


Selected Topics on Induced Chromosome Changes in Tissue-cultured Cells

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There is ample evidence that chromosomal changes can be induced in cultured cells. These changes can involve either an increase or decrease in chromosome number or a change in chromosome structure and can be brought about by both physical and chemical agents.

Increase in chromosome number

Cells that contain more than the normal number of chromosomes are classed as polyploids or aneuploids. Polyploids contain one or more additional chromosome complements, whereas aneuploids occur through either a gain or loss of one or more individual chromosomes. There are many reasons for inducing polyploidy in cultured cells. If one is interested in gene regulation and secondary metabolism, polyploid cells might offer several advantages. Polyploid cells are larger than their counterparts of lower ploidy and, in several instances, have been shown to produce more secondary metabolites. For example, in four induced tetraploid clones of Phlox drummondii Hook, the activity of alcohol dehydrogenase was about two times higher than that of the diploid (14). In two other tetraploid clones, however, the enzyme activity was slightly less. Polyploidy also can change end products of biochemical pathways. In Phlox drummondii, 15 induced tetraploid clones were analyzed for glycolflavones (15). In 14 clones, flavonoids that were not present in the diploid were found in the tetraploid. The opposite was true in eight clones; flavonoids present in the diploid were absent in the tetraploid. In addition, many changes in tissue-specific expression were found. In Britza media L., induced tetraploids produced only C-glycosyl-luteolin derivatives, whereas the parental diploids produced only C-glycosyl-apigenin derivatives (19). Hybrids between the two produced only luteolin derivatives. These limited examples demonstrate that induced polyploidy can disrupt and change regulatory mechanisms associated with gene expression.

The previous discussion on the effects of polyploidy center on autopolyploids, which contain one or more extra sets of homologous chromosomes. Another class of polyploids, allopolyploids, also can be quite useful. These contain one or more extra sets of homoeologous chromosomes from different parental genomes. Allopolyploid hybrids are quite important in plant improvement. Wide hybrids,
created either sexually or somatically, are frequently sterile at the diploid level but fertile at the tetraploid level (3).

There are several ways of increasing the chromosome number of cultured cells. In many instances, polyploid cells predominate in established cultures. In *Haplopappus gracilis* Nutt. callus cultures started from a diploid explant, 13% of the cells were triploid after only 4 months of culture (26). The auxin (2,4-dichlorophenoxy) acetic acid (2,4-D) induced a more rapid increase in chromosome number than did 1-naphthaleneacetic acid (NAA). There are several examples of 2,4-D being a potent agent for increasing chromosome number (29). In 1-year-old tobacco (*Nicotiana tabacum* L.) callus, 26% of the cells were tetraploid, 20% were octoploid, and 54% were aneuploid (17). After 6 years, all of the cells were aneuploid. Morphogenetic potential of the callus was inversely correlated with the degree of aneuploidy. Aneuploidy is a common occurrence in cultured cells. Undifferentiated cultured cells appear to tolerate aneuploidy more than differentiated cells (18).

There are several drugs that have been used to increase chromosome number. The most widely used chemical is colchicine. Colchicine inhibits formation of the spindle by blocking the assembly of microtubules (23). If cells are treated for more than one cell cycle, then the chromosomes will double without the cell dividing. Once the drug is removed, the new polyploid cells will start and complete cell division. Frequencies approaching 100% are common for colchicine-induced polyploidy of cultured cells.

### Decreases in chromosome number

There are several reasons why a decrease in the number of chromosomes would be useful. For example, the production of monosomic lines results in cells missing a single chromosome from the diploid complement. In effect, this loss allows for expression of recessive genes located on the monosomic chromosome. Monosomic lines are used for determining linkage groups and in the production of substitution lines, where one chromosome from a genome is substituted with a foreign chromosome.

Another example involves the production of haploids with only one chromosome complement, which can be used for mutagenesis and selection. Recessive mutations in a haploid cell are expressed immediately, whereas they are masked by the other normal allele in a diploid cell. Haploids also can be used for rapidly creating homozygous diploid lines, which can be used to produce hybrids.

There are several methods for reducing chromosome numbers. The most common involves anther or pollen culture (11). Cell lines can be created that lack a complete chromosome complement through the culture of meiotic cells. In many instances, however, it is not possible to obtain haploid cells or plants from anther or pollen cultures of a desired plant.

Chromosome reduction can also result from hybridization. Sexually produced hybrids between *Hordeum vulgare* L. and *H. bulbosum* Koch. routinely and randomly eliminate *H. bulbosum* chromosomes, leading ultimately to haploid *H. vulgare* (12). In somatic hybrid cell lines between *Arabidopsis thaliana* L. and *Brassica campestris* L., two forms were found (8). One form resulted from the spontaneous loss of all the *Arabidopsis* chromosomes. Even though whole intact *Arabidopsis* chromosomes were not present, several genes from this parent were expressed in the hybrid. In the case of cells of somatic hybrids between *Nicotiana glauca* Graham and *Glycine max* L., all but one or two of the *Nicotiana* chromosomes were lost (10).

There are several chemicals that can be used to decrease the number of chromosomes. One of the first drugs used was p-fluorophenylalanine. *Ribes* sp. cuttings that were treated with this drug produced roots in which about 25% of the cells has a reduced chromosome number (13). *Fragaria × ananassa* Duch. callus that was treated with this drug exhibited a wide range in cellular chromosome number (20). Forty-five percent of the plants regenerated from this callus had their chromosome number reduced by one-half.

Griseofulvin also can be used to reduce chromosome number. When applied to cultured alfalfa (*Medicago sativa* L.) cells (2n = 32), this drug caused both an increase and a decrease in chromosome number (24). About 30% of the cells had a reduced chromosomal complement. Cells were found that had a chromosome number well below 16. Griseofulvin also has been applied to cultured cells of a somatic hybrid (2n = 5x = 35) between *Petunia parodii* Juss. and *P. hybrida* Vilm (6). About 4% of the plants regenerated from the treated callus had a reduced chromosome number; they were all aneuploid with numbers between 23 and 34. Griseofulvin appears to act by producing abnormally shaped cells that have extensive cytoplasmic extrusions and irregular cell plates during mitoses (21). Griseofulvin also acts as a mitotic-arresting agent at metaphase (23). This combination results in the production of minicells containing varying numbers of chromosomes and a mother cell with a reduced chromosome complement. Almost all chromosome reducing agents also increase chromosome number. In the case of griseofulvin, chromosome numbers of an equal number of cells were increased as the diploid level (6).

Another technique for reducing chromosome number relies on centrifugation of metaphase cells on a gradient (4, 25). A suitable material is Percoll, which self-forms a continuous gradient upon high-speed centrifugation (1, 7). When metaphase cells are spun in Percoll, small microcells can be pinched off. These microcells in many instances carry with them one or two chromosomes. The parent cell then is reduced in chromosome number. The extent of chromosome reduction can be controlled by varying the speed or length of centrifugation of metaphase cells.

### Changes in chromosome structure

There are four major types of changes in chromosome structure. These are translocations, deletions, duplications, and inversions. Each one of these events can bring about changes in gene expression. By changing the total amount of repetitive DNA or heterochromatin or the relative position of an active gene near heterochromatin, one can change the gene’s activity. Position-effect variegation is a well-known example, where the transcription of a gene can be turned off when it is placed near heterochromatin (9).

Chromosome structural changes also can allow the incorporation of foreign genes into a host genome. A translocation or interchange between homoeologous chromosomes introduces new genes into a chromosome.

Cells that have been in culture for a long time can accumulate a wide range of chromosomal structure changes. About 50% of the plants regenerated from 12-month-old callus cultures of *Avena sativa* cv. Lodi were cytogenetically abnormal (16). About 10% of the regenerants showed one or more interchanges. About 20% of the regenerants showed one or more deletions or duplications. In diploid *Nicotiana tabacum* callus, a wide range in DNA content was detected (2). The lowest line had only 5 pg of DNA per cell, whereas the highest line had 40 pg. The diploid value is expected to be 7.8 pg. Some of the changes in DNA content could be accounted for by changes in chromosome numbers. However, many of the differences in DNA content were probably due to duplications and deletions. In a highly aneuploid cell line of tobacco, most of the chromosomes were of different lengths than that for a normal karyotype (27). Changes in length were the result of interchanges and translocations.

The growth regulator 2,4-D is known to induce somatic crossing-over (31). This increase in the frequency of somatic crossing over could account for many of the spontaneously occurring variations in chromosome structure seen in tissue cultured cells. There are many chemicals that can alter chromosome structure (30). DNA itself can even lead to chromosome changes. *Vicia faba* L. cells that were treated with mouse DNA exhibited translocations, deletions, duplications, and interchanges (28).

There are several drugs that can induce somatic crossing over between homologous, heterologous, and homoeologous chromosomes. Most drugs that inhibit DNA synthesis, such as hydroxyurea, fluorodeoxyuridine, and mitomycin, can induce somatic-crossing-over. Mitomycin C was very effective in inducing interchanges in *Vicia* root cells (22). Pairing of the somatic chromosomes was initiated in regions of heterochromatin.

Physical treatments that induce chromosome breaks can lead to structural chromosome changes. Ethidium bromide, maleic hydra-
zide, ultraviolet light, \( \gamma \)-irradiation, and x-ray are very efficient at inducing changes (30). A dose of 1600 rad of x-ray increased the frequency of somatic crossing more than 280-fold in *Glycine max* cells (5).

**Conclusion**

Tissue-cultured cells spontaneously accumulate many changes in both the number and structure of chromosomes. Such changes could be due to the culture environment or due to naturally occurring events. It appears that cultured cells are more tolerant of chromosomal aberrations than are differentiated cells of the whole plant. Besides the spontaneous changes, many drugs and physical agents can be used to induce specific chromosome changes.

**Literature Cited**


Summary and Future Direction: Chemical Regulation in Tissue Culture

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This series of papers has expanded upon Skoog’s milestone discovery regarding the roles of cytokinins and auxins in morphogenesis of in vitro systems. One of the effects of adenine-based cytokinins in culture systems is the formation of adventitious shoots or the expression of axillary shoots. This symposium reported that a cytokinin response also can be observed by using phenylurea-based cytokinins. Two phenylurea compounds, thidiazuron and 4PUCl, were illustrated as having strong cytokinin activity, equivalent to or surpassing the adenine-based compounds. One of the effects of these diphenylureas is the formation of autonomous callus. These observations were used to speculate on the mode of action of the phenylureas as being stimulation of endogenous cytokinin biosynthesis (or metabolism), resulting in altered cytokinin levels. Other studies indicate that the phenylureas and adenine cytokinins may have similar action sites, hence similar effects.

In addition to documenting the effects of cytokinin in culture, Skoog showed that low auxin levels enhance root formation and high levels promote callus formation. This symposium suggested that the naturally occurring indole auxin \( \text{IAA} \) can have several forms in culture systems. It can be left in its free state, which is thought to be its biologically active form, and allowing transport through tissues. Hydrolysis of the conjugates would free IAA, causing a biological response. The conjugation–hydrolysis balance may be a means to regulate auxin levels and responses of tissues.

Information reported in the preceding papers dealt briefly with the biochemistry of the auxin–cytokinin response. However, progress has not been rapid since the early work of Skoog and associates. Our information contributes to answering the puzzle of cytokinin–