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Yeast chromatin is uniformly digested by DNase I

(DNA reassociation/cDNA/single copy DNA)

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ABSTRACT **The DNase I (EC 3.1.21.1) sensitivity of transcribed yeast chromatin has been examined. We find that, in contrast to chromatin from higher eukaryotes, transcribed yeast chromatin and total yeast chromatin are equally sensitive to DNase I digestion. We interpret these results to mean that the entire yeast genome exists in a state that represents a restricted proportion of total chromatin in higher eukaryotes.**

Recent work has led to a greatly enhanced understanding of the organization of histones and DNA in the eukaryotic genome. The basic unit of chromatin, the nucleosome, consists of a core particle (1–3) that is separated from adjacent core particles by a species-dependent (4–6) intragenomically variable (6) length of "spacer" DNA. In the core particle itself, about 145 base pairs (7) of DNA wrap about a complex of two each of the four inner histones, H2A, H2B, H3, and H4 (8, 9). One might reasonably assume that a discontinuous, compacted structure like the nucleosome would be inhibitory to transcriptional activity. However, nucleosomes seem to be present on transcriptionally active regions of chromatin (cf. refs. 10 and 11).

Because nucleosomes are not absent from transcriptionally active regions, there must be other, more subtle, characteristics of such regions. Such a characteristic is presumably the basis of a recently developed assay for transcriptionally active chromatin. Weintraub and Groudine (12) and Garel and Axel (13) have shown clearly that chicken globin and ovalbumin genes are especially sensitive to DNase in nuclei isolated from tissues in which these genes are or have been expressed but they are not sensitive in other tissues. It is likely that the sensitivity does not depend upon the rate of transcription because Garel *et al.* (14) have also shown that genes that code for rare oviduct mRNAs [approximately 10,000 different sequences, each of which is present at 1/20,000th the concentration of ovalbumin mRNA (15)] are as sensitive to DNase I as is the ovalbumin gene. Furthermore, the adult globin genes remain DNase I sensitive in mature erythrocytes which have stopped synthesizing RNA (12). These latter findings imply that increased DNase I sensitivity is monitoring chromatin in a state that is necessary for transcription but whose maintenance is not dependent upon the presence of RNA polymerase molecules. If ongoing transcription is not a prerequisite for increased DNase I sensitivity, the possibility exists that enhanced DNase I sensitivity may extend over much larger regions of chromatin than the actual RNA coding sequences. On the other hand, the recent work of Flint and Weintraub (16) suggests that the region of DNase I sensitivity is closely limited to the transcribed sequences for integrated adenovirus genes.

The budding yeast *Saccharomyces cerevisiae* has a genome that is particularly suited for examining the limits of DNase I sensitivity. An unusually high proportion of the yeast genome is transcribed: approximately 20% of yeast single-copy DNA (sc-DNA) is complementary to mRNA, indicating that at least 40% of the genome is transcribed (17). Furthermore, the sequence complexity of polysomal mRNA is indistinguishable from that of total RNA, suggesting that little additional sequence complexity is present as precursors of mature cytoplasmic mRNA (17). Therefore, the remaining 60% of the sc-DNA is likely to represent nontranscribed chromatin. This being the case, we have asked whether DNase I preferentially digests that 40% of the chromatin that is transcribed into mRNA. This has been accomplished by hybridizing DNA from yeast nuclei digested to varying extents with DNase I in vast DNA excess with tracer quantities of radiolabeled sc-DNA and cDNA. The prediction is that, if transcribed sequences are preferentially digested by DNase I, 100% of the tracer cDNA but only 40% of the tracer sc-DNA should show an increased $C_0t_{1/2}$[¶] when the driver DNA is from DNase I-digested nuclei. Furthermore, the $C_0t_{1/2}$ of this 40% should be identical to that of tracer cDNA which measures only transcribed sequences. The results clearly indicate that the total sc-DNA and cDNA always reassociate with similar, if not identical, kinetics. We conclude that the entire yeast genome, or at least the single-copy fraction thereof, is equally DNase I sensitive.

MATERIALS AND METHODS

Cell Growth and Isolation of Nuclei. Strain A364a was grown in modified Ym-1 (18) which contained 2% Bactopeptone, 1% yeast extract, 2% glucose, 1% succinic acid, 0.6% NaOH, and 40 mg each of adenine and uracil per liter; the pH was 5.8. Growth was at 25°C to a density of 2×10^7 cells per ml for logarithmic phase cultures or 30×10^7 cells per ml for stationary cultures. Cells were harvested, spheroplasts were made, and nuclei were isolated as described (19).

DNase I Digestion and DNA Extraction. Nuclei normally were resuspended in 1 M sorbitol, pH 6.3/0.5 mM Mg^{2+} for digestion. In some experiments, nuclei were adjusted to 10 mM Tris, pH 7.4/10 mM NaCl/3 mM Mg^{2+} according to Weintraub and Groudine (12). DNase I digestion was performed with nuclear DNA concentrations of approximately 300 μg/ml and DNase I concentrations of 55 units/ml. Acid-soluble DNA was measured with 3,5-diaminobenzoic acid as described (19). DNA was isolated from nuclei as described (19) except that all DNA preparations were run over SP50-Chelex columns (17) to remove non-DNA UV-absorbing material.

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Abbreviations: sc-DNA, single-copy DNA which is DNA from which all repeated DNA has been removed; cDNA, complementary DNA synthesized with reverse transcriptase and poly(A)-containing RNA.

¶ $C_0t_{1/2}$, one-half of initial concentration of total DNA (moles of nucleotide/liter) × time (sec).

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DNA Sizes. DNA was treated with bacterial alkaline phosphatase, extracted with phenol, precipitated with ethanol, and then ^{32}P-labeled by using T_4 polynucleotide kinase and [γ-^{32}P]ATP as described (20); the product was collected by ethanol precipitation. Single-strand DNA sizes were determined on formamide gels as described (19) except that the acrylamide concentration was 5.5% and the ratio of acrylamide to bisacrylamide was 20:1. The autoradiograms were scanned on an Ortec densitometer.

Preparations of cDNA and sc-DNA. [^{3}H]cDNA was synthesized as described (17). ^{32}P-Labeled sc-DNA was synthesized as follows. High molecular weight nuclear DNA was labeled by nick translation (21). The labeled DNA was hybridized at a low concentration with yeast ribosomal DNA [from yeast plasmid pY1rA12 (22)] to remove fold-back DNA and ribosomal DNA. The single-stranded fraction was recovered by chromatography on hydroxylapatite and then desalted on a Sephadex G-50 column equilibrated with 0.2 M NaCl/10 mM Tris, pH 7.5/1 mM EDTA. The DNA was precipitated by the addition of 20 μg of *Escherichia coli* tRNA and 2 vol of ethanol. The DNA obtained by this procedure contained no detectable repeated DNA (see Fig. 3A). The specific activities of the cDNA and sc-DNA were 4×10^7 and 5×10^7 cpm/μg, respectively. The single-stranded size of both the cDNA and the sc-DNA was approximately 600 nucleotides as determined by alkaline sucrose gradients (23).

Hybridization. Hybridizations were performed as described (17). Because duplex formation was determined by S1 nuclease digestion, the data were corrected by the formula derived by Morrow (24): $(\%\ \text{single-stranded})^{0.44} = 1/(1 + kC_0t)$. Corrections for effects of driver and tracer sizes were those suggested by Smith *et al.* (25). Although better corrections currently exist (26, 27), the use of these corrections does not appreciably alter any of the data.

RESULTS

Kinetics of DNase I Digestion. The time course of DNase I digestion of isolated yeast nuclei as measured by DNA solubility is shown in Fig. 1. The extent of solubization (90%) is comparable to that originally observed by Weintraub and Groudine (12) for chicken erythrocyte nuclei. The single-stranded sizes of the DNA fragments generated during digestion are shown in Fig. 2. Because the analysis was performed on end-labeled DNA, the size distributions represent the number average distribution rather than the weight average obtained by the usual method of ethidium bromide staining. Again, the results are comparable to those obtained with other eukaryotes. DNase I rather quickly decreases the single-strand DNA size, producing the characteristic ladder of 10-base integral fragments below 140 nucleotides. Further digestion resulted in the loss of the larger fragments and gain of the intensity in the smaller bands (<50 base pairs) of the ladder.

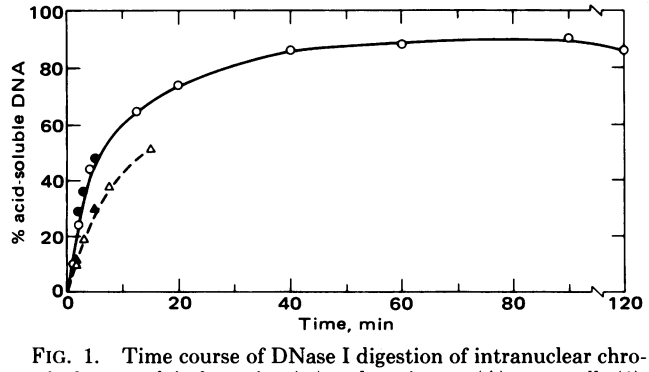

FIG. 1. Time course of DNase I digestion of intranuclear chromatin from nuclei of growing (O) and stationary (Δ) yeast cells (6). The solid symbols are results with nuclei digested according to the conditions described by Weintraub and Groudine (12). Percent acid-soluble DNA is calculated as 100× [acid-soluble DNA/(acid-soluble DNA + insoluble DNA)]. For the data in this figure, cells were grown from the same inoculum, growing and stationary nuclei were isolated, and digestions were done in parallel at 290 μg of DNA per ml for "stationary" digestions (Δ,▲) and 320 μg/ml for "growing" digestions (O,●). DNase I concentration was 55 units/ml.

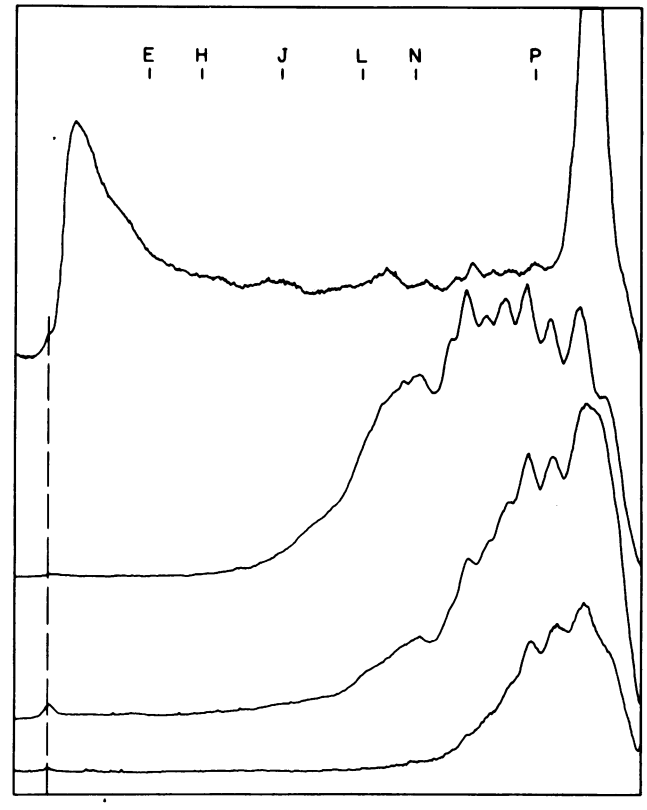

FIG. 2. DNA fragments produced during DNase I digestion. The DNA fragments generated by DNase I digestion of growing nuclei were ^{32}P-end-labeled and electrophoresed (from left to right) on a 5.5% polyacrylamide/98% formamide denaturing gel; then, the gel was autoradiographed. The scans show tracks containing DNA digested to various extents of acid solubility (from top to bottom: 1.6% solubility; 10% solubility; 30% solubility, 48% solubility) according to the conditions described by Weintraub and Groudine (12). Some of the PM2 *Hae* III restriction fragments run in the same gel are shown (E = 820 bases, H = 520 bases, J = 290 bases, L = 166 bases, N = 119 bases, P = 50 bases). The material at the end of the top curve is at the gel front and is due to an artifact of the kinase reaction of this sample.

Hybridization of DNase I Digested DNA to cDNA and sc-DNA. To determine whether there was preferential DNase I digestion of transcribed chromatin, we directly compared the reassociation kinetics of sc-DNA and cDNA hybridized to DNA isolated from DNase I digests of nuclei. Both probes hybridized indistinguishably to nuclear DNA sheared to approximately 600 nucleotides as a single second-order component with $C_0t_{1/2}$ of 10 (Fig. 3A), in good agreement with previously published data (17). In Fig. 3 *B–E* are shown the kinetics of hybridization with DNA digested to 2, 10, 30 and 70% solubility, respectively. cDNA and sc-DNA showed no detectable difference in either the rate or extent of hybridization. The only effects were an increase in the apparent $C_0t_{1/2}$ of both probes and a change in the shape of the curve. Both of these phenomena are accounted for by observed changes in the single-stranded size of the DNA during the course of DNase I digestion (see Fig. 2). Early in the

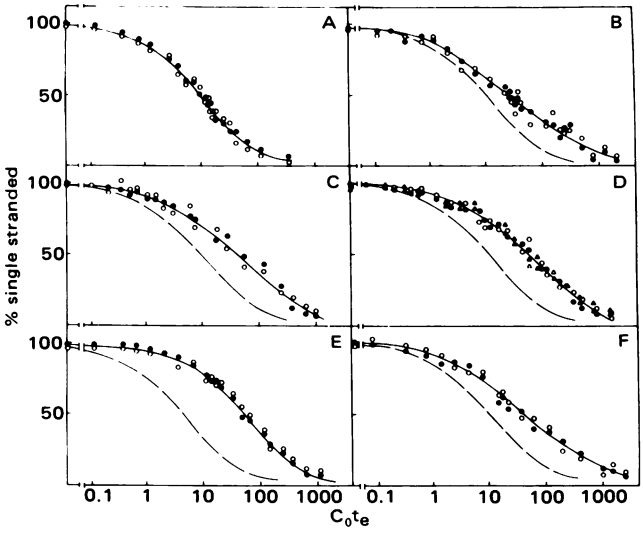

FIG. 3. Reassociation of sc-DNA and cDNA to DNase I-digested DNA, [^{3}H]cDNA (○) and sc-[^{32}P]DNA (●) were reannealed to growing-phase intranuclear chromosomal DNA solubilized to varying extents with DNase I: (*A*) no DNase; (*B*) 1.6% solubility; (*C*) 10% solubility; (*D*) 30% solubility, and [^{3}H]cDNA (△) and sc-[^{32}P]DNA (▲) annealed to DNA digested to 30% solubility by using the conditions of Weintraub and Groudine (12); (*E*) 67% solubility; (*F*) 19% solubility, stationary phase. Dashed lines in *B–F* are tracings of the curve in *A*. Curve in *A* is an ideal S1 kinetic time course.

digestion, the fragment sizes were quite heterogeneous. Because the C_0t_{12} is dependent upon driver length, it is expected that the corresponding hybridization kinetics will also be heterogeneous (Fig. 3*B*). After very long times of digestion, the DNA is of more unform size and the hybridization once more takes place with ideal S1 kinetics (24) (Fig. 3*E*). It can be argued that the digestion of yeast DNA is such that, at low levels of DNA solubility, renaturation kinetics are not sufficiently sensitive to distinguish between subtle differences in the size of transcribed and nontranscribed DNA (Fig. 3 *B–D*). In Fig. 3*E* the DNA has been digested to 70% solubility. At this level of DNA solubility, we would expect that, if differential DNase I sensitivity does exist, all transcribed sequences should be solubilized, in which case the relative extent of hybridization of the two probes would be affected—i.e., none of the cDNA and only 60% of the sc-DNA should be reactive. At the very least, we would expect the relative rate of hybridization of the two probes to be affected because both a concentration difference and a size difference would contribute to a differential rate of hybridization. This also is not the case, again arguing that transcribed yeast chromatin is not preferentially DNase I sensitive.

In Fig. 3*C* we compare the hybridization of DNA solubilized to 30% by using either our conditions of digestion or the conditions originally reported by Weintraub and Groudine (12). There is no difference in the hybridization kinetics. We have also noticed no difference in the rate of digestion (Fig. 1) or the size of the fragments generated when we compare these two conditions of digestion (not shown). Therefore, it is unlikely that our results are due to differences in such conditions.

DISCUSSION

These results clearly show that DNase I does not preferentially digest the transcribed DNA sequences of the yeast genome. However, DNase I does preferentially digest the ovalbumin gene from oviduct nuclei (13) and the globin gene from erythrocyte nuclei (12). Thus, the yeast results are somewhat surprising.

One interpretation is that transcriptional mechanisms in yeast may differ from those of higher eukaryotes. However, yeast mRNA is capped (28) and is polyadenylylated (29). There are three fundamental classes of DNA-dependent RNA polymerases (30–32) with a similar division of labor as in multicellular organisms (cf. ref. 33). Furthermore, yeast nucleosomal structure has been rigorously compared to multicellular eukaryote nucleosomal structure, especially from chicken erythrocytes (6). The major difference lies in the amount of DNA spacing among the species-invariant cores, which is reflected in the small yeast repeat size. Because repeat size varies between cell types (34, 35), between species (4–6), and within the genome (6), this difference is probably not fundamental. In addition, yeast histones have recently been shown to be compositionally similar to other eukaryotic histone and to interact with one another and with heterologous histones in the well-known patterns (36). Thus, the explanation that yeast chromatin is atypical does not seem satisfactory.

A second interpretation is based on differences in experimental design. Our results compare the susceptibility of transcribed sequences to total genome sequences in the same cell type by simultaneous hybridization of probes for transcribed and total sequences to the same DNase I-digested driver DNA. Previous workers have compared susceptibilities of genes expressed in one (37) or more (12–14) different cell types. They have not compared the susceptibility of these active sequences to bulk genomic sequence susceptibility in the same digestion, as we have done. Thus, these results and previous results on metazoans may differ because two slightly different comparisons have been made: in yeast, the structure of active vs. total sequences; in chicken, the structure of a single sequence in an active (expressed) vs. inactive (repressed) cell.

Our preferred explanation is that, in contrast to metazoans, in yeast the entire genome is in the same conformational state as assayed by DNase I sensitivity. Consistent with this notion are the data presented in Figs. 1 and 3 which show the DNase I sensitivity of stationary phase chromatin. As growing yeast cells enter stationary phase and cease to grow, a rather drastic decrease in transcription takes place. There is a decrease in the rate of RNA synthesis (by all three polymerases) in stationary phase nuclei to 1/30th of that in growing nuclei (38). This decrease in transcription is consistent with the decreased rate of DNase I digestion of chromatin isolated from stationary phase cells as compared to chromatin isolated from growing cells (Fig. 1). Moreover, the entire genome participates in the change from growing to stationary chromatin—as assayed by the rate of DNase I digestion—because the transcribed sequences are digested at the same rate as the nontranscribed sequences in both cases (Fig. 3*F*). Thus, as expected from a uniform genome hypothesis, during the transition from the transcriptionally active growing state to the inactive stationary phase, both the transcribed and nontranscribed sequences undergo the chromatin conformational change, assayed by decreased DNase I sensitivity.

Also consistent with this notion are experiments designed to fractionate active and inactive chromatin (39, 40). When such procedures are applied to rat chromatin, about 20% of the chromatin is found in the active fraction. When we apply the solubility criterion of this method to yeast, we find that most (>90%) of the DNA behaves like active chromatin (unpublished observations). Again, this behavior differs from that in multicellular eukaryotes and suggests that the entire yeast genome exists in a uniform activated configuration.

A major difference between yeast and higher eukaryotes lies in DNA content. Yeast DNA has a sequence complexity only 3 times that of *E coli* and ≈1/200th that of most vertebrates. A corollary of this relatively small genome size is the fact that

yeast devotes a large proportion of its sequence complexity (≈40%) to mRNA whereas in higher eukaryotes transcriptionally active sequences represents a more exceptional state. In these latter organisms, mechanisms such as heterochromatization have evolved to package and transcriptionally repress a discrete fraction of the genome. These mechanisms, under temporal and developmental control, presumably involve additional structural components of chromatin. In the absence of such components, chromatin may exist in a state that is uniformly DNase I sensitive. This state would include both transcribed and nontranscribed chromatin, the distinction between these two fractions depending on more subtle structural differences undetectable by differential DNase I sensitivity. It is of some interest that neither heterochromatinization nor mitotic condensation has been observed in yeast (41).

We cannot exclude the possibility that the previous data which measured the fraction of the yeast genome that is actively transcribed are the result of very rapid posttranscriptional processing of RNA. Were this the case (e.g., were 100% of the yeast genome transcribed), this would adequately explain the results presented in this communication. However, we favor the interpretation that only a fraction of the yeast genome is transcribed (≈40%) and, therefore, that DNase I sensitivity monitors a necessary but not sufficient condition for transcription. This interpretation suggests that the fraction of metazoan chromatin that is preferentially DNase I sensitive may include nontranscribed but "active" chromatin. These data and this interpretation also suggest that yeast chromatin is particularly well-suited for studies of the organization and structure of active chromatin.

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