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DNA POLYMERASE ACTIVITY AND DNA SYNTHESIS IN ROOTS OF PEA (*PISUM SATIVUM*) SEEDLINGS

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Abstract—Soluble DNA polymerase (DNA polymerase-α) and chromatin-bound DNA polymerase (DNA polymerase-β) have been assayed in serial sections cut from the roots of 5-day-old pea seedlings. The activity of DNA polymerase-α is high in regions of the root which exhibit high rates of DNA replication, and declines during cell differentiation and maturation. The activity of DNA polymerase-β is low in cells which show high rates of DNA replication, and increases during differentiation and maturation.

INTRODUCTION

The possession of multiple forms of DNA polymerase (EC 2.7.7.7) appears to be a universal feature of both prokaryotic and eukaryotic organisms [1–4]. The existence of more than one type of DNA polymerase raises questions about the roles of the different types of enzymes. In prokaryotes, and particularly in the bacterium *Escherichia coli*, the existence of mutants lacking one or more of the DNA polymerases, and of mutants with temperature-sensitive DNA polymerases has led to a partial elucidation of the roles of the different enzymes [1]. In eukaryotes, however, such genetic evidence exists only for one of the DNA polymerases of the simple fungus *Ustilago maydis* [5]. In the absence of suitable mutants, the most widespread experimental approach to understanding the roles of the DNA polymerases has been the measurement of the activities of the polymerases in cells undergoing DNA replication, and in cells not undergoing DNA replication [3]. This is essentially the rationale of the experiments described in this paper.

In cells of higher plants, two major types of DNA polymerase, other than those in the plastids and mitochondria, have been described. These are (i) a soluble DNA polymerase, similar to DNA polymerase-α of vertebrates [6–12] and (ii) a chromatin-bound DNA polymerase, similar in some respects to the DNA polymerase-β of vertebrates [7, 13–16]. There is also some evidence for the existence of a minor soluble DNA polymerase, which is similar to DNA polymerase-γ of vertebrates in showing a marked preference for poly(A).oligo(dT) as a template-primer [7, 11]. In this paper we have confined our attention to the major soluble polymerase (polymerase-α) and the chromatin-bound polymerase (polymerase-β). Very little is known about the relationship of the activities of these two enzymes to DNA replication in higher plants, although a limited amount of preliminary evidence suggests that the activity of the soluble polymerase is better correlated with DNA replication than is the activity of the chromatin-bound polymerase [7, 17]. In order to follow up these preliminary observations, we have assayed the two enzymes in sections cut from roots of 5-day-old pea seedlings, and have compared the distribution of the enzymes with the distribution of cells undergoing DNA synthesis and mitosis.

RESULTS AND DISCUSSION

The general features of the root sections used in our experiments are shown in Table 1. It is clear that the highest labelling indices (i.e. percentages of cell which incorporate thymidine into DNA) are observed in the apical 4 mm of the roots, after which the labelling index declines. The highest mitotic indices (percentage of cells in division) are observed in the apical 2 mm of the roots. The maintenance of a high labelling index after the rate of mitosis has declined is consistent with the observation that a high proportion of mature cells in pea roots are arrested in the G2 phase of the cell cycle, i.e. have undergone DNA replication without mitosis [18]. The data in Table 1, together with data on cell cycle kinetics [19], have been used to calculate approximate rates of DNA replication in different regions of the root (Fig. 1a). The rates shown in Fig. 1a are consistent with the average rates of DNA accumulation recorded for the apical 10 mm of root in an earlier series of experiments [20].

The data in Fig. 1a may be compared with the activities of the DNA polymerases (Fig. 1b, c). It is clear that the activity of the soluble DNA polymerase (polymerase-α) is highest in the regions of the root which exhibit the highest rates of DNA replication and lower in the regions of the root exhibiting a low rate of DNA replication. These changes in the activity of soluble DNA polymerase are apparent whether the results are expressed per unit of DNA or per unit of protein, and are thus taken to represent genuine changes in enzyme activity per cell, rather than just changes in protein content. Further, experiments in which homogenates from different root sections are mixed together provide no evidence for the presence of any activators or inhibitors of soluble DNA

Table 1. General characteristics of pea root sections

	Distance from tip, mm					
	0–1	1–2	2–4	4–5	5–10	10–15
Labelling index, %	31.9 ±2.0	34.8 ±4.0	33.4 ±5.7	19.3 ±4.9	10.5 ±1.9	7.6 ±1.2
Mitotic index, %	6.6 ±0.6	6.15 ±0.7	3.8 ±1.55	2.35 ±0.45	1.0 ±0.4	1.5 ±0.45
DNA content, μg	0.75 ±0.05		1.59 ±0.15		0.95 ±0.06	1.02 ±0.07
Protein content, μg	8.0 ±1.0		19.0 ±3.0		19.0 ±5.0	36.0 ±7.0

polymerase activity. Thus, the activity of the soluble DNA polymerase shows a general correlation with the ability of cells to replicate DNA.

By contrast, the activity of the chromatin-bound DNA polymerase is lowest in the regions of the root which exhibit the highest rates of DNA replication, and highest in the mature regions of the root. Expression of these data in terms of protein content is not meaningful, since the large amounts of histones in chromatin mask any changes in non-histone protein content. As with the soluble DNA polymerase, there is no evidence for the presence of activators or inhibitors in the different root sections, and the data are thus taken to reflect genuine changes in catalytic activity. Thus, the activity of the chromatin-bound DNA polymerase is inversely correlated with DNA replication.

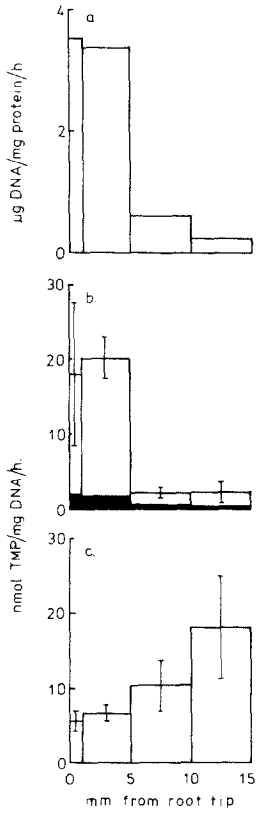

Fig. 1. DNA synthesis and DNA polymerase activity in roots. (a) Approximate rate of DNA synthesis (calculated as described in text). (b) Soluble DNA polymerase (polymerase-α) activity. (c) Chromatin-bound DNA polymerase (polymerase-β) activity. Vertical bars are standard errors of mean. In (b), the solid histograms represent the data expressed per mg protein.

In a previous paper [17] we have attempted to compare the *in vitro* activities of the soluble DNA polymerase with *in vivo* rates of DNA synthesis. However, although we are confident that very little loss of enzyme activity occurs during extraction (see Experimental), we do not now believe that it is possible to compare directly the assayed activity of the enzyme with the rate of DNA accumulation. In particular, the exact state of the template DNA *in vivo* at the time of polymerization is not known, and it is very clear that very marked changes in enzyme activity *in vitro* can be brought about by very small changes in the state of the template DNA [21]. Further, it is impossible *in vitro* to mimic the complex interactions with stimulatory proteins which probably occur during DNA replication *in vivo* [2]. However, in general terms, the activity of the soluble DNA polymerase in the apical regions of the root is of the order of magnitude expected if the enzyme was bringing about DNA replication.

The finding that the majority of the DNA polymerase activity in cells which are replicating DNA is the soluble DNA polymerase-α is in agreement with our earlier findings on the embryonic axes of germinating peas [17] and with the observations of Srivastava on cultured tobacco cells [7]. The data are also very similar, in qualitative terms, to data obtained with a variety of mammalian and avian cells, and in particular to data obtained with cultured baby hamster kidney cells [3]. In the latter, the activity of polymerase-α is highest in exponentially dividing cells, where it makes up *ca* 90% of the total activity. In quiescent cells, the activity of polymerase-α is only 40% of the total, and further, the activity of polymerase-β is higher in quiescent than in exponential cells. Thus, the data from higher plants provide circumstantial evidence that in higher plants, as in vertebrates, the soluble DNA polymerase is involved in DNA replication. Our data and those of Srivastava [7] strongly suggest that the chromatin-bound DNA

polymerase of higher plants is not involved in DNA replication, and by analogy with data from vertebrates, it is suggested that it is involved in DNA repair. Indirect evidence for this view comes from data obtained with ageing barley seeds, where a decrease in the ability to repair damaged DNA is correlated with a decrease in the activity of chromatin-bound DNA polymerase [22]. The three-fold increase in the activity of chromatin-bound DNA polymerase during cell maturation observed in our experiments is, however, unexpected. If this enzyme is indeed involved in DNA repair, then these data suggest that older cells may have a greater need for DNA repair activity than younger cells.

EXPERIMENTAL

Growth of plants. Undamaged pea seeds (*Pisum sativum* L. cv Feltham First) were surface-sterilized for 20 min in an aq. soln of NaClO (2.5% available chlorine), and then washed and soaked in H_2O for 5 hr. The soaked seeds were germinated between sheets of filter paper wetted with sterile distilled H_2O in a large autoclaved glass culture vessel. After 4 days, the seedlings were transferred onto a raft, floating on autoclaved distilled H_2O through which air was bubbled and into which the roots of the seedlings projected. One day later, the seedlings were harvested.

Root sections. The roots were sectioned with a hand-operated guillotine to give batches of sections as indicated in Table 1.

DNA polymerase assays. Soluble and chromatin-bound DNA polymerases were assayed under optimal conditions, as described previously [10, 16]. In crude extracts, both enzymes are stable for several hours at 1–4° [21], and it is thus very unlikely that loss of activity occurred during prepn of the extracts. Data presented are means of between three and five determinations.

Labelling index and mitotic index. Prior to excision from the seedling, the roots were supplied with methyl-[^{3}H]-thymidine (50 Ci/mmol; 1 μCi/ml) for 1 hr. We have previously shown that radioactivity from methyl-labelled thymidine is incorporated specifically into DNA in pea roots, with no prior metabolism of the nucleoside [20]. After labelling, the roots were fixed in EtOH–HOAc (3:1), and sectioned as described above. Permanent autoradiographs were prepared as described previously [23]. From each slide, the labelling index and the mitotic index (sum of prophase, metaphase, anaphase and telophase) were determined. A total of 500 nuclei per slide was scored. Six slides for each root zone taken from three replicate experiments were used, representing a total of nuclei scored of 3000 per root zone.

Estimation of DNA content. Total nucleic acids were extracted from the root sections essentially as described by Guinn [24]. DNA was estimated according to Burton [25].

Estimation of protein. Protein was estimated by the method of Lowry [26], using bovine serum albumin as a standard.

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