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S-Nitrosylation of nuclear proteins: New pathways in regulation of gene expression

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Abstract

Nitric oxide (NO) is a reactive free radical with pleiotropic function that is not only involved in regulation of plant growth and development, but also in response reactions to biotic and abiotic stressors. It mainly acts by post-translationally modifying proteins. The most important mode of action of NO is protein S-nitrosylation, the covalent attachment of a NO group to the thiol side of protein cysteine. Other major types of NO-dependent modifications are metal nitrosylation and tyrosine nitration. NO can regulate gene expression at different levels. On one side, it can initiate signaling cascades or modify proteins involved in signal transduction pathways. On the other side, redox-sensitive transcription factors can be also targets for S- nitrosylation and NO can also affect redox-dependent nuclear transport of some proteins. This suggests that NO plays a pivotal role in regulating transcription and/or general nuclear metabolism in plants.

Keywords: Protein S-nitrosylation, nuclear proteins, gene expression, posttranslational modification, transcriptional regulation

1. Introduction

Developmental programs and environmental factors affect and/or regulate the gene expression machinery. Because not all genes are active in all cells at all times, the regulation of a proper gene set needs a very precise aligned mechanism. Developmental and environmental signals are perceived by regulatory regions of genes that react by initiating or suppressing gene expression. Inside the regulatory region, the gene promoter contains different sequence elements, which are specific for binding transcription factors and RNA polymerase II to initiate transcription.

Important signaling molecules are reactive oxygen species, salicylic acid, abscisic acid, jasmonate and ethylene – dependent on the stage of development or type of environmental stimuli. Since several years we know that nitric oxide (NO) is also a key player in stress response signaling, but also an important signaling molecule during plant development. Despite the extensive studies on NO function in different processes in plants, the whole picture of NO impact on living cells, including production, activity and metabolism of NO still has to be completed. Different mode of action mechanisms of NO signaling have also been reported in plants. The most studied mode of action of NO is protein S-nitrosylation, the covalent attachment of a NO group to the thiol side of protein cysteine. Protein S-nitrosylation, as a reversible posttranslational modification, can affect protein activity (activation or inhibition), translocation and protein function. In addition, other major types of modifications of NO have been also reported, such as metal nitrosylation or tyrosine nitration (Astier & Lindermayr, 2012; Martinez-Ruiz, Cadenas, & Lamas, 2011; Toledo & Augusto, 2012). Later one is an irreversible reaction of a nitrating agent with a tyrosine residue of a target protein.

NO is able to influence gene expression at multiple levels. Since NO is a diffusible gas, it can be present in all extra- and intracellular spaces, where it easily interacts with the surrounding environment (Figure 1). In this way NO can initiate signaling cascades or modify proteins involved in signal transduction pathways, which results in altered gene expression. The other possibility of NO to affect gene expression is through a direct regulation of transcription factors or other regulatory elements on the gene promoters. Moreover, the NO-dependent modification of the chromatin

structure, affecting the accessibility of the DNA, is described. These different possibilities and their effect on gene transcription will be discussed in this chapter.

2. Regulation of gene expression via modification of signaling pathways

The first evidence of the signaling function of NO in plants demonstrated the role to mediate defense responses against pathogens (Delledonne, Xia, Dixon, & Lamb, 1998; Durner, Wendehenne, & Klessig, 1998). The underlying connection between NO signals and immune responses was presented by the induction of defense-related genes in several studies (Delledonne et al., 1998; Durner et al., 1998; Feechan et al., 2005; Zeidler et al., 2004). For example, NO donor treatments of tobacco plants or suspension cells induced the expression of pathogenesis-related 1 (PR1) and phenylalanine ammonia lyase (PAL) genes (Durner et al., 1998). This induction of defense genes was a result of NO-mediated signal involving cyclic GMP (cGMP) and cyclic ADP-ribose as a second messengers similarly to mammals. In parallel it was shown that NO promotes the induction of hypersensitive cell death in soybean cells leading to the induction of the protective genes like *PAL* or chalcone synthase (Delledonne et al., 1998). Similarly, lipopolysaccharide (LPS)-induced rapid NO production has been shown to activate various defense or stress-related genes including glutathione S-transferases, cytochrome P450, and PR genes in *Arabidopsis thaliana* (Zeidler et al., 2004). The activated gene expressions were abolished in the NO-deficient *Atnos1* mutant suggests a functional link between LPS-induced NO production and gene induction. The loss-of function mutation of AtGSNOR1 (*gsnor1-3*) provided the first genetic evidence for the crucial role of S-nitrosothiols (SNOs)

in plant defense (Feechan et al., 2005). The *gsnor1-3* plants with elevated SNO levels displayed disease susceptibility (in basal, R-mediated defense and non-host resistance), which correlated with reduced and delayed expression of salicylic acid-dependent PR1 gene. However, enhanced basal resistance against *Peronospora parasitica* Noco2 (oomycete) was detected using antisense GSNOR plants, which accompanied by constitutive activation of PR-1 (Rusterucci, Espunya, Diaz, Chabannes, & Martinez, 2007). Systemic immunity has been also shown to be affected by SNO/NO in a concentration dependent manner (Espunya, De Michele, Gomez-Cadenas, & Martinez, 2012; Wang et al., 2014). Salicylic acid (SA) as an immune activator coordinates gene transcription networks to induce defense signaling. The central player in the SA-dependent response is the non-expresser of pathogenesis-related genes 1 (NPR1) protein. The function of NPR1 is regulated by different factors at multiple levels in response to pathogens (Pajerowska-Mukhtar, Emerine, & Mukhtar, 2013). As a redox-regulated protein, NPR1 undergoes conformational changes dependent on the redox environment, S-nitrosylation or on the activity of thioredoxin proteins (Mou, Fan, & Dong, 2003; Tada et al., 2008). S-Nitrosylation of cysteine residue-156 facilitates oligomer formation of NPR1 as an inactive form of protein to maintain protein homeostasis in the case of pathogen challenge, while the reduction of oligomer to the active monomer is catalyzed by thioredoxins (TRX-H5 and TRX-H3) (Tada et al., 2008). Then, the monomeric form of NPR1 is translocated to the nucleus, where as a transcriptional co-activator interacts with the TGACG motif binding factor (TGA) family of basic domain/Leu zipper (bZIP) transcription factors and regulates their DNA-binding activity to induce a set of defense genes (Despres et al., 2003; Lindermayr, Sell, Muller, Leister, & Durner, 2010). The connection between NO signaling and the gene transcription was demonstrated by the GSNO-mediated S-nitrosylation of TGA1 transcription factor, which enhanced its DNA-binding activity in the presence of NPR1 (Lindermayr et al., 2010). Underlying this positive effect, GSNO was shown to induce expression of PR genes resulting an induced resistance of *Arabidopsis thaliana* against *Pseudomonas syringae* (Kovacs, Durner, & Lindermayr, 2015). The NO/GSNO signaling was linked to glutathione, a major thiol compound, which concentration is crucial to maintain redox balance in the

cell. The NO/GSNO-induced induction of GSH level resulted in an increase in free SA and consequently in the induction of SA-dependent defense genes (Kovacs et al., 2015).

Similarly, increasing evidences indicate an important role of NO in response to abiotic stressors in plants including drought, salt, heat and cold stress (Corpas et al., 2011; Yu, Lamattina, Spoel, & Loake, 2014). Several candidate proteins for S-nitrosylation have been identified by proteomic studies (Abat & Deswal, 2009; Camejo et al., 2013; Lin et al., 2012; Puyaubert, Fares, Reze, Peltier, & Baudouin, 2014; Tanou et al., 2012); however, the connection to the change of gene expression is less known. Exogenous application of NO resulted in increased cold tolerance in various plants including wheat, maize and tomato (Neill, Desikan, & Hancock, 2003). Furthermore, NO was shown to be produced rapidly following cold exposure on a nitrate reductase (NR)-dependent manner in *Arabidopsis* (Cantrel et al., 2011; Zhao, Chen, Zhang, & Zhang, 2009). Transcriptomic data have revealed that low temperature induces a complex response by reprogramming of gene expression in plants to adapt to cold stress, which includes a high number of transcription factors (Thomashow, 2010). Impaired NO level in the *nia1nia2* NR mutant and in *Arabidopsis* plant overexpressing the non-symbiotic haemoglobin 1 (AHb1) inhibited the expression of specific cold-responsive genes, like a C-repeat-binding factors *CBF1* and *CBF3* (Cantrel et al., 2011). They are members of the AP2/ERF family of transcription factors binding to the CRT/DRE regulatory element in the promoter region of cold-responsive genes. The expression of CBF target genes, like Cold Regulated 15a, Low Temperature Induced gene 30 and 78, were also compromised in NO-deficient plants exposed to cold stress and indicates that NO regulates gene expression through a CBF-dependent pathway in response to low temperature (Cantrel et al., 2011). Salinity composes a major problem in the agriculture worldwide. Pretreatment with NO donor sodium nitroprusside (SNP) or H_2O_2 showed enhanced tolerance to salt stress in citrus plants and 49 S-nitrosylated proteins were identified in response to salinity (Tanou et al., 2009). Moreover, transcriptional study of NO-related genes in leaves and roots from citrus plants exposed to salt stress disclosed complex tissue- and time-specific mechanisms regulating NO homeostasis (Tanou et al., 2012). Heat sensitive mutant *hot5* (sensitive

to hot temperature 5) was identified by a forward genetic screen to identify genes involving in thermotolerance (Lee et al. 2008). The characterized mutation in this line corresponded to the gene coding for GSNOR protein and the heat sensitivity of *hot5* alleles was associated with increased NO species. The consequence of the higher endogenous NO level on gene expression in the *hot5* mutant was investigated by microarray analysis (Xue et al. 2013).

2.1 Large scale transcript profiling studies of NO-treated plants

Based on the various effects of NO in response to multiple environmental and developmental processes, a large number of changes in the gene expression profile were expected in plants following exposure to NO. An early transcriptional analysis of NO-induced genes was performed by cDNA-amplification fragment length polymorphism (AFLP) technique from *Arabidopsis* infiltrated with NO donor SNP (Polverari et al., 2003). Altered expression of 120 cDNA could be observed in genes involving in signal transduction, disease resistance, ROS generation and removal, photosynthesis, cellular trafficking, and basic metabolism. A cDNA microarray experiment on *Arabidopsis* cell culture treated with NO donor NOR-3 has revealed several defense-related and antioxidant genes, that were regulated by NO (Huang, von Rad, & Durner, 2002). Among these genes, the alternative oxidase 1a (AOX1) showed a highest expression level, which could be suppressed by removal of NO adding the NO scavenger cPTIO. The NO-induced activity of AOX1 protein has been shown to play a role in NO tolerance of *Arabidopsis* (Huang et al., 2002). The first large-scale whole genome microarray analysis was performed on *Arabidopsis* plants treated with 0.1 mM and 1 mM NO donor SNP (Parani et al., 2004). 342 upregulated and 80 downregulated genes were identified following SNP treatment, 162 of them showed dose-dependent induction to SNP. Interestingly, 10 % of the NO-regulated genes encoded transcription factors, like members of ethylene response factor (ERF) family, WRKY-type transcription factors, zinc finger proteins and Myb-related transcription factors. Additionally, genes involved in cellular detoxification, plant

defense, signal transduction, and biosynthesis of ethylene, jasmonic acid, lignin and alkaloids were differentially expressed illustrating diverse biological function of NO in plant. The NO-responsive genes from early studies have been discussed in details in regard to stress responses induced by wounding, plant-pathogen interaction and to non-stress related pathways like flowering, symbiosis and iron homeostasis (Besson-Bard et al., 2009; Grun, Lindermayr, Sell, & Durner, 2006). The impact of the cross-talk between NO and H_2O_2 on gene expression was studied by cDNA-AFLP analysis in catalase-deficient tobacco plant (CAT1AS) treated with NO donor SNP (Zago et al., 2006). The enhanced H_2O_2 levels induced by high light exposure strongly potentiated the NO-mediated cell death in CAT1AS plants, but not in WT leaves. However, only 16 transcripts were identified whose induction were dependent on the presence of both H_2O_2 and NO. Among them were ethylene and inositol pathway elements. Thirty-six transcripts induced specifically by NO were involved in signal transduction, defense responses and metabolism processes. Similarly, the role of NO in the O_3-induced cell death was investigated in a study using a whole genome microarray of SNP-treated and O_3-treated *Arabidopsis* plants (Ahlfors, Brosche, Kollist, & Kangasjarvi, 2009). Most of the SNP-regulated genes were also regulated by O_3 in the same manner and the gene enrichment analysis showed over-represented genes involved in various biotic and abiotic stress or hormone signaling. The combination of SNP and O_3 treatment resulted in a reduced gene expression of SA- and defense-related genes in relation to the O_3-induced expression values. The enhancement of the O_3-induced cell death phenotype by NO, together with the attenuated gene expression levels, indicated that NO could directly affect the cell death program. A cDNA-AFLP analysis-based study revealed 999 NO-responsive genes from *Medicago truncatula* roots treated with two NO donor SNP and GSNO, which were used to monitor gene expression changes under pathogenic and symbiotic conditions (Ferrarini et al., 2008). Interestingly, the comparison of the transcriptional response to the different NO donors in leaves and in roots indicated only a low correlation between the two NO donor treatment in the same organ. This is probably due to the different nature of the reactive nitrogen molecules released by NO donors, like NO^+ by SNP and NO^- by GSNO. Moreover, the by-products after NO

release are different. The authors suggested that these differences need to be considered by the interpretation of the NO-responsive genes. Moreover, a combined treatment with NO donors and NO scavengers as control is suggested.

A robust change in the abundance of NO-related genes has been detected during hypersensitive response or in symbiotic nodule formation in *Medicago truncatula* underlying the significant role of NO in these processes (Ferrarini et al., 2008). A root specific microarray experiment was performed in *Arabidopsis* plant treated with SNP (Badri et al., 2008). Only 87 regulated genes were identified from root tissues belonged to similar functional categories as classified in the previous studies; however the comparison of microarrays from root treated with SA or JA showed a few overlapping genes regulated commonly by NO, SA and JA. GSNO-responsive genes were identified in the large-scale study by RNA sequencing (RNA-seq) from roots and leaves of *Arabidopsis* plant treated with 1 mM GSNO (Begara-Morales et al., 2014). All together 3263 genes were regulated by GSNO including WRKY and MYB family transcription factors and genes participate in disease resistance. Moreover, genes related to abiotic stress like wounding, heat and oxidative stress also responded to GSNO. Interestingly, several non-coding miscellaneous RNAs were identified from root in response to GSNO treatment, suggesting the role of NO in controlling the level of mRNAs through chromatin remodeling and silencing processes. The impact of endogenous NO on the gene transcription was investigated in a GSNOR null mutant (*hot5-2*) (Xu, Guerra, Lee, & Vierling, 2013), which contains higher level of S-nitrosothiols than WT plant (Feechan et al., 2005; Lee, Wie, Fernandez, Feelisch, & Vierling, 2008). A microarray analysis has identified 99 up- and 170 down-regulated genes, which were enriched in “Stress Response”, “Redox”, and “Signaling” categories. Six members of ROXY-class glutaredoxins related to redox signaling and three basic helix-loop-helix (bHLH) transcription factors playing role in iron homeostasis were upregulated. In correlation with pathogen sensitivity of GSNOR null mutant, 56 of 170 down-regulated genes could be linked to pathogen responses (Xu et al., 2013).

A bioinformatics approach was performed to analyze the promoter elements of genes that co-expressed in response to NO (Palmieri et al., 2008). Using the microarray data of NO-treated *Arabidopsis* plants and cell culture, eight families of transcription factor binding site (TFBS) were identified that were at least 15% more enriched in the promoter region of NO-regulated genes (Palmieri et al., 2008). WRKY-, GBOX-, OCSE-, L1BX-, MYCL- and OPAQ-elements were overrepresented in the up-regulated genes, while TBPF- and MIIG-motifs were detected with a higher rate among the down-regulated genes. The GBOX-, OCSE-, and OPAQ-elements contain a core motif for basic region/leucine zipper motif (bZIP) transcription factors, which are involved in biotic and abiotic signaling and in different developmental processes in plants (Schutze, Harter, & Chaban, 2008). Similar to bZIP family members, genes of WRKY family members have been also reported to be regulated by different NO donors, suggesting that NO might directly affect transcription factors or transcriptional regulators probably by S-nitrosylation. However, an indirect regulation of gene expression via modification of signaling pathway cannot be excluded.

2.2 Protein S-nitrosylation-mediated nuclear translocation

NO can influence protein activity, localization and thereby induce or inhibit downstream signaling pathways. S-Ntrosylation of the cytosolic glycolytic enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) resulted in inhibition of protein activity *in vitro* (Holtgrefe et al., 2008; Lindermayr, Saalbach, & Durner, 2005). Two cysteine residues (Cys-155 and Cys-159) were identified by mass spectrometry to be S-nitrosylated, which were located in the active center of *Arabidopsis* cytosolic GAPDH (Holtgrefe et al., 2008). Interestingly, the cytosolic isoforms of GAPDH has been found also in the nucleus and recently has been shown to localize to the plasma membrane and the endomembrane system (Henry, Fung, Liu, Drakakaki, & Coaker, 2015; Vescovi et al., 2013). *In vivo* evidence for S-nitrosylation was presented in tobacco BY-2 cells exposed to salt stress, which transiently increased the S-nitrosylation level of GAPDH (Wawer et al., 2010). Two tobacco isoforms

of GAPDH (NtGAPCa and NtGAPCb) interact with the *Nicotiana tabacum* osmotic stress-activated protein kinase (NtOSAK) either in the cytosol (NtGAPCa) or in the cytosol and the nucleus (NtGAPCb). Although, S-nitrosylation of GAPDHs did not influence their interactions, cysteine mutations in the site for S-nitrosylation abolished the nuclear localization of the NtOSAK/NtGAPCb complex (Wawer et al., 2010). Similar mechanisms for nuclear translocation of GAPDH in response to different stressors have been also reported in animal field (Tristan, Shahani, Sedlak, & Sawa, 2011). Stress-induced S-nitrosylation of GAPDH allowed the interaction with an E3 ubiquitin ligase Siah1, leading to nuclear translocation of SNO-GAPDH-Siah1 complex. Then, the stabilized SNO-GAPDH-Siah1 complex facilitated the degradation of the nuclear co-repressor N-CoR resulting cell death (Hara et al., 2005). Further study showed that SNO–GAPDH in the nucleus is able to transnitrosylate nuclear proteins, like the deacetylating enzyme sirtuin-1, the histone deacetylase-2 and the DNA-activated protein kinase (DNA-PK) (Kornberg et al., 2010). By these unique mechanisms SNO-GAPDH can regulate downstream gene expression changes under different stress conditions. Another glycolytic enzyme, cytosolic aldolase 2 (cALD2), was identified by proteomic study of S-nitrosylated proteins from *Arabidopsis* (Lindermayr et al., 2005). S-Nitrosylation by GSNO or SNP and S-glutathionylation by GSSG was demonstrated to inactivate cALD2 enzyme *in vitro* (van der Linde et al., 2011). Similarly to GAPDH, cytosolic ALD2 was also detected in the nucleus in plant and in various animal tissues (Saez & Slebe, 2000; van der Linde et al., 2011). Interestingly, GAPDH and cALD2 were both identified by a yeast one hybrid technique as an interaction partner of the gene coding for NADP-malate dehydrogenase (Hameister et al., 2007).

3. **Regulation of gene expression via modification of transcription factors**

Despite the growing number of identified target proteins for S-nitrosylation in different organisms, still only very few examples are known, where NO directly regulates transcription factors and consequently gene expression. In *E. coli* for example endogenous S-nitrosylation induced by

anaerobic respiration was shown to activate the transcription factor OxyR resulting in induction of genes involved in protection of endogenous nitrosative stress. Moreover, aerobic condition-induced oxidative stress was shown to activate OxyR by oxygen-dependent thiol modifications. Interestingly, the NO- and oxygen-dependent mechanisms resulted in induction of distinct regulons and thereby activate different sets of genes (Seth, Hausladen, Wang, & Stamler, 2012). More evidences for a direct regulation of gene expression by NO are reported in mammals. Several transcription factors (e.g. NF-κB, hypoxia-inducible factors, tumor suppressor p53, or different zinc finger transcription factors) were identified to be modulated by S-nitrosylation with a consequent effect on gene transcription (Sha & Marshall, 2012). In higher plant, the immune coactivator NPR1 is involved in NO signaling (as discussed above) and controls the transcription of SA-dependent defense genes by interacting with a TGA1 transcription factor in the nucleus. The TGA transcription factors are members of a bZIP family and some core elements for DNA binding of these proteins were shown to be enriched in the NO-regulated genes (Palmieri et al., 2008). Two cysteine residues of TGA1 (Cys-260 and Cys-266) form an intramolecular disulfide bridge under oxidizing condition, that prevents its interaction with NPR1 (Despres et al., 2003). Furthermore, TGA1 was reported to be S-nitrosylated and S-glutathionylated at Cys260 and Cys266 and this posttranslational modification increased the DNA binding activity of TGA1 to the activation sequence-1 (as-1) element located in the promoter of several defense-related genes (Lindermayr et al., 2010). The DNA binding activity of TGA1 was increased further in the presence of NPR1. The authors suggested that S-nitrosylation of TGA1 might protect the protein from an oxidative modification and allow a more efficient NPR1-TGA1 interaction. The plant R2R3 MYB domain proteins are redox-regulated transcription factors containing 2 cysteine residues that need to be reduced for their transcriptional activity (Heine, Hernandez, & Grotewold, 2004). S-Nitrosylation of the Cys-53 by SNP and GSNO inhibited the DNA binding activity of AtMYB2 transcription factor from *Arabidopsis* provided the first *in vitro* evidence for the direct regulation of the activity of MYB transcription factors by NO (Serpa et al., 2007). Similar inhibition triggered by S-nitrosylation at the same Cys-53 position was demonstrated in the

case of AtMYB30 (Tavares et al., 2014). AtMYB30 is a positive regulator of plant defense and hypersensitive responses to activate genes related to the lipid biosynthesis pathway (Marino et al., 2013). In the non-infected plants, MYB30 was shown to interact with an E3 ubiquitin ligase MIEL1, which directed it to proteasomal degradation. After bacterial infection, repression of MIEL1 resulted in an accumulation of MYB30 and the execution of the hypersensitive response to arrest bacterial growth. To study the interplay between ubiquitination and S-nitrosylation of MYB30 in response to cell death can provide evidences in the future for the cross-regulation of different posttranslational modifications. A new mechanism based on targeted degradation of group VII ERF transcription factors via the N-end rule pathway was recently proposed to sense NO *in vivo* in plant (Gibbs et al., 2014). This small group of ERF transcription factors was found to be destabilized by NO through the N-end rule pathway of proteolysis, while they were stabilized in the lack of NO. The authors proposed a mechanism that regulates seed germination by group VII ERFs through the regulation of the Abscisic Acid Insensitive 5 transcription factor indicating a crosstalk between NO and ABA. Similar effect of NO on the stability of transcription factor was described in response to iron deficiency. The basic helix-loop-helix Fer-like Fe Deficiency Induced Transcription Factor (FIT) controls iron uptake regulating downstream gene expressions, like Ferric Reductase Oxidase2 and Iron-Regulated Transporter 1 (Meiser & Bauer, 2012). FIT protein was shown to be a subject for proteasomal degradation, however NO could counteract on FIT turnover (Meiser, Lingam, & Bauer, 2011). Moreover, FIT interacts with the ethylene-dependent transcription factor EIN3 that stabilized FIT protein providing a link between iron uptake and ethylene signaling (Lingam et al., 2011). Both signaling component, NO and ethylene was shown to promote the production of each other and to be necessary for upregulation of Fe-acquisition genes (Garcia, Suarez, Romera, Alcantara, & Perez-Vicente, 2011). An overview of S-nitrosylation-medited regulation of gene expression is shown in Figure 2.

4 Regulation of gene expression via modification of chromatin structure

Modifications of DNA and histones, the core components of chromatin, affect the gene expression pattern by alteration of the chromatin structure. Important modifications are cytosine DNA methylation and posttranslational modifications (PTMs), such as acetylation, methylation, phosphorylation, ubiquitinylation, sumoylation, ubiquitination, and ADP-ribosylation at specific amino acid residues on the N-terminal tails of core histones (S. Feng & Jacobsen, 2011; Johnson et al., 2004; Zhang, Sridhar, Zhu, Kapoor, & Zhu, 2007). Genes encoding for DNA and histone modifying enzymes and the regulation of epigenetic processes in plants are extensively reviewed by Pikaard and Mittelsten Scheid (2014).

4.1 NO-dependent regulation of histone acetylation

PTMs play an important role in modification of chromatin structure, because they allow a cell to quickly reply to internal or external appeals. Acetylation of lysine residues of histone tails plays a key role in regulation of gene expression. The transfer of acetyl groups from acetyl-coenzyme A on lysine residues of histone tails is catalyzed by histone acetyltransferases (HATs). This leads to neutralization of the positive charge of the lysine residue and a decreased interaction between histones and negatively charged DNA. This results in a relaxed structure of chromatin making it accessible for transcription factors. Conversely, histone deacetylases (HDACs) remove an acetyl group and are recruited to maintain the chromatin in an inactivated and condensed state (Hollender & Liu, 2008). However, Kurdistani and Grunstein (2003) report that these enzymes are not only part of the transcriptional machinery, but also involved in other chromosomal processes such as DNA replication, repair and heterochromatin formation.

There is increasing evidence that catalytic activity of HDACs can be inhibited by redox-molecules, such as NO resulting in alteration of the chromatin structure (Figure 3). Until now results about redox-regulation of histone modifications are mainly based on research in the human/animal field. A. Nott, Watson, Robinson, Crepaldi, and Riccio (2008) reported that stimuli in neurons induce nuclear accumulation of NO and S-nitrosylation of many nuclear proteins. They also identified, that human HDAC2 is a target of the brain-derived neurotropic factor (BDNF) causing NO synthesis in neurons. It was demonstrated that S-nitrosylation occurs at the cysteine residues, Cys-262 and Cys-274, and doesn't inhibit the deacetylase activity of HDAC2, but causes its release from CREB-regulated gene promoter. This process induces an increase of histone acetylation at neurotrophin-dependent promoter regions and an activation of gene transcription. It was also shown that histone acetylation is inhibited and gene transcription is down-regulated, when Cys-262 and Cys-274 were changed to alanine. Moreover, it was demonstrated that mutated HDAC2 didn't dissociate from gene promoters even after treatment of neurons with NO donors. S-Nitrosylation of HDAC2 was also determined in muscle of dystrophin-deficient MDX mice (Colussi et al., 2008). Evidence was found that enzymatic activity of HDAC2 in muscle cells is impaired by NO, while it remains to be unchanged in neurons, suggesting that S-nitrosylation in muscles occurs at different cysteines then in neurons (Alexi Nott & Riccio, 2009). J. Feng, Jing, Fang, Gu, and Xu (2011) showed that recombinant HDAC8 is S-nitrosylated by NO the donor GSNO *in vitro*. The enzymatic activity of this enzyme is significantly reduced by GSNO and SNO-Cys in a time- and concentration-dependent manner and can be restored by treatment with DTT. Interestingly, another NO-releasing molecule, sodium nitroprusside, has no effect on S-nitrosylation of HDAC8, suggesting that a special structure for transferring of NO is required. Illi et al. (2008) also reported about indirect regulation of class II HDAC by NO. They demonstrated that NO activates PP2A (protein phosphatase 2) which binds to the pCamkIV/ HDACs complex and dephosphorylates it. This process induces the transport of class II HDAC4 and 5 to the nucleus of endothelial cells and deacetylation of histones which in turn leads to the regulation of chromatin folding.

In plants exist three different HDAC families. The largest and widely studied one is homologous to the yeast RPD3. This type of HDACs consists of 12 members that all possess a characteristic histone deacetylase domain, which requires zinc (Zn) ion for a catalytic activity. Based on their structure they can be further divided into three classes. The members of the second family, HD-tuins (HD2), have been originally found in maize and seem to be plant specific proteins, although their homolog *cis-trans* prolyl isomerases are present in other eukaryotes (Aravind, 1998; Lusser, Brosch, Loidl, Haas, & Loidl, 1997). Moreover HD2 proteins show structural differences compared to other HDAC proteins, but display a sequence similarity to the FK506-binding protein. They are composed of three domains: the N-terminal domain which possesses a conserved pentapeptid MEFWG region, required for gene repression activity, a high charged acidic domain and variable C-terminal domain (Dangl M, 2001). Interestingly, six of eight HD-tuins contain a zinc-finger motif on the C-terminus that is probably involved in protein-protein interaction (Ma, Lv, Zhang, & Yang, 2013). The third family of HDACs in plants is represented by unique NAD-dependent SIR2-like proteins. These proteins are not sensitive to any histone deacetylase specific inhibitors such as TSA or sodium butyrate (Hollender & Liu, 2008). So far little is known about the function of sirtuins in plants.

Bourque et al. (2011) demonstrated that type-2 HDACs act as negative regulator of cryptogein induced cell death in *Nicotiana tabacum*. This elicitor is known to trigger NO production (Lamotte et al., 2004) suggesting a connection between NO-signaling and type-2 HDAC function. In a proteomic study of Chaki et al. (2015) 117 nuclear proteins were identified as potential target for NO. Among those four plants specific HDT proteins have been demonstrated to be S-nitrosylated *in-vitro*. So far very little is known about NO regulation of plant HDACs, though. Notably, there is evidence that some members of the RPD3-like family possess highly condensed cysteine residues. Moreover, Cys-262 and Cys-274 of HDAC2, that are shown to be NO-regulated, could be identified in some *Arabidopsis* HDACs. Moreover, Liu, Zhang, Yu, Xiong, and Xia (2015) reports about a redox-sensitive cysteine residue that has been recently identified in *Arabidopsis* HDAC19. Therefore plant histone

deacetylases can be considered as interesting candidates for further studies (Mengel, Chaki, Shekariesfahlan, & Lindermayr, 2013).

4.2 NO-dependent regulation of histone and DNA methylation

Redox processes might also play a role in methylation-dependent regulations of gene expressions. In *Arabidopsis*, non-genic DNA methylation takes place in all three sequence contexts (CG, CHG, and CHH, where H = A, T, or C) on transposable elements (TEs) and other repetitive DNA elements resulting in transcriptional silencing. Whereas, CG-sequence gene body methylation is also found in transcribed genes (S. Feng & Jacobsen, 2011).

The key players for cytosine methylation in *Arabidopsis* are four methyltransferases, their antagonizing demethylases, as well as chromatin remodeling ATPases and methyl cytosine binding proteins (Furner and Matzke (2011) and references cited therein). Histone methylation can be associated with transcriptionally active or repressed regions (reviewed in S. Feng and Jacobsen (2011)). In *Arabidopsis,* the following lysine residues on the N-terminal tail of histone H3 are subject to methylation: K4, K9, K27, and K36 (Johnson et al., 2004; Zhang et al., 2007). SET domain proteins are responsible for histone lysine methylation, of which 49 are encoded in the *Arabidopsis* genome. Demethylation is catalyzed by two classes of proteins: the lysine-specific histone demethylase-LIKE proteins (LDL), and the Jumonji C (JmjC)-domain containing proteins (Pikaard & Mittelsten Scheid, 2014).

Until now, S-nitrosylation of DNA or histone methyltransferase or demethylase has not been reported in plants. However, based on the studies on human JmjC-domain containing demethylase KDM3A (Hickok, Vasudevan, Antholine, & Thomas, 2013), Fe(II)-dependent plant JmjC-domain proteins might be targets for metal nitrosylation by the formation of a nitrosyliron complex with the non-heme Fe(II) coordinated by a 2-histidine-1-carboxylate facial triad in their catalytic pocket.

Further, the application of the NO donor SNP causes DNA hypomethylation (mainly in CHG sites) in *Orzya sativa L. Spp Japonica* cultivars going along with altered expression of chromatin remodeling and DNA methylation modifying enzymes (Ou et al., 2015). To this end, DNA methylation might be regulated via differential expression of DNA methyl modifying proteins rather than by their inhibition through NO-based PTMs.

Apart from that, NO might be an issue in the supply of S-adenosylmethionine (SAM), the major methyl donor, and in the removal of the by-product inhibitor S-adenosylhomocysteine (SAH) of transmethyl reactions. DNA and histones are subjected to methylation by specific S-adenosylmethionine (SAM)-dependent methyltransferases (MTs). In each methyltransfer reaction SAH is formed, which is further converted into homocysteine (Hcy) and adenosine (Ado) by S-adenosylhomocysteine hydrolase (SAHH). The equilibrium of this reversible reaction favoring SAH synthesis (de la Haba & Cantoni, 1959; Palmer & Abeles, 1976) is driven towards hydrolysis of SAH due to removal of its products by down streaming enzymes (Poulton & Butt, 1976). Methionine synthase converts Hcy to methionine, which is in turn adenylated to SAM by S-adenosylmethionine synthetase (SAMS) and Ado is metabolized in the adenosine salvage cycle. The levels of SAM and SAH are considered to be important regulators of cellular methylation processes. Interestingly, several proteomic studies revealed key enzymes of the methylation cycle as targets for S-nitrosylation (Abat & Deswal, 2009; Hu et al., 2015; Lindermayr et al., 2005; Puyaubert et al., 2014): Cobalamin-independent methionine synthase (MT), methionine s-adenosyltransferase (SAMS), and S-adenosylhomocysteine hydrolase (SAHH). In *Arabidopsis* different SAMS isoforms exist, which are differentially inhibited by protein S-nitrosylation (Lindermayr, Saalbach, Bahnweg, & Durner, 2006). Only the isoform SAMS1, but not SAMS2 or SAMS3 was reversibly inhibited by GSNO. It was demonstrated, that S-nitrosylation of the Cys-114 of SAMS1, which is located next to the catalytic center as part of the active site loop, is responsible for inhibition. A similar differential regulation of SAMS activity was observed in mammals. Here two genes encode different SAMS isoforms. While SAMS1A is reversibly inactivated by NO, SAMS2A is not affected (Perez-Mato, Castro, Ruiz, Corrales,

& Mato, 1999). All these results suggest that NO plays a regulatory role in the synthesis of the major methyl-group donor in the cell.

In *Arabidopsis* two genes encode SAHC isoforms, but only SAHC1 is supposed to play a role DNA-methylation processes (Rocha et al., 2005; Vriet, Hennig, & Laloi, 2015). Beside S-nitrosylation, tyrosine nitration has been observed in SAHC of sunflower *(Helianthus annuus L.)*. This type of modification decreased the activity of this enzyme (Chaki et al., 2009). S-Nitrosylation of *Arabidopsis* SAHC1 upon cold stress was reported, but the physiological consequence of cold stress induced S-nitrosylation of SAHC1 is not yet investigated (Puyaubert & Baudouin, 2014; Puyaubert et al., 2014). Previous studies demonstrated the importance of SAHC activity towards chromatin modifications (reviewed in Pikaard and Mittelsten Scheid (2014) and Vriet et al. (2015)): Mutations in the *AtSAHC1* gene lead to reduced cytosine methylation and the release of transcriptional gene silencing (Jordan, West, Bottley, Sheikh, & Furner, 2007; Mull, Ebbs, & Bender, 2006; Rocha et al., 2005). Further, the expression of antisense RNA of SAHC in tobacco plants resulted in a loss of DNA methylation in repetitive elements (Tanaka et al., 1997) and the application of the SAHC inhibitor dihydroxypropyladenine (DHPA) reduces levels of DNA and histone methylation at endogenous repeats in *Arabidopsis* (Baubec et al., 2010). In sum, SAHC plays an important role in chromatin modification (Baubec et al., 2010) and NO-dependent regulation of this enzyme might be an important mechanism to regulate gene expression.

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Figure legends

Figure 1. NO-signaling in response to biotic and abiotic environmental stressors. Biotic or abiotic stress induced NO production affects gene expression and results in stress response.

Figure 2. S-Nitrosylation-mediated regulation of gene expression. S–Nitrosylation of proteins can change a signaling pathway and /or results in protein translocation (A, B). Moreover, NO can directly modify DNA binding proteins (C). Both results finally in transcriptional changes. SH: thiol group, SNO: S-nitrosothiol, dashed arrow: signaling pathways are affected.

Figure 3. Histone acetylation/deacetylation alters chromatin structure. Acetylation of histones catalyzed by HATs results in opening chromatin and making it accessible for initiation of gene transcription. Conversely, deacetylation catalyzed by HDACs leads to maintain chromatin the closed structure. NO might affect HDACs activity, resulting in the hyperacetylation of lysine residues of

histone tails and a loosen chromatin structure. HAT: Histone acetyltransferase, HDAC: Histone Deacetylase, blue: Histone complexes.

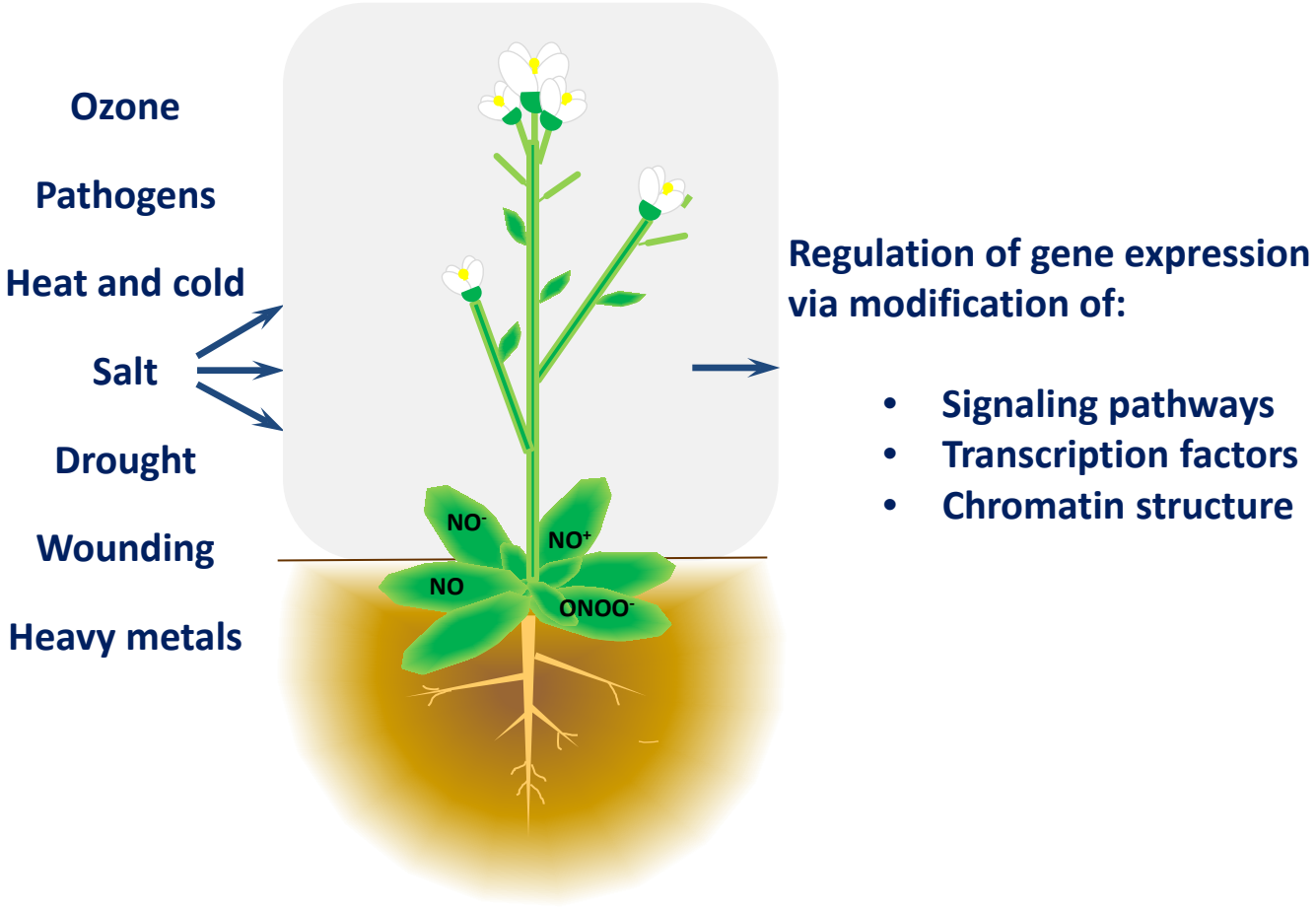
Ozone
Pathogens
Heat and cold
Salt
Drought
Wounding
Heavy metals
NO-
NO+
NO
ONOO-
Regulation of gene expression via modification of:
• Signaling pathways
• Transcription factors
• Chromatin structure

Figure 1

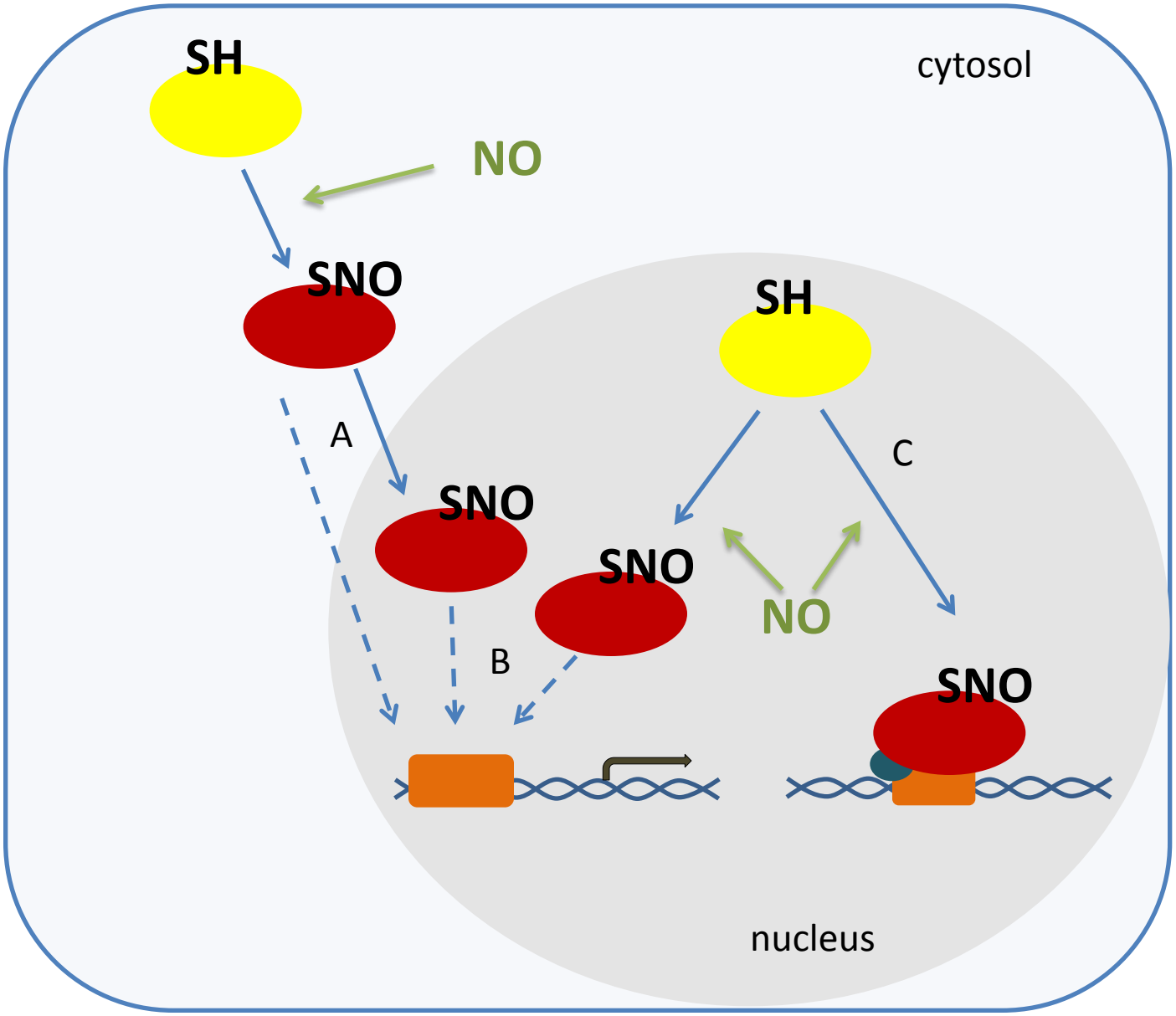

Figure 2

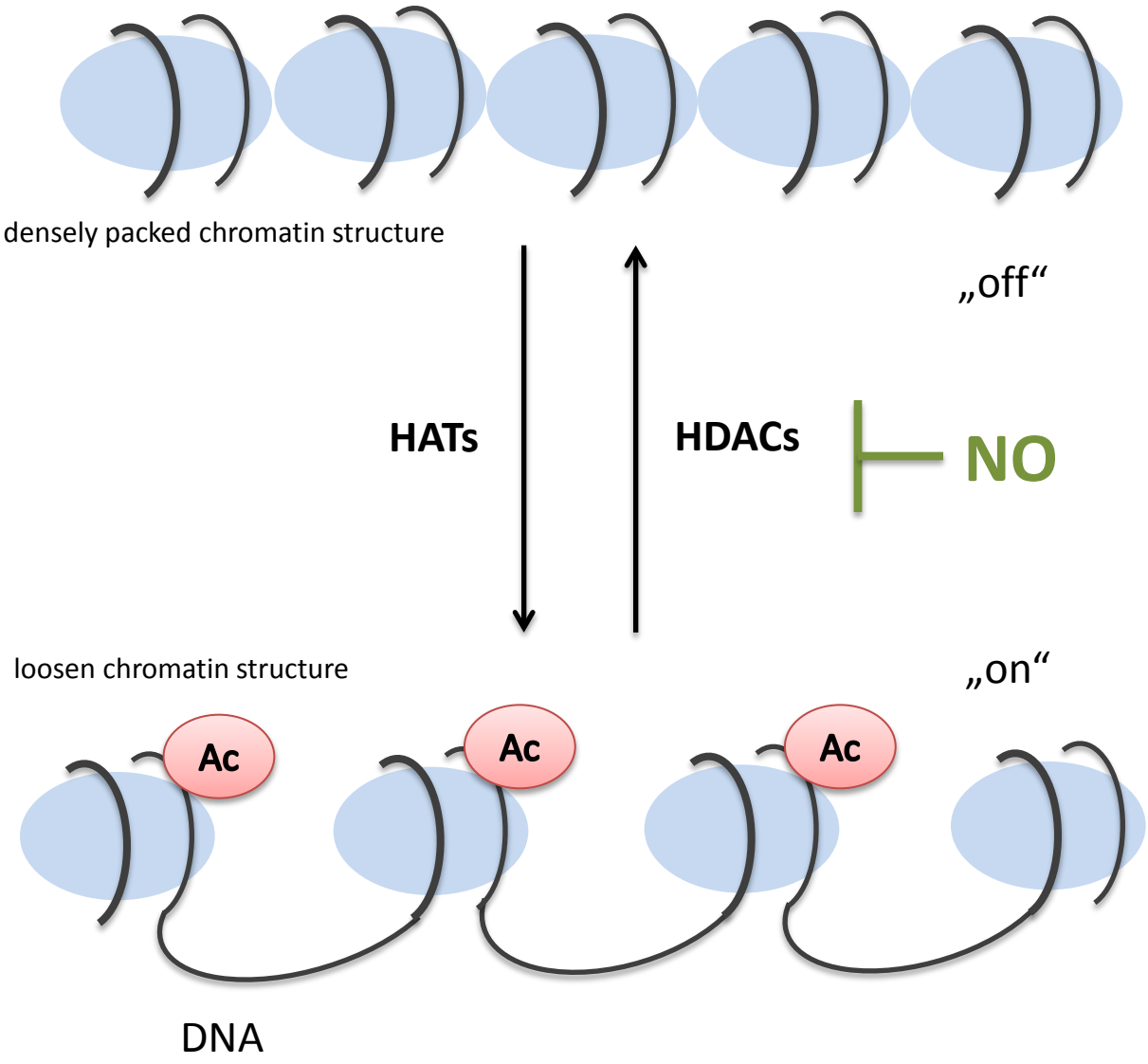
densely packed chromatin structure
„off“
HATs
HDACs
NO
loosen chromatin structure
„on“
Ac
Ac
Ac
DNA

Figure 3