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ISOLATION AND CHARACTERIZATION OF NUCLEOSOMES FROM NUCLEOLAR CHROMATIN OF 2,4-D TREATED SOYBEAN HYPOCOTYL

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Abstract

Nucleolar chromatin obtained from nucleoli of soybean hypocotyl was digested with micrococcal nuclease, DNase I or DNase II. Partially digested nucleolar chromatin was fractionated into at least 5 discrete bands by centrifugation in 5% to 20% linear sucrose density gradients. These were found corresponded to nucleosome monomers, dimers, trimers, tetramers and pentamers. Fractions containing more than pentamers were also found, but were not clearly resolved on sucrose gradients. Analysis of DNA extracted from these bands on 2.0% agarose gels indicates the length of DNA in the nucleosomes was multiples of 180 base pairs. Nucleolar chromatin from 2,4-D treated hypocotyl was more sensitive to micrococcal nuclease digestion than that from untreated hypocotyl. These results suggest that nucleolar chrmatin as nuclear chromatin be made up of nucleosome with the size of 180 base pairs of DNA and the stimulation of rRNA synthesis by 2,4-D in soybean seedlings is probably related to the nuclease sensitivity of nucleolar chromatin.

Key words: Agarose gel electrophoresis; nuclease digestion; nucleolar chromatin; nucleoli; nucleosomes; soybean seedlings.

Introduction

When the synthetic auxin, 2,4-dichlorophenoxyacetic acid (2,4-D), is applied to young etiolated soybean seedlings, cells of the mature hypocotyl enlarge radially and proliferated. The abnormal proliferation of the mature hypocotyl is preceded by a large increase in RNA, especially ribosomal RNA (rRNA) (Key, 1969). This large accumulation of RNA in the mature tissue is associated with α-amanitin insensitive RNA polymerase activity which is localized in nucleoli (Lin *et al.*,

1975) indicating that relates to a nucleolar type RNA polymerase (RNA polymerase I). The enhanced rRNA synthesis by 2,4-D also accompanied by enlargement of nucleolar volume (Chen *et al.*, 1975). The large increase in nucleolar volume seems to relate directly to the large accumulation of preribosomal particles composed rRNA and ribosomal proteins within the structure (Chen *et al.*, 1983). The relatively small change in the organization/composition of the nucleolar chromatin may contribute to a limited extent to the enlarged nucleolus. A significant enhancement of rRNA synthesis is shown to be related to increased levels of RNA polymerase I enzymes and to increased activity of those RNA polymerase I molecules (Guilfoyle *et al.*, 1980). However these alone could not account for the enhancement of rRNA synthesis by as much 8- to 10-fold on a per unit DNA or 15- to 20-fold on a per unit of tissue basis (Guilfoyle *et al.*, 1980). Recently we were able to fractionate nucleoli into nucleolar chromatin (Chen *et al.*, 1983). In this study we investigate the sensitivity of this active nucleolar chromatin (rRNA genes) to nuclease and the structure of nucleosome particles by nuclease digestion analysis.

Materials and Methods

Plant Materials

Soybean seeds (*Glycine max* cv. Kaoshiung No. 3) that had been pretreated with 1% sodium hypochlorite solution were planted in moist vermiculite and germinated in the dark at 28°C. After 72 h of germination the seedlings were sprayed with 2.5 mM 2, 4-D (pH 6.0), and the hypocotyl tissue was harvested 24 h after spraying.

Chemicals

Spermine, Spermidine, Phenylmethylsulfonyl flouride (PMSF), 2-(N-Morpholino) ethane-sulfonic acid (MES), micrococcal nuclease, DNase I and DNase II were purchased from Sigma. Tris, 2-mercaptoethanol (2-MSH), triton x-100, SDS, proteinase K, were purchased from Merck. DTT, agarose, ϕX174 DNA Hae III fragments were from Bethesda Research Labortories (USA). Percoll was purchased from Pharmacia. All other organic chemicals were reagent grade.

Preparation of Nucleoli

The procedure for preparation of nucleoli was based on the method of Lin *et al.* (1975) with some modifications (Chen *et al.*, 1975; Hamilton *et al.*, 1972). Eighty grams of precooled tissue were chopped with razor blade and stirred with 160 ml of prechilled ethyl ether for 2 to 3 min. After removing the ethyl ether the tissues were blotted dry with paper towel. They were mixed with 3 to 4 volumes of grinding buffer containing 0.6 M sucrose, 25 mM MES-NaOH (pH 6.0),

10 mM $MgCl_2$, 10 mM KCl, 10 mM 2-MSH, 1 mM PMSF and 30% glycerol (v/v) (buffer A) and homogenized with the Willems polytron (Brinkman PS-20-ST) for 2 min at low setting. The homogenate was filtered through four layers of cheese cloth and then two layers of miracloth. Triton X-100 was added to the filtrate to final concentration of 1% (v/v) followed by gentle stirring with the Willems polytron, and then centrifugation for 15 min at 6,000 g. The pellet was resuspended in 20 mM MES buffer (pH 6.0) containing 20 mM KCl, 5 mM $MgCl_2$ 1.2 M sucrose and 10 mM 2-MSH (buffer B). The crude nucleolar suspension was loaded on percoll gradients prepared by layering 7 ml each of 40% and 60% percoll in buffer B. The gradients were centrifuged in a Sorvall HB-4 rotor for 30 min at 10,000 g. The interface between the 40% and 60% percoll layers was removed, mixed with equal volume of buffer B, and centrifuged at 8,000 g for 10 min. This pellet was washed with buffer B by repeated suspension and centrifugation.

Preparation of Nucleolar Chromatin

The purified nucleoli were suspended with a small volume of 10 mM Tris-HCl (pH 8), 20 mM KCl, 1 mM $Mg Cl_2$, 250 mM sucrose, and 20 mM DTT and gently stirred at room temperature for 20 min (Takahashi *et al.*, 1972). After centrifugation at 20,000 g for 30 min the nucleolar chromatin were obtained as pellet (Chen *et al.*, 1983).

Enzymatic Digestion of Nucleolar Chromatin

1. Micrococcal nuclease: Nucleolar chromatin suspended in 0.3 M sucrose, 60mM KCl, 15 mM NaCl, 0.15 mM spermine, 0.5 mM spermidine, 15 mM 2-MSH, 1 mM PMSF and 15 mM Tris-HCl (pH 7.4) was treated with micrococcal nuclease (15 units/OD_{260}. nucleolar chromatin) 37°C according to Noll *et al.* (1977).

2. DNase I: Nucleolar chromatin suspended in 10 mM Tris-HCl (pH 7.5), 3 mM $MgCl_2$, 10 mM NaCl and 1 mM PMSF was treated with DNase I (3 Kunitz/OD_{260} nucleolar chromatin) at 4°C according to Giri *et al.* (1980).

3. DNase II: Nucleolar chromatin in 0.1 mM Na_2-EDTA and 1 mM PMSF (adjusted to pH 7.0 with Tris-base) was treated with DNase II at 4°C according to Oosterhof *et al.*, (1975). Reaction of micrococcal nuclease and DNase I was terminated by adding Na_2-EDTA to the final concentration of 25 mM and reaction of DNase II was terminated by addition of 50 mM Tris-base and 0.1 mM Na_2-EDTA to increase pH higher than 7.0

Sucrose Density Gradient Fractionation of Nucleosomes

Fractionation of nuclease partially digested nucleolar chromatin was performed with 5 to 20% sucrose density gradient centrifugation according to Oosterhof *et al.* (1975). Nuclease digested materials loaded on 5 to 20% linear sucrose gradient

containing 12.5 mM Tris-HCl (pH 7.5), 5 mM Na_2-EDTA and 0.5 μg ethidium bromide per ml were centrifuged at 26,000 rpm with Beckman SW 28 rotor at 4°C for 16 h and fractionated with ISCO density gradient fractionator.

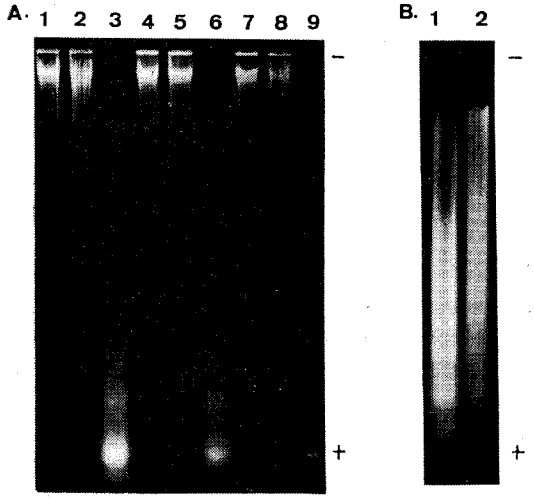

Fig. 1. Integrity and digestion by nuclease of nucleolar chromatin during different steps of purification. (A). DNA from nucleoli purified through sucrose gradients (columns 1 to 3), purified through percoll gradients once (columns 4 to 6) and three times (columns 7 to 9) was analyzed by 1.4% agarose gel electrophoresis. Columns 1, 4 and were control group. Columns 2, 5, and 8; addition of 1 mM $CaCl_2$ to test the activity of contaminated nuclease. Columns 3, 6 and 9; addition of micrococcal nuclease (30 units/OD_{260} of nucleoli). Each nucleolar suspension was incubated at 37°C for 30 seconds. (B). Micrococcal nuclease digestion (15 units/OD_{260}) of nuclear chromatin (column 1) and precoll purified nucleoli (column 2). Enzyme reactions were at 37°C for 30 seconds.

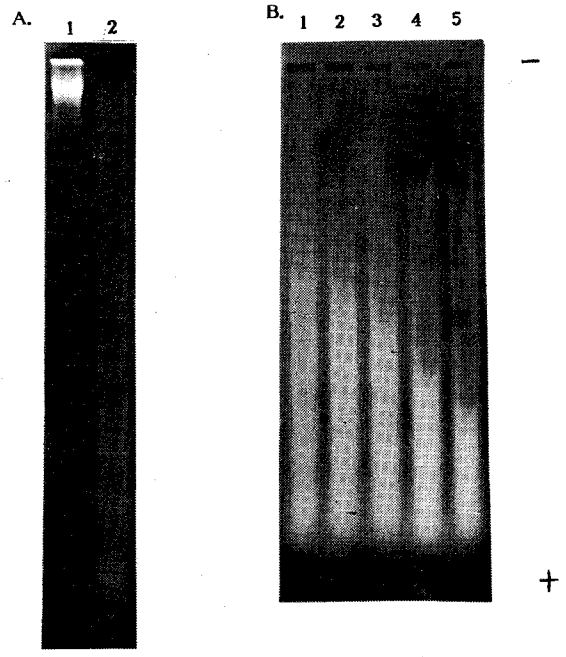

Fig. 2. Agarose gel electrophoresis of DNA from nucleolar chromatin after treatment with different nucleases. Nucleolar chromatin was treated with micrococcal nuclease (A) or DNase I (B) as indicated. Enzyme reaction was terminated by 25 mM Na_2-EDTA. DNA extracted from the enzyme digests was analyzed by 1.6% agarose gel electrophoresis. (A). Column 1 shows no treatment as a control. Column 2 shows after micrococcal nuclease treatment (15 units/OD_{260}) at 37°C for 30 seconds. (B). DNase I treatment (3 Kunitz units/OD_{260}) at 4°C for 6 min (column 1), 7 min (column 2), 8 min (column 3), 9 min (column 4) and 10 min (column 5).

DNA Extraction

DNA extraction from nucleolar chromatin, nucleoli and nucleosome fractions from sucrose gradient centrifugation of nuclease treated nucleolar chromatin was mainly followed the method of Kislev *et al.* (1980) by phenol-chloroform-isoamylalcohol extraction after proteinase K digestion of protein.

Agarose Gel Electrophoresis

One to two grams of agarose was dissolved in 100 ml of 40 mM Tris-acetate (pH 7.9) containing 2 mM Na_2-EDTA. Electrophoresis was done in 40 mM Tris-acetate (pH 7.9), 2 mM Na_2-EDTA plus 0.5 μg/ml ethidium bromide. ϕX174 RF DNA digested with Hae III restriction endonuclease was used as standard base pairs of DNA fragments.

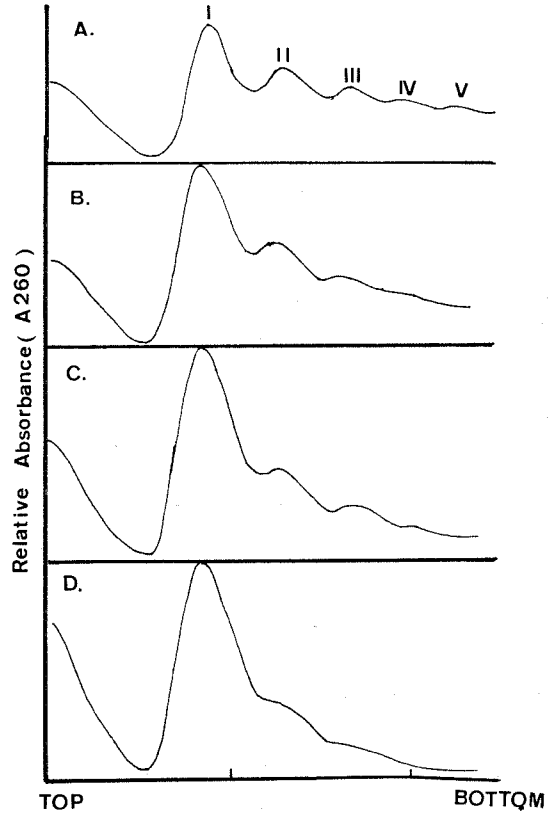

Fig. 3. Sedimentation profile of nucleosomes from nucleolar chromatin of 2,4-D treated soybean seedlings. Nucleolar chromatin was incubated with microccal nuclease (15 units/OD_{260}) at 37° for 30 seconds (A), for 1 min (B), for 1.5 min (C), and for 2 min (D), and followed by 5-20% sucrose gradient centrifugation. The sucrose gradients were fractionated from top to bottom as indicated on the abscissa.

Results

Nucleoli and nucleolar chromatin were often degraded by nuclease in the crude extracts. It was necessary to develop a rapid purification procedure to elemmate nuclease contaminations. Fig. 1A shows the integrity of DNA in nucleoli through different steps of purification. DNA from nucleoli purified through percoll once (columns 4 and 5) was almost as intact as DNA from nucleoli purified three times through percoll (columns 7 and 8). However DNA from nucleoli purified by sucrose gradient was partially degraded as shown in columns 1 and 2. Nucleoli purified by either sucrose or percoll could digested with micrococcal nuclease with the concentration of 30 units/OD_{260} at 37°C for 30 seconds to small fragments (see columns 3, 6 and 9). In our earlier reports we were able to fractionate uncleoli into preribosomal particles and nucleolar chromatin (Chen *et al.*, 1983). So that we test the possibility whether the fractionated nucleolar chromatin is more susceptible to micrococcal nuclease digestion than intact nucleoli. The results of this study with 15 units of micrococcal nuclease/OD_{260} material incubated at 37°C for 30 seconds are shown in Fig. 1B. As it is predictable that fractionate uncleolar chromatin is more susceptible to nuclease digestion than intact nucleoli (column 1 ver. 2). We also tested the sensitivity of nucleolar chromatin to DNase I or DNase I Iother than micrococcal nuclease. Fig. 2B shows gel electrophoresis of DNA extracted from chromatin after treatment with 3 Kunitz units/OD_{260} of DNase I at 4°C for 6 min, 7 min, 8 min, 9 min and 10 min (columns 1, 2, 3, 4, and 5). DNase II treatments showed similar electrophoresis patterns. All three nucleases tested showed digestion of nucleolar chromatins into unit structure of nucleosomes. Based on these experiments we selected micrococcal nuclease for isolation of nucleosome particles in the partial digestion study, we used 15 units of enzyme per 1 OD_{260} nucleolar chromatin material for 0.5, 1, 1.5, and 2.0 min incubation at 37°C. After the termination of the reaction, the mixtures were layered on 5 to 20% linear sucrose gradient for fractionation.

Figure 3 shows the results of separation of nucleosomes after sucrose gradient centrifugation. When the incubation time was 30 seconds, the partially digested nucleolar chromatin was separated into at least 5 discrete bands (Fig. 3A), corresponding to nucleosome monomer (band I), dimer (band II), trimer (band III), tetramer (band IV) and pentamer (band V). By increasing the time of incubation with the enzyme, bands II to V on sucrose gradients were found to shift to band I, the nucleosome monomers (Fig. 3B, 3C, and 3D). Analysis of DNA extracted from these bands on 2.0% agarose gels indicated the DNA of nucleosomes monomer has unique length of 180 base pairs (Fig. 4). Figure 4 also shows the various bands on sucrose gradients corresponding to nucleosome dimers, trimers, tetramers, and pentamers which were composed of 360, 540, 720, and 900 base pairs of DNA.

However, some bands were found to be contaminated with smaller size of nucleosome. Fractions containing more the pentamers were also found but they were not clearly resolved on sucrose gradients. For further purification of nucleosome particles, the various separated bands on the gradients were concentrated and layered on 5 to 20% sucrose gradient individually for centrifugation at 35,000 rpm

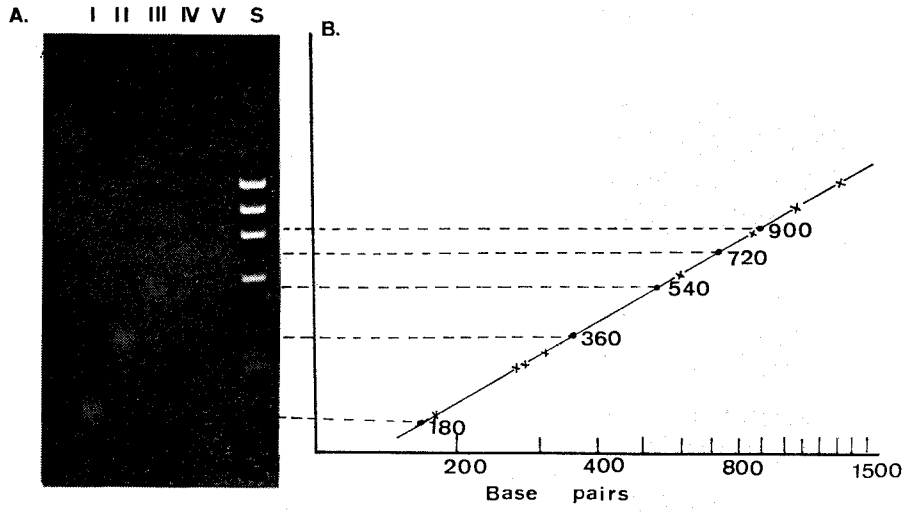

Fig. 4. Agarose gel electrophoresis of DNA extracted from nucleosomes which have been fractionated by sucrose density gradient centrifugation. Nucleolar chromatin digested with micrococcal nuclease was fractionated on sucrose gradient centrifugation as indicated in Fig. 3. DNA extracted from nucleosomes isolated from band I to band V (Fig. 3) was loaded on 2.0% agarose gel for electrophoresis. Columns I to V were nucleosome monomer, dimer, trimer, tetramer and pentamer, respectively. Column S, standard molecular weight marker of ϕX174 RF DNA/Hae III fragments.

for 5 h in a Beckman SW 41 rotor. Each nucleosome band was shown to separate into distinct individual band corresponding to nucleosome monomer, dimers, trimers, etc. without contamination of the others (Fig. 5).

Nucleolar chromatin obtained from 2,4-D treated soybean hypocotyl was more sensitive to micrococcal nuclease digestion than from control (Fig. 6 column 2 ver. 4).

Discussion

We previously reported a quick isolation method with a sufficient yield of nucleoli from soybean hypocotyl for biochemical work (Lin *et al.*, 1975). However, we found in this study that they were contaminated with some nuclease activity.

The additional method with percoll procedure (Chen *et al.*, 1983) allowed the purification of not only morphologically intact nucleoli substantially free of starch

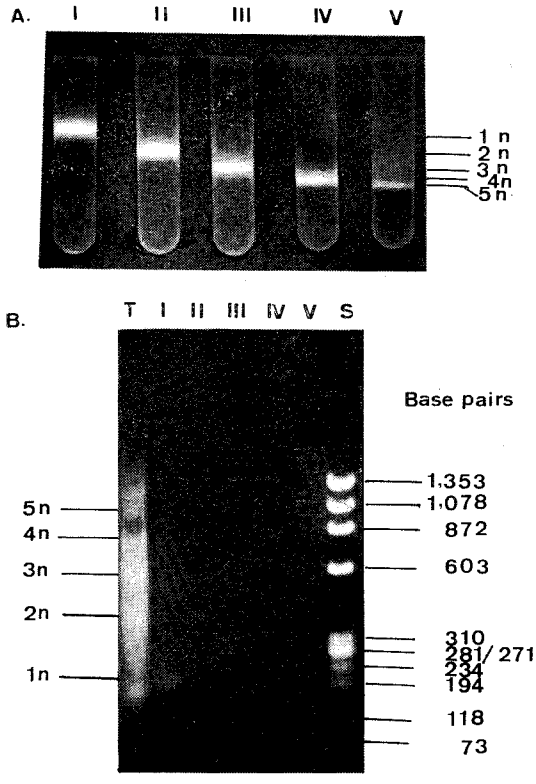

Fig. 5. Purification of nucleosomes by sucrose density gradients and analysis of nucleosomal DNA. Nuclease digested chromatin was fractionated by sucrose gradient centrifugation into five bands, bands I to V as indicated in Fig. 3, corresdonding to nucleosome monomer, dimer, trimer, tetramer and pentamer, respectively. Each band was collected and recentrifuged individually on sucrose gradient for repurification (A). After centrifugation, gradients were fractionated and subjected to DNA extraction and agarose gel electrophoresis (B). Column S is a DNA marker (Hae III fragments of ϕX174 RF DNA), column T is total DNA extracted from micrococcal nuclease digested chromatin before fractionated on sucrose gradient centrifugation.

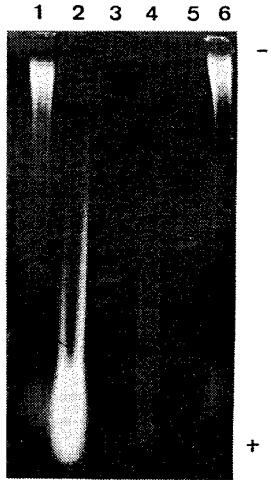

Fig. 6. Sensitivity of nucleolar chromatin from nontreated (control) and 2,4-D treated soybean seedlings to micrococcal nuclease digestion. DNA isolated from nucleolar chromatin after micrococcal nuclease digestlon was analyzed on 1.0% agarose gel electrophoresis. Columns 1 and 2 are 2, 4-D treated nucleolar chromatin; column 4, after micrococcal nuclease treatment; columns 3 and 5 are a marker of DNA fragment from ϕX174 RF DNA digested with restriction endonuclease Hae III and Hae II, respectively.

grains, cytoplasmic and nucleoplasmic contamination of nuclease activity. We also found nucleolar chromatin fractionated from nucleoli by DTT treatment without

the other nucleolar components, i.e. preribosomal particles, was better compared intact nucleoli for isolation of nucleosomes.

Nucleolar chromatin is sensitive to all three nuclease, i.e. micrococcal nuclease, DNase I and DNase II tested in this study. Based on partial digestion of nucleolar chromatin with micrococcal nuclease and separation on sucrose gradient, soybean nucleolar chromatin is made up of nucleosome particles with 180 base pairs of DNA, which is in close agreement with other reports on plant nucleosomes (McGhee and Engel, 1975; Schöffer *et al.*, 1984). The unit length of DNA in a nucleosome so far reported was 160 to 240 base pairs (Kornberg, 1977). Also by prolonged nuclease treatment the monomer band was shown to be about 140 base pairs (McGhee *et al.*, 1975).

Isolation of nucleosomes reported in most of animal systems (Igo-Kemenes *et al.*, 1982), and in several plant systems (McGhee and Engel, 1975; Schöffer *et al.*, 1984) were either from nuclei or from chromatin preparations.

The most important aspect of our study is using nucleolus, whih is a rRNA synthetic site to investigate whether nucleolar chromatin is also composed of multiple units of nucleosomes as nuclear chromatin. When the synthetic auxin, 2,4-D is applied to young etiolated soybean seedlings, cells of the mature hypocotyl enlarge radially and proliferate. The abnormal proliferation of the mature hypocotyl is proceeded by a large increase in RNA, especially rRNA (Key, 1969) and with an enlarged nucleoli (Chen *et al.*, 1975).

The large increase in nucleolar volume following auxin treatment related directly to the large accumulation of preribosomal particles composed of rRNA and ribosomal proteins within this structure (Chen *et al.*, 1983). A significant proportion of this increased synthesis of rRNA in response to auxin relates to increased levels of RNA polymerase I enzyme and to increased activity of those RNA polymerase I molecules (Gulifoyle *et al.*, 1980). A number of interesting features were discovered with respect to nuclease sensitivity of active genes. DNase II, the first nuclease to be used in the study of active chromatin (Gottesfeld *et al.*, 1974), is now largely replaced by other nucleases. DNase I was introduced in a study of transcribed and nontranscribed chicken globin genes (Weintraub and Groundine, 1976).

The chromatin structure of *Drosophila melanogaster* HSP 70 genes in the repressed and actively transcribing states has been investigated by Levy and Noll (1981). The coding regions in the repressed stated were found to be more resistant to micrococcal nuclease digestion than the bulk of chromatin. In the active state the coding regions became more susceptible and yielded products without any apparent nucleosomal periodicity. In contract transcribed chromatin from yeast (Lohr 1981) the expressed herpes thymidines kinase gene (Camerini-Otero and Zasloff, 1980) introduced into mouse cells and chromatin from suspen-

sion cultured cells of *Nicotiana tabacum* var. white Burley which were transformed by *Agrobacterium tumefaciens*, strain T37 (Schöffer *et al.*, 1984) were found to be in compact nucleosomes.

The transcribed genes for ribosomal RNA in amphibian oocytes are not compacted at all and the non-transcribed spacers have a beaded structure that reflect a low degree of compaction (Igo-Kemenes *et al.*, 1982). Another study consider it to be a supranucleosomal structure with a compaction factor of at least 20 (Pruitt and Graing, 1981). A detailed study on *Xenopus laevis* nucleolar chromatin supports the view that active ribosomal RNA genes are not compact into nucleosomes (Kornberg, 1977). In *Tetrahymena pyiformis* the rRNA genes themselves lack compact nucleosomes, while the destal spacers appear to be compacted into nucleosomes (Igo-Kemenses *et al.*, 1982). In this study we used micrococcal nuclease to demonstrate that nucleoli from 2,4-D treated tissues were much more sensitive than those from non-treated tissues. We also found that nucleolar chromatin was constructed by nucleosome particles with the unit length of 180 base pairs DNA.

Literature Cited

Camerini-Otero, R. D. and M. A. Zasloff. 1980. Nucleosomal packaging of the thymidine kinase gene of herpes simplex virus transferred into mouse cells: An actively expressed single-copy gene. Proc. Natl. Acad. Sci. USA **77**: 5079-5083.

Chen Y. M., C. Y. Lin, H. Chang, T. J. Guilfoyle, and J. L. Key. 1975. Isolation and properties of nuclei from control and auxin-treated soybean hypocotyl. Plant Physiol. **56**: 78-82.

Chen, Y. M., D. H. Huang, S. F. Lin, C. Y. Lin, and J. L. Key. 1983. Fractionation of nucleoli from auxin-treated soybean hypocotyl into nucleolar chromatin and preribosomal particles. Plant Physiol. **73**: 746-753.

Giri, C. P. and M. A. Gorovsky. 1980. DNase I sensitivity of ribosomal genes in isolated nucleosomes core particles. Nucleic Acids Res. **8**: 197-214.

Gottesfeld, J. M., W. R. Garrard, G. Bagi, R. F. Wilson, and J. Bonner. 1974. Partial purification of the template-active fraction of chromatin: A preliminary report. Proc. Natl. Acad. Sci. USA **71**: 2193-2197.

Guilfoyle, T., N. Olszewski, and L. Zurfluh. 1980. RNA polymerases and transcription during developmental transitions in soybean. *In* C. J. Leaver (ed.), Genome Organization and Experession in Plants. Plenum Press, New York, pp. 93-103.

Hamilton, R. H., U. Kunsch, and A. Temperli. 1972. Simple rapid procedure for isolation of tobacco leaf nuclei. Anal. Biochem. **49**: 48-57.

Igo-Kemenes. T., W. Hörz, and F. Zachau. 1982. Chromatin. Ann. Rev. Biochem. **51**: 89-121.

Key, J. L. 1969. Hormones and nucleic acid metabolism. Ann. Rev. Plant Physiol. **20**: 449-474.

Kislev, N. and I. Rubenstein. 1980. Utility of ethidium bromide in the extraction from whole plants of high molecular weight maize DNA. Plant Physiol. **66**: 1140-1143.

Kornberg, R. D. 1977. Structure of chromatin. Ann. Rev. Biochem. **46**: 931-954.

Labhart, P. and R. Koller. 1982. Structure of the active nucleolar chromatin of *Xenopus laevis* oocytes. Cell **28**: 279-292.

Levy, A. and M. Noll. 1981. Chromatin fine structure of active and repressed genes. Nature **289**: 198-203.

Lin, C. Y., T. J. Guilfoyle, Y. M. Chen, and J. L. Key. 1975. Isolation of nucleoli and localization of RNA polymerase I from soybean hypocotyl. Plant Physiol. **56**: 850-852.

Lohr, D. E. 1981. Detailed analysis of the nucleosomal organization of transcribed DNA in yeast chromatin. Biochem. **20**: 5966-5972.

McGhee, J. D. and J. D. Engel. 1975. Subunit structure of chromatin is the same in plants and animals. Nature **254**: 499-450.

Noll, M. and R. D. Kornberg. 1977. Action of micrococcal nuclease on chromatin and the location of histone H1. J. Mol. Biol. **109**: 393-404.

Ooeterhof, D. K., J. C. Hozier, and R. L. Rill. 1975. Nuclease action on chromatin: Evidence for discrete, repeated nucleoprotein units along chromatin fibrils. Proc. Natl. Acad. Sci. USA **72**: 633-637.

Pruitt, S. C. and R. M. Grainger. 1981. A mosaicism in the higher order structure of *Xenopus* oocyte nucleolar chromatin prior to and during ribosomal gene transcription. Cell **23**: 711-720.

Schöffer, W., K. Weising, and G, Kahl. 1984. T-DNA of a crown gall tumor is organized in nucleosomes. J. Europ. Mol. Biol. **3**: 373-376.

Takahashi, N., J. Shimada, J. Higo, S. Sigo, and S. Tanifugi. 1972. Ribosomal precursor particles from plant nucleoli. Biochim. Biophys. Acta **262**: 502-513.

Weintraub, H. and M. Groundine. 1976. Chromosomal subunits in active genes have an altered conformation. Science **193**: 848-856.

由大豆幼苗下胚軸的核仁染色質分離核小體及其特性的研究

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由 2,4-D 處理過的大豆幼苗下胚軸，先分離出核仁，再把它瓦解並且分離核仁染色質。核仁染色質先用 micrococcal nuclease 部分水解，再用 5%～20% 等差蔗糖梯度離心後，可分成五個區帶 (band)，每一個區帶相當於核小體的單體 (monomer)，雙體 (dimer)，三體 (trimer)，四體 (tetramer) 及五體 (pentamer) 等。雖然在等差蔗糖梯度離心後，五體以上的聚合物也可能發現，但其解析力不佳。從上述各區帶所得到的樣品，經抽取其 DNA，再用 2.0% agarose 膠質電泳分析，由結果顯示大豆幼苗的核小體是由 180 鹽基對 (base pairs) 的 DNA 所組成的。由 2,4-D 處理過的大豆幼苗下胚軸中所分離出的核仁染色質對 micrococcal nuclease 是比對照組染色質來得敏感的。由上述結果可推知大豆下胚軸的核仁染色質與一般細胞核染色質一樣，是由 180 鹽基對 DNA 所組成的，而且 2,4-D 處理過後的大豆幼苗會促進其 rRNA 的合成，可能與核仁染色質對核酸水解酶 (nuclease) 的敏感性有關。