IN VIVO LABELLING OF PROTEINS ASSOCIATED WITH FOLDED CHROMOSOMES OF YEAST

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Proteins associated with the pre-replicative (g_1) and post-replicative (g_2) folded chromosomes of *Saccharomyces cerevisiae* can be labelled in vivo by growing cells in acetate vegetative medium containing [³⁵S]methionine. In both sporulating (*MATa/MATα*) and non-sporulating (*MATa/MATa*, *MATα/MATα*) diploids proteins associated with the resting stage genome (g_0) can be labelled with [³⁵S]methionine during nitrogen starvation and in sporulation medium. In addition, in *MATa/MATα* diploids proteins associated with the meiotic replication form (r) can also be labelled. SDS-polyacrylamide gel electrophoresis and autoradiography of the labelled proteins from the various folded genome forms showed that the g_1 and g_2 patterns are, with the exception of one polypeptide band, essentially identical. Several differences distinguished the r and g_0 patterns from those of the g_1 and g_2 structures. At least four polypeptide bands distinguish the r and g_0 patterns. No significant differences were observed between the g_0 proteins of sporulating and non-sporulating diploids.

1. INTRODUCTION

Nuclear DNA from the yeast, *Saccharomyces cerevisiae*, can be isolated as a fast-sedimenting structure associated with protein and RNA. Different forms of these structures, termed

folded chromosomes, can be distinguished by band sedimentation in sucrose. Recent studies have shown that these various forms are associated with different functional states of the yeast cell. Two structures, isolated from expo-

Abbreviations: PSP2 = acetate vegetative medium, PSP2-N = nitrogen starvation medium, SPM = sporulation medium, SDS = sodium dodecyl sulfate.

nential cultures of diploid cells, are characterized by sedimentation coefficients of 3000 S and 4600 S. They are termed the g_1 and g_2 forms and represent genomes from pre-replicative (G_1) and post-replicative (G_2) nuclei, respectively. Mitotic replication forms (r) are distributed between the g_1 and g_2 forms, but are not easily isolated (6). Folded chromosomes from stationary phase or nitrogen-starved cells have a sedimentation coefficient of 1700 S. This structure is termed g_0 and is characteristic of cells in a resting, or non-cyclic state (G_0) (5).

The g_0 form also appears as an intermediate in the meiotic pathway, since in cells heterozygous for the mating type locus, $MATa/MAT\alpha$, folded chromosomes accumulate in g_0 before the initiation of meiotic DNA synthesis. Meiotic DNA synthesis can be observed as a shift from the g_0 to a meiotic replication form (r), which is indistinguishable by sedimentation from the mitotic r form. At the end of meiotic DNA synthesis a significant reduction in the sedimentation coefficient is observed. This change appears to be correlated with the entry of cells into meiotic prophase. In meiosis inducing medium strains homozygous for mating type, $MATa/MATa$ or $MAT\alpha/MAT\alpha$, meiotic r forms are not observed and chromosomes remain in the g_0 form (4).

These observations have suggested that the various folded genome forms might provide useful sources of material for studying proteins involved in the structural and functional organization of the yeast genome. As an initial step in the characterization of this class of proteins the present study describes a gel-electrophoretic analysis of proteins associated with mitotic and meiotic folded genomes. We report conditions under which proteins associated with both forms can be radioactively labelled, and present a comparison of the mitotic versus meiotic labelling patterns.

2. MATERIALS AND METHODS

2.1. Yeast strains

Strain 131 of *Saccharomyces cerevisiae* is a $MATa/MAT\alpha$ diploid, sporulating strain (7). Strains X45 and X46 are $MATa/MATa$ and $MAT\alpha/MAT\alpha$ diploids, respectively, derived

from 131 (7), and serve as non-sporulating control strains.

2.2. Sporulation procedure

The sporulation procedure was that described by SIMCHEN et al. (8). Strain 131 was grown exponentially with shaking at 30 °C in 5–10 ml of presporulation medium (PSP2) supplemented as required with 40 $\mu\text{g}\cdot\text{ml}^{-1}$ adenine or 40 $\mu\text{g}\cdot\text{ml}^{-1}$ uracil. At a density of $0.6\text{--}1.5\cdot 10^7$ cells $\cdot\text{ml}^{-1}$, the cells were harvested, washed and transferred to an equal volume of sporulation medium (SPM).

Nitrogen-starved cultures were obtained by transferring exponentially growing cells to a nitrogen starvation medium (PSP2-N) (5).

Vegetative DNA was labelled by adding [6- ^3H]uracil to PSP2 medium at a concentration of 10 $\mu\text{Ci}\cdot\text{ml}^{-1}$. Meiotic DNA was labelled by adding [2,8- ^3H]adenine to SPM at a concentration of 10 $\mu\text{Ci}\cdot\text{ml}^{-1}$.

Proteins associated with folded chromosomes were labelled by adding [^{35}S]methionine to PSP2, PSP2-N, or SPM. Radioactive isotopes were obtained from New England Nuclear.

2.3. Preparation of folded chromosomes

Folded chromosomes were prepared from $1\text{--}5\cdot 10^7$ cells as described by PIÑÓN and SALTS (6) and PIÑÓN (4) with the following modifications. After the cells were converted to spheroplasts, they were lysed in 0.25–0.5 ml of lysing buffer (1 mM- KH_2PO_4 , 1 mM-EDTA, 0.2% Nonidet P-40, 25 mM-2-mercaptoethanol, 1 mM-phenylmethylsulfonyl fluoride, pH 8.5). The lysate was layered on 10 ml of a 10–30% (w/v) sucrose gradient (containing 1 mM- KH_2PO_4 , 1 mM-EDTA, 25 mM-2-mercaptoethanol, 1 mM-phenylmethylsulfonyl fluoride, pH 8.5). Centrifugation was performed at 4 °C in a Beckman SW 41 rotor at 5,000 rpm for 45–60 min when vegetative genomes were isolated, and for 70–100 min when genomes from nitrogen-starved or meiotic cells were isolated. The gradients were fractionated by dripping from the bottom into a cold Falcon 3040 tissue culture plate. Thirteen drops (approximately 0.25 ml) were collected in each fraction. When analysing for alkali-stable, trichloroacetic acid precipitable counts, aliquots

of 0.175 ml from each fraction were analyzed as described in (7). [^{35}S]methionine counts were monitored by spotting 0.01 or 0.05 ml aliquots from each fraction on to Schleicher and Schuell glass fiber filters (25 mm diameter), which were dried under infrared light, and counted in toluene with 0.4 % Omnifluor (New England Nuclear).

2.4. Gel electrophoresis and autoradiography

For gel electrophoresis of [^{35}S]methionine labelled chromosomes, the appropriate fractions, located by spotting and counting, were pooled. Then 0.02 ml of bovine serum albumin (1 mg·ml $^{-1}$) were added as carrier protein, and the chromosomes precipitated by adding 100 % (w/v) trichloroacetic acid to a final concentration of 20 % (w/v), and incubating the suspension for 2 hours on ice. The precipitate was collected by centrifugation at 4 °C in a Sorvall HB-4 rotor at 7,000 rpm for 20 min, then washed once with acetone-0.1 M-hydrochloric acid (6:1 v/v), and twice with acetone, and finally air dried. The precipitate was resuspended in approximately 0.1 ml of SDS-sample buffer (2). The radioactivity in the SDS resuspended fraction constituted 60–100 % of the radioactivity present in the pooled fractions from the sucrose gradients. Aliquots of 20,000–60,000 cpm were then separated in 8–18 % acrylamide gradient gels containing SDS (2). Autoradiography of dried gels was performed by exposing Kodak X-Omat RP Film XRP-5 at –70 °C for 10 to 30 days.

Molecular weight estimates of ^{35}S -labelled polypeptides were based on comparisons with unlabelled molecular weight standards (Bio-Rad Laboratories) run in the same gel (myosin 200,000, β -galactosidase 130,000, phosphorylase B 94,000, bovine serum albumin 68,000, ovalbumin 45,000, carbonic anhydrase 30,000, soybean trypsin inhibitor 21,000, and lysozyme 14,300).

3. RESULTS

Double-labelling experiments were performed in order to determine whether chromosomal proteins could be labelled under mitotic, starvation, and meiotic conditions.

Figure 1 shows the sedimentation profile of folded chromosomes isolated from an exponen-

Figure 1. Sedimentation profile of folded chromosomes (g_1 and g_2) from an exponential culture of strain 131.

Cells were grown at 30 °C in 5 ml PSP2 medium containing 2.9 $\mu\text{Ci}\cdot\text{ml}^{-1}$ [^{35}S]methionine and 10 $\mu\text{Ci}\cdot\text{ml}^{-1}$ [^3H]uracil for 18 hours to a density of $9\cdot 10^6$ cells·ml $^{-1}$, and folded chromosomes were isolated as described in Materials and Methods. Centrifugation was for 40 min. Ordinate, left: cpm (^{35}S) per 0.05 ml from each fraction. Ordinate, right: Alkaline-resistant trichloroacetic acid precipitable cpm (^3H) per 0.175 ml from each fraction. Sedimentation was from right to left.

tial culture of strain 131 uniformly labelled with [^3H]uracil and [^{35}S]methionine during vegetative growth. The g_1 and g_2 structures are seen to be labelled with both isotopes.

Figure 2 depicts the sedimentation profile of folded chromosomes isolated from strain 131 after 4 hours in starvation medium. Cells were labelled with [^3H]uracil during exponential growth, and then transferred to PSP2-N with [^{35}S]methionine. The g_0 structure is clearly labelled with [^{35}S]methionine.

In Figure 3 the profile of folded chromosomes isolated from a sporulating culture of strain 131 in SPM at 7 hours is presented. Unlabelled,

Figure 2. Sedimentation profile of folded chromosomes (g_0) from a nitrogen-starved culture of strain 131.

Cells were grown exponentially in 5 ml of PSP2 containing $10 \mu\text{Ci} \cdot \text{ml}^{-1}$ [^3H]uracil to $1 \cdot 10^7$ cells $\cdot \text{ml}^{-1}$, and then transferred to 5 ml of PSP2-N containing $2.9 \mu\text{Ci} \cdot \text{ml}^{-1}$ [^{35}S]methionine, 30°C . Folded chromosomes were isolated after 4 hours. Centrifugation was for 75 min. The right and left ordinates are as described in the legend to Figure 1.

Figure 3. Sedimentation profile of chromosomes from strain 131 during meiotic DNA replication.

Exponential cells from a 5 ml PSP2 culture ($1 \cdot 10^7$ cells $\cdot \text{ml}^{-1}$) were transferred to 5 ml SPM containing $2.9 \mu\text{Ci} \cdot \text{ml}^{-1}$ [^{35}S]methionine and $10 \mu\text{Ci} \cdot \text{ml}^{-1}$ [^3H]adenine, and incubated at 30°C . After 7 hours, cells were harvested and chromosomes isolated. Centrifugation was for 75 min. The right and left ordinates are as described in the legend to Figure 1.

exponentially growing cells were transferred to SPM with [^{35}S]methionine and [^3H]adenine. As expected the [^3H]adenine is incorporated into the meiotic replication form, r , which is also labelled with [^{35}S]methionine. A small shoulder of [^{35}S]methionine incorporated into the g_0 form is also seen.

These experiments show that chromosomal proteins associated with the various folded chromosome forms can be labelled in vivo by [^{35}S]methionine. We estimate that the proportion of [^{35}S]methionine incorporated into proteins associated with the $g_1 + g_2$, g_0 or r forms, represents about 1% of the total radioactivity incorporated into protein. It should be noted that in these experiments the g_1 and g_2 proteins were labelled over several cell generations, while the

labelled g_0 and r proteins represent those synthesized by non-dividing cells during nitrogen starvation or by cells exposed to meiosis inducing conditions.

Labelled chromosomal proteins suitable for autoradiography were prepared by using higher concentrations of [^{35}S]methionine ($19\text{--}28 \mu\text{Ci} \cdot \text{ml}^{-1}$). Figure 4 shows the sedimentation of different folded chromosome forms isolated for this purpose from sporulating and non-sporulating cells. The fractions which were pooled and analyzed by SDS-polyacrylamide gel electrophoresis and autoradiography are indicated for each gradient.

In Figure 5 is presented an autoradiogram of the 6 chromosome preparations given in Figure 4. In all chromosome preparations a large

Figure 4. Preparative isolations of [^{35}S]methionine labelled chromosomes for gel electrophoresis and autoradiography.

In all experiments 5 ml cultures ($1 \cdot 10^7$ cells $\cdot$ ml $^{-1}$) containing 19.2 $\mu\text{Ci} \cdot$ ml $^{-1}$ [^{35}S]methionine were used. After the gradients were fractionated, aliquots of 0.01 ml were analyzed for ^{35}S -counts. (a) Exponentially growing strain 131 in PSP2; sedimentation 45 min. (b) Strain 131 in SPM at 7 hours; sedimentation 70 min. (c) Strain 131 in SPM at 3.5 hours; sedimentation 100 min. (d) Strain 131 in PSP2-N at 3.5 hours; sedimentation 100 min. (e) Strain X45 in SPM at 3 hours; Sedimentation 100 min. (f) Strain X46 in SPM at 3 hours; sedimentation 100 min. The fractions indicated for each gradient were pooled for electrophoretic analysis.

number of ^{35}S -labelled polypeptide bands are observed. Under our electrophoretic conditions the yeast histones migrate close to the bottom of the gel. Since yeast histones contain few methionine residues, they are not efficiently

labelled with [^{35}S]methionine (9). Only one possible histone band, tentatively identified as histone H2B by comparison with isolated, unlabelled histones is observed.

A number of differences were observed

Figure 5. Autoradiogram patterns of ^{35}S -labelled polypeptides associated with the folded chromosomes of yeast.

Lane 1: The g_1 form from strain 131, corresponding to Figure 4a; 49,450 cpm. Lane 2: The g_2 form from strain 131, corresponding to Figure 4a; 32,450 cpm. Lane 3: Meiotic r chromosomes from a 7 hour culture in SPM, corresponding to Figure 4b; 44,550 cpm. Lane 4: Another 7 hour isolate of meiotic r ; 46,900 cpm. Lane 5: The g_0 form of strain 131 in SPM, corresponding to Figure 4c; 44,550 cpm. Lane 6: The g_0 form of strain 131 in PSP2-N, corresponding to Figure 4d; 44,440 cpm. Lane 7: The g_0 form from strain X45 in SPM, corresponding to Figure 4e; 44,850 cpm. Lane 8: The g_0 form from strain X46 in SPM, corresponding to Figure 4f; 44,850 cpm. The X-ray film was exposed to the dried SDS-polyacrylamide gel for 24 days. Arrows mark the polypeptides mentioned in Results. The position of yeast histone H2B is indicated.

among the autoradiogram patterns of the polypeptides from the various folded chromosome forms. The g_1 and g_2 patterns (lanes 1 and 2, respectively) are very similar. One polypeptide with an apparent molecular weight of 95,000 (indicated with an arrow) seems either to be present in an increased concentration in g_1 , as compared to g_2 , or it is more efficiently labelled in g_1 . Comparison of the g_1 and g_2 patterns with those of the r and g_0 forms (lanes 3–8), shows that a number of bands are absent in g_1 and g_2 . In addition, three bands with molecular weights of 120,000, 118,000 and 53,000 (marked with arrows) seem to be present in much higher concentrations in g_1 and g_2 , than in the other forms.

Polypeptides of the r form isolated from strain 131 in SPM at 7 hours are shown in lanes 3 and 4. Four polypeptides with molecular weights of 150,000, 143,000, 27,000 and 17,000 (marked with arrows in lane 4) are found in much higher concentrations in the meiotic r form than in the g_0 isolated from a 3.5 hour culture of strain 131 in SPM (lane 5). One band with a molecular weight of 95,000 (arrow in lane 5) in the g_0 form is absent or present in much lower concentration in r . This protein may be the same protein found to be present in g_1 , but found only in very low concentration in g_2 . In another experiment the r forms were taken from cells which had been in SPM for various times between 4 and 8 hours. Consistently the same pattern as that found for 7 hours was obtained.

The polypeptide patterns from g_0 chromosomes isolated from strain 131 in SPM and PSP2-N (lanes 5 and 6, respectively) are, with a few exceptions, very similar. Two high molecular weight proteins (molecular weights 150,000 and 143,000; arrows in lane 5) appear in high concentrations in g_0 from cells in SPM medium, while the g_0 from cells in PSP2-N is characterized by two prominent bands with a molecular weight of 100,000 and 29,000 (arrows in lane 6).

No differences were noted between the banding patterns of proteins from the g_0 structures isolated from non-sporulating diploids X45 and X46 incubated for 3 hours in SPM (lanes 7 and 8, respectively). The 150,000 molecular weight band in the g_0 of both X45 and X46 is found to be low when compared to the g_0 pattern from

strain 131 kept under the same conditions (lane 5).

4. DISCUSSION

The results presented above show that it is possible to label in vivo a large number of proteins associated with the folded chromosomes of yeast. Labelling can be done not only with cells in exponential cultures, but also with cells in meiosis inducing and under nitrogen starvation conditions. Moreover, only small volumes and relatively low concentrations of [35 S]methionine are required.

We have carried out a preliminary analysis of these labelled proteins by SDS-gel electrophoresis and autoradiography. While the large majority of labelled polypeptides extracted from the different forms of the folded genome of yeast are the same, a few differences are observable. We interpret this to mean that the overall protein composition of the different folded genomes is very similar, and that only relatively few proteins may distinguish one form from another one. The differences that we did observe may be characteristic of the various structures. Both the similarities as well as the differences in the labelling patterns appear consistent with the sedimentation behaviour of the different forms of folded genomes. According to previous studies the differences in the sedimentation coefficient of the g_1 , mitotic r , and g_2 forms are primarily due to DNA content, while g_0 and g_1 differ in conformation (4, 5). We might expect, therefore, to see more differences between g_0 and the other 3 forms than between g_1 and g_2 , if differences in conformation are due to differences in protein composition. In agreement with this view, our data shows that the g_1 and g_2 polypeptide patterns with one exception are identical, while several differences are found between the g_0 and g_1 or g_2 polypeptides. It is possible that these polypeptides play an active role in the conformational change of the yeast genome as cells enter the G_0 state.

Since one of the motivations for undertaking a study of this type was to try to identify meiotic specific proteins, it was of special interest to us to analyze the proteins isolated from chromosomes of meiotic yeast cells. Previous attempts to identify such proteins have been based on a

comparison of total proteins from meiotic and non-meiotic cell lysates (1, 3, 10, 11). Very few meiotic specific proteins have been identified in these studies. The analysis of proteins associated with folded chromosomes, although a very restricted subset of the total cell proteins, offered the possibility that in this group, meiosis specific chromosomal proteins might be identified and distinguished from chromosomal proteins associated with the response to nitrogen starvation (4). At the present time we can isolate two types of structures from cells in meiosis inducing medium, the g_0 and the r forms (4). We can detect in the r form at least four [^{35}S]methionine labelled polypeptide bands which are either absent or present in much lower concentrations in the g_0 structures. Such proteins may be specific for meiosis, although we cannot exclude the possibility at this time that they may also be found in mitotic replicating chromosomes.

We have not yet been able to isolate chromosomes from later stages of meiosis. An earlier study suggests that when cells enter meiotic prophase, the chromosomes undergo a conformational change and are no longer in a fast-sedimenting form (4). In future studies it will be important to develop methods to isolate chromosomes from cells in meiotic prophase since they are likely to be good sources of proteins functioning specifically in meiotic chromosome pairing and recombination.

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REFERENCES

1. HOPPER, A. K., P. T. MAGEE, S. K. WELCH, M. FRIEDMAN & B. D. HALL: Macromolecule synthesis and breakdown in relation to sporulation and meiosis in yeast. *J. Bacteriol.* 119, 619-628 (1974)
2. LAEMMLI, U. K.: Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227, 680-685 (1970)
3. PETERSEN, J. G. L., M. C. KIELLAND-BRANDT & T. NILSSON-TILLGREN: Protein patterns of yeast during sporulation. *Carlsberg Res. Commun.* 44, 149-162 (1979)
4. PIÑON, R.: Folded chromosomes in meiotic yeast cells: Analysis of early meiotic events. *J. Mol. Biol.* 129, 433-447 (1979)
5. PIÑON, R.: Folded chromosomes in non-cycling yeast cells: Evidence for a characteristic g_0 form. *Chromosoma (Berl.)* 67, 263-274 (1978)
6. PIÑON, R. & Y. SALTS: Isolation of folded chromosomes from the yeast *Saccharomyces cerevisiae*. *Proc. Nat. Acad. Sci. USA*, 74, 2850-2854 (1977)
7. PIÑON, R., Y. SALTS & G. SIMCHEN: Nuclear and mitochondrial DNA synthesis during yeast sporulation. *Exp. Cell Res.* 83, 231-238 (1974)
8. SIMCHEN, G., R. PIÑON & Y. SALTS: Sporulation in *Saccharomyces cerevisiae*: Premeiotic DNA synthesis, readiness and commitment. *Exp. Cell Res.* 79, 207-218 (1972)
9. SOMMER, A.: Yeast chromatin: Search for histone H1. *Mol. Gen. Genet.* 161, 323-331 (1978)
10. TREW, B. J., J. D. FRIESEN & P. B. MOENS: Two-dimensional protein patterns during growth and sporulation in *Saccharomyces cerevisiae*. *J. Bacteriol.* 138, 60-69 (1979)
11. WRIGHT, J. F. & I. W. DAWES: Sporulation-specific protein changes in yeast. *FEBS Letters* 104, 183-186 (1979)