Role of Polyteny in Plant Epigenetic Variability

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Received: June 30, 2015   Accepted: September 8, 2015   Online Published: September 30, 2015

http://dx.doi.org/10.17830/j.eaj.2015.02.073

Abstract

This paper discusses the available data on polyteny in plants and data on the structural and functional organization of the plant genome and its role in epigenetic variability and inheritance. Also discussed the results of our studies on marker enzymes in agamospermous (apomictic) progenies of sugar beet, pointing to the influence of chromosome polyteny on the epigenetic variability of enzyme genes and on the ratios of phenotypic classes of marker enzymes. Comparison of the known with our experimental data enabled the consideration of differential polyteny of plant chromosomes, not only as a factor of epigenetic variability of gene expression, but also as a factor of evolution of the genome structural organization. Polyteny is considered to be an element of the system of recording hereditary information about epigenetic changes arising during ontogenesis.

Keywords: plant polytene chromosomes, epigenetic variability, genome spatial organization, enzyme genes, agamospermy


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1. Introduction

Epigenetic changes refer to changes in the phenotypic expression of a gene that are not associated with changes in its nucleotide sequence and can be transmitted and preserved during mitotic and meiotic cell divisions (Bird, 2002; Rapp & Wendel, 2005). Epigenetic changes play an important role in all aspects of the plant life cycle: including genome integrity, transgene silencing, nucleosome arrangement, nucleolar dominance, paramutations, flowering, parents imprinting, hybrid dysgenesis, uniparental disomy, and many others (Preuss & Pikaard, 2007; Ishikawa & Kinoshita, 2009; Erhard & Hollick, 2011; Groszmann et al., 2011; He et al., 2011; Levites & Kirikovich, 2013a). There are various mechanisms underlying the wide range of phenomena associated with epigenetic changes, but the best-understood mechanisms underlying epigenetic changes are DNA methylation-demethylation (Bender, 2004; Cokus et al., 2008; Law & Jacobsen, 2010) and histone acetylation and methylation which lead to gene repression or activation (Fuchs et al., 2006; He et al., 2011).

Currently, there is a growing understanding that chromatin structure plays a key role in gene expression and that all epigenetic mechanisms are probably realized at the level of spatial DNA organization within the nucleus, while DNA methylation just fixes the tightly packed inactive chromatin (Razin, 2006). On the other hand, what determines the spatial organization of the genome? Vyskot’s (2000) review presents a long list of genomic changes and accompanying epigenetic effects. Important genomic changes include changes in the level of ploidy and the number of chromosomes leading to significant heritable changes of phenotypic traits (Vyskot, 2000). Such changes are referred to as epigenetic changes (Matzke et al., 1999; Vyskot, 2000). This indicates that dose ratios play an important role in epigenetic changes.

A comparison between known genomic changes and their associated epigenetic effects to our experimental data made it possible to suggest that dose ratios in the genome can be determined not only by the addition or loss of separate chromosomes, but also by the level of chromosome polyteny that leads to changes in gene expression and to changes in structural and functional organization and evolution of the genome (Levites, 2010a). In this article, we further develop views on the role of polytene chromosomes in genome evolution and processes of epigenetic variability.

2. Plant Polytene Chromosomes

Polytene chromosomes are multi-filamentous. Polyteny is the presence of a large number of DNA strands in a chromosome. Plant polytenes chromosomes in antipodes have been studied in detail in the Ranunculaceae and Papaveraceae families (Tschermak-Woess, 1957; Hasitschka-Jenschke, 1959), in the species Hordeum vulgare (Odenbach, 1965; Petrova et al., 1985; Pushkina et al., 1989; Khrustaleva et al., 2010), Triticum durum (Ivanovskaya, 1973), and Aconitum neomontanum (Nagl, 1981). Polytene chromosomes were also found at the same time in synergids, e.g., Allium (Hakansson, 1957; Nagl, 1978), in endosperm nuclei of the Allium nutans (Hakansson, 1957), Bryonia dioica (Hasitschka-Jenschke, 1961), Zea mays (Tschermak-Woess, Enzenberg-Kunz, 1965), Hordeum vulgare (Ivanovskaya, 1968), Rhinanthus alectorophorus, and Thesium alpinum (Nagl, 1981). A number of papers are devoted to the study of polytene chromosomes in suspensor cells of Phaseolus (Nagl, 1970, 1981).

A considerable number of papers devoted to the study of polytene chromosomes in anther tapetum cells (using optical and electronic microscopy) are discussed in the review by Carvalheira (2000). In
several papers, it was demonstrated that both nuclei with polyploid amounts of chromosomes and nuclei with polytene chromosomes can be present in tapetal cells of the same plant (D’Amato, 1984; Guerra & Carvalheira, 1994; Carvalheira & Guerra, 1994, 1998). In these papers, a strong association between sister chromatids in plant polytene chromosomes was not observed; this has also been confirmed elsewhere. For example, during the study of chromosomes in root cells of diploid and tetraploid Brassica oleracea using the FISH method, it was demonstrated that in many cells, chromosomes are represented by a bunch of chromatids [i.e., they contain more than two divided chromatid strands (Sesek et al., 2005)].

Most studies of polyteny have been performed on somatic cells; however, the study of polyteny in generative cells is also necessary for clarifying the role of this phenomenon in genetic processes and evolution. A high degree of similarity in many traits and properties of synergids, antipodes, and egg cells [e.g., the ability of these cells to follow the path of embryogenesis (Czapik, 1999; Batygina et al., 2003)] indicate that polyteny is possible not only in synergids and antipodes, but also in female gametes in many plant species.

For example, the chromosomes of the common pine (Pinus silvestris) are 32 times larger in volume during the first zygotic division than chromosomes of somatic cells (Nagl, 1967). It was also demonstrated using different methods that the DNA content in the egg cell nuclei of the species P. sibirica Du Taur exceeds that in a somatic diploid cell by 16 times and is 32C (Ermakov et al., 1981); the DNA contents in the egg cell of Ornithogalum caudatum and Haemanthus albiflos are 4C and 3–4C, respectively (Morozova, 2002). The high DNA content in zygotes of barley and petunia, which gradually decrease during the first zygotic divisions and reach a diploid level at later stages of embryogenesis, is also indirect evidence for chromosome polyteny in the egg cell nuclei (Mericle, & Mericle, 1970; Vallade et al., 1978). The possibility of polytenization during gamete generation in plants was observed in a study conducted on Allium tuberosum (Kojima & Nagato, 1992); it was demonstrated that endoreduplicated meiosis is observed with a frequency of 80% in female gametes but only in 3.9% of male gametes. The wealth of data on the presence of polyteny in the cells of the embryo sac, and the indirect evidences of polyteny in egg cells, indicate a possible role for polyteny in epigenetic variability and heredity in plants.

3. Factors Affecting Polyteny of Chromosomes

It is known that the degree of polyteny in plants is affected by both internal and external factors. For example, the degree of polyteny differs significantly in the cells of different organs and in the cells of the same organ. The degree of polyteny in plants varies from 2C up to 8192C (Brady, 1973); in this case, the highest degree of polyteny corresponds to 12 cycles of the reduplication of two initial diploid sets. Different regions of the same chromosome can be endoreduplicated to a different degree (Cionini et al., 1982) which is due to the fact that eukaryotic chromosomes have many independent points at the beginning of reduplication (Van’t Hof, 1985, 1988; Bryant et al., 2001). The presence of under-reduplicated regions of chromosomes and regions with high levels of polyteny has been demonstrated in many animal and plant species (Nagl, 1976). During the assembly of multiple DNA strands into a single polytene chromosome, at least one guiding DNA strand (which would be intact over its entire length) is required (Kirikovich & Levites, 2013). Puff-like nodules with a clear cross striation (similar to those observed in polytene chromosomes of Drosophila melanogaster) were detected in maize, and it has been demonstrated that the size of
nodular regions of nucleolar organizer in one or both homologous chromosomes of the maize meiocytes changes during two to three inbred generations (Pokhmel'nykh & Shumnyi, 1984).

Polyteny in plants is affected by temperature. For example, polytene chromosomes in the *Phaseolus coccineus* haricot are pompon-like when the plant develops at 20–22°C but acquire a classical appearance when development occurred at 12°C during daytime and at 8°C at night (Nagl, 1970). Daylight duration also influences the degree of polyteny; in a light–dark cycle of 8–16 h, polytene chromosomes in *Phaseolus vulgaris* suspensor cells are thin but acquire a clearer band pattern than at other combinations of light and dark (Nagl, 1973). Treatment of the haricot (*P. Vulgaris*) with actinomycin D results in chromosome condensation, and shortening of the suspensor cells; the chromosome band pattern also becomes clearer (Nagl, 1969).

Conclusions about the influence of external factors on polytenization were based on genetic analysis on the agamospermous offspring of sugar beet derived from different twigs of the same plant with the use of different isolators (parchment and bleached calico; Levites & Kirikovich, 2013b). In the two progeny groups from plant No. 2-7, phenotypic classes ratios differed significantly (\(G=220.8436; P<0.001\)), which allowed us to determine individual polygenotypes for each seed group. To characterize the polyteny state of this or that chromosome site, we proposed the term “polygenotype of locus” and the notation \(F_nS_m\) to describe its allelic composition and the number of chromosomes and chromatides carrying each of the alleles (Levites & Kirikovich, 2011).

Polygenotype \(F_2S_2\) \((\chi^2=2.2)\) mostly corresponds to the ratio on ME1, 1FF:16FS:3SS, as revealed in the seeds obtained under the parchment isolator, and polygenotype \(F_8S_7\) \((\chi^2=1.4539)\) mostly corresponds to the ratio 10FF:14FS:7SS in the seeds from the bleached calico isolator. A low level of polyteny in cells of flowering shoots obtained under the parchment isolator \(F_2S_2\), as compared to that detected in cells under the bleached calico isolator \(F_8S_7\), indicates that external conditions influence polytenization in the cells of a mother plant capable of reproducing by agamospermy.

Dependence of polyteny on external and internal factors is in good agreement with the dependence of epigenetic variability on external and internal factors. Therefore, the close connection between these phenomena will be considered in the following section of this review.

### 4. Genome Structural-Functional Peculiarities Affecting Plant Epigenetic Variability

A decisive role of the cell nucleus in heredity and variability raises a question about the role these or other peculiarities of the nucleus structure have in epigenetic processes. Since a cell’s genome is in the interphase stage during the performance of its specific functions, the structure of the interphase nucleus is of particular interest. As mentioned in the monograph by Stegni (1993), this problem arose almost as soon as the chromosome theory of heredity was advanced. Initial evidences of an ordered arrangement of chromosomes in the cell nucleus were presented in the works of Rabl (1885) and Boveri (1909), which were subsequently supplemented by others such as Navashin (1947), Comings (1968), and Mosolov (1968). During this period, it was shown that the attachment of the chromosomes to the nuclear membrane plays a key role in the orderly spatial arrangement of the interphase nucleus (Comings, 1968; Mosolov, 1968). The structure of interphase nuclei was then investigated in ovarian trophocytes from malarial mosquitoes (Stegniy, 1979, 1993, 2006). This work showed that the spatial arrangement of polytene chromosomes was specific for every species. This species specificity, manifested as the “fingerprint” attachment of chromosomes to the nuclear
membrane, can be viewed as the participation of the spatial genome structure in the encoding of genetic information.

Detailed studies conducted over recent years have provided additional evidences for the non-random location of chromosomes in the interphase nucleus. In interphase nuclei, each chromosome has its own territory (Cremer & Cremer, 2001; Schubert et al., 2006; Branco & Pombo, 2007; Allen, 2009; Shubert & Shaw, 2011) due to its attachment to the nuclear membrane. This attachment is dynamic, so the level of gene activity and time of gene activation depend on the location of the gene within the chromosome territory and the location of the gene relative to the periphery and center of the nucleus (Taddei et al., 2004). The types of DNA interactions with the nuclear membrane are rather stable and reproducible after mitosis in all the cells of the organism, which implies that nuclear spatial organization can be viewed as a part of an epigenetic system defining long-lasting gene activation or repression underlying differentiation (Razin, 2006). Epigenetic activation or repression of genes is associated with the degree of chromatin condensation, which can persist throughout mitosis (Therizols et al., 2014). This is a strong indication that the spatial structure of the genome carries an encoding function that plays an important role in the transfer of genetic information across cell generations.

Spatial organization of the genome was studied in the cells of somatic tissues or in stem cells. The lack of similar studies on generative cells impedes progress toward gaining an understanding of the role of genomic spatial organization in inheritance. The idea about the role of polyteny in genetic and epigenetic processes has been developed as a result of investigations into the agamospermous progenies of sugar beet plants (Levites, 2005, 2007, 2010b).

For example, during the study of the sugar beet progeny generated from somatic cells by mitotic agamospermy, which must theoretically be monomorphous, the complete uniformity by the heterozygous spectrum of the marker alcohol dehydrogenase (ADH1) enzyme and polymorphism (two phenotypic classes) by another enzyme (isocitrate dehydrogenase, IDH3) were detected (Levites et al., 1999) and a hypothesis explaining this phenomenon was proposed (Levites, 2005, 2007, 2010b). According to this hypothesis, inherited information is encoded not only by the nucleotide sequence, but also by differential polyteny of chromosomes. Chromosomes are polytenized in the cells of tissues surrounding the embryo sac. The degree of polyteny of different sites within one chromosome and the homologous sites of homologous chromosomes can differ. Hence, the alleles of heterozygous loci in a genome can be presented by a different number of copies (i.e., in different doses). A somatic cell that is entering into embryogenesis by means of agamospermy can contain only one copy of an allele in each homologous chromosome. Therefore, excessive copies of alleles must be eliminated from the genome. Elimination of excessive alleles copies together with the combinatorial process of randomly selecting two allele copies from the multitude of copies present. In the nucleus, each selected pair of allele copies are preserved through attachment to the nuclear membrane.

If, for instance, one allele of a marker locus is polytenized and present as three copies in the genome and the second one is present as a single copy (genotype FFFS), then the theoretical genotype ratio will be 1FF:1FS. Such genotype ratios were observed in our experiments (Levites et al., 1999; 2000). These genotypes represent the variants of choice for two elements from a given set of elements. The genotype ratios (after the diminution of excessive allele copies) can be found by using the formulas of hypergeometric distributions, which were proposed by Haldane (1930) for calculating genotype
frequencies (i.e., the frequencies of different allele combinations) in polyploid progeny. The absence of diversity of the Adh1 enzyme in the investigated progeny is explained by the fact that the Adh1 locus was not polytenized and, for this reason, was not exposed to diminution and combinatorial processes. Thus, there was no combinatorial process between alleles of the Adh1 locus. The results obtained in this experiment allowed us to conclude that the process of polytenization of different loci occurs independently and affects the ratio of phenotypic classes differently for different genes in an agamospermous progeny.

In another experiment, polymorphism detected in agamospermous progenies from triploid sugar beet plants is also well described based on ideas about differential polyteny of chromosomes in the cells of the maternal plant (Levites & Kirikovich, 2011) obtained from the triploid plants displaying GPI2 polymorphism with a specific set of phenotypic classes and specific phenotype ratio. This polymorphism was represented by homozygous (FF and SS) and heterozygous (FS, FFS, FSS, FSSM, SS, and SSMSM) phenotypes. The standard heterozygous phenotype (FS) was represented by a symmetric three-banded pattern in which the heterodimeric isozyme was the most intensive. This is a basic type of heterozygous GPI2 spectra with a frequency among heterozygotes higher than 90%. We classified the spectra with asymmetric intensity as “trisomic” spectra. Such classification is explained by the fact that these spectra are similar to the spectra found in trisomic plants heterozygous for a marker enzyme gene. By accepting this classification, we assume that stronger expression of an allele is due to its double dose: FFS or FSS.

It is also of interest that (in these progenies) there are isozyme spectra containing products of the modified expression of the S allele along with the usual GPI2 phenotypes. The allele with modified expression was denoted Gpi2-SM. Among the spectra with the modified expression of the allele (FSSM, SSMSM, SSMSM), the frequency of trisomic phenotypes in all three analyzed progenies constituted 50% (13/26); however, among the normal heterozygous spectra, the fraction of trisomic phenotypes was less than 5% (7/145). This means that a change in the dose of one allele of the enzyme locus plays a decisive role in the epigenetic change of its expression, which is reflected in the appearance of the abnormal GPI2 spectra. However, an increase in the dose of an allele can occur not only due to trisomy, but the presence in agamospermous progeny of the seeds with a disomic phenotype SS indicates that a change in the allele expression may be caused by its increased dose due to polyteny.

Based on the concept of differential polytenization (endoreduplication) of chromosomes and the fact that gene expression depends on contact between interphase chromosomes and the nuclear membrane, it is possible to advance a hypothesis on the mechanisms of epigenetic changes as follows. Chromosomes compete for contact with the nuclear membrane which has a limited surface area, tending to a minimum according to physical laws. Gene functioning can lead to an increase in the degree of polyteny in the chromosome sites carrying these genes and, accordingly, to enhanced competition of these sites for contact with the nuclear membrane (Levites, 2010a). An increase in the degree of polyteny in a small number of loci may have no effect on the genome size or surface area of the nuclear membrane. If the number of endoreduplicated regions exceeds a critical value, this may result in increased nucleus surface area and, consequently, may lead to a new activity ratio for different loci, which is manifested as an epigenetic change. If such processes affect cells of generative organs, epigenetic changes can be transferred to the next generation.

Our hypothesis is in good agreement with the notion of the important role that gene dosage has on genome functioning. Effects of gene dosage can be interpreted in terms of chemical law which is
evolutionarily conserved at the supermolecular level. According to this law, the rate of chemical reaction is determined by the concentration of the agents entering the reaction. In this case, gene dosage is analogous to agent concentrations in elementary chemical reactions (Levites, 2010b).

By comparing the two causes of epigenetic changes (dosage changes in the genome and a change in chromosome spatial distribution) one can hypothesize that a change in chromosome spatial distribution is also mediated by dosage effect, since chromosome sites with different degrees of polyteny may compete for contact with the nuclear membrane. Changes in the sites of chromosome attachment to the nuclear membrane may lead to changes in chromosome competitive interactions and to modifying of gene expression. The hypothesis is based on a preponderance of evidence in support of the important role of gene dosage (or changes in the degree of ploidy) in genome reorganization. Such changes depend on external factors and for this reason can serve as an effective means of epigenetic changes and a powerful mechanism for acceleration of evolutionary processes.

The influence of the degree of polyteny on phenotype ratios in the progeny allows us to consider differential polytenization as a factor influencing the transfer of hereditary information to the next generations.

It was previously suggested that genetic information recorded in a nucleotide sequence can be referred to as 1D encoding, while the information recorded in differential polytenization can be considered as 2D encoding (Levites, 2005, 2007, 2010b). The species specificity of the spatial genome architecture (Stegniy, 1993, 2006) suggests that specific chromosome arrangements in a cell nucleus carry information which defines the properties of the organism. Since the cell nucleus generally carries hereditary information, it can be concluded that the genome architecture is also a carrier of hereditary information encoded in 3D (Levites, 2005, 2007, 2010b). This viewpoint and conjecture are unusual since genetics has only begun to develop formal parameters to describe the involvement of genome spatial organization in the encoding of hereditary information. The processes designated as “encoding in 2D and 3D” are of non-rigid nature and react to environmental changes. This enables us to treat such processes as elements of epigenetic variability and changes in such processes as records of hereditary information about the acquired traits (Levites, 2005, 2007, 2010b).
5. Conclusions

The data presented in this review are indicative of the complex and multidimensional nature of the organization and functioning of the hereditary process. The complexity of genome organization makes one review inadequate for covering all operational aspects. Together with the cited results and the conjectures of other authors, this review reflects our subjective viewpoint on the role of differential polyteny, attachment of chromosomes to the nuclear membrane, and chromatin diminution in the processes of inheritance; this is based on our own experience with genetic studies in the field of reproductive biology of plants.

These results (obtained on the phenotypic level, which characterizes the expression of enzyme genes) can serve as a basis for further molecular studies. By considering the data on the influence of external and internal factors on chromosome polytenization (as well as the influence of differential polyteny on the dosage of genes, gene expression, and the phenotypic ratio of the progeny), one can hypothesize the existence of specific, powerful mechanisms that are sensitive to environmental influences that lead to epigenetic changes and genomic evolution. Our hypothesis can serve as a basis for undertaking research in a new areas pertaining to structural and functional organization of the eukaryotic genome and to investigating properties of living organisms that are most important for the evolutionary process and namely such as heredity and variability.

6. Acknowledgements

This work was supported by the budget project № VI.60.1.3. “Genetic and epigenetic mechanisms regulating differentiation, transdifferentiation and reprogramming”.

References


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