A Comparison of the Digestion of Nuclei and Chromatin by Staphylococcal Nuclease†

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ABSTRACT: We have followed the kinetics of staphylococcal nuclease digestion of duck reticulocyte nuclei and chromatin from early stages to the digestion limit. We confirm that partial digestion of nuclei produces discrete DNA bands which are multiples of a monomer, 185 base pairs in length. The multimers are shown to be precursors of the monomer, which is next digested to a homogeneous, 140 base pair fragment. This fragment in turn gives rise to an array of nuclear limit digest DNA bands, which is almost identical with the limit digest pattern of isolated chromatin. As in the case of chromatin, half the DNA of nuclei is acid soluble at this limit. While the DNA limit digest patterns of nuclei and chromatin are similar, the large multimeric structures present as intermediates in nuclear digestion are absent in chromatin digestion. Alternate methods of chromatin gel preparation appear to leave more of the higher order structure intact, as measured by the production of these multimeric bands. Our results are consistent with the "beads on a string" model of chromatin proposed by others.

Studies in several laboratories of the partial digestion of nuclei by staphylococcal nuclease or an endogenous endonuclease have shown that the resulting DNA consists of a series of fragments of discrete size, which are multiples of a fundamental unit approximately 200 base pairs in length (Hewish and Burgoyne, 1973; Noll, 1974). With sufficient digestion, most of the starting nuclear DNA may be recovered as the 200 base pair fragment (Noll, 1974). It has been suggested (Hewish and Burgoyne, 1973; Kornberg, 1974; Noll, 1974) that this behavior reflects a regular arrangement of chromatin proteins along the nuclear DNA, probably related to the "beads on a string" structure of nucleoprotein seen in the electron microscope (Olins and Olins, 1974).

Earlier work in this laboratory (Clark and Felsenfeld, 1971, 1974) has demonstrated that 50% of the DNA of isolated chromatin is resistant to staphylococcal nuclease digestion. We have shown (Axel et al., 1974) that the resistant material is a series of double-stranded DNA fragments ranging in size from about 130 to 45 base pairs. In the course of the digestion the fragments arise simultaneously, passing through none of the discrete higher molecular weight intermediates which are seen during digestion of nuclei.

In this paper we examine the intermediates of nuclear digestion in detail, and show the relationship of these fragments to those produced by digestion of chromatin.

Materials and Methods

Nuclei from frozen duck reticulocytes were prepared from thawed cells suspended with a Dounce homogenizer, in 10 m*M* Tris-HCl (pH 8)–1 m*M* $CaCl_2$–0.25 *M* sucrose. The nuclei were collected by centrifugation and resuspended in the same buffer containing 0.5% Triton X-100. Washing with Triton was repeated three times, and was followed by two washes in 1 m*M* Tris-HCl (pH 8)–0.1 m*M* $CaCl_2$–0.25 *M* sucrose (0.08 *M* sucrose was used for preparations to be layered on sucrose gradients). Nuclei have also been prepared by omitting the Triton steps and by replacing the 1 m*M* $CaCl_2$ with 1 m*M* $MgCl_2$ in the initial washes, and both preparations behave identically with those prepared as described above. Nuclei were used immediately after preparation.

Chromatin was prepared as described previously (Axel et al., 1973). Nuclei were prepared as described above with 1 m*M* $MgCl_2$ replacing the 1 m*M* $CaCl_2$. The nuclei were then washed in the same buffer without Triton and then with 0.25 *M* NaCl–0.025 *M* NaEDTA (pH 6). They were then washed with a series of Tris buffers of gradually decreasing ionic strength, in five steps going from 50 to 0.5 m*M* Tris-HCl (pH 8). This gel was then sheared in a Virtis homogenizer and centrifuged at 12,000*g* for 10 min to remove membrane debris. Staphylococcal nuclease (6000 U/mg) was obtained from Worthington Biochemical Corp.

Digestion of nuclei was carried out in 1 m*M* Tris-HCl (pH 8), 0.1 m*M* $CaCl_2$, 0.05–0.25 *M* sucrose, and 1 to 100 μg/ml of nuclease at a DNA concentration of approximately 750 μg/ml. The reactions were stopped with an excess of NaEDTA (pH 7). DNA concentrations of nuclei were determined by suspending an aliquot of freshly homogenized nuclei in 2 *M* NaCl–5 *M* urea and measuring A_{260}. Nuclei incubated similarly but without added enzyme yielded no DNA of a size smaller than 5000 nucleotides long under denaturing conditions.

DNA was prepared from the digests as described previously (Axel et al., 1974).

Nucleoprotein fragments were separated by sucrose gradient sedimentation. After halting the digestion reaction, the mixture was layered onto a 5–20% linear sucrose gradient in 1 m*M* Tris-HCl (pH 8)–0.1 m*M* NaEDTA (pH 7) with a 70% sucrose shelf in the same buffer and centrifuged 16 hr at 82,000*g* (SW 27 rotor, 25,000 rpm, 4°).

Electrophoresis of DNA fragments on 4 and 6% acrylamide gels was performed as described previously (Axel et al., 1974). Three percent acrylamide gels were run either as discs, or as slabs with 0.5% agarose added. Most gels were

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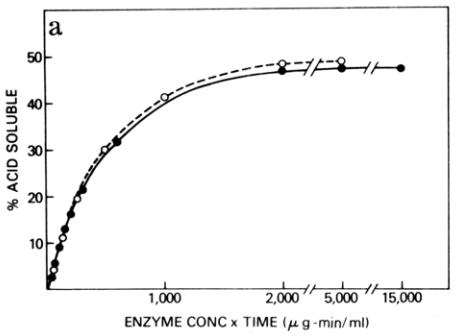

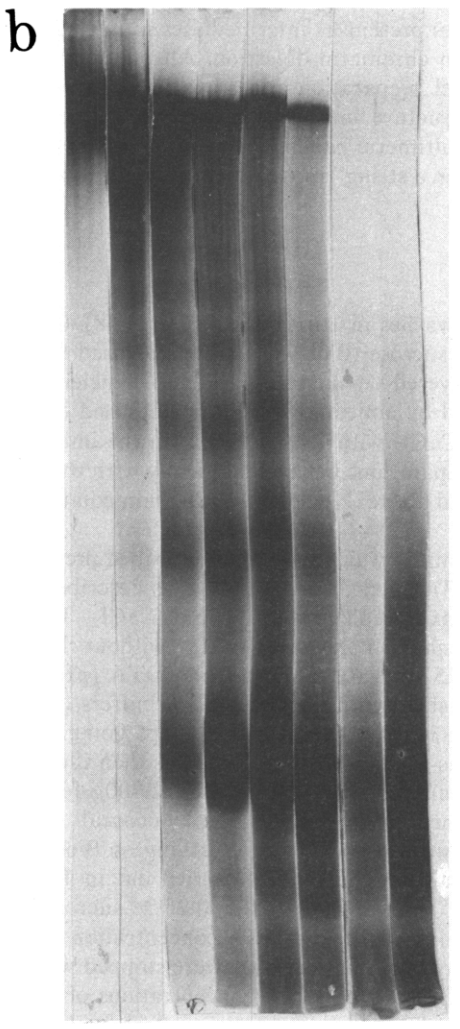

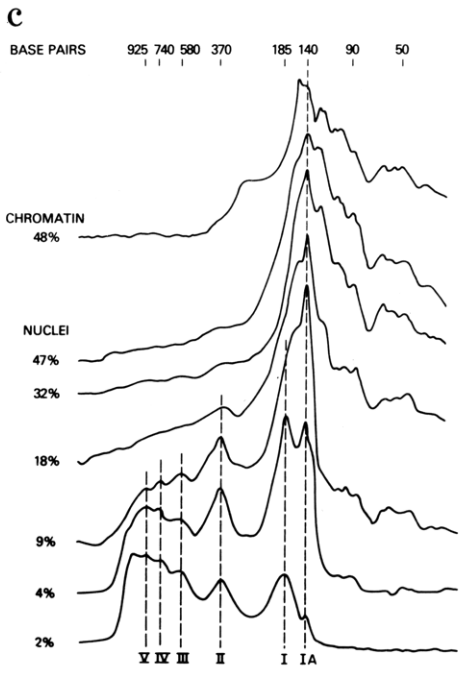

FIGURE 1: Kinetics of nuclear digestion. (a) Release of acid-soluble products. Duck reticulocyte nuclei (●) and duck reticulocyte chromatin (○) were digested with 1–60 μg/ml of staphylococcal nuclease for 10 min to 1 hr in 1 m*M* Tris (pH 8)–0.1 m*M* $CaCl_2$ at 1 mg of DNA/ml. The fraction of the DNA soluble in 0.4 *M* perchloric acid–0.4 *M* NaCl was measured. RNA was shown to contribute <3% of the A_{260}. (b) Polyacrylamide gel electrophoresis. Nuclei were digested and the isolated DNA was run on 3% disc gels as described in Materials and Methods. From left to right are 1, 2, 3, 8, 16, 25, and 48% acid-soluble digest. The last gel is a chromatin limit, 48%, digest. (c) Polyacrylamide gel electrophoresis. Nuclei were digested as in a, run on a 4% slab gel, stained, and photographed. Negatives were scanned with a Joyce-Loebl microdensitometer. These scans have been shown to be linear in DNA concentration. Migration is from left to right. From bottom to top are: nuclear digests at 2, 4, 9, 18, 32, and 47%, and a chromatin digest at 48% acid-soluble DNA. Assigned sizes (see Figure 4) are shown across the top.

stained with "Stains-all" (Eastman) but occasionally the gels were stained in 1 μg/ml of ethidium bromide for 1 hr and photographed using ultraviolet light. Electrophoresis of nucleoprotein fragments was performed as described above, except that the ionic strength of the sample, 4% acrylamide gel, and running buffer was reduced to 10 m*M* Tris-borate.

To determine the size of the DNA in individual bands, the unstained gels were sliced and the DNA was extracted and repurified on hydroxylapatite. Sedimentation velocity measurements of these DNA fragments were performed in the analytical ultracentrifuge, and the molecular weight distribution determined using the equation of Studier (1965). DNA fragments were also sized by coelectrophoresing the DNA sample with total *Hemophilus influenza* restriction enzymes digests of λ and SV40 DNA.

Results

Kinetics of Nuclear Digestion. The kinetics of staphylococcal nuclease digestion of DNA in duck reticulocyte nuclei were followed by measuring the amount of DNA rendered acid soluble. The digestion of nuclei follows the same kinetics as reported earlier (Clark and Felsenfeld, 1974) for the digestion of isolated chromatin. There is a fairly linear rise in acid-soluble products and in both cases a limit is reached when 48% of the DNA has been digested (Figure 1a). At various points in the digestion of nuclei, the size distribution of the remaining DNA was analyzed by polyacrylamide gel electrophoresis. Typical results are shown in Figure 1b. When only 2% of the DNA is acid soluble, the characteristic pattern of the nuclear digest emerges, and as the digestion proceeds, the DNA passes from high molecular weight through "multimers" to "monomer".[1] It will later be shown that the higher molecular weight DNA bands are true multiples of the monomer, 185 base pairs in length. The monomer size, however, is not the limiting molecular weight reached by the DNA. If the digest is examined on a 4% acrylamide gel (Figure 1c) it is apparent that with increasing digestion the 185 base pair monomer is cleaved to form a 140 base pair fragment (band IA). A shoulder which

[1] The term "monomer" is used in this paper to describe both the nucleoprotein subunit and the DNA contained in it.

forms in the region between these sizes is a probable intermediate in this process. As the digestion proceeds toward the 50% limit, band IA digests further, and produces a final series of still smaller DNA pieces. These limit digest bands (except the 140 base pair band) appear simultaneously, and increase in intensity at the expense of the monomers I and IA. At the digestion limit, the size distributions of the DNA fragments produced from nuclei and from chromatin are nearly identical (Figure 1c).[2] All of this DNA is double stranded as judged by thermal denaturation and resistance to nuclease digestion under single-strand specific conditions (Kacian and Spiegelman, 1974).

Our results are consistent with a digestion mechanism in which high molecular weight DNA is digested to multimers, then to monomer, and finally to limit bands. To verify this mechanism and to study the intermediates of digestion, we isolated nucleoprotein containing discrete sizes of DNA. Digestion of nuclei was stopped at a point where only a small percentage of the DNA was acid soluble, and the nucleoprotein fragments were sedimented in sucrose gradients (Figure 2a). The indicated fractions were pooled, and their DNA was analyzed on polyacrylamide gels.

It is seen that nucleoprotein fragments fractionated on the gradient consist of classes whose sedimentation coefficients are monotonically related to the size of the component DNA and thus the individual DNA bands may be isolated in association with protein (Figure 2b). More rapidly sedimenting nucleoprotein particles contain the higher DNA multimers. By digesting the above isolated monomer, dimer, or trimer nucleoprotein particles one can get a clear picture of the intermediates in the digestion of a discrete size class of nucleoprotein. With increasing amounts of digestion of isolated band I (fraction 5), the DNA is found to pass through IA to the limit bands. Similarly, when dimer nucleoprotein (fraction 4) is digested, the DNA size passes from dimer through I to IA and finally to the limit bands. Trimer nucleoprotein (fraction 3) was also digested, and while less pure than the other nucleoprotein fractions, its DNA was found to pass through a mixture of dimer and monomer, to IA, and to the final limit bands.

From the kinetics of nuclear digestion it is apparent that little of the DNA becomes acid soluble when monomer is excised and that most of the starting DNA passes through monomer. Fifty percent of the DNA is monomer size when only 4% of the DNA is acid soluble (Figure 1c), and virtually all of the DNA in the larger nucleoprotein complexes can be shown to produce monomer after only slight amounts of further digestion. Under our conditions, staphylococcal nuclease makes only double-strand cuts in DNA (Clark and Felsenfeld, 1974). The monomer nucleoprotein is presumably produced by this "clipping" action of the nuclease, and thus should be very similar to the multimers, and to whole nuclei, in its chemical properties. The sucrose gradient isolated nucleoprotein fractions described above have an average protein:DNA ratio of 1.39 throughout the gradient. This is close to the 1.35 ratio measured for isolated chromatin. The susceptibility of DNA within the various

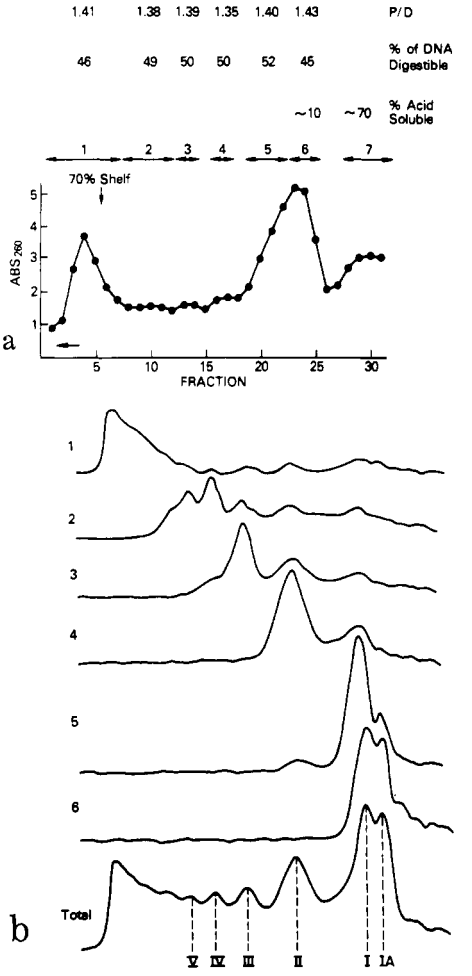

FIGURE 2: (a) Sucrose gradient centrifugation of partially digested nuclei. Nuclei were partially digested (7% acid-soluble DNA), the reaction was stopped by the addition of NaEDTA to 0.3 m*M*, and the mixture was layered onto sucrose gradients as described in Materials and Methods. Sucrose was removed from the pooled fractions by dialysis. Protein concentration was determined by the method of Lowry et al. (1951). DNA concentration was determined using A_{260}. Percent DNA digestible was measured by adding enough $CaCl_2$ to react with EDTA in the gradient and leave a final free Ca^{2+} concentration of 0.1 m*M*. Direction of sedimentation is indicated by an arrow. (b) Scans of 3% acrylamide gels of DNA from sucrose gradient fractions pooled as shown in a.

nucleoprotein fractions to staphylococcal nuclease is also constant for particles of monomer size and larger, and the same as whole nuclei (see Figure 2a).

It should be noted that the DNA of monomer (band I) and of the higher multimers is not homogeneous, but rather displays a distribution of sizes centered around 185 base pairs and multiples of this size. In our hands there is always some DNA which migrates on gels at the nodes between peaks, when judged by Stains-all staining.[3] Truly homogeneous DNA fragments, such as those produced by the action of *H. influenza* restriction enzyme on λ or SV40 DNA, migrate as sharp bands, with a width of 1 mm or less when electrophoresed under our standard conditions.

Kinetics of Chromatin Digestion. Unlike nuclei, our purified and sheared chromatin preparations do not yield the

[2] It should be noted that the largest band of chromatin limit digest (160 base pairs) is barely visible in nuclear digests. We have no explanation for this difference. We now believe that this 160 base pair band from chromatin, in addition to appearing in the limit digest, can serve as an intermediate in the production of all the smaller limit digest bands. In nuclear digestion, the 140 base pair band (1A) appears to play a corresponding role, acting as a precursor of the smaller limit digest bands while also appearing intact as the largest limit digest band.

[3] We have shown Stains-all to produce a response which is linear in DNA concentration. Ethidium bromide staining may or may not reveal this material at the nodes, depending on the staining conditions. We have been unable to find staining conditions such that ethidium bromide staining increases linearly with DNA concentration.

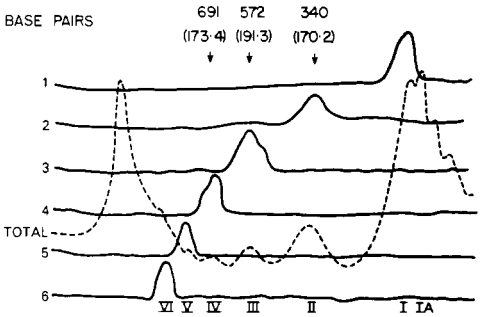

FIGURE 3: Acrylamide gel electrophoresis of isolated DNA bands. The DNA from single nuclei digest bands was purified from 3% acrylamide gels by homogenizing the cut out band in the VirTis in 2 *M* NaCl–5 *M* urea–0.1 *M* $NaPO_4$ (pH 7). Acrylamide fragments were removed by passing this suspension over hydroxylapatite, and DNA was recovered by a 0.5 *M* $NaPO_4$ wash. These fragments were run on 3% acrylamide gels; numbers on the left correspond to fraction numbers. The original digest (- - - -) is used as a marker. Fragment sizes indicated above were obtained from sedimentation velocity measurements (see text).

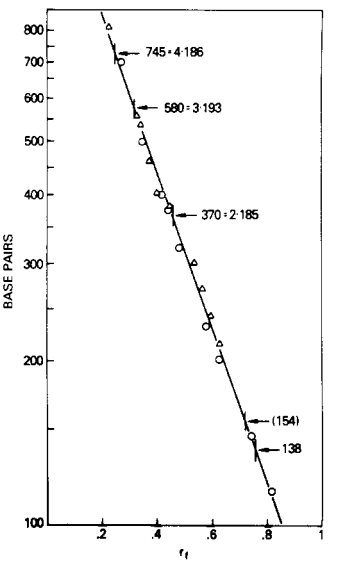

FIGURE 4: Calibration of 4% acrylamide gels; 4 and 7% nuclear digest DNA and SV40 hin and λ hin fragments were run on a 4% acrylamide slab gel. Nuclear digest bands were visualized by staining, hin fragments by autoradiography. SV40 hin fragment sizes were calculated from the fraction of SV40 in each band (Danna et al., 1973) taking the molecular weight of SV40 as 3.6×10^6 (Tai et al., 1972). λ hin fragment sizes were determined by Maniatis (Maniatis et al., 1973).

monomer and multimer DNA bands as intermediates in staphylococcal nuclease digestion. The intermediates of chromatin digestion consist of DNA with a very broad continuous size distribution (Axel et al., 1974). Discrete monomer and multimer DNA bands are observed as nuclear digestion intermediates only because the monomer is not internally attacked by the nuclease before it is clipped free from the remaining nucleoprotein (see Discussion). It is possible that monomer and multimer nucleoprotein structures do exist in sheared chromatin, but that the monomer bead is attacked internally before it is excised, so that no discrete monomer and multimer DNA bands are observed. Sahasrabuddhe and Van Holde (1974) do find 11S nucleoprotein intermediates in chromatin digestion, and Weintraub (personal communication) has confirmed this result. This is about the size of the monomer nucleoprotein fragment from nuclear digests as measured both by Noll (1974) and in our laboratory.

We have investigated this phenomenon further by using polyacrylamide gels formed and run at low ionic strength to separate the nucleoprotein oligomers. Using 4% polyacrylamide, and conditions similar to those for DNA gels except for low ionic strength (one-tenth the buffer concentration), we find that purified nucleoprotein oligomers from partial nuclear digests migrate as distinct bands, but more slowly than the corresponding protein-free DNA fragments they contain. If the electrophoresis is now repeated with partial digests of sheared chromatin, bands appear at positions corresponding to the dimer, monomer, and IA nucleoprotein fragments. Thus, the same subunit structures present in nuclei are also present in chromatin. In the case of chromatin the "bead" and "string" are attacked at comparable rates. Nevertheless, so long as the protein remains attached to DNA, the oligomers are stable entities, and can be observed on gels. Removal of protein liberates the broken DNA, which no longer reveals the pattern of structure present in the chromatin.

We have varied our methods of chromatin preparation to determine the point at which the monomer is no longer excised intact, that is, the point at which the monomer and multimer DNA bands are no longer observed. If we eliminate the preparative steps (see Materials and Methods) in which nuclei are slowly swelled and then sheared, and instead form a chromatin gel by an abrupt ionic strength decrease, partial digestion of the unsheared gel yields multimer and monomer DNA bands identical with those obtained from nuclei. Partial shearing of this gel eliminates multimer DNA bands in the digest, but monomer, I and IA, DNA band formation still occurs at a reduced level. Apparently, some of the monomers are no longer excised intact. When extensively sheared, this chromatin, like our normal sheared chromatin, produces no discrete digestion intermediates. It is thus possible to preserve the higher order structure present in the nucleus by very gentle treatment of the nucleoprotein. Other modifications of the chromatin preparative procedure yield somewhat different results. If the nuclei are swelled by gradual ionic strength decrease (see Materials and Methods), the resulting gel produces no multimer and reduced amounts of monomer DNA digestion intermediates, even in the absence of a separate shearing step. The "monomer" intermediate derived from this material is 160 base pairs in length, half-way between the sizes of I and IA (see footnote 2). It should be emphasized that all of the above chromatin preparations, and all of the nuclear preparations, yield the same DNA limit digest pattern, regardless of which stable intermediates are formed.

It seems likely that the band patterns we observe reflect structure present in the native nucleoprotein. We believe that rearrangement is not a major factor in the production of either the multimer bands or those present in the limit digest. When sheared chromatin is digested by staphylococcal nuclease at low ionic strength, histones do not exchange freely onto added DNA nor are they liberated into solution (Clark and Felsenfeld, 1971, 1974). Formaldehyde cross-linked chromatin can be digested to produce a limit digest with the same percentage digestion and of similar DNA size (as judged by sedimentation coefficient distribution) to that obtained with normal chromatin. Virtually all of the histones remain cross-linked to the DNA at the end of the digestion process (B. Sollner-Webb et al., manuscript in preparation). We conclude that no removal or major rearrangement of protein in occurring within nuclei or chromatin

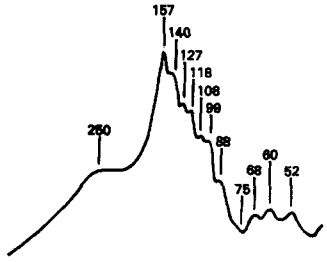

FIGURE 5: Calibration of 6% acrylamide gels. Chromatin limit digest DNA and sequenced λ hin fragments were run on 6% acrylamide gels and developed as described in Figure 4. A densitometer tracing of a negative of chromatin limit DNA run on a 6% gel is shown, and the assigned size in base pairs of each band is indicated.

during staphylococcal nuclease digestion.

Size of the DNA Fragments. The size of the DNA fractions from the nuclear digest was determined by sedimentation velocity measurements (see Materials and Methods), and by calibration of the acrylamide gels with sized marker fragments. Acrylamide gels of the isolated DNA bands used for sedimentation velocity studies are shown in Figure 3, together with their calculated weight average molecular weights. This method shows that the DNA multimers are multiples of a monomer about 185 base pairs in length.

An independent size determination was achieved by electrophoresing nuclear digest bands together with double-stranded DNA fragments of known size. The size standards were total digests of SV40 and bacteriophage λ DNAs produced by the *Hemophilus influenza* (hin) restriction enzymes, and λ hin fragments some of which had been sequenced by Dr. T. Maniatis (Mauer et al., 1974). These known molecular weight standards were run in 3, 4, and 6% polyacrylamide gels along with partial (4 and 7% acid soluble) digests of nuclei and limit digests of chromatin (Figure 4). The dimer, trimer, and tetramer are seen to be multiples of a unit 185–190 base pairs in length, in good agreement with Williamson's (1970) and Noll's (1974) sizings, and with the sedimentation measurements above. Band IA (Figure 1c) is about 140 base pairs long.[4]

The λ hin fragments run on a 6% gel similarly provide a calibration for the chromatin (and nuclear) limit digest bands. This new calibration yields slightly larger sizes for the limit digest fragments (Figure 5) than we reported previously (Axel et al., 1974).

We have also determined the median sedimentation coefficient of the isolated nucleoprotein monomer in the analytical ultracentrifuge. The value of s_{20} was 10.2 measured in 1 m*M* Tris-HCl–0.1 m*M* NaEDTA (pH 8). This is slightly smaller than the value reported by Noll (1974) using direct measurement on sucrose gradients. The relatively small amount of nonhistone protein in our preparations, or the differences in measurement technique, could account for this discrepancy.

Discussion

Evidence for a subunit structure of the nucleoprotein within nuclei was first presented by Hewish and Burgoyne (1973) who extended the work of Williamson (1970) and showed that autolysis of nuclei could lead to the formation of DNA fragments that are multiples of a basic unit about 200 base pairs long. Noll (1974) showed that similar results could be obtained by treating nuclei with staphylococcal nuclease. Van Holde and his collaborators (Sahasrabuddhe and Van Holde, 1974) have independently demonstrated that limited treatment of purified chromatin with staphylococcal nuclease results in formation of small nucleoprotein particles containing DNA between 100 and 200 base pairs in length, and Noll (1974) has isolated similar particles from nuclei. These findings, combined with the electron microscopic and physicochemical studies of Olins and Olins (1974), provide strong support for a model in which protein clusters or "beads" are more or less regularly arranged along the DNA, leaving regions of DNA between the beads that are relatively accessible to nuclease action. Such a model has been proposed by several investigators.

The nucleoprotein monomer which is excised by nuclease digestion must be derived from the larger nuclear structure by clipping at relatively susceptible points between subunits. The initial attack does not involve extensive digestion of DNA in the "interbead" region, since considerable monomer is generated at early stages in the digestion, and the monomer nucleoprotein has about the same protein–DNA ratio and the same percentage digestibility as the whole chromatin. This monomer DNA is slightly heterogeneous in size, centered on 185 base pairs. On further digestion monomer loses about 45 base pairs to produce a somewhat smaller fragment, IA, which is rather homogeneous, with a very sharp lower molecular weight cut off at 140 base pairs (Figure 1c). It seems likely that the nicking action that produces monomers results in the liberation of homogeneous size "beads" (140 base pairs) with attached "tails" of accessible double-stranded DNA of various lengths deriving from the nicked interbead regions. Such tails have been observed in the electron microscope by Van Holde and Van Bruggen (Van Holde et al., 1974).

Further nuclease action presumably results in preferential digestion of the tails, to produce the homogeneous band IA. If the average interbead region is 45 base pairs long, DNA isolated from monomers and multimers would decrease in size by this amount as digestion proceeded. Under suitable conditions, the DNA from the nucleoprotein multimers also shows a slow downward shift in size as digestion proceeds, consistent with a loss of about 45 base pairs (Figure 6). Whether this is observed or not depends on the rate of removal of the tails relative to further interbead nicking, and varies with different nuclear preparations. This observation is also consistent with a model in which excised multimers are terminated by accessible tails of 45 base pairs average total length. However, while the results suggest a uniform size DNA within the bead, the fact that DNA is observed in the node region of the gels (see Results) is inconsistent with a uniform interbead separation of 45 base pairs. Rather, there appears to be a distribution of interbead sizes centered on 45 base pairs, or perhaps some of the DNA (10–20%) is involved in some other structure.

It should be noted that virtually all of the nuclear limit bands pass through the homogeneous band IA. Therefore, almost no digestion takes place within the IA nucleoprotein fragment before it is excised from the larger nucleoprotein pieces. Apparently there are constraints within nuclei which hold the intramonomeric sites inaccessible to staphylococcal nuclease until the monomer, itself, is removed from this higher order nucleoprotein structure. With further diges-

[4] In the accompanying paper, Axel (1975) finds the "IA" DNA from rat liver nuclei to be the 160, rather than 140, base pair fragment. This may be due to differences in the tissue being examined or to some minor differences in preparation or sizing. It seems possible that our band IA is equivalent to the 170 base pair fragment reported by Noll (1974), whose gel calibration differs slightly from ours.

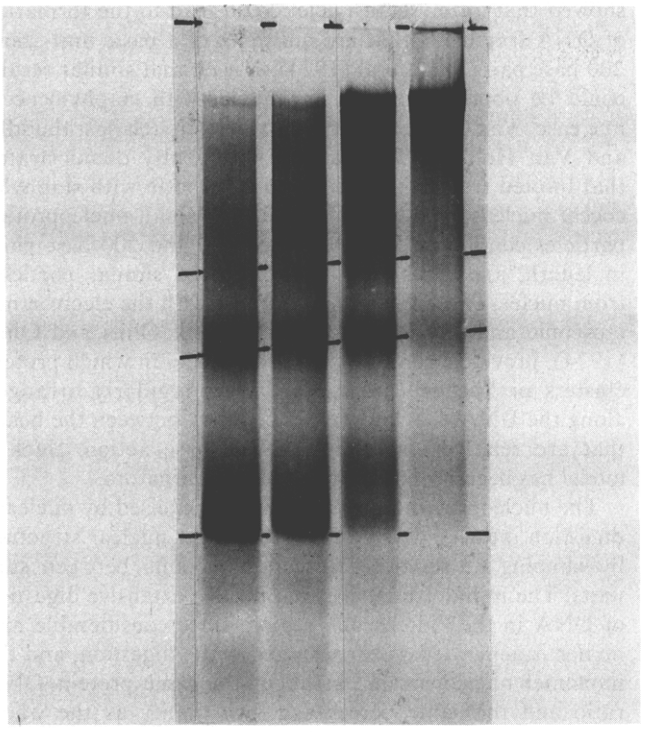

FIGURE 6: Acrylamide gel electrophoresis. Nuclei were digested and the isolated DNA was run on a 4% slab gel. From right to left are 2, 4, 9, and 17% acid-soluble digests. Lines indicate the decrease in dimer and trimer sizes. [Size calibrations (in Figures 3, 4, and 5) were performed on digests with multimers of full length.]

tion, IA produces a final set of DNA bands, ranging in size from 140 to 40 base pairs. These nuclear limit bands are extremely similar, both in sizes and relative amounts, to the sharply defined limit digest bands generated from the staphylococcal nuclease digestion of isolated chromatin. The substructure within the nuclear monomer is thus seen to be preserved during isolation of chromatin. As we have described above, certain preparative methods for chromatin also result in preservation of the entire nuclease digestion pattern seen with intact nuclei. Studies of higher order structure can presumably be carried out with such preparations. However, shearing sufficient to abolish higher order structures appears to have little effect upon the specific transcription of globin genes from chromatin templates isolated from tissues actively synthesizing globin message. Quantitatively similar results have been obtained with both sheared and unsheared chromatin preparations (Axel et al., 1973; Gilmour and Paul, 1973; Barrett et al., 1974).

Earlier work in this and other laboratories (Clark and Felsenfeld, 1971, 1974; Axel et al., 1974; Weintraub and Van Lente, 1974) has shown that extensive digestion of chromatin with staphylococcal nuclease results in a limit digest containing half the DNA, and that this DNA consists of discrete double-stranded fragments of sizes ranging from about 130 base pairs to 45 base pairs. The purpose of the studies described in this paper is to relate these fragments to the larger ones produced by limited digestion of nuclei. Our results demonstrate that the large multiple nucleoprotein subunits obtained from nuclei are true precursors of the monomer nucleoprotein subunits, and that this monomer is in turn degraded to form the limit digest pattern we described earlier (Axel et al., 1974). Results very similar to those we describe here for reticulocyte nuclei and chromatin have been obtained with material isolated from rat liver as shown in the accompanying paper (Axel, 1975).

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