Factors Affecting the Onset of Deoxyribonucleic Acid Synthesis During Wheat Embryo Germination

STUDY OF THE CHANGES IN DNA POLYMERASES A, B AND C AND THE POOL OF DNA PRECURSORS

By Michel CASTROVIEJO, Danièle THARAUD, Bernard MOCQUOT* AND Simon LITVAK†

*Department de Biochimie, UER-BBC, Université de Bordeaux II, 351 Cours de la Libération, 33405 Talence, France, and *Laboratoire de Physiologie Végétale, I.N.R.A., 33 Pont de la Maye, France*

(Received 24 November 1978)

DNA synthesis starts about 12h after water imbibition in wheat embryos. We have determined that noticeable amounts of labelled thymidine are found inside the embryo only after 6h of germination. DNA polymerase C from ungerminated wheat embryos decreased markedly in activity during the first hours of germination, whereas the activities of DNA polymerases A and B increased, having a maximum at about 15h of germination. Serological evidence has suggested a clear antigenic relationship between DNA polymerases A and C. Although the pool of ATP increases rapidly after water imbibition, the increase in the pool of dNTP species was much slower.

The wheat embryo is a graphic example of a quiescent organism that under certain conditions (water imbibition) triggers an array of molecular events leading to the biosynthesis of macromolecules and, thus, to an active growing stage (Mayer & Shain, 1974; Taylorson & Hendricks, 1977; Bray, 1976). An active protein-synthesizing system is present 30min after the start of wetting in wheat embryos (Marcus *et al.*, 1966). Translation of early proteins in the germinating embryo is considered to be programmed by mRNA already present in the dry seed (Brooker *et al.*, 1977). RNA synthesis also starts very soon after water imbibition (Sen *et al.*, 1975; Spiegel *et al.*, 1975; Rejman & Buchowicz, 1973). However, DNA synthesis cannot be observed in wheat seeds before 12–15h of germination (Chen & Osborne, 1970). DNA synthesis seems to be dependent on protein synthesis during the first hours of germination. On the basis of studies with blastidine S, an inhibitor of eukaryotic protein synthesis, it has been proposed that the limiting factor in the start of DNA synthesis is the biosynthesis of DNA polymerase (Mory *et al.*, 1972, 1975).

Concerning the variation of ribonucleoside triphosphates, an increase in ATP was found 15min after water was placed on non-dormant lettuce seeds (Pradet & Prat, 1976). Similar results have been found with wheat embryos (Cheung & Marcus, 1975). Very little information is available with respect to deoxynucleoside triphosphates in higher

plants. By using plant tissue cultures it has been shown that the deoxyribonucleoside triphosphate pool is much lower than the ribose analogue (Nygaard, 1972).

We have chosen the wheat embryo as a differentiating system for the study of the factors controlling the onset of DNA synthesis. As a first step we have purified and characterized three DNA polymerases from the soluble cytoplasmic fraction, as well as a mitochondrial DNA polymerase (Castroviejo *et al.*, 1975; Tarrago-Litvak *et al.*, 1975; the preceding paper, Castroviejo *et al.*, 1979). In the present paper we describe the changes in DNA polymerases A, B and C during wheat embryo germination, the concentrations of ATP and dNTP species, as well as the thymidine-phosphorylating activity (ATP- and AMP-dependent). A preliminary account of this work has already been published (Litvak *et al.*, 1978).

Materials and Methods

Materials

Wheat seeds and materials used throughout the present work are described in the preceding paper (Castroviejo *et al.*, 1979).

Methods

Germination of wheat embryos. Embryos isolated as described in the preceding paper (Castroviejo

† To whom reprint requests should be addressed.

et al., 1979) were grown on Petri dishes with 1% (w/v) gelose, 1% (w/v) glucose plus 0.2% streptomycin. Seeding was done under a sterile hood; 4g of wheat embryo per Petri dish (160mm diameter) was used. Germination took place at room temperature. After different times, embryos were harvested and processed immediately. No bacterial or mould contamination was observed before 72h of germination.

DNA synthesis during germination. The 'radio-active' incubation mixture contained in a total volume of 1ml: 100–150 isolated embryos, germinated as described above for different lengths of time, 50mM-Tris/HCl, pH7.5, 10 μ g of chloramphenicol and 1 μ Ci of [methyl-³H]thymidine (10Ci/mmol). Incubation was carried out for 1h at 25°C. It was stopped by filtration of embryos through Whatman GF/C glass-fibre filters, which were then washed with 50mM-thymidine. Nucleic acids were recovered and counted for radioactivity as described by Yadav (1976) by using HClO₄. DNA was determined by the diphenylamine method (Burton, 1967).

Thymidine transfer into the embryos. The method is essentially as described above for DNA synthesis. After the incubation had been stopped, and the embryos washed extensively with unlabelled thymidine, they were resuspended in water, homogenized in a mortar and a portion counted for radioactivity in a dioxan/PPO (2,5-diphenyloxazole)/POPOP [1,4-bis-(5-phenyloxazol-2-yl)benzene] scintillation mixture (Bray, 1960).

DNA polymerase purification. The method is fully described in the preceding paper (Castroviejo *et al.*, 1979). The three DNA polymerases are purified from the soluble cytoplasmic fraction. Similar results were obtained with commercial wheat-germ or ungerminated isolated wheat embryos. By phosphocellulose chromatography all DNA polymerase activity is separated from the bulk of the protein. On DEAE-cellulose, enzyme C is not retained and can be separated from DNA polymerases A and B. The latter two enzymes are separated by phosphocellulose chromatography.

DNA polymerase assays. Determination *in vitro* of DNA polymerase activity is described in detail in the preceding paper (Castroviejo *et al.*, 1979).

Serological reactions. (a) Rat immunization. Rats were immunized with purified DNA polymerase C (before immunization, about 1ml of serum was removed from each rat to be used as control). The rats were inoculated with 100 μ g of purified enzyme, emulsified in an equal volume of complete Freund's adjuvant and inoculated subcutaneously. Three additional 100 μ g boosters of DNA polymerase C were inoculated at 3-week intervals. Test bleeds were taken 1 week after each boost. Rats were completely bled 1 week after the last boost of antigen.

(b) Purification of immunoglobulin G. Immuno-

globulin G fractions were prepared essentially by the method reported by Robert-Guroff & Gallo (1977). The final immunoglobulin G fractions were divided into small portions and stored at -20°C.

(c) Immunological assays. The preincubation mixture contained DNA polymerase (A, B or C) plus antibody and it was incubated for 16h at 4°C. After the preincubation, the amount of active DNA polymerase remaining was determined.

Nucleoside triphosphate measurement. Deoxy-nucleoside triphosphates were measured enzymically with *E. coli* DNA polymerase I by the method of Solter & Handschumacher (1969), as modified by de Petrocellis & Rossi (1976). An internal standard of 50pmol of dNTP is introduced in the samples to determine the extent of inhibition of polymerization due to the components of the plant extract. By this method we are able to detect down to 2–5pmol of dNTP species.

For determination of dNTP 100 embryos germinated for different lengths of time were homogenized in a Polytron homogenizer in a solution of trichloroacetic acid in ether (98g/l). Nucleotides are extracted with an aqueous solution of 100mM-trichloroacetic acid. The final volume at neutral pH and after low-speed centrifugation was 4ml.

ATP was measured by using the luciferin-luciferase method (Pradet & Prat, 1976) in a portion of the whole extract.

Thymidine kinase and nucleoside phosphotransferase assays. Embryos (75 in number) were harvested after germinating for different lengths of time and homogenized in a Potter homogenizer with 1.5ml of 100mM-Tris/HCl, pH8.0, plus 5mM-DTT. The homogenate was centrifuged for 15min at 10000g and the resultant supernatant was used as the source of enzyme. All preparative steps were carried out at 4°C. Thymidine kinase was assayed as described by Weinstock & Dju (1971). In a final volume of 0.25ml the assay mixture contained 5mM-ATP, 5mM-MgCl₂, 15mM-NaF, 2 μ Ci/ml of [methyl-³H]-thymidine (18Ci/mmol) and 0.2ml of wheat extract. To assay nucleoside phosphotransferase, AMP replaced ATP and NaF was omitted. The reaction was carried out at 37°C and stopped by heating the samples at boiling temperature for 5min. After 15min in a cold-water bath the mixtures were centrifuged for 10min at 3000g. A 0.1ml portion of the supernatant was spotted on a disc (24mm diameter) of DEAE-cellulose paper (Whatman DE-81). After the filters were dry, they were washed in 1 litre of 1mM-ammonium formate for 10min, then twice in water and finally in 300ml of ethanol. Phosphorylated thymidine retained in the paper discs was eluted by shaking the filters with 1ml of 0.1M-HCl containing 200mM-KCl in counting vials and counted for radioactivity with 10ml of Bray's (1960) scintillation mixture.

Results

DNA synthesis and thymidine entry into wheat embryos

After only 12h of germination, a noticeable incorporation of thymidine into DNA was detected. DNA content measured by the diphenylamine method was doubled after 16–20h of germination, as Fig. 1 shows. Not shown in the present paper are results indicating that all the trichloroacetic acid-insoluble radioactivity was sensitive to pancreatic deoxyribonuclease I treatment. The total labelled thymidine entry in embryos germinated for different times was determined, as shown in Fig. 1.

It can be seen that only after 6h of germination, radioactive thymidine was detected inside the embryo, a plateau being attained after 20h of germination.

Changes in DNA polymerases A, B and C during germination

Wheat embryos (10g) were germinated for 0, 10, 20 and 40h as described under 'Methods'. The embryos were harvested and total DNA polymerase activity was purified by homogenization, high-speed centrifugation, salt fractionation, phosphocellulose chromatography and dialysis as described in the preceding paper (Castroviejo *et al.*, 1979). As mentioned under 'Methods', enzyme C can be distinguished from A and B, since the former is not retained in a DEAE-cellulose column. Total DNA polymerase activity from embryos germinated for

different times was chromatographed in small DEAE-cellulose columns (1.5cm × 4.5cm). As Fig. 2 shows the activity in the wash-out of the DEAE-cellulose column decreased markedly during germination, being almost absent after 40h of germination. DNA polymerases A and B can be separated in a phosphocellulose column (1cm × 4cm). To avoid the partial overlapping of both enzymes when using a continuous gradient, we have used an elution procedure described in the preceding paper (Castroviejo *et al.*, 1979): the first peak from such a column corresponds to DNA polymerase A and the second to enzyme B. The fractions having DNA polymerase activity from each peak were pooled, and precipitated with $(\text{NH}_4)_2\text{SO}_4$. The pellet was redissolved in 50mM-Tris/HCl (pH 7.9)/1mM-EDTA/20% (v/v) glycerol and dialysed against the same buffer in 50% glycerol. The procedure was repeated for each germination time. The same concentration procedure was used with DNA polymerase C after DEAE-cellulose. Table 1 shows that although enzyme C activity decreases, enzyme A and B activities increase markedly during the first hours of germination.

Antigenic relationship between wheat DNA polymerases

The antibody against DNA polymerase C was prepared as described under 'Methods', and its inhibitory power was tested *in vitro* against DNA polymerases A, B and C. As Fig. 3 shows, the activity of enzyme C is strongly inhibited, as well as

Fig. 1. DNA synthesis and thymidine entry into wheat embryos

The conditions for the thymidine incorporation into DNA during wheat germination and the thymidine transfer into the embryos are described under 'Methods'. ▲, Thymidine entry (1h uptake); ●, thymidine incorporation into DNA (1h uptake); ■ DNA determined. Values are means of three determinations. The s.d. in all cases was less than 10% of the mean.

Fig. 2. Changes of wheat DNA polymerase C during embryo germination

Wheat embryos (10g) were germinated for (a) 0h, (b) 10h, (c) 20h and (d) 40h; DNA polymerase C was extracted and purified as described in the Results section. Chromatography on DEAE-cellulose was performed under the same conditions for the different samples; DNA polymerase activity was determined in the presence of activated DNA, and the incubation time was 30 min.

Table 1. Changes of wheat DNA polymerases A, B and C during germination

Wheat embryos were germinated for the indicated times, and DNA polymerases extracted and purified as indicated in the Results section. DNA polymerase C was purified up to the DEAE-cellulose step. Fractions shown in Fig. 2 were pooled and concentrated by $(\text{NH}_4)_2\text{SO}_4$ fractionation. DNA polymerases A and B were purified up to the second phosphocellulose step and eluted with a continuous gradient between 200 and 800mM-KCl. Values are means $\pm$ s.d. for three determinations. The DNA polymerase activity was measured in the presence of activated DNA.

DNA polymerase	Activity (pmol of TMP incorporated/h per mg of protein)			
	Time (h) ... 0	10	20	40
A	240 $\pm$ 38	870 $\pm$ 95	560 $\pm$ 80	380 $\pm$ 49
B	190 $\pm$ 25	620 $\pm$ 51	340 $\pm$ 45	250 $\pm$ 47
C	750 $\pm$ 83	180 $\pm$ 32	105 $\pm$ 22	25 $\pm$ 12

the activity of DNA polymerase A, whereas DNA polymerase B activity is only slightly affected by the antibody. No effect was observed with antibodies from control animals.

Fig. 3. Neutralization of DNA polymerase A (Δ), B ($\blacktriangle$) and C ($\circ$) against antiserum to DNA polymerase C. One unit of DNA polymerase A, B or C was preincubated for 16h at 0°C in 0.1 ml of solution containing 50mM-Tris/HCl, pH 7.8, 0.2% bovine serum albumin and dilutions of anti-(DNA polymerase C) serum. After the preincubation the remaining DNA polymerase activity was tested as described under 'Methods', with activated DNA as template. The incubation time was 30 min.

Variation of nucleoside triphosphate pools, thymidine kinase and nucleoside phosphotransferase

The concentration of ATP, determined by the luciferin-luciferase method, increases dramatically in wheat embryos after water imbibition (Fig. 4a), whereas the deoxynucleoside triphosphate concentrations increase much more slowly, a plateau being attained for dCTP and TTP only after 16–20h of germination. dCTP was the most abundant DNA precursor, although its concentration is 50 times lower than that of ATP, as Fig. 4(b) shows.

Fig. 5 shows the changes in the capacity of phosphorylation of thymidine at different germination times. Two sources of phosphate were used. In the presence of ATP, the enzyme activity (thymidine kinase) showed a net increase 6h after water imbibition. By using AMP as source of phosphate (nucleoside phosphotransferase), the increase of enzyme activity was observed later, as compared with thymidine kinase, since enzyme induction was seen 10h after the start of germination.

Discussion

Although DNA polymerases are a key part of the DNA-synthetic machinery, there are many other protein factors involved in this process. No DNA polymerase is able to use an intact native double-stranded DNA as template, nor can DNA synthesis be initiated on single-stranded DNA by DNA polymerases. Thus for DNA synthesis to operate

Fig. 4. Change in ATP (a) and dNTP (b) content during germination

Samples of 100 embryos were germinated for different times and the dNTP pool extracted and measured as indicated under 'Methods'. Values are means of three determinations. Δ , dCTP; $\bullet$, dTTP; $\triangle$, dATP, and $\blacksquare$, dGTP.

in vivo, factors and enzymes other than DNA polymerases, like unwinding proteins, gyrases, RNA polymerases, primases, etc. must be present (see Molineux & Kohiyama, 1978; Litvak *et al.*, 1978). Moreover, replication of DNA requires the continuous supply of building blocks, the four deoxynucleoside triphosphates originating from the reduction of ribonucleotides. It has been increasingly evident that the concentration of dNTP species can control the rate of DNA synthesis (Reichard, 1978). Thus DNA synthesis can be regulated not only at the polymerization level, but also at the level of precursor biosynthesis. We are perfectly aware that by studying the changes in DNA polymerases, in dNTP pools, and the variation of thymidine kinase during wheat embryo germination, we are dealing only with a small fragment of the DNA-synthetic system.

Fig. 5. Thymidine phosphorylation during wheat embryo germination

Samples of 75 embryos were germinated for different lengths of time. Thymidine kinase and nucleoside phosphotransferase were extracted and assayed as described under 'Results'. Δ , ATP as source of phosphate (thymidine kinase activity); $\blacksquare$, AMP as source of phosphate (nucleoside phosphotransferase activity). Values are means $\pm$ S.D. for three determinations.

We have chosen the germinating-wheat-embryo system as a model for the study of the control of DNA synthesis, since it has been claimed that the onset of DNA synthesis cannot be observed before 15h of germination (Chen & Osborne, 1970). Fig. 1 confirms those results. It can be seen that labelled thymidine starts being incorporated into DNA at about 10h of germination. A decrease in thymidine incorporation is observed after 20h of germination. We do not have an explanation for this observation, although it may be related to the fact that we are using isolated embryos and not intact grains. We have not observed the rhythmic increase in thymidine incorporation described by Yadav (1976). A noteworthy observation is that although it could have been expected that the passage of thymidine into the embryo would be a passive phenomenon, hence instantaneous, we were not able to detect the labelled precursor inside the embryo before 6h of germination (Fig. 1). These results can be explained by the 'organization' of a thymidine-transport system during the first hours after the start of germination.

When the activities of DNA polymerases A, B and C were studied during the first 40h of germination, we observed the decrease of enzyme C and the increase of enzymes A and B (Fig. 2 and Table 1). The intriguing decrease in DNA polymerase C can be tentatively explained by assuming that this enzyme is a vestige from the embryogenesis stage, kept

intact during desiccation, not utilized during germination, and henceforth degraded after water imbibition. On the other hand, we can also speculate that there is a relation of the precursor-product type between enzyme C and enzymes A and B. While the activity of the former is decreasing, that of the two others increases. In order to answer this question, the experiments whose results are shown in Fig. 3 were performed. Extensively purified DNA polymerase C was used for preparing antibodies able to inhibit the activity *in vitro* of the enzyme. These antibodies against DNA polymerase C were active in neutralizing not only the activity of the enzyme C, but also the activity of DNA polymerase A, whereas only a very weak inhibition of DNA polymerase B was observed. It is difficult to explain the relationship between DNA polymerases C and A in terms of the maturation of a larger protein, as described for other enzymes, since DNA polymerase C has a smaller size than enzyme A (see the preceding paper, Castroviejo *et al.*, 1979). The main difference between enzymes C and A concerns the template-specificity in the assay *in vitro*. It is possible that a subunit conferring a new template-specificity synthesized during the first hours of germination becomes associated to enzyme C to give DNA polymerase A. The increase of DNA polymerase A and B activities (Table 1) coincides with a period of active DNA synthesis (Fig. 1). An increase in DNA polymerase- α during the S-phase in animal cells has been considered as evidence of the involvement of this enzyme in DNA replication (Spadari & Weissbach, 1974). Similarly, we can ascribe such a role to DNA polymerases A and B. We do not have an explanation for the decrease of both activities after 10h of germination (Table 1). However, a similar pattern in the case of DNA polymerase- γ of HeLa cells has been interpreted as the indication of a role in DNA initiation (Spadari & Weissbach, 1974). The fact that we were germinating isolated embryos and not whole seeds must also be taken into consideration.

Other authors have proposed that DNA polymerase from wheat embryos is synthesized during the first hours of germination (Mory *et al.*, 1975). For the moment we have dealt exclusively with enzymes purified from the soluble fraction. Some preliminary experiments from our laboratory indicate that DNA polymerase activity increases in purified nuclei during wheat embryo germination, but the low yield of purified nuclei has avoided further studies. It has been claimed, in the case of pea germination, that two DNA polymerase activities, one soluble and the other associated with chromatin, increase during germination, the soluble activity increase being more important (Robinson & Bryant, 1975; Stevens & Bryant, 1976).

The profound influence that the biosynthesis of DNA precursors may have on overall DNA synthesis

is not often fully appreciated (Bjursell & Reichard, 1973). In virtually all cells, two fundamentally different kinds of pathways are used for the synthesis of nucleotides. One is the pathway *de novo* where nucleotides are synthesized without passing through a pool containing free bases or nucleosides. A second pathway is called the 'salvage pathway', in which bases and nucleosides are salvaged from intracellular and extracellular sources. Breakdown of nucleic acids within bacterial, plant and animal cells is a major source for this pathway (Kornberg, 1974). We have studied two aspects related to the biosynthesis of DNA precursors during wheat germination. One concerns the concentration of nucleoside triphosphates during germination and the other is the study of thymidine kinase, a key enzyme of the salvage pathway. The increase in ATP concentration after water imbibition is extremely fast, as already shown by Pradet *et al.* (1968) for lettuce seeds and by Cheung & Marcus (1975) for wheat embryos. The increase of dNTP species, on the other hand, is much slower, and in the case of dCTP and TTP, a plateau is reached only after 20h of germination (Fig. 4). No dNTP pool was detected in ungerminated embryos. The high concentration of dCTP must be taken with caution, since it has been shown that, in animal cells, dCDP-acylglycerol can be synthesized from dCTP, the incorporation of dCTP into the phospholipid precursor being 10–20 times faster than the incorporation of dCTP into DNA *in vitro* (Yamada *et al.*, 1976). The low concentration of dNTP species in the first hours of germination may constitute a partial explanation of the delay in the onset of DNA synthesis in germinating wheat embryos. The same important role of dNTP concentration on DNA synthesis has been shown in the case of rice seeds incubated in the absence of oxygen (Mocquot *et al.*, 1977).

Thymidine kinase catalyses the ATP-mediated phosphorylation of thymidine to give dTMP. This enzyme is found in all growing cells and is induced or increased to much higher activity during certain virus infections or under conditions of increased cell proliferation. We considered it worthwhile to study such an enzyme during wheat embryo germination. Several investigators have reported that tissue extracts from various plants lack thymidine kinase activity and that thymidine is phosphorylated by a nucleoside phosphotransferase catalysing the exchange of phosphate between a nucleotide, such as AMP, and thymidine (Arima *et al.*, 1971; Deng & Ives, 1972). The controversy about which mechanism of thymidine phosphorylation operates in higher plants still exists, since it has been claimed, on the one hand that thymidine kinase is present in plants (Brunori *et al.*, 1974) and on the other hand, in radish radicles, that only nucleoside phosphotransferase seems to be active (Delseny *et al.*, 1976). We

have tested the phosphorylation of thymidine by using ATP and AMP as source of phosphate. As Fig. 5 shows, there is in both cases a lag period after water imbibition. However, the increased rate in both cases seems to be different, since the nucleoside phosphotransferase increase is delayed as compared with thymidine kinase activity. One of the arguments against the presence of thymidine kinase is that, in the crude extracts used as source of enzyme, the presence of adenosine triphosphatase would lead to the transformation of the added ATP to AMP. Our results would argue against this possibility, since the phosphorylation of thymidine with ATP precedes the AMP-dependent activity.

A key enzyme in dNTP biosynthesis is nucleotide reductase. Unfortunately all our attempts to detect this activity in wheat embryos have failed. Recently Schimpff *et al.* (1978) measured the activity of wheat seeds nucleotide reductase *in vivo*, since it was extremely difficult to detect the enzyme *in vitro*. They reported that enzyme activity is observed at 20–30h after imbibition, only in aged seeds (2–4 years old). By using a different approach, those authors found, as in our case, no deoxyribonucleoside triphosphate pool in dry seeds.

We are greatly indebted to Dr. J. Duplan and to Dr. A. Pradet, Dr. A. Marcus and Dr. A. Weissbach for helpful discussions. This work was supported by C.N.R.S. and the University of Bordeaux II.

References

- Arima, T., Masaka, M., Shiosaka, T., Okuda, H. & Fujii, S. (1971) *Biochim. Biophys. Acta* **246**, 184–193
- Bjursell, G. & Reichard, P. (1973) *J. Biol. Chem.* **248**, 3904–3909
- Bray, C. M. (1976) in *Molecular Aspects of Gene Expression in Plants* (Bryant, J. A., ed.), pp. 109–138, Academic Press, London and New York
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Brooker, J. D., Cheung, C. P. & Marcus, A. (1977) in *The Physiology and Biochemistry of Seed Dormancy and Germination* (Khan, A. A., ed.), pp. 347–356, North-Holland, Amsterdam
- Brunori, H., Hotta, Y. & Stern, H. (1974) *Plant Cell Physiol.* **15**, 421–427
- Burton, K. (1967) *Methods Enzymol.* **12B**, 163–164
- Castroviejo, M., Tarrago-Litvak, L. & Litvak, S. (1975) *Nucleic Acids Res.* **2**, 2077–2090
- Castroviejo, M., Tharaud, D., Tarrago-Litvak, L. & Litvak, S. (1979) *Biochem. J.* **179**, 183–191
- Chen, D. & Osborne, D. (1970) *Nature (London)* **225**, 336–340
- Cheung, C. P. & Marcus, A. (1975) *Anal. Biochem.* **69**, 131–139
- Delseny, M., Got, A. & Guitton, Y. (1976) *Physiol. Veg.* **14**, 159–178
- Deng, Q. & Ives, D. H. (1972) *Biochim. Biophys. Acta* **277**, 235–244
- de Petrocellis, B. & Rossi, M. (1976) *Dev. Biol.* **48**, 250–257
- Kornberg, A. (1974) *DNA synthesis*, W. H. Freeman & Co., San Francisco
- Litvak, S., Mocquot, B. & Castroviejo, M. (1978) in *DNA Synthesis: Present and Future* (Molineux, I. M. & Kohiyama, M., eds.), pp. 629–639, Plenum Press, New York
- Marcus, A., Feely, J. & Volcani, T. (1966) *Plant Physiol.* **41**, 1167–1172
- Mayer, A. M. & Shain, Y. (1974) *Annu. Rev. Plant Physiol.* **25**, 167–193
- Mocquot, B., Pradet, A. & Litvak, S. (1977) *Plant Sci. Lett.* **9**, 365–371
- Molineux, I. M. & Kohiyama, M. (eds.) 1978 *DNA Synthesis: Present and Future*, Plenum Press, New York
- Mory, Y. Y., Chen, D. & Sarid, S. (1972) *Plant Physiol.* **49**, 20–23
- Mory, Y. Y., Chen, D. & Sarid, S. (1975) *Plant Physiol.* **55**, 437–442
- Nygaard, P. (1972) *Physiol. Plant.* **26**, 29–33
- Pradet, A., Narayanan, A. & Vermeersch, J. (1968) *Bull. Soc. Fr. Physiol. Veg.* **14**, 107–114
- Pradet, A. & Prat, C. V. (1976) in *Etudes de Biologie Végétale* (Jacques, R., ed.), p. 561, Louis-Jean, Paris
- Reichard, P. (1978) *Fed. Proc. Fed. Am. Soc. Exp. Biol.* **37**, 9–14
- Rejman, E. & Buchowicz, J. (1973) *Phytochemistry* **12**, 271–276
- Robert-Guroff, M. & Gallo, R. C. (1977) *Biochemistry* **16**, 2874–2880
- Robinson, N. E. & Bryant, J. A. (1975) *Planta* **127**, 69–75
- Schimpff, G., Müller, H. & Follmann, H. (1978) *Biochim. Biophys. Acta* **520**, 70–81
- Sen, S., Payne, P. I. & Osborne, D. J. (1975) *Biochem. J.* **148**, 381–387
- Solter, A. W. & Handschumacher, R. E. (1969) *Biochim. Biophys. Acta* **174**, 585–590
- Spadari, S. & Weissbach, A. (1974) *J. Mol. Biol.* **86**, 11–20
- Spiegel, S., Obendorf, R. L. & Marcus, A. (1975) *Plant Physiol.* **56**, 502–507
- Stevens, C. & Bryant, J. A. (1976) *Biochem. Soc. Trans.* **4**, 803–806
- Tarrago-Litvak, L., Castroviejo, M. & Litvak, S. (1975) *FEBS Lett.* **59**, 125–130
- Taylorson, R. B. & Hendricks, S. B. (1977) *Annu. Rev. Plant Physiol.* **28**, 331–354
- Weinstock, I. M. & Dju, M. Y. (1971) *Biochim. Biophys. Acta* **232**, 5–13
- Yadav, S. P. (1976) *Planta* **129**, 87–89
- Yamada, M., Aucker, J. & Weissbach, A. (1976) *Arch. Biochem. Biophys.* **177**, 461–467