Induced Meiotic Reductions in Root-tips

IV. Concluding remarks

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Accepted April 27, 1985

The experiments on induction of "meiotic reductions" in somatic cells were initiated on observing that caffeine, besides producing chromosomal aberrations, also induced "meiotic reductions" (simulating the first meiotic division in sporecytes) in the root-tip cells of *Pterotheca falconeri*. As stated in the earlier papers of the series this species is ideal for such investigations in that the haploid chromosome number is only 3 and each chromosome is a marker chromosome. Since caffeine is a purine derivative it was considered desirable to study the effect of as many as possible purines and pyrimidines or their derivatives, as well as the nucleic acids, DNA and RNA, to arrive at some meaningful conclusions. A range of concentrations of each, therefore, were tried at suitable temperature and pH. The results obtained so far are tabulated in a consolidated form in Table 1 wherein only the optimal concentration of each of these physiological substances in inducing maximum percentage of "meiotic reductions" is presented. Together with this, the percentage of reductional groupings of all types involving varied distribution of homologues is given

The percentages of "meiotic reductions" and reductional groupings were calculated in relation to the anaphase stage. Huskins (1948) calculated the percentage of "meiotic reductions" under the name of "somatic meiosis" in relation to the number of dividing cells in his material of root-tips of onion. This to us seems not entirely satisfactory since this does not clearly portray the point under discussion.

*Pterotheca falconeri* possesses a diploid set of 6 chromosomes and at the time of mitotic division 6:6 chromosomes move to each pole. It is also pertinent to mention that out of several hundreds of dividing cells analysed in the control root-tips not even a single instance of "meiotic reduction" of 3:3 homologous chromosomes was ever observed, nor any haploid cell with 3 characteristic chromosomes of the complement.

To us it now seems certain that all the above mentioned physiological substances definitely induce "meiotic reductions" causing segregation of homologous chromosomes towards the opposite ends without the intervention of spindle. A comparison of the percentages of "meiotic reductions" vis a vis the sum total of

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The reductional groupings included 3+3 non-homologous chromosomes (AAC/BBC, AAB/BCC, ABB/ACC), 4+2 (AABB/CC, AACC/BB, BBCC/AA, AABC/BC, ABC/AC, ABCC/AB), and 5+1 (AABBC/C, AABCC/B, BBBC/A) separation. These are merely random segregations.
Table 1. Percentage of "meiotic reductions"/reductional groupings at anaphase on treatment with optimal concentrations of purine and pyrimidine derivatives and nucleic acids DNA and RNA on *P. falconeri* root-tips

<table>
<thead>
<tr>
<th>Name of the chemical</th>
<th>Optimal treatment</th>
<th>&quot;Meiotic reductions&quot;</th>
<th>Reductional groupings</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>I</td>
<td>II</td>
</tr>
<tr>
<td><strong>Purines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caffeine</td>
<td>57 mM/1 HT/72 HR</td>
<td>54.54</td>
<td>9.99</td>
</tr>
<tr>
<td>Xanthosine</td>
<td>60 mM/1 HT/0 HR</td>
<td>33.33</td>
<td>8.33</td>
</tr>
<tr>
<td>Xanthine</td>
<td>57 mM/1 HT/6 HR</td>
<td>25.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Aminophylline</td>
<td>51 mM/1 HT/0 HR</td>
<td>12.50</td>
<td>—</td>
</tr>
<tr>
<td>Theophylline</td>
<td>54 mM/1 HT/0 HR</td>
<td>6.25</td>
<td>—</td>
</tr>
<tr>
<td>Adenine</td>
<td>40 mM/1 HT/72 HR</td>
<td>21.96</td>
<td>8.33</td>
</tr>
<tr>
<td>Adenosine</td>
<td>54 mM/1 HT/12 HR</td>
<td>16.66</td>
<td>—</td>
</tr>
<tr>
<td><strong>Pyrimidines</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thymine</td>
<td>50 mM/1 HT/24 HR</td>
<td>21.74</td>
<td>—</td>
</tr>
<tr>
<td>Cytosine</td>
<td>57 mM/2 HT/48 HR</td>
<td>25.00</td>
<td>—</td>
</tr>
<tr>
<td>Cystidine</td>
<td>57 mM/1 HT/48 HR</td>
<td>21.47</td>
<td>—</td>
</tr>
<tr>
<td>Uracil</td>
<td>60 mM/1 HT/24 HR</td>
<td>20.69</td>
<td>—</td>
</tr>
<tr>
<td>Uridine monophosphate</td>
<td>50 mM/2 HT/24 HR</td>
<td>23.53</td>
<td>—</td>
</tr>
<tr>
<td><strong>Nucleic acids</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DNA</td>
<td>6%/1 HT/72 HR</td>
<td>10.00</td>
<td>5.00</td>
</tr>
<tr>
<td>RNA</td>
<td>5%/1 HT/24 HR</td>
<td>44.86</td>
<td>—</td>
</tr>
</tbody>
</table>

Reductional groupings of all types taken together leave us in no doubt that these are not just random separations but have a deeper meaning. That spindle is not involved becomes clear when we find that not unoften such "meiotic reductions" do take place even at prophase when the chromosomes are yet long and convoluted. The absence of any specific orientation of the centromeres of the homologues further obviates any suggestion of the operation of a spindle. A feature of unusual interest is that in a few instances were observed what looked like chiasmata but which we have termed as pseudochiasmata since they do not conform exactly to the chiasmata as found in the sporocytes during normal meiosis. However, pairing of the homologues was quite often observed. Furthermore, the two haploid complements at the opposite ends never organised to form resting nuclei but underwent an equational division resulting in the formation of 4 haploid sets of chromosomes comparable to those found in the sporocytes at the end of 2nd meiotic division. Interestingly during this process spindles were definitely organised being either parallel or at right angles to one another as in sporocytes. It is also worth emphasising here that such equational divisions were noticed as a rule after 12–24 hours of recovery period, indicating that they are the outcome of a second division cycle following the first. Haploid cells with the characteristic chromosome complement of the species were noticed after treatment in very many cases. In RNA treatment, haploid cells were observed in the highest percentage in 5%/1 HT treatment and in the same treatment highest percentage of first "meiotic reductions" were also scored.

We would like to add here that we have tried the effects of various other chemicals on the root tips of this species but have never found anything similar to what has been described above suggesting thereby that the physiological substances
employed do play a definite role in the determination of "meiotic reductions".

Similar meiotic reductions have also come to light on the treatment of root-tips of another species Tragopogon gracile with caffeine and many pyrimidines. This species has a chromosome complement of \( n = 6 \) chromosomes and two of these are marker chromosomes which can easily be identified. The details along with illustrations will be presented in a subsequent paper.

The phenomenon of "meiotic reduction" involving segregation of homologous chromosomes was referred to as "somatic meiosis" by Huskins, as stated earlier. In his experiments of treatment of onion root-tips with sodium salt of RNA it could not be made clear, whether \( 8+8 \) separation was merely numerical separation or qualitative also. According to Kodani (1948) it was a matter of chance that the homologous chromosomes reached the opposite poles or to the same pole. Wilson and Cheng (1949), however, established in Trillium sps. that the two phenomena, separation of chromosomes into two numerically equal groups and segregation of homologues, occurred with much greater frequency than as a purely random phenomenon.

Basing their premises on these observations, Wilson et al. (1951) finally concluded that the "various forms of reductional and segregational separations are intermediate between normal and complete lack of spindle organisation". Srinivasachar and Patau (1958) also agreed with this, stating that "reductional groupings arise de novo due to disturbances in spindle formation."

"Meiotic reductions", according to us, cannot be explained by Wilson's suggestion of spindle disturbances. If it is simply a phenomenon of spindle disturbance, why should "meiotic reductions" occur at a higher frequency than even the sum total of reductional groupings of all sorts. Secondly, careful scrutiny of some of the figures suggest that spindle did not play any part in their separation. "Meiotic reductions" initiated at prophase also prove that this phenomenon is not connected with the spindle. In sodium nucleate induced segregational mitoses of Huskins, he also stated that spindle has no role.

According to Kodani (1948) sodium nucleate prevents duplication of chromosomes and the unduplicated chromosomes reach the opposite poles. We are unable to support this contention which seems to us to be a misinterpretation of the actual division of a haploid cell.

In our experiments chromosome duplication was not inhibited, only spindle was not formed, and the homologous chromosomes segregated to the opposite 'poles'. Thereafter they underwent equational division.

The appearance of equational division, however, was rather interesting. Such division always appeared after 12-24 hrs recovery, showing that these must have been preceded earlier by "meiotic reductions." The absence of other combinations of equal or unequal chromosome distribution at the four poles except ABC/ABC/ABC/ABC is also otherwise inexplicable.

**Some Theoretical Considerations**

At this stage I would like to call attention to a feature universally present in the plant kingdom. In all the vascular land plants, be they cryptogams or phanero-
gams, a tapetum is invariably present in the microsporangia surrounding the sporogenous tissue. Near the time that the sporocytes round off and initiate meiotic divisions, the tapetum cells either degenerate forming a periplasmodium in which the sporocytes are bathed, or the tapetum is of the secretory type. In either case the nucleic acids or their degradation products are invariably liberated into the cavity of the microsporangia around the sporocytes. In many cases the nuclei of tapetal cells undergo divisions prior to degeneration so as to increase the nucleic acid content. In the ovules during the onset of megasporogenesis the cells surrounding the enlarging megaspore mother-cell are crushed, thus making available the nucleic acid contents. It is also significant that in vascular cryptogams like *Isoetes* the massive micro- and mega-sporangia are partitioned by trabeculae which degenerate to supply nucleic acid products to the sporocytes undergoing meiosis. It is further worthy of note that similar trabeculae were present partitioning the micro- and mega-sporangia in the allied fossil members like *Lepidodendron*, *Bothrodendron*, *Sigillaria* etc. In the hepatics, there are the elater mother-cells whose contents degenerate during elater organisation thus making available the products to the sporocytes which are to undergo meiosis. In forms like *Riccia* and allied genera where elaters are absent some of the cells of the sporogenous tissue degenerate, while the rest undergo meiotic divisions. Thus meiotic reductions in nature are intimately associated with the liberation of nucleic acids or their degradation products. Could it be that they play a significant role in bringing about meiotic reduction?

It may also be useful to recall a significant observation of Cooper (1952). He observed that in many plant species the tapetum of anther secreted specific substances and nutrients for the growth of sporogenous tissue. When these cells were at early leptotene stage some Feulgen +ve bodies migrated from the tapetal nuclei to the vicinity of PMC's. Then they moved into the cells containing the loosely paired chromosomes and got associated with them. Such chromatin bodies were also observed by a number of workers during microsporogenesis (Gregory 1905, West and Lachmere 1944, Sparrow and Hammond 1949). Though various functions have been ascribed to these, Darlington and LaCour (1946) were the first to suggest that these materials were being utilised by the nucleus at the onset of meiosis. Painter (1940) observed that the cytoplasm of both tapetal cells and sporogenous tissues in anthers of *Rhoeo discolor* were extremely rich in RNA and their diminution occurred during meiotic process. Montalenti *et al.* (1950) in course of study of spermatogenesis of the isopod *Asellus aquaticus* belonging to animal kingdom, observed that RNA was secreted from a layer of big secretory cells surrounding each testicular lobe or follicle. This RNA was presumably converted into DNA and utilised in meiosis. Furthermore, some cases of chromosome pairing have been found in animal tumour cells whose cytoplasm was heavily charged with RNA (Evans and Swezy 1929, Barigozzi and Cusmano 1946, 1947). All these observations may help in a better understanding of the induction of "meiotic reductions" in root-tip cells in response to nucleic acids or their components.

In view of the above and on the basis of our experiments with the physiological substances we are led to postulate the following hypothesis:
There seems to be some reserve pool in the root-tip cells of purines and pyrimidines or their precursors. When root-tips are treated with any of the purines, pyrimidines, or their derivatives in optimal doses there is a spurt in the synthesis of nucleic acids specific to the species and when the saturation point reaches in the homologues they repel bringing about "meiotic reductions". This is inherent in the nature of nucleic acids. The presence of spindle fibres under no circumstances is a compelling necessity, it only helps in the movement of the homologues in normal meiosis of sporocytes to the two poles for organisation of semi-resting nuclei before these undergo the second meiotic division to form tetrad nuclei. Meiosis is a two step process, repulsion of the homologues and their movement along the spindle fibres to the two poles.

This in essence is the Saturation—Repulsion Hypothesis underlying the basis of "Meiotic reductions".

Let it also be made clear that every species has a specific type of DNA. This has to be synthesised first from the ingredients of the precursors. The DNA (or RNA) as commercially available is not specific to the particular species under experimentation. It has to be degraded first (by enzymatic action) and then re-synthesised to conform to the specific type before it can bring about saturation followed by repulsion.

Finally a question arises as to why are not other stages of meiosis seen preceding the "meiotic reductions" in root-tip cells. Meiosis in sporocytes is governed by a series of closely integrated biochemical events of which the concentration of nucleic acid at a particular point of time is one such event which helps to bring about segregation of homologues. We do not know of the other biochemicals which may be responsible for the onset of earlier prophase stages like pachytene and diplotene etc. The whole process of meiosis is subtle being dependent not only on the variety of chemicals liberated through successive gene actions but also on their concentrations as well as interactions at certain points of time leading to different meiotic stages. However, in our experiments pairing of homologues or even the semblance of chiasma formation were sporadically seen which is not without significance.

The effect of purines and pyrimidines on mitotic index and "meiotic reductions" depends upon 3 variables, 1) concentration, 2) time of action and 3) recovery period. Lower concentration and higher treatment period is recompensed by higher concentration and lower treatment period in their effect and this is understandable. In such cases the recovery period is delayed from the usual 12 hrs to 24 hrs or even more. It is also to be realised that the decrease in mitotic index in greater recovery period is further influenced by the aberrant cells i.e. those in which structural chromosomal abnormalities have occurred because of which they become incapacitated for further divisions. The mitotic index thus decreases in greater recovery periods until it levels off in time.
Literature cited