Epigenetic regulation of sulphur homeostasis in plants

Xin-Yuan Huang[1*], Mengzhen Li[1], Rongjian Luo[1], Fang-Jie Zhao[1], David E Salt[2]

[1] State Key Laboratory of Crop Genetics and Germplasm Enhancement, College of Resources and Environmental Sciences, Nanjing Agricultural University, Nanjing 210095, China

[2] Future Food Beacon of Excellence and the School of Biosciences, University of Nottingham, Sutton Bonington Campus, Loughborough, Leicestershire LE12 5RD, UK

Highlight statement: We summarize and discuss recent findings on the epigenetic regulation of sulphur homeostasis and response to sulphur deficiency in plants, including DNA methylation, histone modifications and noncoding RNA mediated gene silencing.

Author emails:

Xin-Yuan Huang: xinyuan.huang@njau.edu.cn

Mengzhen Li: 1335593013@qq.com

Rongjian Luo: 915497646@qq.com

Fang-Jie Zhao: fangjie.zhao@njau.edu.cn

David E Salt: david.salt@nottingham.ac.uk

***Correspondence author**:

Xin-Yuan Huang, email: xinyuan.huang@njau.edu.cn; Tel: +86(0)25 84399562

Abstract

Plants have evolved sophisticated mechanisms for adaptation to fluctuating availability of nutrients in soil. Such mechanisms are of importance for plants to maintain homeostasis of nutrient elements for their development and growth. The molecular mechanisms controlling the homeostasis of nutrient elements at the genetic level have been gradually revealed, including the identification of regulatory factors and transporters responding to nutrient stresses. Recent studies have suggested that such responses are not only controlled by genetic regulation but also by epigenetic regulation. In this review, we present recent studies on the involvement of DNA methylation, histone modifications and noncoding RNA mediated gene silencing in the regulation of sulphur homeostasis and response to sulphur deficiency. We also discuss the potential effect of sulphur containing metabolites such as *S*-adenosylmethionine (SAM) on the maintenance of DNA and histone methylation.

Keywords: sulphur, epigenetics, DNA methylation, histone modifications, non-coding RNAs, miRNAs, *S*-adenosylmethionine

Introduction

As one of the essential macronutrients, sulphur (S) plays a pivotal role in plant growth and development. Plants take up S from the rhizosphere in the form of inorganic sulphate. In *Arabidopsis thaliana* (Arabidopsis), this process is mainly driven by two root-specific high-affinity sulphate transporters, SULTR1;1 and SULTR1;2 (Rouached *et al.*, 2008; Takahashi *et al.*, 2000; Yoshimoto *et al.*, 2002). After being transported into root cells, sulphate is either transported into the plastids by SULTR3 sulphate transporters (Cao *et al*., 2013; Chen *et al*., 2019), where it is assimilated into organic sulphur compounds, or transported into the vacuoles for storage. In the sulphur assimilation pathway, sulphate is first activated by ATP sulfurylase (ATPS) to adenosine 5′-phosphosulfate (APS), which is either reduced to sulphite in the primary assimilation pathway or phosphorylated to form 3′-phosphoadenosine 5′-phosphosulfate (PAPS) (Takahashi *et al.*, 2011). These two reactions are catalyzed by APS reductase (APR) and APS kinase (APK), respectively. PAPS provides an activated sulphate for many sulfation reactions, while sulphite is further reduced to sulphide by sulphite reductase (SiR). Sulphide is condensed with *O*-acetylserine (OAS) by *O*-acetylserine (thiol) lyase (OAS-TL) to form the S-containing amino acid cysteine (Cys). Cys can be used directly for protein biosynthesis or serves as a precursor for the biosynthesis of methionine (Met) and glutathione (GSH). These two molecules can be used for biosynthesis of many sulphur containing derivatives such as glucosinolates and phytochelatins, which are important for plants to alleviate biotic stress and detoxify heavy metals, respectively (Cobbett, 2000; Halkier and Gershenzon, 2006).

The transporters responsible for sulphate uptake, and enzymes involved in the S assimilation pathway have been well-characterized (Leustek *et al.*, 2000; Takahashi *et al.*, 2011). The regulation of S homeostasis at the genetic level in plants has also been gradually revealed. Such regulation includes the modulation of sulphate acquisition and distribution, S assimilation and the biosynthesis and recycling of sulphur containing compounds at both the transcriptional and posttranscriptional levels. In term of the regulation of sulphate uptake and distribution, several *trans*-acting factors and *cis*-elements have been identified. The most important regulatory factor identified so far is the transcription factor SLIM1 (SULFUR LIMITATION 1). SLIM1 regulates the

expression of *SULTR1;1* and *SULTR1;2* to activate sulphate uptake in roots, and *SULTR4;2* to release sulphate from vacuoles (Maruyama-Nakashita *et al.*, 2006). Several *cis*-elements responsive to S deficiency have been identified, including the sulphur-responsive element (SURE) in the promoter of *SULTR1;1* (Maruyama-Nakashita *et al.*, 2005), a SURE-like element in the promoter of the wheat *Sulfur deficiency-induced-1* (*sdi-1*) gene (Howarth *et al.*, 2009), the UPE-box in tobacco *UP9C* gene (Wawrzynska *et al.*, 2010), and SURE21A and SURE21B in the 3'-untranslated region of *SULTR2;1* (Maruyama-Nakashita *et al.*, 2015). It appears that SLIM1 does not target directly to the SURE element in the promoter of *SULTR1;1* and *SULTR1;2* though it regulates the expression of these two gens. Rather, SLIM1 forms a homodimer and binds to the UPE-box, which also exists in the promoters of sulphur deficiency induced genes in Arabidopsis, such *LSU*, *APR* and *SULTR2;1* (Wawrzynska *et al.*, 2010; Wawrzynska and Sirko, 2016).

Similar to the complex regulation of sulphate uptake and distribution, sulphate assimilation is also tightly controlled, being highly regulated by the demand for reduced sulphur, in a regulatory system known as the 'demand-driven' regulatory pathway (Davidian and Kopriva, 2010; Lappartient and Touraine, 1996; Lappartient *et al.*, 1999). However, the molecular mechanisms underlying the regulation of sulphate assimilation remain largely unclear. SLIM1 is likely involved in regulating the expression of *ATPS4* and *SERAT3;1* as these two genes are downregulated in the *slim1* mutant (Maruyama-Nakashita *et al.*, 2006). The transcriptional factor LONG HYPOCOTYL5 (HY5) has been shown to regulate the expression of *APR1* and *APR2* in Arabidopsis by directly targeting the promoters of these two genes (Lee *et al.*, 2011). However, HY5 seems to not regulate the expression of *APR3*, suggesting multiple genetic pathways for the regulation of the reduction of APS. The regulation of the biosynthesis of sulphur containing secondary metabolites such as glucosinolates is much more complex. Many transcription factors, including at least eight MYBs, six MYC-bHLHs, two WRKYs, and a DNA-binding-with-one-finger (DOF) transcription factor OBP2, have been shown to be involved in this process (Frerigmann, 2016). Recently, two repressors controlling glucosinolate biosynthesis, *sulfur deficiency induced 1* (*SDI1*) and *SDI2* have been identified in Arabidopsis (Aarabi *et al.*, 2016). Under sulphur limited conditions the

nuclear localized SDI1 interacts with MYB28, a major transcription factor that promotes glucosinolate biosynthesis, to suppress the biosynthesis of glucosinolates and prioritize sulphate utilisation for primary metabolites (Aarabi *et al.*, 2016). The catabolic recycling of organic S compounds such as glucosinolates and GSH is essential for plants to adapt to sulphur limiting conditions. Glucosinolates are thought to function as a sulphur storage pool in plants in the Brassicaceae as their levels fluctuate according to the environmental sulphur status (Falk *et al.*, 2007; Maruyama-Nakashita, 2017; Maruyama-Nakashita *et al.*, 2006). Although the catabolic enzymes of glucosinolates and GSH have been identified and well characterized (Bachhawat and Yadav, 2018; Kumar *et al.*, 2012; Kumar *et al.*, 2015; Ohkama-Ohtsu *et al.*, 2008; Paulose *et al.*, 2013; Wittstock and Burow, 2010), the genetic regulation of the breakdown of these compounds is largely unknown. Except SLIM1 which functions as a central transcriptional regulator in the degradation of glucosinolates under sulphur limited conditions (Maruyama-Nakashita *et al.*, 2006), other transcription factors and corresponding targeting *cis*-elements involved in the degradation of glucosinolates and GSH remain to be identified.

It is well recognized that the regulation of S homeostasis is under complex genetic control. Emerging evidence suggests that epigenetic regulation of gene expression plays an important role in the adaptive response to S deficiency and the maintenance of S homeostasis (Huang *et al.*, 2016). Epigenetic changes refer to heritable genetic changes resulting from modification of a chromosome without alteration of the DNA sequence (Berger *et al.*, 2009). Epigenetic regulation of gene expression in response to biotic and abiotic stresses, and adaptation to environmental cues, has been gradually revealed (Alonso *et al.*, 2019; Chinnusamy and Zhu, 2009; Lamke and Baurle, 2017; Sahu *et al.*, 2013; Secco *et al.*, 2017). Epigenetic regulation mainly occurs at three levels; DNA methylation, histone modifications, and noncoding RNA regulation. Perhaps the most direct link between S homeostasis and DNA and histone methylation is the fact that S-adenosylmethionine (SAM), a major methyl donor required for many transmethylation reactions, is a sulphur containing compound. In this review, we discuss what is currently known about the regulation of S homeostasis at these three epigenetic levels.

DNA methylation

DNA methylation is one of the most well studied epigenetic modifications, playing an important role in the regulation of gene expression, transposon silencing, and imprinting. DNA methylation generally refers to the transfer of a methyl group from SAM to the 5' position of cytosine to form 5-methylcytosine. In plants, DNA methylation occurs in three different sequence contexts CG, CHG and CHH (where H is A, C or T). A specific DNA methylation state in a given genomic region is determined by the dynamic regulation of *de novo* DNA methylation, maintenance of DNA methylation and DNA demethylation (Law and Jacobsen, 2010; Zhang *et al.*, 2018). In plants, *de novo* DNA methylation is mediated by the RNA-directed DNA methylation (RdDM) pathway, which requires DNA methyltransferase DOMAINS REARRANGED METHYLASE 2 (DRM2), and many other proteins. The maintenance of DNA methylation during DNA replication depends on the cytosine sequence context, and different DNA methyltransferases are involved. The methylation of symmetric CG is maintained by METHYLTRANSFERASE 1 (MET1), and CHG is maintained by DNA methyltransferase CHROMOMETHYLASE 2 (CMT2) and CMT3, whilst the asymmetric CHH is maintained by DRM2 at RdDM target regions or CMT2 at histone H1-containing heterochromatin. DNA demethylation can be divided into passive and active demethylation, with the former refering to the failure of maintenance of methylation during DNA replication. Such passive DNA demethylation can be due to the shortage of the methyl donor, or loss of function of DNA methyltransferase. Active DNA demethylation is mediated by a base excision repair pathway which requires different bifunctional 5-methylcytosine DNA glycosylases, including REPRESSOR OF SILENCING 1 (ROS1), TRANSCRIPTIONAL ACTIVATOR DEMETER (DME), DEMETER-LIKE PROTEIN 2 (DML2) and DML3. A detailed description of *de novo* DNA methylation, maintenance of methylation, and DNA demethylation, can be found in recent reviews (Law and Jacobsen, 2010; Zhang *et al.*, 2018).

Nutrient stresses, such as phosphate starvation (Secco *et al.*, 2015; Yong-Villalobos *et al.*, 2015) and zinc deficiency (Chen *et al.*, 2018), have been shown to change the global DNA methylation at the whole genome level. Using whole genome bisulphite sequencing (BS-Seq), changes in DNA methylation, at base level resolution throughout the genome,

have been revealed in rice (Secco *et al.*, 2015) and Arabidopsis (Yong-Villalobos *et al.*, 2015). Under phosphate starvation conditions widespread changes in DNA methylation were observed in the rice genome, and such changes mainly derive from the hypermethylation of transposable elements in the vicinity of phosphate starvation inducible genes (Secco *et al.*, 2015). Similarly, extensive remodelling of global DNA methylation also occurs in Arabidopsis plants, with some of this DNA methylation remodelling being correlated with changes in the expression of phosphate starvation inducible genes (Yong-Villalobos *et al.*, 2015). Although a limited number of changes in DNA methylation were reported in Arabidopsis under phosphate starvation (Secco *et al.*, 2015), this may be due to different treatment conditions and/or different approaches in the identification of differentially methylated regions (Secco *et al.*, 2017). Zinc deficiency also triggers genome-wide differential DNA methylation, with prominent changes in transposable elements (Chen *et al.*, 2018). Depletion of the macronutrient nitrogen alters locus-specific DNA methylation patterns, although the changes on genome-wide DNA methylation are currently unknown due to the limitation of the technique used (Kou *et al.*, 2011).

Sulphate deficiency is assumed to affect genome-wide DNA methylation in plants because the universal methyl donor for DNA methylation SAM is synthesized from Cys, the first organic sulphur compound in the primary sulphate assimilation pathway. The biosynthesis of SAM can be initiated by the condensation of Cys and *O*-phosphohomoserine (OPH) to form cystathionine (Cyst), which is further converted to homocysteine (Hcy) by cystathionine γ-synthase (CGS) and cystathionine β-lyase (CBL), respectively (Fig. 1) (Hesse and Hoefgen, 2003). Methionine synthase (MS) subsequently converts Hcy to Met using the methyl group from 5-methyltetrahydrofolate (5-CH3-THF), and ultimately Met is converted to SAM catalysed by SAM synthetase (SAMS). The biosynthesis of SAM is tightly controlled and the concentration of SAM is affected by the availability of sulphate. Under sulphate deficient condition, SAM concentration decreases (Nikiforova *et al.*, 2005). Recently, using BS-seq to investigate genome-wide changes in DNA methylation in response to sulphur deficiency, we observed that cytosine methylation levels in all three sequence contexts CG, CHG and CHH decreased in both roots and shoots under sulphate depletion conditions (Fig. 2A). This might be due to a

shortage of the methyl donor SAM which potentially lead to enhanced passive DNA demethylation (Zhang *et al.*, 2018). Interestingly, DNA methylation levels tend to increase under phosphate starvation (Fig. 2B) (Yong-Villalobos *et al.*, 2015), suggesting distinct mechanisms in the modulation of genome-wide DNA methylation under different nutrient stresses.

During DNA methylation, the methyl group of SAM is transferred to cytosine by a specific DNA methyltransferase, and results in the production of a molecule of *S*-adenosylhomocysteine (SAH). SAH is a strong inhibitor of all known SAM-dependent methyltransferases and is thus rapidly hydrolyzed into Hcy and adenosine by *S*-adenosylhomocysteine hydrolase (SAHH) (Hoffman *et al.*, 1979; Moffatt and Weretilnyk, 2001). This reaction is reversible, and the equilibrium is largely driven towards SAH hydrolysis by the rapid removal of Hcy and adenosine. The by-product adenosine is phosphorylated to adenosine monophosphate (AMP) by adenosine kinase (ADK) (Moffatt *et al.*, 2002). Hcy can be re-methylated to Met for biosynthesis of SAM to complete the SAM cycle (Fig. 1). The SAM cycle, as well as the SMM (*S*-methylmethionine) cycle, are two Met recycling systems essential for sustaining the high demand of Met for SAM-dependent transmethylation reactions and also for maintaining the optimized ratio of SAM to SAH (Sauter *et al.*, 2013). The SAM to SAH ratio is generally termed the 'methylation potential' and can be used as a metabolic indicator for the methylation status in cells. The alteration of the SAM to SAH ratio usually leads to changes in global methylation patterns. Partial loss-of-function of *SAHH1* (also known as *HOMOLOGY-DEPENDENT GENE SILENCING1*, *HOG1*) leads to increased SAH levels and a decreased SAM to SAH ratio resulting in DNA hypomethylation in Arabidopsis (Ouyang *et al.*, 2012; Rocha *et al.*, 2005). A subset of genes is up-regulated in the hypomethylated *hog1* mutant, which shows a dramatic growth defect (Jordan *et al.*, 2007; Rocha *et al.*, 2005). Reduction of ADK activity in Arabidopsis also increases SAH levels and reduces DNA methylation (Moffatt *et al.*, 2002). Both SAHH1 and ADK1 are targeted to the nucleus, and form a complex with a methyltransferase CMT (Lee *et al.*, 2012). Such a protein complex may facilitate the rapid removal of SAH and adenosine to avoid the inhibition of methyltransfereases by SAH.

The impairment of SAM biosynthesis itself could also lead to global DNA methylation changes. Mutation of *SAMS3* (also called *METHIONINE ADENOSYLTRANSFERASE 4*, *MAT4*) reduces whole-genome DNA methylation mostly in the CHG and CHH sequence contexts (Meng *et al.*, 2018). The null mutant of *SAMS3* is lethal, and the weak allele mutants accumulate extremely high levels of Met and SAH, and lower levels of SAM (Goto *et al.*, 2002; Meng *et al.*, 2018). Four isoforms of SAMS in Arabidopsis interact with each other and may form homo- and/or hetero-polymers to fulfill the biosynthesis of SAM (Meng *et al.*, 2018). A similar genome-wide DNA hypomethylation was also observed for the other three *SAMS* mutants in Arabidopsis (Meng *et al.*, 2018). Knockdown of three *SAMS* genes in rice by RNA interference reduces DNA methylation at several flowering related genes, and lead to a late-flowering phenotype (Li *et al.*, 2011). Although the effect of the Met and Hcy biosynthesis defect on DNA methylation is largely unexplored in plants, it is assumed that the perturbation of Met and Hcy levels may change SAM levels, and thus modulate the DNA methylation pattern. Indeed, increased plasma Hcy is associated with the elevation of plasma SAH levels, and results in DNA hypomethylation in human (Castro *et al.*, 2003; Yi *et al.*, 2000). This might be due to the fact that high levels of Hcy suppress the expression of *SAHH* and thus elevates the level of SAH (Jiang *et al.*, 2007a; Jiang *et al.*, 2007b), which inhibits the activity of most of the SAM-dependent methyltransferases (Hoffman *et al.*, 1979)., Such lines of evidences have suggested that interruption of the SAM cycle alters the genome-wide DNA methylation. However, it is still unclear how global DNA methylation is affected by sulphate assimilation or which step in the assimilation pathway plays the key roles in epigenetic regulation.

The one-carbon metabolism pathway plays an important role in epigenetic modifications including DNA methylation. This is because the one-carbon unit carrier 5-methyl tetrahydrofolate (5-CH_3-THF) provides the methyl group for the biosynthesis of Met (Fig. 1). 5-CH_3-THF is converted from 5,10-methylenetetrahydrofolate (5,10-CH_2-THF) by methylenetetrahydrofolate reductase (MTHFR) in a NADH-dependent manner (Roje *et al.*, 1999). Although the impact of MTHFR on epigenetic modifications is unclear, mutation of MTHFR in maize has been shown to reduce lignin levels which is likely due to a shortage of the methyl donor SAM (Tang *et al.*, 2014). In fact, defects in several

steps of folate biosynthesis or turnover have been shown to affect SAM levels and thus change genome-wide DNA methylation (Fig. 1). Suppression of folate biosynthesis by treatment with sulfamethazine, which is a structural analog and competitor of the folate synthesis precursor *p*-aminobenzoic acid (*p*ABA), decreases folate pool size and SAM level, and thus causes a reduction in DNA methylation (Zhang *et al.*, 2012). Inhibition of dihydrofolate reductase (DHFR), which catalyses the conversion of DHF to THF, by methotrexate also decreases the level of SAM, and is thought to lead to genome-wide DNA hypomethylation (Loizeau *et al.*, 2008). The interruption of folate turnover also changes the methylation potential, and alters global DNA methylation. Mutation in the cytoplasmic bifunctional methylenetetrahydrofolate dehydrogenase/methenyltetrahydrofolate cyclohydrolase (MTHFD1), which is required for the turnover of 5,10-CH_2-THF to THF, causes a strong genome-wide decrease in DNA methylation (Groth *et al.*, 2016). The *mthfd1* mutant accumulates a higher level of Hcy due to impaired folate metabolism, and the increased Hcy level leads to decreased SAHH activity and accumulation of SAH, which competitively inhibits SAM-dependent DNA methylation. Even though both SAM and SAH are increased, the stronger increase in SAH levels leads to an overall decrease in methylation potential, resulting in DNA hypomethylation. Folate polyglutamylation, which is carried out by folylpolyglutamate synthetase (FPGS), is essential for folate affinity, stability and subcellular compartmentation (Hanson and Gregory, 2011; Matherly and Goldman, 2003; Shane, 1989). Folate-dependent enzymes prefer polyglutamylated folates to the monoglutamyl form (Shane, 1989). Mutation of *FPGS1* in Arabidopsis dramatically reduces DNA methylation, and releases chromatin silencing at a genome-wide scale (Zhou *et al.*, 2013). Similar to the *mthfd1* mutant, the Hcy level also significantly increases in the *fpgs1* mutant, following elevation of the SAH level, and the reduction of the methylation potential.

We recently identified a high S Arabidopsis mutant and identified the casual gene as *MORE SULPHUR ACCUMULATION1* (*MSA1*) (Huang *et al.*, 2016). MSA1 was previously annotated as serine hydroxymethyltransferase 7 (SHM7). Although MSA1 is catalytically inactive *in vitro* and might require other co-factors to facilitate activity, SHM family proteins are believed to catalyse the reversible conversion of serine and THF

to glycine and 5,-10-CH2-THF (Schirch and Szebenyi, 2005). Mutation of *MSA1* leads to a reduction of cytosine methylation levels in roots and increased levels in shoots, which may be due to lower levels of SAM in roots but slightly increased SAM levels in shoots (Huang *et al.*, 2016). Interestingly, a large number of differentially methylated genes (DMGs) were found between the mutant and wild-type (Huang et al., 2016), even though the detailed mechanism underlying the opposite effect of *msa1* on genome-wide DNA methylation between roots and shoots is unclear. Several S-deficiency responsive genes and genes involved in glucosinolate and anthocyanin metabolisms are differentially methylated in *msa1*, including *SULTR1;1*, *SULTR1;2*, *APR3* and *ATPS4*. Methylation in the promoter region of a gene usually inhibits its expression (Zilberman *et al.*, 2007). In Huang et al., (2016) we found that a 258-bp genomic region 118-bp upstream of the sulphur responsive element (SURE) in the promoter of *SULTR1;1*, which is essential for the S deficiency response (Maruyama-Nakashita *et al.*, 2005), is hyper-methylated under S sufficient condition but is hypo-methylated under S deficiency (Huang *et al.*, 2016). This is correlated with the low expression level of *SULTR1;1* under S sufficient condition and its strong induction by S deficiency. In the *msa1-1* mutant, the upstream region of SURE in the promoter of *SULTR1;1* is hypo-methylated and is associated with the elevation of its expression level and the increase of S levels in shoots (Huang *et al.*, 2016). Similar hypo- and hypermethylations in the vicinity of *cis*-acting elements, such as MBS, P1BS and W-box, in the promoter of phosphate–responsive genes have also been shown to correlate with increased or decreased expression of phosphate responsive genes (Yong-Villalobos *et al.*, 2016). Therefore, dynamic DNA methylation particularly in the gene promoter region may represent an important mechanism in regulation of the expression of nutrient deficiency responsive genes.

Promoter DNA methylation could repress transcription in two ways (Domcke *et al.*, 2015). First, methylation in the promoter could inhibit the binding of transcriptional activators thus hindering the activation of gene expression. Second, DNA methylation in the promoter could present an epigenetic mark that recruits the binding of transcriptional repressors to the promoter, thus repressing gene expression. Therefore, for nutrient deficiency induced genes such as *SULTR1;1*, DNA methylation in the promoter would inhibit the binding of a transcriptional activator (Fig. 3A) or promote the binding of a

transcriptional repressor (Fig. 3B), thus keep gene expression at a low level under nutrient sufficient conditions. However, under nutrient deficient condition, the cytosines in the promoter would be demethylated, allowing binding of a transcriptional activator, or releases a transcriptional repressor, leading to the activation gene expression (Fig. 3).

Histone modifications

Histones are the protein components of nucleosomes and fundamental units of chromatin. Canonical histones include, histone 2A (H2A), H2B, H3 and H4. A typical nucleosome contains an octameric protein complex consisting of two of these four core histones which are wrapped with 147 base pairs of DNA (Kouzarides, 2007). Histone modifications refer to posttranslational covalent modifications on the amino-terminal tails of these core histones, including methylation, acetylation, phosphorylation, ubiquitination, and many other less investigated modifications (Bannister and Kouzarides, 2011; Kouzarides, 2007; Liu *et al.*, 2010). Such modifications are carried out by specific modifying enzymes ('the writers') to establish different histone marks, which can be recognized and translated by regulatory proteins (the readers/effectors) to trigger downstream signaling events. In certain cases, these histone marks can be removed by particular enzymes ('the erasers') (Liu *et al.*, 2010). Histone modifications alter the accessibility of DNA to the transcriptional machinery, and influence gene expression. In general, histone acetylation and phosphorylation are associated with transcriptional activation, whereas the effect of histone methylation on gene expression is more complicated (Berger, 2007). Histone methylation occurs on lysine and arginine residues at different amino acid positions of H3 and H4, in which lysine can undergo mono-, di- or tri-methylation while arginine may be mono-, or di-methylated symmetrically or asymmetrically. Among these diverse histone methylations, methylations on histone H3 lysine-4 (H3K4) and H3K36 are typically associated with active gene transcription, whereas methylation on H3K9 and H3K27 generally leads to gene repression (Bannister and Kouzarides, 2011; Liu *et al.*, 2010; Xiao *et al.*, 2016). Dynamic histone modifications maintained by various 'writers' and 'erasers' play critical roles in regulation of gene

expression during development, and responding to environmental stimuli including nutrient stresses.

Several studies have demonstrated the involvement of histone modifications in modulating the expression of nutrient responsive genes. For example, at the gene body of the Arabidopsis nitrate transporter gene *NRT2.1*, the level of tri-methylation of lysine 27 on histone H3 (H3K27me3) is much higher at high N supply compared to the low N supply, whereas the levels of H3K4me3 and H3K36me3 showed an opposite response (Widiez *et al.*, 2011). As mentioned above, H3K27me3 is associated with gene repression while H3K4me3 and H3K36me3 leads to gene activation. Therefore, the deposition of H3K27me3 on the *NRT2.1* locus mediated by HNI9/AtIWS1 is essential for feedback repression of *NRT2.1* by high N supply. The involvement of H3K4me3 in regulation of gene expression under phosphate deficiency was also reported. The H3K4me3 mark can be recognized and bound by a plant homeodomain (PHD)-containing putative transcription factor AL6 which acts as a histone mark reader (Lee *et al.*, 2009). Under phosphate deficient condition, the H3K4s at the promoter-proximal nucleosomes of the MYB transcriptional factor gene *ETC1* are likely tri-methylated. AL6 then binds to the H3K4me3 at the *ETC1* locus through its PHD domain and activates the expression of *ETC1*, which might further regulate downstream gene expression and promote root hair elongation during phosphate deficiency (Chandrika *et al.*, 2013a; Chandrika *et al.*, 2013b). Not only methylation on histone 3 is involved in the nutrient stress response, the symmetric dimethylation on histone 4 arginine-3 (H4R3sme2) was also reported to be involved in regulation of Fe homeostasis. Global H4R3sme2 level increase under excess Fe but decrease in the absence of sufficient Fe supply, which requires the Shk1 binding protein 1 (SKB1/AtPRMT5), a histone modification 'writer' catalyzing the symmetric dimethylation of histone H4R3 (Fan *et al.*, 2014). SKB1 targets the chromatin of the Ib subgroup bHLH genes (*AtbHLH38*, *AtbHLH39*, *AtbHLH100* and *AtbHLH101*) to regulate their transcription by deposition of H4R3sme2. Although SKB1 does not response to Fe status, the association of SKB1 to the chromatin of Ib subgroup *bHLH* genes and the H4R3sme2 levels on these loci decrease under limited Fe supply, and thus enhance the expression of these genes in order to enhance Fe uptake (Fan *et al.*, 2014). Besides histone methylation, histone acetylation might also regulate expression under phosphate

starvation. Knockdown of a histone deacetylase HDA19, which acts as a histone acetylation 'eraser', alters the expression of a subset of genes involved in the phosphate starvation response (Chen *et al.*, 2015).

Although there is no direct evidence to support histone modifications involvement in regulation of sulphur homeostasis, histone methylations and acetylations are found in many genes involved in sulphate uptake and assimilation in Arabidopsis (Table 1), including H3K27me3 (Zhang *et al.*, 2007), H3K4me3 and H3K36me3 (Luo *et al.*, 2013), H3K23ac and H4K16ac (Lu *et al.*, 2015), and H3K9ac (Zhou *et al.*, 2010). Therefore, it can be assumed that histone modification may also play a role in maintaining sulphur homeostasis. In fact, the interruption of the SAM cycle, which leads to abnormal SAM to SAH ratio, affects histone methylation (Fig. 1). Mutations of *FPGS1*, *MTHFD1* and *SAMS3*, which all lead to lower SAM to SAH ratios, not only reduce global DNA methylation but also decrease H3K9me2 levels (Groth *et al.*, 2016; Meng *et al.*, 2018; Zhou *et al.*, 2013). Furthermore, elevation of SAH has been shown to decrease the methylation of histone H3 at the arginine 8 (H3R8me2a) site in brain of hyperhomocysteinemic rats (Esse *et al.*, 2013), and methylation of H4R3me2a in the liver of cystathionine β-synthase-deficient mice (Esse *et al.*, 2014).

Noncoding RNA regulation

Noncoding RNAs (ncRNAs) refer to functional RNA transcripts that do not code for proteins. ncRNAs comprise different groups of transcripts, including the ribosomal RNAs, transfer RNAs, and regulatory ncRNAs that play critical roles in transcriptional and post-transcriptional regulation in eukaryotes. According to their length, ncRNAs can be divided into small ncRNAs (sRNAs), and long ncRNAs (lncRNAs). The micro RNAs (miRNAs), and small interfering RNAs (siRNAs), are two main groups of small regulatory RNAs with different biogenesis processes and functions (Axtell, 2013). ncRNAs that are longer than 200 nucleotides are generally considered as lncRNAs (Kapranov *et al.*, 2007). Many lncRNAs function as regulators of gene expression during development and responses to environmental stimuli (Kim and Sung, 2012), though very recent studies suggest some individual lncRNAs may not function as previously thought

(Goudarzi *et al.*, 2019). An example of lncRNA responding to nutritional stress is *INDUCED BY PHOSPHATE STARVATIONA* (*IPS1*), which prevents the cleavage of *PHO2* by miRNA399 through a target mimicry mechanism (Bari *et al.*, 2006). lncRNAs responsive to sulphur deprivation have been identified in microalgae *Chlamydomonas reinhardtii* (Li *et al.*, 2016). However, lncRNAs are largely unexplored in plants, and their function in regulation of S homeostasis is still unknown though some of lncRNAs are conserved among species (Li *et al.*, 2016). Similarly, the involvement of siRNAs in the response to S deficiency and maintenance of S homeostasis is less studied in plants. Here, we focus on the functions of miRNAs in regulation of gene expression in the maintenance of S homeostasis.

miRNAs are major post-transcriptional regulators of gene expression through guiding the degradation of target mRNAs and/or inhibiting the translation of target genes (Axtell, 2013; Jones-Rhoades et al., 2006). More than three hundred miRNAs have been identified in Arabidopsis by computational and experimental approaches, including those responding to nutrient deprivation (Kozomara and Griffiths-Jones, 2011). Among these miRNAs, the expression of 32 miRNAs was found to be down- or up-regulated under S deficient condition, accounting for approximately 10% of the total miRNAs identified in Arabidopsis so far (Liang *et al.*, 2015). miR395 is one of the most well investigated miRNAs in response to S deficiency, and plays a central role in sulphate assimilation and allocation. miR395 was first identified by a computational approach, and was confirmed experimentally to be highly induced by sulphur starvation (Jones-Rhoades and Bartel, 2004). Such induction requires redox signalling as the S deprivation induction of miR395 is compromised in the GSH biosynthesis mutant *cad2* and the thioredoxin reductase double mutant *ntra ntrb*, which are defective in glutaredoxin- and thioredoxin-dependent redox signaling, respectively (Jagadeeswaran *et al.*, 2014). Furthermore, external supplementation of GSH suppresses the induction of miR395 by S deprivation.

miR395 was predicted to target three ATP sulfurylase genes (*ATPS1*, *ATPS3* and *ATPS4*), and a low-affinity sulphate transporter *SULTR2;1* in Arabidopsis (Jones-Rhoades and Bartel, 2004). The cleavage of these four target genes by miR395 was validated experimentally in different tissues (Allen *et al.*, 2005; Jagadeeswaran *et al.*, 2014; Jones-Rhoades and Bartel, 2004; Kawashima *et al.*, 2009). Overexpression of the *MIR395* gene

strongly suppresses the accumulation of transcripts of these four genes and increases the sulphate level in shoots. Furthermore, knockout of *ATPS1* and *SULTR2;1*, and knockdown of *ATPS4*, simultaneously phenocopies the high sulphate level of miR395-over-expressing plants, supporting the notion that miR395 targets to *ATPS1*, *ATPS4* and *SULTR2;1* (Liang *et al.*, 2010). Although the cleavage of target genes by miR395 is clear, the transcript levels of the four target genes are not always negatively correlated with the level of miR395. miR395 is strongly induced by sulphate starvation in both roots and shoots, whereas the transcript levels of the four target genes show distinct responses to sulphate deficiency in roots and shoots. *ATPS4* shows a canonical regulation by miR395 as its expression decreases in both roots and shoots following the induction of miR395 by sulphate starvation (Jagadeeswaran *et al.*, 2014; Liang *et al.*, 2010). Target mimics of miR395 also leads to over-accumulation of *ATPS4* transcripts under both sulphate sufficient and deficient conditions (Kawashima *et al.*, 2011). The transcript levels of *ATPS1*, *ATPS3* and *SULTR2;1* in shoots decrease in response to sulphate deficiency as expected (Jagadeeswaran *et al.*, 2014). However, in roots under sulphate deficient condition, *ATPS1* and *ATPS3* maintain consistent expression levels (Jagadeeswaran *et al.*, 2014), or are slightly induced, depending on the period of sulphate deficiency (Kawashima *et al.*, 2011; Liang *et al.*, 2010). *SULTR2;1* is consistently strongly induced by sulphate deficiency in roots, even though miR395 is also induced (Jagadeeswaran *et al.*, 2014; Kawashima *et al.*, 2011; Liang *et al.*, 2010). The positive correlation between miR395 and *SULTR2;1* expression in roots is due to their non-overlapping expression pattern in the root vascular tissues. *SULTR2;1* is specifically expressed in the xylem parenchyma and pericycle cells, whereas the expression of miR395 is restricted in phloem companion cells, which leaves the target mRNA of *SULTR2;1* intact (Kawashima *et al.*, 2009).

There are four *ATPS* genes in the Arabidopsis genome. *ATPS1*, *3* and *4* encode the plastid-localized isoforms, whereas *ATPS2* dually encodes plastidic and cytosolic isoforms (Hatzfeld *et al.*, 2000; Rotte and Leustek, 2000; Bohrer *et al.*, 2015). The plastidic isoforms function in the initial activation of sulphate for assimilation into cysteine, while the cytosolic ATPS2 is involved in sulphation reaction for biosynthesis of glucosinolates (Hatzfeld *et al.*, 2000). Interestingly, miR395 only targets plastidic

isoform genes, but not the cytosolic *ATPS2*, indicating that miR395 specifically regulates sulphate assimilation in plastids, but not in the cytosol. Therefore, miR395 plays an important role in sulphate assimilation and root-to-shoot translocation of sulphate by regulating mRNA levels of ATP sulfurylase genes and *SULTR2;1*. Such regulation seems to be conserved among different species, such as rice (Guddeti *et al.*, 2005; Jagadeeswaran *et al.*, 2014; Yuan *et al.*, 2016) and *Brassica napus* (Huang *et al.*, 2010). miR395 is also induced in response to heavy metals such as arsenic (As) and copper (Cu), and is suppressed by nitrogen and carbon deficiency, suggesting broad functioning of miR395 in the regulation of gene expression in response to nutrient stresses (Jagadeeswaran *et al.*, 2014; Liang *et al.*, 2015). Interestingly, under phosphate limiting conditions miR399 is involved in the regulation of phosphate uptake and translocation through the targeting of *PHO2* to maintain phosphate homeostasis (Chiou *et al.*, 2006; Fujii *et al.*, 2005), further highlighting the importance of miRNAs in regulation of adaptation in response to nutrient deficiency.

Conclusions and future perspectives

Emerging evidence is starting to indicate the important roles of epigenetic regulation in controlling responses to nutrient stresses, and the maintenance of nutrient homeostasis in plants. miRNAs mediated gene silencing which is well-established to participate in the regulation of sulphate uptake and assimilation, whereas the examples of the involvement of DNA methylation and histone modifications in regulation of S homeostasis are still limited. Given that the universal methyl group donor SAM is derived from sulphate in plants, the reduction of SAM levels either due to impairment of its biosynthesis, or the interruption of folate metabolism, all leads to alterations in genome-wide DNA methylation, and in some cases also changes in histone methylation (Fig. 1). Therefore, a tight link between sulphur metabolism and DNA and histone methylation appears to exist in plants. Indeed, mutation of *MSA1*/*SHM7* leads to a reduction of SAM levels and alters global DNA methylation, including the methylation level of several S homeostasis related genes, which triggers S deficiency response and enhances sulphate uptake and assimilation in the *msa1-1* mutant (Huang *et al.*, 2016). Such enhancement of sulphate

uptake and assimilation may be a feedback response to the reduction of SAM levels observed in this mutant. It is therefore necessary to detect whether a similar S deficiency response occurs in those folate metabolism related mutants with alteration of DNA and histone methylation due to the shortage of SAM. Several enzymes involved in SAM biosynthesis or metabolism have isoforms localized to the nucleus, including SAMS1/2/3 (Mao *et al.*, 2015; Meng *et al.*, 2018) and MSA1 (Huang *et al.*, 2016) for SAM biosynthesis, and SAHH1 and ADK1 for recycling SAM (Lee *et al.*, 2012). It is therefore likely that SAM is synthesized in the nuclei to locally sustain the methyl group for DNA and histone methylation (Huang et al., 2016). The perturbation of such a nuclear SAM pool may then trigger S deficiency responses through unknown signalling pathways.

Several studies have demonstrated that dynamic DNA methylation at *cis*-elements in promoter regions may influence the expression of nutrient responsive genes such as *SULTR1;1* (Huang *et al.*, 2016) and several phosphate starvation responsive genes (Yong-Villalobos *et al.*, 2016; Yong-Villalobos *et al.*, 2015). Such a relationship between gene expression and DNA methylation is largely based on their correlation, which might not necessarily reflect causality. With the development of epigenome editing tools that enable the specific methylation or demethylation of targeted cytosine residues in the promoter of the genes of interest (Gallego-Bartolome *et al.*, 2018; Gallego-Bartolome *et al.*, 2019), it is now possible to reliably establish the causality of DNA methylation status and transcriptional activity. Furthermore, most studies usually assess DNA methylation in whole roots and shoots or even in whole plants, which may mask functionally important heterogeneity among different cell types. Unique patterns of DNA methylation in specific cell types, or in a single cell, have been revealed (Kawakatsu *et al.*, 2016; Li *et al.*, 2019). It is thus necessary to determine cell-type specific or even single cell DNA methylation profiles to link DNA methylation and gene expression. The application of single cell methylome analysis techniques and precise epigenome editing tools will enable functional analyses of DNA methylation in gene expression, and allow the direct demonstration of its role in response to nutrient stresses.

Acknowledgements

This work was supported by Natural Science Foundation of Jiangsu Province for Distinguished Young Scholars (BK20180023), the Natural Science Foundation of China (31772382), Fundamental Research Funds for the Central Universities (KYZ201714), and grant to DES from the U.S. National Science Foundation Arabidopsis 2010 Program (IOB 0419695) and the U.S. National Science Foundation Plant Genome Research Program (Grant IOS 0701119).

Reference

Aarabi F, Kusajima M, Tohge T, *et al.* 2016. Sulfur deficiency-induced repressor proteins optimize glucosinolate biosynthesis in plants. Science Advances **2**, e1601087.

Allen E, Xie Z, Gustafson AM, Carrington JC. 2005. microRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell **121**, 207-221.

Alonso C, Ramos-Cruz D, Becker C. 2019. The role of plant epigenetics in biotic interactions. New Phytologist **221**, 731-737.

Axtell MJ. 2013. Classification and comparison of small RNAs from plants. Annual Review of Plant Biology **64**, 137-159.

Bachhawat AK, Yadav S. 2018. The glutathione cycle: Glutathione metabolism beyond the gamma-glutamyl cycle. IUBMB Life **70**, 585-592.

Bannister AJ, Kouzarides T. 2011. Regulation of chromatin by histone modifications. Cell Research **21**, 381-395.

Bari R, Datt Pant B, Stitt M, Scheible WR. 2006. PHO2, microRNA399, and PHR1 define a phosphate-signaling pathway in plants. Plant Physiology **141**, 988-999.

Berger SL. 2007. The complex language of chromatin regulation during transcription. Nature **447**, 407-412.

Berger SL, Kouzarides T, Shiekhattar R, Shilatifard A. 2009. An operational definition of epigenetics. Genes & Development **23**, 781-783.

Bohrer AS, Yoshimoto N, Sekiguchi A, Rykulski N, Saito K, Takahashi H. 2015. Alternative translational initiation of ATP sulfurylase underlying dual localization of sulfate assimilation pathways in plastids and cytosol in *Arabidopsis thaliana*. Frontier in Plant Science **5**, 750.

Cao MJ, Wang Z, Wirtz M, Hell R, Oliver DJ, Xiang CB. 2013. SULTR3;1 is a chloroplast-localized sulfate transporter in *Arabidopsis thaliana*. Plant Journal **73**, 607-616.

Castro R, Rivera I, Struys EA, Jansen EE, Ravasco P, Camilo ME, Blom HJ, Jakobs C, Tavares de Almeida I. 2003. Increased homocysteine and S-adenosylhomocysteine concentrations and DNA hypomethylation in vascular disease. Clinical Chemistry **49**, 1292-1296.

Chandrika NN, Sundaravelpandian K, Schmidt W. 2013a. A PHD in histone language: on the role of histone methylation in plant responses to phosphate deficiency. Plant Signaling & Behavior **8**, e24381.

Chandrika NN, Sundaravelpandian K, Yu SM, Schmidt W. 2013b. ALFIN-LIKE 6 is involved in root hair elongation during phosphate deficiency in *Arabidopsis*. New Phytologist **198**, 709-720.

Chen CY, Wu K, Schmidt W. 2015. The histone deacetylase HDA19 controls root cell elongation and modulates a subset of phosphate starvation responses in *Arabidopsis*. Scientific Reports **5**, 15708.

Chen Z, Zhao PX, Miao ZQ, *et al.* 2019. SULTR3s function in chloroplast sulfate uptake and affect ABA biosynthesis and the stress response. Plant Physiology. DOI: 10.1104/pp.18.01439

Chen X, Schonberger B, Menz J, Ludewig U. 2018. Plasticity of DNA methylation and gene expression under zinc deficiency in *Arabidopsis* roots. Plant & Cell Physiology **59**, 1790-1802.

Chinnusamy V, Zhu JK. 2009. Epigenetic regulation of stress responses in plants. Current Opinion in Plant Biology **12**, 133-139.

Chiou TJ, Aung K, Lin SI, Wu CC, Chiang SF, Su CL. 2006. Regulation of phosphate homeostasis by MicroRNA in Arabidopsis. Plant Cell **18**, 412-421.

Cobbett CS. 2000. Phytochelatin biosynthesis and function in heavy-metal detoxification. Current Opinion in Plant Biology **3**, 211-216.

Davidian JC, Kopriva S. 2010. Regulation of sulfate uptake and assimilation--the same or not the same? Molecular Plant **3**, 314-325.

Domcke S, Bardet AF, Adrian Ginno P, Hartl D, Burger L, Schubeler D. 2015. Competition between DNA methylation and transcription factors determines binding of NRF1. Nature **528**, 575-579.

Esse R, Florindo C, Imbard A, *et al.* 2013. Global protein and histone arginine methylation are affected in a tissue-specific manner in a rat model of diet-induced hyperhomocysteinemia. Biochimica et Biophysica Acta (BBA) - Bioenergetics **1832**, 1708-1714.

Esse R, Imbard A, Florindo C, *et al.* 2014. Protein arginine hypomethylation in a mouse model of cystathionine beta-synthase deficiency. FASEB Journal **28**, 2686-2695.

Falk KL, Tokuhisa JG, Gershenzon J. 2007. The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. Plant Biology (Stuttgart, Germany) **9**, 573-581.

Fan H, Zhang Z, Wang N, *et al.* 2014. SKB1/PRMT5-mediated histone H4R3 dimethylation of Ib subgroup bHLH genes negatively regulates iron homeostasis in *Arabidopsis thaliana*. Plant Journal **77**, 209-221.

Frerigmann H. 2016. Chapter Four - Glucosinolate regulation in a complex relationship – MYC and MYB – no one can act without each other. In: Kopriva S, ed. Advances in Botanical Research, Vol. 80: Academic Press, 57-97.

Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK. 2005. A miRNA involved in phosphate-starvation response in *Arabidopsis*. Current Biology **15**, 2038-2043.

Gallego-Bartolome J, Gardiner J, Liu W, Papikian A, Ghoshal B, Kuo HY, Zhao JM, Segal DJ, Jacobsen SE. 2018. Targeted DNA demethylation of the *Arabidopsis* genome using the human TET1 catalytic domain. Proceedings of the National Academy of Sciences of the United States of America **115**, E2125-E2134.

Gallego-Bartolome J, Liu W, Kuo PH, *et al.* 2019. Co-targeting RNA Polymerases IV and V promotes efficient de novo DNA methylation in *Arabidopsis*. Cell. DOI: 10.1016/j.cell.2019.01.029

Goto DB, Ogi M, Kijima F, Kumagai T, van Werven F, Onouchi H, Naito S. 2002. A single-nucleotide mutation in a gene encoding S-adenosylmethionine synthetase is associated with methionine over-accumulation phenotype in *Arabidopsis thaliana*. Genes & Genetic Systems **77**, 89-95.

Goudarzi M, Berg K, Pieper LM, Schier AF. 2019. Individual long non-coding RNAs have no overt functions in zebrafish embryogenesis, viability and fertility. Elife **8**, e40815.

Groth M, Moissiard G, Wirtz M, *et al.* 2016. MTHFD1 controls DNA methylation in *Arabidopsis*. Nature Communications **7**, 11640.

Guddeti S, Zhang DC, Li AL, Leseberg CH, Kang H, Li XG, Zhai WX, Johns MA, Mao L. 2005. Molecular evolution of the rice miR395 gene family. Cell Research **15**, 631-638.

Halkier BA, Gershenzon J. 2006. Biology and biochemistry of glucosinolates. Annual Review of Plant Biology **57**, 303-333.

Hanson AD, Gregory JF, 3rd. 2011. Folate biosynthesis, turnover, and transport in plants. Annual Review of Plant Biology **62**, 105-125.

Hatzfeld Y, Lee S, Lee M, Leustek T, Saito K. 2000. Functional characterization of a gene encoding a fourth ATP sulfurylase isoform from *Arabidopsis thaliana*. Gene **248**, 51-58.

Hesse H, Hoefgen R. 2003. Molecular aspects of methionine biosynthesis. Trends in Plant Science **8**, 259-262.

Hoffman DR, Cornatzer WE, Duerre JA. 1979. Relationship between tissue levels of S-adenosylmethionine, S-adenylhomocysteine, and transmethylation reactions. Canadian Journal of Biochemistry **57**, 56-65.

Howarth JR, Parmar S, Barraclough PB, Hawkesford MJ. 2009. A sulphur deficiency-induced gene, sdi1, involved in the utilization of stored sulphate pools under sulphur-limiting conditions has potential as a diagnostic indicator of sulphur nutritional status. Plant Biotechnology Journal **7**, 200-209.

Huang SQ, Xiang AL, Che LL, Chen S, Li H, Song JB, Yang ZM. 2010. A set of miRNAs from Brassica napus in response to sulphate deficiency and cadmium stress. Plant Biotechnology Journal **8**, 887-899.

Huang XY, Chao DY, Koprivova A, *et al.* 2016. Nuclear localised MORE SULPHUR ACCUMULATION1 epigenetically regulates sulphur homeostasis in *Arabidopsis thaliana*. PLoS Genetics **12**, e1006298.

Jagadeeswaran G, Li YF, Sunkar R. 2014. Redox signaling mediates the expression of a sulfate-deprivation-inducible microRNA395 in *Arabidopsis*. Plant Journal **77**, 85-96.

Jiang Y, Sun T, Xiong J, Cao J, Li G, Wang S. 2007a. Hyperhomocysteinemia-mediated DNA hypomethylation and its potential epigenetic role in rats. Acta Biochim Biophys Sin (Shanghai) **39**, 657-667.

Jiang YD, Zhang JZ, Huang Y, Su J, Zhang JG, Wang SL, Han XQ, Wang SR. 2007b. Homocysteine-mediated expression of SAHH, DNMTs, MBD2, and DNA hypomethylation potential pathogenic mechanism in VSMCs. DNA and Cell Biology **26**, 603-611.

Jones-Rhoades MW, Bartel DP. 2004. Computational identification of plant microRNAs and their targets, including a stress-induced miRNA. Molecular Cell **14**, 787-799.

Jones-Rhoades MW, Bartel DP, Bartel B. 2006. MicroRNAs and their regulatory roles in plants. Annual Review of Plant Biology **57**, 19-53.

Jordan ND, West JP, Bottley A, Sheikh M, Furner I. 2007. Transcript profiling of the hypomethylated hog1 mutant of *Arabidopsis*. Plant Molecular Biology **65**, 571-586.

Kapranov P, Cheng J, Dike S, *et al.* 2007. RNA maps reveal new RNA classes and a possible function for pervasive transcription. Science **316**, 1484-1488.

Kawakatsu T, Stuart T, Valdes M, *et al.* 2016. Unique cell-type-specific patterns of DNA methylation in the root meristem. Nature Plants **2**, 16058.

Kawashima CG, Matthewman CA, Huang S, *et al.* 2011. Interplay of SLIM1 and miR395 in the regulation of sulfate assimilation in *Arabidopsis*. Plant Journal **66**, 863-876.

Kawashima CG, Yoshimoto N, Maruyama-Nakashita A, Tsuchiya YN, Saito K, Takahashi H, Dalmay T. 2009. Sulphur starvation induces the expression of microRNA-395 and one of its target genes but in different cell types. Plant Journal **57**, 313-321.

Kim E-D, Sung S. 2012. Long noncoding RNA: unveiling hidden layer of gene regulatory networks. Trends in Plant Science **17**, 16-21.

Kou HP, Li Y, Song XX, Ou XF, Xing SC, Ma J, Von Wettstein D, Liu B. 2011. Heritable alteration in DNA methylation induced by nitrogen-deficiency stress accompanies enhanced tolerance by progenies to the stress in rice (*Oryza sativa* L.). Journal of Plant Physiology **168**, 1685-1693.

Kouzarides T. 2007. Chromatin modifications and their function. Cell **128**, 693-705.

Kozomara A, Griffiths-Jones S. 2011. miRBase: integrating microRNA annotation and deep-sequencing data. Nucleic Acids Research **39**, D152-157.

Kumar A, Tikoo S, Maity S, Sengupta S, Sengupta S, Kaur A, Bachhawat AK. 2012. Mammalian proapoptotic factor ChaC1 and its homologues function as gamma-glutamyl cyclotransferases acting specifically on glutathione. EMBO Reports **13**, 1095-1101.

Kumar S, Kaur A, Chattopadhyay B, Bachhawat AK. 2015. Defining the cytosolic pathway of glutathione degradation in *Arabidopsis thaliana*: role of the ChaC/GCG family of gamma-glutamyl cyclotransferases as glutathione-degrading enzymes and AtLAP1 as the Cys-Gly peptidase. Biochemical Journal **468**, 73-85.

Lamke J, Baurle I. 2017. Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biology **18**, 124.

Lappartient AG, Touraine B. 1996. Demand-driven control of root ATP sulfurylase activity and SO_4^{2-} uptake in intact canola (the role of phloem-translocated glutathione). Plant Physiology **111**, 147-157.

Lappartient AG, Vidmar JJ, Leustek T, Glass ADM, Touraine B. 1999. Inter-organ signaling in plants: regulation of ATP sulfurylase and sulfate transporter genes expression in roots mediated by phloem-translocated compound. The Plant Journal **18**, 89-95.

Law JA, Jacobsen SE. 2010. Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews: Genetics **11**, 204-220.

Lee BR, Koprivova A, Kopriva S. 2011. The key enzyme of sulfate assimilation, adenosine 5'-phosphosulfate reductase, is regulated by HY5 in *Arabidopsis*. Plant Journal **67**, 1042-1054.

Lee S, Doxey AC, McConkey BJ, Moffatt BA. 2012. Nuclear targeting of methyl-recycling enzymes in *Arabidopsis thaliana* is mediated by specific protein interactions. Molecular Plant **5**, 231-248.

Lee WY, Lee D, Chung WI, Kwon CS. 2009. *Arabidopsis* ING and Alfin1-like protein families localize to the nucleus and bind to H3K4me3/2 via plant homeodomain fingers. Plant Journal **58**, 511-524.

Leustek T, Martin MN, Bick JA, Davies JP. 2000. Pathways and regulation of sulfur metabolism revealed through molecular and genetic studies. Annual Review of Plant Physiology and Plant Molecular Biology **51**, 141-165.

Li H, Wang Y, Chen M, Xiao P, Hu C, Zeng Z, Wang C, Wang J, Hu Z. 2016. Genome-wide long non-coding RNA screening, identification and characterization in a model microorganism *Chlamydomonas reinhardtii*. Scientific Reports **6**, 34109.

Li W, Han Y, Tao F, Chong K. 2011. Knockdown of SAMS genes encoding S-adenosyl-l-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice. Journal of Plant Physiology **168**, 1837-1843.

Li X, Chen L, Zhang Q, Sun Y, Li Q, Yan J. 2019. BRIF-Seq: bisulfite-converted randomly integrated fragments sequencing at the single-cell level. Molecular Plant. DOI: 10.1016/j.molp.2019.01.004

Liang G, Ai Q, Yu D. 2015. Uncovering miRNAs involved in crosstalk between nutrient deficiencies in *Arabidopsis*. Scientific Reports **5**, 11813.

Liang G, Yang F, Yu D. 2010. MicroRNA395 mediates regulation of sulfate accumulation and allocation in *Arabidopsis thaliana*. Plant Journal **62**, 1046-1057.

Liu C, Lu F, Cui X, Cao X. 2010. Histone methylation in higher plants. Annual Review of Plant Biology **61**, 395-420.

Loizeau K, De Brouwer V, Gambonnet B, Yu A, Renou JP, Van Der Straeten D, Lambert WE, Rebeille F, Ravanel S. 2008. A genome-wide and metabolic analysis determined the adaptive response of *Arabidopsis* cells to folate depletion induced by methotrexate. Plant Physiology **148**, 2083-2095.

Lu L, Chen X, Sanders D, Qian S, Zhong X. 2015. High-resolution mapping of H4K16 and H3K23 acetylation reveals conserved and unique distribution patterns in *Arabidopsis* and rice. Epigenetics **10**, 1044-1053.

Luo C, Sidote DJ, Zhang Y, Kerstetter RA, Michael TP, Lam E. 2013. Integrative analysis of chromatin states in *Arabidopsis* identified potential regulatory mechanisms for natural antisense transcript production. Plant Journal **73**, 77-90.

Mao D, Yu F, Li J, *et al.* 2015. FERONIA receptor kinase interacts with S-adenosylmethionine synthetase and suppresses S-adenosylmethionine production and ethylene biosynthesis in *Arabidopsis*. Plant Cell and Environment **38**, 2566-2574.

Maruyama-Nakashita A. 2017. Metabolic changes sustain the plant life in low-sulfur environments. Current Opinion in Plant Biology **39**, 144-151.

Maruyama-Nakashita A, Nakamura Y, Tohge T, Saito K, Takahashi H. 2006. *Arabidopsis* SLIM1 is a central transcriptional regulator of plant sulfur response and metabolism. Plant Cell **18**, 3235-3251.

Maruyama-Nakashita A, Nakamura Y, Watanabe-Takahashi A, Inoue E, Yamaya T, Takahashi H. 2005. Identification of a novel cis-acting element conferring sulfur deficiency response in *Arabidopsis* roots. Plant Journal **42**, 305-314.

Maruyama-Nakashita A, Watanabe-Takahashi A, Inoue E, Yamaya T, Saito K, Takahashi H. 2015. Sulfur-responsive elements in the 3'-nontranscribed intergenic region are essential for the induction of SULFATE TRANSPORTER 2;1 gene expression in *Arabidopsis* roots under sulfur deficiency. Plant Cell **27**, 1279-1296.

Matherly LH, Goldman ID. 2003. Membrane transport of folates. vitamins and hormones, Vol. 66: Academic Press, 403-456.

Meng J, Wang L, Wang J, Zhao X, Cheng J, Yu W, Jin D, Li Q, Gong Z. 2018. METHIONINE ADENOSYLTRANSFERASE4 mediates DNA and histone methylation. Plant Physiology **177**, 652-670.

Moffatt BA, Stevens YY, Allen MS, *et al.* 2002. Adenosine kinase deficiency is associated with developmental abnormalities and reduced transmethylation. Plant Physiology **128**, 812-821.

Moffatt BA, Weretilnyk EA. 2001. Sustaining S-adenosyl-L-methionine-dependent methyltransferase activity in plant cells. Physiologia Plantarum **113**, 435-442.

Nikiforova VJ, Kopka J, Tolstikov V, Fiehn O, Hopkins L, Hawkesford MJ, Hesse H, Hoefgen R. 2005. Systems rebalancing of metabolism in response to sulfur deprivation, as revealed by metabolome analysis of *Arabidopsis* plants. Plant Physiology **138**, 304-318.

Ohkama-Ohtsu N, Oikawa A, Zhao P, Xiang C, Saito K, Oliver DJ. 2008. A gamma-glutamyl transpeptidase-independent pathway of glutathione catabolism to glutamate via 5-oxoproline in *Arabidopsis*. Plant Physiology **148**, 1603-1613.

Ouyang B, Fei Z, Joung JG, *et al.* 2012. Transcriptome profiling and methyl homeostasis of an *Arabidopsis* mutant deficient in S-adenosylhomocysteine hydrolase1 (SAHH1). Plant Molecular Biology **79**, 315-331.

Paulose B, Chhikara S, Coomey J, Jung HI, Vatamaniuk O, Dhankher OP. 2013. A gamma-glutamyl cyclotransferase protects *Arabidopsis* plants from heavy metal toxicity by recycling glutamate to maintain glutathione homeostasis. Plant Cell **25**, 4580-4595.

Rocha PS, Sheikh M, Melchiorre R, *et al.* 2005. The *Arabidopsis* HOMOLOGY-DEPENDENT GENE SILENCING1 gene codes for an S-adenosyl-L-homocysteine hydrolase required for DNA methylation-dependent gene silencing. Plant Cell **17**, 404-417.

Roje S, Wang H, McNeil SD, Raymond RK, Appling DR, Shachar-Hill Y, Bohnert HJ, Hanson AD. 1999. Isolation, characterization, and functional expression of cDNAs encoding NADH-dependent methylenetetrahydrofolate reductase from higher plants. Journal of Biological Chemistry **274**, 36089-36096.

Rotte C, Leustek T. 2000. Differential subcellular localization and expression of ATP sulfurylase and 5'-adenylylsulfate reductase during ontogenesis of *Arabidopsis* leaves indicates that cytosolic and plastid forms of ATP sulfurylase may have specialized functions. Plant Physiology **124**, 715-724.

Rouached H, Wirtz M, Alary R, Hell R, Arpat AB, Davidian JC, Fourcroy P, Berthomieu P. 2008. Differential regulation of the expression of two high-affinity sulfate transporters, SULTR1.1 and SULTR1.2, in *Arabidopsis*. Plant Physiology **147**, 897-911.

Sahu PP, Pandey G, Sharma N, Puranik S, Muthamilarasan M, Prasad M. 2013. Epigenetic mechanisms of plant stress responses and adaptation. Plant Cell Reports **32**, 1151-1159.

Sauter M, Moffatt B, Saechao MC, Hell R, Wirtz M. 2013. Methionine salvage and S-adenosylmethionine: essential links between sulfur, ethylene and polyamine biosynthesis. Biochemical Journal **451**, 145-154.

Schirch V, Szebenyi DM. 2005. Serine hydroxymethyltransferase revisited. Current Opinion in Chemical Biology **9**, 482-487.

Secco D, Wang C, Shou H, Schultz MD, Chiarenza S, Nussaume L, Ecker JR, Whelan J, Lister R. 2015. Stress induced gene expression drives transient DNA methylation changes at adjacent repetitive elements. Elife **4**. DOI: 10.7554/eLife.09343

Secco D, Whelan J, Rouached H, Lister R. 2017. Nutrient stress-induced chromatin changes in plants. Current Opinion in Plant Biology **39**, 1-7.

Shane B. 1989. Folylpolyglutamate synthesis and role in the regulation of one-carbon metabolism. Vitamins and Hormones **45**, 263-335.

Takahashi H, Kopriva S, Giordano M, Saito K, Hell R. 2011. Sulfur assimilation in photosynthetic organisms: molecular functions and regulations of transporters and assimilatory enzymes. Annual Review of Plant Biology **62**, 157-184.

Takahashi H, Watanabe-Takahashi A, Smith FW, Blake-Kalff M, Hawkesford MJ, Saito K. 2000. The roles of three functional sulphate transporters involved in uptake and translocation of sulphate in *Arabidopsis thaliana*. Plant Journal **23**, 171-182.

Tang HM, Liu S, Hill-Skinner S, Wu W, Reed D, Yeh CT, Nettleton D, Schnable PS. 2014. The maize brown *midrib2* (*bm2*) gene encodes a methylenetetrahydrofolate reductase that contributes to lignin accumulation. Plant Journal **77**, 380-392.

Wawrzynska A, Lewandowska M, Sirko A. 2010. Nicotiana tabacum EIL2 directly regulates expression of at least one tobacco gene induced by sulphur starvation. Journal of Experimental Botany **61**, 889-900.

Wawrzynska A, Sirko A. 2016. EIN3 interferes with the sulfur deficiency signaling in *Arabidopsis thaliana* through direct interaction with the SLIM1 transcription factor. Plant Science **253**, 50-57.

Widiez T, El Kafafi el S, Girin T, *et al.* 2011. High nitrogen insensitive 9 (HNI9)-mediated systemic repression of root NO^{3-} uptake is associated with changes in histone methylation. Proceedings of the National Academy of Sciences of the United States of America **108**, 13329-13334.

Wittstock U, Burow M. 2010. Glucosinolate breakdown in *Arabidopsis*: mechanism, regulation and biological significance. Arabidopsis Book **8**, e0134.

Xiao J, Lee U-S, Wagner D. 2016. Tug of war: adding and removing histone lysine methylation in *Arabidopsis*. Current Opinion in Plant Biology **34**, 41-53.

Yi P, Melnyk S, Pogribna M, Pogribny IP, Hines RJ, James SJ. 2000. Increase in plasma homocysteine associated with parallel increases in plasma S-adenosylhomocysteine and lymphocyte DNA hypomethylation. Journal of Biological Chemistry **275**, 29318-29323.

Yong-Villalobos L, Cervantes-Perez SA, Gutierrez-Alanis D, Gonzales-Morales S, Martinez O, Herrera-Estrella L. 2016. Phosphate starvation induces DNA methylation in the vicinity of cis-acting elements known to regulate the expression of phosphate-responsive genes. Plant Signaling & Behavior **11**, e1173300.

Yong-Villalobos L, Gonzalez-Morales SI, Wrobel K, *et al.* 2015. Methylome analysis reveals an important role for epigenetic changes in the regulation of the *Arabidopsis* response to phosphate starvation. Proceedings of the National Academy of Sciences of the United States of America **112**, E7293-7302.

Yoshimoto N, Takahashi H, Smith FW, Yamaya T, Saito K. 2002. Two distinct high-affinity sulfate transporters with different inducibilities mediate uptake of sulfate in *Arabidopsis* roots. Plant Journal **29**, 465-473.

Yuan N, Yuan S, Li Z, Li D, Hu Q, Luo H. 2016. Heterologous expression of a rice miR395 gene in Nicotiana tabacum impairs sulfate homeostasis. Scientific Reports **6**, 28791.

Zhang H, Deng X, Miki D, Cutler S, La H, Hou YJ, Oh J, Zhu JK. 2012. Sulfamethazine suppresses epigenetic silencing in *Arabidopsis* by impairing folate synthesis. Plant Cell **24**, 1230-1241.

Zhang H, Lang Z, Zhu JK. 2018. Dynamics and function of DNA methylation in plants. Nature Reviews: Molecular Cell Biology **19**, 489-506.

Zhang X, Clarenz O, Cokus S, Bernatavichute YV, Pellegrini M, Goodrich J, Jacobsen SE. 2007. Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. PLoS Biology **5**, e129.

Zhou HR, Zhang FF, Ma ZY, Huang HW, Jiang L, Cai T, Zhu JK, Zhang C, He XJ. 2013. Folate polyglutamylation is involved in chromatin silencing by maintaining global DNA methylation and histone H3K9 dimethylation in *Arabidopsis*. Plant Cell **25**, 2545-2559.

Zhou J, Wang X, He K, Charron JB, Elling AA, Deng XW. 2010. Genome-wide profiling of histone H3 lysine 9 acetylation and dimethylation in *Arabidopsis* reveals correlation between multiple histone marks and gene expression. Plant Molecular Biology **72**, 585-595.

Zilberman D, Gehring M, Tran RK, Ballinger T, Henikoff S. 2007. Genome-wide analysis of *Arabidopsis thaliana* DNA methylation uncovers an interdependence between methylation and transcription. Nature Genetics **39**, 61-69.

Figure legend

Fig. 1. The interconnection of sulphate assimilation, folate metabolism and the SAM cycle with the DNA and histone methylation. The sulphate uptake and assimilation pathway, the biosynthesis and turnover of folate and the SAM cycle were shown in the background in light green, light blue and orange, respectively. Interruption of pathways with enzymes highlighted in blue and red alters genome-wide DNA methylation, and mutation of enzymes in red change histone methylation. Abbreviations for enzymes: ADK, adenosine kinase; APK, APS kinase; APR, APS reductase; ATPS, ATP sulfurylase; CBL, cystathionine β-lyase; CGS, cystathionine γ-synthase; DHFR, DHF reductase; DHFS, DHF synthase; DHPS, DHP synthase; γ-ECS, γ-glutamylcysteine synthetase; FPGS, folylpolyglutamate synthase; GSHS, glutathione synthetase; MS, methionine synthase; MTHFD1, bifunctional methylene THF dehydrogenase/methenyl THF cyclohydrolase; OAS-TL, OAS(thiol)lyase; SAHH, SAH hydrolase; SAMMT, SAM-dependent methyltransferase; SAMS, SAM synthetase; SAT, serine acetyltransferase; SHM, serine hydroxymethyltransferase; SiR, sulphite reductase; SOT, sulfotransferase; SULTR, sulphate transporter; SYN, 10-formyl THF synthetase. Abbreviations for compounds: Ado, adenosine; AMP, adenosine monophosphate; APS, adenosine 5'-phosphosulfate; Cys, cysteine; Cyst, cystathionine; DHF, dihydrofolate; DHP, dihydropteroate; Glun, polyglutamate; Hcy, homocysteine; Met, methionine; OAS, O-acetylserine; pABA, UDP-glucose-p-aminobenzoate; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Ser, serine; THF, tetrahydrofolate; γ-GluCys, γ-glutamylcysteine.

Fig. 2. Whole genome methylation levels of *Arabidopsis* under sulphate and phosphate starvation conditions. (**A**) Methylation levels at all cytosines in the genome (Total C) and the CG, CHG and CHH sequence context under +S and -S conditions. Methylation level

was determined by whole genome bisulfite sequencing (BS-Seq) of the shoots and roots of plants grown on MGRL agar media with 1.5 mM sulphate (+S) or without added sulphate (-S) for two weeks. (**B**) Methylation levels at all cytosines in the genome (Total C) and the CG, CHG and CHH sequence context under +Pi and -Pi conditions. Data were derived from Yong-Villalobos et al. (2015) and recalculated based on the raw data. Plants were grown hydroponically with 1 mM phosphate for 7 days and then transferred to hydroponic media containing 1 mM (+Pi) or 5 µM phosphate (-Pi) to be grown subsequently for 16 days. Methylation level was determined by BS-Seq of the shoots and roots, respectively.

Fig. 3. A potential model of dynamic DNA methylation in regulation of gene expression. The nutrient deficiency responsive genes are methylated at the *cis*-element in the promoter under sufficient nutrient condition. The methylation may prevent the binding of the transcriptional activator (TA) [upper panel in (A)] or recruit the transcriptional repressor (TR) [upper panel in (A)] and thus inhibits gene expression. However, under nutrient deficient condition, the hypo-methylated promoter allows the binding of transcriptional activator to promote the transcription.

Table 1. Histone modifications of genes involved in sulphate uptake and assimilation.

Gene ID	Gene symbol	Histone modifications					
		H3K27me3 [a]	**H3K4me3 [b]**	**H3K36me3 [b]**	**H3K23ac [c]**	**H4K16ac [c]**	**H3K9ac [d]**
Sulphate transporter							
At4g08620	*SULTR1;1*						
At1g78000	*SULTR1;2*						
At1g22150	*SULTR1;3*						
At5g10180	*SULTR2;1*						
At1g77990	*SULTR2;2*						
At3g51895	*SULTR3;1*						
At4g02700	*SULTR3;2*						
At1g23090	*SULTR3;3*						
At3g15990	*SULTR3;4*						
At5g19600	*SULTR3;5*						
At5g13550	*SULTR4;1*						
At3g12520	*SULTR4;2*						
ATP sulfurylase							
At3g22890	*ATPS1*						
At1g19920	*ATPS2*						
At4g14680	*ATPS3*						
At5g43780	*ATPS4*						
APS reductase							
At4g04610	*APR1*						
At1g62180	*APR2*						
At4g21990	*APR3*						
APS kinase							
At2g14750	*APK1*						
At4g39940	*APK2*						
At3g03900	*APK3*						
At5g67520	*APK4*						
Sulfite reductase							
At5g04590	*SiR*						
Serine acetyltransferase							
At5g56760	*SERAT1;1*						
At1g55920	*SERAT2;1*						
At3g13110	*SERAT2;2*						
At2g17640	*SERAT3;1*						
At4g35640	*SERAT3;2*						
O-acetylserine (thiol)lyase							
At4g14880	*OASTL-A1*						
At3g59760	*OASTL-C*						
At2g43750	*OASTL-B*						
At3g22460	*OASTL-A2*						
Cysteine synthase							
At3g03630	*CS26*						
At3g04940	*CYSD1*						
At3g61440	*CYSC1*						
At5g28030	*CYSD2*						

Whole genome analysis of histone modifications was carried out by using chromatin immunoprecipitation (ChIP) coupled with high-density whole genome tiling microarrays (ChIP-chip), or ChIP coupled with high throughput sequencing (ChIP-seq). Genes involved in sulphate uptake and assimilation were extracted and shown in Table 1. Cells in grey background mean the presence of histone modifications. Data from: [a] Zhang *et al*., 2007; [b] Luo *et al*., 2013; [c] Lu *et al*., 2015; [d] Zhou *et al*., 2010.

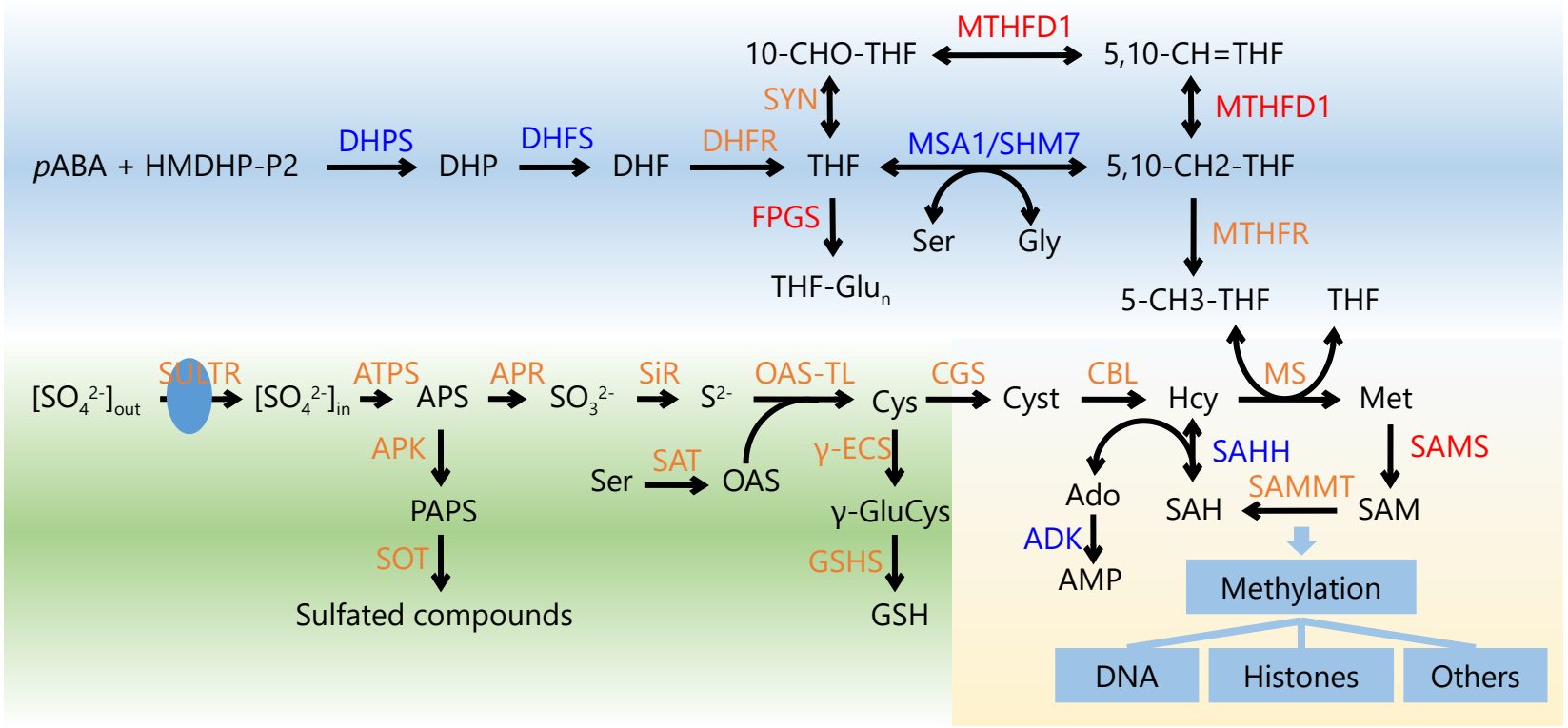

Fig. 1. The interconnection of sulfate assimilation, folate metabolism and the SAM cycle with the DNA and histone methylation. The sulfate uptake and assimilation pathway, the biosynthesis and turnover of folate and the SAM cycle were shown in the background of light green, light blue and orange colors, respectively. Interruption of enzymes in blue and red colors alters genome-wide DNA methylation, and mutation of enzymes in red color change histone methylation.

Abbreviations for enzymes: ADK, adenosine kinase; APK, APS kinase; APR, APS reductase; ATPS, ATP sulfurylase; CBL, cystathionine β-lyase; CGS, cystathionine γ-synthase; DHFR, DHF reductase; DHFS, DHF synthase; DHPS, DHP synthase; γ-ECS, γ-glutamylcysteine synthetase; FPGS, folylpolyglutamate synthase; GSHS, glutathione synthetase; MS, methionine synthase; MTHFD1, bifunctional methylene THF dehydrogenase/methenyl THF cyclohydrolase; OAS-TL, OAS(thiol)lyase; SAHH, SAH hydrolase; SAMMT, SAM-dependent methyltransferase; SAMS, SAM synthetase; SAT, serine acetyltransferase; SHM, serine hydroxymethyltransferase; SiR, sulphite reductase; SOT, sulfotransferase; SULTR, sulfate transporter; SYN, 10-formyl THF synthetase.

Abbreviations for compounds: Ado, adenosine; AMP, adenosine monophosphate; APS, adenosine 5′-phosphosulfate; Cys, cysteine; Cyst, cystathionine; DHF, dihydrofolate; DHP, dihydropteroate; Glun, polyglutamate; Hcy, homocysteine; Met, methionine; OAS, O-acetylserine; pABA, UDP-glucose-p-aminobenzoate ; PAPS, 3′-phosphoadenosine 5′-phosphosulfate; SAH, S-adenosylhomocysteine; SAM, S-adenosylmethionine; Ser, serine; THF, tetrahydrofolate; γ-GluCys, γ-glutamylcysteine.

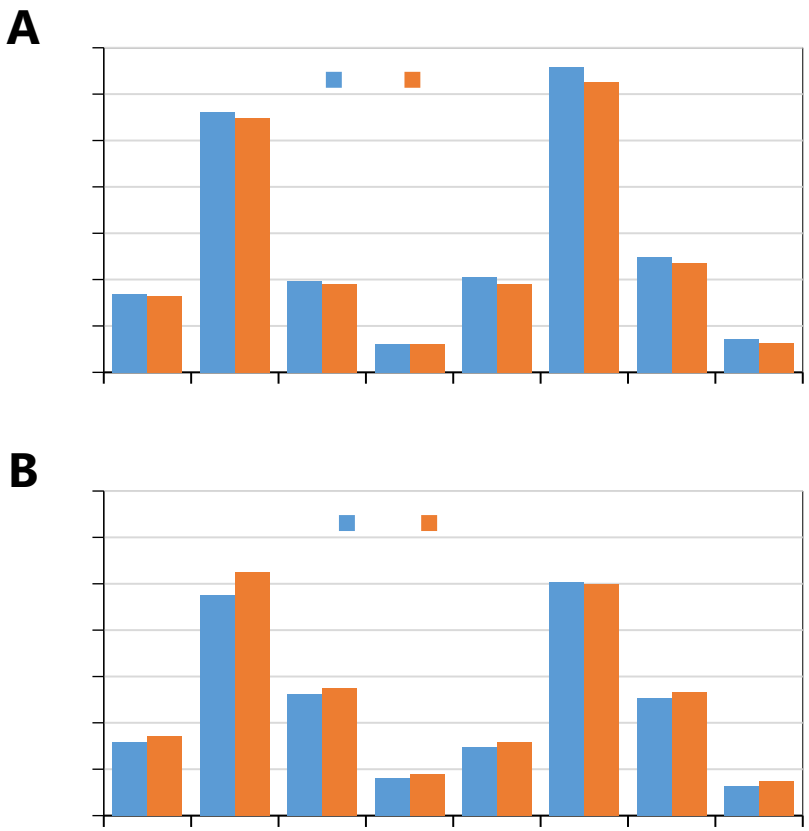

Fig. 2. Whole genome methylation level of *Arabidopsis* under sulfate and phosphate starvation conditions. (**A**) Methylation levels at all cytosines in the genome (Total C) and the CG, CHG and CHH sequence context under +S and -S conditions. Methylation level was determined by whole genome bisulfite sequencing (BS-Seq) on the shoots and roots of plants grown on MGRL agar media with 1.5 mM sulfate (+S) or without added sulfate (-S) for two weeks. (**B**) Methylation levels at all cytosines in the genome (Total C) and the CG, CHG and CHH sequence context under +Pi and -Pi conditions. Data were derived from Yong-Villalobos et al. (2015) and recalculated based on the raw data. Plants were grown hyponically with 1 mM phosphate for 7 days and then transferred to hydropic media containing 1 mM (+Pi) or 5 μM phosphate (-Pi) for 16 days. Methylation level was determined by BS-Seq on the shoots and roots, respectively.

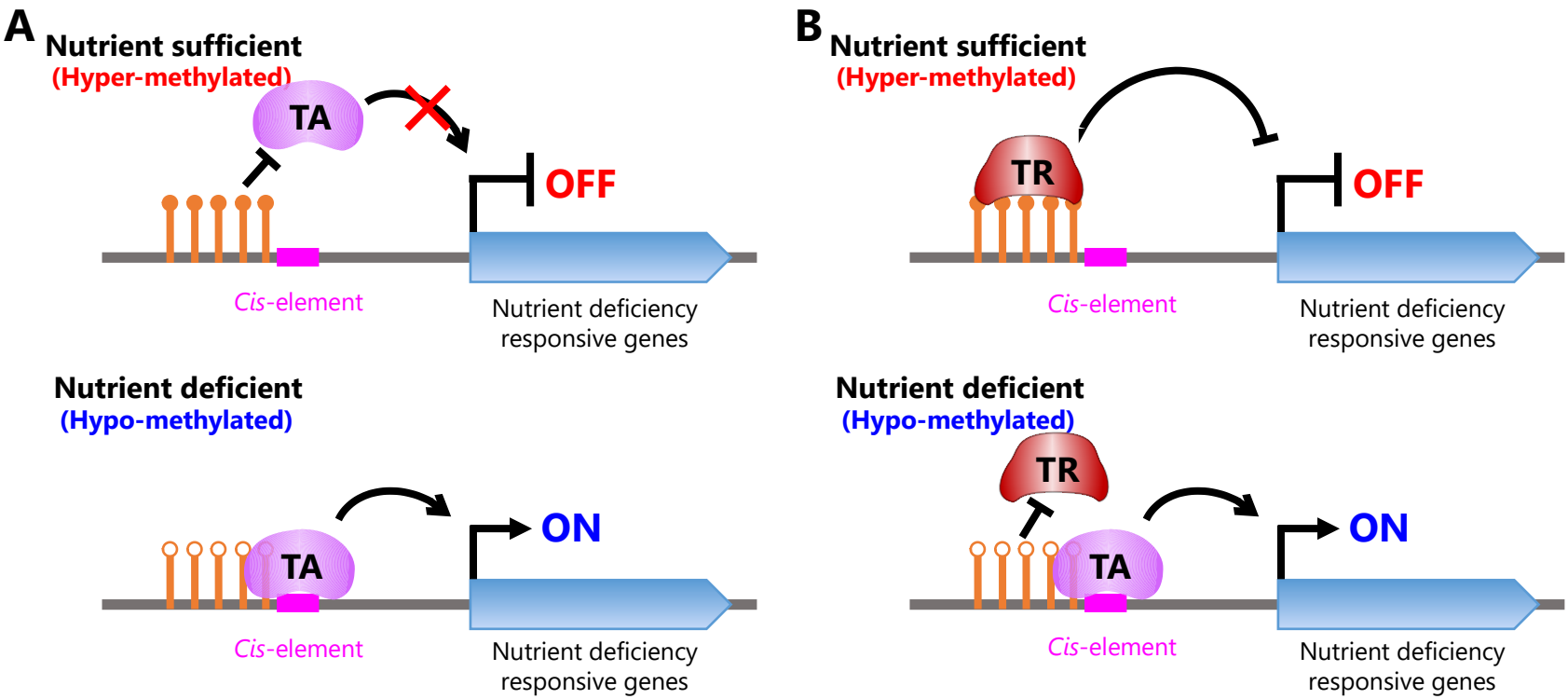

Fig. 3. A potential model of dynamic DNA methylation in regulation of gene expression. The nutrient deficiency responsive genes are methylated at the *cis*-element in the promoter under sufficient nutrient condition. The methylation may prevent the binding of the transcriptional activator (TA) [upper panel in (A)] or recruit the transcriptional repressor (TR) [upper panel in (A)] and thus inhibits gene expression. However, under nutrient deficient condition, the hypo-methylated in the promoter allows the binding of transcriptional activator to promote the transcription.