PRODUCTION OF MITOTIC ABNORMALITIES BY RIBONUCLEASE*

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The normal course of mitosis in plant and animal cells can be disrupted by many kinds of physical and chemical agents. Nuclear transformations essential to the orderly progress of mitosis can be retarded, chromosome structure deformed, spindle mechanisms impaired, and cytokinetic processes inhibited. Such disturbances may be revealed, on cytological examination of the treated tissues, by a low frequency of dividing cells or a preponderance of specific stages of mitosis; by the presence of fragmented, adherent, or excessively contracted chromosomes; or by the occurrence of aneuploid and polyploid chromosome complexes, tripolar and multipolar spindles, binucleate and multinucleate cells.1

Production of these types of aberrations by a variety of agents suggests that the same cellular constituents are ultimately affected, despite possible differences in the nature of the reaction system initiated by the inciting stimulus. In an effort to identify the structural materials involved, we have determined the capacity of some crystalline enzymes to induce mitotic abnormalities in growing roots of onion and lily. Preliminary experiments revealed that ribonuclease (0.01–0.1 per cent aqueous solutions) induced aberrations of divers types with a high frequency but that equimolar concentrations of trypsin, chymotrypsin, and deoxyribonuclease had little effect in modifying chromosome form and distribution.2 Such differences in cellular response may be due, at least in part, to differences in ability of these four enzymes to penetrate the cell; ribonuclease has the lowest molecular weight (about 13,000, as compared with 20,000–60,000 for the others)3 and might for that reason be expected to enter the living cell more readily.

An extensive analysis of the action of ribonuclease on meristematic root-tip cells was accordingly undertaken and forms the basis of the present report. This analysis has led to the conclusion that the production of mitotic abnormalities may be initiated by enzymatic degradation of the ribonucleic acid (RNA) component of ribonucleoproteins.

Experimental Methods.—Growing roots of onion (Allium cepa L.) and lily (Lilium tigrinum Ker.) were treated by immersion in aerated solutions of ribonuclease or other reagents that served as controls, for periods ranging from 15 minutes to 48 hours, at room temperature (ca. 22° C.). Seven different samples of ribonuclease were tested,4 and all were effective in inducing mitotic abnormalities. Control solutions were selected to evaluate the action of the solvents and to determine whether the induced abnormalities could be attributed to properties of the ribonuclease solutions other than their enzymatic activity. Therefore, they included water, potassium phosphate buffer, ribonuclease that had been inactivated by boiling or exposure to ultraviolet radiation, a mixture of amino acids in the proportions characteristic of ribonuclease,5 histone, protamine, egg albumin, sucrose, and a ribonuclease digest of RNA. Cytological diagnosis of the action of the enzyme and
control solutions was made by examination of squashes of the treated roots stained in aceto-orcein and of sections stained in basic and acidic dyes (used independently and in combination) or by the Feulgen method.

Results.—Treatment of roots in a solution of ribonuclease produced gross changes affecting the pattern of growth and of microscopically detectable changes affecting the structure and distribution of the chromosomes.

Alteration of the normal pattern of growth, as evidenced by looping and coiling of the roots, apparently reflected some modification in the distribution of growth-promoting substances. Further indications of such modification were afforded by the appearance of polyploid chromosome complexes in the region of cell elongation of some onion roots during the first few hours of treatment. Since the higher degrees of ploidy observed (8- and 16-ploid) could not have arisen within this short time interval from diploid cells through repeated doublings in successive mitoses, it is assumed that the action of the solution of ribonuclease stimulated division of cells that had attained a polyploid state in the course of differentiation.

The effect of solutions of ribonuclease on cells of the meristematic region was revealed by the appearance of all the types of abnormalities noted in the introductory paragraph of this report. The nature and frequency of the induced alterations and the ability of the cells to resume mitotic activity were found to be related to the pH of the solution, the concentration of the enzyme, and the duration of the treatment. Details of the experiments on which these generalizations are based will be published elsewhere; a summary of the results is presented here.

The effect of pH was tested in the range between 5.6 and 7.9, using a 0.8 mg/ml solution of ribonuclease for 2–9 hours. At the extremes of this pH range aberrations were produced, but the solutions readily killed the cells. At pH's between 6.0 and 7.0, solutions of ribonuclease proved less toxic, although capable of inducing mitotic abnormalities. On the basis of the information gained in this survey, concentration and "recovery" studies were made at pH 6.0; ribonuclease dissolved in tap water gave essentially this pH, and any necessary adjustment was made by adding a trace of 0.001 N sodium hydroxide.

At pH 6.0, concentrations of ribonuclease of 0.001 mg/ml of solvent (8 × 10⁻⁸ M) had no apparent effect on mitotic processes even when treatment was continued over long periods of time. A tenfold increase in concentration did not inhibit mitosis but caused enlargement of the nucleoli and excessive contraction of the chromosomes after 24–48 hours' treatment. Abundant abnormalities were produced by 0.1 mg/ml solutions and by those of higher concentration.

Treatment for 2–4 hours with a 0.1 mg/ml solution caused excessive contraction and "stickiness" of metaphase and anaphase chromosomes. The latter effect was revealed by adhesion of the distal ends of sister-chromatids, which formed bridges between the separating anaphase and telophase groups of chromosomes. After 4–8 hours of treatment, cells were observed in which strongly contracted chromosomes with paired chromatids were scattered at random (the c-type mitosis characteristic of colchicine treatment) or aggregated into two or more groups whose members showed various degrees of stickiness. Prolonged treatment led to inhibition of mitosis and eventually to pycnotic degeneration of the cells.

The course of production of these abnormalities, which proceeds from the periphery across the root to the central axis, parallels the course of movement of the
enzyme, as revealed by a decrease in stainability of the cells with the basic dye pyronin and an increase in stainability with the acidic dye fast green. Thus a 0.1 mg/ml solution modified the colorability of cytosome and nucleus of cells in the outer layers of the root meristem within a few hours and eliminated pyronin stainability of all meristematic cells within 24–48 hours. The marked modifications of stainability effected by long periods of treatment reflect degradational changes of such magnitude that recovery of the cells is seldom possible; but, if the roots are transferred to water after a short exposure to the action of the enzyme, they may continue to grow, as indicated by the presence of dividing cells with various types of abnormalities, including polyploid chromosome complexes, chromatid bridges, multipolar spindles, and multinucleate cells. Chromosome fragments were rarely observed, and this finding suggests that solutions of ribonuclease do not effect direct breakage of chromosomes in cells of the root-tip meristem.

Solutions containing between 0.2 and 1.0 mg. of ribonuclease per milliliter of solvent also produced the types of aberrations noted above, but the time interval between their appearance and the termination of vital activities of the cell was shortened as the concentration of the enzyme was increased. It was thus possible to determine the immediate as well as the delayed responses of the cells at different stages of mitosis by comparing the effects of solutions of ribonuclease at different concentrations.

Among the controls, solutions of histone, protamine, and inactivated ribonuclease induced some types of aberrations, but with much lower frequencies than did equimolar solutions of ribonuclease. A few aberrations were also induced by ultraviolet-irradiated water. Other controls, including water, and solutions of egg albumin, sucrose, the mixture of amino acids in the proportions characteristic of ribonuclease, and the ribonuclease digest of RNA induced very few abnormalities in any of the treated roots.

Discussion.—The reaction pathways involved in the production of mitotic abnormalities by ribonuclease have not been determined. Ribonuclease might conceivably act as a basic protein to disrupt structural complexes or to interfere with synthetic processes.7 The efficacy of histone, protamine, and even inactivated ribonuclease in producing some types of aberrations indicates that this possibility cannot be eliminated. On the other hand, evidence that ribonuclease may act as an enzyme within the living cell to degrade structural complexes containing RNA is afforded by the correlation between the appearance of aberrations and the modifications in stainability of the cells as the enzyme moves across the root. Stainability of RNA is reduced, but that of deoxyribonucleic acid (DNA) is not altered.

The degradation of structural nucleoproteins initiated by the action of ribonuclease represents one possible mechanism for the production of mitotic abnormalities. The need for intact nucleoproteins in maintenance of the structural framework of the cell was demonstrated in studies of the action of trypsin on fixed and unfixed salivary-gland cells of Drosophila.8 Degradation leading to structural deformation might conceivably be initiated in the nucleic acid or protein moiety of either the ribonucleaseprotein or the deoxyribonucleoprotein, but the results obtained in the experiments using ribonuclease focus attention on the RNA component.

Ribonucleoproteins are essential structural constituents of both cytosome and nucleus, and the ribonuclease-induced alterations affect chromosome distribution as
well as form. Many cytoplasmic particles are rich in ribonucleoprotein, and the spindles of onion root-tip cells contain both RNA and protein. Appreciable amounts of RNA are present in the condensed chromosomes and in the nucleolus. Chemical analysis of wheat germ indicates that the concentration of nuclear RNA may be as high as that of DNA in actively dividing tissues. It is thus apparent that on a purely quantitative basis ribonucleoproteins cannot be ignored in assessing the factors involved in the production of chromosomal aberrations and mitotic abnormalities. If, in addition, the native ribonucleoproteins are more labile than the deoxyribonucleoproteins, as seems probable from extraction and turnover studies of RNA and DNA, they would provide a substrate responsive to the action of any agent capable of interfering with mitotic processes. Whether the degradation induced thereby would involve a direct action or an indirect pathway would appear to depend on the properties of the inciting agent. In this connection, the following alternatives must be considered: that modifications in chromosome form and distribution may be initiated by degradation of ribonucleoproteins of cytoplasmic particles (for example, by ribonuclease or by acriflavine) and the consequent functional impairment of their constituent enzymes, or, conversely, that structural modification may be initiated by impairment of enzymatic activity (for example, by enzyme poisons) and the disturbance of processes essential to the metabolism of RNA.

These considerations suggest that the production of mitotic abnormalities by agents other than ribonuclease may be attributable in part to degradation of ribonucleoproteins. A vast number of agents can inhibit spindle mechanisms and cause stickiness or clumping of chromosomes, whereas relatively few can induce chromosome breakage or promote structural rearrangement. The first group of responses characterizes the "primary" or "physiological" effects of ionizing radiations, whereas the second group typifies the "secondary" or "structural" effects. Ribonuclease readily produces responses of the first type, rarely those of the second. It has been suggested, accordingly, that the primary effects can be initiated by changes in RNA, whereas the secondary effects involve more profound alterations in nuclear materials, perhaps directly affecting deoxyribonucleoproteins. In this connection, it has been reported recently that a given dose of X-rays is more effective in inducing changes that result in stickiness and clumping if given at late prophase than if given at metaphase or anaphase. Maximum sensitivity thus seems to occur during the stages when RNA is accumulating in the chromosomes.

Although the ribonucleoproteins appear to afford particularly vulnerable sites for the initiation of structural changes, the results obtained in treatment of roots with control solutions suggest that degradation of any component of the nucleoprotein complex may lead to production of aberrations. The changes effected by such degradation in the colloidal properties of cellular materials could conceivably underlie the concomitant modifications in chromosome form and distribution.

Summary.—Treatment of growing roots of onion and lily with solutions of crystalline ribonuclease stimulated mitotic activity in polyploid cells of the region of cell elongation and modified the form and distribution of chromosomes in the meristematic cells. The production of aberrations by ribonuclease is associated with the degradation of ribonucleoproteins, as is shown by the close correlation between the appearance of abnormalities and the modifications in stainability of the cells with
pyronin and fast green as the enzyme moves across the root. This correlation emphasizes the importance of ribonucleoproteins in determining normal chromosome form and distribution during the course of division of meristematic cells. The findings have been evaluated with relation to the action of other chemical and physical agents in producing similar types of mitotic abnormalities.

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11 W. S. Vincent, these PROCEEDINGS, 38, 139–145, 1952.


