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LETTER TO EDITOR

Theoretical and Practical Aspects of Studies in Epigenetic Variability in Sugarbeet

E.V. Levites

Institute of Cytology and Genetics Siberian Division of Russian Academy of Sciences, Lavrentieva Av. 10, 630090 Novosibirsk, Russia

Factors affecting epigenetic variability have been considered on the basis of experimental and literature data. A concept of multidimensional encoding of inherited information in eukaryotes has been proposed.

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Interest in epigenetic variability has now grown considerably. Epigenetic changes occur during ontogenesis due to external and internal factors and can be transmitted, by the gametes, to the next generations. The scientific importance of studies in genetic variability is because they help in understanding the mechanism of gene expression during ontogenesis. On the other hand, these studies have practical value, because the frequency of epigenetic changes is much higher than that of mutations and can reach upto 50%, which makes them attractive for breeding practices. The first plant, for which induced inheritable epigenetic changes were demonstrated, was flax (Durrant, 1962). They showed that fertilizers applied at certain doses gave rise to vigour tall plants called large genotrophs. Their traits were preserved in the next generations. Later those experiments were performed on wheat with nicotinic acid as an active agent (Bogdanova, 1984, 2003).

It was established that genotrophs have many more repeats in their DNA than the control plants (Cullis, 1985). It was, therefore, hypothesized that genotrophs do not occur due to gene mutations but due to gene amplification (Cullis, 1985).

Another step in understanding the process that underlie epigenetic variability was the finding that the activity of the genes depends on the level of cytosine methylation in the promoters genes. It was demonstrated that such epigenetic variability as gene silencing is due to methylation of DNA bases, whereas gene de-silencing is due to demethylation (Vyskot, 2000). The modified cytosine analog 5-azacytidin is used as a demethylating agent.

Interestingly, the level of methylation of chromosomes and, therefore, the level of activity of the genes in them, is transmitted by the gametes to the next generation (Vyskot, 2000).

The first experiments on sugarbeet using 5-azacytidin were conducted by Maletskaya and the associates (Maletskaya *et al.*, 2002). They showed that the soaking of seeds in 5-azacytidin promotes early flowering, reduces the number of flowerless plants, increases the number of second order branches. Studies in other plants (flax, tobacco) provide evidence that 5-azacytidin also affects flower morphology (Vyskot, 2000). It can therefore be expected that this epimutagen can similarly affect flower structure in sugar beet. The flowering time, branching pattern, and flower morphology are important technological traits in sugarbeet, because they have implications for seed number and seed quality. Thus, it can be proposed that 5-azacytidin can be used as an instrumental tool for studying the processes that lead to the occurrence of the traits, which are important for sugarbeet farming.

However, the formation of complex morphological traits cannot be understood unless simple traits that are easy to detect and inherit have been studied. Some of such traits are isozymes.

Codominant inheritance of isozymes allow each allele of the heterozygous enzyme locus to be traced in the succession of generations. The use of isozymes as genetic markers in sugar beet lead to the following observations :

Author for Correspondence : E.V. Levites
e-mail : levites@bionet.nsc.ru

1. The epigenetic variability of an enzyme locus (allele) depends on which form of reproduction (gamospermy or agamospermy) (Levites *et al.*, 1991, 1998, 2001b) stage of plant development (Levites *et al.*,1991), allele origin (maternal or paternal) (Levites *et al.*, 2001a), gene dose or allele dose, or genome (Levites and Denisova, 1999; Levites *et al.*, 2001a).
2. The pattern of epigenetic variability is specific for each locus (Levites *et al.*, 1998).

The mechanism of epigenetic variability, is quite sophisticated.

Epigenetic variability inevitably leads to the hypothesis that the underlying structures and mechanisms are just bits of genetics information and its encoding. This led to such concepts as a code provided by DNA base methylation, and a histone and a chromatin code. However, a question arises at this point: what determines the level of DNA methylation and histone acetylation.

As is known, epigenetic variability depends on dose effects. For instance, it has been demonstrated that colchicine changes the pattern of epigenetic variability and promotes mitotic agamospermy in sugar beet (Levites *et al.*, 2000). This led to the conclusion that chromosome endoreduplication plays a pivotal role in setting up conditions that favour mitotic agamospermy. In other words, chromosome endoreduplication determines the form of reproduction and epigenetic variability. What do other sugarbeet experiments say, though? As was demonstrated in one of them, progeny resulting from mitotic agamospermy may display no variation for the heterozygous marker locus, *Adh1*, whereas there is variation for the *Idh3* locus located 17 cM away on the same chromosome (Levites *et al.*, 1994; 1998). Essentially, this variation is a case of pseudosegregation, because when there is mitotic agamospermy, there is no meiosis, and the diversity observed only results from epigenetic variability. Genetic analysis of progeny resulting from mitotic agamospermy suggests that variation is not due to the silencing of one of the alleles in the heterozygous locus in part of the progeny, but due to redetermination (Levites, 2002; Levites and Kirikovich, 2003). Following is the explanation that was given to the phenomenon. The genotype (or epigenotype) and the phenotype of any cell entering embryogenesis should depend on the ratio of doses of the alleles in that cell. For example, if the respective numbers of chromatids carrying the *F* and *S* alleles are close, then the cell is heterozygous both genotypically and phenotypically, and so will be the embryo that developed from it. If there are many more chromatids carrying *F* than *S* alleles in the cell, then the cell has an *F* epigenotype and an F phenotype, and so will the embryo developed from it. And vice versa: if there are many more chromatids carrying *S* than *F* alleles in the cell, then the cell has an *S* epigenotype and an S phenotype, and so will the embryo developed from it. However, the fact that the loci located on the same chromosome undergo epigenetic variability differently suggests that endoreduplication is not uniform along the chromosome. Some chromosome regions have a high level of polyteny, some have none. These regions may be too small to be detected in metaphase chromosomes. This hypothesis is supported by data on DNA content in the cells of large (L) and small (S) flax genotrophs. As was shown, L-genotrophs contain 16 % more cell nuclear DNA than S-genotrophs, however, only 0.23 % of this difference can be explained by DNA amplification (Durrant, 1971; Timmis and Ingle, 1974). Therefore, the others are due to different levels of polyteny at different chromosome regions. That such regions do exist is indicated by the existence of independent replication regions (replicons) (Lewin, 1994). Naturally, processes such as DNA base methylation and histone acetylation must be resulting from polyteny in each particular chromosome region.

Based on these data, chromosome polyteny should be regarded as a way of the encoding of inherited information. Polyteny of all the genome chromosomes is a well-known phenomenon observed in some plant tissues (D'Amato, 1985). However, the comparison of gene expression data on genotrophs and plants resulting from mitotic agamospermy, still more importance should be attached to polyteny. Polyteny should be regarded as the encoding of inherited information in the second spatial dimension. The sequence of molecules in a DNA molecule is the encoding in 1-dimensional space, and together with polyteny, they become the encoding in 2-dimensional space. Thus there must be a 3D system for the encoding of inherited information. And this system shows explicitly when the genes in nonhomologous chromosomes start behaving as if they were linked. The many cases of pseudolinkage are another piece of evidence for such a system. 3-dimensional encoding of inherited information should not be confused with the 3-dimensional organization of a cell nucleus, although this organization is undoubtedly implicated in encoding. Three-dimensional encoding is primarily a functional association. Since all the living things exist not only in space but also in time, the encoding of inherited information should include a temporal component. Apparently, the temporal dimension, as far as the encoding of inherited information is concerned, is the frequency of biological processes, which are in concord with diurnal and seasonal rhythms. Obviously, dimensions of encoding of inherited information in question have different degrees of conservatism. Encoding provided in the first dimension by a sequence of DNA bases is the most

conserved. Others are less so. They are oriented at perceiving the influences of the environment and may participate in the inheritance of acquired characters.

The encoding of inherited multidimensional information helps understand a lot of facts. For instance, gene expression in hybrids, self- and cross-incompatibility can be explained in a new way. Different levels of polyteny of separate regions of homologous chromosomes can explain why male and female gametes have different capabilities in transmitting inherited information.

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