Indian J. Genet., 76(1): 24-30 (2016)
DOI: 10.5958/0975-6906.2016.00004.3

Analysis of epigenetic chromatin modification during mitosis in rice

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(Received: July 2015; Revised: December 2015; Accepted: January 2016)

Abstract

DNA methylation and histone modification are two important types of epigenetic modification. In this study, we analyzed the dynamic changes and chromosomal localizations of four different epigenetic modifications, DNA 5-MeC, H3K4me3, H3K9me2, and H3S10Ph, during mitosis in rice. The results showed that signals from DNA 5-MeC and H3K4me3 were distributed in the heterochromatin and euchromatin regions respectively, but no obvious signals from H3K9me2 were detected. There were no significant changes noticed in these three modification patterns at different phases during mitosis. However, the H3S10Ph, which was mainly distributed in the centromeric regions, showed dynamic changes during mitosis that occurred mainly at prophase and anaphase but not at interphase and telophase.

Key words: Rice, mitosis, DNA methylation, histone modifications

Introduction

Epigenetics refers to a heritable change in gene expression and function without any change in the DNA sequence. Such epigenetic changes represent modifications to the genome without changing the nucleotide sequence, and these changes can be transferred to offspring (Bender 2004; Goldberg et al. 2007). There are many types of epigenetic modifications. DNA methylation and histone modifications are two important chemical modifications that can affect gene expression (Zhou et al. 2014; Kouzarides 2007).

DNA methylation is an important way of regulating genome function through an epigenetic modification (Jacobsen and Meyerowitz 1997). In plants, DNA methylation is involved in many biological processes in cells, and plays an important role in regulating plant growth, development, and evolution (Hajkova and Surani 2004). Approximately 30% of the cytosine residues in the genomes of higher plants are methylated (Richards 1997). The extent and pattern of methylation in a species at different developmental stages, or in different tissues or cells at the same stage, can also be different (Esteller 2007).

Histone modification is another important type of epigenetic modification. Histones are one of the essential components of chromatin. The basic structural unit of chromatin is the nucleosome, which is composed of an octamer core containing two molecules of each histone H2A, H2B, H3, and H4 that bind with DNA (Zhang et al. 2015). Many covalent modifications, such as methylation, acetylation, phosphorylation, ubiquitination and sumoylation, the different modifications can regulate chromatin structure and gene expression in various ways (Kouzarides 2007; Li et al. 2008). Researches on methylation, acetylation and phosphorylation of histone in plants are more thorough. Histone methylation mainly at lysine (Lys, K) and arginine residues (Arg, R) in histones H3 and H4 (Cao et al. 2002), and it has been reported to affect heterochromatin formation and related to gene silencing (Martin et al. 2005). Histone acetylation mainly at lysine (Lys, K) in histones H3 and H4, plays an important role in regulating plant growth and development (Tsuji et al. 2006). Phosphorylation of histone proteins occurs primarily at N-terminal serine residues, threonine residues and tyrosine residues (Prigent and Dimitrov 2003). It is reported that histone

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phosphorylation plays an important role on DNA damage repair, transcription regulation and modulating chromatin structure (Rossetto et al. 2012).

Mitosis is an important process in eukaryotes. Mother somatic cell division produces daughter cells through mitosis (Jongsma et al. 2014). Compared to mother cell, each daughter cell contains the same number of chromosomes. In order to divide properly, chromosome morphological changes are important. Previous studies have shown that DNA demethylation could result in chromatin decondensed and normal chromosome can not be formed in the process of mitosis. DNA methylation make chromatin condense to form chromosome and ensure the precise of mitosis (Yang et al. 2010). Histone modification, such as H3K9 methylation with the similar effect to DNA methylation, may contain the information which triggering mitosis. While histone acetylation has the opposite effect, histone deacetylation made chromatin condense to form chromosome in mitosis (Li et al. 2005). Histone phosporylation, such as histone h3 phosporylation, which occurs in heterochromatin regions and correlates with intact centromere and chromosomal condensation, however, the mechanism is largely unknown (Houben et al. 1999).

Rice (*Oryza sativa* L.) is an important model organism and crop, but epigenetic research has lagged behind in this species (Shi et al. 2014). However, the completion of the rice genome sequence, the constantly-expanding databases of rice genomic information, and the rapid development of bioinformatic capabilities are laying the foundation for the study of epigenetics in rice. In the present study, we examined the localization of four different epigenetic modifications, DNA 5-MeC (5-methylcytosine), H3K4me3 (histone H3 lysine 4 trimethylation), H3K9me2 (histone H3 lysine 9 dimethylation), and H3S10Ph (histone H3 serine 10 phosphorylation), in rice chromosomes during mitosis, which will enable further analysis of their characteristics and distribution in the rice genome.

Materials and methods

Materials

The present study used the rice reference genome sequence from *Oryza sativa* L. cv. Nipponbare in this work.

Immunodetection of 5-methyl-cytosine

Roots were harvested from greenhouse grown plants, fixed in methanol acetic acid (3:1), and stored at -20^{o}C until use. Root tips were macerated in 2% cellulose and 1% pectinase at 37^{o}C for 40 min. Chromosome squashes were made in the fixative on a glass slide and flame dried, as described by Kurata (1978).

Methylated cytosine residues were detected with mouse antibodies raised against 5-methylcytosine (5-MeC) (AVIVA, AMM99021) and a goat anti-mouse secondary antibody conjugated with Alexa 488 (Invitrogen, A11001). The immunodetection procedure applied to mitotic chromosomes was as described by Gong et al. (2013). Chromosomes were counterstained with 4',6-diamidino-phenylindole (DAPI) in Vectashield (Vector Laboratories, H-1200).

Immunofluorescence

The immunofluorescence procedure applied to mitotic chromosomes was as described by Gong et al. (2009). Histone modification were detect with rabbit antibodies raised against H3K4me3, H3K9me2, and H3S10Ph (Millipore 07-473, 07-441, 04-1093), and a goat anti-rabbit secondary antibody conjugated with Alexa 488 (Invitrogen, A11008). Chromosomes were counterstained with DAPI in Vectashield (Vector Laboratories, H-1200).

Results

Analysis of DNA methylation in mitotic cells

DNA methylation is an important type of epigenetic modification that occurs after DNA replication and before transcription. DNA methylation can affect transcription, and thus gene function (Li et al. 2008). In order to determine whether there is a difference in DNA methylation during each phase of mitosis, DNA methylation levels were assayed in Nipponbare root tip cells in mitosis. The results show that fluorescent signals from 5-methylcytosine (5-MeC) can be detected in mitotic cells from root meristems of Nipponbare (Fig. 1). According to the chromosome structure, as shown in the mode pattern of Fig. 2, the region adjacent to centromere shows heterochromatin region, which can be dyed deeply by alkaline dyeing, while the distal portion of the chromosome is euchromatin, which has greater transcriptional activity. In chromatin stained with DAPI at interphase, the stronger areas are heterochromatin regions (see arrow Fig. 1-A), while weaker staining indicates euchromatin regions. Lower levels of DNA methylation can be detected in euchromatin regions, while heterochromatin

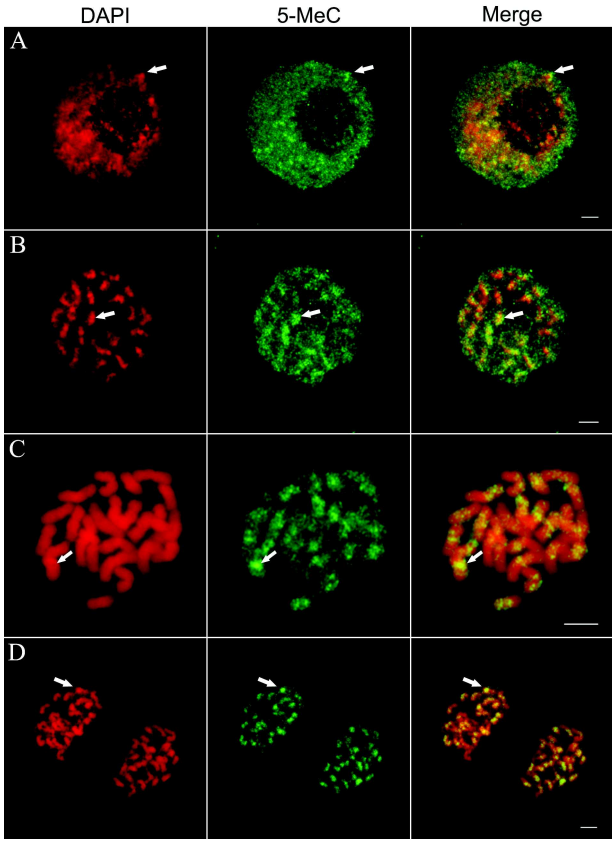

Fig. 1. Fluorescent detection of DNA methylation (5-MeC) in somatic root tip cells of the *japonica* rice cultivar 'Nipponbare'. A: interphase; B: early prophase; C: prophase; D: telophase. All chromosomes were counterstained with DAPI. Scale bars = 5 μm

regions have a relatively higher level of DNA methylation (see arrow in Fig. 1-A). Chromatin assumes a spiral form as the cell enters into early prophase. At early prophase, heterochromatin regions have a higher level of DNA methylation (see arrow in Fig. 1-B), while the distal regions of the chromosome have relatively low methylation levels (Fig. 1-B). The spiral form of chromatin visible in chromosomes at prophase shows a low level of transcriptional activity in heterochromatin regions, and has a high degree of DNA methylation (Fig. 1-C). During telophase, the chromosomes become loose and elongated, and migrate the equatorial region of the spindle, the cell plate is formed, and the process of cytokinesis divides the cell into two daughter cells. In the chromosomes of the two daughter cells, the more intensely stained parts have a higher level of DNA methylation, while the weaker stained euchromatin regions have a lower level of methylation (Fig. 1-D).

As shown in Fig. 1, DNA methylation can be detected as fluorescent signals from 5-MeC in the respective periods of mitosis (Table 1), and the levels of DNA methylation during each period show no significant differences. Heterochromatin regions, stained strongly, have higher levels of DNA methylation than euchromatin regions, where DNA methylation levels are relatively low.

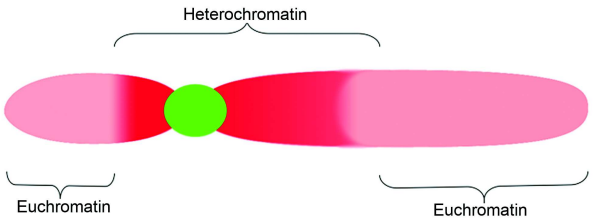

Fig. 2. Chromosome organization. The centromere is shown in green. Regions of heterochromatin (red) are proximal to the centromere, while the distal regions of the chromosome are mainly composed of euchromatin (pink)

Analysis of histone methylation

Histone methylation is an important type of epigenetic modification, and it can include mono-methylation (me1), di-methylation (me2) and tri-methylation (me3) (Klose and Zhang 2007). In this study, using antibodies against H3K4me3 and H3K9me2 to detect histone protein immunofluorescence in Nipponbare mitotic cells, only H3K4me3 gave significant signals during the phases of mitosis (Fig. 3), while signals from H3K9me2 were not detected (Fig. 4). In chromatin stained with DAPI at interphase, heterochromatin regions were stained more intensely (see arrow in Fig. 3-A), with lighter DAPI staining observed in euchromatin regions. As can be seen in Fig. 3-A, less H3K4me3 signal can be detected in heterochromatin regions (see arrow), while in euchromatin regions, which have higher transcriptional activity, the level of H3K4me3 was relatively high. In early prophase, the chromatin fibers coil to form chromosomes. Stronger H3K4me3 signals were detected in the distal regions of the chromosomes, and H3K4me3 levels were relatively low in heterochromatin regions near the centromere (see arrow in Fig. 3-B). At mitotic metaphase, chromosomal centromeres are aligned in the middle of the equatorial plane, and the proximal regions of the chromosomes have higher levels of H3K4me3 (Fig. 3-C). At anaphase, sister chromatids separate, centromeres divide in two, and the chromosomes move to the opposite poles in the cell.

Higher H3K4me3 levels were detected in distal regions of the chromosomes which indicates euchromatin (see arrow in Fig. 3-D), while in the heterochromatin regions, H3K4me3 levels were significantly reduced.

As shown in Fig. 3 and 4, in all observed cells at each period of mitosis, H3K4me3-specific signals can be detected but H3K9me2-specific signals do not (Table 1). H3K4me3 levels showed no significant differences during the four phases of mitosis. Therein, compared to heterochromatin regions, the distal euchromatin regions of the chromosome had brighter signals of H3K4me3, showing higher methylation levels .

Analysis of histone phosphorylation levels

In 1997, Hendzel et al. reported an antibody specific for phosphorylated histone H3 that does not recognize non-phosphorylated proteins. In our experiments, the immunoassay did not detect H3S10Ph signals in interphase (Fig. 5-A). In early prophase, when the chromatin get condensed to form chromosomes, H3S10Ph signals were detected in the heterochromatin regions, and the distal regions of the chromosomes gave no obvious signals of H3S10Ph (Fig. 5-B). At prophase, chromosomes condensed further, and the H3S10Ph signals were more obvious, especially in heterochromatin (see arrow in Fig. 5-C). During mitotic metaphase, the chromosomal centromeres are arranged in the equatorial plate, higher levels of H3S10Ph near the centromeric regions (Fig. 5-D) were detected. At anaphase, the sister chromatids separate, the centromeres divide and the spindle fibers pull the chromosomes to the opposite poles of the daughter

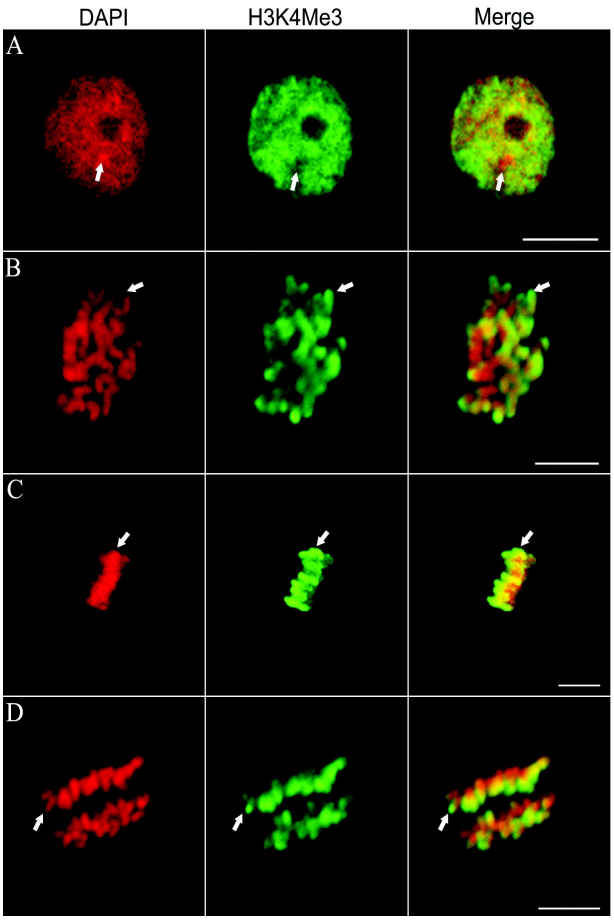

Fig. 3. Fluorescent detection of H3K4me3 in somatic root tip cells of japonica rice cultivar 'Nipponbare'. A: interphase; B: prophase; C: metaphase; D: anaphase. All chromosomes were counterstained with DAPI. Scale bars = 5 μm

Table 1.

Antibody/ mitotic phase	DNA-5MeC	H3K4me3	H3K9me2	H3S10Ph
interphase	high signal level in heterochromatin region	high signal in euchromatin region	no significant signal	no signal
prophase	high signal in heterochromatin region	high signal in euchromatin region	no significant signal	high signal in heterochromatin region
metaphase	-	high signal in euchromatin region	no significant signal	high signal in heterochromatin region
anaphase	-	high signal in euchromatin region	no significant signal	high signal in heterochromatin region
telophase	high signal in heterochromatin region	-	-	no signal

"-" indicates that cells were not observed

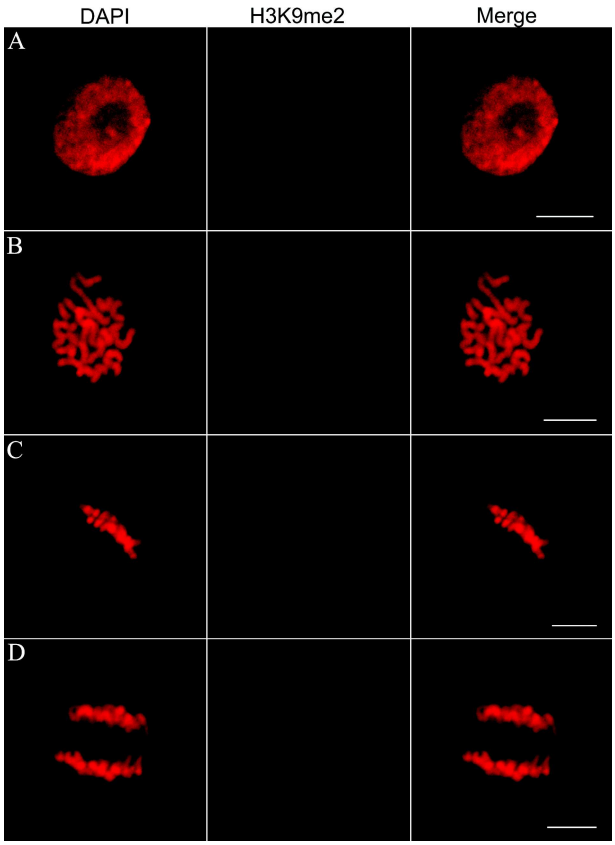

Fig. 4. Fluorescent detection of H3K9me2 in somatic root tip cells of rice cultivar 'Nipponbare'. A: interphase; B: prophase; C: metophase; D: anaphase. All chromosomes were counterstained with DAPI. Scale bars = 5 μm

cells. Spindle fibers attached to the centromeric regions of the chromosomes have higher levels of H3S10Ph (Fig. 5-E). During mitotic telophase, the chromosomes uncoil to become loose and slender and got associated with the newly-formed nuclei in the daughter cells. And we did not detect any signals of H3S10Ph (Fig. 5-F) could be detected.

These results show that H3S10Ph, similar to DNA methylation, is present in heterochromatin region, but the timing of DNA methylation is different; H3S10Ph occurred only in mitosis prophase, metaphase, and anaphase and between interphase and telophase there was no H3S10Ph (Table 1). H3S10Ph was concentrated mainly in heterochromatin regions, while the distal region of the chromosome did not show significant concentration of H3S10Ph.

Discussion

In this study, we found that DNA methylation is mainly found in heterochromatin regions in rice, and is negatively correlated with transcriptional activity. It has been reported that the higher the level of DNA methylation in heterochromatin regions, the lower the relative level of gene expression; conversely, lower levels of DNA methylation are correlated with higher levels of gene expression (Li et al. 2008). DNA methylation is indicative of gene silencing, while unmethylated DNA indicates gene activation (Zemach et al. 2010). Studies have shown that DNA methylation

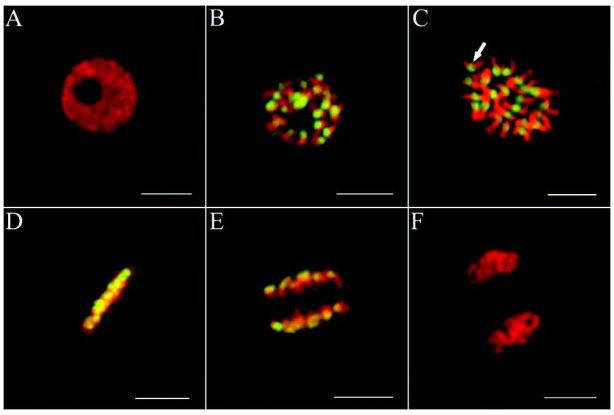

Fig. 5. Fluorescent detection of H3Ser10 phosphorylation in somatic root tip cells of 'Nipponbare'. A: interphase; B: early prophase; C: prophase; D: metaphase; E: anaphase; F: telophase. All chromosomes were counterstained with DAPI. Scale bars = 5 μm

displays cyclical alternations during mitosis, where the level of DNA methylation was low during interphase and increased from prophase to metaphase, and the level of DNA methylation decreased from metaphase to telophase (Yang et al. 2010). However, in present study, these dynamic changes were not observed, which could be related to the relatively small amount of heterochromatin in rice chromosomes; the specific mechanism needs to be studied further.

In rice, the negative correlation between the levels of H3K4me3 and DNA methylation (5-MeC) was found. H3K4me3 shows a positive correlation with gene expression; the higher the level of H3K4me3 in euchromatin, the higher the gene expression activity, and the converse is also true (Yang et al. 2010; Peach et al. 2012). In our study, a similar result was found that H3K4me3 is present in the euchromatin regions of the chromosome, and H3K4me3 signals can be detected during each phase of mitosis. Some studies

have suggested that H3K9me2 is similar to DNA methylation because it shows cyclical changes during mitosis in maize root tip cells; H3K9me2 first appears during interphase, from prophase to metaphase, the relative level of H3K9me2 is high, and from metaphase to telophase, the level of H3K9me2 declines, epigenetic modification presents the reverse change (Yang et al. 2010). However, in present study, H3K9me2-specific signals were not detected during mitosis in rice root tip cells, and hence, further experiments are needed to understand this.

Earlier studies showed that H3S10Ph initiates in defined chromosomal domains during G2, and that H3Ser10 is related to chromosome condensation and separation (Nakayama et al. 2001; Gurley et al. 1978), and H3S10Ph is related to the integrity of centromere (Houben et al. 1999). The study in maize found that H3S10Ph initiates bulk phosphorylation during early prophase, following chromosomal condensation, and is relevant to sister chromatid polymerization, but is unrelated to chromosomal cohesion (Prigent and Dimitrov 2003; Houben et al. 2007). In the current study, H3S10Ph displayed dynamic changes during different phases of mitosis. H3S10Ph initiates in early prophase, reaches a maximum level during metaphase, declines during anaphase, and is lost during telophase. It is mainly distributed in the centromeric regions, and relates to heterochromatin in mitosis. These results are consistent with previous findings. However, the correlation between H3S10Ph and centromere, chromosomal cohesion and the regulatory role of H3S10Ph in rice need to be studied further.

Acknowledgments

This work was supported by grants from the National Natural Science Foundation of China (31370216), a Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions (PAPD) and the Natural Science Foundation of Jiangsu Higher Education Institutions of China (13KJA210003).

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