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Comparison of the structure and transcriptional capability of growing phase and stationary yeast chromatin: a model for reversible gene activation

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ABSTRACT

We have compared the structure of intra-nuclear and isolated chromatin from logarithmically growing yeast cells to chromatin from cells which had entered the stationary phase and ceased growing. Both chromatins show a similar nucleosomal repeat pattern, 160 bp repeat size, with staphylococcal nuclease and similar variability in repeat sizes within the genome. DNase I produces the same ladder (<120 b) and a quite similar extended ladder (120-300 b) which shows that both chromatins have phased nucleosomes. However, the rate of DNase I digestion of growing phase is greater than in stationary. Functionally speaking, growing phase nuclei are 5-20 times as active in the rate of endogenous transcription (all three polymerases are involved). The transcriptional and DNase I susceptibility differences noted in nuclei are maintained in sucrose gradient isolated oligonucleosomes and mononucleosomes from the two states.

INTRODUCTION

Much effort has been directed toward determining the structural basis of differential gene expression in eukaryotes and, recently, its relationship to the nucleosomal organization of chromatin (1). Because of the limited amount of transcription in most cells, systems generally used, for example, chicken reticulocyte-erythrocyte or hen oviduct, probe the structure of a very small amount of active DNA (one or a few genes) in a high background of non-active DNA.

As another system to approach the question of control of gene expression, we have studied the transition of yeast chromatin as cultures grow from the very active, logarithmically growing phase into the stationary phase. In stationary phase, the yeast cells quit growing and DNA replication ceases. Studies of the exact transcription rates in stationary yeast cells have not been done. However, the proportion of active ribosomes (i.e. those bound to mRNA as polysomes) is much higher in growing cells than in stationary (2).

This fact, together with the lack of growth makes it extremely likely that transcription in this quiescent state decreases, probably dramatically, relative to the active growing state of yeast.

The growing/stationary system has several advantages for studying the relation of transcription to chromatin structure: (a) the changes are reversible; upon addition of fresh medium, cell growth resumes after a brief lag phase; (b) the different states can be produced from the very same inoculum without addition of exogenous, potentially perturbatory substances; (c) in growing yeast at least 40% of the rather small yeast genome is transcribed into poly A containing cytoplasmic RNA (3). Thus, studies of bulk yeast would include much information about the structure of genetically active as well as information about inactive chromatin.

However, we have evidence suggesting that a much higher proportion of growing yeast chromatin behaves like active chromatin. Yeast DNA sequences which are transcribed into cytoplasmic poly A^+ mRNA have a DNase I susceptibility which is identical to the remainder of the presumably untranscribed (3) DNA of the yeast genome (Lohr and Hereford, manuscript in preparation). It has been shown in chicken that there is preferential DNase I sensitivity of at least some actively transcribed chromatin compared to at least some non-transcribed sequences (4, 5). Our results suggest that in growing yeast, the entire genome possesses the same conformation with respect to DNase I, so that by analogy the entire genome, including the untranscribed sequences, is in the same overall conformation as the (subset of) transcriptionally active chromatin. Thus, bulk chromatin studies in growing yeast can yield mainly information about the structure of active chromatin. Any structural changes in active chromatin during the transition from growing to stationary phase, correlating with the differences in template capability in the two stages, could be observable as bulk changes in chromatin structure.

With this rationale, we have compared the structure of chromatin from these two states. To look for structural differences, we have used nuclease probes (staphylococcal nuclease, DNase I). We have used endogenous transcription studies to show that functional activity differences between the two states are present in isolated nuclei. We have fractionated staphylococcal nuclease digests of intranuclear chromatin on sucrose gradients and find that the observed structural and functional differences noted in nuclei are preserved in isolated chromatin fragments. Finally, we discuss experiments which attempt to determine the basis for the functional and the structural differences noted between growing phase and stationary chromatin.

EXPERIMENTAL PROCEDURES

Growth of Cells, Isolation and Nuclease Digestion of Nuclei:

Cells (Y55) for "growing" and "stationary" cultures were inoculated from the same inoculum, allowed to grow as described (6) for the same number of doublings and harvested at a density of 4-5 x 10^7 (growing) and 20-30 x 10^7 (stationary). Both sets of cells were washed with H_2O, pretreated in 0.1M Tris - 0.1M EDTA pH = 8.0, 0.5% β-mercaptoethanol for 30 min at 4°C (growing) and 32°C (stationary), spun at 10,000 xg for 10 minutes, washed in S buffer (1.1M sorbitol, 24 mM KH_2PO_4 pH = 6.5) and spun again as before. This difference in pretreatment is unavoidable: unless treated at 32°C, stationary will not spheroplast; if growing are done at 32°C they will spheroplast excessively rapidly, resulting in significant cell lysis during spheroplasting. Cells were resuspended in S buffer plus 0.4 mM Ca^{2+} at 0.25 g/ml. Excellent spheroplasts are obtained in 30 minutes to 1 hour at 32°C. Spheroplasts were isolated by centrifugation at 3000 xg for 10 minutes. Growing and stationary nuclei were isolated as in (6) except: PMSF was added to 1 mM at the 18% Ficoll step, to 0.5 mM at the HM resuspension and to 0.5 mM in the digestion buffer; the lysate in 18% Ficoll was scrubbed by 5-10 up and down strokes with a Teflon pestle in a test tube. We have added the homogenization step because in one of the spins, in 18% Ficoll, in which growing phase nuclei pellet, we and others (7) have observed that most of the nuclei from stationary phase float! This is due to the attachment to the nucleus of a vesicular-like sphere which is probably the vacuole. The attachment can be broken by a few strokes homogenization with a Teflon pestle in a test tube (E. and U. Wintersberger, private communication) with no apparent damage to the nuclei. We now use a homogenization in the growing phase nuclear isolation also, after lysis in Ficoll, so that the two kinds of nuclei are isolated by the same procedure.

Staphylococcal nuclease digestion was done at 200-400 µg/ml DNA, 200 U/ml for 15-20 minutes, EDTA was added to 4 mM and the chromatin dialyzed (for sucrose gradients) or the reaction stopped and DNA isolated and acid solubilities done (9) (for time courses of digestion). DNase I digestions were done at 200-400 µg/ml DNA, 50-60 U/ml enzyme for varying periods of time, the DNA isolated and acid solubility determined as described (6).

Isolation and Analysis of Nucleoprotein Particles:

Staphylococcal nuclease digests, after dialysis, were concentrated and put on 15-30% isokinetic sucrose gradients (11) and spun for 16-18 hours at 38,000 RPM in an SW40 rotor. Fractions were collected dropwise, through the

bottom of the tube.

Gradient samples for SDS gels were made 20% in TCA at 4°C, spun at 10,000 xg for 10 min., washed with ethanol/ether and dissolved in SDS buffer. Samples for DNA gels were treated with RNase (6), made 2.0% in Sarkosyl, 0.15M NaCl, pronase added to 50 µg/ml and put on gels. Samples for determining RNA/DNA ratios were hydrolyzed in 0.3N KOH for 1 hour at 37°C, $HClO_4$ added to 0.3N on ice, spun at 10,000 xg for 10 minutes, the supernatant (S_1) removed and the pellet hydrolyzed by 0.5N $HClO_4$ at 70°C for 20 minutes, spun at 10,000 xg for 10 minutes and the supernatant removed (S_2). The A_{260} values of S_1 (RNA) and S_2 (DNA) were read and compared to known concentrations of RNA and DNA subjected to the same procedure.

Redigestion of Oligomeric Nucleoprotein:

Samples were made 0.5 mM in Mg^{2+}, DNase I added to 15-50 U/ml and digestion done for various times. Reactions were stopped, DNA extracted and acid solubilities determined (6) using a modified DABA procedure (9). SDS gels were according to Laemmli (12), 3% non-denaturing DNA gels (6), 8% polyacrylamide-urea denaturing gels according to (13).

Nuclear and Oligomeric and Monomeric Nucleoprotein Endogenous RNA Synthesis:

RNA syntheses were conducted in a final volume of 37.5λ containing the following components: 10 mM Tris-HCl (pH 7.9, 23°C) 10 mM $MgCl_2$; 240 µM ATP, CTP and GTP. Oligomeric nucleoprotein assays contain 10 µM ^3H UTP (9.6 Ci/m mole); nuclear assays contain 50 µM ^3H UTP (4.8 Ci/m mole). (At these levels 780 cpm equals one picomole for nuclear assays and 1560 cpm equals one picomole for isolated oligomeric nucleoprotein.) Nuclear assays also contained 1 M Sorbitol; 0.5 mM $CaCl_2$; 5 mM phosphoenolpyruvate and 20 µg of pyruvate kinase per ml (14). Isolated nucleoprotein assays contain 0.55 µg DNA (by DABA) as chromatin. Nuclear assays contain 20λ nuclei (~200-400 µg DNA/ml), isolated as described. Assays were incubated at 25°C for 15 min and terminated with 2 ml ice-cold TCA and 10 µg calf thymus DNA (GF/C assays) or by addition of 0.5 vol. 1% Sarkosyl containing 50 mM pyrophosphate (DE81 assays). Acid precipitable radioactivity in RNA was assayed on Whatman GF/C filters as described (15) or by the DE81 filter method (16).

RESULTS

The Basic Nucleosomal Structure:

Digestion of chromatin by the enzyme staphylococcal nuclease (E.C. 2.1.4.7) provides a measurement (cf. 17) of the length of the repeating unit of

chromatin (core + spacer DNA, 18-20). In Figure 1, we show the double-strand sizes of the various oligomeric DNA fragments as a function of % of DNA made acid soluble by staphylococcal nuclease.

Clearly, the DNA produced by staphylococcal nuclease digestion shows the same repeat size and the same course of size decrease with digestion for the stationary and growing phase chromatin. Thus the basic nucleosomal structure with 160 bp repeat size seems to remain intact in stationary yeast chromatin.

We also know that core particles are spaced along the yeast chromatin strand by varying lengths of spacer DNA (9). This conclusion comes from measurements of the width (in bp) of the various oligomer DNA peaks; variably spaced cores produce widths which increase for the larger oligomer peaks because the larger the oligomer the greater the possibility that it contains both large and small spacings and hence has a greater width than a smaller oligomer. In Table I we see that in stationary phase, as in the growing phase, the single-strand widths vary in the order IV > III > II. Thus the cores must still be spaced variably along the chromatin strand.

An alternative way to test for variable intragenome repeat sizes involves Exo III exonuclease trimming of DNA tails of oligomer nucleoproteins. Conditions have been found in which the tails can be digested from the 3' end down to the core particle itself (21, 22). At this point the enzyme clips the 5'

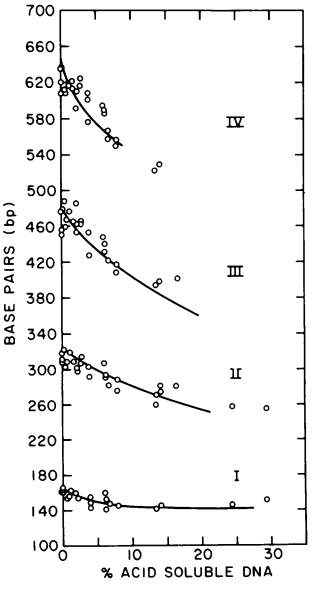


Figure 1. The solid lines are best fits of the double-strand DNA fragment sizes from staphylococcal nuclease digests of nuclei from growing cells, plotted as a function of the % DNA rendered acid soluble. The circles (O) show the double strand sizes of DNA fragments from a digestion of nuclei from stationary cells. All sizes were determined in comparison to PM2-HaeIII restriction fragments run in parallel tracks of the same gel. There has been a recent upgrading of the PM2 size calibration (10) by about 3%. The sizes shown here use the old calibration (9), however, since the calibration makes no relative difference to this work. I, II, III, IV refer to the DNA size classes 1, 2, 3, and 4 times the basic repeat size class.

Table I. Distribution Widths for the DNA Produced by Staphylococcal Nuclease Digestion of Stationary Intranuclear Chromatin

	$W_{3/4h}$
IV	120b
III	73b
II	47b

$W_{3/4h}$, the width of a peak of DNA at 3/4 its maximum height, is shown for dimer, trimer and tetramer size DNA. $W_{3/4h}$ is determined from scans of the negative photograph of an ethidium bromide stained gel. The widths shown are single-strand widths, in bases, from a 5 1/2% polyacrylamide-formamide denaturing gel. The double-strand widths show the same as above (II < III < IV).

end (22) leaving a trimmed core particle. Exo III treatment of sucrose gradient isolated oligomeric nucleoproteins from both growing and stationary yeast chromatin causes the distribution of DNA sizes to sharpen somewhat for dimer, trimer and tetramer (Figure 2). However, there is still a distribu-

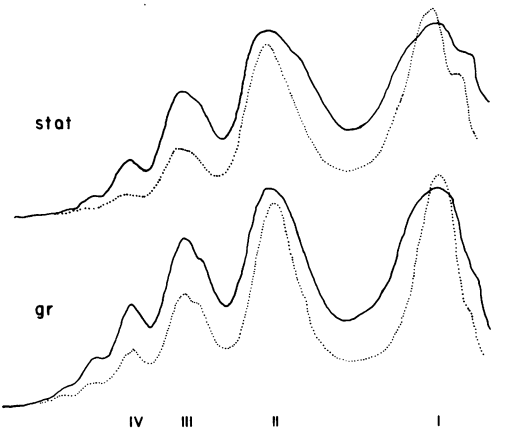


Fig. 2. The "mix" (cf. Table III) fraction from sucrose gradients of staph nuclease digested intranuclear chromatin from growing and stationary phase yeast cells was treated with 0.1 unit of exonuclease III (per 10 µg DNA) as described (22). DNA was extracted (methods) and run on a 5-3/4% polyacrylamide-formamide denaturing gel. Scans are from the photograph of the ethidium bromide stained gel for stationary untreated (——) and Exo III treated (····) and for growing untreated (——) and treated (····) DNA. Electrophoresis is from left to right.

tion of DNA sizes after the exonuclease treatment and the magnitude of the width of this distribution is similar in growing and stationary, confirming similarity in the occurrence of variable intragenome repeat sizes in both chromatin states.

DNase I Digestion:

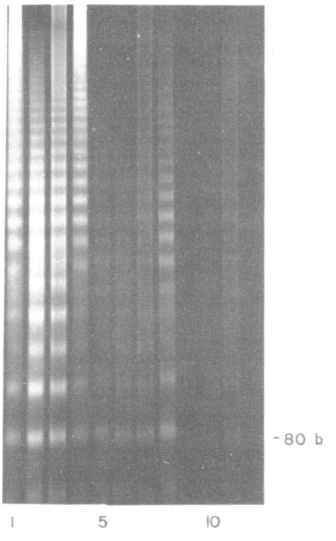
DNase I digestion of intranuclear chromatin from growing cells produces a series of regularly spaced, discrete bands up to at least 300b (23). It is obvious that the ladder (< 110b) and the extended ladder (> 140b) appear quite distinctly in both growing and stationary nuclei (Figure 3).

However, we find that the digestion proceeds more rapidly in the growing phase nuclei (Figure 3). Note for example in Figure 3A that even though the DNA concentration of growing nuclei was ~10% higher than stationary, the digestion of growing phase proceeds faster. Figure 3B shows scans of a DNase I digestion done on nuclei from another yeast strain with similar results. Although quantitative comparison of rate differences of size decrease is difficult, from Figure 3B, for example, it appears that the rate can be several times larger in growing chromatin. For example, 3 or 7-1/2 minutes of stationary digestion seems equivalent to 1 or 2 minutes for growing. These digestion rate differences could be due to differences in chromatin structure or in some other aspect of nuclear structure, such as permeability. DNase I digestion of isolated chromatin fragments (see below) suggests that the differences are due to chromatin structural differences. Also, we have not noted preferential digestion of growing chromatin by staph nuclease, in fact staph acts as fast or faster on stationary chromatin. This also argues against a simple permeability difference as the explanation for the DNase I results.

Comparison of acid soluble DNA evolution also shows that growing phase chromatin is digested faster than stationary, but differences in rate of digestion measured by acid solubility are not nearly as striking as differences shown by the size decrease of DNA (cf. Figure 4 vs. Figure 3A, wells 6, 7).

Acid solubility evolution measures the rate at which DNA smaller than about 15 nucleotides is produced while the gels shown in Figure 3B measure the rate of DNase I attack of longer range structures, principally the chromatin supercoil but eventually the core particle also. It is certainly conceivable that these two kinds of measurements could give non-equivalent magnitudes of digestion rate differences. Thus these results suggest that the major difference in DNase I digestion of growing and stationary chromatin lies in the rate of attack of longer range structure in stationary chromatin

Figure 3



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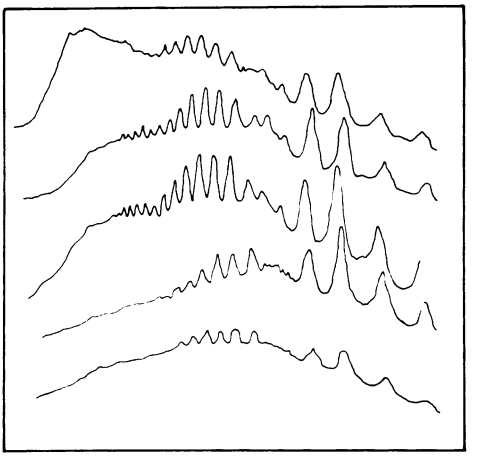
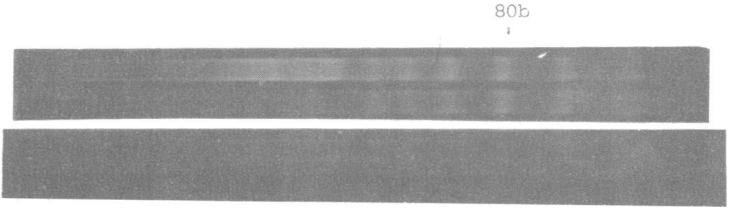


B. Scans of DNase I digests of growing and stationary nuclei from another yeast strain, A364a, a haploid, run on an 8% polyacrylamide -urea denaturing gel. Migration is from left to right. The scans show from top to bottom: stationary,1 min digestion; growing, 1 min digestion; stat., 3 min; growing, 2 min; stat., 7.5 min of digestion all done at the same DNA and DNase I concentrations.

A. DNA fragments from DNase I digestions on an 8% polyacrylamide-urea denaturing gel.Migration is from top to bottom and the wells contain from left to right:30 sec digestion (1-4), 1 minute digestion (5-8),2 minute digestion (9-12), of the following, repeated in the same order: (1) growing nuclei; (2) growing oligomers, (3) stationary oligomers ("Oligo", Table III); (4) stationary nuclei. The DNA and DNase I concentrations used were: nuclei [DNA]=450 µg/ml, [DNase I]=50 units/ml for growing; [DNA]=425 µg/ml, [DNase I]=50 units/ml for stationary. Oligomers: [DNA]=30 µg/ml, [DNase I]= 20 units/ml for growing and stationary. Nuclei and nucleoprotein were from the same experiment. The acid solubility data shown in Figure 4 comes from this oligomer digestion.



C. DNA fragments from a DNase I digestion of monomer nucleoprotein run on an 8% polyacrylamide-urea denaturing gel. Migration is from left to right and the wells contain from top to bottom:(1),(2)stationary,growing monomer digested for 30 seconds;(3),(4)stationary,growing monomer digested for 60 sec. [DNA]=35 µg/ml; [DNase I]=15 units/ml for both growing and stationary digestions.

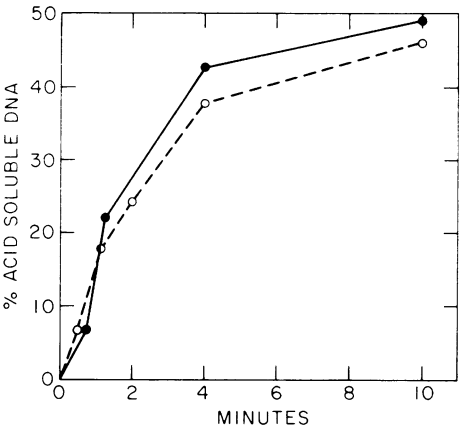


Figure 4. (●) shows the % of the DNA rendered acid soluble versus time of digestion for growing and (O) for stationary phase oligomeric nucleoprotein ("oligo", Table III), digested with DNase I.

and not in the rate of complete solubilization of chromosomal DNA.

Nuclear Transcription

Yeast nuclei can perform endogenous transcription (14). We have reconfirmed these results. We find, as shown in Table II, that nuclei from growing cells incorporate ^{3}H-UTP into an acid insoluble form at several times (5-20 times) the rate of incorporation in nuclei from stationary cells. All four ribonucleoside triphosphates are required for this process and the product is RNase sensitive (Table II) as expected for DNA dependent RNA synthesis. These differences are due to differences in RNA synthesis and not differential degradation because 1) the incorporated counts are stable in the absence of continued incorporation (Table II), and 2) a mixture of stationary and vegetative chromatin (obtained from lysed nuclei) maintains a high level of incorporation and does not show a drastic decrease which would be expected if increased endogenous RNase were the explanation for the low level of incorporation in stationary phase (Table II).

Compared to nuclei from other eukaryotes, growing yeast nuclei synthesize RNA at a very high rate. For example, the growing phase nuclei are even more active than these incorporations show because there are cold UTP pools in the nuclei which dilute the label. This can be shown by increased incorporation using higher levels of radioactivity. Nevertheless, we have measured rates of 35-40 pmole/µg DNA in a 15 minute incorporation. Thus, yeast is an uniquely active system in which to study transcription and the structure

Table II. Endogenous Transcription in Nuclei

	Endogenous	+ RNase	+ ^{3}H-UTP only
Growing	8500	0	0
Stationary	600	0	0
	Endogenous	**+ 30 min. in EDTA**	**Endogenous chromatin**
Growing	10000	13700	10400
Stationary	800	1100	1300
Growing & stationary	-	-	9800

Endogenous transcription was done in isolated nuclei as described in Experimental Techniques. Values are in cpm ^{3}H-UTP incorporated/µg DNA (background subtracted).

(+ RNase) - RNase (300 µg/ml, 15 min.) added subsequent to incorporation.

(^{3}H-UTP only) - all incorporation conditions as usual except only 1 nucleotide triphosphate (UTP) was added.

(+ 30 min. in EDTA) - EDTA was added to the endogenous incorporation and the sample remained at incorporation conditions for 30 minutes.

(Endogenous chromatin) - aliquots of nuclei were pelleted, lysed in low ionic strength buffer and endogenous transcription performed on growing chromatin alone, stationary chromatin alone and a mixture of the two (mixed at nuclei stage).

of transcriptionally active chromatin.

Yeast possesses three classes of RNA polymerases (24, 25) which vary in their sensitivity to the mushroom toxin, α-amanitin. Polymerase II, which is thought to transcribe single copy genes is virtually entirely inhibited by 10 µg/ml of α-amanitin. Polymerase I, which makes the large rRNA precursor is inhibited by high concentrations of α-amanitin (~1 mg/ml); polymerase III, which is responsible for 5S RNA and tRNA and perhaps some single copy genes (14) is insensitive to α-amanitin. Based on the α-amanitin sensitivities determined *in vitro* (24, 25), all three polymerases are probably active in nuclei (14; Bennetzen and Hall, private communication), from both growing and stationary cells (Figure 5A).

The relatively small percentage (10-30%) of II activity compared to higher I + III activities is characteristic of the relative amounts of transcription observed in the intact yeast cell itself (Sripati and Warner, private communication). Thus all three RNA polymerase activities are present in our isolated nuclei with no apparent substantial enrichment or loss of

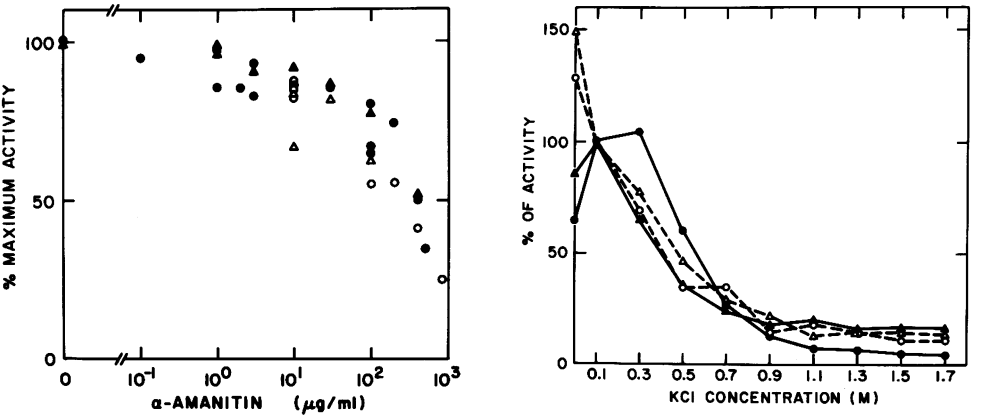


Figure 5. Effect of α-amanitin and KCl concentration on endogenous RNA synthesis.

A. Conditions are as given in experimental procedures with α-amanitin varied as shown. (● and ▲) growing and stationary oligomeric nucleoprotein, the "oligo" fraction (see Table III); (O and Δ) growing and stationary nuclei.
B. Aliquots of nuclei or chromatin were assayed as in experimental procedures, varying KCl concentration as shown. Data was normalized to 100% at 0.1M KCl. The data is an average of a number of preparations. (● and ▲) growing and stationary oligomeric nucleoprotein, the "oligo" fraction (see Table III); (O and Δ) growing and stationary nuclei.

any particular polymerase fraction. Profiles of transcriptional activity as a function of salt are shown in Figure 5B.

Isolation of Oligomeric Nucleoprotein Particles:

Can the kinds of differences noted in stationary vs. growing nuclei be maintained in isolated, fractionated chromatin? To find out, we have fractionated staphylococcal nuclease intranuclear chromatin digests by sedimentation in isokinetic sucrose gradients (8). The kind of resolution we obtain on a sucrose gradient of yeast nucleosomes is shown in Figure 6, compared to the resolution obtained from a sucrose gradient of chicken erythrocyte nucleosomes run and fractionated under identical conditions as the yeast.

This low resolution in the yeast gradient is quite characteristic of both stationary and growing phase chromatin and suggests that some aspect of the yeast nucleosomal structure must be heterogeneous. However, there seems to be only limited heterogeneity in DNA size, for virtually all of the DNA occurs in the nucleosomal repeat pattern.

There is, however, considerable heterogeneity in other components.

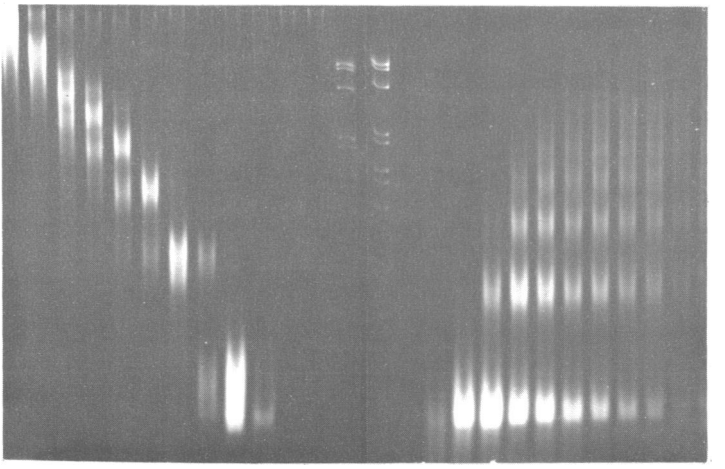


Figure 6. DNA extracted from sucrose gradient fractions of yeast and chicken erythrocyte staph nuclease digests was electrophoresed on 3.5% polyacrylamide gels, electrophoresis from top to bottom. Wells contain from left to right: chicken erythrocyte fractions 1, 3, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15; PM2 DNA; PM2 DNA; yeast fractions 15, 13, 12, 11, 10, 9, 8, 7, 6, 5, 3, 1.

Compared to organisms with less active genomes, there are very large amounts of RNA and non-histone proteins in these gradient fractions.

The RNA, histone and non-histone profiles of nuclei and nucleosomes will however be discussed elsewhere (Lohr and Ide, manuscript in preparation).

DNase I Digestion of Isolated Oligomeric Nucleosomal Particles:

As with nuclei, isolated nucleoprotein particles from growing cells show increased DNase I susceptibility compared to particles isolated from stationary cells. The gels in Figure 3 show that the course of size decrease for digestion of oligomeric and monomeric nucleosomal particles goes visibly faster for growing particles even though DNA, DNase I and Mg^{++} concentrations are the same for both digestions. Thus some structural aspect of growing phase oligonucleosomes and mononucleosomes renders them preferentially DNase I sensitive. We have also followed the evolution of acid soluble DNA by DNase I. This quantity also shows that digestion of chromatin from growing cells proceeds faster than a parallel and identical digestion of chromatin from stationary cells for oligonucleosomes (Figure 4) as well as mononucleosomes (not shown). However, as for nuclei, the difference is generally much less striking than noted by following the size decrease of DNA.

Endogenous Transcription in Chromatin Fragments:

Surprisingly, one can isolate rather small chromatin fragments which still support endogenous transcription and even maintain the differential transcription noted between stationary and growing chromatin (Table III).

^{3}H-UTP is incorporated into RNase sensitive material in growing chromatin fragments at 10-40 times the rate in fragments from stationary. Growing phase chromatin is also more active than naked DNA (Table IIIB) transcribed by wheat germ pol II under template limiting conditions. Incorporation of label is prevented by prior digestion of the chromatin by RNase free DNase I (Table IIIA); there are quite low levels of background incorporation when only the labeled nucleotide is added (Table IIIA). Furthermore, the same sort of differences are observed when other ^{3}H-nucleotides or when α-labeled, ^{32}P-ATP is used as the incorporated isotope instead of ^{3}H-UTP (not shown). Thus the ^{3}H-UTP incorporation must reflect DNA dependent RNA polymerase activity in these isolated fragments.

Not all chromatin fragments from growing nuclei are equally active; generally speaking, the larger the size of the chromatin fragments, the greater the transcription rate (Table III). Furthermore, the ratio of the transcription rates between the various pools remains constant throughout a time course of incorporation from 10 seconds through 15 minutes (Ide and Lohr, unpublished observations), so it must reflect a true rate difference. Thus some aspect necessary for transcription is diminished in smaller oligo or mononucleosome sized particles. We have no explanation for this size dependence.

The α-amanitin and salt profiles (Figure 5) of transcription in these fragments resemble the nuclear patterns. We are clearly obtaining some transcription (10-30% depending on the preparation) by polymerase II, the most sensitive to α-amanitin. The RNA polymerase must be rather firmly attached to the chromatin fragments, for it remains bound through a dialysis and sucrose gradient preparation in the presence of EDTA. The salt profiles show that fairly high levels of salt are required to remove most of the activity. Interestingly, (1) the maximum incorporation for nuclei occurs at 0 salt but at 0.1-0.3M KCl for the isolated nucleosomes; (2) the maximum ratio of activity (growing/stationary nucleosomes) occurs at .3-.5M KCl as if it is easier to dissociate some part of the endogenous polymerase from the stationary chromatin at these lower salts. Increasing levels of salt cause the ratio of incorporated counts (growing/stationary) to decrease (Figure 5). Addition of exogenous RNA polymerase II from wheat germ makes little differ-

Table III. Endogenous Transcription in Isolated Nucleoprotein Particles

A.

Stationary	Endogenous	+ DNase I	+ RNase	^{3}H-UTP only
"Huge"	1000	<100	100	100
"Oligo"	1000	100	<100	200
"Mix"	500	200	<100	200
Growing				
"Huge"	33,500	250	200	100
"Oligo"	16,100	300	250	200
"Mix"	5,800	200	200	200

Nucleoprotein fractions from sucrose gradients were pooled into the following designations: "Huge", containing mainly DNA > 1000 bp (> 6-7 nucleosomes); "oligo", containing mainly DNA 500-1500 bp (3-10 nucleosomes); "mix", containing DNA 140-600 bp (1-4 nucleosomes). (Endogenous) - transcription as described in Experimental Techniques. (+ DNase I) - endogenous transcription after (RNase free) DNase I treatment (130 U/ml; 15 min.). (+ RNase) - endogenous transcription followed by pancreatic RNase A treatment (300 µg/ml, 15 min.). (+ ^{3}H-UTP only) - endogenous transcription as usual but using only the labeled nucleotide, in this case ^{3}H-UTP. Values are in cpm ^{3}H-UTP incorporated/µg DNA per 15 minute assay.

B. Oligomeric Nucleoprotein Compared to DNA

Huge growing	21,400
Huge stationary	2,300
CT DNA + Pol II	10,200

Endogenous transcription of the "huge" nucleoprotein fraction was done as described (Methods). High molecular weight, denatured calf thymus DNA was transcribed by wheat germ polymerase II under template limiting conditions, using the same buffers and nucleotide levels as for endogenous transcription. Values are in cpm ^{3}H-UTP incorporated/µg DNA.

C. Oligomeric Nucleoprotein

	Growing Phase		Stationary Phase	
	Endogenous	+ Pol II	Endogenous	+ Pol II
Huge	40,500	43,000	3000	3500
Oligo	26,900	24,600	3400	3100
Mix	4,700	5,400	1050	800

Endogenous transcriptions of nucleoprotein fractions (see A for DNA sizes) from sucrose gradients were transcribed as described (Methods) in the absence (endogenous) and presence of wheat germ polymerase II. Endogenous incorporations differ in A.,B. and C. because they are from different preparations. However, we have noted the same trends in each of the 14 preparations done to date.

ence in incorporation (Table III) in either growing or stationary so that the chromatin template must not be readily accessible to exogenous polymerase.

Why are the growing phase chromatin fragments so much more active in transcription? One can suggest several explanations. Perhaps there are fewer RNA polymerases bound to the stationary particles. On the other hand, there may be conformational states of the chromatin strand (for example, supercoiled states) which favor (or disfavor) transcription such that the polymerases remain bound to both, but are more active on growing phase chromatin. Treatment of chromatin with the detergent Sarkosyl has been shown to cause the dissociation of chromosomal proteins but leave RNA polymerase (32) which can still synthesize RNA. If comparable amounts of RNA polymerase were bound to growing and stationary chromatin fragments but the stationary fragments are in a conformation which inhibits transcription, treatment with Sarkosyl should release this inhibition and growing and stationary incorporation levels should become more comparable. Table IV shows that this is not the result.

Low Sarkosyl (0.22%) or amounts which have been shown to release most of the chromosomal proteins (0.45%) (31) caused the level of incorporation in stationary to decrease as fast or faster than for growing phase chromatin. Thus we conclude that the most likely explanation for the differences in incorporation levels between growing and stationary chromatin is that there are different amounts of RNA polymerase bound to the chromatin fragments in the

Table IV. The Effect of Sarkosyl on Endogenous Transcription

	Growing Phase		Stationary Phase	
	0.22%	0.44% Sarkosyl	0.22%	0.44% Sarkosyl
Huge	$\left(\frac{13700}{38900}\right) = 0.35$	$\left(\frac{11000}{38900}\right) = 0.28$	$\left(\frac{850}{2600}\right) = 0.33$	$\left(\frac{550}{2600}\right) = 0.21$
Oligo	$\left(\frac{8300}{13900}\right) = 0.6$	$\left(\frac{4600}{13900}\right) = 0.33$	$\left(\frac{900}{3500}\right) = 0.26$	$\left(\frac{950}{3500}\right) = 0.27$
Mix	$\left(\frac{4500}{7100}\right) = 0.63$	$\left(\frac{3400}{7100}\right) = 0.48$	$\left(\frac{450}{1700}\right) = 0.26$	$\left(\frac{450}{1700}\right) = 0.26$

Endogenous transcription of the fractions shown (see Table III for sizes) were done as in Methods except that in some cases the incorporation mix also contained the indicated % Sarkosyl. The numbers in parenthesis are the cpm ^{3}H-UTP incorporated per µg expressed as

$$\frac{\text{cpm/µg DNA indicated Sarkosyl}}{\text{cpm/µg DNA 0 Sarkosyl}}$$

to give the ratio shown to the right of each parenthesis.

two states. It is also possible that the polymerase bound to stationary chromatin is a less active enzyme form than the polymerase on growing chromatin. Experiments to directly count the number of endogenous RNA polymerase molecules are in progress.

DISCUSSION

The basic nucleosomal architecture, as elicited by nucleases, seems similar in both growing and stationary yeast states. Staphylococcal nuclease digestion of both active and inactive states produces size classes of DNA reflecting the presence of nucleosomal repeating units, with repeat length of about 160 bp. There seems to be variability in the exact spacer lengths within the genome of both states. Nucleosome phasing, which refers to the presence of discrete and possibly quantized spacer lengths between core particles, is present in stationary as well as active chromatin. On the other hand, although producing the same patterns, digestion proceeds at different rates on the two chromatins. For example, DNase I digests the more active chromatin faster as measured by the rate of evolution of acid soluble DNA or most strikingly by the rate of the size decrease of the DNA fragments with digestion.

Interestingly, the nuclease digestion of the active state of yeast chromatin compared to the stationary at least qualitatively resembles the results obtained on multicellular eukaryotic chromatin when the structure of the subset of active genes in a genome is compared to the structure of the bulk (inactive) chromatin. Transcriptionally active genes appear to be in nucleosomes for they can be isolated as 11S core particles (28); the nucleosomal repeat size reflects the bulk nucleosomal repeat size (29). Digestion by DNase I produces fragments of the usual submonomer DNase I ladder (30), but the digestion of active chromatin by DNase I proceeds at a distinctively elevated rate (4, 5). Thus in both growing/stationary yeast and in the multicellular eukaryotes studied, the major structural difference noted between active and inactive chromatin is the rate of digestion but not the kinds of digestion products produced.

Our endogenous transcription results are quite important because they suggest that we can isolate in nuclei and more surprisingly, even in nuclease produced oligonucleosomes, chromatin in differential transcriptional states, presumably reflecting functional characteristics of the *in vivo* state. Thus nuclei and oligonucleosomes from growing cells incorporate ^{3}H-UTP into RNase sensitive material at an order of magnitude higher rate than

the same material from stationary cells. The transcriptional differences arise from differences in RNA synthesis, not differences in degradation. α-Amanitin sensitivity suggests that all three polymerases are involved. Thus we feel confident that the nuclei and even the isolated chromatin maintains its in vivo conformation and so constitutes a valuable material upon which to perform structural studies such as these. In fact, these studies clearly illustrate a powerful advantage of this in vitro system. Studies of RNA synthesis cannot easily be done in stationary cells because of the cytoplasmic nucleases and proteases elaborated during this phase. However, nuclei, purified away from such cytoplasmic contaminants, can be used for such studies. The preferential DNase I digestion of growing phase nucleosomes over stationary is further suggestion of the maintenance of the in vivo structure in isolated nucleosomal particles.

One example of structural information about active chromatin obtained from this study is shown by the poor resolution obtained on sucrose gradients. This probably results from the high level of gene activity in yeast and tells us something about the kind of structure to be expected for active chromatin compared to chromatins such as chicken erythrocyte. The presence of RNA in various sizes and non-histone proteins (including RNA polymerase) in various amounts on these particles causes them to run quite heterogeneously in sucrose gradients compared to the rather homogeneous chicken erythrocyte particles. Thus we can find mononucleosome sized DNA throughout the gradient.

There are several types of gene control in eukaryotes. We may divide these into reversible control such as genes involved in hormone induced responses and irreversible control, for example globin genes in adult chicken erythrocytes. The cells used in our experiments have made the transition from stationary to growing at least twice during the course of growth on slants and growth in preinoculation before their final growth phase. So clearly the kind of gene control studied here is reversible. Structural changes associated with this reversible control are quite subtle, involving differences in nuclease susceptibility with little detectable change in the kinds of products produced by the nuclease attack. There is an effect on long-range nucleosomal arrangement, for DNase I rather quickly cleaves down large growing phase chromatin compared to stationary, but yet the relative rates of the endpoint of the process, acid solubilization, are less distinctive. However, since DNase I hypersensitivity extends to the isolated monomer level, there must also be mononucleosome structural differences. Thus both long-range structural (i.e., internucleosomal) and mono-

nucleosome differences are involved in the growing to stationary transition. The longer range changes could of course arise as a result of the nucleosome changes.

The subtle structural changes are associated with striking transcriptional differences between the two states (e.g. 10-30 fold differences in the rate of RNA synthesis). The transcription differences seem to arise from differences in the amount of bound and/or active RNA polymerase in chromatin from the two states. In addition, salt profiles show that polymerase on stationary chromatin is preferentially sensitive to low salt (0.3 - 0.5M KCl) as if it were less tightly bound than to growing chromatin. We suggest that the subtly altered structural state observed in stationary chromatin relates to the functional transcription differences (i.e. the decreased level of bound or active RNA polymerase). The explanation of the increased nuclease susceptibility may thus suggest a structural explanation for transcriptional deactivation in stationary yeast chromatin.

Finally, we have shown that one can isolate manageably sized chromatin fragments which still contain bound RNA polymerase of all three classes, in a conformation capable of performing endogenous transcription. Furthermore, these fragments can be isolated in differentially active states, reflecting the state of activity in the cells of origin. This *in vitro* system should be very useful for studying transcriptional control.

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