1 left). Under the mentioned conditions, numerous Arabidopsis mitochondrial genes, including *nad* genes or genes encoding proteins related to cyt. *c* maturation, were upregulated; fewer plastome loci were affected by both treatments.

On Fig. 1 and discussed below, detailed data on the participation of various miRNAs in plant organellar stress responses is presented.

Nutrient starvation

MiRNAs participate in the starvation response in which SnRK1 protein kinase is activated and various miRNAs cooperate in major transcriptional reprogramming. Organelle functions are postulated to be co-regulated by SnRK1 and miRNAs, where the latter ones are components of a signaling cascade regulating the level of specific targets (Confraria *et al*., 2013).

One of the best examples of participation of miRNAs in the response to nutrient starvation is accumulation of several miRNAs under Cu deprivation conditions (Fig. 1 central panel). This element is critical for basic bioenergetic functions of plastids and mitochondria, like photosynthesis and respiration, respectively. The levels of Arabidopsis miR397, miR408 and miR457 (higher at decreased Cu amounts) negatively correlate with the accumulation of nonessential Cu proteins (plantacyanin and laccase; Abdel-Ghany & Pilon, 2008). In Arabidopsis and *Physcomitrella patens,* Cu regulates the level of miR398 (often by miR398 decrease) and miR1073, respectively; the above mentioned miRNAs negatively control the accumulation of the plastid isoform of Cu/Zn superoxide dismutase (CSD) by cleavage of the target mRNA (Yamasaki *et al*., 2007; Higashi *et al*., 2013). Moreover, miR398 is also responsible for the translational repression of the isoform 2 of CSD (CSD2), in which both AGO1 and AGO10 participate (Brodersen *et al*., 2008).

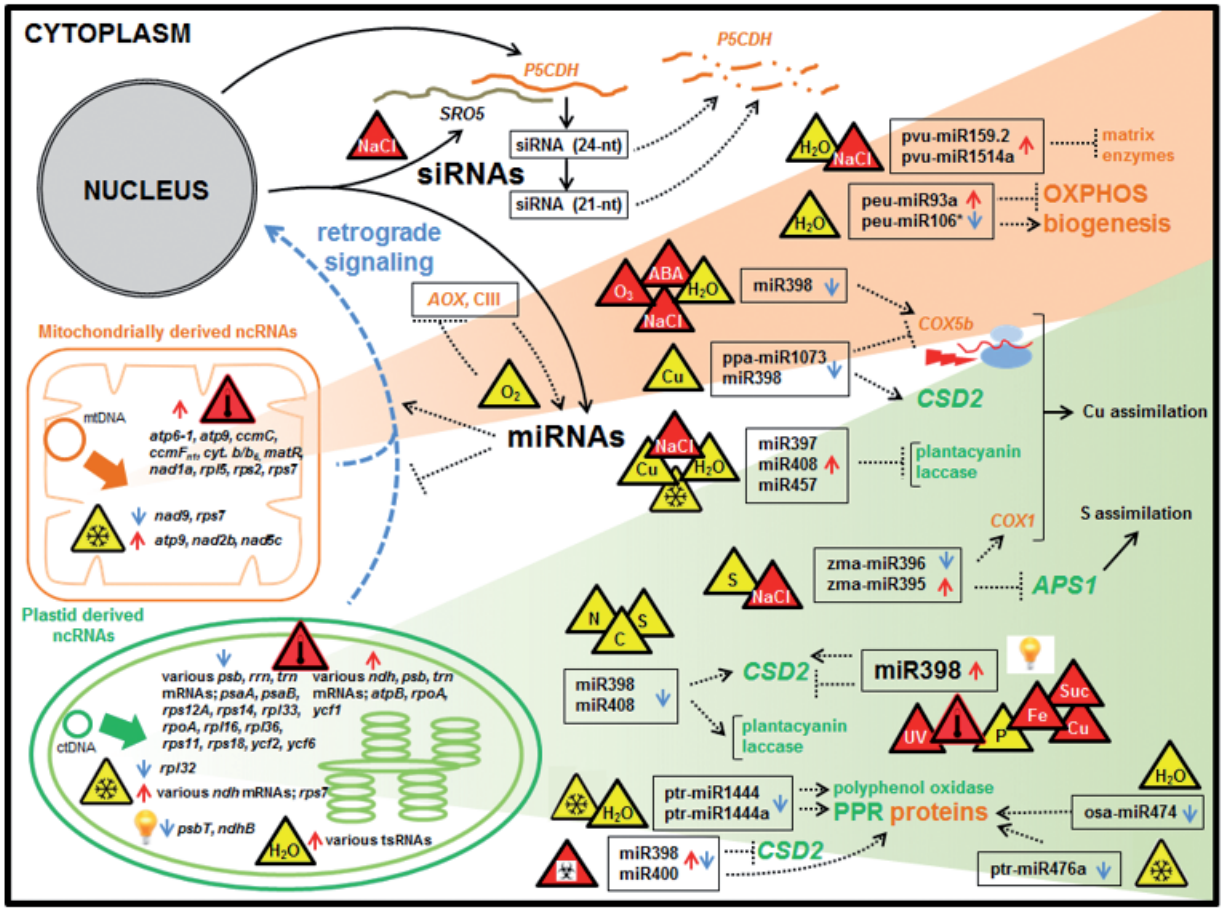
Another role for miRNAs in controlling levels of organellar proteins under nutrient deficiency is control of the level of *APS1* transcripts by miR395 (Fig. 1 right). Since most of the sulfate is assimilated into Cys by plastid APSs, miR395 is preferentially involved in sulfate anabolism in plastids and it is involved in the relevant nutrient stress response because miR395 induction accompanies decreased accumulation of *APS1* messengers (Jones-Rhoades & Bartel, 2004; Chiou, 2007). Other examples of miRNAs involved in the nutrient limitation responses include induction of the above mentioned miR398 by P starvation in tomato leaves (Gu *et al*., 2010). Under N deficiency, miR398a/b showed upregulation in shoots and roots of maize (*Zea mays*). Also, sucrose positively regulates the miR398 level in Arabidopsis and cooperates with the Cu availability in this process (Dugas & Bartel, 2008). However, under C, N and S starvation of Arabidopsis plants grown on MS media, the general downregulation of miR398 and miR408 accompanied an inverse response of target mRNAs; in addition, miR395 responded in a more complex way (Liang *et al*., 2015). Such examples highlight diverse trends of a given miRNA regulation by similar nutrient deficiencies in various plant species (Fig. 1 bottom; Xu *et al*., 2011).

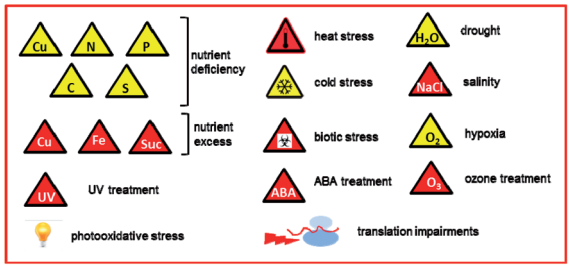
Drought, salinity and temperature treatments

Various ncRNAs play a significant role in responses to water deficit which may result in severe crop production losses. Hackenberg and coworkers (2015) characterized a number of sRNAs derived from plastid tRNA genes (especially *trnH*), that were upregulated under drought in barley; however, several of them were postulated to be nucleus-originated and further imported to plastids.

One of the highly induced miRNAs during drought, as well as under a cold stress, was miR408 (Liu *et al*., 2008) important for Cu protein biogenesis (see section 4.1). In *Populus trichocarpa,* drought may result in the increase of the Cu concentration in the cytoplasm, and therefore the downregulation of miR408, miR1444 and miR1444a (targeting messengers for plantacyanin, plastid polyphenol oxidase and organellar PPR proteins, respectively) allows for Cu utilization by upregulated Cu proteins (Fig. 1 bottom; Lu *et al*., 2008; Shuai *et al*., 2013). Furthermore, Arabidopsis miR408 level and its interplay with plantacyanin mRNA accumulation was investigated by Ma and coworkers (2015) under salinity, cold and oxidative stresses. The authors had pointed out the improved tolerance to the above mentioned adverse conditions at high miR408 levels. On the contrary, elevated miR408 accumulation accompanied enhanced plant sensitivity to drought and osmotic stress. Under miR408 overexpression, increased abundance of *CSD2* messengers was observed. Moreover, miR398a/b and miR408 levels increased in *Medicago truncatula* during drought, which was accompanied by the downregulation of their respective targets (Trindade *et al*., 2010).

Lu and coworkers (2008) had shown that *P. trichocarpa* miR476a level (putatively targeting messengers for organellar PPR proteins) decreased under prolonged cold treatment (Fig. 1 bottom right). Interestingly, miR476 together with miR408 were shown to increase in abundance in the xylem developing under a compression stress in the same poplar species (Lu *et al*., 2005). Another miRNA-miR474, targeting mRNAs for organellar PPR proteins (similarly to miR476a), appeared to be one of the most conserved miRNAs of rice that were down-





Legend to the figure symbols

Figure 1. Participation of ncRNAs in organelle biogenesis under stress.
The upregulations (red arrows up) or downregulations (blue arrows down) of the most relevant miRNAs for plastid (green area) and mitochondrial (brown area) biogenesis under adverse conditions are presented. Pointed arrows and arrows ended with perpendicular lines denote stimulatory or inhibitory effects, respectively, on the relevant biological processes, including accumulation of targeted messengers encoding various organellar proteins (mitochondrial – in brown, plastid – in green). Dual effects of miRNAs on retrograde communication are depicted (middle, on the left). Also, note the general miRNA-dependent regulations in abundance of mRNAs encoding subunits of OXPHOS complexes, PPR proteins and enzymes involved in Cu/S uptake and assimilation. In addition, their participation in oxygen sensing resulting in the altered alternative oxidase and CIII subunit expression is depicted (middle). Under increased salinity, biogenesis of small interfering RNAs (siRNAs) provides regulation of enzymes involved in proline catabolism (top). For the clarity, Arabidopsis miRNAs are not provided with species indication. **Figure abbreviations**: ABA, abscisic acid; AOX, mitochondrial alternative oxidase; APS, ATP sulfurylase; atp, ATP synthase; CIII, mitochondrial complex III; COX, cyt. c oxidase; ccm, cyt. c maturation; CSD, Cu/Zn superoxide dismutase; cyt., cytochrome; mat, maturase; nad, mitochondrial NADH dehydrogenase (CI); ndh, chloroplast NAD(P)H dehydrogenase (chlororespirating); osa, *Oryza sativa*; OXPHOS, oxidative phosphorylation; P5CDH, Δ-pyrroline-5-carboxylate dehydrogenase; peu, *Populus euphratica*; ppa, *Physcomitrella patens*; PPR, pentatricopeptide; *psa*, photosystem I; *psb*, photosystem II; ptr, *Populus trichocarpa*; pvu-*Phaseolus vulgaris*; *rbc*, Rubisco; *rpl*, ribosomal large subunit; *rpo*, RNA polymerase; *rps*, ribosomal small subunit; *rrn*, rRNA; Suc, sucrose; *SRO5*, similar to RCD one 5; *trn*, tRNA; tsRNAs, tRNA-derived small RNAs; *ycf*, hypothetical chloroplast open reading frame; zma, *Zea mays*.

regulated during drought (Zhou *et al.*, 2010). Recently, Li *et al.* (2011), had analyzed new drought-responsive miRNAs of *Populus euphratica,* also including the ones that targeted messengers indispensable for biogenesis of CI and CIV (Fig. 1 top).

Accumulation of two novel miRNAs of common bean (*Phaseolus vulgaris*): miR159.2 and 1514a, increased under salinity and drought stresses (Fig. 1 top right). They were proposed to be involved in the regulation of the level of important mitochondrial enzymes, including

PDH subunit and Mn superoxide dismutase (Arenas-Huertero *et al.*, 2009). Under salt stress, diverse accumulation of miRNA395 and miR396 (targeting mRNAs for *APS*, NADP-dependent malic enzyme and for *COX1*, respectively) was also assayed in two maize (*Zea mays*) lines which varied in salt tolerance. Therefore, the above mentioned regulations play an important part in the control of energy metabolism (Ding *et al.*, 2009).

Hypoxia

Some miRNAs and tasi-RNAs also play a role in plant development and gene regulation under low oxygen availability. As hypoxia often accompanies water submergence, investigation of participation of miRNAs (e.g. miR474) in response to the latter condition is also important (Zhang *et al.*, 2008). Notably, hypoxia- responsive sRNAs target mostly mitochondrial-predicted PPR proteins of the P subfamily. The inhibition of mitochondrial CIII and the alternative oxidase activity leading to the mitochondrial dysfunctions increased the abundance of several miRNAs and tasiR289 (Fig. 1 in the centre). Such effects were due to the oxidative stress, retrograde regulation involving Ca signaling and the fine tuning of organellar-nuclear dialogue (Moldovan *et al.*, 2010). Overall, mitochondrial respiration seems to be regulated by sRNAs under hypoxia, however, further experiments are necessary in order to validate this thrilling possibility.

Biotic stress

Transgenic Arabidopsis plants overexpressing miR400 (which regulates the messenger level of two mitochondrial PPR proteins) were particularly sensitive to *Pseudomonas syringae* and *Botrytis cinerea* infections (Park *et al.*, 2014). Such sensitivity was also displayed by the respective *ppr* mutant plants, accumulating high levels of H_2O_2. Notably, overexpression of the same miRNA and downregulation of one of the discussed PPR protein mRNAs enhanced heat stress sensitivity in Arabidopsis plants (Yan *et al.*, 2012). Biotic stress (infiltration with *P. syringae* avirulent strain) also decreased miR398 accumulation and curiously the *CSD2* mRNA level (Fig. 1 bottom centre; Jagadeeswaran *et al.*, 2009). However, Chen *et al.* (2012) pointed out that some infections by the *Dothiorella gregaria* fungus increased the level of this miRNA by as much as 3 folds in the poplar hybrids (*Populus cathayana* x *Populus nigravaritalica*), despite its relatively low abundance.

Participation of miR398 in multiple stress responses

Overall stress response of the conserved miRNAs may be distinct in various plant species. Very often, it is also habitat and developmental stage-dependent. Currently, it is obvious that miR398 plays a significant role under a plethora of diverse stress conditions (Fig. 1 bottom right). Jia and coworkers (2009) had reported various changes in the accumulation of miR398 under abscisic acid treatment and salinity in *Populus tremula* and Arabidopsis. For instance, the level of poplar miR398 increased under a short salt stress; however, longer exposure to salinity resulted in the appearance of diverse variations in miR398 abundance. In poplar, accumulation of *CSD2* transcripts was negatively correlated with the miR398 level.

Sunkar and coworkers (2006) had investigated impact of other stress conditions, including oxidative stress (resulting from high illumination), as well as excess of Fe and Cu, on the interplay between miR398 and its target accumulation. Transcription of *MIR398* gene under stress conditions is often suppressed, which results in the decrease of miR398-directed cleavage of *CSD2* messengers. Contrary to that, miR398 was induced by drought and salinity in switchgrass (*Panicum virgatum*; Xie *et al.*, 2014). Also Jagadeeswaran *et al.* (2009) had shown that the regulation of miR398 pool in Arabidopsis plants under elevated salt concentration is far more complex. Though ozone and salinity lowered the miR398 accumulation, the level of *CSD2* mRNAs also remained low under those conditions. Apart from *CSD2*, the other organellar target for Arabidopsis miR398 – mRNA encoding subunit of mitochondrial CIV (*COX5b*) appeared not to be drought-responsive in pea (*Pisum sativum*), despite the miRNA decrease (Jones-Rhoades & Bartel, 2004; Sunkar & Zhu, 2004; Yamasaki *et al.*, 2011; Jovanović *et al.*, 2014).

The extent of reciprocal regulation of miR398 and *CSD2* mRNA levels varies even in diverse Arabidopsis cultivars from cold and continental habitats (Juszczak & Baier, 2012). Besides this, miR398 is involved in the regulation of Arabidopsis heat tolerance and also responds to UV-B radiation (Zhou *et al.*, 2007); it is rapidly induced by heat stress and decreases the abundance of *CSD2* messengers. Conversely, transgenic plants expressing miR398-resistant form of *CSD2* were heat sensitive (Guan *et al.*, 2013; Lu *et al.*, 2013).

siRNAs in organellar stress response

Participation of plant siRNAs in organellar stress response was investigated initially by Borsani *et al.* (2005). They had characterized endogenous siRNAs which accumulate under salinity stress in Arabidopsis. Salt induces expression of the nuclear *SRO5* gene, which overlaps with *P5CDH* gene encoding mitochondrial Δ-pyrroline-5-carboxylate dehydrogenase. Pairing of both messengers (leading to dsRNA formation) is followed by the appearance of a 24-nt-long siRNA detectable specifically under salt stress (its biogenesis is controlled by DCL2, RDR6, SGS3 and NRPD1A protein factors). This siRNA cleaves an already present mRNA for *P5CDH* and it is further processed to shorter siRNA (21-nt) by DCL1. As a result, the degradation of *P5CDH* messengers occurs, which results in enzyme downregulation and intracellular accumulation of free Pro necessary for the acquiring of salt tolerance (Fig. 1 top and central panel). In this case, siRNAs generated from duplexes containing messengers for mitochondrial proteins become important components of the stress response regulatory loop in controlling reactive oxygen species production.

Yu and coworkers (2013) had analyzed heat responsive NAT-siRNAs in *B. rapa*, which may be useful for genetic improvement of the crop thermotolerance. Some NAT transcripts necessary for the biogenesis of the above mentioned siRNAs, are derived from the genes encoding important photosynthetic proteins and subunits of the protein complex involved in chlororespiration. Last, but not least, intraorganellar plastid asRNAs, controlling the level of *psbT* sense messengers, were decreased in abundance under high-light stress (Zghidi-Abouzid *et al.*, 2011). Those authors proposed additional function for asRNA – the protection of *psbT* mRNAs from degradation under stress conditions. Recently, Kotakis and coworkers (2015) proposed a global model for the regulation of the level of plant ncRNAs (including organellar ones) by light intensity; in turn, ncRNAs control the abundance of important organellar enzymes (for instance, ATP synthases) and the expression level of redox-responsive genes.

FURTHER PERSPECTIVES AND CONCLUSIONS

One of the most exciting findings regarding ncRNAs' interactions with organellar compartments was the concept of intramitochondrial localization of miRNAs in a number of animal and human cell lines/tissues which can remodel protein complexes and result in mitochondrial dysfunctions. The obtained results were strengthened by RNAse digestion assays in order to remove cytosolic contaminants and miRNA attached to the outer mitochondrial membrane (Das *et al*., 2012; Leung, 2015). In addition, by immunoassays and confocal microscopy, only selected components (AGO2) of miRNA biogenesis machinery were shown to be present inside mitochondria; their abundance varied in the course of muscle differentiation. However, some contradictory results were also reported and it was rather accepted that the putative miRNAs within animal/human mitochondria seem to be rather nuclear-encoded and processed in the cytoplasm before organellar import (Das *et al*., 2012; Ro *et al*., 2013). Also interactions of miRNAs with some mitochondrial transcripts, including *COX* messengers, were experimentally confirmed for animal models. Intraorganellar miRNAs may also increase mitochondrial translation, instead of their expected repressive role (Bandiera *et al*., 2011; Das *et al*., 2012; Zhang *et al*., 2014). How those phenomena could be present in plant mitochondria is still a matter of debate.

As stated above, certain mitochondrial messengers are often predicted targets of several plant miRNAs (Suppl. Table 2a at www.actabp.pl). Therefore, it may be interesting to investigate up to which extent plant miRNAs could target mitochondrial mRNAs encoding OXPHOS proteins (e.g. *nad* transcripts). In the future, more nuclear messengers for mitochondrial and plastid proteins should be also experimentally validated as novel targets of miRNA (Suppl. Table 2b at www.actabp.pl). It should be stressed that the pool of ncRNAs encoded by plant organellar genomes is still far from full assessment, despite application of high-throughput methodologies. It was proposed recently, that the animal mitogenomes also encode dozens of small ncRNAs, often derived from the sense transcripts of mitochondrial genes and generated by uncharacterized RNAses and not by DICER-dependent route (Ro *et al*., 2013).

Due to the limited reports, it is tempting to further determine to what extent ncRNAs can influence organellar biogenesis under selected stress conditions, including cold and heat (Liu *et al*., 2008; Lu *et al*. 2008; Ma *et al*., 2015). Moreover, participation of the stress recovery phase in such processes is largely unexplored. The next important question is: could many organellar activities involving PPRs and other RNA processing factors be really altered under the impact of stress action and recovery in plant cells? The discussion of the relevant physiological consequences of such variations seems to be also crucial.

Finally, the real potential for genetic engineering of plant ncRNAs in order to improve plastid and mitochondrial biogenesis under stress conditions among crop species remains to be further explored. Gómez & Pallás (2010a, b) had shown that a ncRNA with defined secondary structure is able to mediate functional foreign mRNA targeting into plastids. Mitochondrial biogenesis and metabolism can be also influenced by the expression of artificial miRNAs against mRNAs encoding important mitochondrial enzymes (Haas *et al*., 2008; Stoll *et al*., 2014) or chimeric ribozymes for directed knockdown of mtRNA, leading to a genetic manipulation of plant mitochondria (Val *et al*., 2011; Mileshina *et al*., 2015). How such transplastomic/transmitochondrial manipulations could affect plant organellar stress response under the field conditions? How relevant the practical result of those attempts would be? Clearly, many aspects of the fascinating and mysterious interplay between diverse ncRNAs and plant organelles still remain elusive and hopefully they will be broadly characterized in forthcoming years.

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