Proc. Natl. Acad. Sci. USA
Vol. 80, pp. 815–819, February 1983
Genetics

DNase I sensitivity of transcriptionally active genes in intact nuclei and isolated chromatin of plants

(nucleosome/high mobility group protein/wheat/micrococcal nuclease)

STEVEN SPIKER*, MICHAEL G. MURRAY†‡, AND WILLIAM F. THOMPSON†

*Genetics Department, North Carolina State University, Raleigh, North Carolina 27650; and †Carnegie Institution of Washington, Department of Plant Biology, Stanford, California 94305

Communicated by Winslow R. Briggs, November 12, 1982

ABSTRACT **We have investigated the DNase I sensitivity of transcriptionally active DNA sequences in intact nuclei and isolated chromatin from embryos of wheat (*Triticum aestivum* L.). Nuclei or isolated chromatin was incubated with DNase I, and the extent of DNA digestion was monitored as percentage acid solubility. The resistant DNA and DNA from sham-digested controls were used to drive reassociation reactions with cDNA populations corresponding to either total poly(A)$^+$RNA from unimbibed wheat embryos or polysomal poly(A)$^+$RNA from embryos that had imbibed for 3 hr. Sequences complementary to either probe were depleted in DNase I-resistant DNA from nuclei and from chromatin isolated under low-ionic-strength conditions. This indicates that transcriptionally active sequences are preferentially DNase I sensitive in plants. In chromatin isolated at higher ionic strength, cDNA complementary sequences were not preferentially depleted by DNase I treatment. Therefore, the chromatin structure that confers preferential DNase I sensitivity to transcriptionally active genes appears to be lost when the higher-ionic-strength method of preparation is used. Treatment of wheat nuclei with DNase I causes the release of four prominent nonhistone chromosomal proteins that comigrate with wheat high mobility group proteins on NaDodSO$_4$ gels.**

The enhanced sensitivity of transcriptionally competent genes to pancreatic DNase I was first shown to occur in animal genomes for the globin (1) and ovalbumin (2) genes. In nuclei treated with DNase I, the sequences complementary to rare cytoplasmic poly(A)$^+$RNA are also digested more rapidly than bulk DNA (3, 4). The enhanced sensitivity to DNase I is thought to be a function of the chromatin structure of the genes and not due to the presence of transcription complexes.

Similar results have been found in the protist *Tetrahymena*, in which Giri and Gorovsky (5) showed that the DNA of activated ribosomal genes is more susceptible to DNase I digestion than is bulk DNA.

In contrast to the above observations, Lohr and Hereford (6) have shown that, in a fungus (*Saccharomyces cervisiae*), DNA in transcribed chromatin is digested at the same rate as DNA in total chromatin. They have interpreted their results to mean that the entire yeast genome exists in a potentially active state comparable with that of only a restricted proportion of the genome in animals. Sledziewski and Young (7) have come to the same conclusion about the general sensitivity of yeast chromatin to DNase I even though they have found a difference in rate of digestion of genes for two isozymes of alcohol dehydrogenase.

Because of the apparent difference in chromatin organization in at least one fungus as compared with animals and protists, it is important to determine whether enhanced DNase I sensitivity of transcribed genes is a property of chromatin structure in plants, the remaining eukaryotic kingdom. Following procedures similar to those of Garel *et al.* (3), Levy and Dixon (4), and Lohr and Hereford (6), we isolated nuclei from mature wheat embryos and incubated them with DNase I. The DNase I-resistant DNA was then used to drive reassociation reactions with cDNA complementary to cytoplasmic polysomal poly(A)$^+$RNA. Our results with wheat embryo nuclei were similar to those found in chicken and trout nuclei but not similar to those found with yeast nuclei. That is, DNA sequences complementary to mRNA were preferentially digested by DNase I in the wheat nuclei. In several developmentally regulated systems, DNase I sensitivity is maintained after transcription has ceased (8). This appears to be the case for wheat because the preferential sensitivity was found in unimbibed mature embryos and therefore in transcriptionally quiescent nuclei.

Because nuclei are difficult to isolate from many plant tissues, it is useful to know whether the preferential DNase I sensitivity of transcribed genes can be maintained in chromatin isolated directly from tissues by procedures that do not involve isolation of intact nuclei. Chromatin isolated by our modification of the method of Bonner *et al.* (9) maintains a structure in which the transcribed genes are preferentially digested to the same degree as in intact nuclei. Chromatin isolated by the method of Simon and Becker (10), which exposes the chromatin to higher ionic strength, does not maintain a structure in which transcribable genes are preferentially digested by DNase I.

The reasons for enhanced DNase I sensitivity of transcribed genes are not well understood. It appears, however, that the association of high mobility group (HMG) proteins with active chromatin is involved (11–14). The first clue that these well-characterized nonhistone chromosomal proteins might be involved came from the work of Vidali *et al.* (15), who showed that HMG proteins were selectively released from duck erythrocyte nuclei by treatment with DNase I. We show here that among the proteins released from wheat nuclei by DNase I treatment are four prominent proteins that comigrate with wheat HMG proteins on NaDodSO$_4$/polyacrylamide gels.

MATERIALS AND METHODS

Plant Materials. Wheat embryos were obtained from General Mills (Minneapolis, MN) or were mechanically isolated from dry mature grains (*Triticum aestivum* L., var. Yamhill) by using a combination of blending and sieving followed by separation on a sucrose gradient (16). The embryos from both sources gave the same results in these experiments.

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Abbreviations: NT, nucleotides; PhMeSO$_2$F, phenylmethylsulfonyl fluoride; HMG, high mobility group, nonhistone chromosomal proteins.

‡ Present address: Agrigenetics Corp., Agrigenetics Research Park, 5649 E. Buckeye Rd., Madison, WI 53716.

Isolation of Nuclei. All operations were carried out at 0–2°C. Twenty grams of wheat embryos was stirred for 30 min in 200 ml of grinding medium [1 M hexylene glycol/10 mM Pipes, pH 7/3 mM $MgCl_2$/5 mM 2-mercaptoethanol/5 mM sodium butyrate/5 mM NaCl/0.1 mM phenylmethylsulfonyl fluoride ($PhMeSO_2F$)]. The slurry was ground for 30 sec at 40 V in a Waring blender and filtered successively through eight layers of cheese cloth and 150-μm and 100-μm nylon filters. A camel-hair paintbrush was used to prevent clogging of the filters. The filtrate was centrifuged for 10 min at 1,000 × *g* in a swinging bucket rotor. Then, the paintbrush was used to suspend the pellet in 100 ml of washing medium (the same constituents as the grinding medium but with addition of 0.5% Triton X-100 and reduction of the hexylene glycol concentration to 0.5 M). In this suspension process, the hard-packed starch pellet was left behind. The suspension was filtered through a 45-μm nylon filter, centrifuged as above, resuspended in 100 ml of washing medium, and refiltered. The filtrate was applied in aliquots to the top of a step gradient of 30%, 60%, and 90% Percoll (Pharmacia) in washing medium. The Percoll step gradient was formed on top of a pad of sucrose-saturated washing medium (lacking hexylene glycol). The crude nuclear preparation was centrifuged on this gradient in a swinging bucket rotor for 30 min at 100 × *g*. After centrifugation, nuclei were found in the 90% Percoll layer and at the interface of the 90% Percoll layer and the sucrose pad. The wheat nuclei are large (diameter, ≈11 μm); smaller nuclei, such as those from yeast, will be found at a lower density in the gradient.

The nuclei thus purified were gently suspended in 100 ml of washing medium with the aid of the paintbrush. The nuclei were then centrifuged at 100 × *g* in a swinging bucket rotor for 10 min, resuspended, and recentrifuged. Typical yields were 10^8 nuclei from 20 g of dry wheat embryos.

Isolation of Chromatin. Chromatin was isolated directly from tissue by two methods. (*i*) The method of Simon and Becker (10) was used with the following modifications. All solutions contained 0.1 mM $PhMeSO_2F$/12 mM $NaHSO_3$. A Waring blender was used instead of a Sorvall Omnimixer. Purified chromatin was finally obtained by centrifugation through 1.7 M sucrose/15 mM 2-mercaptoethanol/10 mM Tris·HCl, pH 8/0.5% Triton X-100/12 mM $NaHSO_3$/0.1 mM $PhMeSO_2F$ for 30 min at 16,000 × *g*.

(*ii*) The method of Bonner *et al.* (9) was used with the following modifications. Two hundred grams of wheat embryos was homogenized at top speed in a Waring blender for 1 min in 1 liter of grinding medium (0.25 M sucrose/10 mM Tris·HCl, pH 8/5 mM 2-mercaptoethanol/5 mM $MgCl_2$/12 mM $NaHSO_3$/0.1 mM $PhMeSO_2F$). The homogenate was filtered successively through eight layers of cheesecloth and 150-μm and 100-μm nylon filters. The filtrate was centrifuged at 12,000 × *g* for 10 min. The pellets were suspended in washing medium (grinding medium/0.5% Triton X-100) by using a glass/Teflon homogenizer. The suspension was centrifuged at 12,000 × *g* for 10 min and the pellets were resuspended and centrifuged as above. The resulting pellets were suspended in centrifugation medium (1.7 M sucrose/10 mM Tris·HCl, pH 8/5 mM $MgCl_2$/5 mM 2-mercaptoethanol/0.5% Triton X-100/12 mM $NaHSO_3$/0.1 mM $PhMeSO_2F$), layered over an equal volume of centrifugation medium, and centrifuged at 27,000 × *g* for 1 hr to yield a pellet of purified chromatin.

Nuclease Digestion of Nuclei and Chromatin. Micrococcal nuclease digestion was carried out by suspending nuclei or chromatin at 1 mg of DNA per ml in micrococcal nuclease digestion medium (20 mM Pipes, pH 7/1 mM $MgCl_2$/10 mM NaCl/1 mM $CaCl_2$/5 mM 2-mercaptoethanol/0.1 mM $PhMeSO_2F$ containing either 0.25 M sucrose or 1 M hexylene glycol). The nuclei or chromatin was then digested at 37°C with micrococcal nuclease (EC 3.1.31.1; Worthington) at 50 units/ml.

DNase I digestion was carried out by suspending nuclei or chromatin at 1 mg per ml in DNase I digestion medium (0.25 M sucrose/10 mM Pipes, pH 7/10 mM NaCl/3 mM $MgCl_2$/5 mM 2-mercaptoethanol, 0.1 mM $PhMeSO_2F$). The nuclei or chromatin was then digested at 37°C with DNase I (EC 3.1.21.1; Worthington) at 10 units/ml.

When DNA was to be analyzed, reactions were stopped by chilling on ice and adding EDTA to 12 mM. When proteins were to be analyzed, reactions were stopped as described below.

DNA Measurement. Quantitation of DNA and assessment of the extent of digestion in terms of percentage acid solubility were determined by fluorescence with diaminobenzoic acid by the method of Lohr *et al.* (17).

Isolation of Proteins After Nuclease Digestion. Aliquots of DNase I-treated nuclei and chromatin were analyzed for protein released into low-speed supernatants by a method similar to that of Vidali *et al.* (15). After digestion, nuclei or chromatin was chilled on ice and centrifuged immediately (2,000 × *g* in a swinging bucket rotor for 5 min). The supernatant and pellet fractions were extracted with 0.2 M H_2SO_4 and the acid-soluble proteins were precipitated with 3 vol of acetone, washed, and dried for electrophoretic analysis. Aliquots of sham-digested nuclei and chromatin were processed in the same manner as controls.

Electrophoresis. Proteins were analyzed on $NaDodSO_4$-containing gels according to Thomas and Kornberg (18) and run in a minislab apparatus as described by Matsudaira and Burgess (19).

DNA was analyzed on nondenaturing gels (0.1 M Tris/1 mM EDTA/12.5 mM NaOAc/1% agarose adjusted to pH 8.1 with HOAc). The 25-cm "submarine" gels were run at 50 V, 60 mA, for 15 hr at room temperature and the bands were visualized under UV light after staining with ethidium bromide.

DNA Purification. DNA was purified by a modification of the Marmur procedure (20). The resulting EtOH precipitates were dissolved, treated sequentially with RNase A, proteinase K, and $CHCl_3$/octanol (24:1), and again precipitated with EtOH (21). Total wheat reference DNA was isolated directly from unimbibed wheat embryos as described (21) or from chromatin isolated as described above. Unless otherwise noted, DNA used for reassociation studies was sheared to a single-strand mass average length of 250–270 nucleotides (NT) as determined by alkaline agarose electrophoresis (21, 22).

Preparation of cDNA Probes. Total RNA was isolated from unimbibed wheat germ essentially according to Silflow *et al.* (23) with the modification that several cycles of LiCl precipitation were used in place of centrifugation through CsCl. Polysomal RNA was prepared from 3-hr-imbibed wheat embryos. Poly(A)$^+$RNA was purified by using poly(U)-Sepharose and [^{3}H]cDNA was prepared as described (24). cDNA tracers were 350 NT long at the time of use.

Reassociation Kinetics. cDNA reassociation kinetics in excess of that of various DNA preparations was used to assess the relative concentration of poly(A)$^+$RNA-complementary sequences. Reassociation reactions were carried out in 2 M $(NH_4)_2SO_4$/20% EtOH/10 mM Pipes, pH 6.8/0.1 mM EDTA at 45°C (approximately 25°C below the melting temperature; ref. 24). All reassociation mixtures included known quantities of *Escherichia coli* [^{14}C]DNA to serve as an internal standard (21). Reassociation was monitored by using hydroxylapatite (20, 21). Data are presented in the form of C_0t curves and all C_0t values are corrected to the equivalent C_0t for 0.12 M sodium phosphate buffer (pH 6.8) at 60°C (20, 25). Results were analyzed in terms of theoretical second-order components by using the computer program described by Pearson *et al.* (26).

RESULTS AND DISCUSSION

Reassociation Kinetics of cDNA from DNase I-Treated Nuclei. To determine whether transcriptionally active sequences in wheat are preferentially sensitive to DNase I, we used the basic approaches of Garel *et al.* (3) and Levy and Dixon (4). Intact wheat embryo nuclei were incubated with DNase I and digested to various extents as monitored by percentage acid solubility. Sham-digested nuclei (incubated without DNase I) were used as controls. The resistant DNA was then purified and sheared to a single-strand mass average length of 250–270 NT.

The reassociation kinetics of 350-NT-long cDNA tracers was followed in excess of the above DNA preparations to assess the relative concentration of cDNA complementary sequences. The cDNAs were prepared from either total poly(A)$^+$RNA obtained from unimbibed embryos (Fig. 1*A*) or polysomal poly(A)$^+$RNA obtained from embryos that had been imbibed for 3 hr (Fig. 1*B*). Identical reassociation kinetics were obtained with DNA from sham-digested wheat nuclei or with total wheat DNA (data not shown).

cDNA reassociation is slower when driven with resistant DNAs resulting from DNase I digestion than when driven with sham-digested DNA. In analyzing these data, a number of computer solutions were obtained based on the use of one or two second-order components. If we average the results for the various solutions, both cDNA preparations [total poly(A)$^+$RNA and polysomal poly(A)$^+$RNA] reassociate about 40% as fast when driven by the resistant DNA resulting from DNase I treatment to the extent of 14% acid solubility. DNA remaining after digestion to the extent of 37% acid solubility drove cDNA renaturation about 20% as fast as sham-digested DNA. The resistant DNA from digestion to 37% acid solubility was only about 100–150 NT long. However, using the relationship of Hinnebusch *et al.* (27), we estimate that the maximum reassociation rate retardation due to the shorter driver length could be only about 40%.

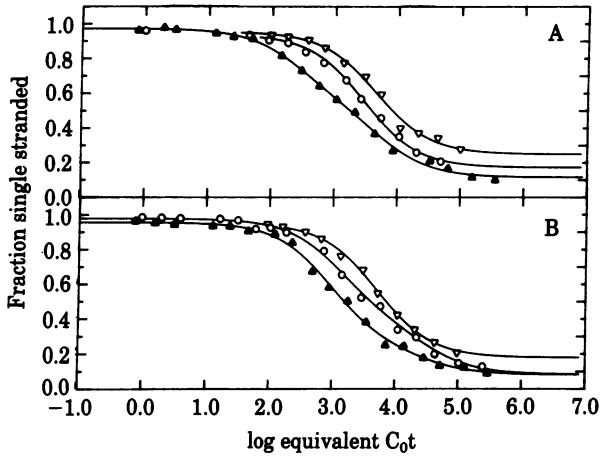

FIG. 1. Preferential DNase I sensitivity of poly(A)$^+$RNA encoding sequences in wheat nuclei. Wheat nuclei were incubated in the presence or absence of DNase I and the resistant DNA was purified. DNAs were sheared to a single-strand fragment length of 250–270 NT if they were not already that length or shorter as a result of DNase treatment. The relative concentrations of poly(A)$^+$RNA-coding sequences in the various samples were determined by following the reassociation kinetics of cDNA tracers in excess of each sample. Reassociation kinetics are shown for 350-NT-long [^{3}H]cDNA in excess 270-NT-long sham-digested DNA (▲), 250-NT-long resistant DNA resulting from DNase I digestion to the extent of 14% acid solubility (○), and 150-NT-long resistant DNA resulting from digestion to 37% acid solubility (▽). (*A*) cDNA was prepared from unimbibed embryo total poly(A)$^+$RNA. (*B*) cDNA was prepared from 3-hr imbibed embryo polysomal poly(A)$^+$RNA. Based on computer solutions to the data, both cDNA preparations reassociate about 40% as fast when driven by DNA remaining after digestion to the extent of 14% acid solubility and about 20% as fast when driven by the DNA remaining after digestion to the extent of 37% acid solubility as when driven by sham-digested DNA.

We conclude that the slower rates of cDNA reassociation in the presence of DNAs remaining after DNase I digestion reflect preferential digestion of the complementary sequences.

The nuclei we have used for this study bear some developmental similarities to the nuclei used by Weintraub and Groudine (1). In their studies, they found that the globin genes of mature chicken erythrocytes are preferentially digested by DNase I even though they are no longer synthesizing RNA. In wheat embryos, development is arrested when desiccation begins about 40 days after anthesis. No further RNA synthesis occurs until after the imbibition of water, the first stage in germination. Thus, in nuclei isolated from desiccated embryos, there are no genes that are actually being transcribed. However, mRNAs synthesized prior to desiccation persist in dry embryos and are involved in directing protein synthesis during the early hours of germination (28, 29). Furthermore, when extensive RNA synthesis begins again a few hours into germination, the proteins synthesized in cell-free medium from bulk mRNA appear to be similar to those synthesized with dry embryo bulk mRNA (30). Thus, it appears that no extensive restructuring of the chromatin has occurred during this period and that most of the genes that are transcribing RNA before desiccation remain "potentially transcribable" in the dry embryos and are again transcribed during the early germination period.

DNase I Digestion of Isolated Chromatin. Large quantities of wheat embryo nuclei such as shown in Fig. 2 can easily be isolated from dry wheat embryos. However, isolation of large quantities of nuclei from many hydrated plant tissues is not always as simple. Therefore, we wished to determine whether the structural features of chromatin that confer preferential DNase I sensitivity to transcriptionally active genes could be maintained in chromatin isolated by procedures that do not involve intact nuclei.

We studied the properties of chromatin prepared by two methods. The method of Simon and Becker (10) was developed for isolating chromosomal proteins from wheat embryos and involves a step in which the chromatin is precipitated with 50 mM $(NH_4)_2SO_4$. We suspect that, at this step (and possibly others in the procedure), rearrangement of chromosomal proteins occurs and the native structure of chromatin with its characteristic pattern of DNase I sensitivity is lost.

Samples of chromatin isolated by the method of Simon and Becker were incubated in the presence and absence of DNase I and the concentration of cDNA-complementary sequences was determined as described above for intact nuclei. The cDNA reassociation kinetics of excess sham-digested DNA and of the resistant DNA resulting from DNase I digestion to the extent

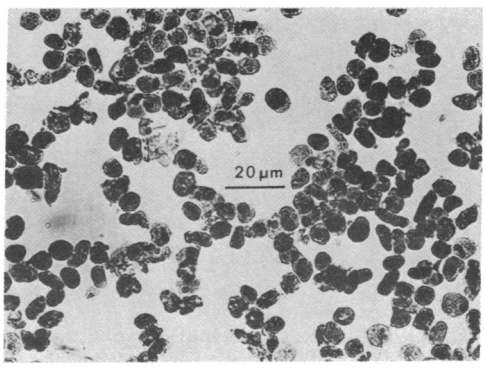

FIG. 2. Wheat embryo nuclei. An aliquot of wheat embryo nuclei isolated as described in the text and stained with acetocarmine.

of 20% acid solubility are shown in Fig. 3A. Since the reassociation curves are virtually superimposable, the cDNA-complementary sequences do not appear to have been preferentially digested by DNase I in this chromatin.

When chromatin is prepared under lower-ionic-strength conditions by our modification of the procedure of Bonner *et al.* (9), the structure that confers preferential DNase I sensitivity to the transcribed genes is maintained. The cDNA reassociation kinetics of excess sham-digested DNA and the resistant DNA resulting from DNase I digestion to the extent of 11% acid solubility are compared in Fig. 3*B*. Based on a variety of one- and two-component solutions to the data, cDNA reassociation is about 33% as fast when driven with the DNA from DNase I-treated chromatin.

Further evidence for a loss of native structure in the chromatin isolated by the Simon–Becker procedure and retention of native structure in the chromatin isolated by the Bonner procedure is shown in Fig. 4. DNA fragments resulting from micrococcal nuclease digestion of nuclei and the two types of chromatin were separated on a native agarose gel. The nucleosome pattern resulting from digestion of "Bonner" chromatin is similar to that resulting from digestion of nuclei. When "Simon–Becker" chromatin is digested with micrococcal nuclease, only a faint nucleosome pattern is discernible against a high background. Time-course digestion experiments were carried out with the Simon–Becker chromatin and the aliquot in which a nucleosome pattern was most noticeable is shown in Fig. 4.

The lack of preferential digestion of the cDNA-complementary sequences in chromatin isolated at higher ionic strength serves as an important control. Previous control experiments

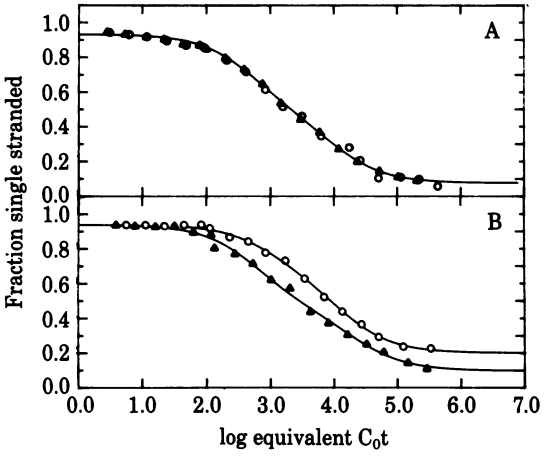

FIG. 3. Relative DNase I sensitivity of poly(A)$^+$RNA encoding sequences in wheat chromatin. Chromatin was prepared by two different methods, samples were incubated in the presence or absence of DNase I, and the relative concentrations of poly(A)$^+$RNA encoding sequences were compared as described in Fig. 1. A 350-NT-long cDNA tracer was prepared from unimbibed embryo total poly(A)$^+$RNA. (*A*) Chromatin was prepared according to Simon and Becker (10). This procedure involved precipitation of chromatin with 50 mM $(NH_4)_2SO_4$. With this chromatin preparation, cDNA reassociation kinetics are identical whether driven by 270-NT-long sham-digested DNA (▲) or by the 150-NT-long resistant DNA resulting from DNase I digestion to the extent of 20% acid solubility (○). Similar results were obtained with resistant DNAs resulting from digestion to 15% or 17% acid solubility (data not shown). (*B*) Chromatin was prepared under conditions of lower ionic strength by our modification of the procedure of Bonner *et al.* (9). cDNA reassociation kinetics in excess 270-NT-long sham-digested DNA (▲) and the 250-NT-long resistant DNA resulting from digestion to the extent of 11% acid solubility (○) are shown. Based on a variety of computer solutions to these data, cDNA reassociation of the resistant DNA resulting from DNase I treatment is about 33% as fast as that of sham-digested DNA.

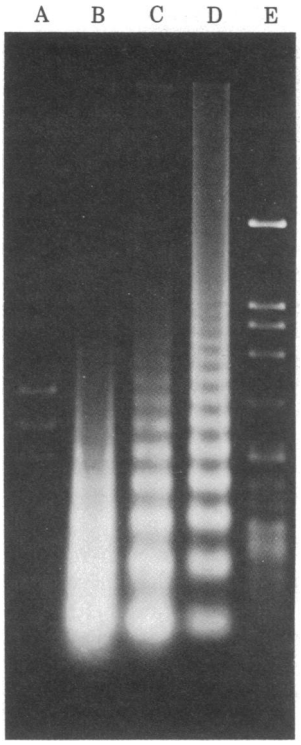

FIG. 4. Micrococcal nuclease digestion of wheat embryo nuclei and chromatin. Nuclei and chromatin substrates were adjusted to 1 mg of DNA/ml and digested with micrococcal nuclease at 50 units/ml at 37°C. Time-course digestion experiments were carried out for all samples. The DNA fragments were then purified and separated electrophoretically on agarose gels (pH 8.1). Lane A: *Hae* III-digested ϕX174 DNA. Lane B: chromatin isolated by the method of Simon and Becker (10). A 16-min digest is shown; this extent of digestion and quantity of DNA applied to the gel shows a nucleosome pattern better than that found under any other conditions although the faint nucleosome pattern is barely noticeable against the background of variable length fragments produced by micrococcal nuclease. Lane C: chromatin isolated by the modified method of Bonner *et al.* (9); a 10-min digest is shown. Lane D: nuclei isolated as described in the text; a 2-min digest is shown. Lane E: *Hae* III-digested λ DNA.

in which naked DNA was digested with DNase I have shown that preferential DNase I sensitivity of potentially active sequences is not directly dependent on nucleotide sequence but is dependent on chromatin structure. (See ref. 31 for review.) We have shown that chromatin structure can be perturbed by a higher-ionic-strength isolation procedure and lose its selective sensitivity to DNase I. This chromatin has also lost the uniform spacing of its nucleosomes, although nucleosomes still exist in the chromatin as judged by the mononucleosome-sized DNA of the smallest abundant digestion product (Fig. 4) and by sedimentation of the mononucleosomes (data not shown). There are no apparent differences in the proteins of chromatin that has lost its selective DNase I sensitivity and those of chromatin isolated by the modified Bonner procedure, which has retained its selective sensitivity (data not shown).

Proteins Released from DNase I-Treated Nuclei. The structural feature that confers a DNase I-sensitive conformation to transcribable genes is not well understood but it appears that HMG proteins are involved (11–14). Vidali *et al.* (15) have observed that HMG proteins are selectively released from duck erythrocyte nuclei treated with DNase I. Although plant HMG proteins are not as yet well characterized, wheat does have four proteins that meet the operational criteria for HMG proteins (31). As shown in Fig. 5, among the proteins released from DNase I-treated wheat embryo nuclei are four prominent bands that comigrate with wheat HMGs on $NaDodSO_4$/polyacrylamide gels. Ten-fold concentrations of proteins released from sham-digested nuclei do not appear to have bands corresponding to the HMG proteins.

These results do not, of course, prove that the putative wheat HMG proteins play the same role in plants that HMG proteins 14 and 17 appear to play in animals. DNase I treatment could cause the release of these proteins for many reasons not connected with the structure of transcriptionally active genes. However, we do believe that the parallels between the plant and animal systems are interesting: under conditions of DNase I digestion that preferentially degrade transcribable genes, four proteins are released from wheat nuclei that meet the operational criteria for HMG proteins.

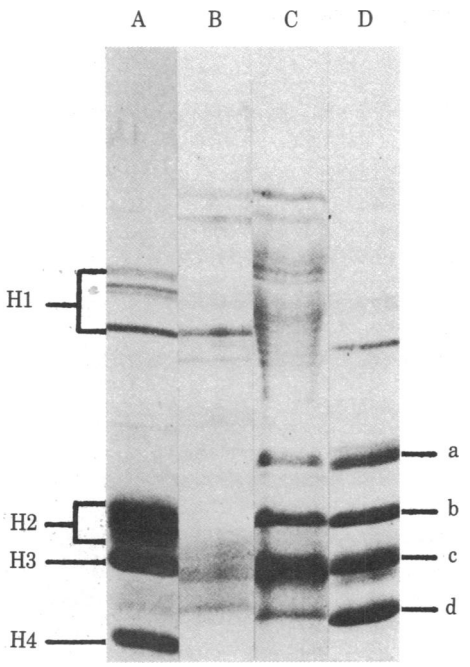

FIG. 5. Proteins released into a low-speed supernatant after digestion of nuclei with DNase I. Nuclei were incubated in the presence of DNase I or under the same conditions in the absence of DNase I (sham digested), low-speed supernatant and pellet fractions were obtained, and each was extracted with 0.2 M H_2SO_4. Acid-soluble proteins were separated by electrophoresis on NaDodSO$_4$/polyacrylamide gels. Lane A: proteins in the pellet fraction of DNase I-digested nuclei. Lane B: proteins in the supernatant fraction of sham-digested nuclei; this lane represents a 10-fold concentration as compared with lane C. Lane C: proteins in the supernatant fraction of DNase I-digested nuclei. Lane D: a crude preparation of wheat HMG proteins [a–d, according to Spiker *et al.* (31)]. In this electrophoresis system, the DNase I preparation we used has a major band that migrates with the minor wheat histone H1 bands. The DNase I preparation also has a minor band that migrates between histone H1 and protein HMGa and two minor bands that migrate faster than histone H4. None of the DNase I bands comigrate with any of the HMG proteins.

APPENDIX

Characterization of cDNA. A substantial fraction of total and polysomal poly(A)$^+$RNA in wheat embryo is repetitive transcript. The best computer solutions to the data we show here (as well as for seven other total DNA-driven cDNA C_0t curves) required two theoretical second-order components. An average of about 60% of the reactable cDNA tracer reassociated in excess total or sham-digested DNA with a rate of 0.00016 liter·mol NT^{-1}·sec^{-1}. This rate is about 0.07% of that of *E. coli* DNA fragments included in the same reassociation mixtures. Assuming these cDNAs to be single-copy transcripts and taking a value of 4.5×10^6 NT pairs for the size of the *E. coli* genome (32, 33), we calculate a kinetic complexity of about 6.3×10^9 NT pairs or about 6.4 pg of DNA per haploid genome. While this value is in good agreement with that of Smith and Flavel (34) it is about 1/3 of that (18 pg) found by Bennett (35) by cytophotometric determination of the 1C DNA content of hexaploid wheat. Smith and Flavell (34) suggest that this apparent discrepancy is best explained if the three constituent genomes comprising modern hexaploid wheat share close homology. Indeed, studies on the thermal stability of cDNA duplexes do indicate a very high degree of homology (unpublished data). The remaining 40% of the cDNA reassociates with a rate of 0.0035 liter·mol NT^{-1}·sec^{-1}, indicating that these sequences are transcribed from members of families of sequences present in about 20 copies per constituent haploid genome—i.e., 60 copies per 1C DNA.

We thank Glenn Ford and Debra Peters for assistance. This work was supported in part by Grant PCM81-05135 from the National Science Foundation (to S.S.), Grant 79-59-2115-0-1-009-1 from the Competitive Grants Office of the Science and Education Administration, U.S. Department of Agriculture (to W.F.T.), and funds from the Carnegie Institution of Washington. This is paper no. 8374 of the Journal Series of the North Carolina Agricultural Research Service and publication no. 780 of the Carnegie Institution of Washington, Department of Plant Biology.

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