The Accumulation of Soluble Deoxyribosidic Compounds in Relation to Nuclear Division in Anthers of *Lilium longiflorum**

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ABSTRACT

Intervals of sporogenous DNA synthesis in lily anthers are associated with marked accumulations of methanol- and acid-soluble deoxyribosidic compounds. There are differences in solubility between compounds accumulating at meiosis and those accumulating at mitosis. The formation of these compounds appears to be related to a breakdown of somatic DNA.

INTRODUCTION

The purpose of this communication is to enlarge upon an earlier report (1) regarding the accumulation of soluble deoxyribosidic compounds in anthers of *Lilium longiflorum* (var. Croft). In that report, a striking correlation was shown to exist between their concentration within the anther and the initiation of DNA (deoxyribonucleic acid) synthesis in the immature pollen. It will now be demonstrated that the compounds in question fall broadly into two chemical classes, each of which is associated with one or both of the two cycles of nuclear division. Furthermore, a probable relationship will be shown between the origin of these compounds and the degradation of somatic DNA in the course of anther development.

The principal events in anther development leading to the production of pollen may be simply described. When the flower buds are about 10 mm. in length, the pollen mother cells or "microsporocytes," which are contained in the central cavity of each anther, are undergoing DNA synthesis. Following this each microsporocyte divides meiotically to yield 4 microspores. When the buds are 58 to 60 mm. in length the microspores double their DNA content and undergo mitosis to become binucleate. Following mitosis, each of the nuclei doubles its DNA content (see Fig. 1). Concomitant with the events just described, a series of changes occurs in the layer of cells surrounding the central cavity. This layer, called the "tapetum," proliferates rapidly after completion of microsporocyte DNA synthesis but disintegrates close to the interval of microspore mitosis. The disintegration is well under way at the time the microspores are increasing their DNA content.

Methods

Anthers of *Lilium longiflorum* were collected and frozen as described in an earlier publication (2). For each set of measurements, 1 to 3 gm. of the frozen material were extracted in an acetone–dry ice bath with a 0.05 M methanolic solution of formic acid. About 3 to 5 ml. of extracting fluid was used per gm. of anther. The extract was evaporated to dryness under reduced pressure; the resultant residue was resuspended in a small volume of water with the aid of a drop of tween 80 and stored at −30°C. until assayed. The tissue was then further extracted with cold 5 per cent TCA (trichloroacetic acid), and after removal of the TCA by means of ethyl ether the extracts were stored along with the methanolic ones.

The acid-insoluble residue was washed with ethanol and ether. The dry powder thus obtained was analyzed for nucleic acid content by the Ogur-Rosen method (3) or by extraction with 2 M NaCl at 100°C. The latter procedure has been used in this laboratory for a variety of plant tissues. In a number of tissues the two methods have been found to yield different values, salt apparently being a more effective extractant than perchloric acid. Fortunately, no such difference was found in the case of lily anthers. The deoxyribose content of nucleic acid extracts was determined by Burton's modified

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diphenylamine method (5). Phosphorus was measured by the method of Allen (6).

Methanol and TCA extracts were assayed microbiologically for deoxyribose content (7). Hycase (Sheffield Chemical Company, Norwich, New York) was used in the test medium instead of the prescribed acid-hydrolyzed casein. (We are indebted to Dr. J. Schultz of the Lankenau Research Institute of Philadelphia for recommending the substitution.) Bacterial growth was approximately linear in the presence of 0.05 to 0.2 micrograms of thymidine; generally, samples assayed were within that range of concentration. For each developmental stage of the anther, bacterial growth was measured in the presence of extracts added as such to the test medium, and also in the presence of extracts previously treated for 17 hours at pH 9 with a commercial preparation of alkaline phosphatase (Worthington Biochemicals, Freehold, New Jersey). The phosphatase mixture consisted of one volume of a 0.04 per cent solution of phosphatase, one volume of 0.6 M ammonium acetate buffer, and two volumes of anther extract. Assays were also run on extracts in the presence of added thymidine; no synergistic effects were observed.

RESULTS

Methanolic and acidic extracts of anthers contain deoxyribosidic compounds which are highest in concentration during intervals of DNA synthesis in the immature pollen (Figs. 1 and 2).

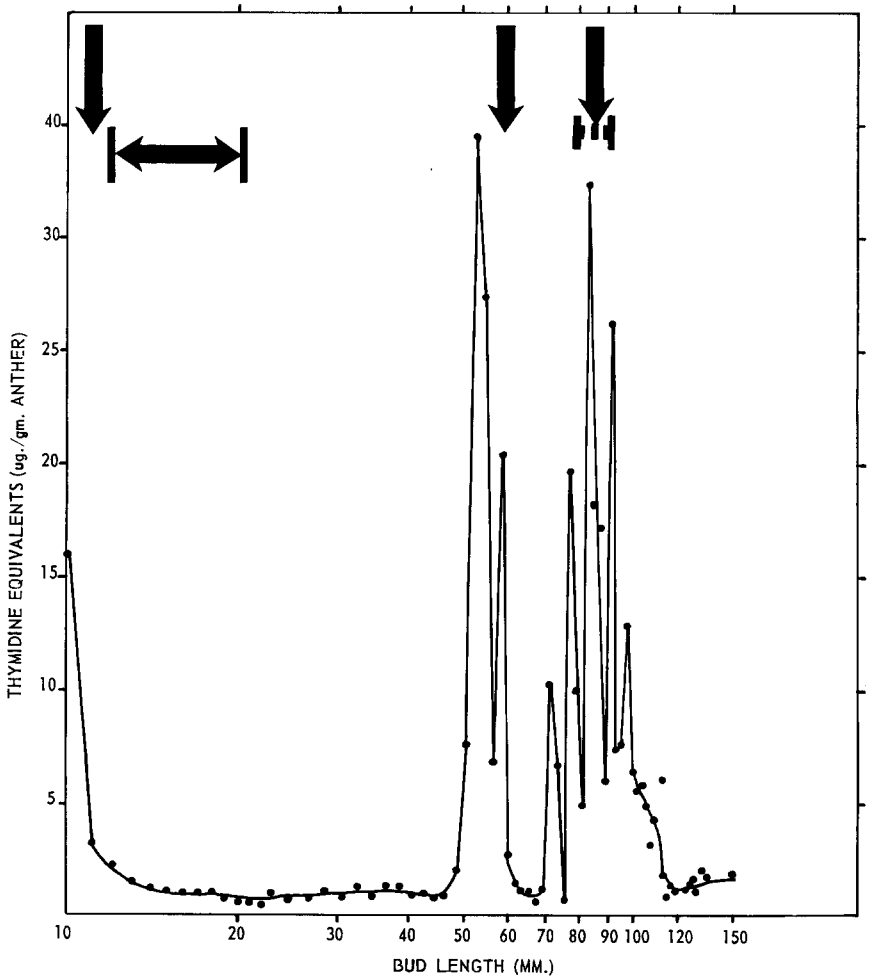

FIG. 1. Microbiologically active deoxyribosides in developing anthers of *Lilium longiflorum*. The values plotted represent the combined concentrations of untreated methanolic and TCA extracts. Vertical arrows indicate intervals of DNA synthesis in microsporocytes and microspores; the horizontal arrow indicates the interval of tapetal DNA synthesis. The broad marker under the third vertical arrow is inserted because of the uncertain timing of DNA synthesis. Location of the arrows is based on the data of Ogur *et al.* (4) and of Taylor and McMaster (9). Our own studies of the DNA content of isolated microspores (11) confirm the conclusions of these workers.

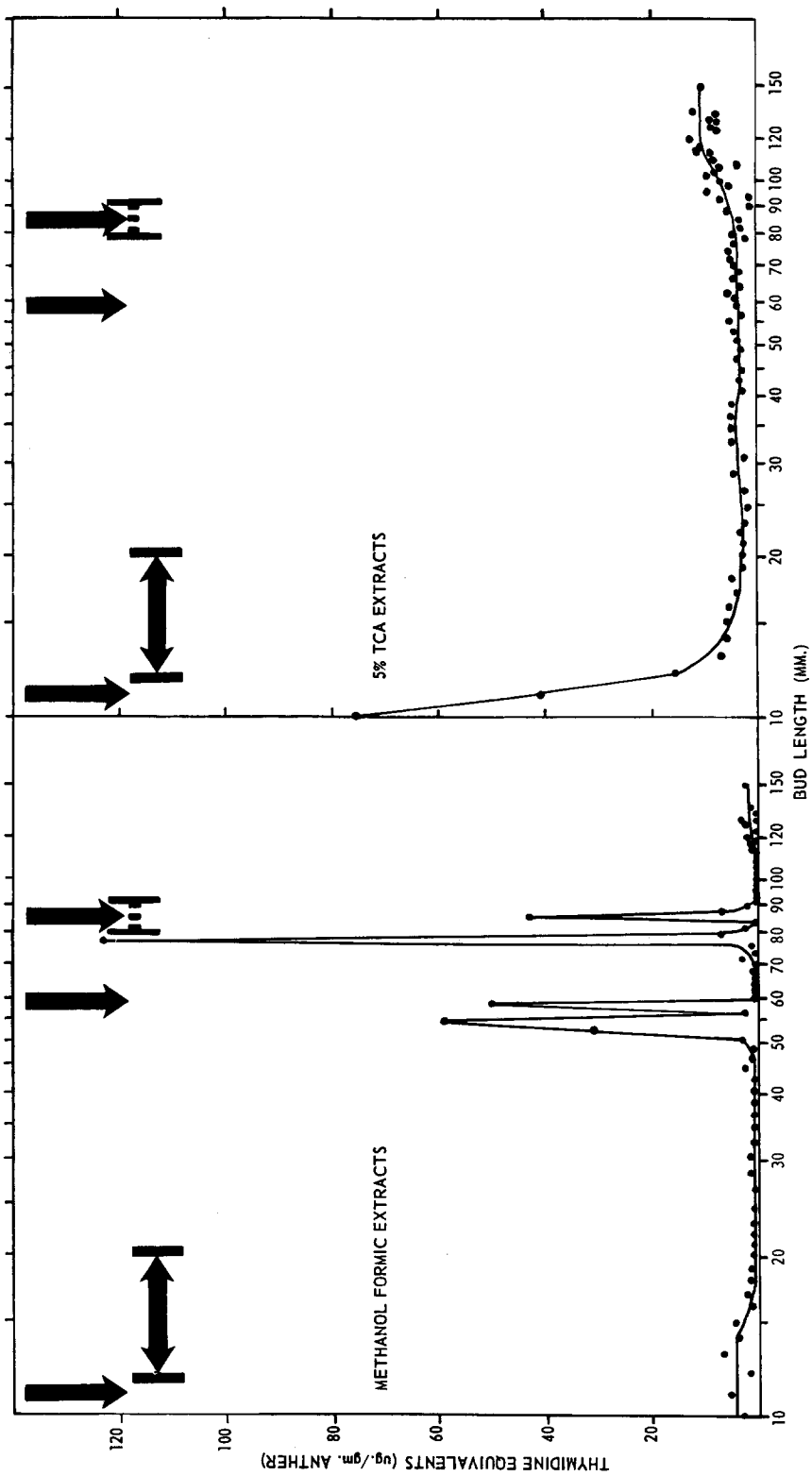

FIG. 2. Phosphatase-activated deoxyribosides in developing anthers. The values represent deoxyribosides released by treating extracts with alkaline phosphatase. In each section the first vertical arrow marks premeiotic DNA synthesis; the second and third vertical arrows enclose microspore mitosis.

There is no evidence respecting the form of deoxyribosides present, other than that afforded by the solvents used and by the effects of treating the extracts with alkaline phosphatase. The solubility of the compounds in methanol or TCA indicates a molecular size smaller than that of DNA. Moreover, some 20 to 50 per cent of the compounds must be comparatively simple deoxyribosidic units since without prior enzymatic degradation they serve as growth factors for the *Lactobacillus* test organism. (Compare Figs. 1 and 2.) The compounds activated by treatment with phosphatase are not mononucleotides. This may be inferred from Schneider and Potter's demonstration that mononucleotides are as effective as nucleosides in growth-promoting activity (8), a result we have confirmed. However, nucleotide pyrophosphates, polynucleotides, or complexes of nucleosides with other cell constituents could all yield microbiologically active products when acted on by the relatively crude phosphatase preparation used.

There is a qualitative difference between the meiotic and mitotic pools of deoxyribosidic material. At meiosis, phosphatase-activated compounds are present only in TCA extracts; at mitosis, such compounds are extracted by acidified methanol (Fig. 2). In the case of untreated extracts the difference is much less marked. During the interval associated with meiosis the deoxyriboside concentrations in the two untreated extracts are approximately equal; beyond a bud length of 50 mm. about 90 per cent of the deoxyriboside material is recovered in the methanolic extracts. It is possible, moreover, that differences other than those of solubility mark the respective deoxyribosidic pools of meiosis and mitosis.

One feature common to all the curves shown is the absence of any rise in deoxyriboside concentration during the interval of tapetal cell mitosis (Figs. 1 and 2). This behaviour cannot be related to low requirements of tapetal cells for DNA precursors since the amount of DNA synthesized in these cells greatly exceeds that synthesized in the microsporocytes and microspores (9). The production of high concentrations of soluble deoxyribosidic compounds would thus appear to be specifically related to chromosome reproduction in the germ cells.

The cause of the abrupt rise in deoxyriboside concentration is undetermined, but some evidence points to DNA catabolism as a source of these compounds. If the DNA content of the anther is plotted as a function of development, it is clear that there is little net change in total DNA between the time preceding microsporocyte meiosis and maturation (Fig. 3). The sharp rise in DNA content during early development coincides with the interval of mitosis in the tapetal cells. Following this interval, there is a progressive decline in DNA content. This decline, which is associated with the breakdown of the tapetum, could provide a rich source of deoxyribosidic material for DNA synthesis in the microspores. If it does, then there must be a periodic channelling of the breakdown process, rather than a gradual accumulation of breakdown products. This has been inferred from the fact that disappearance of anther DNA is a comparatively steady process whereas the appearance of soluble deoxyribosides is a saltatory one.

It is improbable that the pattern of DNA formation and disappearance illustrated in Fig. 3 is entirely due to the tapetal cells. In early development there is a quadrupling of DNA content per anther. Tapetal DNA would have to constitute at least 75 per cent of the total to account for the rise. This is not the case (9). Thus, either the subsequent loss of DNA represents a breakdown of other cells besides the tapetal ones, or the DNA measured is not the chromosomal DNA commonly visualized in autoradiographic studies. Our analyses provide one pointer on this question. In precipitating the salt-extracted nucleic acids with ethanol, it was noted that at developmental stages preceding microspore mitosis, about 12 per cent of the DNA in the anther could not be so precipitated, whereas at later stages (bud lengths greater than 60 mm.) all of the DNA could be. This difference suggests the existence of two types of DNA in early development, one type being metabolically labile and susceptible to degradation without a concomitant breakdown of its host cell. Pelc (10) has already inferred the existence of a labile DNA from autoradiographic analyses of mouse seminal vesicles, and a similar situation may prevail in the anthers. The fact that anther RNA (ribonucleic acid) does not follow a course parallel to the DNA (Fig. 3) would also favor the idea of DNA rather than cellular breakdown. Except for an unexplained dip in concentration surrounding the interval of microspore mitosis, there is a progressive accumulation of RNA in the anther.

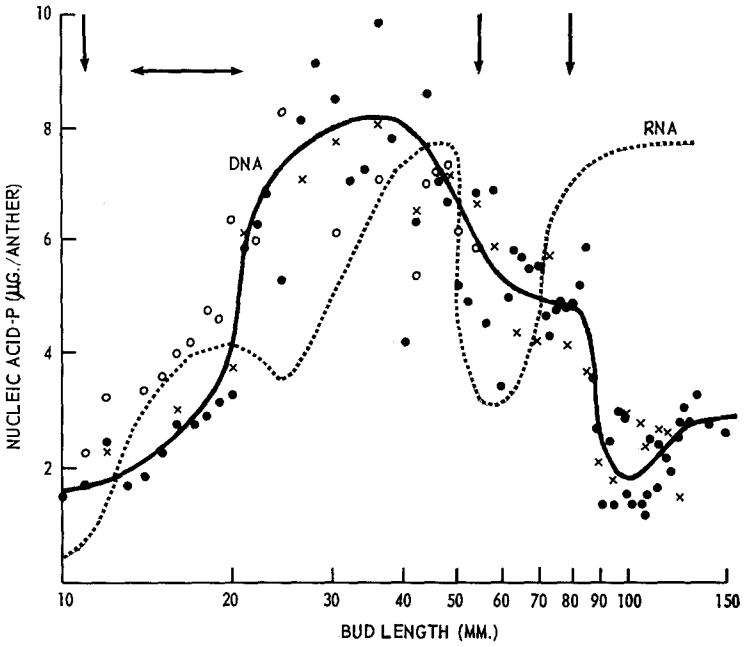

FIG. 3. DNA and RNA contents of developing anthers in *Lilium longiflorum*. Solid dots represent DNA values as determined by the diphenylamine reaction in salt extracts of stored anthers; crosses represent parallel assays on hot 0.5 N perchloric acid extracts. Values marked by open circles have been obtained from perchloric acid extracts of fresh anthers. The DNA curve is drawn through the arithmetic averages of the various developmental stages. The RNA curve is similarly obtained, but the individual determinations are not shown.

CONCLUSIONS

The purpose of these studies has been to throw some light on the immediate cause of chromosome duplication. To the extent that conditions leading to DNA synthesis represent those underlying the reproduction of the chromosome as a whole, the present study has provided two points of information. First, it has indicated that a build-up of low molecular weight precursors precedes duplication; and that, like the process of duplication itself, the build-up occurs over a relatively brief interval of time. The incidence of the peaks of deoxyriboside concentration appears to vary no more from plant to plant than does the incidence of the mitotic process. Anthers of the same bud generally have the same deoxyriboside concentration. When anthers of various bud lengths are compared, the differences in deoxyriboside concentration are all or none. High and low values are common; intermediate ones are rare. It is difficult to avoid the conclusion that these marked accumulations are directly related to chromosomal DNA synthesis. Second, the study has shown that within a reproductive organ such as the anther, synthesis of microspore DNA may occur in an environment rich with products of DNA catabolism. This latter relationship between DNA loss in the whole anther and DNA increase in the microspores has also been found in lily anthers cultured *in vitro* over an interval covering the period of microspore DNA synthesis (11).

The presence of deoxyribosides in non-proliferating tissues and their increase in proliferating ones was first demonstrated by Schneider (12, 13) who obtained his data from a variety of mammalian organs. Our results endorse the generality of conclusions and also indicate that with respect to the life cycle of the cells concerned, the interval of increase is brief, occurring immediately before DNA formation.

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