Characterization of *Saccharomyces cerevisiae* strains displaying high-level or low-level resistance to trichothecene antibiotics

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Biochemical and genetic analyses have been carried out on *Saccharomyces cerevisiae* strains characterized *in vivo* as sensitive, low-level-resistant or high-level-resistant to trichothecene antibiotics. Levels of drug resistance *in vitro* were determined for each strain and for suitable diploids derived from them. Ribosome biogenesis was also studied in selected haploids. It is suggested that resistance in all cases results from a mutation in the gene encoding ribosomal protein L3. If this is indeed the situation, then different mutations in this same gene not only can cause low-level or high-level resistance to trichothecene antibiotics but also can affect the maturation of either 40 S or 60 S ribosomal subunits.

INTRODUCTION

The trichothecene antibiotic trichodermin inhibits peptidyl transferase activity selectively on eukaryotic (80 S) ribosomes (for references see Carter & Cannon, 1977). High-level trichodermin resistance in *Saccharomyces cerevisiae* is controlled by the *tcm1* gene (Grant *et al.*, 1976) that encodes ribosomal protein L3 (Fried & Warner, 1981). Of particular interest are the *S. cerevisiae* mutants CLP-8 and TR-1. They carry allelic mutations giving high-level trichodermin resistance *in vivo* (Grant *et al.*, 1976) and display indistinguishable levels of trichodermin resistance *in vitro* (Carter *et al.*, 1980). Although it remains to be established unequivocally that CLP-8 and TR-1 carry identical alterations in ribosomal protein L3, there are indications that this is so from Threadgill *et al.* (1986), who purified ribosomal protein L3 from both mutants CLP-8 and TR-1 by h.p.l.c. and analysed the CNBr-cleavage products of the proteins. From this work an equivalent covalent modification was deduced for the two protein preparations.

Despite the above considerations, however, CLP-8 and TR-1 differ in at least one important respect. The former, but not the latter, shows a defect in the maturation of its 40 S ribosomal subunits (Carter & Cannon, 1980). However, although the trichodermin-resistant trait in CLP-8 can be segregated genetically from the lesion responsible for the defect in ribosome biogenesis, the reverse situation does not apply (see Cannon, 1982). By inference, therefore, the phenotypic differences shown by CLP-8 and TR-1 with respect to ribosome biogenesis seem likely to reflect the differences in the genotypic backgrounds of the two strains (Cannon, 1982), which must indeed be the case if the two mutants have exactly the same alteration in ribosomal protein L3. This interpretation is given further support by the work of Mitlin & Cannon (1984), in which ribosome biogenesis is perturbed by a shift in growth temperature from 30 °C to 36 °C in A224A, the parent of CLP-8, but not in Y166, the parent of TR-1.

In the present work we have studied these genetic influences more fully. We first isolated a new mutant from *S. cerevisiae* strain Y166, designated MC-1, that displays low-level resistance to trichothecene antibiotics *in vivo*. Genetic and biochemical analyses indicate that the mutation in MC-1 is allelic with those present in CLP-8 and TR-1. We have determined the effect of the

mutation in MC-1 on ribosome biogenesis for comparison with this process in the mutants CLP-8 and TR-1. Finally, we have applied the classical genetic techniques of diploid formation and tetrad analysis to assess more completely how the different genetic backgrounds of the *S. cerevisiae* strains Y166 and A224A can modulate the effect of *tcm1* gene mutations on ribosome biogenesis.

MATERIALS AND METHODS

Sources of chemicals

[2-¹⁴C]Uracil (50–60 mCi/mmol), [methyl-³H]methionine (10 Ci/mmol) and L-[U-¹⁴C]phenylalanine (522 mCi/mmol) were purchased from Amersham International. Sources of all other chemicals are cited in Mitlin & Cannon (1984) and Carter *et al.* (1980). Antibiotics were dissolved in 50% (v/v) dimethyl sulphoxide (Carter & Cannon, 1978).

Yeast cells and their maintenance and growth

The following strains of *S. cerevisiae* were from our laboratory culture collection: A224A (*a, leu-2, can-1*), CLP-8 (*a, leu-2, can-1, tcm-1*), Y166 (*α, trp-5, his-4*), TR-1 (*α, trp-5, his-4, tcm-1*). Strains were maintained at 28 °C on YEPD plates (Mitlin & Cannon, 1984) and cultured at the same temperature in liquid YEPD medium (Mitlin & Cannon, 1984).

Mutant isolation and studies on drug resistance *in vivo*

Strain MC-1 was a spontaneous mutant of *S. cerevisiae* obtained by plating Y166 cells on YEPD plates containing 5 µg of trichodermin/ml. The mutants MC-1, CLP-8 and TR-1 were grown in liquid YEPD medium containing various concentrations of trichodermin to check drug-resistance levels.

Genetic analysis of yeast strains

Matings were performed essentially as described by Mortimer & Hawthorne (1969). Diploid strains were formed by mixing suitable haploid cells (*a/α*) on YEPD plates, followed by selection on minimal medium (Udem & Warner, 1972) containing no amino acid additions. The diploids were sporulated by incubation for 3 days at 28 °C on pre-sporulation plates. Asci were treated with glusulase to digest cell walls, and the four sister spores were

Abbreviations used: pre-rRNP, pre-ribonucleoprotein; pre-rRNA, ribosomal precursor RNA.

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separated with a Singer Micromanipulator Mark III (Singer Instrument Co., Roadwater, Somerset, U.K.). The offspring from each haploid spore were subsequently cultured on YEPD plates and screened for trichodermin resistance and amino acid requirements *in vivo*. Cells were used as appropriate for biochemical analyses (see below).

Preparation of ribosomes and supernatant fractions for use in yeast cell-free systems

High-salt-washed 80 S ribosomes were prepared from both haploid and diploid yeast strains (see the text) and polyphenylalanine synthesis was assayed at 28 °C in reconstituted yeast cell-free systems (Carter *et al.*, 1980). Incorporation levels were in line with those reported by these authors.

Preparation of yeast spheroplasts

Yeast cells were grown and harvested and converted into metabolically active spheroplasts, at an incubation temperature of 28 °C (Carter *et al.*, 1980). Spheroplasts were allowed to recover at 28 °C for 2.5 h in YEPD medium containing 0.4 M-MgSO₄ for osmotic support.

Sucrose-gradient analysis of native ribosomal subunits from yeast spheroplasts

Conditions for these experiments are described in Mitlin & Cannon (1984), for spheroplasts previously allowed to recover in YEPD medium as described above.

Preparation of total cellular RNA from spheroplasts and its analysis by polyacrylamide-gel electrophoresis

Conditions for these experiments were essentially as described in Mitlin & Cannon (1984), except that incubation was at 28 °C and 'pulse' and 'chase' times were as indicated in the relevant Figure legend. Spheroplasts were initially allowed to recover in complete synthetic medium at 28 °C (cf. above sections), and subsequent operations were carried out in this same medium.

RESULTS AND DISCUSSION

The *S. cerevisiae* strains CLP-8 (Schindler *et al.*, 1974), TR-1 (Jimenez *et al.*, 1975) and MC-1 (the present work) are spontaneous trichodermin-resistant mutants. CLP-8 and TR-1 show, *in vivo*, essentially complete resistance to 20 µg of antibiotic/ml, whereas this same drug concentration inhibits growth of MC-1 by approx. 50% (results not shown). Accordingly, we consider CLP-8 and TR-1 as high-level-resistant mutants and MC-1 as a low-level-resistant mutant.

We first ascertained if resistance *in vivo* to trichodermin in MC-1 resulted from a ribosomal modification, by checking the ability of trichothecene antibiotics to inhibit protein synthesis *in vitro* not only in MC-1 but also in other *S. cerevisiae* strains, both haploid and diploid. The diploids A224A/Y166, A224A/TR-1, A224A/MC-1, CLP-8/TR-1, Y166/CLP-8 and CLP-8/MC-1 were formed, allowed to sporulate where required, and tetrads were dissected to yield haploid progeny. Cell-free systems, prepared from appropriate cells, were assayed for poly(U)-directed polyphenylalanine synthesis. To determine drug-resistance levels, various concentrations of fusarenon X rather than trichodermin were selected. CLP-8, TR-1 and MC-1 are all cross-resistant *in vivo* to this trichothecene antibiotic (results not shown). Furthermore, although trichodermin inhibits the poly(U) system in yeast relatively poorly (Schindler, 1974; Jimenez *et al.*, 1975), this system is, nevertheless, particularly sensitive to inhibition by fusarenon X, although the drugs are both selective inhibitors of the peptidyl transferase centre on eukaryotic ribosomes (Carter & Cannon, 1978). The results of

Fig. 1. Resistance to fusarenon X by ribosome preparations from haploid and diploid strains of *S. cerevisiae* used for synthesizing polyphenylalanine *in vitro*.

The sources of high-salt-washed 80 S ribosomes are indicated. Diploid cells are identified as A224A/TR-1 etc. Ribosome preparations were assayed for polyphenylalanine synthesis after addition of supernatant fraction from *S. cerevisiae* strain Y166 in the absence and presence of the fusarenon X concentrations indicated. All methods are described in the Materials and methods section or in relevant references provided therein. Results are expressed as percentage incorporation relative to 100% incorporation in control samples containing no added drug. Samples were assayed for polyphenylalanine synthesis after 10 min incubation at 28 °C.

these experiments are recorded in Fig. 1. High resistance levels *in vitro* to fusarenon X are shown by ribosomes from CLP-8, TR-1 and the diploid CLP-8/TR-1, as expected (Carter & Cannon, 1978; Carter *et al.*, 1980). Predictably, ribosomes from Y166, A224A and the diploid Y166/A224A are inhibited by the antibiotic. In line with our earlier results *in vivo*, MC-1 ribosomes show a lower resistance level to fusarenon X than that observed for ribosomes from either CLP-8 or TR-1, thus confirming that this strain is low-level-resistant to trichothecenes both *in vivo* and *in vitro*. Of particular importance is the result for the CLP-8/MC-1 diploid, which shows a resistance level, *in vitro*, intermediate between those of the constituent haploid strains. This indicates strongly that a changed structural gene for a ribosomal component confers fusarenon X resistance not only in CLP-8 but also in MC-1; resistance mediated by enzymic modification of a ribosomal structural component is not, apparently, involved.

It has been shown conclusively by Fried & Warner (1981) that high-level trichodermin resistance in *S. cerevisiae* is controlled by the gene (*tcml*) that encodes ribosomal protein L3. The mutation causing high-level trichodermin resistance in each of the strains CLP-8 and TR-1 maps in the *tcml* gene (Grant *et al.*, 1976), and these strains carry a covalent modification in ribosomal protein L3 (Threadgill *et al.*, 1986). We have now shown that MC-1 is low-level-resistant to trichothecene antibiotics by virtue of its possession of an altered ribosomal component. Alteration of either a rRNA or a ribosomal protein by a modification enzyme does not appear to be involved in MC-1 (see above), and a mutation within a rRNA gene is almost certainly excluded, since in yeast these are arranged as 100 tandemly repeated copies on chromosome XII (Petes, 1979); the *tcml* gene is located on chromosome XV (Grant *et al.*, 1976). It seems likely, therefore, that low-level trichodermin resistance in MC-1 is controlled, as in CLP-8 and TR-1, by an amino acid substitution in one ribosomal protein.

We proceeded to ascertain if there was an allelic relationship between the trichothecene-resistance genes in CLP-8 and MC-1. We dissected 100 complete tetrads from a CLP-8/MC-1 cross and tested the progeny for trichodermin-resistance levels *in vivo* by using liquid cultures containing 20 μ g of antibiotic/ml. For every complete tetrad tested, two spore cultures showed low-level trichodermin resistance and two spore cultures showed high-level trichodermin resistance. Although we concede that such genetic analyses do not provide absolute proof of allelism, our results strongly suggest that the trichothecene-resistance genes in CLP-8 and MC-1 are indeed allelic. Additional support for this interpretation is provided by published work on the genomic organization of yeast ribosomal-protein genes. These are not arranged in operons like those in *Escherichia coli*, and only two examples of physical linkage of yeast ribosomal-protein genes have been described (for references see Planta *et al.*, 1986). The *tcm1* gene shows no such linkage, and would thus be expected to segregate independently of all other ribosomal-protein genes in our genetic tests. Additionally, we have carried out experiments on a further mutant of *S. cerevisiae* designated NAR2b. This mutant is derived from Y166 by chemical mutagenesis, and like MC-1 is low-level-resistant to trichothecene antibiotics (Gonzalez *et al.*, 1981). These authors have claimed an allelic relationship between the low-level trichothecene-resistance gene in NAR2b and the *tcm1* gene of high-level trichothecene-resistant mutants. We have now shown (M. Cannon, unpublished work) that NAR2b and MC-1 have identical properties with respect to trichothecene-resistance levels both *in vivo* and *in vitro*. Resistance levels of the diploids CLP-8/MC-1 and CLP-8/NAR2b are identical, and spore progeny from a CLP-8/NAR2b cross give the same 2:2 segregation pattern for low-level and high-level trichothecene resistance as was observed for a CLP-8/MC-1 cross (see above). Furthermore, NAR2b shows the same defect in ribosome biogenesis that we shall shortly describe for MC-1 (see the next section). Taken together, all these considerations support the concept that all the trichothecene-resistant mutants described here are allelic, and if this is indeed the case then all the mutants have an alteration in ribosomal protein L3.

We next studied ribosome biogenesis in selected yeast strains. In CLP-8, the pre-rRNP particle that contains 20 S pre-rRNA has a slowed transport from nucleus to cytoplasm and a slowed cytoplasmic cleavage of 20 S pre-rRNA to mature 18 S rRNA, both defects causing a delay in the appearance of mature 40 S ribosomal subunits (Carter & Cannon, 1980). This phenomenon

is conveniently revealed by studying, on sucrose gradients, the cytoplasmic distribution of native ribosomal subunits. In CLP-8, there is a large excess of material sedimenting at 60 S, with little or no material sedimenting at 40 S (Carter & Cannon, 1980), and Fig. 2 illustrates this along with typical profiles of native ribosomal subunits from the other four haploid yeast strains, A224A, Y166, TR-1 and MC-1. Since Mitlin & Cannon (1984) showed that the profile for A224A was markedly affected by a temperature shift to 36 °C, a temperature of 28 °C was chosen for the present experiments. The profiles for A224A, CLP-8 and TR-1 confirm, essentially, those published previously by Carter *et al.* (1980), and the large relative excess of 60 S subparticles in the CLP-8 preparation is strikingly apparent. The profile for A224A is taken as normal, and that for TR-1 differs from it consistently with a small relative increase in the 60 S peak. In contrast with the situation in CLP-8, no defect in ribosome biogenesis is observed for TR-1 (the present results; M. Cannon, unpublished work). The ribosomal-subunit profile of MC-1 is particularly interesting. Both 60 S and 40 S peaks are clearly resolved by sucrose-gradient centrifugation, but there is a marked imbalance, with the 40 S peak considerably higher than normal (cf. profiles for its parent Y166 and strain TR-1). Because of this result we carried out additional experiments designed to pinpoint a possible defect in the biogenesis of MC-1 ribosomes.

The initial pre-rRNA transcripts of both MC-1 and its parent Y166 were labelled with [*methyl*-³H]methionine, and the flow of label through the various pre-rRNA species to mature rRNA was monitored during selected 'chase' periods with unlabelled methionine. The results are illustrated in Fig. 3. Compared with the control strain Y166, there is a slowed maturation, in MC-1, of 27 S pre-rRNA throughout the 'chase'. This does not, however, cause any detectable instability in this or in any other pre-rRNA or rRNA species. These observations provide an explanation for the data shown in Fig. 2. Thus in MC-1 the defect in ribosome biogenesis presumably slows the release of 60 S ribosomal subunits from nucleus to cytoplasm. The result is a relative build-up of 40 S subparticles, which await their partners before their combination into 80 S ribosomes. Furthermore, the defect in MC-1 contrasts completely with the defect in CLP-8, although we have argued above that each mutant carries an alteration in the structure of ribosomal protein L3. Strikingly, however, despite slowed processing affecting either subunit, the pre-rRNA and RNA components remain stable in both mutants. The fact that an altered component in a 60 S subparticle can

Fig. 2. Sucrose-gradient analysis of cytoplasmic ribosomal subunits from spheroplasts of various strains of *S. cerevisiae*

Cells of the various *S. cerevisiae* strains indicated were grown, and spheroplasts were prepared and recovered at 28 °C. Spheroplasts were then collected by centrifugation and lysed, and lysates were analysed on sucrose gradients. All techniques are described or referenced in the Materials and methods section. Gradients were monitored by using an Isco density-gradient fractionator, over an absorbance range of 0–0.5. The horizontal arrow under the abscissa represents the direction of sedimentation of the particles.

Fig. 3. Electrophoretic analysis of total cellular RNA prepared from spheroplasts of the yeast strains Y166 and MC-1

Total RNA was extracted and analysed on gels as described or referenced in the Materials and methods section. RNA was from spheroplasts of strains Y166 and MC-1 previously pulsed at 28 °C with [methyl-³H]methionine for 5 min. Chase times, using unlabelled methionine, were 0, 5, 10 and 20 min for Y166 [(a), (b), (c = ●), (d = ○)] and for MC-1 [(e), (f), (g = ●), (h = ○)]. In order to locate positions of the mature rRNA species, these were radiolabelled with [¹⁴C]uracil (Mitlin & Cannon, 1984) and samples were mixed with the ³H-labelled total cellular RNA before electrophoretic analysis. For clarity, ¹⁴C radioactivity (■) is shown only in the graphs for the 0 min chase samples. ³H radioactivity is shown by (●) for (a), (b), (c), (e), (f) and (g), and by (○) for (d) and (h).

affect maturation of 40 S ribosomal subunits in CLP-8 has never been satisfactorily explained, although several possibilities that might account for this indirect effect have been considered (Mitlin & Cannon, 1984). Perhaps the defect in MC-1 is more easily rationalized. It seems entirely feasible that an altered component within a particular pre-rRNP particle could alter the properties of that particle, and, indeed, exquisitely affect its maturation. If, as we suggest, the altered component is ribosomal protein L3, it is clear that the nature of any amino acid change within this protein is crucial, with respect to the above phenomena, as indicated by the very different responses shown by CLP-8 and MC-1 and also TR-1.

In an attempt to assess more completely how different genetic backgrounds can modulate effects caused by *tcm1* gene mutations, we set out to introduce this gene into A224A from either TR-1 or MC-1 by using classical genetic techniques. Diploids were formed between A224A/TR-1 and A224A/MC-1 and, after sporulation, tetrads were dissected. Individual spores were cultured and the cells analysed to reveal their ribosomal-subunit profiles. The profiles are not reproduced here, since those shown in Fig. 2 are adequate for reference. Instead, the data are summarized in tabular form (Table 1). From the A224A/TR-1 cross, two sister spores generate cells that show the high resistance

to trichothecenes, both *in vivo* and *in vitro*, that is characteristic of both CLP-8 and TR-1. The two drug-resistant progeny have ribosomal-subunit profiles corresponding to those of CLP-8 and TR-1 respectively. In contrast, the two drug-sensitive strains have profiles corresponding to those of Y166 and A224A respectively. An identical segregation pattern was reported by Carter & Cannon (1980) from an analysis of the spore progeny of a Y166/CLP-8 diploid. Analysis of the A224A/MC-1 diploid also produced two drug-sensitive and two drug-resistant spore progeny, both of the latter showing the low-level resistance phenotype of MC-1. The two drug-sensitive progeny again displayed ribosomal-subunit profiles corresponding to those of Y166 and A224A respectively. In contrast, the drug-resistant progeny both provide profiles that are essentially indistinguishable from that obtained from MC-1. No abnormal profile corresponding to that of CLP-8 is generated by the A224A/MC-1 cross. Finally, this analysis was extended by analysing spore progeny from a CLP-8/MC-1 diploid. As predicted, two of the progeny showed low-level resistance to the trichothecenes, and both gave ribosomal-subunit profiles corresponding to those of MC-1. The two other progeny were high-level drug resistant and had ribosomal-subunit profiles that corresponded to those of CLP-8 and TR-1 respectively.

Table 1. Analysis of tetrads from diploids formed between selected strains of *S. cerevisiae*

Diploids were formed between the yeast strains indicated, allowed to sporulate and subjected to tetrad analysis. The spore progeny were cultured and analysed for drug-resistance levels and ribosomal-subunit profiles all as described in the Materials and methods section. For each cross, seven complete tetrads were dissected and analysed. Profiles for A224A and Y166 were distinguished by carrying out the experiment at 36 °C rather than 28 °C (Mitlin & Cannon, 1984).

Diploid	Trichothecene-resistance phenotype	Type of ribosomal-subunit profile
A224A/TR-1		
Spore a	Sensitive	A224A
Spore b	Sensitive	Y166
Spore c	High-level resistant	TR-1
Spore d	High-level resistant	CLP-8
A224A/MC-1		
Spore a	Sensitive	A224A
Spore b	Sensitive	Y166
Spore c	Low-level resistant	MC-1
Spore d	Low-level resistant	MC-1
CLP-8/MC-1		
Spore a	Low-level resistant	MC-1
Spore b	Low-level resistant	MC-1
Spore c	High-level resistant	CLP-8
Spore d	High-level resistant	TR-1

Our results show that the trichothecene-resistance gene (encoding ribosomal protein L3) from TR-1 can substitute for that in CLP-8 and induce the same lesion in ribosome biogenesis, thus providing further evidence that the change in the L3 proteins of these two strains is the same and providing further support for the presence of a 'silent' lesion in A224A (Carter *et al.*, 1980; Mitlin & Cannon, 1984; Threadgill *et al.*, 1986). In contrast, the trichothecene-resistance gene from MC-1 has a completely different effect, and slows the maturation of 60 S ribosomal subunits in the genetic backgrounds of both A224A and Y166. Clearly, further experiments are required to elucidate completely the precise molecular basis underlying these phenomena. L3 is the largest of the yeast ribosomal proteins (Fried & Warner, 1981), and the *tml* gene has, atypically for ribosomal-protein structural genes, no introns (see Schultz & Friesen, 1983). This

property may be important in the remarkable co-ordinate regulation that is required to control the synthesis of the multiple components of the eukaryotic ribosome. L3 is apparently involved, too, in the regulation and maintenance of the killer double-stranded RNA genome of *S. cerevisiae* (Wickner *et al.*, 1982), and its importance in controlling trichothecene resistance has been well documented here. Our present work stresses further the central importance of this interesting ribosomal protein with respect to its effects on the structure, function and biosynthesis of *S. cerevisiae* ribosomes.

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REFERENCES

- Cannon, M. (1982) *Biochem. Soc. Symp.* **47**, 79–93
 Carter, C. J. & Cannon, M. (1977) *Biochem. J.* **166**, 399–409
 Carter, C. J. & Cannon, M. (1978) *Eur. J. Biochem.* **84**, 103–111
 Carter, C. J. & Cannon, M. (1980) *J. Mol. Biol.* **143**, 179–199
 Carter, C. J., Cannon, M. & Jimenez, A. (1980) *Eur. J. Biochem.* **107**, 173–183
 Fried, H. M. & Warner, J. R. (1981) *Proc. Natl. Acad. Sci. U.S.A.* **78**, 238–242
 Gonzalez, A., Santamaria, F., Vazquez, D. & Jimenez, A. (1981) *Mol. Gen. Genet.* **181**, 140–146
 Grant, P., Schindler, D. & Davies, J. (1976) *Genetics* **83**, 667–673
 Jimenez, A., Sanchez, L. & Vazquez, D. (1975) *Biochim. Biophys. Acta* **383**, 427–434
 Mitlin, J. A. & Cannon, M. (1984) *Biochem. J.* **220**, 461–467
 Mortimer, R. K. & Hawthorne, D. C. (1969) in *The Yeasts* (Rose, A. H. & Harrison, J. S., eds.), vol. 1, pp. 385–460, Academic Press, New York
 Petes, T. (1979) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 410–414
 Planta, R. J., Mager, W. H., Leer, R. J., Woudt, L. P., Raue, H. A. & El-Baradi, T. T. A. L. (1986) in *Structure, Function, and Genetics of Ribosomes* (Hardesty, B. & Kramer, G., eds.), pp. 699–718, Springer-Verlag, New York
 Schindler, D. (1974) *Nature (London)* **249**, 38–41
 Schindler, D., Grant, P. & Davies, J. (1974) *Nature (London)* **248**, 535–536
 Schultz, L. D. & Friesen, J. D. (1983) *J. Bacteriol.* **155**, 8–14
 Threadgill, G. J., Conrad, R. C., Changchien, L.-M., Cannon, M. & Craven, G. R. (1986) *Biochem. J.* **237**, 421–426
 Udem, S. A. & Warner, J. R. (1972) *J. Mol. Biol.* **65**, 227–242
 Wickner, R. B., Porter Ridley, S., Fried, H. M. & Ball, S. G. (1982) *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4706–4708

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