Genome-wide nucleosome dynamics under heat stress in *Arabidopsis thaliana*

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Kashif Nawaz

aus Dhanbad, India

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Prüfungsvorsitzender:	Prof. Dr. Martin Hülskamp
Berichtstatter:	Prof. Dr. George Coupland Prof. Dr. Stanislav Kopriva

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ABSTRACT

Heat is a common stress, causing many changes in plant physiology and growth, leading to major economic losses in crops. There is growing evidence that heat stress also affects chromatin architecture. However, genome-wide patterns of these changes and its relationship with transcription of genes are poorly understood. Previously, it was shown that under heat stress nucleosomes are depleted prior to, and strongly enriched at the transcription start site (TSS) of heat shock protein coding genes in *Arabidopsis thaliana*. Aim of this work is the analysis of changes in nucleosome occupancy under ambient, heat stress and post-stressed (recovery) conditions in *Arabidopsis thaliana*. Therefore, a genome-wide map of nucleosome positions was generated using the approach of Micrococcal nuclease sequencing (MNase-seq). This revealed that unlike intergenic region, nucleosomes are abundantly present in genic regions and further, are more prominent in exons than introns. Some of these signals were very strong, indicating precise nucleosome positioning at specific genes over many plant tissues in *Arabidopsis*. Further observations, have shown substantial changes in the nucleosome occupancy including both nucleosome gain and loss in response to heat-stress. In particular, loss of nucleosome occupancy has been observed around TSS region of genes, which were highly transcriptionally up-regulated during heat stress response. The opposite, but weaker, trend was observed in strongly down-regulated genes, showing gain in nucleosome occupancy. In conclusion, this study suggests a correlation between the nucleosome occupancy and expression of heat stress responsive genes.

ZUSAMMENFASSUNG

Hitze ist eine häufige Belastung, die viele Veränderungen in der Pflanzenphysiologie verursacht und zu großen wirtschaftlichen Einbußen bei Pflanzen führt. Es gibt zunehmend Hinweise darauf, dass Hitzestress auch die Chromatinarchitektur beeinflusst. Genomweite Muster dieser Veränderung und ihre Beziehung zur Transkription von Genen sind jedoch kaum verstanden. Zuvor wurde gezeigt, dass unter Hitzestress Nukleosomen vor der Transkriptionsstartstelle (TSS) von Hitzeschockprotein-kodierenden Genen in *Arabidopsis thaliana* abgereichert und stark angereichert werden. Innerhalb dieser Arbeit sollten die Veränderungen der Nukleosomenbelegung im Genom von *Arabidopsis thaliana* unter Normalbedingungen, Hitzestress und Post-Stress Erholunguntersucht werden. Dabei wurden genomweite Karten von Nukleosomenpositionen mit Hilfe des Micrococcal Nuclease Sequencing (MNase-seq) Ansatzes generiert. Dies zeigte, dass Nukleosomen im Gegensatz zu Bereichen zwischen den Genen, in den genischen Regionen reichlich vorhanden sind und in Exons mehr hervortreten als in Introns. Einige dieser Signale waren sehr stark, was auf eine genaue Positionierung der Nukleosomen an spezifischen Genen gegenüber vielen Pflanzengeweben in *Arabidopsis* hinweist. Als Reaktion auf Hitzestress konnten wesentliche Veränderungen in der Nukleosomenbelegung, einschließlich der Verstärkung und des Verlusts von Nukleosomen beobachtet werden. Insbesondere wurde ein Verlust der Nukleosomenbelegung um die TSS-Region herum beobachtet, die den Genen entspricht die während Hitzestress stark transkriptionell hochreguliert waren. Eingegenteiliger, aber schwächere Trend wurde in stark herunterregulierten Genen beobachtet, was einen Anstieg der Nukleosombelegung zeigte. Zusammenfassend zeigt diese Studie eine Korrelation zwischen der Nukleosomenbelegung und der Expression entsprechender hochregulierter Gene.

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LIST OF ABBREVIATIONS

5S rDNA	5S ribosomal DNA
A	Adenine
Arg/R	Arginine
bp	Basepair
C	Cytosine
Cen-H3	Centromere-specific histone H3
Chr/chr	Chromosome/chromosome
DE	Differentially expressed
DNA	Deoxyribonucleic acid
fastq files	Specialized file containing sequencing reads
FPKM	Fragments per kilobase of transcript per million mapped reads
G	Guanine
GCN5 HAT	Histone acetyltransferase GCN5
H	A, C or T
H1	Histone H1
H2A	Histone H2 subunit A
H2A.B	H2A histone family, member B
H2A.W	H2A histone family, member W
H2A.X	H2A histone family, member X
H2A.Z	H2A histone family, member Z
H2B	Histone H2 subunit B
H3	Histone H3
H3.1	H3 histone family, member 1
H3.2	H3 histone family, member 2
H3.3	H3 histone family, member 3
H4	Histone H4
HS	Heat stressed
HSEs	Heat shock elements
HSFs	Heat shock factors
HSGs	Heat shock genes
HSPs	Heat shock proteins
Kb	Kilobase
log10FC	log10 fold change

log2FC	log2 fold change
Lys/K	Lysine
Mb	Megabase
MNase	Micrococcal Nuclease
MNase-seq	Micrococcal nuclease sequencing
MPIPZ	Max Planck Institute for Plant Breeding Research
NFR	Nucleosome free region
PTMs	Post-translational modifications
p-value/p-val	Probability of rejecting the null hypothesis
p_{adj}/q-val	Adjusted p-value for correction of false positive
RNA	Ribonucleic acid
RNA-seq	RNA sequencing
RSB	Resuspension buffer
SUMO	Small ubiquitin-related modifier
T	Thymine
TAIR	The *Arabidopsis* Information Resource
TSS	Transcription start site
TTS	Transcription termination site

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1. INTRODUCTION

Plants are sessile organisms, and as such are frequently subjected to variety of abiotic and biotic stresses without possibility for escape. For the reason that, unlike animals, plants do not have locomotion ability to escape from unfavorable condition. Most common natural stresses include high/low temperatures, high salinity, water deficit, and bacterial, fungal and animal pathogens [Mittler, 2006; Prasad et al,. 2011; Atkinson eat al., 2013; Narsai et al., 2013; Prasch et al., 2013; Suzuki et al., 2014; Mahalingam, 2015; Pandey et al., 2015; Ramegowda et al., 2015]. Exposure to these stresses provoke an impeding effect on physiological, morphologicaland molecular processes of plants, eventually leading to reduction in fitness and productivity [Hell et al., 2002; Swarbrick et al., 2006; Bolton et al., 2009; Massad et al., 2012; Shao et al., 2008]. Abiotic stresses are one of the major concerns and have huge impact on growth and productions. It has been reported that abiotic stress alone can be collectively responsible for more than 50% growth reduction in most crops [Wang et al., 2003]. In addition, continuous increasing temperature after post-industrialization era causes major crop losses worldwide and the speed of this change seems to be still accelerating, often most severaly in developing countries. As a protection, plants have developed stress-specific response mechanisms, which ultimately help them to adapt morphologically, physiologically, and biochemically and thus survive and reproduce under many unfavorable conditions [Bohnert et al., 1995].

1.1. Effect of heat stress on plants

Heat stress (HS) causes numerous effects on plants. Higher temperature often results in deformation in plant growth, primarily in areal parts, and interference with developmental and physiological processes [Hasanuzzaman et al., 2012, Hasanuzzaman et al., 2013]. The damages vary from mild, such as change in plant respiration rate to adverse at molecular and genetic level. Severity of damage depends upon exposure duration and intensity of temperature. Plant response to HS may vary with species, temperature and exposure time. During HS, cellular damage or cell death may occur very rapidly, which could lead to a dreadful collapse of cellular organizations [Ahuja et al., 2010]. At the molecular level, HS has initial effect on loss of membrane integrity, which eventually leads to disruption in maintaining water balance. Protein denaturation is also one of the most prominent initial changes under HS [Chang et al., 2007]. It has been reported that HS may affect all stages of plant development starting from germination, vegetative growth, organ development, reproduction and finally the yield [Hasanuzzaman et al., 2013, Mittler et al., 2010, Lobell et al., 2011, McClung et al., 2010]. HS has been reported to alter the structure of various proteins,

RNA species and cytoskeletons structures, and remolds the efficiency of various enzymes, which leads to metabolic imbalance [Ruelland et al., 2010, Suzuki et al., 2011, Suzuki et al., 2012, Pagamas et al., 2008].

Most of the morphological, biochemical and physiological changes for adaption against HS conditions occur via modulation and coordination of specific genes linked with the heat sensing and adaptation pathways [Vinocur et al., 2005].

1.2. Heat shock proteins (HSPs): The wizards of heat stress tolerance

Plants are equipped with various heat sensing and response specific genes commonly called "heat shock genes" (HSGs). These genes, encoding heat shock proteins (HSPs), are vital for the survival of plants under HS [Chang et al., 2007]. With rise in temperature, intracellular proteins start changes in the structure and conformations leading to the denaturation. To protect them, HSPs acts as chaperones and provide stability through the process known as protein folding [Baniwal et al., 2004]. There are five major families of HSPs in plants: HSP100, HSP90, HSP70, HSP60 and HSP20 [Swindell et al., 2007; Table-1]. Among them, HSP70 and HSP60 are the most conserved proteins in nature and considered to play a pivotal role in heat tolerance [Kültz et al., 2003]. On the other hand, small HSPs (HSP20), which have low molecular mass of 12-40 kDa have been shown to represent the highest diversity in the structure [Wang et al., 2004, Morrow et al., 2012].

Major HSPs Class	***Functions***
HSP100	ATP-dependent dissociation and degradation of aggregate protein
HSP90	Co-regulator of heat stress linked signal transduction complexes and manages protein folding. Requires ATP for its function
HSP70, HSP40	Primary stabilization of newly formed proteins, ATP-dependent binding and release
HSP60, HSP10	ATP-dependent specialized folding machinery
HSP20 or small HSP (sHSP)	Formation of high molecular weight oligomeric complexes, which serve as cellular matrix for stabilization of unfolded proteins. HSP100, HSP70 and HSP40 are needed for its release

Table 1. Molecular functions of the major classes of heat shock proteins (HSPs) for heat stress tolerance in plant system.

Promoter regions of most HSGs have specific motifs, which act as binding sites for specific transcription factors (TFs). These motifs are known as heat shock elements (HSEs) and the TFs are called heat shock factors (HSFs). It has been shown, that under HS condition, HSGs showed significantly higher gene expression in comparison to ambient condition [Nover et al., 2001]. Also, promoters of the genuine HSGs contain HSEs in one or more copies and present in the form of specific palindromic nucleotide sequence. HSFs recognize these specific elements and subsequently bind to the promoter of such genes which eventually contributes to its regulation [Nover et al., 2001]. Apart from the promoters of HSGs, HSEs are also present in promoters of several other genes and other genomic locations [Guo et al., 2008, Storozhenko et al., 1998] or even in transposable elements (Pietzenuk et al., 2016).

1.3. Chromatin classification, gene regulation and epigenetics

Eukaryotic nuclear DNA is present in the form of chromatin, which is complex of mainly DNA and proteins. This chromatin structure is the result of step-by-step supercoiling of DNA, which requires the assistance of many structural organization and maintenance proteins [Rosa et al., 2013, Wilson, 2002]. Chromatin is present in two basic forms: euchromatin and heterochromatin based on the staining intensity [Heitz, 1928; Bennetzen et al., 2000]. Heterochromatin is dominated by repetitive sequences, repressed genes and silenced transposons and varies from moderate to high stain intensity, while euchromatin (also known as open chromatin) is less intense. High intensity of heterochromatin indicates its tightness of packing. Because heterochromatin is tightly packed, it was believed to be inaccessible to transcription proteins and therefore unable to transcribe the genes present in such region of the genome. Although, several studies showed that some of these genes are transcribed by Pol-IV [Volpe et al., 2002, Hediger et al., 2006, Värv et al., 2010]. Despite of this early classification, recent study also suggested that there more than two chromatin state [Roudier et al., 2011]. In *Arabidopsis thaliana* it is present in four different forms and each marked with different mixture of epigenetic markers [Roudier et al., 2011].

On the basis of helical organization, DNA can be classified in three different forms inside the cell; A, B and Z [Rosalind, 1953, Dickerson, 1992, Harvey, 2015]. They mainly differ in terms of rotation of their helix; A and B are right handedly helically twisted while Z is twisted left handedly [Mitsui et al., 1970]. B-form is the most common DNA form in eukaryotes [Leslie et al., 1980]. Recently, a new kind DNA was reported in human nuclei, known as I-motif DNA [Zeraati et al., 2018]. They organized themselves in such a way by assembling two loops, which leads to formation DNA with four strands [Zeraati et al., 2018]. However, inside the cell A, B and Z DNA are present with histones in the form of nucleosome assembly,

and also without the histones in the form of linker DNA [Szerlong et al., 2011, Bradbury et al., 1989]. Despite the increase in complexity in eukaryotic transcription machinery, transcription of linker DNA is very much similar to prokaryotic DNA, [Hahn, 2004]. In the typical case, RNA polymerase II (RNA Pol II), one of the major components of transcription process, binds to the core promoter region, which activates the whole machinery of transcription. It binds to the defined region of promoter, specifically rich in alternate sequence of adenine and thymine and hence commonly known as TATA box. This activation facilitates the attachment of other important transcription factors (TFs) to the promoter with the assistance of RNA Pol II. The process starts with the binding of RNA Pol II to the core promoter in combination with transcription factor II A (TFIIA), transcription factor II B (TFIIB) and transcription factor II D (TFIID) to form pre-initiation complex [Hahn, 2004]. Transcription factor II H (TFIIH) then denatures about 11 to 15 base long DNA, double strand into two single strands to initiate the transcription at RNA Pol II attachment site. Carboxyl terminus of RNA Pol II is phosphorylated by TFIIH during 30 base elongation process. This leads to the detachment of RNA Pol II from other TFs before the elongation proceeds. After that, RNA Pol II accumulates other factors needed for elongation stage. Transcription process then ends with termination stage [Hahn, 2004].

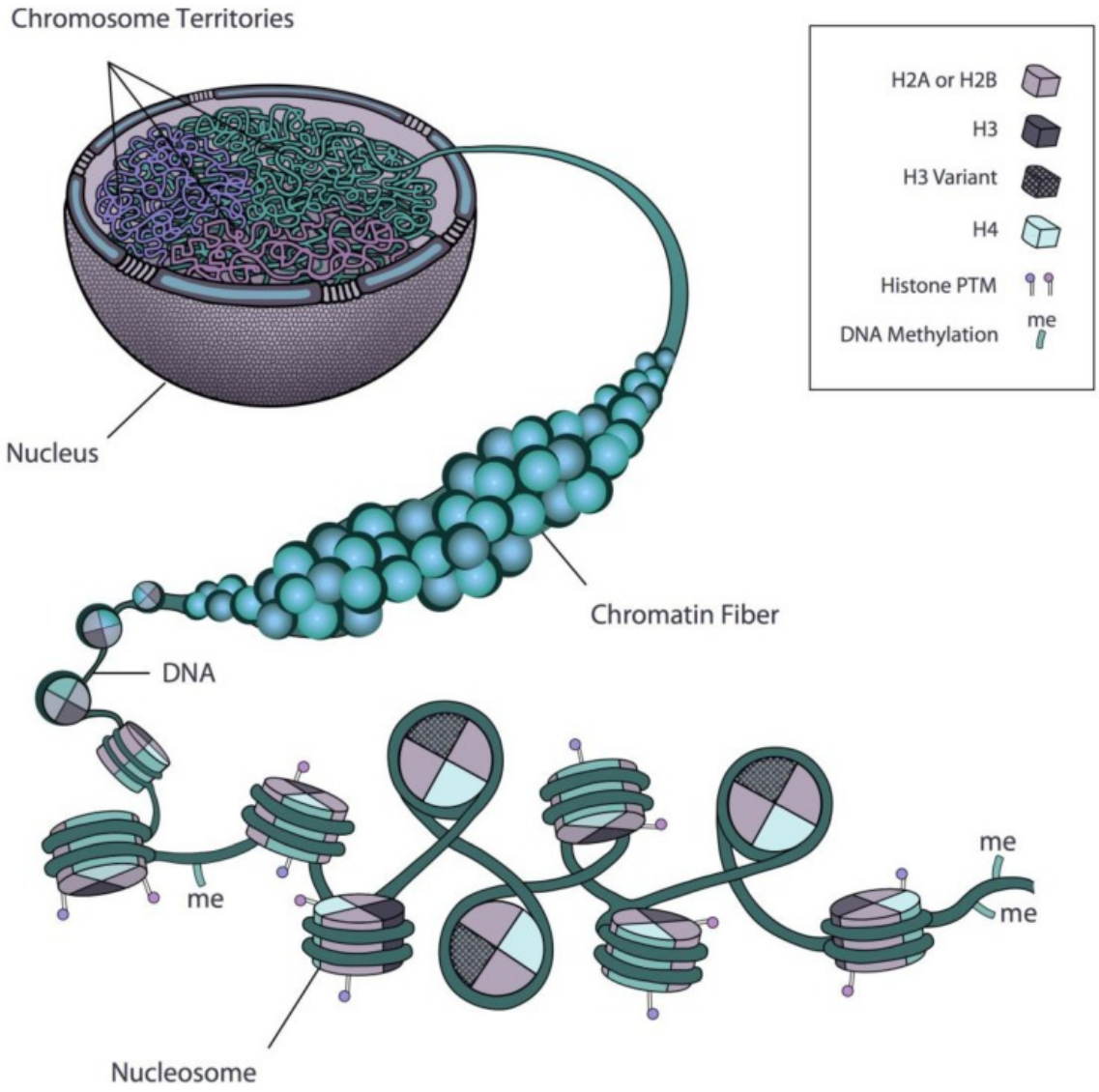


Figure 1. Organization of chromatin and nucleosomes in the cell nucleus. Scheme depicting different means of chromatin regulation. PTM, post-translational modification. Chromosome territories within the nucleus, shown in different colors, are composed of chromatin fibers, which, in turn, contain supercoiled DNA with nucleosomes beads. [Adapted from Rosa et al., 2013]

Epigenetics markers are the features which are not directly governed by DNA sequence [Dupont et al., 2009]. This broadly includes DNA (cytosine) methylation and modifications in histone proteins. Both of them are discussed in detail, later in separate sections. Here, nucleosome is explained first, which is important to understand the histone proteins. Nucleosomes are the basic structural packaging units of the eukaryotic chromatin. A nucleosome consists of eight histone proteins, which includes pair of each H2A, H2B, H3 and H4, generally denoted as octamer. This octamer is wrapped with a stretch of 147 bp (corresponding to 1.67 turn) of double stranded DNA [Rosa et al., 2013] (Figure 1). An additional protein H1, belts the whole nucleosome structure from outside to provide complete

stability to the nucleosome [Rosa et al., 2013] (Figure 2). Nucleosomes are present in repetitive manner throughout the genome, spaced by a linker or naked region of varied length. Length of the linker region varies depending upon the location on the genome (~30 bp) [Szerlong et al., 2011, Bradbury, 1989]. The positioning and maintaining occupancy of the nucleosomes are important for the proper functioning of biological processes through regulating the gene expression in the cell [Kornberg, 1974].

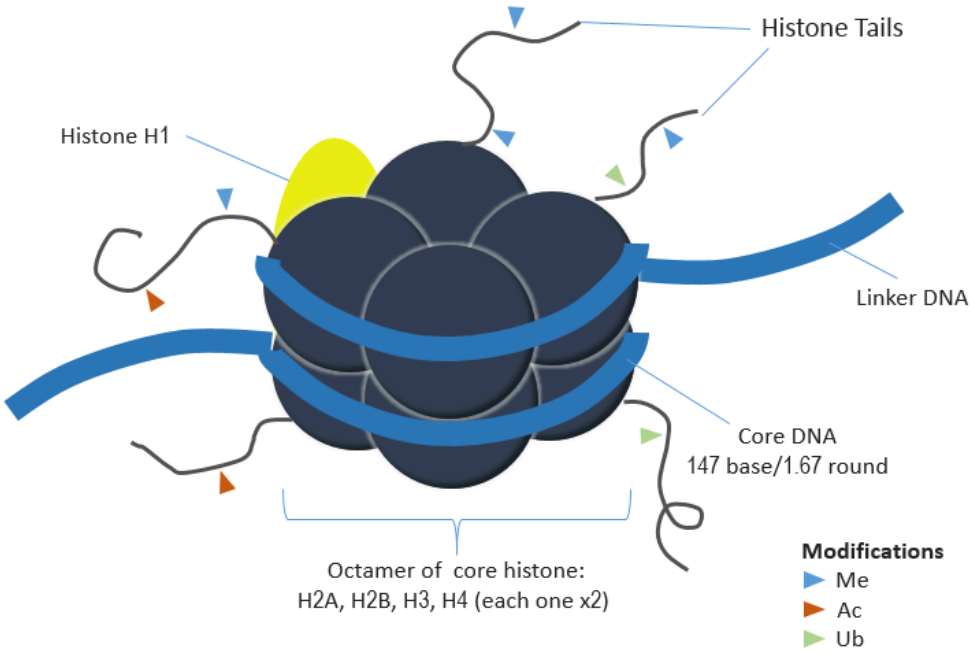


Figure 2. Schematic structure of a nucleosome; consisting of histone octamer; a pair of H2A, H2B, H3 and H4, core DNA of length approx. 147 bp (wrapped around histone octamer) and one external histone H1. Linker DNA, also called naked DNA, lies outside the nucleosome structure. Histone tails are the extended amino acid sequence of histone proteins, which usually undergo different modification such as methylation (Me), acetylation (Ac) and ubiquitination (Ub).

1.3.1. Post-translational modifications in histone proteins

Histones contain flexible N-terminal tail of amino acids that lies outside from the histone core [Davey et al., 2002]. These tails are extensively modified post-translationally through the addition of methyl, acetyl, phosphoryl and ADP-ribose groups. Also, addition of small peptides, such as SUMO and ubiquitin, has been observed. Whole genome profiling of these modifications showed that they co-occur in the genome in different combination, and each of these modifications can be broadly classified as being associated with transcribed genes and transposons [Li et al., 2007; Rando and Chang, 2009; Berger, 2007]. Specifically, methylation and acetylation of H3 lysine (K) acts predominantly as the repressor or activator of gene expression, respectively, in plants. Apart from some exceptions, types of histone

modifications and their functions are generally conserved in both animals and plants [Fuchs et al., 2006].

Acetylation of histones revamps the structure of nucleosomes directly by loosening the bond between DNA and histones. While other PTMs, such as methylation, generally create biding sites for chromatin-based effect processing proteins. PTMs which are bound by specific effector proteins, are involved either in the repression of gene transcription essentially through compacting nucleosome array [Eskeland et al., 2010; Francis et al., 2004], or can induce transcription by engaging chromatin remodeling complexes, or complexes involved in splicing or elongation [Pray-Grant et al., 2005; Sims et al., 2007].

In *Arabidopsis*, methylation of histone protein is considered as one of the most influential epigenetic marks, which occur as mono-, di-, and tri-methylation [Liu et al., 2010]. The usual sites for its occurrence are e.g. Lys4 (K4), Lys9 (K9), Lys27 (K27), Lys36 (K36), and Arg17 (R17) of histone H3, and Arg3 (R3) of histone H4 [Liu et al., 2010]. Trimethylation of H3 lysine 4 (H3K4me3), dimethylation of H3 lysine 9 (H3K9me2) and trimethylation of H3 lysine 27 (H3K27me3) are three the most studied H3 methyl modification, which associate with trancriptional activation, constitutive silencing and transient silencing, respectively. H3K4me3 is deployed at the 5' end of highly transcribed genes by the enzyme trithorax group (trxG) protein complexes associated with the activation RNA polymerase [Santos-Rosa et al., 2002]. In contract, H3K27me3 is associated with silencing of many developmental regulator genes in plants. It appears to be deposited by PRC2 (Polycomb Repressive 2) complex. H3K27me3 is generally present in promoters and gene bodies, and is found in about 15 to 20% of genes in *Arabidopsis* [Zhang et al., 2007; Deal et al., 2010], and 30 to 40% in Maize [He et al., 2010; Wang et al., 2009]. Moreover, in *Arabidopsis* H3K27me3 is bound by LHP1 (LIKE HETEROCHROMATIN PROTEIN 1) [Zhang et al., 2007; Turck et al., 2007], which with several other proteins forms PRC1 silencing complex [Xu et al., 2008]. Furthermore, EMF1 (EMBROYNIC FLOWER 1), a plant specific protein, also plays a PRC1 like role in silencing of floral developmental gene *AGAMOUS (AG)* [Calonje et al., 2008].

1.3.2. Histone variants

Beside of the canonical histones, which are incorporated during DNA replication, all eukaryotes have variant type H3 and H2A and can confer unique properties to the nucleosome they deposited [Talbert and Henikoff, 2010]. H3 and H2A variants act like on/off switch for the genes and have been shown to play other roles like DNA repair, meiotic recombination and chromosome segregation [Yelagandula et al., 2014; Coleman-Derr et al., 2012; Zilberman et al., 2008; Weber et al., 2014; Moggs et al., 2000; Kirik et al., 2006; Ravi et al., 2011;

Fukagawa et al., 2014]. H2AX and H2A.Z are the variants of H2A found in plants, along with their multiple isoforms [Gendler et al., 2008]. Furthermore, H2AX is phophorylated on its C-terminal serine at sites of DNA damage and involved in recognition of the sites of DNA damage [van Attikum and Gasser, 2009]. While H2A.Z variant differs from H2A throughout the protein chain by many amino acid substitutions, especially at C-terminal α-helical region [Suto et al., 2000]. This variant has been extensively studies in yeast, animals, and plants, and found to be involved in several genomic processes such as transcriptional regulation, formation of heterochromatin boundaries and maintenance of genome integrity [Raisner and Madhani, 2006]. Genome-wide mapping of this variant by ChIP-chip (chromatin immunoprecipitation coupled to microarrays) revealed its extent of wide deposition through genome, especially flanking around TSS [Raisner et al., 2005; Zilberman et al., 2008; Mavrich et al., 2008], where it appears to contribute in transcriptional regulation, by aiding to prevent DNA methylation [Zilberman et al., 2008; Conerly et al., 2010]. Swr1 ATP-dependent nucleosome remodeling complex of plants and animals is responsible for incorporation of H2A.Z into nucleosome. During the process nucleosome is being partially unwrapped and an H2A/H2B dimer is replaced by an H2A.Z/H2B dimer [Mizuguchi et al., 2004]. Moreover, except H4, all of the core histones possess a number of variants, which probably have emerged by gene duplication event [Malik et al., 2003].

H3.3 and CenH3 are the two variants of H3 found in al eukaryotes. CenH3 variant is deposited at centromere and plays role in chromosome segregation [X et al]. H3.3 variant differ from H3 in only three to four amino acids [Malik and Henikoff, 2003] and is incorporated outside of DNA replication site through different histone chaperons, such as HirA and Daxx, depending upon genomic location [Tagami et al., 2004; Drane et al., 2010; Goldberg et al., 2010]. H3.3 chaperone homolog HirA in *Arabidopsis* was showed to mediate silencing of *KNOX* genes during development of leaves [Phelps-Durr et al., 2005], apparently through H3.3 deposition. Its deposition occurs predominantly at promoters, gene regulatory elements, and at transcribed region of expressed genes, where nucleosomes are constantly being distorted and replaced [Mito et al., 2005; Deal et al., 2010]. Apart from above discussed variants, in *Arabidopsis*, H3 also have H3.1 and H3.2 variants, while H2A histone also have H2A.X and H2A.W [Ingouff et al., 2010; Kawashima et al., 2015].

1.3.3. Methylation of DNA

In plants, cytosine can be methylated at its fifth carbon position of the aromatic ring, and this can occur at any cytosines regardless of the DNA sequence context. Generally, 5-methylcytosine is associated with transcriptional silencing by triggering chromatin compaction and thus directly blocking trancriptional factor binding and hence preventing

transcription [Zilberman et al., 2007, Hohn et al., 1996; Chawla et al., 2007]. Although DNA methylation affects the accessibility of underlying sequence towards the regulatory machinery, it does not alter the genetic code of the original genomic sequence [Bernatavichute et al., 2008; Law et al., 2010]. Cytosine is methylated in three functional contexts; CG, CHG and CHH, where H is A, C or T. CHHs are asymmetrically methylated sites, while CG and CHG are symmetrically methylated sites [Du et al., 2015; Matzke et al., 2014]. *Arabidopsis* genome is covered with 24% of CG sites, 6.7% of CHG and 1.7% of CHH [Cokus et al., 2008]. Genome-wide studies showed that the preferred location of methylation is at repetitive DNA sequence, including two major tandem repeats centromere and 45S rDNA repeats [Pikaard, 2002]. DNA methylation in all contexts occurs also locally in transposons located in otherwise euchomaticcontext [Lippman et al., 2004; Zhang et al., 2006; Zilberman et al., 2007]. Individual genes also have considerable amount of methylated cytosine, exclusively in CG sequence context [Tran et al., 2005a; Zhang et al., 2006; Zilberman et al., 2007; Vaughn et al., 2007; Cokus et al., 2008].

Methyl group is added to cytosine is performed by DNA methyltransferases. All known cytosine 5-methyltransferases are from single large family (which three sub-families; DNMT1, CMT and DRM) [Rangwala and Richards, 2004; Chan et al., 2005; Goll and Bestor, 2005]. Although, in *Arabidopsis*, there are three subfamilies of DNA methyltransferases: CG maintenance methyltransferases (METHYLTRANSFERASE1 (MET1)), chromomethylases (CHROMOMETHYLASE3 (CMT3)), and the de novo methyltransferases (DOMAINS REARRANGED METHYLTRANSFERASES (DRMs)) [Finnegan and Kovac, 2000; Kankel et al., 2003; Goll and Bestor, 2005].

1.3.4. Nucleosome organizing proteins

Replication, transcription, recombination and repair require brief and continuing modification in the nucleosome positioning and their alliance with DNA. Thus, chromatin related processes are not limited to DNA or protein modifications, but include also changes in occupancy and composition of nucleosomes, and DNA accessibility to other proteins. Nucleosome relocation and dissociation can be accompanied by chromatin-remodeling ATPases such as the SWI/SNF complex in plants (reviewed in Jerzmanowski 2007; Becker and Workman 2013). The first identified chromatin remodeler in *Arabidopsis* was DECREASE IN DNA METHYLATION 1 (DDM1) [Jeddeloh et al., 1999]. Loss of DDM1 function reduces DNA methylation and H3K9me2, dysregulates numerous genes and transcriptionally activates repetitive elements [Teixeira et al. 2009]. Like the SWI2/SNF2 ATPase protein, DDM1 shows ATP-dependent nucleosome repositioning activity in vitro [Brzeski and Jerzmanowski 2003].

1.3.5. Nucleosome variants, PTMs, DNA methylation and their correlation with gene expression

Over the recent years, many studies elaborated the role of histone vraianst like H2A.Z and PTMs like H3K4me3 and H3K27me3 in plant gene regulation. Supposedly the best understood illustration for this phenomenon is of the *Arabidopsis FLOWERING LOCUS C (FLC*) gene, which is responsible for vernalization. The *FLC* gene is expressed in vegetative phase and act as repressor of flowering process through reducing the expression of FLOWERING LOCUS T (FT) that promotes flowering. During the vegetative phase *FLC* expression is promoted through the deposition of H3K4me3 [He et al., 2004; Oh et al., 2004; Tamada et al., 2009], and recruitment of H2A.Z by the SWR1-like complex [Deal et al., 2007; Lázaro ae al., 2008; March-Díaz ae al., 2007; March-Díaz et al., 2008]. Loss of H2A.Z with other deposition complexes leads to reduced expression of *FLC*. Moreover, other modifications to *FLC* chromatin, including acetylation of H3, ubiqutination of H2B, and methylation of H3K36 are also involved in maintaining expression of *FLC*, hence repressing flowering [He, 2009]. On attaining the maturity under optimal environmental condition, *FLC* must be silenced for flowering. This switch from active to silent requires incorporation of H3K4demethylase enzymes and a PRC2 complex that deposits H3K27me3, which leads to silencing of the gene. Additionally, arginine methylation of H3 and H4 along with methylation of H3K9 has also role in stable silencing of *FLC* [He, 2009]. Strikingly, H2A.Z remains present and showed increase in abundance in silenced *FLC* chromatin, suggesting that it is necessary but insufficient for *FLC* expression [Deal et al., 2007]. To resume the vegetative phase in next generation, *FLC* revertes to an active state during embryonic development [Sheldon et al., 2008; Choi et al., 2009].

Methylation has been shown for many roles in *Arabidopsis*, it is widely associated to regulate the development by regulating transposons and developmental genes. Methylation is the default state for the majority of the methylated sequences in *Arabidopsis* genome. Moreover, there is very few evidences that shows the status of methylated sequences changes in non-imprinted genes during growth and development. Methylation surely has effects on genome repeats, such as centromeric and 45S rDNA repeats, as impacted when methylation is removed, but does not appear to regulate them. In addition to the location specific functions, DNA demethylation delivers a housekeeping function by deleting genic RNA-directed DNA methylation [Penterman et al., 2007a; Gong et al., 2002]. This keep transposable elements silent and genes are protected from silencing induced by methylation. CG methylation found in genic regions also show similar housekeeping activity. *met1* mutant plants shows overall increase in expression of body-methylated genes in comparison to non-methylated genes in

wild type [Zilberman et al., 2007]. This indicates, methylation in gene body reduces their expression, but there is no proof of direct regulation.

With the advent of high throughput sequencing, genome-wide nucleosome estimation helped to understand its behavior globally [Zaugg and Luscombe, 2012; Field et al., 2008; Wei et al., 2012]. Genome-wide nucleosome profiles have been developed in several model species, including *Saccharomyces cerevisiae*; [Yuan et al., 2005; Lee et al., 2007], *Arabidopsis thaliana* [Chodavarapu et al., 2010; Li et al. 2014], rice, *Oryza sativa* [Wu et al. 2014], maize, *Zea mays* [Fincher et al. 2013; Vera et al. 2014], fruit fly, *Drosophila melanogaster* [Mavrich et al., 2008], *Caenorhabditis elegans* [Johnson et al., 2006; Valouev et al., 2008], and humans, *Homo sapiens* [Schones et al., 2008; Valouev et al., 2011].

In plants, many of these studies showed the relationship between nucleosome location and DNA methylation, TF binding sites (TFBSs), and gene expression [Chodavarapu et al. 2010; Fincher et al. 2013; Li et al. 2014; Vera et al. 2014; Wu et al. 2014]. Specifically, it was shown that the nucleosome density around TSSs and in gene bodies correlates with gene expression in *A. thaliana* and maize [Li et al. 2014; Vera et al. 2014]. It has also been examined that at TFBSs and DNase I hypersensitive sites (DHSs) in rice nucleosome occupancy varies, which are likely bound to regulatory proteins [Wu et al., 2014]. Additionally, *A. thaliana's* DHSs showed the relationships between chromatin accessibility and tissue-specific or environmental sensitive DNA elements [Zhang et al., 2012; Sullivan et al., 2014].

1.4. Effects of HS on the nuclear structure dynamics

1.4.1. Transcription regulation by nucleosome change

The transcription initiation and subsequent elongation are affected by presence of nucleosomes in the genomes of eukaryotes [Workman et al., 1998]. These nucleosomes have 14 interaction points of DNA-protein (histone) interface [Luger, 2003; Muthurajan et al, 2003]. The interactions are due to the presence of different charges in amino-acid and DNA molecules and represent one the most stable DNA-protein interaction in genome, which eventually facilitates a very efficient packaging unit [Davey et al, 2002].

Previously, nucleosomes were believed to take part only in DNA packaging [Hagerman, 1990; Widom, 1985; Travers et al., 1987]. But these nucleosomal structures obstruct the access to polymerases and transcriptional factors. To provide access to DNA, nucleosomes can be dislodged or evicted, histone variants can be incorporated by altering the its compositions, or histone proteins can be modified post-trancriptinally to loosen the histone-DNA interactions. These processes require chromatin remodeling complexes, which are

implicated under stress responses, for instance ATCHR12, a SNF2/Brahma-type chromatin remodeling protein which facilitates growth responses in stressful condition [Mlynarova et al., 2007; Folta et al., 2014]. Mechanisms, such as intrigation of chromatin remodelers with stress signals remains to be understand.

1.4.2. HS effect on chromatin and chromosomes

Evidences of direct effect of abiotic stresses on nuclear organization has been reported in varied plant species, where decondensation of centromeric repeat, 5S rDNA and 45S rDNA loci under heat stress has been observed [Santos et al., 2011; Tomas et al., 2013; Pecinka et al., 2010]. At interphase, chromosomes of *Arabidopsis* form well defined chromocenters [Pecinka et al., 2004], from which several euchomatin loops emerge out [Fransz et al., 2002]. These chromatin organizational changes could either denote the effects of HS on its global arrangement or the result of change in expression of genes, which are involved in maintaning their structural organization.

Reduction in the nucleosome density in response to long heat stress did not automatically trigger transcriptional activation [Pecinka et al., 2010]. However, heterochromatic genes activated by heat stress were silenced slower in the mutants of the CHROMATIN ASSEMBLY FACTOR 1 (CAF-1) subunits, providing indication that histones and their chaperones are involved in this process. CAF-1 is a type of histone chaperone, which facilitates histone storage, assembly, and eviction [Zhu et al., 2012; Otero et al., 2014]. Gene expressions of several histone chaperones in *Arabidopsis* are differentially regulated under abiotic stress [Zhu et al., 2012; Tripathi et al., 2015], suggesting their role in chromatin structural response to stress. Conversely, stress responsive genes are up-regulated in mutants of CAF-1 or ANTI-SILENCING FUNCTION 1 (ASF1) [Weng et al., 2014; Schönrock et al., 2006]. Plants which express truncated NUCLEOSOME ASSEMBLY PROTEIN 1 (NAP1), an H2A-H2B chaperone, or lacking ASF1, a small histone chaperone protein show hypersentivity to stress [Liu et al., 2009; Weng et al., 2014]. ASF1s bind to certain heat shock genes under stress condition, where they may ease gene activation and simultaneous nucleosome dissociation [Weng et al., 2014].

1.4.3. HS regulates gene expression through epigenetic mechanisms in plants?

Most of the research till date has focused on adaptation of plants under temperature variability, such as heat and cold stress (reviewed in Penfield, 2008). In low temperature-sensitive plants, prolonged cold exposure can trigger metabolic adapation and signaling cascade that strengthen plant survival under subzero conditions. Stressful high temperature exposures can

intiate the HSPs synthesis that assist protection against denaturation of proteins and maintenance of celluar functions. Little fluctuations in ambient temperature can also have significant effects on development of plant. Delayed flowering and compact architecture have been observed when plants were grown at cooler temperature [Balasubramanian et al., 2006]. However, under high temperature, plants show axes elongation and increase in transition to reproductive development through expression of *FLOWERING TIME (FT*) [Balasubramanian et al., 2006].

Kumar and Wigge (2010) showed that chromatin has an essential role in detecting the changes in ambient temperature. They exploited the thermal response of *HSP70* gene expression through mutant screening to show its thermosensitivity. In this strategy, they isolated multiple alleles of *arp6*. ARP6 is the part of SWR1 chromatin-remodeling complex, which is deposits the histone variant H2A.Z in nucleosomes [Li et al., 2005]. Results showed that temperature directly modifies promoter accessibility and nucleosome composition. They also demonstrated that temperature-enhanced eviction of H2A.Z occurs independent of direction of the transcription response. Hence, they concluded that temperature-mediated responses in nucleosome composition are not merely consequences of higher gene expression. They proposed, at cooler temperature H2A.Z occupancy represses gene expression by hindering or prevention the binding of protein complex that activates transcription. Expulsion of H2A.Z under high temperature would hence facilitate transcription of the following genes. For the downregulated genes, it is suggested that H2A.Z expulsion may ease the binding of repressors, hence limiting transcription. Thus, occupancy of H2A.Z provides a mechanism to detect strategic changes in the ambient temperature and regulate gene expression accordingly.

1.4.4. HS memory at transcriptional and protein level

Plants have the ability to inherit stress at certain extent. Although, on account of allocation of large amount of energy and resources for stress tolerance function, stress adaptation is often coupled with growth reduction [Huot et al., 2014]. Most of the stress-related changes generally revert to intitial state shortly after stress exposure, which permits plants to forget stressful conditions and re-appoint resourses to growth and reproduction mechanisms. Intrestingly, certain changes persist long after removal of stressful environment and empower the formation of a “stress memory”, which equiped plants with a highly effective and specific defence to the future stress [Crisp et al., 2016].

Previous experiments suggest that plants have memory for heat stress, known as thermomemory. Pre-administration of plants to moderately high temeperature enable them to act more effectively upon fututе HS, with the help of attained memory. Although, the

molecular mechanism that underlies in memory building under HS is largly not understood. Recent eveidenaces show that the transcripts of many genes and their protein amounts remain high even after removal of HS in plants [Banzet et al., 1998; Dat et al., 1998; Schett et al., 1999; Lee et al., 2000]. Some genes maintain their very high expression levels even for days. Non-epigenetic mechanisms like RNA metabolism and maintanace of quality-stabilizing proteins are important for short-term memory regulation [Crisp et al., 2016; Crisp et al., 2017]. For example, elevated level of HSPs has been reported, which represents to play crutial role in memory formation, both in mutants and wild type [Charng et al., 2007; Charng et al., 2006; Lin et al., 2014; Sedaghatmehr et al., 2016; Weng et al., 2014; Wu et al., 2013]. Experimental evidences also suggest the involvement of epigenetic factors such as histone modification pattern [Lämke et al., 2016]. It was reported that prolonged induction of the memory genes was associated with sustained accumulation of H3K4me3 and H3K4me2, which persisted even after active transcription terminates [Ding et al., 2012; Sani et al., 2013]. Further, to understand the adaption against heat stress in plants, chromatin-based detailed mechanisms of HS memory has been extensively studied. [Bäurle, 2016; Bäurle, 2017; Lämke et al., 2017].

Nevertheless, there are several questions in connection to the relationships between nucleosome occupancy, TFBSs sequences, and gene expression under heat stress that are yet to be studied in plants and other model organisms. The first is, at what extent the understanding of nucleosome occupancy can explain the gene expression under heat stress. Second, however TFBSs and nucleosome occupancy have convulsion in certain extent in plants [Wu et al. 2014], it remains unclear if there are heat stress specific sequence motifs that tend to located in nucleosome-depleted around TSSs, how these motifs influence gene expression under heat stress condition, also at the location of these motif whether the nucleosome showing any depletion. Third, the extent of nucleosome dynamics under heat stress in plants remains to be determined.

2. AIMS OF THE PROJECT

- Estimation of genome–wide nucleosome profile in control, heat stress and recovery conditions in *Arabidopsis thaliana*.
- Comparison of individual nucleosome dynamics under heat stress and recovery with respect to control conditions.
- Perform correlation analysis between gene expressions with corresponding nucleosome occupancies.
- Estimation of HSEs distribution in genes differentially expressed under heat stress.

3. MATERIALS AND METHODS

3.1. Plant materials and growth conditions

Arabidopsis thaliana plants of Columbia (Col-0) accession were used. *A. thaliana* seeds were placed on wet standard soil and stratified for one week at 4°C before they were moved to the greenhouseand grown until plants reached a five-leaf rosette stage. Subsequently, plants were transferred to a growth chamber (Percival) maintained at 21°C during the day and 16°C during the night (16h light/8h dark with illumination by 100 µmol m^{-2} sec^{-1} white light).

3.2. Heat stress treatment and post-stress recovery

After two days of acclimation, part of the plants were placed in a growth chamber (Percival) pre-heated to 37°C for 6h. For nuclei extraction, plant samples were harvested at control and heat stressed (HS) conditions and frozen to -80°C after treatmentwith liquid nitrogen. The remaining stressed plants were re-transferred to growth chamber with control conditions and harvested after two days (48h) (Figure 3).

3.3. RNA-Seq materials

Our lab previously performed the identical experiment for extraction of RNA from control, heat and recovery stages [Pietzenuk et al., 2016]. Raw RNA-seq reads from this experiment is present at the public repository NCBI GEO archive with accession number GSE69077. So, the raw sequencing data from this work were used for studing gene expressions in this study. The reads were single ended form each condition with two biological replicates.

3.4. Nuclei extraction and nucleosome preparation for MNase-Seq

Nuclei extraction and MNase treatment was performed after Ricardi et al (2010) with minor adjustments. First, 3 g of tissue were harvested for each replicate and immediately frozen in liquid nitrogen and stored in -80°C until used. Tissues were grinded into fine powder in liquid nitrogen using mortar and pestle. Each sample powder was suspended in 30 – 40 ml of cold extraction buffer I (10 ml/g of tissue). Whole experiment was conducted in 0 – 4°C.

Extraction buffer I (100 ml)

Final	Stock	Volume
0.44 M sucrose	2 M	22 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	1 ml

5 mM b-ME	14,3 M	35.7 µl
1× protease inhibitor cocktail:		
1mM PMSF (in 96% ETOH)	100mM	1 ml
5 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets		

Subsequently, solutions were filtered using miracloth and spun for 20 min at 2,800 x g. Supernatant was removed and the pellet was resuspended in 10 ml of extraction buffer II.

Extraction buffer II (100 ml)

Final	Stock	Volume
0.25 M sucrose	2 M	12,5 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	1 ml
10 mM $MgCl_2$	1 M	1 ml
1% Triton X-100	100%	1 ml
5 mM b-ME	14,3 M	35.7 µl
1× protease inhibitor cocktail:		
1mM PMSF (in 96% ETOH)	100mM	1 ml
2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets		

Next, the solutions were incubated on ice for 10 min to lyse the chloroplasts. And the suspension was spun for 20 min at 2,100 x g. Again, the supernatant was removed and pellet was resuspended in 4 ml of extraction buffer II without Triton X-100. Then, solutions were spun for 20 min at 2,100 x g and the pellets were resuspended in 4 ml of Percoll extraction buffer.

Percoll extraction buffer (50 ml)

Final	Stock	Volume
95% V/V Percoll	100%	45 ml
0.25 M sucrose	2.5 M	5 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	0.5 ml
10 mM $MgCl_2$	1 M	0.5 ml
5 mM b-ME	14.3 M	17.8 µl

1× protease inhibitor cocktail:		
1mM PMSF (in 96% ETOH)	100mM	0.5 ml
2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets		

The solution was spun for 10 min at 12,000 x gand stopped without activated break to avoid rough shaking of the extract. Afterwards, ~300 µl of the upper phase (white-greenish flocks/cloud) were carefully extracted and diluted in 1,200 µl of nuclei extraction buffer.

Nuclei extraction buffer (100 ml)

Final	Stock	Volume
10% Glycerol	100%	10 ml
50 mMTris (pH 8.0 adj by HCl)	1 M	5 ml
5 mM $MgCl_2$	1 M	0.5 ml
10 mM b-ME	14.3	70.1 µl
1× protease inhibitor cocktail		
1mM PMSF (in 96% ETOH)	100mM	1 ml
2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets		

Then, the solution was spun repeatedly for 10 min at 12,000 x g. The supernatant was discarded and the pellet was resuspended into 1-1.5 ml of nuclei resuspension buffer.

Now, the pellets were dissolvedin 500 µl of Micrococcal nuclease buffer.

Micrococcal nuclease buffer (10 ml)

Final	Stock	Volume
50 mMTris-HCl, pH 8.5	1 M	0,5 ml
5 mM Mg acetate	1 M	50 µl
25% glycerol	100%	2,5 ml
1 mM $CaCl_2$	1M	10 µl

Aliquots of 100 µl were digested for 20 min at 37°C with 0, 2.5, 5, 10 and 20 U of MNaseI. Digestion were stopped immediately, by adding 10 µl of 0.5 M EDTA, 20 µl of 1 M Tris-HCl pH 6.8 and 1.5 µl of 14 mg/ml proteinase K to the eluate and incubated for 1 h at 45°C.

Digested DNA was extracted with equal volume of phenol/chloroform (1:1). The solution was centrifuged for 5 min at 5,000 x g to separate the phases. Then, the upper phase, containing DNA, was transferred into a new tube. To this, 0.1 volume of 3 M sodium acetate with pH 5.3 (adjusted by glacial acetic acid) was added and precipitated with 0.7 volumes of isopropanol (-20°C) in the presence of yeast tRNA (1 µg/ml final concentration). Subsequently, the pellet was washed with 300 µl of 70% ethanol and centrifuged for 5 min in 12,000 x g. Then, the supernatant was removed dried under a fume hood. Afterwards, the DNA pellets were resuspended overnight at 4°C in 20 µl of Tris pH 8 with 10 µg/ml RNase A.

Recovered DNA was analyzed on a 2.5 % Agarose-Gel (Bioline) for 2 h at 70 mA. Since I targeted on mononucleosomal DNA fragments only, bands at an approximate size of 150 bp were excised from the gel and cleaned up using DNA Gel Extraction Kit (Qiagen).

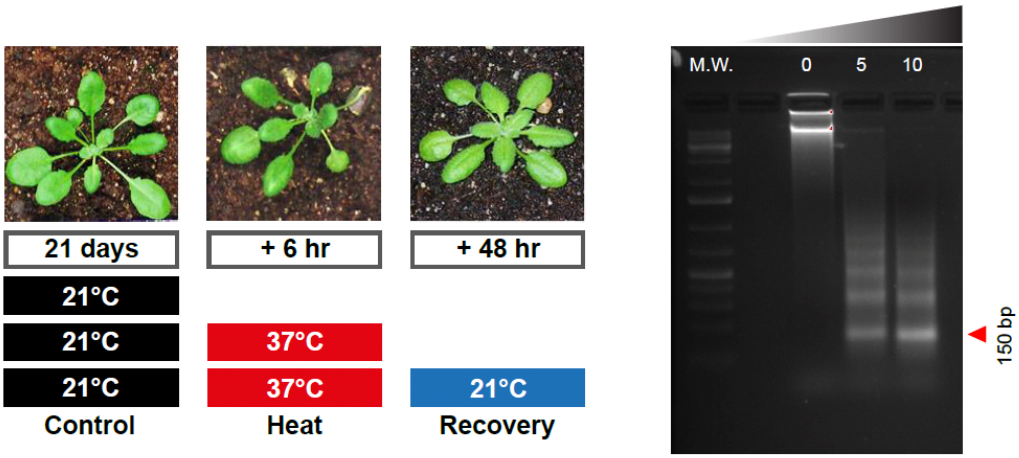


Figure 3. Experimental setup scheme for HS treatment and recovery in *A. thaliana*. Control conditions were grown in 21°C for 21 days. Heat shock of 37°C were subjected to two third of the samples for 6 hours. From this two third HS treated one third has been re-subjected to initial control condition for 48 hours (3 days). Extracted chromatin has been run on agarose gel after digestion with MNase (Micrococcal Nuclease) enzyme. Around 150 bp fragments extracted from the gel for sequencing. (M.W.: Molecular weight, 0, 5, 10 are MNase enzyme Unit)

3.5. Library preparation and deep sequencing for MNase-seq

Sequencing libraries of mono-nucleosomal DNA were constructed using the TruSeq^{TM} DNA Sample Preparation v2 (Illumina Ltd.), starting with the "End repair"-step as DNA was already fragmented using MNase I. Library preparation was started by using at least 500 ng of mononucleosomal DNA with adjusted volumes of Resuspension Buffer (RSB) and AMPure XP Beads (Beckmann Coulter Inc.). Further library preparation was performed after protocol.

Illumina Hiseq2100 sequencer (MPIPZ genome centre; Cologne, Germany) has been used to generate paired-end (PE) reads of 100 bp size.

3.6. Mapping of RNA-Seq and differential expression analysis

RNA-seq raw reads obtained fromPietzenuk et al., 2016. All single end fastq files were filtered for per base sequence quality above 25 (Phred score) using FastQC, a quality control tool for high throughput sequencing data [Andrews, 2010]. The single-end reads from control, HS and recovery (two replicate each) were mapped on *A. thaliana* TAIR10 genome using TopHat [Trapnell et al., 2009] with maximum mis-match of two per alignment. Differential gene expression analysis was performed usingCuffDiff [Trapnell et al., 2012], which estimates the expression value of each genein FPKM (Fragments per Kilobase per million). Subsequently, CuffDiff was used forstatistical testingthe comparison of the expression of each corresponding gene, performing the two-tailed hypothesis testing with t-test for each set of genes. For each such comparison, the p-value (probability of rejecting the null hypothesis) is calculatedand adjusted (q-value) by Benjamin Harrison multiple test correction method [Benjamini et al., 1995]. Further analyses were performed by custom made scripts written in python and R. Scripts are present in GitHub Gist repository online (https://gist.github.com/kashiff007). Over representation Analysis (ORA) of biological functions, pathway and locations of differentially expressed gene groups were performed by GOrilla: GO annotation tool [Eden et al., 2009].

3.7. Mapping of MNase-Seq and peak calling

For MNase-seqanalysis, three biological replicates from control, HS and recoverywere paired-end reads sequenced with 100bp size. This has been done to get more precise location of each nucleosomes and reducing the error of single-end reads bias. The raw reads were filtered for per base sequence quality above 25 using FastQC tool [Andrews, 2010]. The paired-end reads from control, HS and recovery were then concordantly and uniquely mapped on *A. thaliana*

TAIR10 genome using Bowtie version 1.1.2 with maximum mis-match of two per alignment [Langmead et al., 2009].

Mapped reads (bam-file) were sorted andconverted into normalized nucleosome occupancy profile (bigwig file) using DANPOS2 [Chen et al., 2013]. DANPOS2 uses genome-wide normalization method to normalize the uniquely mapped reads. For this, a bin is created on the genome and mapped reads are calculated in the bin and divided by the total number of uniquely mapped reads in the genome. Then, the bin is run across the genome.

$$\text{Nucleosome score in a bin} = \frac{N_b}{\frac{N}{10^6} \text{ X } \frac{L_b}{10^3}}$$

N_b = Number of uniquely mapped reads in a bin

N = Number of uniquely mapped reads in the whole genome

L_b = Length of the bin

After estimating the nucleosome score, DANPOS2 has smoothen the peaks for better visualization. It also performsthe determination of differential nucleosome occupancy among control, HS and recovery conditions, by using Poisson test by default to perform statistical comparison for each defined nucleosome. The criteria of$p_{val} \leq 10^{-5}$were used for calling each nucleosome, while $q_{val} \leq 0.05$ for differences in the nucleosome occupancy (Figure 4). Downstream analysis of DANPOS2 output has been performed by deepTools [Ramírez et al., 2014] and various in-House scripts and R packages (AppendixIII).

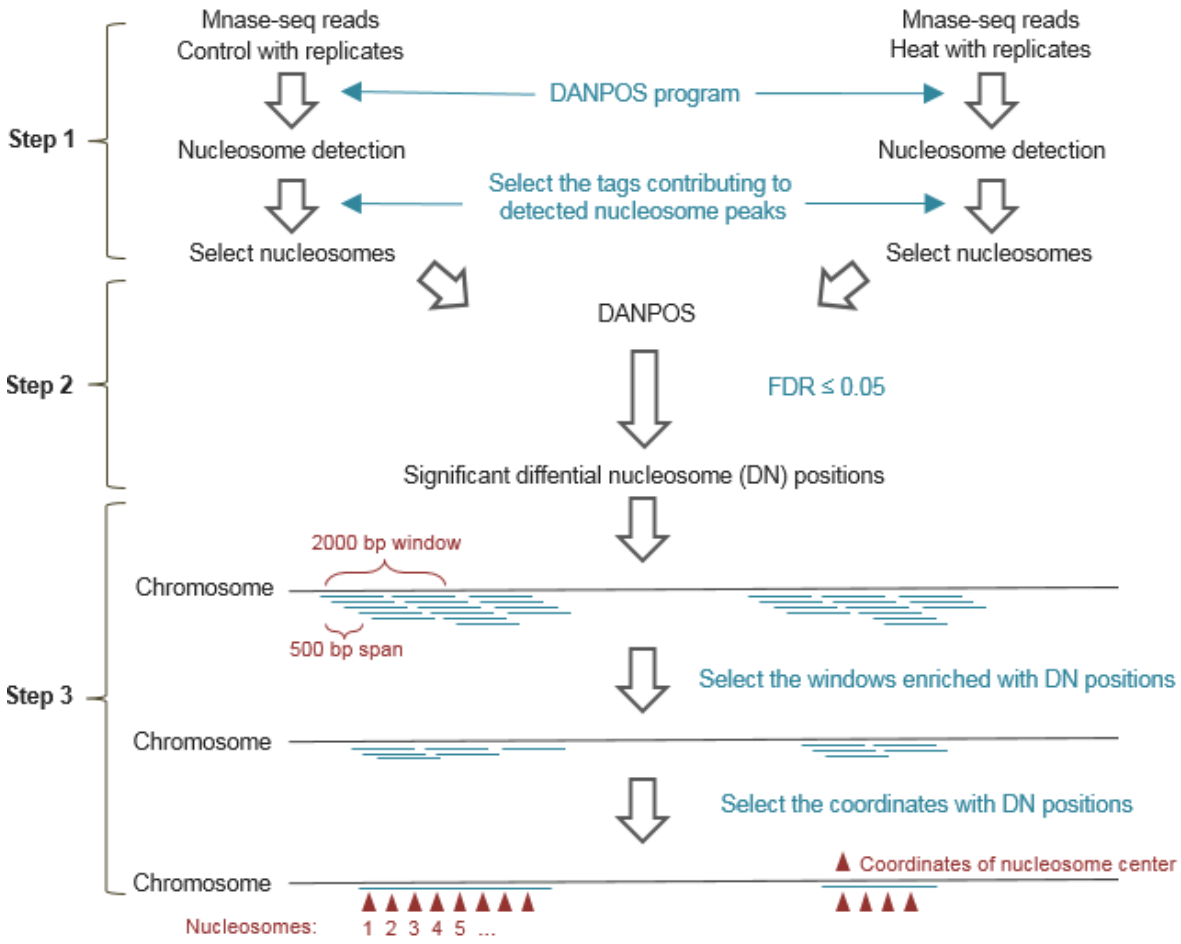


Figure 4. Workflow of MNase-seq downstream analysis. Step 1: Determination of nucleosome position on genome with the help mapped reads and normalization. Step 2: Statistical differential nucleosome analysis for comparison between two or more condition. Step 3: Selection of differential nucleosome area after scanning the genome with a window (bin).

3.8. Scanning for heat shock elements (HSEs) motifs in the promoter regions

For searching the HSEs associated with genes, 1500bp upstream sequences from transcription start site (TSS) were selected as promoters for each protein-coding gene within *Arabidopsis* genome (n=27420). Previous publications suggest four major kinds of HSEs present in *A. thaliana* [Sakurai et al., 2010]. All four types of motifs are shown in table 5. FIMO [Grant et al., 2011], MEME suit [Bailey et al., 2009] were used for searching these four types of motifs in the promoter regions of all protein coding genes with $p_{val} <= 0.0001$.

4. RESULTS

4.1. Recovery of global transcript level to control

Transcript expression of all protein coding genes in *Arabidopsis thaliana (*n=27,420, TAIR10) were analyzed for control, HS and recovery conditions with RNA-seq. Differential gene expression was performed for the HS and recovery with respect to control. I found that from the 27,420 protein-coding genes, 13,354 genes (48.70%) were expressed with FPKM > 5 in at least one of the three conditions, and this group was considered as *all expressed genes*. While 14,066 genes having FPKM < 5 in all conditions were grouped as *all non-expressed genes*. From the all expressed genes category, 5,323 genes were significantly changing their expression under HS in comparison to control, with the difference in their FPKM greater than 10 ($p_{adj} \leq 0.05$). Hence, these genes were considered as *heat responsive genes (*Table 2). Among these heat responsive genes, 2,819 and 50 were up-regulated with $\log_2$FC > 1 in HS and recovery conditions, respectively, while 1,332 and 52 were down-regulated with $\log_2$FC < -1 in HS and recovery, respectively. Venn diagram of both these conditions are shown in Figure 5A (right). In order to get the global transcript change I estimated the expression density of all protein-coding genes, which shows that it change under HS (t test; $p_{val} = 5.286 \times 10^{-07}$) and then regain its original state under recovery condition (t test; $p_{val} = 0.07802$) (Figure5B; left). This result was again confirmed by correlation plot, which was generated by distance calculation using Pearson method (Figure5C; left). Gene density and correlation plot showed the overall expressions of all protein coding genes in control and recovery were very much similar with $p_{val} = 0.07802$, and with Pearson's correlation score of more than 0.99, which suggest that the gene expressions were reverting to its original state after removal of heat for 48 hr.

Genes Categories	***Definition***	***Number***
Total Protein Coding Gene	Total Protein coding genes from TAIR10	27,420
All Expressed Genes	Number of genes at least expressed in one of the conditions with minimum 5 FPKM expression value	13,354
All Non-Expressed Genes	Number of genes with less than 5 FPKM in all three condition	14,066
Heat responsive Genes	Genes which shows fold change significantly with $p_{adj} \leq 0.05$ and FPKM difference > 10	5,323

Table 2. Showing different categories of genes based on their expressions with their respective numbers.

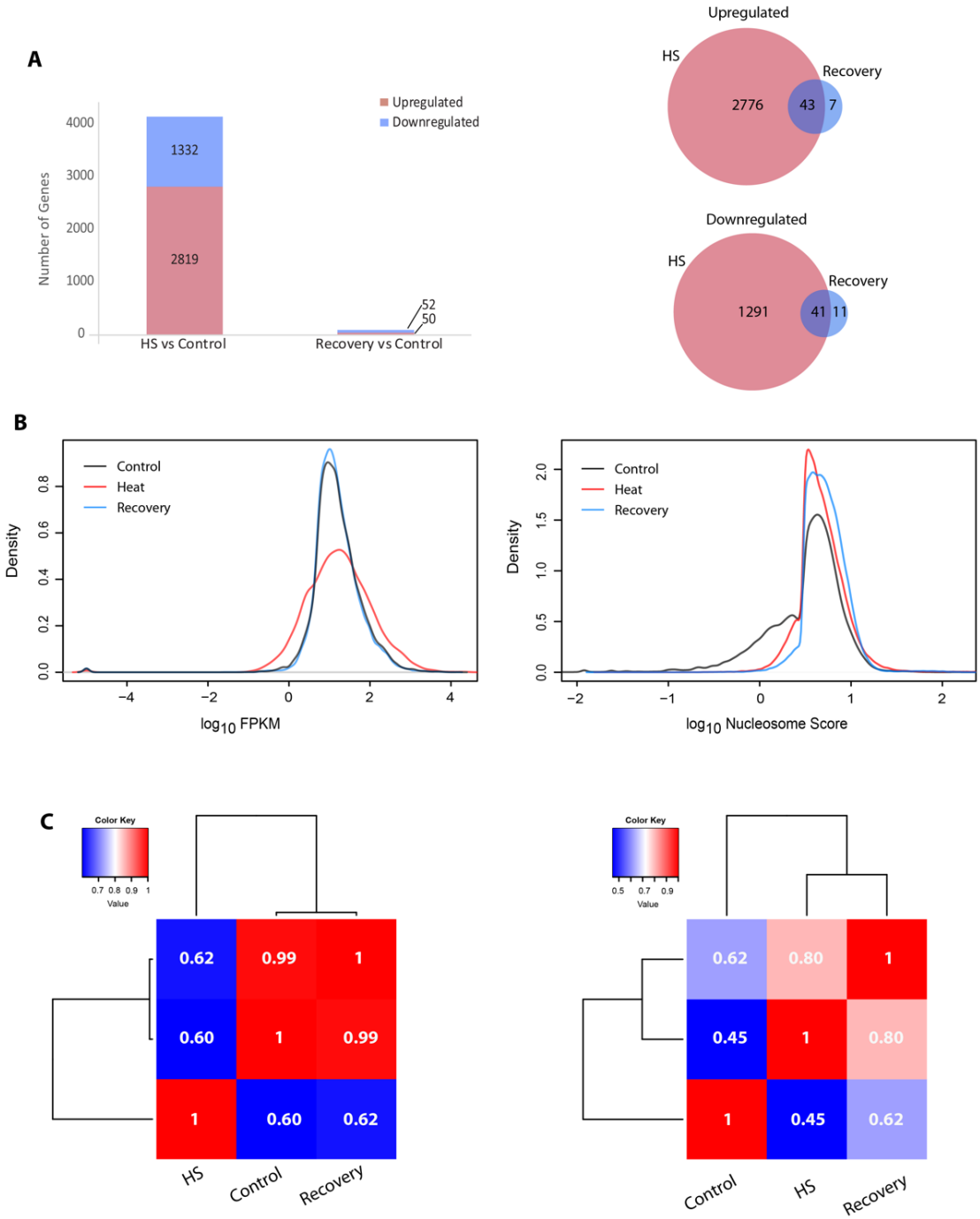


Figure 5. A: Bar plot on the right showing the significant number of up-regulated and down-regulated genes in HS vs control and recovery vs control. Venn diagram on the right showing number of up-regulated ($log_2FC > 1$) and down-regulated ($log_2FC < -1$) genes with and $p_{adj} \leq 0.05$ under HS (pink) and recovery (blue) compare to control. **B:** Left side plot displaying gene density of all protein-coding genes from control (black), HS (red) and recovery (blue).

x-axis showing $\log_{10}$ FPKM for genes. Right plot is the density plot of all the nucleosome across the genome in all three conditions. x-axis showing $\log_{10}$ nucleosome occupancy score. **C:** Correlation values among the different conditions of genome wide gene expression (left) and nucleomse occupancy score (right) with dendrogram showing the hierarchical relationship.

4.2. High temperature induces global changes in nucleosome occupancy

For the comparison of nucleosome occupancy of HS and recovery with control, I performed the downstream analysis of MNase-seq reads. Reads from all replicates were mapped on TAIR10 genome of *A. thaliana* using Bowtie [Langmead et al., 2009]. Three mismatches were allowed per read and reads with multiple alignment possibilities were suppressed to one. The main purpose of supression of multiple alignment to single location is to mitigate the effects of PCR amplification bias introduced during library. These mapped read files had each read's address and its abundance information on the genome. Table 3 is showing the sequenced and mapped reads summary from MNase-seq experiment. As suggested in many previous studies, I used mapped percentage of MNase-seq reads to the genome coverage at least more than 10-fold [Flores et al., 2014].

$$C = \frac{L \times N}{G}$$

Where C = Coverage (X),

L = Read length (bp),

G = Haploid genome size (bp),

and N = Number of reads

This information was used to predict the nucleosome occupancy in genome-wide level through DANPOS2 tool. First, DANPOS2 predicted all significant nucleosomes with a score and position in control, HS and recovery separately using all replicates. Then, it compared HS and recovery with control for the estimation of differential nucleosome.

From the predicted nucleosomes score, I plotted density of the $\log_{10}$ value of nucleosome score of each nucleosome from every condition (Figure 5B; right). For verifying the significance of changes in nucleosome occupancy among control, HS and recovery, I performed paired-end Wilcoxon-Mann-Whitney-Test. I found that p-value in each comparison were highly significant (p_{val} = 2.2e-16). To confirm it further, I also compared the

genome-wide nucleosome scores among the conditions and plotted the Pearson's correlation heatmap with dendrogram (Figure 5C; right).

Sample	***Replicates***	***Reads***	***Mapped***	***Genome coverage (x)***
Control	Rep 1	21241326	6007581 (28.28%)	15.13
	Rep 2	21129986	8399548 (39.75%)	21.15
	Rep 3	99409816	43697810 (43.96%)	110.03
HS	Rep 1	132305458	47904312 (36.21%)	120.62
	Rep 2	38483612	26025518 (67.63%)	65.53
	Rep 3	115113942	7774222 (6.75%)	19.57
Recovery	Rep 1	71256582	32479789 (45.58%)	81.78
	Rep 2	37721353	23300243 (61.77%)	58.67
	Rep 3	123486970	17607094 (14.26%)	44.33

Table 3. Summary of MNase-seq read mapping from control, HS and recovery conditions with its replicated from *A. thaliana*.

It is evident from the density plots, that under HS gene expression and nucleosome occupancy both changes, and after the heat removal for 48 h, the gene expression reverted back to control state but not the nucleosome occupancy in same extent. This suggests that nucleosomes may require more time to regain their original positions.

To analyze the nucleosome size distribution, I plotted the density of the mononucleosomal fragment size (Figure 6A). As I intentionally extracted reads for mononucleosomes, fragment size was varying from 100 to 250 bp with the median as shown in Figure 6A for all three

conditions. There was a sharp increase in the number of fragments with size between 140 bp to 170 bp under HS, and further small decrease was observed in recovery after HS removal. Fragment size with more than 180 bp showed opposite trend i.e. a greater number of such reads were present in control, while comparably less in HS and recovery. Changes in fragment distributions were statistically supported by using paired-end Wilcoxon-Mann-Whitney-Test. I found the fragments distribution control was differing from HS with p_{val}= 0.02328, and control from recovery with p_{val} = 0.09208.

However, differences in HS and recovery fragment distributions were not much significant, which was also supported by p_{val} = 0.8402. To analyze it further, I first defined the significant genome-wide locations of all nucleosomes, and then calculated the finding possibility of each nucleosome in a defined position, or how well a nucleosome is positioned to its location. This possibility of finding a nucleosome at a position is termed as fuzziness, and it is reported as the standard deviation, calculated with the help of replicates. This means, more the fuzziness-score higher the standard deviation and *viceversa* [Jiang et al., 2009] (Table 4; Figure 6B). Further, the fuzziness-score was statistically compared among all three conditions using paired-end Wilcoxon-Mann-Whitney-Test, and all comparison yield p_{val} = 2.2e-16.

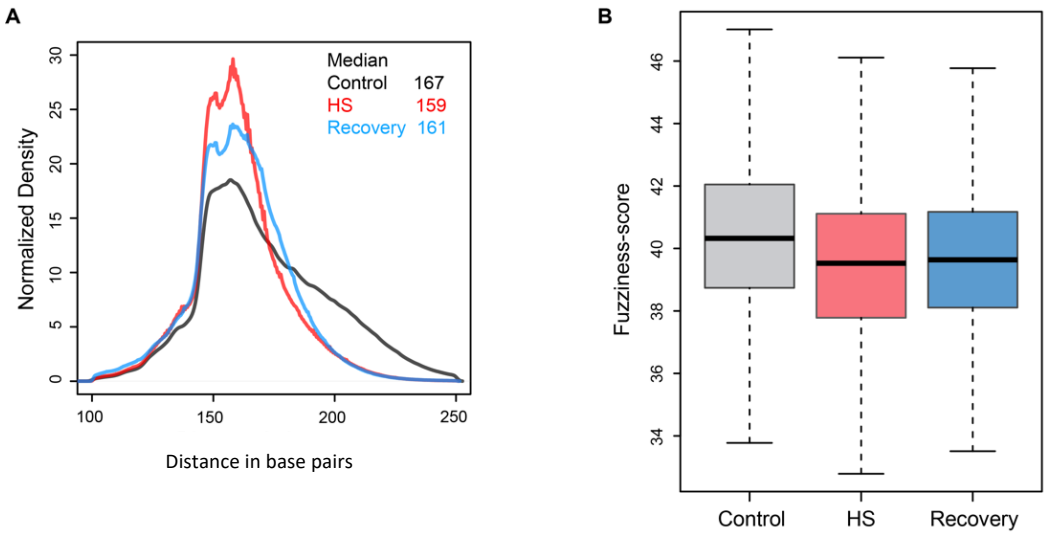


Figure 6. **A**: Distance between beginning and end (width) of predicted nucleosomes in control, HS and recovery. X axis represents the distance in basepairs, and Y axis shows the normalized density. Nucleosome width reflects the length of the mononucleosomal DNA fragments used for sequencing. **B**: The deviation of nucleosome positions within each unit in a cell population is referred to as fuzziness. Boxplot showing fuzziness score in y axis from control, HS and recovery.

Sample	*Number of Nucleosomes predicted with pval ≤ 10^{-5}*	*Mean Fuzziness score of all predicted Nucleosomes*
Control	118378	40.46
HS	118773	39.49
Recovery	117923	39.70

Table 4. Number and fuzziness-score of nucleosomes in each sample and calculated significantly with all three replicates using DANPOS2 with $p_{val} \leq 10^{-4}$. Fuzziness is reported as the standard deviation, calculated by all three replicates.

4.3. Transcription and chromatin modification-dependent nucleosome spacing

I then analyzed how the nucleosome organization regionally affected by transcription and chromatin functions. For each temperature condition, I used deep RNA-seq data, previously generated by our lab [Pietzenuk et al., 2016]. Genes were pooled into groups according to their expression values. The average nucleosome spacing showed more or less the same pattern in all three conditions, and was smallest among silent genes (varying between 250-275 bp in all three conditions, Figure 7A). It significantly increased by as much as 144 bp as the expression levels went up (t-test p-value 2.894×10^{-3}) and reached to 500 bp at the expression of 10-30 FPKM. It starts decreasing with further increase in expression. This suggest that transcription-induced cycle of nucleosome eviction and reoccupation prompt rarer nucleosome packing and slight increment in nucleosome occupancy till certain extent of expression then the pattern reverts (Figure 7C). Based on these results, we postulate that higher-order chromatin organization as entailed by specific modifications of chromatin might be associated with specific pattern of spacing. Utilizing previous studies of ChIP-seq data of control condition, we characterized regions of enrichment for histone modification that are present within gene-body euchromatin (H3K27me3 and H3K4me1) [Veluchamy et al., 2016; Inagaki et al., 2017], euchromatin associated with promoters and enhancers (H3K9ac, H3K4me2 and H3K4me3) [Zhu et al., 2017; Inagaki et al., 2017], or heterochromatin (H3K9me2 and H3K27me1) [Inagaki et al., 2017; Roudier et al., 2015], and estimated nucleosome spacing for each of these epigenetic domains. I observed that heterochromatin associated domains contained the shortest spacing of 328- 363 bp, followed by a greater spacing of 393-597 bp within promoter-associated domains, while gene-body domain spacing was largest at 696-819 bp (Figure 7B). These findings unveiled remarkable heterogenicity in

nucleosome organization across the genome that depends on local gene activity, regional regulatory and metabolic state, and global cellular identity.

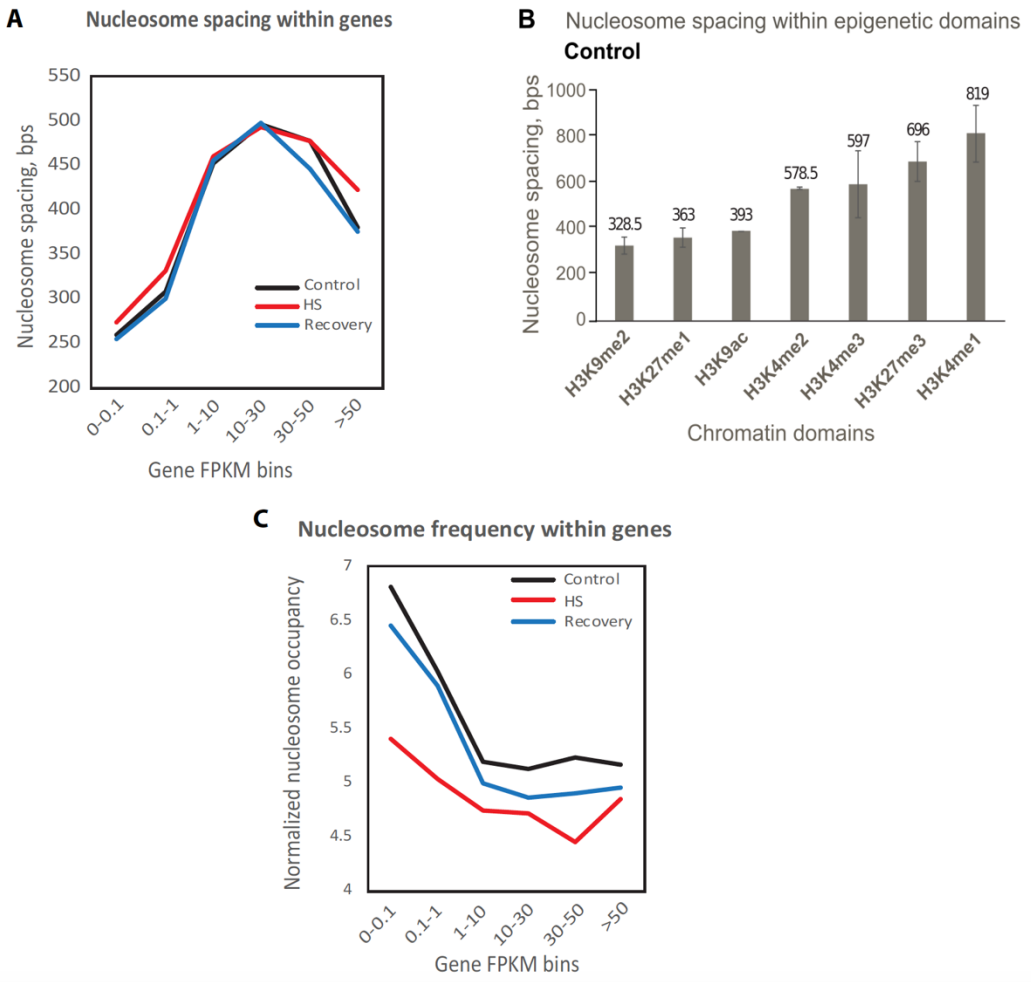


Figure 7. **A**: Nucleosome spacing as a function of transcriptional activity. X axis represents gene expression values binned according to FPKM values. Internucleosome spacing is plotted along the Y axis. **B**: Nucleosome spacing within genomic regions marked by specific histone marks in control condition in plants. Bar height plots estimated nucleosome spacing for each histone modification. Bar colors differentiate chromatin types (euchromatin vs heterochromatin). **C**: Association between transcriptional levels and measured nucleosome occupancy. X axis represents gene expression values binned according to their RPKM values. Y axis represents normalized frequencies of observed nucleosome coverage within the regions occupied by genes in each bin.

4.4. Sequence signals that drive nucleosome positioning

To identify the DNA signals accountable for consistent nucleosome positions, I identified more than 0.1 million sites occupied by nucleosome in all three conditions at high stringency (p-value < 10^{-5}, Methods). I observed region occupied by the nucleosome centre (dyad) exhibits a remarkable high G/C content in all three

conditions (p-value < 10^{-100}, Figure 8A). To associate the dyad sequence composition with how well a nucleosome is positioned, I calculated the fuzziness score (Figure 8B) for each nucleosome on the genome and found that in well positioned nucleosome the boundaries of the dyad are well protected with higher A/T as well as lower G/C frequency. While loosely positioned nucleosome does not have such protected boundaries (Figure 8B). Further, to associate the dyad sequence composition with change in nucleosome occupancy among different conditions (control, HS and recovery), I estimated the differential nucleosomes at genome wide level. I found ~ 43,000 differential nucleosomes when compare HS condition to control with adjusted p-value < 0.05. Less differential nucleosome (0 to -1 fold and 1 to 0 fold) exhibits similar pattern of sequence distribution as in genome wide at dyad (Figure 8C). As the fold change increases to 2-fold, the sequence distributions getting distorted both, at boundary and core of the dyads. On the contrary, when fold change decreases to -2 fold, the A/T content get higher both, at boundary and core, while G/C content get lower specifically at flanking region of the dyads. To further check the role of G/C and A/T sequence content at dyad, I grouped the sequences into subsets based on stringency cut-off. This revealed that decrease in G/C (Figure 8D) at the core region as the position strength increases. These change in terms of sequence content reveals the importance of flanking repelling elements for the positioning of nucleosome in all three conditions, and also in nucleosomes which are changing with hest stress. Such elements with strong G/C cores and A/T flanks are emphasized with proposed positioning mechanism (Figure 8E). Unlike 10 bp dinucleotide periodicity of nucleosomal population in various species [Satchwell et al., 1986; Segal et al., 2006], these positioning signals are proposed to be contributing factor in precise positioning and/or rotational topography of DNA over the nucleosomes [Segal et al., 2006] on a small but significant scale.

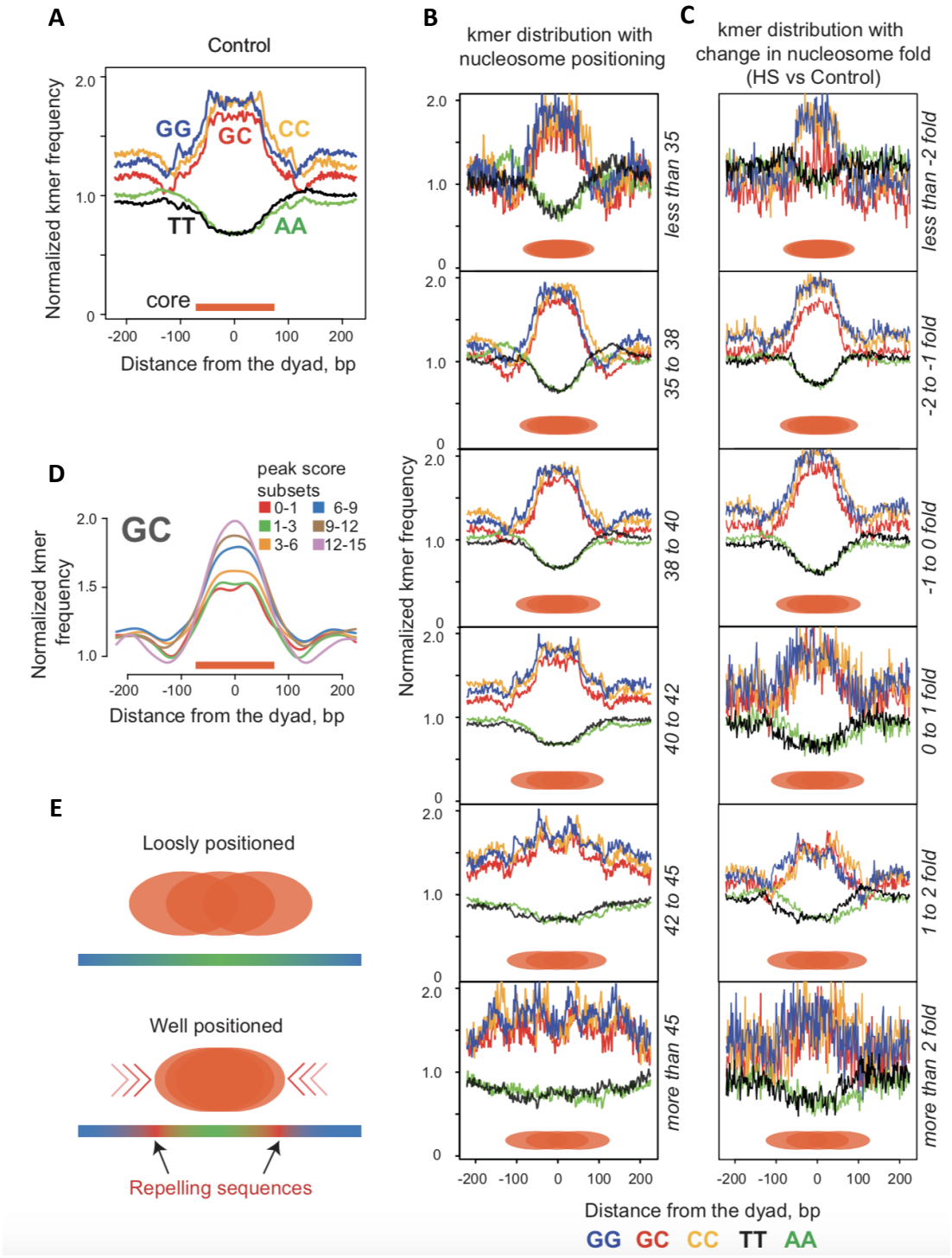


Figure 8. **A**: Sequence signals within sites containing moderately positioned nucleosomes. Distance from the dyad to a given dinucleotide are plotted along X-axis; Y-axis denotes frequency of a given k-mer divide by its genome-wide occurence. Orange band indicates the 147 bp footprint of a nucleosome. **B**: Change in k-mer distribution with increasing fuzziness score of nucleosomes (top: well positioned, bottom: loosely positined). X and Y axes as in (a). **C**: Change in k-mer distribution within differential nucleosome occupancy; HS vs control. Top three plots showing k-mer distribution in loss in occupancy while bottom three showing for increase in occupancy. X and Y axes same as in (a). **D**: Changes in GC dinucleotide usage with increasing positioning stringency. X and Y axes same as in (a). Shown are curves of GC

usage within the sites of increasing occupancy of nucleosome dyads (Peak score subset of 0-1, 1-3, 3-6, 6-9, 9-12 and 12-15). **E**: Schematic depiction of the nucleosome dyad site positioning mechanism. The C/G-rich core area (green) favors occupancy, but does not precisely position the nucleosome (top). Adding flanking A/T-rich repelling elements (purple, bottom) restricts the position of the nucleosome.

DANPOS2 also yield a wig file which can be used to see the nucleosome profile across the genome. Figure 9 is showing the screenshot of nucleosome profile from genome browser of the first Mb region of chromosome 1.

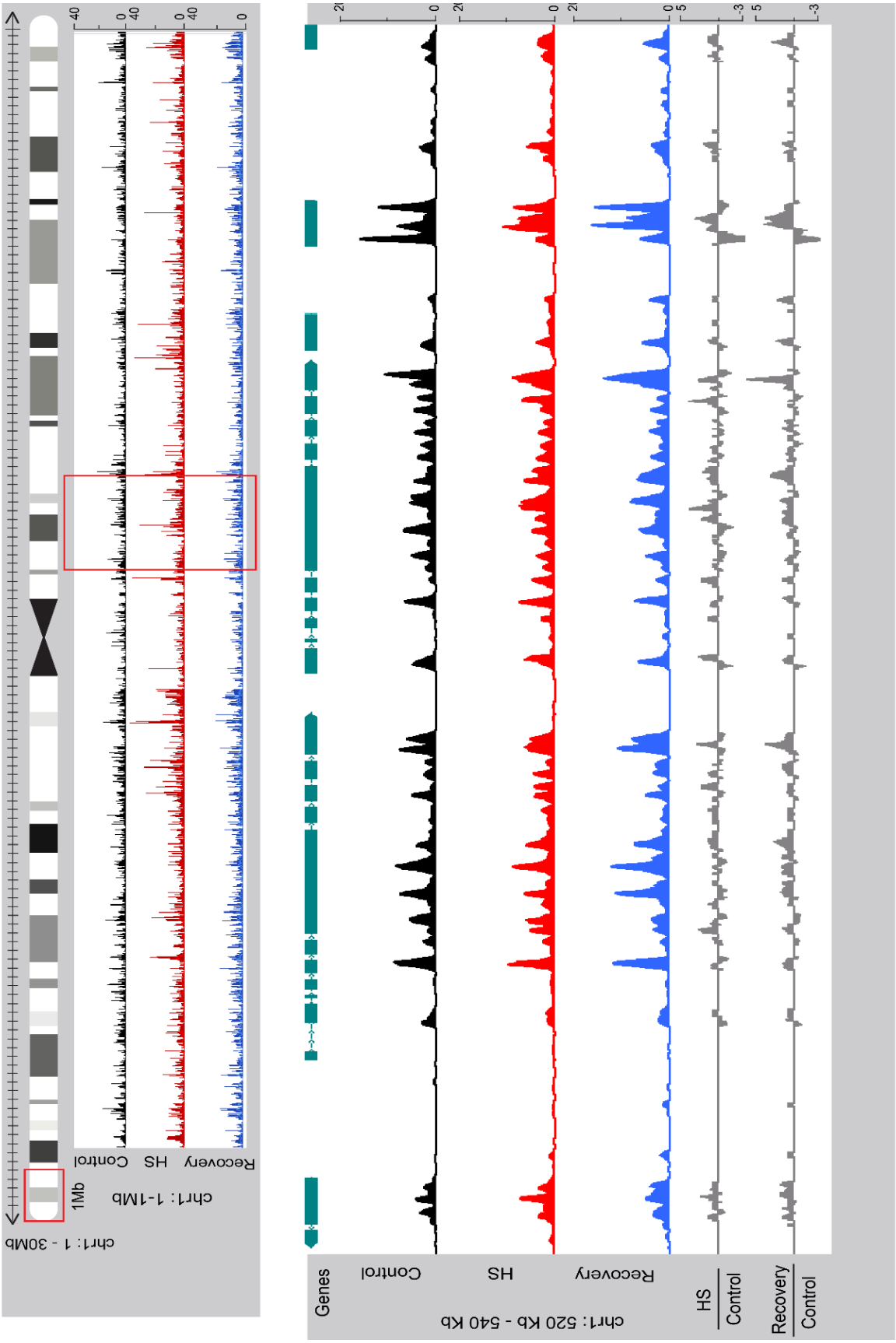


Figure 9. Genome browser screenshot from chromosome 1 of *A. thaliana.* Upper part is showing from chr1:0 to 1 Mb and lower part is showing 520kb to 540 kb

4.5. Nucleosome enrichment at gene body and promoter under heat stress

For analyzing the nucleosome occupancy around genes, I plotted the average nucleosome signal for all protein coding genes, all protein coding expressed genes (all expressed genes) and all protein coding non-expressed genes. I chose the range from 1 kb upstream of transcription start site (TSS) to 1 kb downstream of TSS and 1 kb upstream of transcription termination site (TTS) to 1 kb downstream of TTS. I found thatnucleosome occupancy of all protein-coding genesshowed difference in average plots under HS and recovery with respect to thecontrol condition (Figure 10A). The average plot showedoverall nucleosome enrichment throughout the genes, and in upstream (promoter) and downstream of genes. Around TSS, there is depletion or no nucleosome present in all three conditions and this region is called nucleosome free region (NFR). Under HS, once the nucleosome occupancy gained over the regions, it remained more or less same in recovery condition. In all expressed genes, there was enrichment in +1 nucleosome (first nucleosome after TSS) in all three conditions. Under HS, enrichment was observed in further nucleosomes after +1 also (Figure 10B). On the contrary, nucleosome occupancy appeared flatter in genes with less than 5 FPKM expression value (all non-expressed genes) in comparison to all expressed genes (Figure 10C). Average nucleosome occupancy of all heat responsive genes showed highest enrichment throughout the gene body. Specially, the first and last few nucleosomes showed sharp increment in the occupancy (Figure 10D). I performed the t-test for the significance of difference among control, HS and recovery for each category, and found p_{val} were very close to zero for HS vs control and recovery vs control comparison. While p_{val} was not significant for HS vs recovery.

From this observation, it is clear that nucleosomes are showing enrichment under high temperature (37°C) and the change remains stable for at least 48 h during which the plants were subjected to ambient conditions (recovery). These enrichments are normally present throughout the genebodies, including upstream and downstream locations, but they are more prominent in starting and ending nucleosomes of genes. This also correlatesmy previous results (Figure 5 and6) where individual nucleosomelocations are less strictly associated with fixed position (high fuzziness-score), and has highernucleosome occupancy under HS and recovery. Observations from heat responsive genes suggest that change in nucleosomes are more associated the differentially expressed genes. Keeping this in mind, I moved further to analyze nucleosomes occupancy in differentially expressed genes.

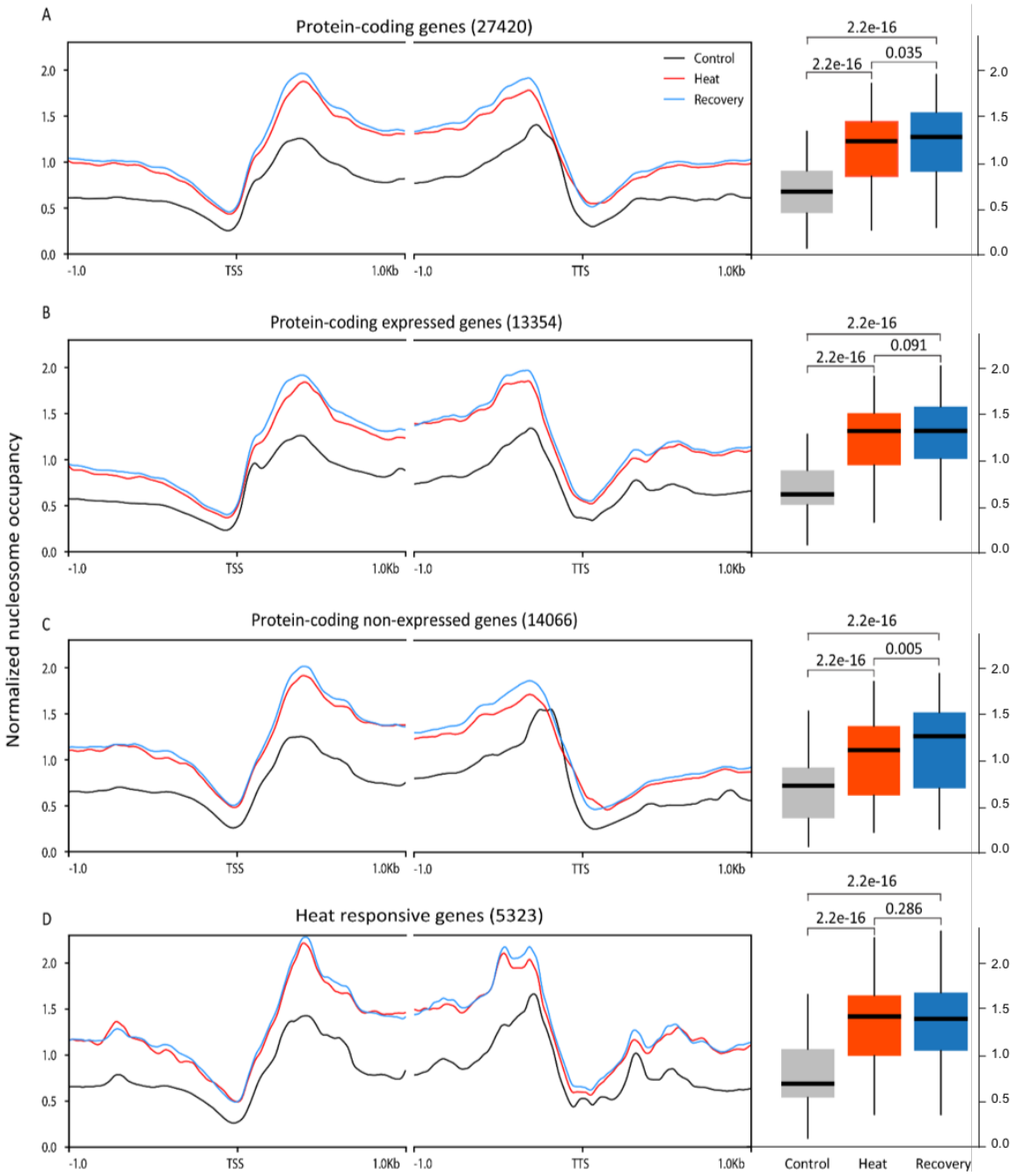


Figure 10. Average nucleosome occupancy plot of control (black), Heat (red) and recovery (blue) in gene body. Left half showing region from 1000 bp downstream to transcript start site (TSS) to 1500 bp upstream, while right half showing region from 1500 bp downstream to transcript termination site (TTS) to 1000 bp upstream. Boxplot of average nucleosome occupancy from same region of control (black), Heat (red) and recovery (blue) with t-test p_{val}. **A:** All 27420 protein coding genes from *TAIR10*; HS vs control p_{val}< 2.2e-16, recovery vs control p_{val}< 2.2e-16 and HS vs recovery p_{val} = 0.0352. **B**: Protein coding genes with minimum 5 FPKM transcript expression level in at least one of the samples (13354 genes); HS vs control p_{val}< 2.2e-16, recovery vs control p_{val}< 2.2e-16 and HS vs recovery p_{val} = 0.09112. **C:** Protein coding genes with less than 5 FPKM transcript expression in all three samples (14066 genes); HS vs control p_{val}< 2.2e-16, recovery vs control p_{val}< 2.2e-16 and HS vs recovery p_{val} = 0.005515. **D:** Heat responsive genes with significantly differentially expressed (5312 genes);

HS vs control p_{val} < 2.2e-16, recovery vs control p_{val} < 2.2e-16 and HS vs recovery p_{val} = 0.2861. y-axis is normalized nucleosome occupancy of *MNase-seq* reads.

4. 6. Categorization of the genes based on expression fold change

To analyze the relation between gene expression and nucleosome change, I categorized the heat responsive genes into six groups based on the amplitude of the change in their transcription. These six groupswere, log_2FC 1 to 2 (n = 1743), 2 to 4, n = 892, more than 4, n = 184 for up-regulated, from -2 to -1, n = 584, from -4 to -2, n = 554, less than-4, n = 194 for down-regulated (Table 5).

log_2FC Up-regulated	***Name***	***Number of genes***	***Number of genes with HSE***
1 to 2	**Group** I	1743	1050 (60.24%)
2 to 4	**Group** II	892	572 (64.12%)
Above 4	**Group** III	184	127 (69.02%)
log_2FC Down-regulated			
-2 to -1	**Group** IV	584	305 (52.22%)
-4 to -2	**Group** V	554	296 (53.43%)
Below -4	**Group** VI	194	97 (50%)

Table 5. Heat responsive genes were subcategorized into six groups based on fold change.

For all six groups, I performed GO term analysis using Gene Ontology enrichment analysis and visualization tool GOrilla (http://cbl-gorilla.cs.technion.ac.il/). The list of *protein-coding expressed genes* was used as a background list for the enrichment analysis. Background set is group of all the genes which is used as a reference for estimating the enrichments of user provided genes subset. Figure 11 is showing the overview of all six groups with their GO process and cellular locations (component).

Group I (log_2FC = 1 to 2, n = 1743) contain the genes that are enriched in common metabolic processes and located in all cellular components, which were likely to be over expreesed with lower degree under HS. **Group II (**log_2FC = 2 to 4, n = 892) include genes which were majorly involved in RNA processing and their location was predominantly nuclear, suggesting the involvement directly in transcriptional dynamics or indirectly aiding the genes, which were extremely differential expressed. **Group III (**log_2FC > 4, n = 184) had the genes which were

immensely up-regulated under HS. These genes were enriched in biological process GO terms of heat and light responses, and includedHSPs, HSFs, and protein folding chaperones. **Group IV (**log_2FC = -2 to -1, n = 584) had slightly down-regulated genes with functional enrichment in cell death. It may be interpreted as at the time of HS; the cell needs to reduce their normal death process as a dosage compensation under stress. **Group V (**log_2FC = -4 to -2, n = 554) contained the genes with moderately down-regulated expression and mainly involved in process of carbohydrate metabolism. **Group VI (**$log_2FC < -4$, n = 194) has the genes which were extremely down-regulated, with function related to nucleosome assembly and chromatin organization. In addition, their locations were dominated in nucleosome, DNA packaging complex and chromosomal part, indicating that at high temperature, the DNA loosens or forms more open structure and also there may be reduction in nucleosome signal at some specific places.

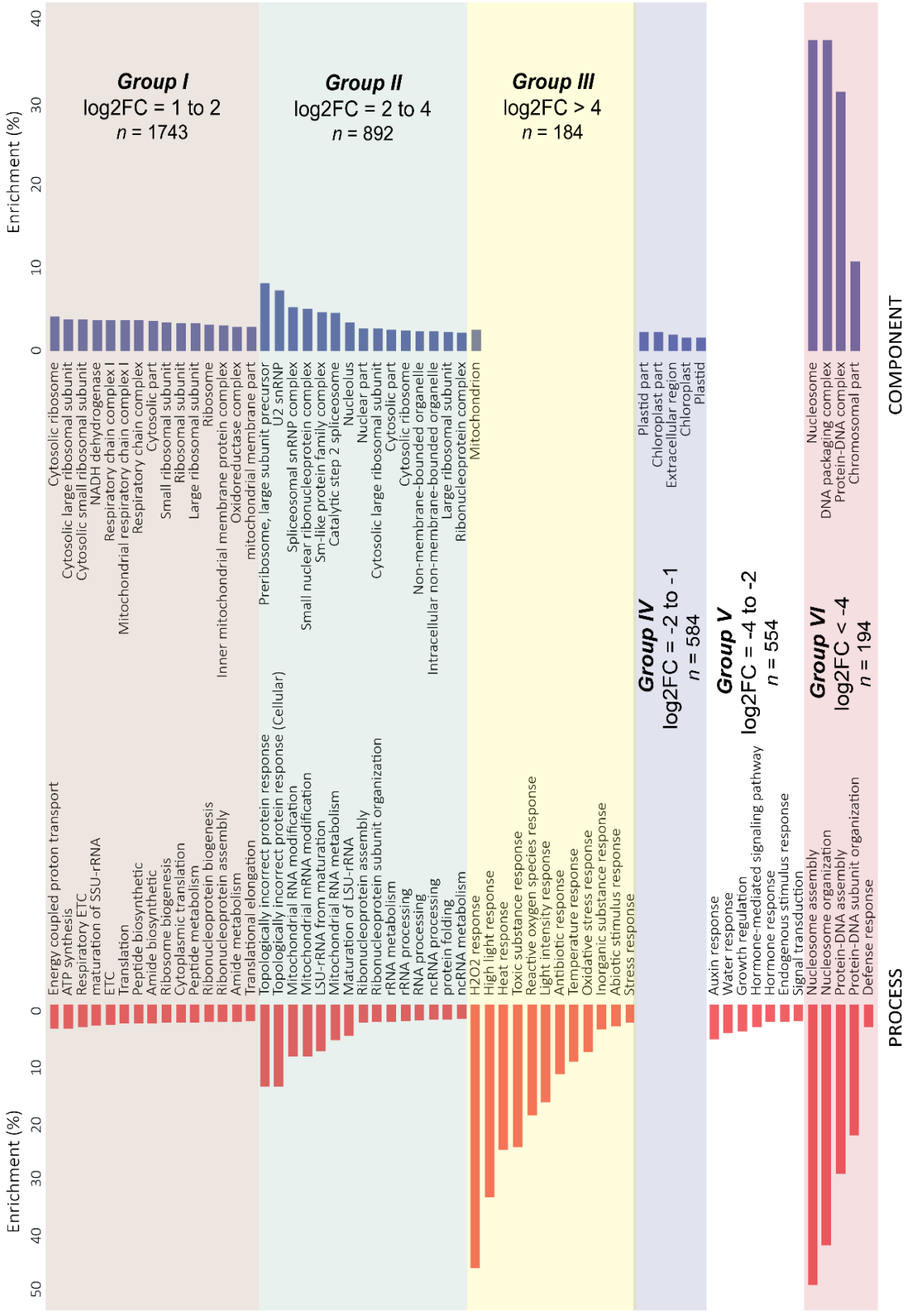


Figure 11. GO enrichment of all six groups compare to background set, here the background is represented by all protein coding genes. Genes involved in cellular components and different process are shown in blue and red bars, respectively.

4.7. Heat shock element motif identification

I found from my results that both nucleosome occupancy and gene expression are changing under HS. It has been extensively studied that the major protein groups, which were induced under HS, are heat shock proteins (HSPs). Along with HSPs, there are many genes which are also induced under HS. Heat shock elements (HSEs) are the most important motifsin promoters of such genes, where HSFs bind to activate the transcription process under HS. It has been shown in yeast that these HSEs region in promoter of heat-induced genes must expose to HSFs or in other words, these regions should be devoid of nucleosomes under HS.

I found from literature that there are four major kinds of HSEs present in *A. thaliana* [Raxwal et al., 2012]. Therefore, for getting clear insight of HS effects, I searched HSEs in all genes from the six groups. I used FIMO, Meme suit for searching these four motifs against 1500 base upstream, promoter region (p_{val}<= 0.00001). The number of genes with HSEs in each groupare enlisted in Table 5. Here, I am reporting gene with at least one HSEs in their promoter region.

Apart from the six gene expression groups, I also searched for HSEs in all protein-coding genes. In addition, I found 15,846 genes had HSEs in their promoter.

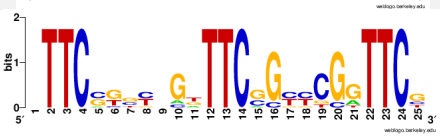
HSE Types	***Sequence***	***Logo***	***Number of protein-coding genes with HSE***
4P type	nTTCnnGAAnnTTCnnGAAn		4227
3P type	nTTCnnGAAnnTTCn		6983
Gap type	nTTCnnGAAnnnnnnnGAAn		6632
Step type	nTTCnnnnnnnTTCnnnnnnnTTCn		8353

Table 6. Four major types Heat shock elements (HSEs) with sequence and logo. Number of occurrence in 1500 base of promoter of all protein-coding genes (Total number of protein coding genes with one or more HSE are 15846).

4.8. Nucleosome profiles of differentially expressed genes

For analyzing the nucleosome profile around differentially expressed genes, I plotted the average nucleosome occupancy for all six groups (Figure 12). The analyzed regions were 1000 bp upstream/downstream of TSS and TTS. From all these six plots, Group III (highly upregulated genes) showed very significant difference in nucleosome occupancy under HS (t-test; pval < 6.109×10^{-6}). While in other five groups (I, II, IV, V and VI), nucleosome occupancy was more or less similar to all expressed genes in control, HS and recovery.

Group III plot suggested that there was clear relative difference in nucleosome occupancy in HS and recovery with respect to control. Itshowedthat for highly up-regulated genes ($log_2FC > 4$) in high temperature, there is arelative loss in nucleosome occupancyunder HS and it remains the same in 48hr of recovery condition. This loss in nucleosome was observed at its maximum at the TSS region, suggesting that in control conditions, there is higher occupancy of nucleosomes at the start site of such genes. With the application of heat, these nucleosomes lose their occupancy and at the same time the expression of such genes gets up-regulated. This nucleosome architecture remains same in recovery condition. To analyze it further, we performed correlation between nucleosome occupancy and corresponding gene expression for each group.

In order to get clearer image at the individual gene level, few of the examples from highly up-regualted category (Group III) has been shown in figure 13. In all four examples the nucleosome occupancy is decresing around TSS under HS, and the same time there is increase in gene expression at very high degree. While, in the recovery condition, some nucleosome recovers very minutely or it might be the starting of the recovery. But, at the same time gene expression under recovery is fully reverted.

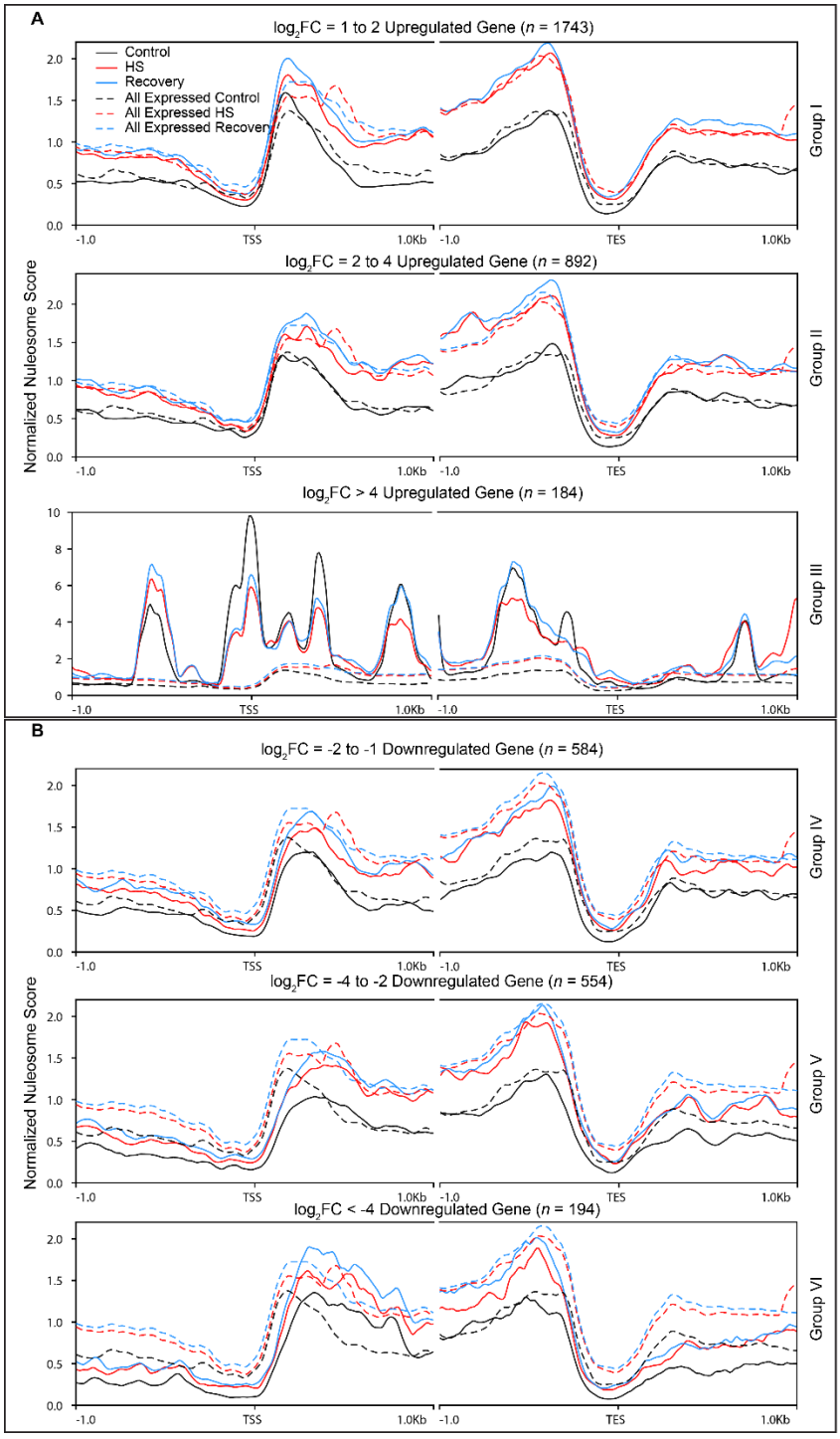


Figure 12. Nucleosome occupancy around gene body of six groups of genes; control (black), Heat (red) and recovery (blue) in gene body; **A:** Up-regulated genes from first three groups i.e. $log_2FC = 1$ to 2, $log_2FC = 2$ to 4 and $log_2FC > 4$. **B**: Down-regulated genes from last three groups i.e. $log_2FC = -2$ to -1, $log_2FC = -4$ to -2 and $log_2FC < -4$. Control, HS and recovery from each group are shown in black, red and blue respectively. Dashed lines are shown from all expressed genes as control.

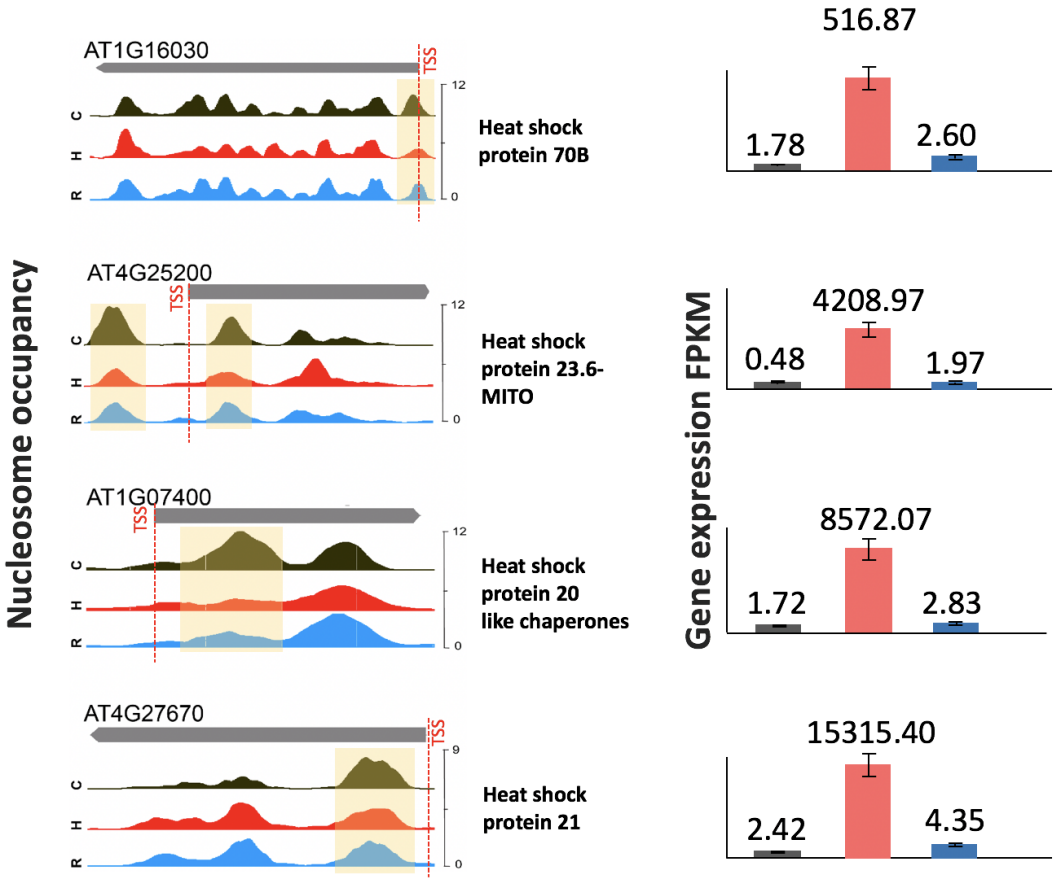


Figure 13. Nucleosome occupancy of few genes from highly up-regulated expression category (Group III) and their corresponding gene expressions in control, HS and recovery. Here, all belongs to heat shock family and showing loss in occupancy around TSS.

4.9. Correlation of nucleosome occupancy and gene expression

For analyzing the relationship between nucleosome occupancy with gene expression, I have selected the region around TSS (100 bp upstream and downstream) for each gene and averaged the nucleosome score from control, HS and recovery for this region. Although, there are several other regions of genes which shows change in nucleosome occupancy upon hest stress, I selected the TSS region because it shows maximum variability. Then I correlated the change in gene expression (HS vs control) with corresponding averaged nucleosome scores using Spearman method (Figure 14A). Each Spearman correlation score is denoted by a score "rho" with p-value. It was found that the p-values of correlation from Groups III and VI were significant ($p_{val} \leq 0.05$). Group III, which has highly HS up-regulated genes shows negative correleation score, while Group VI which has genes from highly HS down-regulated genes shows positive correlation in all three conditions. This suggests that highly up-regulated genes

under HS has lower nucleosome occupancy around TSS region. In contrast, highly down-regulated genes bear higher nucleosome occupancy around TSS.

In order to examine the extent of nucleosome occupancy changes from control to HS, and then to recovery condition among differentially expressed genes, I performed the regression analysis. This has been done by plotting a sctter plot of all the gene expression from a condition with averaged nucleomse occupancy score around TSS from the same condition, which predict the direction of pattern with a line of regression, and then p-value is estimated for these comparions. In figure 14B, top three plots are from correlation of gene expression with their corresponding nucleomse occupancy from control, HS and recovery respectively. It has been observed a negative correlation in all three conditions but moving from control to HS the extent of slope of regression line increases. Which suggests that in highly up-regulated genes, nucleosomes occupancy in HS condition tends to decrease with higher degree, and recoveres its occupancy in recovery stage. This might be the reason that in up-rulated genes under HS various transcription elements requires for elevation of transcription needs to occupy TSS region, resulting in lowering of nucleomse occupancy in order to allow these attachments. Further, as in the recovery stage when transcription acquires its original state the nucleosome occupancy also does so.

While for highly down-regulated genes (Group VI), show a weak positive correlation in all three conditions but moving from control to HS the extent of slope of regression line decreases (figure 14B; bottom). This suggests that in highly down-regulated genes the nucleomse occupancy around TSS is control condition is lower, then under heat stress becomes further lower but in very minute degree, then regains its occupancy in recovery. There might be some transcription mechanism in down-regulated genes which requires much more time to adjust its transcription elements.

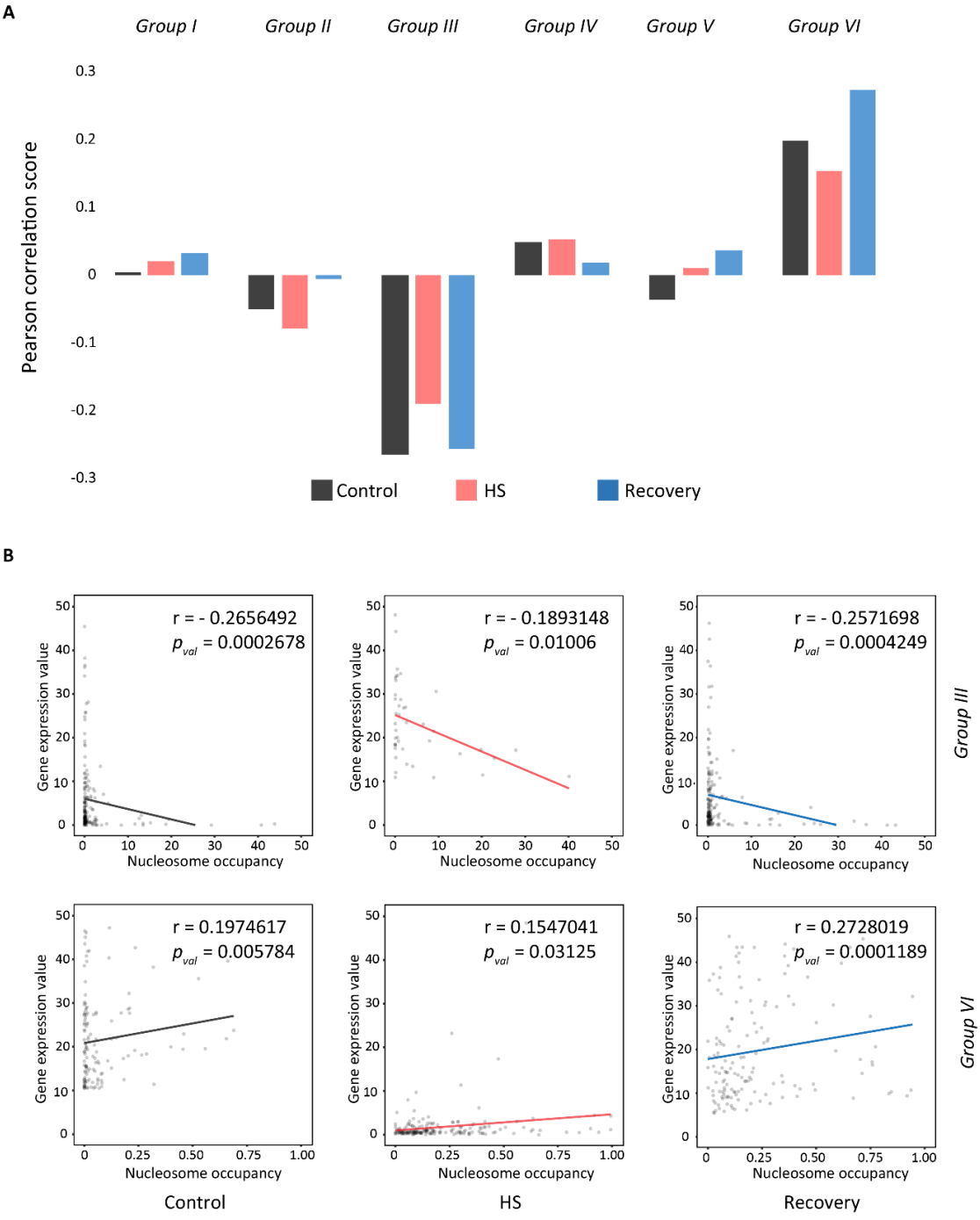


Figure 14. **A:** Spearman correlation score of nucleosome occupancy from 100bp upstream and downstream of TSS with corresponding genes expression from each of the six groups. **B:** Scatter plots of nucleosome occupancy against its corresponding gene expression in control, HS and recovery from Group III (highly up-regulated genes) and Group VI (highly down-regulated genes). **r**: Spearman correlation value with p_{val}.

5. DISCUSSION

Genome wide identification of nucleosome positions has been reported under control condition in *Arabidopsis thaliana* in previous studies [Chodavarapu et al., 2010; Li et al. 2014]. This study facilitates the initial characterization of determinats of genome-wide nucleosome organization under heat stress (HS; 37°C for 6 hours) and recovery (HS + 25°C for 48 hours) condition in areal tissues of *A. thaliana*. Under HS, many previous studies reported the analysis of change in gene expression, most of them reported the list of genes which changes [Yángüez et al., 2013; Rasmussen et al., 2013]. Here, I showed how these differentially expressed genes regained its original expression after the recovery condition (Figure 5).

5.1. Nucleosome landscape enrichement under HS and recovery condition

Here with the help of fragments size from each nucleosome, I reported global change as nuclesome density in terms of its fragment size under HS, which remains the same after recovery state (unlike gene expression) (Figure 5 and Figure 6A). Nucleosome-free region (NFR) (also called nucleosome-depleted region (NDR)) has been illustrated before [Zilberman et al., 2008], while here I showed the NFR in HS and recovery which are transcriptionally active gene promoter regions characterized by presence of NFR (Figure 10). Such comparison has not been reported before under HS and recovery conditions. This nucleosome depletion region in control ensures that DNA is accessible to proteins, which includes various chromatin regulators, and transcription and replication machineries. The most well positioned and highly regulated nucleosomes in all three conditions (control, HS and recovery) are the first nucleosomes downstream of TSS, also known as +1 nucleosome (Figure 15). Before NFR or NDR, nucleosome -1 also shows high occupancy, but comparatively less than +1 nucleosomes. Moving downstream after +1 nucleosome into the gene body, nucleosome positioning starts dissipating (Figure 15a). This position and histone composition of +1 nucleosome are important to transcription, because these factors affect RNA polymerase passage via the gene body and also binding of TFs.

Nucleosome dynamics can be explained as the interplay among nucleosome occupancy and positioning. Nucleosome occupancy is the average nucleosome number within a defined genome location in a cellular population. Hence, it is considered as probability of nucleosome being find at the analyzed site. On the other hand, nucleosome positioning is the probability of a nucleosome existing at a specific genomic location relative to being present at its

surroundings, in a given cell population. Thus, the nucleosome organization can be explained by the combination of occupancy and positioning (Figure 15b).

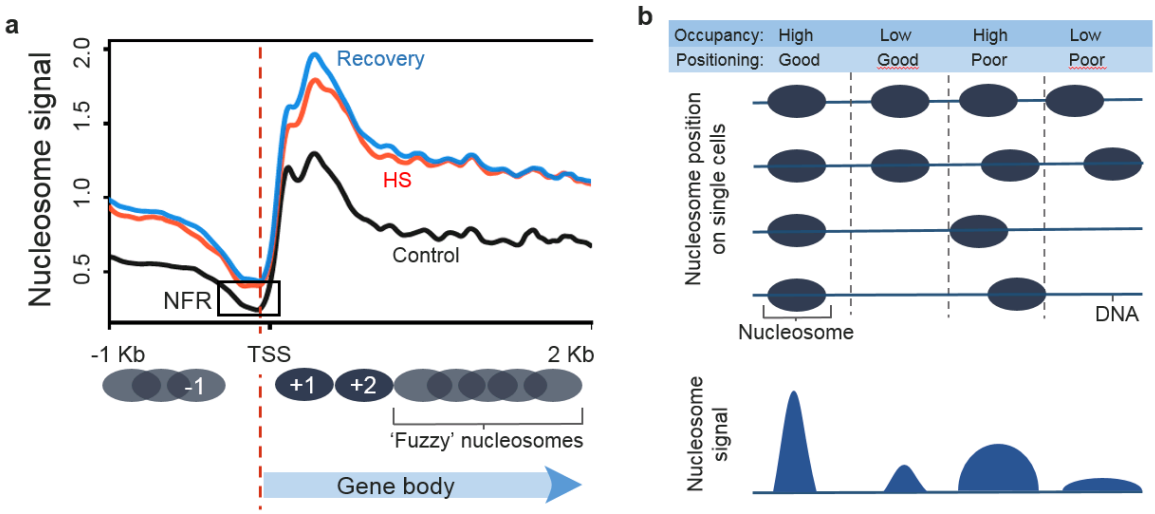


Figure 15. A. Nucleosomes can be found before and after TSS. The plotted lines (control: black, HS: red and recovery: blue) represents the distinctive occupancy and positioning of nucleosomes in common genomic locations. The peaks correspond to variation in nucleosome occupancy. A nucleosome free region (NFR)/nucleosome depleted region (NDR) is found at TSS in all genes in control, HS and recovery. Just downstream of NFR, a well-positioned nucleosome, called '+1' is situated. Before the NFR, -1 nucleosome is present, but the occupancy and positioning are less and poor, respectively in comparison to +1 nucleosome. This positioning dissipates with downstream region after +2 nucleosome and nucleosomes become fuzzier. **B.** Nucleosome occupancy is defined as probability of nucleosomes being present in a genomic region in a population of cells. It was estimated using MNase-seq reads mapping. Nucleosome positioning is the probability of nucleosome reference point being present at a specific genomic location relative to surrounding coordinates.

5.2. Variation in nucleosome spacing and occupancy with transcription level

Nucleosome has an active role in gene expression regulation through allowing the ability to TFs to access their *cis* elements in promoters [Lam et al., 2008, Workman et al., 1998; Kumar et al., 2010]. Access to the proteins to DNA wrapped with histone is facilitated with systemic un-wrapping and wrapping events [Chien and Heijden, 2014]. Spacing of nucleosomes remains same among different temperature conditions, but differs among different histone modification domains, and is influenced by the activity of transcription (Figure 7A and 7B). Unlike spacing, we reported negative correlation between gene expression and nucleosome occupancy (Figure 7C). With the increasing gene expression, there is drecrease in nucleosome ccupancy. At the same time, occupancy is always less under HS. To confirm it further, I divided the protein cooding genes to get differentially expressed gene categories (Figure 12), and found highly upregulated genes shows again negative correlation with expression (Figure

14). This result contrasts with the global phenomenon, where nucleosome profile show gain in occupancy (Figure 6A), because there might be various intergic nucleosome which could have very high occupancy under HS and recovery conditions. This could be associated by previous work where it was shown that nucleosomes play crucial roles by controlling the genes under different stress conditions [Liu et al., 2015]. In addition, specific nucleosome modification domains are associated with different stress conditions [Wang et al., 2003].

Interestingly, all the HSPs are included in the highly up-regulated genes. Apart from HSPs the group also contain few other genes which were associated with heat acclimatization and other abiotic stress (Figure 11). From these 184 highly up-regulated genes 127 (70%) contains at least one HSE in their promoter regions (Table 5). On the other hand, most of highly down-regulated genes belongs to categories of nucleosomal assembly and organizations (Figure 11). Here, 50% of the genes has at least one HSE in their promoter (Table 6).

This can be explained as, for these highly up-regulated genes under HS, nucleosomes lose their occupancy by un-wrapping of the DNA around the TSS region, so that RNA polymerases and TFs can easily access the DNA around promoter region. Consequently, these genes get transcribed more profoundly under HS (Figure 16). At the same time, all these genes belong to heat response and acclimatization. In the recovery condition I found the gene expression of highly up-regulated genes were regained to their original state but nucleosome occupancy remains like HS condition. This could be explained in relation of epigenetic memory establishment under HS. Studies found that prolonged exposure of HS leads to accumulation of H3K4me3 and H3K4me2 that persisted even after active transcription [Ding et al., 2012; Sani et al., 2013]. Under recovery, this histone methylation may remain intact as an epigenetic memory stored in genome [Bäurle et al., 2017], for countering sudden re-exposure of HS in near future.

For highly down-regulated genes, the situation may not be opposite. I found in my correlation analysis of these down-regulated genes, there is decrease in nucleosome occupancy at TSS region with decrease in gene expression under HS (Figure 14). This could be explained in context of repressor proteins. Repressors are DNA/RNA binding proteins that inhibits the gene expression of one of several genes through binding to the associated operators. This binding of repressor blocks the attachment of RNA polymerase with other transcription factors to the promoter region, results in preventing transcription of the genes into mRNA [Neidhardt et al., 1984]. In control, this highly down-regulated group has empty region (nucleosome free) around promoter where transcription machinery can attach and thus the normal transcription can happen. Under HS, the region called operator become devoid of nucleosome so that repressor protein can bind and hence the decreases the transcription

(Figure 16). Further, most of these highly down-regulated genes belongs to nucleosome assembly suggesting that under HS decrease in nucleosomes and its assembler, that could be associated with loss of nucleosome in highly up- and down-regulated genes.

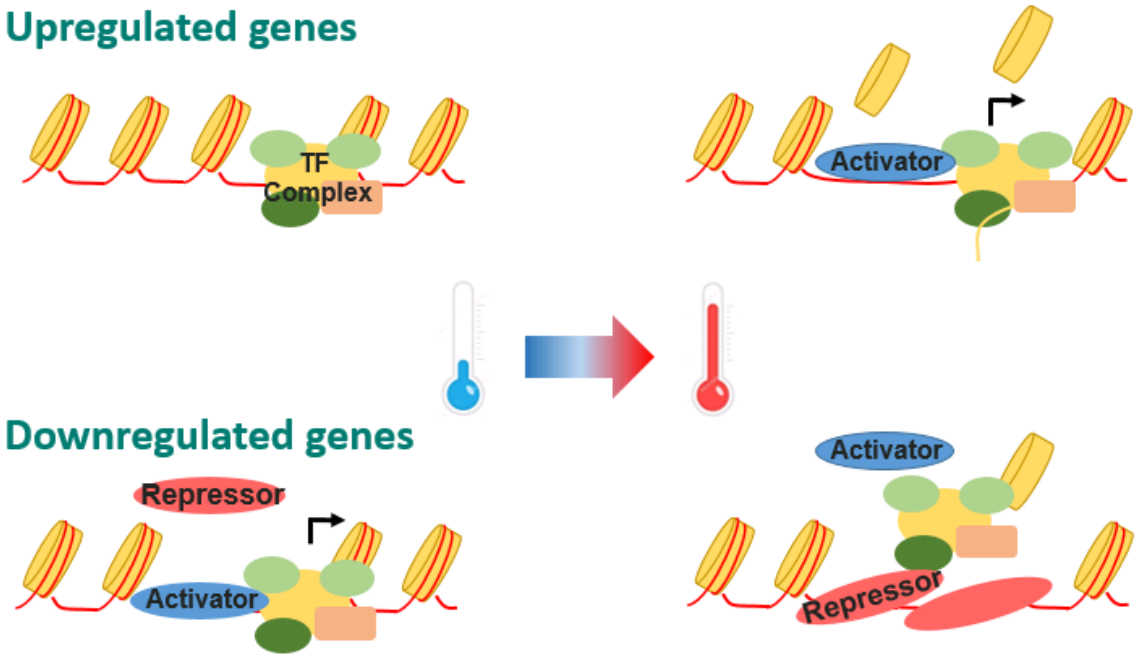


Figure 16. Model depiction of events occurring with nucleosome and gene expression under control (left) and HS (right) condition for highly up-regulated and down-regulated genes.

5.3. Nucleosome positioning influenced by their sequence composition

Apart from the nucleosome occupancy and positioning, there are several other factors, such as subnucleosomal structure, histone variants and modifications, which contribute to nucleosomal organization. Sequence pattern in the core and flanking region of the nucleosomes played a crucial role in nucleosomal organization, and have been shown in animal [Mavrich et al., 2008; Valouev et al., 2011] and plants [Zhang et al., 2015]. The influence of the sequence composition on positioning preferences of nucleosomes is self-deprecating but detectable (Figure 8A). I confiremed from previous analyses how the A/T and G/C contents varied in core and boundaries of the nucleosome under control [Zhang et al., 2015], and the pattern of its varation with function of nucleosome postioning (Figure 8B). This work also confirms the variation in nucleosome sequence in relation to change in nucleosome occupancy under HS (Figure 8C). Despite DNA sequence being a powerful driver of nucleosome organization on genome landscape, the external temperature conditions often

override sequence signals and can direct nucleosomes to occupy intrinsically unfavorable DNA elements or evict nucleosomes from intrinctically favorable locations.

6. SUMMARY:

In conclusion, this study revealed that under HS, the average genome-wide nucleosome occupancy at its position was increased, and remained more or less the same in recovery conditions in *A. thaliana*. Probability of finding a nucleosome at a position also increased under HS and recovery. Average nucleosome occupancy in a gene body of all protein-coding genes was also enhanced under HS and recovery, but when occupancy was measured with increasing gene expression, it was found to be less in HS in each bin. In highly up-regulated genes, there was a relative decrease in nucleosome occupancy especially around TSS. Under HS, relative loss of nucleosomes around TSS was correlated with relatively higher gene expression. On the other hand, relative gain of nucleosomes around TSS correlated with relative lower gene expression.

REFERENCES

1. **Ahuja, I., de Vos, R. C., Bones, A. M., Hall, R. D.** (2010). Plant molecular stress responses face climate change. Trends in Plant Science**15 (12)**:664-774.
2. **Andrews S.** (2010). FastQC: a quality control tool for high throughput sequence data. Babraham Bioinformatics.
3. **Atkinson, N.J., Lilley, C.J., Urwin, P.E.** (2013). Identification of genes involved in the response of *Arabidopsis* to simultaneous biotic and abiotic stresses. Plant Physiology**162 (4)**:2028-2041.
4. **Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., Ren, J., Li, W. W., Noble, W. S.** (2009). MEME SUITE: tools for motif discovery and searching. Nucleic Acids Research **37** (Web Server issue):W202-208.
5. **Balasubramanian, S., Sureshkumar, S., Lempe, J., Weigel, D.** (2006). Potent Induction of *Arabidopsis thaliana* Flowering by Elevated Growth Temperature. PLoS Genet., **2**:106
6. **Baniwal, S. K., Bharti, K., Chan, K. Y., Fauth, M., Ganguli, A., Kotak, S., Mishra, S. K., Nover, L., Port, M., Scharf, K. D., Tripp, J., Weber, C., Zielinski, D., von Koskull-Döring, P. J.** (2004). Heat stress response in plants: a complex game with chaperones and more than twenty heat stress transcription factors. Journal of Biosciences**29 (4)**:471-487.
7. **Bäurle I** (2017). CAN'T REMEMBER TO FORGET YOU: Chromatin-based priming of somatic stress responses. Seminars in Cell and Developmental Biology. Semin Cell Dev Biol. **83**:133-139
8. **Bäurle I.** (2016). Plant Heat Adaptation: priming in response to heat stress. F1000Res. **5**: 694.
9. **Becker PB, Workman JL.** (2013). Nucleosome remodeling and epigenetics. Cold Spring Harb Perspect Biol. **5 (9)**
10. **Benjamini, Y., Hochberg, Y.** (1995). Controlling the False Discovery Rate: A Practical and Powerful Approach to Multiple Testing. Journal of the Royal Statistical Society**1**:289-300.
11. **Bennetzen, J., L.** (2000). The many hues of plant heterochromatin. Genome Biol. **1 (1)**:REVIEWS107
12. **Berger, S. L.** (2002). Histone modifications in transcriptional regulation. Current Opinion in Genetics & Development**12 (2)**:142-148.
13. **Berger, S. L.** (2007). The complex language of chromatin regulation during transcription. Nature. **447 (7143)**:407-412.
14. **Bohnert, H. J., Nelson, D. E., Jensen, R. G.** (1995). Adaptations to Environmental Stresses. Plant Cell**7 (7)**:1099-1111.
15. **Bolton, M. D.** (2009). Primary metabolism and plant defense--fuel for the fire. Molecular Plant-Microbe Interactions.**22 (5)**:487-497.
16. **Bradbury, E. M.** (1989). Chromatin Series in molecular biology. Journal of Molecular Recognition **2 (3)**:530.
17. **Brzeski, J., Jerzmanowski, A**. (2003). Deficient in DNA methylation 1 (DDM1) defines a novel family of chromatin-remodeling factors. J Biol Chem. **278 (2)**:823-828.

18. **Calonje, M., Sanchez, R., Chen, L., Sung, Z. R.** (2008). EMBRYONIC FLOWER1 participates in polycomb group-mediated AG gene silencing in *Arabidopsis*. Plant Cell.**20**:277–291.
19. **Chan, S. W., Henderson, I. R., Jacobsen, S. E.** (2005). Gardening the genome: DNA methylation in *Arabidopsis thalianaArabidopsis thaliana*. Nat Rev Genet. **6 (5)**:351-360.
20. **Chang, H. C., Tang, Y. C., Hayer-Hartl, M., Hartl, FU.** (2007). SnapShot: molecular chaperones, Part I. Cell**128 (1)**:212.
21. **Charng, Y. Y., Liu, H. C., Liu, N. Y., Chi, W. T., Wang, C. N., Chang, S. H., & Wang, T. T.** (2007). A heat-inducible transcription factor, HsfA2, is required for extension of acquired thermotolerance in *Arabidopsis*. Plant Physiology, **143**:251–262.
22. **Charng, Y. Y., Liu, H. C., Liu, N. Y., Hsu, F. C., & Ko, S. S.** (2006). *Arabidopsis* Hsa32, a novel heat-shock protein, is essential for acquired thermotolerance during a long recovery period after acclimation treatment. Plant Physiology, **140**:1297–1305.
23. **Chawla, R., Nicholson, S. J., Folta, K. M., Srivastava, V.** (2007). Transgene-induced silencing of *Arabidopsis* phytochrome A gene via exonic methylation. Plant J. **52 (6)**:1105-1118.
24. **Chen, K., Xi, Y., Pan, X., Li, Z., Kaestner, K., Tyler, J., Dent, S., He, X., Li, W.** (2013). DANPOS: dynamic analysis of nucleosome position and occupancy by sequencing. Genome research **23 (2)**:341-351.
25. **Choi, J., Hyun, Y., Kang, M. J., In Yun, H., Yun, J. Y., Lister, C., Dean, C., Amasino, R. M., Noh, B., Noh, Y. S., et al.** (2009). Resetting and regulation of Flowering Locus C expression during *Arabidopsis* reproductive development. Plant J. **57**:918–931
26. **Choi, K., Zhao, X., Kelly, K. A., Venn, O., Higgins, J. D., Yelina, N. E., et al.** (2013). *Arabidopsis* meiotic crossover hot spots overlap with H2A.Z nucleosomes at gene promoters. Nature Genetics **45**:1327-1336.
27. **Cokus, S. J., Feng, S., Zhang, X., Chen, Z., Merriman, B., Haudenschild, C. D., Pradhan, S., Nelson, S. F., Pellegrini, M., Jacobsen, S. E. (2008)**. Shotgun bisulphite sequencing of the *Arabidopsis* genome reveals DNA methylation patterning. Nature **452 (7184)**:215-219.
28. **Conerly, M. L., Teves, S. S., Diolaiti, D., Ulrich, M., Eisenman, R. N., Henikoff, S.** (2010). Changes in H2A.Z occupancy and DNA methylation during B-cell lymphomagenesis. Genome Res. **20 (10)**:1383-1390.
29. **Cosgrove, M. S., Wolberger, C.** (2005). How does the histone code work? Biochemistry and Cell Biology **83 (4)**:468-476.
30. **Creyghton, M. P., Markoulaki, S., Levine, S. S., Hanna, J., Lodato, M. A., Sha, K., Young, R.A., Jaenisch, R., Boyer, L.A.** (2008).H2AZ is enriched at polycomb complex target genes in ES cells and is necessary for lineage commitment. Cell **135 (4)**:649-661.
31. **Crisp, P. A., Ganguly, D., Eichten, S. R., Borevitz, J. O., & Pogson, B. J.** (2016). Reconsidering plant memory: Intersections between stress recovery, RNA turnover, and epigenetics. Science Advances, **2**:e1501340.
32. **Crisp, P. A., Ganguly, D., Smith, A. B., Murray, K. D., Estavillo, G. M., Searle, I., Ford E, Bogdanović O, Lister R, Borevitz JO, Eichten SR, Pogson, B. J.** (2017). Rapid recovery gene downregulation during excess-light stress and recovery in *Arabidopsis*. Plant Cell, **29**:1836–1863

33. **Deal, R. B., Henikoff, S.** (2010). A simple method for gene expression and chromatin profiling of individual cell types within a tissue. Dev Cell. **18**:1030–1040
34. **Deal, R. B., Henikoff, J. G., Henikoff, S.** (2010). Genome-wide kinetics of nucleosome turnover determined by metabolic labeling of histones. Science. **328**:1161–1164.
35. **Deal, R. B., Topp, C. N., McKinney, E. C., Meagher, R. B.** (2007). Repression of flowering in *Arabidopsis* requires activation of FLOWERING LOCUS C expression by the histone variant H2A.Z. Plant Cell **19 (1)**:74-83.
36. **Del Prete, S., Arpon, J., Sakai, K., Andrey, P., Gaudin, V.** (2014). Nuclear architecture and chromatin dynamics in interphase nuclei of *Arabidopsis thalianaArabidopsis thaliana*. Cytogenetic and Genome Research **143**:28-50.
37. **Dickerson, Richard, E.** (1992). DNA Structure From A to Z. Methods in Enzymology. **211**: 67–111.
38. **Drane, P., Ouararhni, K., Depaux, A., Shuaib, M., Hamiche, A.** (2010). The death-associated protein DAXX is a novel histone chaperone involved in the replication-independent deposition of H3.3. Genes Dev. **24**:1253–1265.
39. **Du, J., Johnson, L. M., Jacobsen, S. E., Patel, D. J.** (2015). DNA methylation pathways and their crosstalk with histone methylation. Nature Reviews Molecular Cell Biology **16 (9)**:519-532.
40. **Dupont, C., Armant, D. R., Brenner, C. A.,** (2009). Epigenetics: Definition, Mechanisms and Clinical Perspective. Semin Reprod Med. **27 (5)**: 351–357.
41. **Eden, E., Navon, R., Steinfeld, I., Lipson, D., Yakhini, Z.** (2009). GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. BMC Bioinformatics **3**:10:48.
42. **Eskeland, R., Leeb, M., Grimes, G. R., Kress, C., Boyle, S., Sproul, D., Gilbert, N., Fan, Y., Skoultchi, A. I., Wutz, A., et al.** (2010). Ring1B compacts chromatin structure and represses gene expression independent of histone ubiquitination. Molecular Cell. **38**:452-464.
43. **Fan-Tso, C., Thijn. van der.H.** (2014). Characterization of Nucleosome Unwrapping within Chromatin Fibers Using Magnetic Tweezers. Biophysical Journal **107 (2)**: 373–383.
44. **Field, Y., Kaplan, N., Fondufe-Mittendorf, Y., Moore, I., K., Sharon, E., Lubling, Y., Widom, J., Segal, E.** (2008). Distinct modes of regulation by chromatin encoded through nucleosome positioning signals. PLoSComput Biology. **4 (11):**e1000216.
45. **Fincher, J. A., Vera, D. L., Hughes, D. D., McGinnis, K. M., Dennis, J. H., Bass, H. W.** (2012). Genome-wide prediction of nucleosome occupancy in maize reveals plant chromatin structural features at genes and other elements at multiple scales. Plant Physiology **162 (2)**:1127-41.
46. **Finnegan, E. J., Kovac, K. A.** (2000). Plant DNA methyltransferases. Plant Mol Biol. **43 (2-3)**:189-201
47. **Flores, O., Deniz, Ö., Soler-López, M., Orozco, M.** (2014). Fuzziness and noise in nucleosomal architecture. Nucleic Acids Research **42 (8)**:4934-4946.
48. **Folta, A., Severing, E. I., Krauskopf, J., van de Geest, H., Verver, J., Nap, J. P., Mlynarova, L. (**2014). Over-expression of *Arabidopsis* AtCHR23 chromatin remodeling ATPase results in increased variability of growth and gene expression. BMC Plant Biol, **14**:76
49. **Francis NJ, Kingston RE, Woodcock CL.** (2004). Chromatin compaction by a polycomb group protein complex. Science. **306 (5701)**:1574-1577.

50. **Fransz, P., de Jong, H.** (2011). From nucleosome to chromosome: a dynamic organization of genetic information. Plant Journal**66**:4-17.
51. **Fransz, P., de Jong, J. H., Lysak, M., Castiglione, M. R., Schubert, I.** (2002). Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. PNAS**99**:14584-14589.
52. **Fuchs, J., Demidov, D., Houben, A., Schubert, I.** (2006). Chromosomal histone modification patterns--from conservation to diversity. Trends Plant Sci. **11 (4)**:199-208.
53. **Gendler, K., Paulsen, T., Napoli, C.** (2008). ChromDB: the chromatin database. Nucleic Acids Res. **36**:D298–302.
54. **Goldberg, A. D., Banaszynski, L. A., Noh, K. M., Lewis, P. W., Elsaesser, S. J., Stadler, S., Dewell, S., Law, M., Guo, X., Li, X., et al.** (2010). Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell. **140**:678–691.
55. **Goll, M. G., Bestor, T. H.** (2005). Eukaryotic cytosine methyltransferases. Annu Rev Biochem. **74 ()**:481-514.
56. **Gong, Z., Morales-Ruiz, T., Ariza, R. R., Roldán-Arjona, T., David, L., Zhu, J. K.** (2002). ROS1, a repressor of transcriptional gene silencing in *Arabidopsis*, encodes a DNA glycosylase/lyase. Cell. **111 (6)**:803-814.
57. **Grant, C. E., Bailey, T. L., Noble, W. S. (**2011). FIMO: scanning for occurrences of a given motif. Bioinformatics**27 (7)**:1017-1018.
58. **Guo, T., Fang, Y.** (2014). Functional organization and dynamics of the cell nucleus. Frontiers in Plant Science **5**:378.
59. **Guo. L., Chen, S., Liu, K., Liu, Y., Ni, L., Zhang, K., and Zhang, L.** (2008). Isolation of Heat Shock Factor HsfA1a-binding Sites in vivo Revealed Variations of Heat Shock Elements in *Arabidopsis* Thaliana. Plant Cell Physiol. **49 (9):** 1306–1315
60. **Hahn, S.** (2004). Structure and mechanism of the RNA polymerase II transcription machinery. Nature Structural & Molecular Biology **11**:394-403.
61. **Harvey, S. C.** (2015). The scrunchworm hypothesis: Transitions between A-DNA and B-DNA provide the driving force for genome packaging in double-stranded DNA bacteriophages. Journal of Structural Biology. **189**: 1–8.
62. **Hasanuzzaman, M., Hossain, M. A., da Silva, J. A. T., Fujita, M.** (2012). Plant Responses and Tolerance to Abiotic Oxidative Stress: Antioxidant Defenses is a Key Factor. Crop Stress and its Management: Perspectives and Strategies 261-316.
63. **Hasanuzzaman, M., Nahar, K., Fujita, M.** (2013). Extreme Temperatures, Oxidative Stress and Antioxidant Defense in Plants. Abiotic Stress - Plant Responses and Applications in Agriculture 169–205.
64. **He, G., Zhu, X., Elling, A. A., Chen, L., Wang, X., Guo, L., Liang, M., He, H., Zhang, H., Chen, F., et al.** (2010). Global epigenetic and transcriptional trends among two rice subspecies and their reciprocal hybrids. Plant Cell. **22**:17–33
65. **He, Y.** (2009). Control of the transition to flowering by chromatin modifications. Molecular Plant. **2**:554–564.
66. **He, Y., Doyle, M. R., Amasino, R. M.** (2004). PAF1-complex-mediated histone methylation of FLOWERING LOCUS C chromatin is required for the vernalization-responsive, winter-annual habit in *Arabidopsis*. Genes Dev. **18**:2774–2784
67. **Hediger, F., Gasser, S. M.** (2006). Heterochromatin protein 1: don't judge the book by its cover! Curr Opin Genet Dev.**16 (2)**:143-150.

68. **Heil, M., Bostock, R. M.** (2002). Induced systemic resistance (ISR) against pathogens in the context of induced plant defences. Annals of Botany**89 (5)**:503-512.
69. **Heitz, E.** (1928). Das Heterochromatin der Moose. Jahrb Wiss Botanik. **69**:762–818.
70. **Hodges, C., Bintu, L., Lubkowska, L., Kashlev, M., Bustamante, C.** (2009). Nucleosomal fluctuations govern the transcription dynamics of RNA polymerase II. Science**325 (5940):**626-628.
71. **Hohn, T., Corsten, S., Rieke, S., Müller, M., Rothnie, H.** (1996). Methylation of coding region alone inhibits gene expression in plant protoplasts. Proc Natl Acad Sci U S A. **93 (16)**:8334-8339
72. **Huot, B., Yaoa, J., Montgomery, B. L., & He, S. Y.** (2014). Growth–defense tradeoffs in plants: A balancing act to optimize fitness. Molecular Plant, **7**:1267–1287.
73. **Inagaki, S., Takahashi, M., Hosaka, A., Ito, T., Toyoda, A., Fujiyama, A., Tarutani, Y., Kakutani, T.** (2017). Gene-body chromatin modification dynamics mediate epigenome differentiation in *Arabidopsis*. The EMBO Journal **e201694983**
74. **Jeddeloh, J. A., Stokes, T. L., Richards, E. J.** (1999). Maintenance of genomic methylation requires a SWI2/SNF2-like protein. Nat. Genet., **22**:94-97
75. **Jenuwein, T., Allis, C. D.** (2001). Translating the histone code. Science**293 (5532)**:1074-1080.
76. **Jerzmanowski, A.** (2007). SWI/SNF chromatin remodeling and linker histones in plants. Biochim Biophys Acta. **1769 (5-6)**:330-345.
77. **Jiang, C., Pugh, B. F.** (2009). A compiled and systematic reference map of nucleosome positions across the Saccharomyces cerevisiae genome. Genome Biology **10 (10)**:R109.
78. **Johnson, S. M., Tan, F. J., McCullough, H. L., Riordan, D. P., Fire, A. Z.** (2006) Flexibility and constraint in the nucleosome core landscape of *Caenorhabditiselegans* chromatin. Genome Research 16: 1505–1516.
79. **Kankel, M. W., Ramsey, D. E., Stokes, T. L., Flowers, S. K., Haag, J. R., Jeddeloh, J. A., Riddle, N. C., Verbsky, M. L., Richards, E. J.** (2003). *Arabidopsis* MET1 cytosine methyltransferase mutants. Genetics. **163 (3)**:1109-1022.
80. **Khorasanizadeh, S.** (2004). The Nucleosome. Cell **116**:259-272.
81. **Kim, D. H., Doyle, M. R., Sung, S., Amasino, R. M.** (2009) Annu. Rev. Cell Dev. Biol., **25**:277-299
82. **Kobor, M. S., Venkatasubrahmanyam, S., Meneghini, M. D., Gin, J. W., Jennings, J. L., Link, A. J., Madhani, H. D., Rine, J.** (2004). A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. PLoS Biology **2 (5)**:E13
83. **Kornberg, R. D.** (1974). Chromatin Structure: A Repeating Unit of Histones and DNA. Science **184**:868-871.
84. **Krogan, N. J., Keogh, M. C., Datta, N., Sawa, C., Ryan, O.W., Ding, H., Haw, R. A., Pootoolal, J., Tong, A., Canadien, V., et al.** (2003). A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. Molecular Cell **12 (6)**:1565-1576.
85. **Kültz, D.** (2003). Evolution of the cellular stress proteome: from monophyletic origin to ubiquitous function. Journal of Experimental Biology **206 (18)**:3119-3124.
86. **Lämke, J., and Bäurle, I.** (2017). Epigenetic and chromatin-based mechanisms in environmental stress adaptation and stress memory in plants. Genome Biology **18**:124

87. **Langmead, B., Trapnell, C., Pop, M., Salzberg, S. L.** (2009). Ultrafast and memory-efficient alignment of short DNA sequences to the human genome. Genome biology **10 (3)**:R25.
88. **Law, J.A., Jacobsen, S.E.** (2010). Establishing, maintaining and modifying DNA methylation patterns in plants and animals. Nature Reviews Genetics **11**:204-220.
89. **Lázaro, A., Gómez-Zambrano, A., López-González, L., Piñeiro, M., Jarillo, J. A.** (2008). Mutations in the *Arabidopsis* SWC6 gene, encoding a component of the SWR1 chromatin remodelling complex, accelerate flowering time and alter leaf and flower development. J Exp Bot.**59**:653–666
90. **Lee, W., Tillo, D., Bray, N., Morse, R. H., Davis, R. W., Hughes, T. R., Nislow, C.** (2007). A high-resolution atlas of nucleosome occupancy in yeast. Nature Genetics**39**: 1235–1244.
91. **Leslie, A. G., Arnott, S., Chandrasekaran, R., Ratliff, R. L.** (1980). Polymorphism of DNA double helices. Journal of Molecular Biology. **143 (1):** 49–72.
92. **Li, B., Carey, M., Workman, J. L.** (2007). The role of chromatin during transcription. Cell. **128 (4)**:707-719.
93. **Li, G., Liu, S., Wang, J., He, J., Huang, H., Zhang, Y., Xu, L.** (2014). ISWI proteins participate in the genome-wide nucleosome distribution in *Arabidopsis*. Plant Journal.**78 (4)**:706-14.
94. **Lin, M. Y., Chai, K. H., Ko, S. S., Kuang, L. Y., Lur, H. S., & Charng, Y. Y.** (2014). A positive feedback loop between HEAT SHOCK PROTEIN101 and HEAT STRESS-ASSOCIATED 32-KD PROTEIN modulates long-term acquired thermotolerance illustrating diverse heat stress responses in rice varieties. Plant Physiology, **164**:2045–2053
95. **Lippman, Z., Gendrel, A. V., Black, M., Vaughn, M. W., Dedhia, N., McCombie, W. R., Lavine, K., Mittal, V., May, B., Kasschau, K. D., Carrington, J. C., Doerge, R. W., Colot, V., Martienssen, R.** (2004). Role of transposable elements in heterochromatin and epigenetic control. Nature **430 (6998)**:471-476.
96. **Liu, C., Lu, F., Cui, X., Cao, X.** (2010). Histone methylation in higher plants. Annual Review of Plant Biology**61**:395–420.
97. **Liu, J., Feng, L., Li, J., He, Z.** (2015). Genetic and epigenetic control of plant heat responses. Frontiers in Plant Science **24**:6:267.
98. **Liu, Z. Q., Gao, J., Dong, A. W., Shen, W. H. (**2009). A truncated *Arabidopsis* NUCLEOSOME ASSEMBLY PROTEIN 1, AtNAP1;3T, alters plant growth responses to abscisic acid and salt in the Atnap1;3-2 mutant. Mol Plant, **2**:688-699
99. **Lobell, D.B., Schlenker, W., Costa-Roberts, J.** (2011). Climate trends and global crop production since 1980. Science**333**:616–620.
100. **Lu, P. Y., Lévesque, N., and Kobor, M. S.** (2009). NuA4 and SWR1-C: two chromatin-modifying complexes with overlapping functions and components. Biochemistry and Cell Biology **87**:799-815.
101. **Mahalingam, R.** (2015). Consideration of combined stress: a crucial paradigm for improving multiple stress tolerance in plants, in Combined Stresses in Plants. Springer International Publishing. 1–25.
102. **Mahdi, Z., David, B. L., Peter, S., Aaron, L. M., Romain, R., William, E. H., Tracy, M. B., Marcel, E. D., Daniel, C.** (2018). I-motif DNA structures are formed in the nuclei of human cells. Nature Chemistry**10**: 631–637.

103. **Malik, H. S., Henikoff, S.** (2003). Phylogenomics of the nucleosome. Nature Structural & Molecular Biology **10 (11)**:882-891.

104. **March-Díaz, R., García-Domínguez, M., Florencio, F. J., Reyes, J. C.** (2007). SEF, a new protein required for flowering repression in *Arabidopsis*, interacts with PIE1 and ARP6. Plant Physiol. **143**:893–901.

105. **March-Díaz, R., García-Domínguez, M., Lozano-Juste, J., León, J., Florencio, F. J., Reyes, J. C.** (2008). Histone H2A.Z and homologues of components of the SWR1 complex are required to control immunity in *Arabidopsis*. Plant J. **53**:475–487

106. **Massad, T. J., Dyer, L. A., Vega, C. G. (**2012). Costs of defense and a test of the carbon-nutrient balance and growth-differentiation balance hypotheses for two co-occurring classes of plant defense. PLoS One**7 (10)**:e47554.

107. **Matsunaga, S., Katagiri, Y., Nagashima, Y., Sugiyama, T., Hasegawa, J., Hayashi, K., Sakamoto, T.** (2013). New insights into the dynamics of plant cell nuclei and chromosomes. International Review of Cell and Molecular Biology**305**:253-301.

108. **Matzke, M. A., Mosher, R. A.** (2014). RNA-directed DNA methylation: an epigenetic pathway of increasing complexity. Nature Reviews Genetics**15 (6)**:394-408.

109. **Mavrich, T. N., Jiang, C., Ioshikhes, I. P., Li, X., Venters, B. J., Zanton, S. J., Tomsho, L. P., Qi, J., Glaser, R. L., Schuster, S. C., et al**. (2008). Nucleosome organization in the Drosophila genome. Nature **453**: 358–362.

110. **McClung, C.R., Davis, S.J.** (2010). Ambient thermometers in plants: From physiological outputs towards mechanisms of thermal sensing. Current Biology **20**:1086-1092.

111. **Meneghini, M. D., Wu, M., Madhani, H. D.** (2003). Conserved histone variant H2A.Z protects euchromatin from the ectopic spread of silent heterochromatin. Cell**112 (5):**725-736

112. **Miller, M. J., Barrett-Wilt, G. A., Hua, Z., and Vierstra, R. D.** (2010). Proteomic analyses identify a diverse array of nuclear processes affected by small ubiquitin-like modifier conjugation in *Arabidopsis*. PNAS **107**:16512-16517.

113. **Miller, M. J., Scalf, M., Rytz, T. C., Hubler, S. L., Smith, L. M., and Vierstra, R. D.** (2013). Quantitative proteomics reveals factors regulating RNA biology as dynamic targets of stress-induced SUMOylation in *Arabidopsis*. Molecular & Cellular Proteomics**12**:449-463.

114. **Mito, Y., Henikoff, J. G., Henikoff, S.** (2005). Genome-scale profiling of histone H3.3 replacement patterns. Nat Genet. **37**:1090–1097.

115. **Mitsui, Y., Langridge, R., Shortle, B. E., Cantor, C. R., Grant, R. C., Kodama, M., Wells, R. D.** (1970). Physical and enzymatic studies on poly d (I–C)·poly d (I–C), an unusual double-helical DNA. Nature. **228 (5277):** 1166–1169.

116. **Mittler, R.** (2006). Trends Abiotic stress, the field environment and stress combination. Plant Science **11 (1)**:15-19.

117. **Mittler, R., Blumwald, E.** (2010). Genetic engineering for modern agriculture: challenges and perspectives. Annual Review Plant Biology**61**:443-462

118. **Mizuguchi, G., Shen, X., Landry, J., Wu, W. H., Sen, S., Wu, C.** (2004)**.** ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. Science **303 (5656)**:343-348.

119. **Mlynarova, L., Nap, J. P., Bisseling, T. (**2007). The SWI/SNF chromatin-remodeling gene AtCHR12 mediates temporary growth arrest in *Arabidopsis thaliana* upon perceiving environmental stress. Plant J, **51:**874-885

120. **Morrow, G., Tanguay, R. M.** (2012). Small heat shock protein expression and functions during development. The International Journal of Biochemistry & Cell Biology**44 (10)**:1613-1621.
121. **Narsai, R., Wang, C., Chen, J., Wu, J., Shou, H., Whelan, J.** (2013). Antagonistic, overlapping and distinct responses to biotic stress in rice (Oryza sativa) and interactions with abiotic stress. BMC Genomics**12**:14-93.
122. **Neidhardt, F. C., VanBogelen, R. A., and Vaughn, V.** (1984). The genetics and regulation of heat-shock proteins. Annual Review of Genetics. **18**:295-329
123. **Nover, L., Bharti, K., Döring, P., Mishra, S. K., Ganguli, A., Scharf, K. D.** (2001). *Arabidopsis* and the heat stress transcription factor world: how many heat stress transcription factors do we need? Cell Stress Chaperones**6 (3)**:177-89.
124. **Oh, S., Zhang, H., Ludwig, P., van Nocker, S.** (2004). A mechanism related to the yeast transcriptional regulator Paf1c is required for expression of the *Arabidopsis* FLC/MAF MADS box gene family. Plant Cell. **16**:2940–2953.
125. **Okada, T., Endo, M., Singh, M. B., and Bhalla, P. L.** (2005). Analysis of the histone H3 gene family in *Arabidopsis* and identification of the male-gamete-specific variant AtMGH3. Plant Journal **44**:557-568.
126. **Otero, S., Desvoyes, B., Gutierrez, C.** (2014). Histone H3 dynamics in plant cell cycle and development. Cytogenetic Genome Res, **143**:114-124
127. **Pagamas, P., Nawata, E.** (2008). Sensitive stages of fruit and seed development of chili pepper (Capsicum annuum L. var. Shishito) exposed to high-temperature stress. ScientiaHorticulturae**117**:21-25.
128. **Pandey, P., Ramegowda, V., Senthil-Kumar, M.** (2015). Shared and unique responses of plants to multiple individual stresses and stress combinations: physiological and molecular mechanisms. Front Plant Science**16**:6-723.
129. **Pass, D. A., Sornay, E., Marchbank, A., Crawford, M. R., Paszkiewicz, K., Kent, N. A., Murray, J. A. H.** (2017). Genome-wide chromatin mapping with size resolution reveals a dynamic sub-nucleosomal landscape in *Arabidopsis.* PLoS Genetics **e1006988**
130. **Pecinka, A., Dinh, H.Q., Baubec, T., Rosa, M., Lettner, N., Mittelsten Scheid, O.,** (2010). Epigenetic regulation of repetitive elements is attenuated by prolonged heat stress in *Arabidopsis*. Plant Cell **22**:3118-3129.
131. **Penfield, S.** (2008). Temperature perception and signal transduction in plants. New Phytologist **179**: 615-628
132. **Penterman, J., Uzawa, R., Fischer, R. L.** (2007). Genetic interactions between DNA demethylation and methylation in *Arabidopsis*. Plant Physiol. **145 (4)**:1549-1557.
133. **Petrovská, B., Šebela, M., Doležel, J.** (2015). Inside a plant nucleus: discovering the proteins. Journal of Experimental Botany**66**:1627-1640.
134. **Phelps-Durr, T. L., Thomas, J., Vahab, P., Timmermans, M. C.** (2005). Maize rough sheath2 and its *Arabidopsis* orthologue ASYMMETRIC LEAVES1 interact with HIRA, a predicted histone chaperone, to maintain knox gene silencing and determinacy during organogenesis. Plant Cell. **17**:2886–2898.
135. **Pietzenuk, B., Markus, C., Gaubert, H., Bagwan, H., Merotto, A., Bucher, E., Pecinka, A.** (2016). Recurrent evolution of heat-responsiveness in Brassicaceae COPIA elements. Genome Biology**17**:209.

136. **Pikaard, C. S.** (2002). Transcription and tyranny in the nucleolus: the organization, activation, dominance and repression of ribosomal RNA genes. *Arabidopsis* Book. **1 ()**:e0083.

137. **Prasad, P. V. V., Pisipati, S. R., Momcilovic, I., Ristic Z.** (2011). Independent and combined effects of high temperature and drought stress during grain filling on plant yield and chloroplast EF-Tu expression in spring wheat. Journal of Agronomy and Crop Science. **197**:430–441.

138. **Prasch, C. M., Sonnewald, U.** (2013). Simultaneous application of heat, drought, and virus to *Arabidopsis* plants reveals significant shifts in signaling networks. Plant Physiology. **162 (4)**:1849-66.

139. **Pray-Grant, M. G., Daniel, J. A., Schieltz, D., Yates, J. R. 3rd, Grant, P. A.** (2005). Chd1 chromodomain links histone H3 methylation with SAGA- and SLIK-dependent acetylation. Nature. **433 (7024)**:434-438.

140. **Raisner, R. M., Hartley, P. D., Meneghini, M. D., Bao, M. Z., Liu, C. L., Schreiber, S. L., Rando, O. J., Madhani, H. D.** (2005). Histone variant H2A.Z marks the 5′ ends of both active and inactive genes in euchromatin. Cell. **123**:233–248.

141. **Raisner, R. M., Madhani, H. D.** (2006). Patterning chromatin: form and function for H2A.Z variant nucleosomes. Curr Opin Genet Dev. **16 (2)**:119-124.

142. **Ramakrishna, K. C., Suhua,F., Yana, V. B., Pao-Yang,C., Hume,S., Yanchun, Y., Jonathan, H., Frank, K., Jin, K., Shawn, J. C., David, C., Maria, B., Peter, H., Amander, T. C., Ute, K., Sabeeha, S. M., Xiaoyu, Z., Steven, E. J., and Matteo, P.** (2010).Relationship between nucleosome positioning and DNA methylation. Nature. **466 (7304)**: 388–392.

143. **Ramegowda, V., Senthil-Kumar, M. J.** (2015). The interactive effects of simultaneous biotic and abiotic stresses on plants: mechanistic understanding from drought and pathogen combination. Plant Physiology **176**:47-54.

144. **Ramírez, F., Dündar, F., Diehl, S., Grüning, B. A., Manke, T.** (2014). deepTools: a flexible platform for exploring deep-sequencing data. Nucleic Acids Research **42** (Web Server issue):W187-191.

145. **Rando, O. J., Chang, H. Y.** (2009). Genome-wide views of chromatin structure. Annu Rev Biochem. **78 ()**:245-271.

146. **Rangwala, S. H., Richards, E. J.** (2004). The value-added genome: building and maintaining genomic cytosine methylation landscapes. Curr Opin Genet Dev. **14 (6)**:686-691.

147. **Rasmussen, S., Barah, P., Suarez-Rodriguez, M. C, Bressendorff, S., Friis, P., Costantino, P., Bones, A. M., Nielsen, H. B., Mundy, J.** (2013) Transcriptome Responses to Combinations of Stresses in *Arabidopsis.* Plant Physiology. **161**:1783–1794

148. **Raxwal, V., Katiyar-Agarwal, S., Agarwal, M.** (2012). Structural and Functional Diversity of Plant Heat Shock Factors. Plant Stress **6 (SI1)**:89-96.

149. **Rosa, S., Shaw, P.** (2013). Insights into Chromatin Structure and Dynamics in Plants. Biology (Basel) **2 (4)**:1378-1410.

150. **Rosalind, F.** (1953). The Structure of Sodium ThymonucleateFibres. I. The Influence of Water Content. ActaCrystallographica. **6**: 673-677.

151. **Roudier, F., Ahmed, I., Bérard, C., Sarazin, A., Mary-Huard, T., Cortijo, S., Bouyer, D., Caillieux, E., Duvernois-Berthet, Al-Shikhley, L., Giraut, L., Després, B.,Drevensek, S., Barneche, F., Dèrozier, S., Brunaud, V., Aubourg, S.,**

Schnittger E., Bowler, C., Martin-Magniette, M., Robin, S., Caboche, M., Colotb, V. (2011). Integrative epigenomic mapping defines four main chromatin states in *Arabidopsis*. EMBO J. **30 (10)**: 1928–1938.

152. **Roudier, F., Colot, V., Szarzynska, B., China, C.** (2015). Genome-wide profiling of H3K27me1 and 5mC enrichment in young leaves of *Arabidopsis thaliana*. NCBI PRJNA238067.
153. **Ruelland, E., Zachowski, A.** (2010). How plants sense temperature. Environmental and Experimental Botany **69**:225–232.
154. **Sakurai, H., Enoki, Y.** (2010). Novel aspects of heat shock factors: DNA recognition, chromatin modulation and gene expression. The FEBS Journal **277 (20)**:4140-4149.
155. **Santos, A. P., Ferreira, L., Maroco, J., Oliveira, M. M. (**2011). Abiotic stress and induced DNA hypomethylation cause interphase chromatin structural changes in rice rDNA loci. Cytogenetic Genome Res, **132**:297-303
156. **Santos-Rosa, H., Schneider, R., Bannister, A. J., Sherriff, J., Bernstein, B. E., Emre, N. C., Schreiber, S. L., Mellor, J., Kouzarides, T.** (2002). Active genes are tri-methylated at K4 of histone H3. Nature. **419 (6905)**:407-411.
157. **Satchwell, S. C., Drew, H. R., Travers, A. A. (**1986). Sequence periodicities in chicken nucleosome core DNA. J Mol Biol. **191 (4):**659-75.
158. **Schones, D. E., Cui, K., Cuddapah, S., Roh, T. Y., Barski, A., Wang, Z., Wei, G., Zhao, K.** (2008) Dynamic regulation of nucleosome positioning in the human genome. Cell **132**: 887–898.
159. **Schönrock, N., Exner, V., Probst, A., Gruissem, W., Hennig, L. (**2006). Functional genomic analysis of CAF-1 mutants in *Arabidopsis thaliana*. J Biol Chem, **281**:9560-9568
160. **Sedaghatmehr, M., Mueller-Roeber, B., & Balazadeh, S.** (2016). The plastid metalloprotease FtsH6 and small heat shock protein HSP21 jointly regulate thermomemory in *Arabidopsis*. Nature Communications, **7**:12439.
161. **Segal E, Fondufe-Mittendorf Y, Chen L, Thåström A, Field Y, Moore IK, Wang JP, Widom J. (**2006) A genomic code for nucleosome positioning. Nature. **442 (7104):**772-8.
162. **Shao, H. B., Chu, L. Y., Jaleel, C. A., Zhao, C. X.** (2008). Water-deficit stress-induced anatomical changes in higher plants. ComptesRendusBiologies**331 (3)**:215-25.
163. **Sheldon, C. C., Hills, M. J., Lister, C., Dean, C., Dennis, E. S., Peacock, W. J.** (2008). Resetting of FLOWERING LOCUS C expression after epigenetic repression by vernalization. Proc Natl Acad Sci USA. **105**:2214–2219.
164. **Sims, R. J. 3rd, Millhouse, S., Chen, C. F., Lewis, B. A., Erdjument-Bromage, H., Tempst, P., Manley, J. L., Reinberg, D.** (2007). Recognition of trimethylated histone H3 lysine 4 facilitates the recruitment of transcription postinitiation factors and pre-mRNA splicing. Mol Cell. **28 (4)**:665-676.
165. **Storozhenko, S., Pascal De Pauw, P., Marc Van Montagu, M., Inzé, D., and Kushnir, S.** (1998). The Heat-Shock Element Is a Functional Component of the *Arabidopsis* APX1 Gene Promoter. Plant Physiology **118 (3):** 1005–1014.
166. **Strahl, B. D., Allis, C. D.** (2000). The language of covalent histone modifications. Nature**6; 403 (6765)**:41-45.
167. **Sullivan, A. M., Arsovski, A. A., Lempe, J., Bubb, K. L., Weirauch, M. T., Sabo, P. J., Sandstrom, R., Thurman, R. E., Neph, S., Reynolds, A. P., Stergachis, A.**

B., Vernot, B., Johnson, A. K., Haugen, E., Sullivan, S. T., Thompson, A., Neri, F. V., Weaver, M., Diegel, M., Mnaimneh, S., Yang, A., Hughes, T. R,,Nemhauser, J. L., Queitsch, C., Stamatoyannopoulos, J. A. (2014). Mapping and dynamics of regulatory DNA and transcription factor networks in *A. thaliana*. Cell Report **8 (6):**2015-2030.

168. **Suto, R. K., Clarkson, M. J., Tremethick, D. J., Luger, K.** (2000). Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. Nat Struct Biol. **7 (12)**:1121-1124.
169. **Suzuki, N., Koussevitzky, S., Mittler, R., Miller, G.** (2012). ROS and redox signalling in the response of plants to abiotic stress. Plant, Cell & Environment **35**:259-270.
170. **Suzuki, N., Miller, G., Morales, J., Shulaev, V., Torres, M.A., Mittler, R.** (2011). Respiratory burst oxidases: The engines of ROS signaling. Current Opinion in Plant Biology **14 (6)**:691-699.
171. **Suzuki, N., Rivero, R. M., Shulaev, V., Blumwald, E., Mittler, R.** (2014). Abiotic and biotic stress combinations. New Phytologist **203 (1)**:32-43.
172. **Swarbrick, P. J., Schulze-Lefert, P., Scholes, J. D.** (2006). Metabolic consequences of susceptibility and resistance (race-specific and broad-spectrum) in barley leaves challenged with powdery mildew. Plant Cell Environment**29 (6)**:1061-1076.
173. **Swindell, W. R., Huebner, M., Weber, A. P.** (2007). Transcriptional profiling of *Arabidopsis* heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. BMC Genomics**8**:125.
174. **Szerlong, H. J., Hansen, J. C.** (2011). Nucleosome distribution and linker DNA: connecting nuclear function to dynamic chromatin structure. Biochemistry and Cell Biology **89**:24-34.
175. **Tagami, H., Ray-Gallet, D., Almouzni, G., Nakatani, Y.** (2004). Histone H3.1 and H3.3 complexes mediate nucleosome assembly pathways dependent or independent of DNA synthesis. Cell. **116**:51–61.
176. **Talbert, P. B., Henikoff, S.** (2010). Histone variants--ancient wrap artists of the epigenome. Nat Rev Mol Cell Biol. **11 (4)**:264-275.
177. **Tamada, Y., Yun, J., Chul Woo, S., Amasino, R.** (2009). ARABIDOPSIS TRITHORAX-RELATED7 Is Required for Methylation of Lysine 4 of Histone H3 and for Transcriptional Activation of FLOWERING LOCUS C. Plant Cell. **21 (10)**: 3257–3269
178. **Teixeira, F. K., Heredia, F., Sarazin, A., Roudier, F., Boccara, M., Ciaudo, C., Cruaud, C., Poulain, J., Berdasco, M., Fraga, M. F., Voinnet, O., Wincker, P., Esteller, M., Colot, V.** (2009). A role for RNAi in the selective correction of DNA methylation defects. Science. **323 (5921)**:1600-1604.
179. **Tiang, C. L., He, Y., Pawlowski, W. P.** (2012). Chromosome organization and dynamics during interphase, mitosis, and meiosis in plants. Plant Physiology**158**:26-34.
180. **Tomas, D., Brazao, J., Viegas, W., Silva, M. (**2013). Differential effects of high-temperature stress on nuclear topology and transcription of repetitive noncoding and coding rye sequences. Cytogenetic Genome Res, **139**:119-127
181. **Tran, R. K., Henikoff, J. G., Zilberman, D., Ditt, R. F., Jacobsen, S. E., Henikoff, S.** (2005). DNA methylation profiling identifies CG methylation clusters in *Arabidopsis* genes. Curr Biol. **15 (2)**:154-159.
182. **Trapnell, C., Pachter, L., Salzberg, S. L.** (2009). TopHat: discovering splice junctions with RNA-Seq. Bioinformatics **25 (9)**:1105-1111.

183. **Trapnell, C., Roberts, A., Goff, L., Pertea, G., Kim, D., Kelley, D. R., Pimentel, H., Salzberg, S. L., Rinn, J. L., and Pachter, L. (**2012). Differential gene and transcript expression analysis of RNA-seq experiments with TopHat and Cufflinks. Nature Protocol. **7 (3)**:562-578.

184. **Tripathi, A. K., Singh, K., Pareek, A., Singla-Pareek, S. L.** (2015). Histone chaperones in *Arabidopsis* and rice: genome-wide identification, phylogeny, architecture and transcriptional regulation. BMC Plant Biol, **15**:414

185. **Turck, F., Roudier, F., Farrona, S., Martin-Magniette, M. L., Guillaume, E., Buisine, N., Gagnot, S., Martienssen, R. A., Coupland, G., Colot, V.** (2007). *Arabidopsis* TFL2/LHP1 specifically associates with genes marked by trimethylation of histone H3 lysine 27. PLoS Genet. **3 (6)**:e86.

186. **Turner, B. M.** (2002). Cellular memory and the histone code. Cell **111 (3)**:285-291.

187. **Valouev, A., Ichikawa, J., Tonthat, T., Stuart, J., Ranade, S., Peckham, H., Zeng, K., Malek, J. A., Costa, G., McKernan, K., et al.** (2008) A high-resolution, nucleosome position map of *C. elegans* reveals a lack of universal sequence-dictated positioning. Genome Research**18**: 1051–1063.

188. **Valouev, A., Johnson, S. M., Boyd, S. D., Smith, C. L., Fire, A. Z., Sidow, A.** (2011) Determinants of nucleosome organization in primary human cells. Nature **474**: 516–520.

189. **van Attikum, H., Gasser, S. M.** (2009). Crosstalk between histone modifications during the DNA damage response. Trends Cell Biol. **19 (5)**:207-217.

190. **Värv, S., Kristjuhan, K., Peil, K., Lõoke, M., Mahlakõiv, T., Paapsi, K., Kristjuhan, A.** (2010). Acetylation of H3 K56 is required for RNA polymerase II transcript elongation through heterochromatin in yeast. Mol Cell Biol. **30 (6)**:1467-1477.

191. **Vaughn, M. W., Tanurdzić, M., Lippman, Z., Jiang, H., Carrasquillo, R., Rabinowicz, P. D., Dedhia, N., McCombie, W. R., Agier, N., Bulski, A., Colot, V., Doerge, R. W., Martienssen, R. A.** (2007). Epigenetic natural variation in *Arabidopsis thaliana*. PLoS Biol. **5 (7)**:e174.

192. **Veluchamy, A., Jégu, T., Ariel, F., Latrasse, D., Mariappan, K. G., Kim, S., Crespi, M., Hirt, H., Bergounioux, C., Raynaud, C., Benhamed, M.** (2016). LHP1 Regulates H3K27me3 Spreading and Shapes the Three-Dimensional Conformation of the *Arabidopsis* Genome. PLoS One. **11 (7)**: e0158936.

193. **Vera, D. L., Madzima, T. F., Labonne, J. D., Alam, M. P., Hoffman, G. G., Girimurugan, S. B., Zhang, J., McGinnis, K. M., Dennis, J. H., Bass, H. W.** (2014).Differential nuclease sensitivity profiling of chromatin reveals biochemical footprints coupled to gene expression and functional DNA elements in maize. Plant Cell. **26 (10)**:3883-93.

194. **Vinocur, B., Altman, A.** (2005). Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. Current Opinion in Biotechnology. **16 (2):**123-132.

195. **VinodKumar, S., Wigge, A. P. (**2010). H2A.Z-Containing Nucleosomes Mediate the Thermosensory Response in *Arabidopsis*. Cell **140 (1)**:136-147.

196. **Volpe, T. A., Kidner, C., Hall, I. M., Teng, G., Grewal, S. I. S., Martienssen, R. A.** (2002). Regulation of Heterochromatic Silencing and Histone H3 Lysine-9 Methylation by RNAi. Science **297 (5588)**:1833-1837

197. **Wang, W., Vinocur, B., Altman, A.** (2003). Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta. **218 (1):**1-14.

198. **Wang, W., Vinocur, B., Shoseyov, O., Altman, A.** (2004). Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends in Plant Science. **9 (5)**:244-252.

199. **Wei, G., Hu, G., Cui, K., Zhao, K.,** (2012). Genome-wide mapping of nucleosome occupancy, histone modifications, and gene expression using next-generation sequencing technology. Methods Enzymol. **513 ():**297-313.

200. **Weng, M., Yang, Y., Feng, H., Pan, Z., Shen, W. H., Zhu, Y., & Dong, A.** (2014). Histone chaperone ASF1 is involved in gene transcription activation in response to heat stress in *Arabidopsis thaliana*. Plant, Cell & Environment, **37**: 2128–2138.

201. **Wilson, J.** (2002). Molecular biology of the cell: a problems approach. New York: Garland Science. **78 (3)**:355.

202. **Workman, J. L., Kingston, R. E.** (1998). Alteration of nucleosome structure as a mechanism of transcriptional regulation. Annual Review of Biochemistry **67**:545-79.

203. **Wu, T. Y., Juan, Y. T., Hsu, Y. H., Wu, S. H., Liao, H. T., Fung, R. W., & Charng, Y. Y.** (2013). Interplay between heat shock proteins HSP101 and HSA32 prolongs heat acclimation memory posttranscriptionally in *Arabidopsis*. Plant Physiology, **161**:2075–2084.

204. **Wu, Y., Zhang, W., Jiang, J.** (2014). Genome-wide nucleosome positioning is orchestrated by genomic regions associated with DNase I hypersensitivity in rice. PLoS Genetics. **10 (5)**:e1004378.

205. **Xu, L., Shen, W. H.** (2008). Polycomb silencing of KNOX genes confines shoot stem cell niches in *Arabidopsis*. Curr Biol. **18**:1966–1971.

206. **Yángüez, E., Castro-Sanz, A. B., Fernández-Bautista, N., Oliveros, J. C., Castellano, M. M.** (2013). Analysis of Genome-Wide Changes in the Translatome of *Arabidopsis* Seedlings Subjected to Heat Stress, PLosOne **10.1371**-0071425

207. **Yuan, G. C., Liu, Y. J., Dion, M. F., Slack, M. D., Wu, L. F., Altschuler, S. J., Rando, O. J.** (2005) Genome-scale identification of nucleosome positions in S. cerevisiae. Science **309**: 626–630.

208. **Yuan, L., Tang, L., Zhu, S., Hou, J., Chen, G., Liu, F., Liu, S., Wang, C.** (2017). Influence of heat stress on leaf morphology and nitrogen–carbohydrate metabolisms in two wucai (Brassica campestris L.) genotypes. ActaSocietatisBotanicorumPoloniae. **86**:2.

209. **Zaugg, J. B., Luscombe, N. M.** (2012). A genomic model of condition-specific nucleosome behavior explains transcriptional activity in yeast. Genome Research. **22 (1):**84-94.

210. **Zhang, K., Sridhar, V. V., Zhu, J., Kapoor, A., Zhu, J. K.,** (2007). Distinctive core histone post-translational modification patterns in *Arabidopsis thaliana*. PLoS One. **2 (11)**:e1210.

211. **Zhang, T., Zhang, W., Jiang, J.** (2015). Genome-Wide Nucleosome Occupancy and Positioning and Their Impact on Gene Expression and Evolution in Plants. Plant Physiology. **168(4)**: 1406–1416.

212. **Zhang, W., Zhang, T., Wu, Y., Jiang, J.** (2012). Genome-wide identification of regulatory DNA elements and protein-binding footprints using signatures of open chromatin in *Arabidopsis*. Plant Cell. **24 (7)**:2719-2731.

213. **Zhang, X., Clarenz, O., Cokus, S., Bernatavichute, Y. V., Pellegrini, M., Goodrich, J., Jacobsen, S. E.** (2007). Whole-genome analysis of histone H3 lysine 27 trimethylation in *Arabidopsis*. PLoS Biol. **5 (5)**:e129.

214. **Zhang, X., Germann, S., Blus, B. J., Khorasanizadeh, S., Gaudin, V., Jacobsen, S. E.** (2007). The *Arabidopsis* LHP1 protein colocalizes with histone H3 Lys27 trimethylation. Nat Struct Mol Biol. **14 (9)**:869-871.

215. **Zhang, X., Jacobsen, S. E.** (2006). Genetic analyses of DNA methyltransferases in *Arabidopsis thaliana*. Cold Spring Harb Symp Quant Biol. **71 ()**:439-447.

216. **Zhou, X., Graumann, K., Meier, I.** (2015). The plant nuclear envelope as a multifunctional platform LINCed by SUN and KASH. Journal of Experimental Botany**66**:1649-1659.

217. **Zhu, A., Greaves, I. K., Dennis, E. S., Peacoock, W. J.** (2017). Genome-wide analyses of four major histone modifications in *Arabidopsis* hybrids at the germinating seed stage. BMC Genomics. **18**: 137.

218. **Zhu, Y., Dong, A., Shen, W. H. (**2012). Histone variants and chromatin assembly in plant abiotic stress responses. Biochim Biophys Acta-Gene Regul Mech, 1819:343-348

219. **Zilberman, D., Coleman-Derr, D., Ballinger, T., Henikoff, S.** (2008). Histone H2A.Z and DNA methylation are mutually antagonistic chromatin marks. Nature. **456**:125–129

220. **Zilberman, D., Henikoff, S.** (2007). Genome-wide analysis of DNA methylation patterns. Development. **134 (22)**:3959-3965.

APPENDIX

APPENDIX I

MNaseI sensitivity assay
Based on protocol by Ricardi et al., Plant Methods, 2010, 6:11

Chromatin isolation

Important notes before:
- Reducepipetting. Pipette the solutions just to get rid of the Pellets from the wall. Then resuspend pellets by shaking.
- Keep solutions and samples on 4°C (ice)

6- Harvest ~3g of tissue and shock freeze in liquid nitrogen. Store at -80°C until use.

6- Grind the tissue to a fine powder in liquid N2 cooled 50ml falcon with 3 metal beads for 2x30 sec (alternatively use mortar and pestle).

7- Resuspend the powder in 30-40 ml of cold extraction buffer 1 (10 ml/1 g of tissue). Unless otherwise specified, all of the following steps should be done at 0-4°C.

Extraction buffer 1 (100 ml)

Final	Stock	Volume
0.44 M sucrose	2 M	22 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	1 ml
5 mM b-ME	14,3 M	35,7 µl
1× protease inhibitor cocktail:		
1mM PMSF (in 96% ETOH)	100mM	1 ml

5 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets

8- Filter sequentially through miracloth.

9- Spin the filtered solution for 20 min at 2,880 × g.

10- Remove the supernatant and resuspend the pellet in 10 ml of extraction buffer 2.

Extraction buffer 2 (100 ml)

Final	Stock	Volume
0.25 M sucrose	2 M	12,5 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	1 ml
10 mM $MgCl_2$	1 M	1 ml
1% Triton X-100	100%	1 ml
5 mM b-ME	14,3 M	35,7 µl
1× protease inhibitor cocktail		
1mM PMSF (in 96% ETOH)	100mM	1 ml

2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets

11- Incubate for 10 min on ice to lyse chloroplasts and spin for 20 min at 2,100 × g.

12- Remove the supernatant and resuspend the pellet in 4 ml of extraction buffer 2 without Triton X-100.

13- Spin for 20 min at 2,100 × g and resuspend the pellet in 4 ml of Percoll extraction buffer. Divide 4 ml per sample into two 2 ml Eppis

Percoll extraction buffer (50 ml)

Final	Stock	Volume
95% V/V Percoll	100%	45 ml
0.25 M sucrose	2,5 M	5 ml
10 mMTris (pH 8.0 adj by HCl)	1 M	0,5 ml
10 mM MgCl2	1 M	0,5 ml
5 mM b-ME	14,3 M	17,8 µl
1× protease inhibitor cocktail		
1mM PMSF (in 96% ETOH)	100mM	0,5 ml

2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets

14- Spin for 10 min at 12,000 × g, without the breaks. SWITCH OF BREAKS FROM CENTRIFUGE!
15- Carefully take 300 µl of the upper phase (looks like white-greenish (pale) flocks/cloud) into a fresh 1.5 ml eppi and dilute it by adding 1200 µl nuclei resuspension buffer.

Nuclei resuspension buffer (100 ml)

Final	Stock	Volume
10% Glycerol	100%	10 ml
50 mMTris (pH 8.0 adj by HCl)	1 M	5 ml
5 mM MgCl2	1 M	0,5 ml
10 mM b-ME	14,3	70,1 µl
1× protease inhibitor cocktail		
1mM PMSF (in 96% ETOH)	100mM	1 ml

2 tablets of EDTA Free Complete Mini Protease Inhibitor Cocktail tablets

16- Spin for 10 min at 12,000 × g.

17- Discard the supernatant and resuspend the pellet in 1 – 1,5 ml of nuclei resuspension buffer. (This step you can freeze the sample in liquid Nitrogen and store at -80°C)

18- Spin for 10 min at 12,000 × g.

Micrococcal nuclease digest

Dissolve pellet in 500 µl Micrococcal nuclease buffer

Micrococcal nuclease buffer (10 ml)

Final	Stock	Volume
50 mMTris-HCl, pH 8.5	1 M	0,5 ml
5 mM Mg acetate	1 M	50 µl
25% glycerol	100%	2,5 ml
1 mM CaCl2	1M	10 µl

Digest 100 µl aliquots for 20 min at 37°C with 0, 2.5, 5, 10 and 20 U of MNaseI

35- Stop digestion by Adding 10 µl of 0.5 M EDTA, 20 µl of 1 M Tris-HCl pH 6.8 and 1.5 µl of 14 mg/ml proteinase K to the eluate and incubate for 1 h at 45°C.

DNA recovery

36- Extract DNA with equal volume of phenol/chloroform (for making mix – take 1000 µl phenol/960 µl chlorophorm). Centrifuge 5 min at 5,000 g to separate the phases (commercial DNA clean-up columns may alternatively be used). Transfer the upper phase into a new tube.

37- Add 0.1 volume of 3 M sodium acetate pH 5.3 (adjusted by glacial acetic acid) to the aqueous phase and precipitate with 0.7 volumes of isopropanol (-20°C) in the presence of tRNA (1 µg/ml final concentration) (= recommended incubation overnight @ -20°C). Centrifuge 20 min at max speed.

38- Wash pellet with 300 µl of 70% ethanol. Centrifuge 5 min at 12,000 g. Remove supernatant by pipette, dry by speedvac or under hood. Resuspend the DNA pellet overnight at 4°C in 20 µl of Tris pH 8 or TE supplemented with 10 µg/ml RNase A.

39- DNA is now ready for analysis by PCR.

APPENDIX II

TopHat alignment Command for single end RNA-seq reads

```
tophat --bowtie1 -o R1 -p 20 -G ../../../Genome_New_1/TAIR10_1_5.gtf
../../../Genome_New_1/TAIR10_1_5.fas
../../../RNA-seq_Reads/Recovery_1.fastq
```

CuffDiff gene expression calculation and different expression calculation of RNA-seq reads

```
cuffdiff -p 40 -o DE_analysis/CvHvR/ -L Control,HS,Recovery
../Genome_New_1/TAIR10_1_5.gtf
Tophat/TopHat_output/C1/accepted_hits.bam,Tophat/TopHat_output/C2/acce
pted_hits.bam
Tophat/TopHat_output/H1/accepted_hits.bam,Tophat/TopHat_output/H2/acce
pted_hits.bam
Tophat/TopHat_output/R1/accepted_hits.bam,Tophat/TopHat_output/R2/acce
pted_hits.bam
```

Bowtie alignment command for nucleosome occupancy and location estimation of MNase-seq paired-end.

```
bowtie --fr -p 30 -v 3 TAIR10_1_5.fas
-1 ../../MNase-seq_Reads/ambient_rep4_R1.fastq
-2../../MNase-seq_Reads/ambient_rep4_R2.fastq
-S ../Control_sam_bam/Control_4.sam
```

SAM to sorted BAM

```
samtools view -u unsorted.sam | samtools sort -o sorted.bam
```

DANPOS script command line for nucleosome calling, normalization and differential nucleosome estimation.

```
python ../../Documents/software/danpos-2.2.1/danpos.py dpos -t 0.00001
-f 1 -p 0.00001 -n N -a 1 -z 25 -q 3 -jd 75 -o Heat-Control/
Heat danpos/norm/:Control danpos/norm/ -s 1
```

Supplemental Table 1. Spearman correlation value with p-value of nucleosome occupancy score with gene expression in all six groups. C: control, H: heat stress and R: recovery conditions

GROUPS	Spearman	*pval*
GROUP1_C	0.004518	0.8505
GROUP1_H	0.020624	0.3895
GROUP1_R	0.031557	0.1879
GROUP2_C	-0.04957	0.139
GROUP2_H	-0.07965	0.01735
GROUP2_R	-0.00537	0.8728
GROUP3_C	-0.26565	0.000268
GROUP3_H	-0.18931	0.01006
GROUP3_R	-0.25717	0.000425
GROUP4_C	0.04872	0.2398
GROUP4_H	0.052354	0.2065
GROUP4_R	0.018563	0.6544
GROUP5_C	-0.03659	0.39
GROUP5_H	0.010192	0.8108
GROUP5_R	0.036937	0.3855
GROUP6_C	0.197462	0.005784
GROUP6_H	0.154704	0.03125
GROUP6_R	0.272802	0.000119

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LEBENSLAUF

PERSÖNLICHE INFORMATIONEN

Name: Kashif Nawaz
Adresse: Carl-von-Linne Weg 10, 50829 Köln, Deutschland
Staatsbürgerschaft: Indisch
Geburtsdatum: 04.05.1986
Geburtsort: Jamshedpur, India

AUSBILDUNG

Seit Juli 2015	Promotion	Max Planck Institut für Pflanzenzüchtungsforschung / Universität zu Köln

Titel der Abschlussarbeit: „Genome-wide nucleosome dynamics under heat stress in *Arabidopsis thaliana*“. Finanziert durch Deutsche Forschungsgemeinschaft (DFG): ADAPTOMICS. Forschungsförderung für eine Doktorarbeit in Deutschland

06/18/2012 06/28/2014	M. Tech in Computer- und Systembiologie	Jawaharlal Nehru Universität Neu Delhi, Indien

Titel der Abschlussarbeit: “Assemble, Annotation and Differential Expression Analysis from RNA-seq Data using TopHat/Cufflinks: Application to Cancer and Rice Genome”

06/17/2008- 06/08/2012	B. Tech in der industriellen Biotechnologie,	Dr. MGR Universität, Chennai, Indien

Titel der Abschlussarbeit: “An integrated multicellular network map of ghrelin signaling pathway”

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Köln, 05.07.19

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