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# The yeast DNA polymerase I transcript is regulated in both the mitotic cell cycle and in meiosis and is also induced after DNA damage

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ABSTRACT

Using mitotic cultures synchronised by a feed-starve protocol or by elutriation, we have shown that the yeast DNA polymerase I gene is periodically expressed with its transcript increasing at least 100-fold in late G1 with a peak around the G1/S phase boundary. This is precisely the same interval of the cell cycle in which three other yeast DNA synthesis genes, CDC8, CDC9 and CDC21, have been found to be periodically expressed (White et al 1987. Expl. Cell. Res., in press). The polymerase I transcript is also regulated in meiosis, showing an overall fluctuation in level of some 20-fold, with a peak at about mid-S phase. In addition, following irradiation with 50J/$m^2$ ultraviolet light, there was a 20-fold increase in the transcript, starting after 30 minutes and reaching a peak two hours later. These results indicate that DNA polymerase I is subject to a complex control and imply that it has a role in both DNA synthesis and DNA repair.

## INTRODUCTION

Many of the processes of DNA metabolism are discontinuous in nature, for instance DNA synthesis occupies only a small proportion of either the mitotic cell cycle or meiosis, and DNA repair is only required occasionally. Therefore the enzymes involved in these processes could be subject to some form of regulation which allows their synthesis only when the gene product is required. In certain cases this regulation could be highly complex since a number of enzymes function in several different aspects of DNA metabolism. A prominent example is DNA ligase which acts in DNA synthesis to join Okazaki fragments and also in repair and recombination to complete the final sealing step in nicked duplex DNA. We have studied the regulation of the DNA ligase gene, CDC9, in yeast and found that in both meiosis and the mitotic cell cycle there is a brief but marked increase in CDC9 transcript levels just prior to DNA synthesis (1,2), suggesting that transcription of this gene is confined to a short interval immediately before S phase. Similarly, there is an increase in levels of both DNA ligase message and

enzyme after exposure of yeast cells to ultraviolet (UV) light, γ-irradiation or methyl methane sulphonate (3).

Another enzyme involved in many aspects of DNA metabolism is DNA polymerase. In yeast there are two species of nuclear DNA polymerase and although their respective roles are not clearly established, their biochemical properties suggest that DNA polymerase I is responsible for DNA replication(4,5,6). There have, in fact, been two previous attempts to study expression of yeast DNA polymerase, each involving the mitotic cell cycle alone (7,8). Synchronised cells were used in these experiments and total DNA polymerase activity was assayed throughout the cell cycle. No attempt was made to differentiate between species of DNA polymerase and, in summary, the results suggest that overall polymerase activity fluctuates with a peak occurring at approximately early S phase.

Possibly a more direct means of examining gene expression is to follow fluctuations in level of the transcript by hybridisation with a DNA probe. This is particularly useful when several species of an enzyme exist in a cell since hybridisation is highly specific for a particular transcript. The yeast DNA polymerase I gene has recently been cloned (9,10) thus providing a specific DNA probe for monitoring its transcript. In the experiments described here, we have exploited this to examine regulation of the polymerase I gene not only in the mitotic cell cycle but also in meiosis and after UV-irradiation.

## MATERIALS AND METHODS

### Strains and Media

The genotypes of the Saccharomyces cerevisiae strains were as follows; MC16 MATα leu2-3 his4-712 ade2-1 lys2-1 SUF2, NCYC239 and SK-1 are prototrophic diploids, while details of the cdc4-3 diploid MH14 have been given previously (11). Construction of the SK-1 related a/a and α/α diploids has also been described previously (1).

YPD (1% Difco yeast extract, 2% Bacto peptone and 2% glucose) or YPA (1% potassium acetate in place of the 2% glucose) were used as rich media and Difco Yeast Nitrogen Base (0.67%; 0.5% glucose) as minimal medium with the appropriate nutritional supplements.

### Synchronisation procedures

Mitotic cultures of NCYC239 were synchronised by the feed-starve protocol of Williamson and Scopes (12) and the use of the Beckman JE10X Elutriator Rotor to synchronise cells of budding yeast has been described previously (2).

For meiotic cultures, strain SK-1 was grown to mid-log phase in YPA, filtered, washed, resuspended in 1% potassium acetate (SPM) and incubated at 30°C with vigorous shaking (1).

UV-irradiation

Mid-log phase cells (~$10^7$/ml) of strain MC16 grown at 30°C were filtered, washed and resuspended at $10^7$ cells/ml in 0.9% saline before UV-irradiation with 50J/$m^2$ (254nm) at room temperature (3). After irradiation, cells were again filtered and resuspended at $10^7$/ml in pre-warmed fresh YPD at 30°C and incubated in the dark for the duration of the experiment.

RNA preparation

Samples of $10^8$ cells were harvested, washed in saline and frozen rapidly in dry ice. Total RNA was extracted using the hot phenol method of Aves et al (13) and RNA concentrations were determined by measuring the $A_{260}$ and all were adjusted to 1 mg/ml.

Northern hybridisation analysis

5 µg samples of total RNA were denatured with glyoxal, size separated by electrophoresis in 1.5% agarose gels and transferred to GeneScreen membrane (New England Nuclear) according to manufacturers instructions. Subsequent hybridisation, washing and rehybridisation was also carried out as recommended by the manufacturer.

The probe DNA was a 3.2kb HindIII-SalI fragment from the cloned polymerase I gene (9) and was labelled with $^{32}$P-dTTP (3000 Ci/mMol; New England Nuclear) by an oligolabelling protocol (14) to a specific activity of approximately $10^9$ cpm/µg.

Autoradiography was carried out at -70°C using Fuji RX X-ray film or Kodak XAR X-ray film with X-Ograph Hi-Speed-X intensifying screens. Several exposures were made to ensure that the signal was within the exposure range of the film. A Joyce Loebl Chromoscan 3 densitometer was used to scan autoradiographs for quantitation.

## RESULTS

### DNA polymerase I is regulated in the mitotic cell cycle

Regulation of polymerase I in the cell cycle was investigated by sampling synchronised cells and analysing transcript levels by probing an RNA blot with an appropriate DNA fragment ('Northern hybridisation'). Three different methods of synchronising cells were used and the degree of synchrony obtained in each case was assessed by budding profiles, together with the cell cycle dependent transcription of the histone H2A. In addition, the

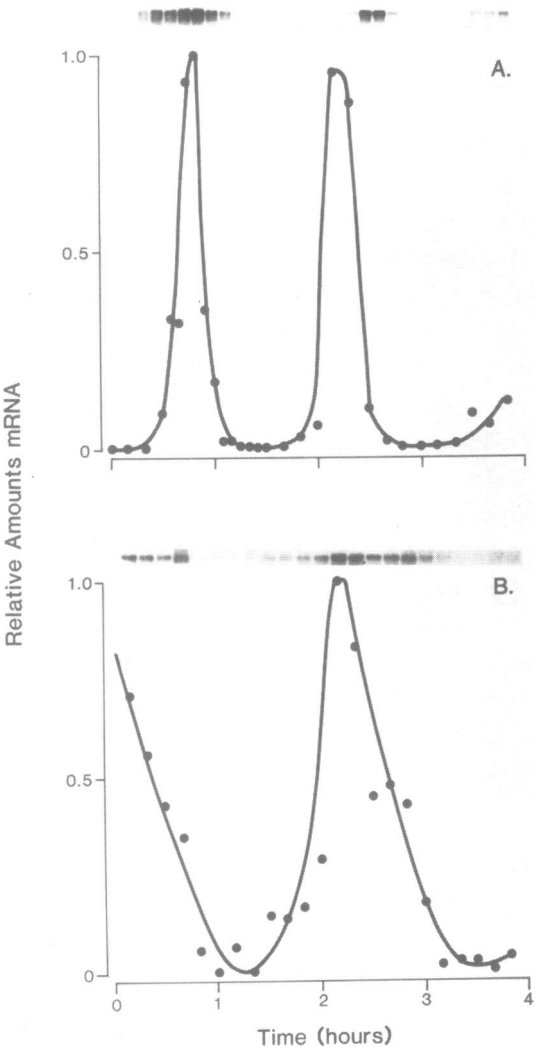


Figure 1. Regulation of the DNA polymerase I transcript in mitotic cells synchronised by (A) feed/starve or (B) by elutriation. Data relating to budding and DNA synthesis in these synchronous cultures of strain NCYC 239 has been presented in a previous publication (2). Two synchronous cell cycles were followed in each case and samples were removed at intervals. However, with the elutriation synchrony, sampling commenced only after the start of the first cycle. Total RNA was extracted from each sample and after electrophoresis, a Northern blot was prepared. This was probed with a labelled DNA fragment from the poylmerase I gene and suitably exposed autoradiographs were quantitated using densitometry. The levels of two control transcripts (the cell cycle regulated histone H2A and the invariant protein 1 as a loading control) on the blots used here have already been described (2).

transcript level of 'Protein 1', a gene adjacent to histone H2A, but which is not cell cycle regulated (15) was used as an internal control. In each case the cultures displayed a high degree of synchrony which has been described in detail in a previous publication (2). On the RNA blots derived from them, the histone H2A transcript showed a sharp fluctuation in level, while the constant level of the protein 1 transcript indicated that the gel had been evenly loaded and that there were no major blotting artefacts (2). It is these same blots which have been used in the experiments described below. Two cell cycles were followed in each culture and Figure 1 shows the result obtained using cells synchronised by the feed-starve (12) and elutriation procedures. The level of the 5.2 Kb polymerase I transcript (9) fluctuated sharply in both cultures and in each two fluctuations can be seen approximately one cell cycle apart. Similar results were also obtained using α-factor synchrony (results not shown) and since these three quite different methods give essentially identical results, the observed periodic fluctuations of polymerase I are most unlikely to be due to a synchronisation artefact. The fluctuation in transcript levels in the feed-starve synchrony is at least 100-fold, though it is difficult to accurately quantitate the fluctuation using autoradiography alone because of the limited linear exposure range of photographic film.

The peak in the polymerase I transcript occurred at the same time as the peak in the CDC9 DNA ligase transcript at the G1/S phase boundary and before the peak in the histone H2A message (2). Significantly, we have recently shown that the CDC8, CDC9 and CDC21 gene transcripts all fluctuate at the same point in the cell cycle (11) and hence, this point appears to coincide with that for the DNA polymerase I transcript. In the experiments on the cell cycle expression of these three CDC genes, we examined their transcript levels in various cell cycle mutants (cdc28, cdc4 and dbf4 (16))which at the restrictive temperature (37°C) block cells in G1, at about the time when these genes are expressed. The most interesting result was obtained with cdc4-blocked cells. No histone H2A expression was detected in these but the three CDC genes showed a single complete fluctuation in transcript levels, indicating that they may be co-ordinately regulated.

To determine whether the polymerase I transcript is regulated in the same place, we probed the same blots from the cdc4-blocked cells that had been used with CDC8, 9 and 21. In this experiment a 25°C culture of cdc4-3 was synchronised by elutriation and then split into two, with one half being transferred to 37°C and the other half remaining at the permissive temper-

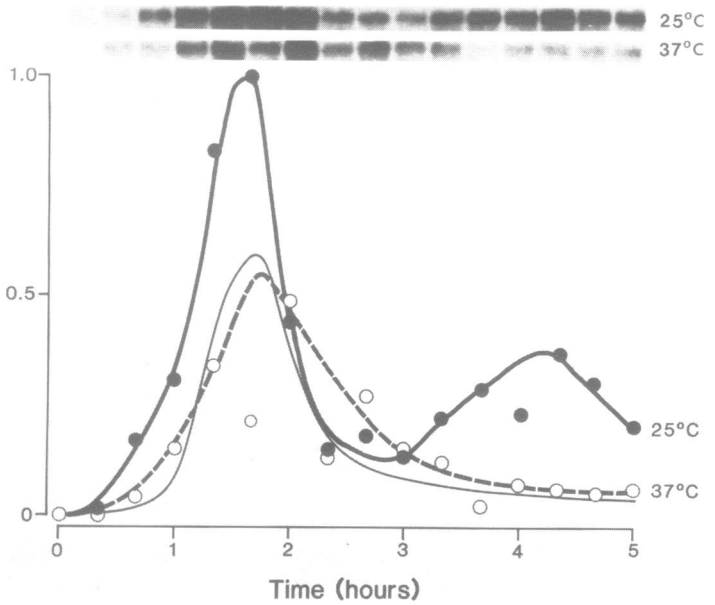


Figure 2. Expression of DNA polymerase I in cdc4-blocked cells synchronised by elutriation. After synchronisation of cells growing at 25°C, the culture was immediately divided into two and one half was incubated at 25°C, while the other half was transferred to 37°C and incubation was continued for a period encompassing two cell cycles. Budding data and the levels of histone H2A and protein I transcripts in these cultures and the blots derived from them have been described elsewhere (11). For further details see the legend to Fig. 1. Note that the peak sample from the 37°C culture was spoiled in preparation. The thin unbroken line shows the profile of the CDC9 transcript at 37°C (11).

ature of 25°C (11). Incubation was continued for the equivalent of two cell cycles, samples were removed at intervals and the RNA blots prepared which, as described above, were probed with histone H2A and protein 1 as controls (11). When probed with polymerase I the 25°C culture showed the expected two peaks of polymerase I message (Fig.2). In contrast, at 37°C, only one complete fluctuation was seen. This mirrors the results obtained with the three CDC genes and, more importantly, the polymerase I peak at 37°C occurred at precisely the same time, for comparison an outline of the result obtained with CDC9 is included in Fig. 2. The polymerase I transcript is therefore expressed in the same interval of the cell cycle as CDC8, CDC9 and CDC21 and therefore the regulatory pathways of all four genes are in some way related.

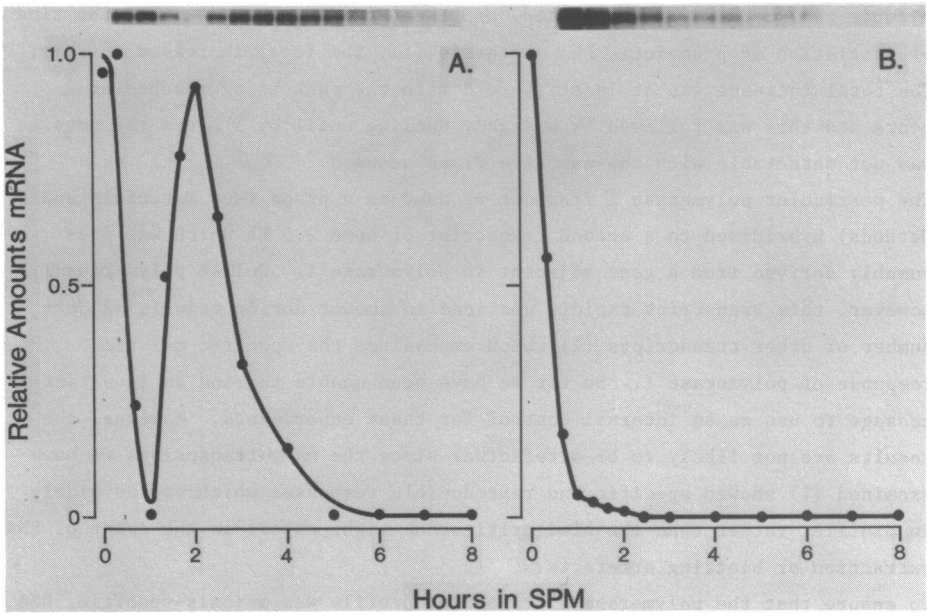


Figure 3. Regulation of the DNA polymerase I transcript during meiosis. Meiotic cultures of (A) strain SK-1 and (B) of a SK-1 related MATa/MATa diploid (1) were established by transferring mid-log cells into sporulation medium (SPM). The cultures were sampled at intervals and a Northern blot prepared. See legend to Fig. 1 for further details.

The DNA polymerase I transcript is regulated during meiosis

As well as growing vegetatively, diploid yeast can undergo the alternative developmental pathway of meiosis. A considerable amount of DNA metabolism takes place during meiosis, one of the early events being a single round of DNA synthesis and this is followed by synaptonemal complex formation and recombination. The regulation of DNA metabolism enzymes is therefore of interest in meiosis and in the case of polymerase I is also relevant for purposes of comparison between meiosis and mitosis.

We have previously used strain SK-1 to examine the meiotic expression of CDC8 CDC9 and CDC21 (1) as it can be induced to undergo meiosis with a reasonable degree of synchrony. For instance, premeiotic DNA synthesis occupies some 65 minutes in individual cells (17) and in the meiotic culture we used to examine expression of the CDC genes, premeiotic S started at 1 hour and was essentially complete by 4 hours (1). The RNA samples derived from this culture have now been used to evaluate levels of the polymerase I transcript (Fig. 3). The level of the transcript initially declined, presumably as

mitotic cell cycles were completed, and then after 1 hour, at about the time of initiation of premeiotic DNA synthesis (1), the level increased sharply. The total increase was at least 10-fold with the peak being reached at 2 hours and this was followed by a steady decline until by 5 hours the message was not detectable with the exposure times we used.

The particular polymerase I fragment we used as a probe (see Materials and Methods) hybridised to a second transcript of some 2.5 Kb which was presumably derived from a gene adjacent to polymerase I. Unlike polymerase I, however, this transcript rapidly declined in amount during meiosis as do a number of other transcripts (1) which emphasises the specific meiotic response of polymerase I. So far we have been unable to find an invariant message to use as an internal control for these experiments. However, the results are not likely to be artefactual since the many transcripts we have examined (1) showed specific and reproducible responses which varied widely in profile, rather than the similarities one might expect in the event of RNA extraction or blotting artefacts.

To ensure that the polymerase I transcript profile was meiosis-specific, RNA was also analysed from asporogeneous MATa/MATa and MATα/MATα diploids. Both showed quite different patterns of polymerase I expression from those in SK-1 itself. In the first case the polymerase I message declined from the outset and had virtually disappeared by about 1 hour (Fig. 3B) while in the MATα/MATα it was present at a constant but rather low level throughout the experiment (result not shown). We have previously noted differences of this sort between MATa/MATa and MATα/MATα diploids (1) and it is interesting to note that the unknown 2.5 Kb transcript also remains at a constant level in the MATα/MATα but declines in the MATa diploid, albeit at a slower rate than polymerase I. Taken together, these results clearly confirm that the expression of polymerase I is under some form of meiotic control.

The DNA polymerase I transcript is induced by UV-light

A polymerase is required for the post-excision gap filling in DNA repair and we therefore determined whether polymerase I is induced after DNA damage. We have previously shown that the induction of DNA ligase was most dramatic in yeast cells irradiated in stationary phase (3), however the polymerase I transcript is barely detectable in stationary phase cells and mid-log cells were therefore UV-irradiated. After irradiation, the cells were returned to fresh medium, sampled at intervals and the RNA extracted for Northern hybridisation analysis (Fig.4). A marked increase in the level of the polymerase I transcript was observed, starting some 30 minutes after irradiation and

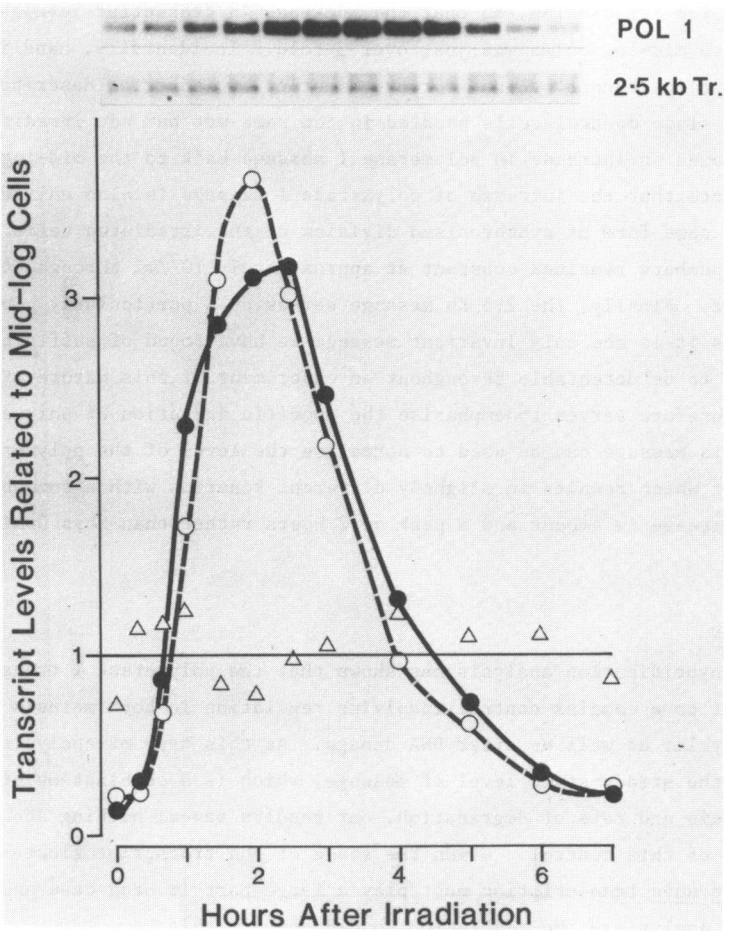


Figure 4. Behaviour of the DNA polymerase I transcript after UV-irradiation. Mid-log phase cells of strain MC16 were irradiated with 50J/$m^2$ and after resuspension in fresh medium, they were incubated in the dark. Samples were removed at intervals over a seven hour period and a Northern blot was prepared. See the legend to Fig. 1. ● DNA polymerase I transcript relative to the level in mid-log cells; △ unknown 2.5 Kb transcript relative to the level in mid-log cells; ○ DNA polymerase I transcript normalised with respect to levels of the 2.5 Kb transcript.

reaching a peak at 2.5 hours. This was followed by a decline to basal levels indicating that the increase was a specific response to the irradiation. The absolute increase in polymerase I transcript levels was estimated to be approximately 20-fold. However, the polymerase I transcript appears to be rather unstable and a marked drop in level occurred during preparation of

the cells for irradiation, so that the increase in transcript levels relative to mid-log cells was just over 3-fold. Incidentally, handling of the cells alone cannot account for the kinetics of induction described in Figure 4, since control cells handled in the same way but not irradiated simply showed an increase in polymerase I message back to the mid-log phase level. Note that the increase of polymerase I message is also unlikely to be due to some form of synchronised division of the irradiated cells since the cell numbers remained constant at approximately $10^7$/ml throughout the experiment. Finally, the 2.5 Kb message serves as a particularly good control as it is the only invariant message we have found of sufficient stability to be detectable throughout an experiment of this nature (Fig.4) and it therefore serves to emphasise the specific induction of polymerase I. Indeed this message can be used to normalise the level of the polymerase I transcript which results in slightly different kinetics with a somewhat larger increase in amount and a peak at 2 hours rather than 2hrs 30mins (Fig. 4).

## DISCUSSION

Northern hybridisation analysis has shown that the polymerase I transcript is subject to a complex control involving regulation in both meiosis and the mitotic cycle, as well as after DNA damage. As this type of analysis only measures the steady state level of message, which is a combination of rate of synthesis and rate of degradation, our results reveal nothing about the mechanism of this control. Given the scale of the transcript fluctuations, however, *de novo* transcription must play a large part in each case and a molecular analysis of the regulation should confirm this.

In the mitotic cell cycle the polymerase I message showed a sharp fluctuation in amount with a peak at around the G1/S phase boundary. Altogether six genes essential for ongoing DNA synthesis are now known to be regulated in the cell cycle (Table 1). In addition to polymerase I, they include the three *CDC* genes mentioned above, together with genes for DNA primase (unpubl. obs.) the enzyme required for starting synthesis of Okazaki fragments, and ribonucleotide reductase (18). In the latter case only enzyme assay data is available, but with the other five genes we have used RNA prepared from the same synchronous cultures to show that their expression occurs in the same interval of the cell cycle (Figs. 1 and 2; ref.11; unpubl. obs.). The fact that in the *cdc4*-block experiment all five genes showed a similar profile emphasises their simultaneous expression and

Table 1. Regulation of genes essential for DNA synthesis in the mitotic cell cycle.

| Gene | Gene product | Periodically expressed in late G1/S | Reference |
|---|---|---|---|
| CDC8 | Thymidylate kinase | + | 11 |
| CDC9 | DNA ligase | + | 2,19 |
| CDC21 | Thymidylate synthase | + | 11,20 |
| POL1 | DNA polymerase I | + | This paper |
| PRI1 | DNA primase | + | Unpubl. obs. |
| DBF1 | Not known | - | J. Chapman pers. comm. |
| - | Ribonucleotide reductase[a] | + | 18 |

a. Based on enzyme assays alone (see text).

suggests that they may be co-regulated. However, whether the regulatory pathways are identical for each of them is uncertain. We have compared the DNA sequences of CDC8, CDC9 and CDC21 and found few similarities between the upstream regions of the genes and therefore different regulatory proteins may be involved. Indeed preliminary gel retardation assays suggest that at least some of the proteins binding upstream of the CDC genes are different (unpubl. obs.). Possibly, therefore, DNA synthesis genes in yeast may have independently evolved to respond to the same physiological signal.
It is important to note, however, that not all DNA synthesis genes are regulated in this way. Thus, the DBF1 gene, essential for ongoing DNA synthesis(21), has recently been examined in this laboratory and its transcript does not fluctuate during the cell cycle, but is present at a constant level (J. Chapman, pers.comm.). The six DNA synthesis genes which are cell cycle regulated (Table 1) include enzymes required for most aspects of replication (precursor production, polymerisation, ligation of intermediates) so it is puzzling that other DNA synthesis genes are not similarly regulated.

The DBF1 gene product has not yet been identified, but one possibility is that it is essential at several points in the cell cycle rather than in DNA synthesis alone, so that it is produced constitutively. Alternatively, proteins from periodically expressed genes could all be part of the same replication complex.

Polymerase I message also shows a fluctuation in level during meiosis as do the three CDC genes (1). Studies on the corresponding cdc mutants have shown that these genes are all essential for meiosis (22) and while there is at present no such data for polymerase I, our results strongly suggest that polymerase I is responsible for premeiotic DNA synthesis. It should be emphasised that although polymerase I and the three CDC genes all show transcript fluctuations in meiosis, the kinetics differ from one another (Fig. 3 and ref. 1) in contrast to the situation in vegetative cells. This might reflect different control mechanisms in meiosis or simply differences in message half-life.

The polymerase I message clearly responds to DNA damage, there being a 20-fold increase after UV-irradiation at $50J/m^2$. This implies that it is induced by the lesions introduced into the DNA and there are two main possibilities to account for the increase. One is the existence of a mechanism specific for activation of the gene in response to DNA damage, quite independent of its regulation in the cell cycle. A number of other yeast genes also seem to be induced after DNA damage, including the CDC9 DNA ligase (3,19), RAD2 (23), the DIN genes (24) and the DDR genes (25). However, no evidence has yet been obtained for an inducible repair system in yeast analogous to the SOS response in E.coli and these genes are therefore possibly triggered by separate mechanisms, independently of each other. In the case of genes regulated in the cell cycle such as DNA polymerase I and CDC9, expression after DNA damage may in some way utilise the existing cell cycle controls. Indeed a repair specific stage in the cell cycle has been postulated (26,27), although this is believed to occur in G2, well after the normal expression of polymerase I. Conceivably, however, conditions in irradiated cells might somehow resemble those which occur in G1/S phase cells thus allowing the normal cell cycle expression of polymerase I. In this event the apparent induction of polymerase I following irradiation could equally be an indirect consequence of the DNA damage, simply mediated by a cell cycle block, and not associated with DNA repair at all. In an attempt to eliminate this possibility we have irradiated a cdc28 mutant held at 37°C and therefore blocked early in G1 before the point at

which the usual cell cycle expression of polymerase I occurs. This resulted in a normal induction of the polymerase I message (data to be presented elsewhere), strongly suggesting that the increase due to DNA damage is independent of the cell cycle regulation. Moreover, we have recently found that DNA primase, which is also cell cycle regulated in late G1 (see above), is not induced by UV-irradiation (unpubl. obs.). It therefore seems likely that the induction of polymerase I by UV-light is a specific and direct response to DNA damage rather than simply being due to a secondary cell cycle effect.

The induction of an enzyme (or its transcript) in a given process surely implies a role in that process. By this criterion DNA polymerase I is involved not only in DNA repair but also in DNA replication, both in mitosis and meiosis. What then can be the role of DNA polymerase II? Of course we have not investigated all aspects of DNA metabolism and it may play a specific part in, for example, recombination or in other types of DNA repair, such as excision of mismatched bases (it has an associated exonuclease activity). Alternatively, there may be be considerable overlap in the function of eukaryotic DNA polymerases. However, at present there is no firm information on this in any eukaryote, but the cloned yeast polymerase I gene will allow the isolation of conditional mutants which should help resolve this question.

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