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Nuclease accessibility of chromatin from a heterotic hybrid and from parental inbreds

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Abstract

The DNAase II, Mg^{2+} procedure was used to fractionate the seedling chromatin of a heterotic maize hybrid and its parental inbreds FRM017 and FRN28. The hybrid and the more vigorous maize inbred FRN28 have 14 and 11 percent soluble chromatin (euchromatin) respectively, while the less vigorous FRM017 contains 30 percent. The unfractionated chromatin of the hybrid contains less protein than either inbred. The RNA contents of the unfractionated chromatin of the hybrid and of FRN28 are similar and are one-half that of less vigorous FRM017. Hybrid euchromatin contains relatively more protein and RNA than DNA as well as higher proportions of protein and RNA than heterochromatin, unfractionated chromatin, or inbred euchromatin; this suggests a more "efficient" type of activity, as reflected by the low amount of euchromatin and a high proportion of RNA and chromosomal proteins.

Introduction

Increases in the level of nucleotides and RNA synthesis have been found in heterotic maize hybrids as compared to their inbred parents (Mino and Inoue 1980, Amero *et al.* 1983, Nebiolo *et al.* 1983). Biochemical studies of hybrids during development reveal that some parameters are correlated with heterosis and others are not. The liver chromatin of heterotic rats manifested no consistent correlation in the RNA/DNA, nonhistone/DNA and histone/DNA ratios (Tallman *et al.* 1977, 1978, 1979). Although nonhistone electrophoretic patterns of inbreds and hybrids were strain-specific less variation was observed in the patterns of the hybrid (Tallman *et al.* 1979). Chromatographed hybrid histone H1 showed quantitative and qualitative variation over development, but when electrophoresed, inbred H1 patterns were more variable (Tallmann *et al.* 1978, 1979, Amero *et al.* 1983). In maize, DNA and histone contents were equivalent or intermediate to those of inbreds (Chang *et al.* 1979). Gilyazetdinov *et al.* (1978) found no quantitative of qualitative differences in nonhistones among any of the strains studied.

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This investigation approaches heterosis by probing the accessibility of maize heterotic hybrid and inbred chromatin to nuclease digestion through the use of a modified DNAase II, Mg^{2+} procedure (Gottesfeld and Bonner 1977). The use of this system is believed to provide information on chromatin conformation, template accessibility and chromatin activity.

Material and methods

The preparation of etiolated seedlings and isolation of nuclei from maize (*Zea mays* L.) has been described (Palmer and Ulrich 1988) as has the demonstration of the heterotic phenotypes of the hybrid from maize inbred strains FRM017 and FRN28 (Nebiolo *et al.* 1983). DNAase II was purchased from *Worthington Diagnostic Systems, Inc.*, Freehold, N.J. Other reagents and equipment were as previously described (Palmer and Ulrich 1988). The UV absorption spectrum of sheared aliquots of the Mg^{2+}-soluble fraction has a distinct peak at 260 nm and an absorption at 300-320 nm less than 0.1 of that at 260 nm. This indicates low turbidity and a lack of chromatin aggregates and nonchromosomal protein (Bonner *et al.* 1968).

The chromatin isolation procedure was a modification of an intermediate ionic strength method described by Garrard and Hancock (1978). Immediately upon isolation of nuclei all supernatants were aspirated. The isolated nuclei were suspended in 40 cm^3 of 75 mM NaCl, 24 mM Na_2EDTA, pH 8.0 and homogenized in a Teflon-glass tissue grinder (*Kontes*, size D, 0.01 to 0.014 mm clearance) by one stroke with the motor drive at the lowest setting. Fragmentation of chromatin was prevented by minimal homogenization. The material was centrifuged at 1500 *g* for 10 min. Pellets were resuspended by homogenization in 40 cm^3 of Buffer G (10 mM Tris, 2 mM EDTA, and 0.1 % Triton X-100 (v/v), pH 8.0 and centrifuged at 4500 *g* for 10 min. Solubilization of chromatin proteins was avoided by minimizing exposure to NaCl or detergent. Pellets were again washed in Buffer G via homogenization and centrifuged at 12 000 *g* for 10 min. A final washing, as recommended by Garrard and Hancock (1978), was unnecessary for plant chromatin. Pellets were suspended in 10 cm^3 of Buffer G without Triton X-100, homogenized by one stroke of a tissue grinder (*Thomas*, size B), and stirred on ice for 1 h with a micro-stir bar at the slowest setting. Five cm^3 of the chromatin suspension were layered over 32 cm^3 of 1.7 M sucrose, 10 mM Tris, 2 mM EDTA, and 0.5 mM phenylmethylsulfonyl fluoride (PMSF), pH 8.0. The upper $^2/_3$ of each tube was slowly mixed with a spatula, and centrifuged at 70 000 *g* for 3 h. The gelatinous pellets were gently resuspended with a spatula in 10 cm^3 of buffer H (25 mM sodium acetate, pH 6.6), placed in dialysis tubing (6000 - 8000 MW cutoff), and dialyzed overnight against 2 liters of Buffer H with 0.1 mM PMSF, pH 6.6. The dialysate was sedimented for 15 min at 27 000 *g*. Pellets were suspended in 4 cm^3 of Buffer H containing 25 mM sodium acetate, pH 6.6, by one stroke of a glass-Teflon homogenizer (*Kontes*, size D).

Chromatin purity was assayed by an UV absorption spectrum (Bonner *et al.* 1968). An aliquot was diluted 1:9 with buffer H without PMSF and sheared for 3 min at 30 V with a *VirTis "45"* homogenizer to solubilize the chromatin completely.

Sheared material was stirred on ice for 30 min and centrifuged at 10 000 *g* for 30 min. The supernatant was scanned from 230 to 330 nm. Scattering correction for chromatin turbidity was determined by extrapolation from the higher wavelengths (Bonner *et al.* 1968).

Aliquots of the chromatin suspension were tested for proteolytic activity by the *Bio-Rad Protease Substrate Gel Tablet Method* according to the protocol given by the supplier. Trypsin activity in 50 mM Tris-HCl and 50 mM CaCl, pH 7.5, was used as a standard, and sensitivity was tested from 0.5 to 35 μg cm^{-3}.

DNA concentration was measured in 0.5 M $HClO_4$ by the diphenylamine reaction (Burton 1968), with facilitation of color development by heating tubes for 15 min in boiling water. Calf thymus DNA was used as a standard. RNA was separated from DNA by alkaline hydrolysis (Munro and Fleck 1966), since DNA interferes with the RNA assay. RNA concentrations were determined by the orcinol assay (Schneider 1975), with standard concentrations prepared from *Torula* yeast RNA. Proteins were quantified by comparison to bovine serum albumin standards using the Folin phenol method (Lowry *et al.* 1951).

Chromatin fractions were isolated by a modification of the method of Gottesfeld and Bonner (1977) and chromatin was prepared for digestion within 8 h of isolation following the overnight dialyses. Following centrifugation at 27 000 *g* for 15 min the pellet was suspended in Buffer H, pH 6.6, with 1 mM PMSF, by one stroke of a glass-Teflon homogenizer (clearance 0.1 to 0.15 mm). Chromatin in Buffer G plus 1.0 mM PMSF, pH 6.6 was adjusted to 75 μg cm^{-3} DNA as determined by colorimetric assay. DNAase II (specific activity 50 000 U mg^{-1}) was added at 100 U cm^{-3}. The reaction proceeded at 24 °C, and was terminated by placing the tubes on ice and adding 0.1 M Tris-HCl, pH 11. The digestion-resistant chromatin was pelleted at 27 000 *g* for 15 min. The supernatant (S1) was further fractionated by addition of 0.2 M $MgCl_2$ to a concentration of 2 mM in order to precipitate a small condensed fraction released by DNAase digestion. After 30 min of slow agitation with a micro-stir bar, the precipitate was collected by centrifugation as before. The first pellet (P1) and second pellet (P2) were pooles to constitute the inactive fraction or heterochromatin fraction, while S2 was the putative active fraction or euchromatin. Fresh and frozen (-80 °C) aliquots of (P1 + P2) and S2 were retained to determine endogenous protease activity.

Results and discussion

The final chromatin preparations contained no detectable endogenous protease activity. The assay system used as a control displayed considerable digestion sensitivity by the trypsin standard even at 0.5 μg cm^{-3} trypsin.

The UV absorption spectrum of sheared aliquots of the Mg^{2+}-soluble fraction had a distinct peak at 260 nm and an absorption at 300 - 320 nm less than 0.1 of that at 260 nm. This indicates low turbidity and a lack of chromatin aggregates and nonchromosomal protein (Bonner *et al.* 1968).

DNAase II cleaves the less condensed regions of chromatin which corresponds to transcriptionally-active regions of the genome. The digestion released a reproducible Mg^{2+}-soluble fraction by 90 min, after which only a few more percent of the total DNA was released (data not shown). Extensive characterization of hybrid fractionated chromatin shows the presence of histones, nonhistones, and HMG-like proteins in all fractions, while unique H1 subfractions and nonhistones appeared in the Mg^{2+}-soluble fraction. Therefore, this fraction is a suitable model for the euchromatin portion of the genome, both in quantity released (a measure of chromatin condensation via nuclease accessibility) and in the quantitative and qualitative analysis of chromatin components. Furthermore, the small Mg^{2+}-insoluble fraction released was pooled with the considerably digestion-resistant portion of the maize genome to serve as a model for heterochromatin.

Table 1. Composition of chromatin fractions from parental maize inbred and F1 heterotic hybrid. Means ± S.E. [µg per 100 µg (total DNA)]. Hybrid $n = 6$, inbred $n = 4$.

Strain	Chromatin fraction	DNA	Protein	RNA
FRM017 × FRN28	Unfractionated	100 ± 7.9	465 ± 31.4	171 ± 24.4
	Mg^{2+}-soluble	14 ± 1.5	86 ± 9.9	43 ± 5.4
	Digestion-resistant	86 ± 8.0	400 ± 37.5	137 ± 12.3
FRM017	Unfractionated	100 ± 9.5	620 ± 66.0	330 ± 26.3
	Mg^{2+}-soluble	30 ± 3.8	18 ± 1.8	31 ± 2.4
	Digestion-resistant	66 ± 7.2	593 ± 43.1	285 ± 13.1
FRN28	Unfractionated	100 ± 7.8	670 ± 44.9	160 ± 11.7
	Mg^{2+}-soluble	11 ± 0.5	19 ± 2.3	17 ± 1.8
	Digestion-resistant	98 ± 8.8	640 ± 72.2	141 ± 10.2

The composition of the chromatin fractions isolated from parental inbreds and heterotic hybrids after 90 min of DNAase II digestion is given in Table 1. The results are difficult to interpret due to the absence of strong correlations with the hybrid fractions. If the Mg^{2+}-soluble "euchromatin" fraction was the largest one (as measured by amount of DNA) in the hybrid, the increase in the amount and rate of transcription observed with heterotic maize (Nebiolo 1983) could be explained by a conformational mechanism. This would imply an increased availability of gene sequences to polymerase and other regulatory proteins via a more relaxed chromatin structure. Alternately, if the amount of euchromatin was smaller in the hybrid, this could be regarded as evidence for the premise that the hybrid is more efficient in its genetic metabolism than the inbreds. Greater efficiency could also occur via greater stability of RNA and/or by increased transcription at certain DNA sites.

The amount of Mg^{2+}-soluble "euchromatin" released from the hybrid chromatin was intermediate to those of the inbreds. Hybrid euchromatin constituted approximately 14% of the total DNA digested, while the FRM017 and FRN28 inbred chromatins were approximately 30% and 11% euchromatin, respectively. The hybrid

fraction was similar to that of FRN28, which is the most vigorous inbred (Nebiolo *et al.* 1983). The less vigorous parental (FRM017) strain contained more than twice as much DNA in the less condensed configuration. Therefore, this would indicate that an association between vigor and smaller euchromatin fraction may exist.

Unfractionated hybrid chromatin contains less protein than either of the inbreds, which are similar in their protein content (Table 1). RNA content in the unfractionated hybrid and FRN28 inbred are also similar and are one-half the content of the less vigorous FRM017. The contents of individual fractions are better expressed as DNA:protein and DNA:RNA ratios (Table 2). The relative proportions of components in unfractionated and digestion resistant chromatin are similar within each strain; the latter fraction constitutes the major portion of chromatin. The Mg^{2+}-

Table 2. DNA:protein:RNA ratio of chromatin fractions from parental maize inbred and F1 hybrid.

Strain	Chromatin fraction	Ratios to DNA		
		DNA	Protein	RNA
FRM017 × FRM28	Unfractionated	1.00	4.65	1.71
	Mg^{2+}-soluble	1.00	6.06	3.03
	Digestion-resistant	1.00	4.66	1.59
FRM017	Unfractionated	1.00	6.20	3.30
	Mg^{2+}-soluble	1.00	0.60	1.03
	Digestion-resistant	1.00	8.89	4.32
FRM28	Unfractionated	1.00	6.70	1.60
	Mg^{2+}-soluble	1.00	1.73	1.55
	Digestion-resistant	1.00	6.53	1.44

soluble chromatin contains DNA:protein and DNA:RNA ratios which are different from other fractions within each strain. The heterotic hybrid "euchromatin" fraction contains relatively more protein and RNA than DNA, and also higher proportions of protein and RNA than the heterochromatin or unfractionated hybrid chromatin. This type of macromolecular distribution is also found in nuclease digestion studies of animal chromatins (Mathis *et al.* 1980). However, the inbred euchromatin fractions contain much less protein and RNA than unfractionated and digestion-resistant chromatins from the same strain. This finding suggests that the increased protein and RNA content of hybrid euchromatin may reflect the presence of increased amounts of polymerases, regulator proteins, nascent RNA, or possibly chromosomal RNA. However, the validity of this view awaits testing by additional studies. Overall, this implies, for the system studied, that the hybrid euchromatin and that of the most vigorous inbred are of a similar size and conformation. The heterotic hybrid may contain a more "efficient" type of activity, as reflected by a higher proportion of RNA and protein that the euchromatin of inbred parents. This may account for the superior poly (A)+RNA content of the hybrid as reported by Nebiolo *et al.* (1983).

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