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Periodic transcription as a means of regulating gene expression during the cell cycle: contrasting modes of expression of DNA ligase genes in budding and fission yeast

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Using cultures synchronised by three independent procedures, we have shown that the *CDC9* gene, coding for DNA ligase, is periodically expressed in the *Saccharomyces cerevisiae* cell cycle. The level of *CDC9* transcript increases many fold in late G1 reaching a peak at about the G1/S phase boundary and preceding the peak in histone message by some 20 min. The level of DNA ligase itself also fluctuates, showing the expected pattern for a stable enzyme synthesised periodically. In contrast, the transcript from the DNA ligase gene (*CDC17*) of *Schizosaccharomyces pombe* is present at a constant level throughout the cell cycle, and no fluctuation in amount was detected, although the histone H2A showed the expected periodic synthesis. Furthermore, DNA ligase activity remains at a constant level during the *S. pombe* cell cycle showing that there is unlikely to be any form of translational control. These contrasting modes of expression of the DNA ligase genes in the two organisms suggests that when periodic transcription is observed from an essential cell cycle gene, it may have no particular significance for regulating progress through the cell cycle. Also, regulatory circuits may be less well conserved between organisms than the processes they control and thus different organisms may utilise quite different modes of control to achieve the same ends.

Key words: cell cycle regulation/DNA ligase genes/*Saccharomyces cerevisiae*/*Schizosaccharomyces pombe*/RNA blot

Introduction

The yeast cell cycle has been subjected to an extensive genetic analysis which has resulted in the isolation of a large number of cell cycle (*CDC*) mutants (for a review, see Pringle and Hartwell, 1981). Although the role of the great majority of these genes is not yet known, they presumably provide essential functions in various discontinuous processes of the cell cycle. It is also likely that the products of many of them will be required during only a limited period of the cycle. Progress through the cycle may therefore depend on the orderly function of these *CDC* gene products which, in turn, may be reflected in the pattern of *CDC* gene expression. Periodic transcription provides one means by which such genes could be controlled with gene activation confined to a short period prior to, or during, the phase of the cell cycle for which the gene product is required.

DNA ligase is an example of a well characterised enzyme which might be expressed in this way since it is required principally in S phase. Temperature-sensitive mutants defective in this enzyme have been identified amongst *CDC* mutants in two yeasts, *cdc9* in *Saccharomyces cerevisiae* and *cdc17* in *Schizosaccharomyces pombe*. Both of these mutants have a defect in joining Okazaki fragments, which gives rise to the characteristic *cdc* phenotype and both are sensitive to agents which damage DNA. This indicates that the enzyme fulfils the same role in both organisms which is underlined by the ability of the cloned *CDC9* gene to complement the *cdc17* defect (Barker and Johnston, 1983). However, the two yeasts differ substantially in the organisation of their cell cycles (Nurse, 1985) and molecular studies have revealed significant differences in gene organisation. In view of this, we decided to investigate whether the similarities between DNA ligase in the two yeasts would extend to their regulation and, in particular, whether both would be periodically transcribed. Peterson *et al.* (1985) used synchronous cultures prepared using α-factor and size selection in sucrose gradients to infer that *CDC9* may be periodically transcribed. We have now confirmed this by using elutriation and feed-starve synchrony, and have gone on to accurately time *CDC9* transcription with respect to the S phase and histone transcription. In marked contrast, we find that the *CDC17* gene in fission yeast is not periodically transcribed or translated, a result which raises interesting questions concerning the significance of periodic transcription as a means of regulating events in the eukaryotic cell cycle.

Results

Expression of CDC9 in the S. cerevisiae cell cycle

To confirm the periodic transcription of the *CDC9* message, initially reported by Peterson *et al.* (1985), and to establish its precise timing, three different synchronisation methods were used. Firstly, *S. cerevisiae* cells were synchronised using the feed-starve procedure of Williamson and Scopes (1962) (Figure 1). Altogether two highly synchronous rounds of division were followed and the high degree of synchrony obtained is reflected in the budding profile, which shows a sharp increase in the number of buds from 0% to 95% over a 30 min interval. Budding commenced at 50 min into the experiment and, as expected, was exactly coincident with the onset of DNA synthesis in the first cell cycle (Williamson, 1973). Total RNA was extracted at intervals throughout the experiment and the relative abundance of specific mRNAs assessed by Northern hybridisation techniques plus densitometric quantitation. The *CDC9* transcript is indeed periodic showing an enormous increase in amount with two sharp peaks evident, exactly one cell cycle apart, and even the beginnings of a third fluctuation is evident. The peak occurs in late G1, at about the onset of DNA synthesis, although this timing is somewhat obscured in the second cycle, due to a loss of synchrony.

The same Northern blot was probed with the histone gene H2A, together with an adjacent gene of unknown function 'Protein 1'

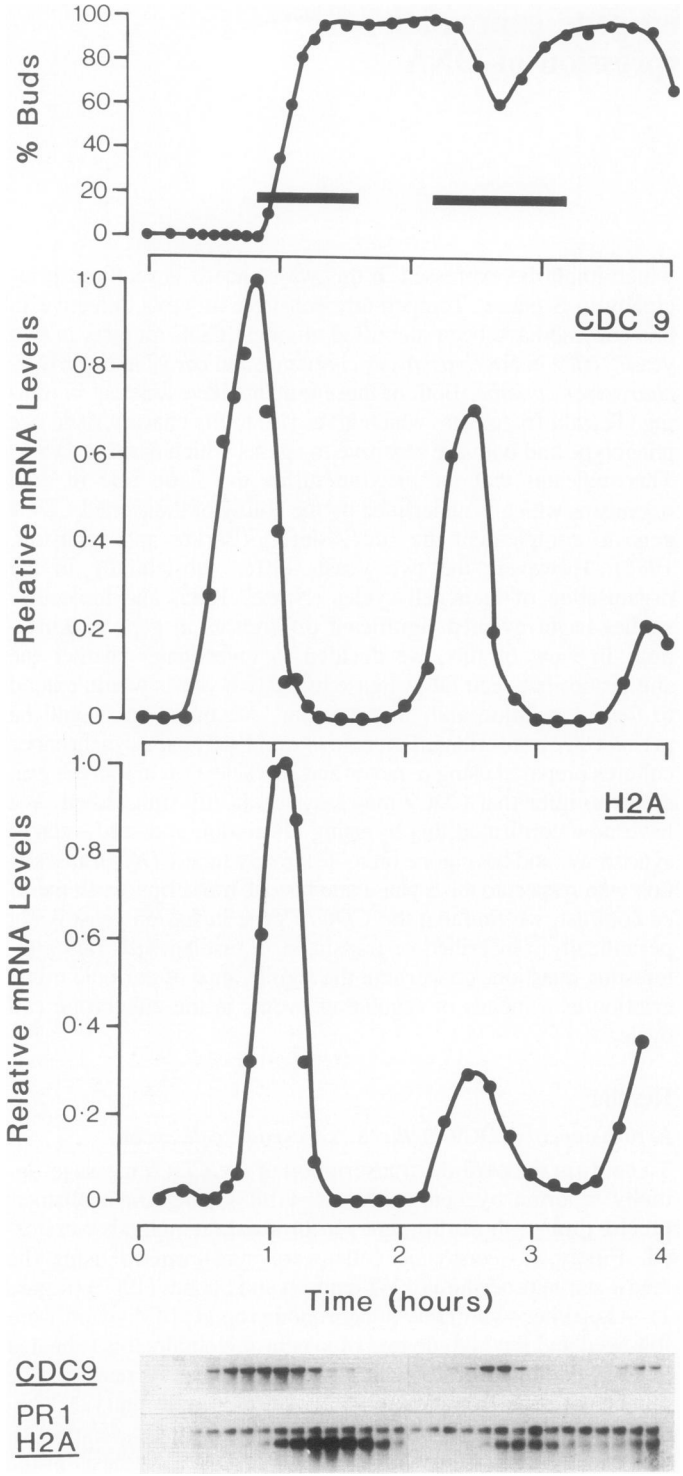

Fig. 1. Expression of the *CDC9* gene transcript in the cell cycle: feed–starve synchrony. Cells were harvested at a number of points through the experiment. Total RNA was extracted from them, and a Northern blot prepared (see Materials and methods). The blot was then probed with ^{32}P-oligolabelled fragments of *CDC9* and H2A/Protein 1, and the resulting autoradiographs were quantitated using densitometry. The upper panel shows the budding profile and the period of DNA synthesis (▬▬) in the culture determined by incorporation of [^{3}H]adenine. The lower panels show the Northern blots and their quantitation.

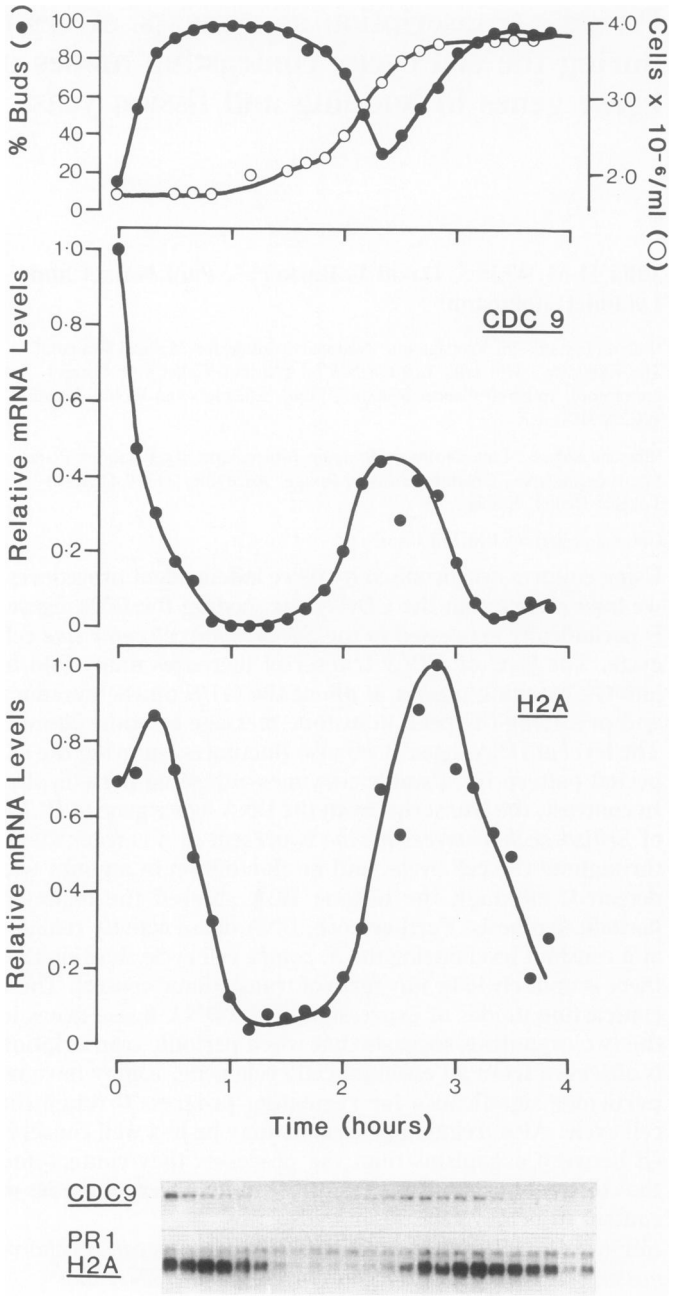

Fig. 2. Expression of the *CDC9* gene transcript in the cell cycle: elutriation synchrony. The upper panel shows bud counts (●) and cell numbers (○), while the Northern blots and profiles of the *CDC9* and H2A transcript levels are shown in the lower panels. As can be seen from the *CDC9* message, sampling of the culture commenced slightly after the start of the first cycle.

(Hereford *et al.*, 1979). H2A is already known to be periodically transcribed, reaching a peak in mid S-phase, whilst the Protein 1 transcript remains at a constant level (Hereford *et al.*, 1981). Our results confirm these observations and, more importantly, show that the *CDC9* message peaks earlier in the cycle than H2A. Moreover, the fact that Protein 1 remains invariant throughout the cell cycle rules out the possibility that our results are due to an experimental artefact such as uneven loading of the gel.

Despite the excellent synchrony obtained using the feed–starve method, it could be argued that this technique leads to physiologically perturbed cells. Two additional cell cycle experiments were therefore carried out using alternative methods of synchronisation, namely size selection by elutriation, and α-factor

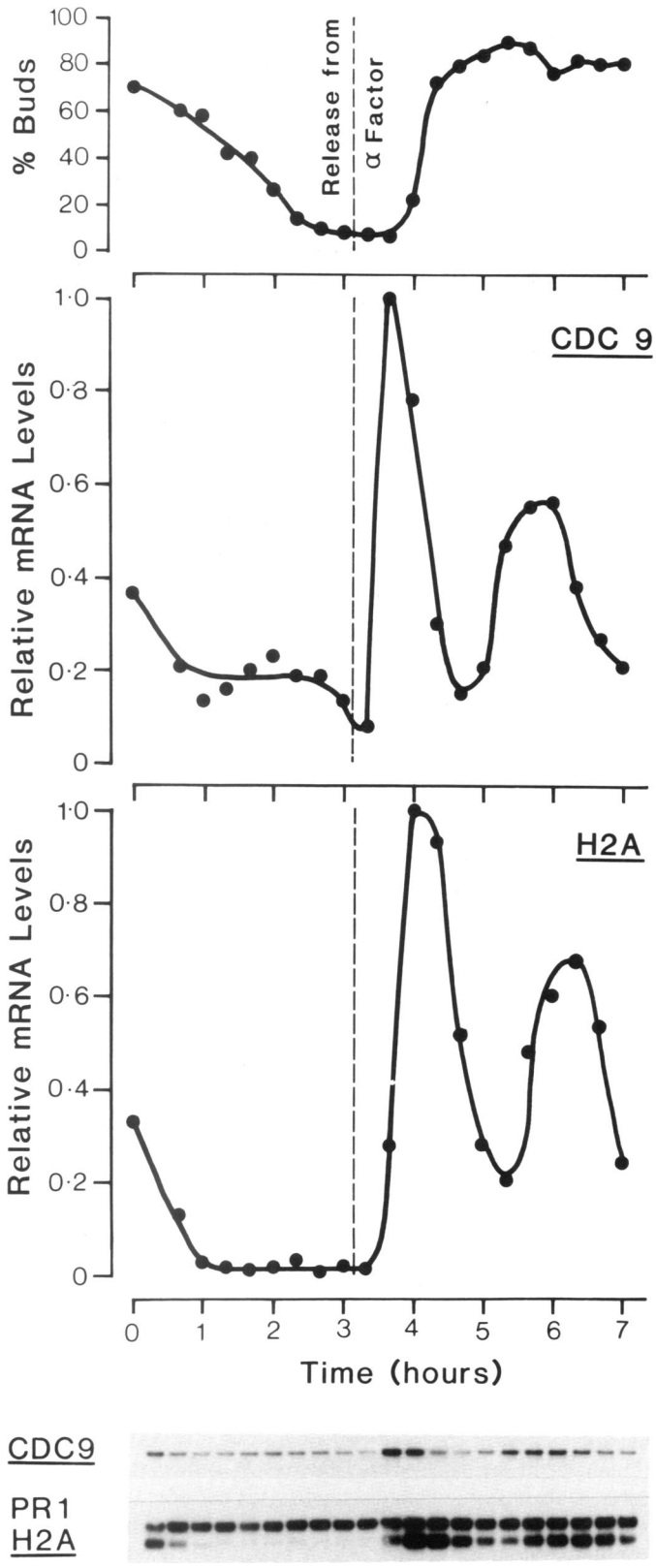

Fig. 3. Expression of the *CDC9* gene transcript in the cell cycle: α-factor release synchrony. The upper panel shows the budding profile of the culture, whilst the lower panels show the Northern blots and the *CDC9* and H2A transcript profiles.

release. Of all the synchronisation methods used, elutriation probably imposes least stress upon cells, whilst with α-factor, the cells were held in the pheromone for only one generation to

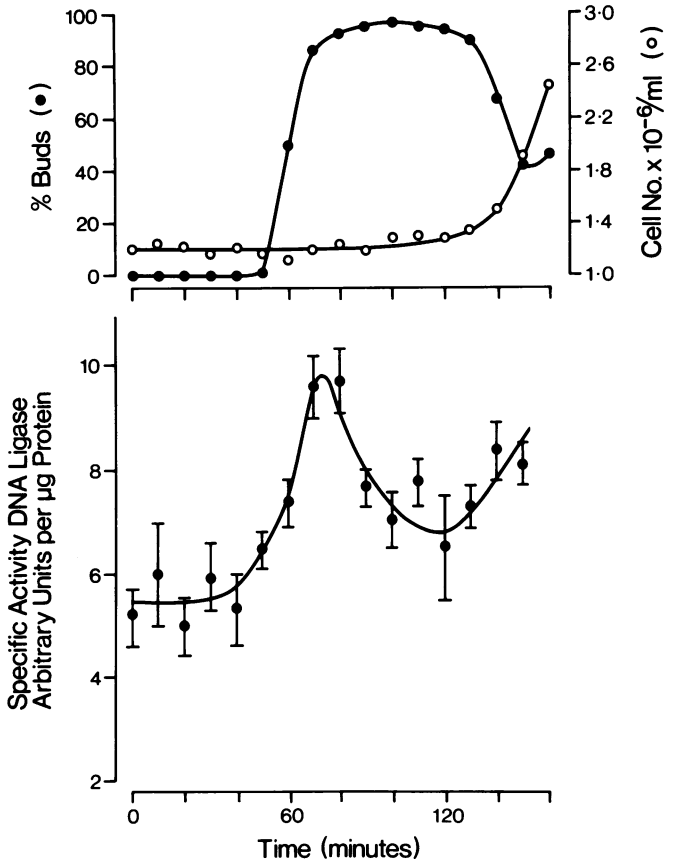

Fig. 4. DNA ligase levels in the *S. cerevisiae* cell cycle. Cells were lysed and crude extracts assayed for DNA ligase activity as described in Materials and methods. The upper panel shows the budding profile of the culture, while the lower panel shows the levels of DNA ligase. The error bars indicate the range of values obtained from three experiments.

minimise perturbations. Both methods produced good synchronous cultures, analysis of which confirmed the earlier results, again over two cell cycles (Figures 2 and 3). The *CDC9* and H2A messages showed sharp fluctuations, with the *CDC9* peak preceding that of H2A in each case. Both experiments gave a lower overall increase in *CDC9* message than the feed–starve technique, reflecting the lesser degree of synchrony attained. While it seems most plausible that the abrupt increase in *CDC9* mRNA is due to transcription, our experiments do not rule out a sudden change in message half-life and we hope to investigate this using *in vivo* labelling at a later date.

Levels of DNA ligase in the S. cerevisiae cell cycle

Having characterised levels of *CDC9* message in the cell cycle, we next examined levels of the *CDC9* gene product, DNA ligase. Using a sensitive assay, providing accurate quantitation in crude extracts of yeast (D.G.Barker *et al.*, 1985), the enzyme was assayed at intervals throughout the first cycle of a feed–starve synchronised culture (Figure 4). The *CDC9* ligase is extremely stable *in vivo* (unpublished observations) so that the initial level of the enzyme was high in this experiment. The specific activity of the enzyme then remained approximately constant until the beginning of S phase, when a 1.8-fold increase occurred, followed by a steady decline in specific activity for the remainder of the cycle. This increase is less than the expected doubling in specific activity for a stable enzyme which is periodically synthesised reflecting the less than perfect synchrony in the culture.

Expression of CDC17 in the S. pombe cell cycle

To assess the significance of the periodic transcription of *CDC9*

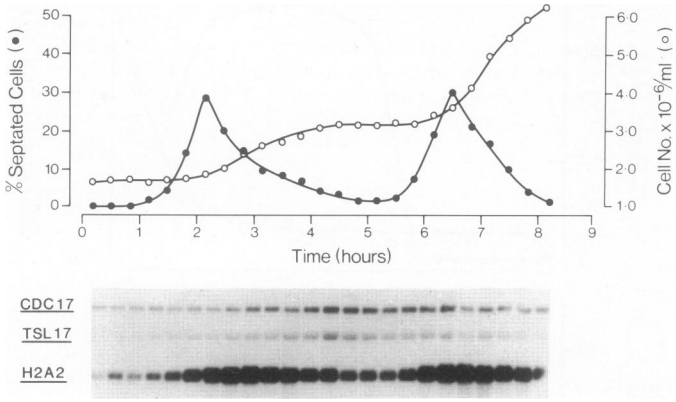

Fig. 5. *CDC17* transcript levels throughout the *S. pombe* cell cycle at 25°C. *S. pombe* cells were synchronised by means of an elutriator rotor as described by Aves *et al.* (1985). Cells were harvested at a number of points throughout the experiment, RNA extracted, and a Northern blot prepared. The upper panel shows the cell numbers and the percentage septated cells. The lower panel shows the result of probing the Northern blot with ^{32}P-oligolabelled fragments of *CDC17*/*TSL17* and H2A genes.

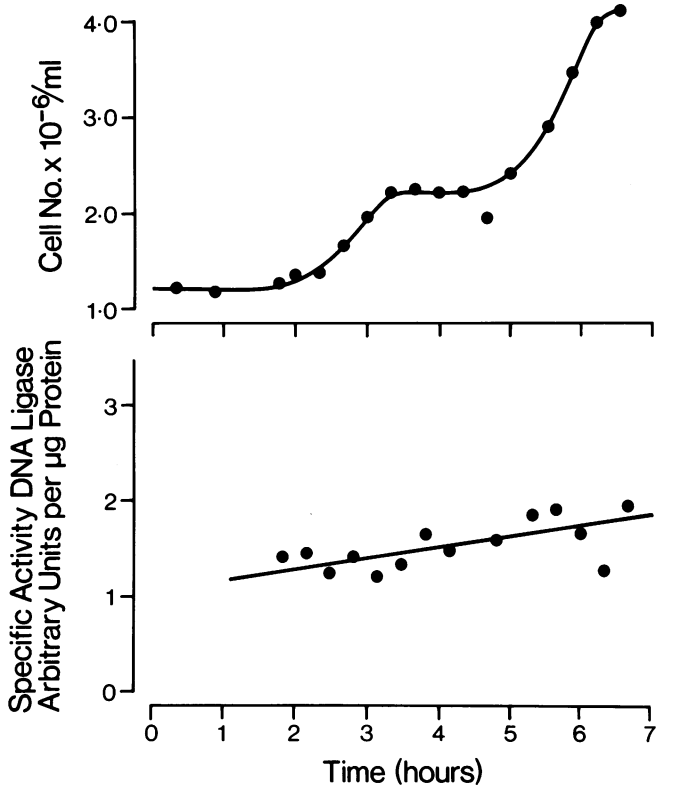

Fig. 6. DNA ligase activity during the *S. pombe* cell cycle at 30°C. A synchronous culture of *S. pombe* cells were prepared using an elutriator rotor. Samples were removed and the DNA ligase activity was assayed as described in Materials and methods. The upper panel shows the increase in cell numbers in the culture and the lower panel shows the levels of DNA ligase activity per unit soluble protein in the crude cell extracts.

described above, the expression of the corresponding DNA ligase gene, *CDC17*, in fission yeast was examined. Cells were synchronised by elutriation and the level of *CDC17* and histone H2A messages were followed over two cell cycles (Figure 5). As expected, H2A is clearly under cell cycle control and showed two sharp fluctuations in level, thus confirming the high degree of synchrony in this culture. Yet, surprisingly, the *CDC17* transcript showed no fluctuation in level comparable with *CDC9*. Instead the message increased slightly during the first cell cycle and then maintained a constant level throughout the second. We believe this profile of *CDC17* mRNA reflects the slight perturbation of the cells during passage through the elutriator rotor, and their subsequent recovery after one cycle to give a constant level of message. Moreover, the *CDC17* transcript profile is followed precisely by the transcript from *TSL17*, a gene of unknown function adjacent to the *CDC17* gene (D.G.Barker, J.H.M.White and L.H.Johnston, in preparation), thus providing further evidence that *CDC17* is not under any form of specific cell cycle control.

The CDC17 DNA ligase in S. pombe is not translationally controlled

In view of the difference in transcription pattern from the DNA ligase genes in the two yeasts, it was of particular interest to determine whether the enzyme in *S. pombe* is subject to periodic translational control. The enzyme was therefore assayed over two synchronous cell cycles and a gradual increase in specific activity was observed (Figure 6). We believe this to be due to the re-establishment of normal levels of DNA ligase after perturbation of the cells during elutriation as was the case with the mRNA. Certainly there was no evidence of periodicity in the enzyme and in a normal cell cycle it is likely to be present at a constant level. Therefore in *S. pombe*, DNA ligase does not appear to be subject to any form of specific transcriptional or translational control.

Discussion

Three different techniques of synchronisation, feed – starve, α-factor and size selection by elutriation have been used to show that the *CDC9* message is periodically synthesised in the mitotic cell cycle of *S. cerevisiae*. All have given consistent results showing clearly that the *CDC9* transcript reaches a sharp peak in late G1 just before the onset of DNA synthesis. At this stage in the cell cycle there are few, if any, breaks in the DNA (Johnston and Williamson, 1978), so that DNA strand breaks *per se* cannot be acting as the trigger for the induction.

The timing of this periodic transcription of *CDC9* was confirmed by comparing it with histone gene transcription, which is known to occur in early to mid S phase (Hereford *et al.*, 1981). With cells synchronised using all three methods, the *CDC9* fluctuation obviously preceded that of histone H2A, so presumably the two genes are subject to different control circuits.

In our synchronised cultures of *S. pombe* the fluctuating levels of histone mRNA can also be very clearly followed throughout two cell cycles. Unexpectedly, however, the *S. pombe* DNA ligase message is not periodic but is present at a constant level throughout both cycles. Moreover, in *S. pombe* the specific activity of DNA ligase itself does not change dramatically during the cell cycle, whereas in *S. cerevisiae* it increases shortly after the fluctuation in message and then gradually declines for the remainder of the cycle. This latter result is the expected pattern for a stable enzyme which is synthesised periodically. Thus our results are consistent with *CDC17* being transcribed and translated continuously in *S. pombe*, which is in marked contrast to the behaviour of *CDC9* in *S. cerevisiae*.

This difference is all the more striking considering that DNA ligase fulfils the same function in the two yeasts (see Introduction) and that both are relatively fast growing organisms with similar lifestyles in the wild. These findings strongly suggest that periodic transcription is not necessarily of particular significance as a means of controlling expression of essential cell cycle genes. In this respect, it is worth noting that several genes governing

START in the yeast cell cycle, and whose products are presumably required transiently, also do not show periodic changes in mRNA level, so that their activity must be controlled in some other way.

Indeed, it is not clear why a stable enzyme such as the DNA ligase in *S. cerevisiae* should be periodically transcribed at all. In experiments to be reported elsewhere we will show that *CDC9* in *S. cerevisiae* is periodically transcribed at the same time as two other genes required for DNA synthesis, *CDC8* and *CDC21*. Possibly all DNA replication genes are controlled in this way and *CDC9* may simply be regulated as part of this system. In *S. pombe* this association could have been broken (if *CDC9* were negatively regulated, mutation would lead to constitutive expression) or possibly DNA synthesis genes are not co-ordinately controlled in this organism.

This difference between *CDC9* and *CDC17* also suggests that regulatory circuits may be less well conserved between organisms than the processes they control, at least for stable enzymes/proteins. Thus different organisms may utilise quite different modes of control to achieve the same ends. It is of interest to know whether a particular control mode can be tolerated in an organism which normally utilises a different mode. This can now be investigated by studying the pattern of expression of *CDC9* in *S. pombe* and *CDC17* in *S. cerevisiae*.

Materials and methods

Strains and media

S. cerevisiae NCYC 239 is a prototrophic diploid and *S. cerevisiae* L129-13C is *Mata his7 lys2 trp1*. The *S. pombe* strain used was 972 h^-.

Strains were grown in YEPD (2% yeast extract, 1% peptone and 2% glucose) as rich medium. Minimal medium was either Wickerham's minimal salts (Wickerham, 1951), or Difco yeast nitrogen base (YNB) without amino acids (0.67% YNB, 0.5% glucose), with appropriate nutritional supplements at 20 μg/ml. *S. pombe* strains were grown on the minimal medium EMM2 as modified by Nurse (1975). Bud counts in *S. cerevisiae* and septated cell counts in *S. pombe* were determined microscopically and cell numbers were monitored by means of a Coulter counter.

Synchronization procedures

S. cerevisiae 239 cells were used for synchronisation by the protocol of Williamson and Scopes (1962), which essentially involves alternatively feeding and starving cells from a stationary phase culture. The same strain was also used for synchronisation by passage through a Beckman JEIOX Elutriator rotor, according to the method described by Aves *et al.* (1985). Four litres of culture at 1×10^7 cells/ml in YNB at 25°C were pumped into the rotor, which was spinning at 2100 r.p.m. During the separation, cells were maintained at 25°C and continually flushed with medium. After loading and separation the pump rate was increased and a fraction of small G1 cells at the beginning of the cell cycle were eluted off. A total of 1 litre of cells at 2×10^6 cells/ml were collected.

S. cerevisiae L129-13C was synchronised by release from an α-factor-induced cell cycle block. Cells were grown in Wickerhams minimal medium to a density of 1×10^7 cells/ml at 25°C. α-factor (Sigma) was added to a final concentration of 3 μg/ml, and the cells incubated for a further 190 min. The α-factor was removed by rapid filtration and washing of the cells, and finally resuspending them in an equal volume of fresh medium.

S. pombe cells were synchronised using the elutriation method alone, exactly as described by Aves *et al.* (1985).

DNA synthesis measurements

10 ml of culture (i.e. 10^8 cells) were removed immediately after synchronisation, and [8-^{3}H]adenine (Amersham; 22 Ci/mmol) was added to a final concentration of 10 μCi/ml. Incorporation of the label into DNA was determined as described previously (Johnston and Game, 1978).

Preparation of total RNA

10^8 cells were harvested, washed in saline and rapidly frozen in dry ice. Total RNA was extracted using a hot phenol protocol, as described by Aves *et al.* (1985). The extracted RNA was quantitated by measuring the $O.D._{260}$, and the concentration was adjusted to 1 mg/ml.

Northern blot analysis

5 μg of total RNA was denatured with glyoxal, fractionated according to size by electrophoresis in 1.5% agarose gels in 10 mM sodium phosphate buffer (pH 6.5) and then transferred to 'Genescreen' hybridisation transfer membrane (New England Nuclear) according to the manufacturers instructions. Subsequent treatment of the filter, hybridisation, washing and rehybridisations was also carried out according to the manufacturer's instructions. Probe DNA was labelled with [^{32}P]dTTP (3000 Ci/mmol; New England Nuclear) to an approximate specific activity of 10^9 c.p.m./μg using the oligolabelling protocol of Feinberg and Vogelstein (1983). The 1.69-kb *PstI*–*SacI* *CDC9* probe was derived from the plasmid pR12Sclig2 (Barker and Johnston, 1983). The *S. cerevisiae* H2A/Protein 1 probe was produced from the 2.3-kb *SacI* fragment of the plasmid TRT1 (Hereford *et al.*, 1979). The 4.2-kb *Hin*dIII–*Bgl*II fragment from the plasmid H2-YEp13 (Johnston *et al.*, 1986) was used to probe for *CDC17/TSL17* mRNA of *S. pombe*, whilst the H2A2 probe was derived from a 1.14-kb *Hin*dIII fragment from the plasmid pSJM211 (Matsumoto and Yanagida, 1985).

After hybridisation, the blots were autoradiographed at −70°C for between 1 and 3 days using Fuji RX X-ray film, together with 'X-ograph' Hi-Speed-X intensifying screens. Several exposures were carried out for each experiment so that every RNA of interest was within the exposure range of the film. Densitometric quantitation of the resulting autoradiographs was carried out using a Joyce Loebl Chromoscan 3 Densitometer.

DNA ligase assays

DNA ligase activity was measured in crude cell extracts of yeast, prepared from $\sim 2 \times 10^8$ cells, disrupted by vigorous mixing with glass beads (500 μm) on a Whirlimixer. The protein concentration was determined by the method of Lowry *et al.* (1951), and DNA ligase activity was assayed according to the method of Barker *et al.* (1985). In this method, a single-stranded nick is generated in a region of double-stranded DNA by annealing two adjacent oligonucleotides (15-mer and ^{32}P 5′-end labelled 17-mer) with a single-stranded M13 template. DNA ligase catalyses the sealing of the nick and thus generates a ^{32}P-labelled 32-mer oligonucleotide, which is separated from the substrate by gel electrophoresis. Activity is quantitated by excising the region of the gel containing the 32-mer and Cerenkov counting. All assays were carried out at 25°C, and under conditions in which there is a linear relationship between enzyme activity and yield of 32-mer product.

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References

Aves,S.J., Durkacz,B.W., Carr,A. and Nurse,P. (1985) *EMBO J.*, **4**, 457–463.
Barker,D.G. and Johnston,L.H. (1983) *Eur. J. Biochem.*, **134**, 315–319.
Barker,D.G., Johnson,A.L. and Johnston,L.H. (1985) *Mol. Gen. Genet.*, **200**, 458–462.
Feinberg,A.P. and Vogelstein,B. (1983) *Anal. Biochem.*, **132**, 6–13.
Hereford,L., Fahrner,K., Woolford,J.,Jr, Rosbash,M. and Kaback,D.B. (1979) *Cell*, **18**, 1261–1271.
Hereford,L.M., Osley,M.A., Ludwig,J.R.,II and McLaughlin,C.S. (1981) *Cell*, **24**, 367–375.
Johnston,L.H. and Game,J.C. (1978) *Mol. Gen. Genet.*, **161**, 205–214.
Johnston,L.H. and Williamson,D.H. (1978) *Mol. Gen. Genet.*, **164**, 217–225.
Johnston,L.H., Barker,D.G. and Nurse,P. (1986) *Gene*, **41**, 321–325.
Lowry,O.H., Rosebrough,N.J., Farr,A.L. and Randall,R.J. (1951) *J. Biol. Chem.*, **193**, 265–275.
Matsumoto,S. and Yanagida,M. (1985) *EMBO J.*, **4**, 3531–3538.
Nurse,P. (1975) *Nature*, **256**, 547–551.
Nurse,P. (1985) *Trends Genet.*, **1**, 51–55.
Peterson,T.A., Prakash,L., Prakash,S., Osley,M.A. and Reed,S.I. (1985) *Mol. Cell. Biol.*, **5**, 226–235.
Pringle,J. and Hartwell,L. (1981) *The Molecular Biology of the Yeast Saccharomyces*, Cold Spring Harbor Laboratory Press, NY.
Wickerham,L.J. (1951) *Technical Bulletin of the US Department of Agriculture*, 1029.
Williamson,D.H. (1973) *Biochem. Biophys. Res. Commun.*, **52**, 731–740.
Williamson,D.H. and Scopes,A.W. (1962) *Nature*, **193**, 256–257.

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