nation for the reported blocking effect of GnRH on various estrogen-dependent processes, including ovulation, ovum transport, ovum implantation, and mammary tumorigenesis. Moreover, the inhibitory effect of GnRH and agonists on FSH-induced progesterone production in cultured granulosa cells could offer a possible explanation for the interference by GnRH and its agonists in such a progesterone-dependent process as pregnancy. Our results, however, do not exclude the possibility that administration in vivo of pharmacological doses of GnRH in intact animals may also cause imbalances in pituitary gonadotropin production that also result in the inhibition of various reproductive functions (*7*).

The mechanisms by which GnRH exerts inhibition on ovarian granulosa cells are not known. Although GnRH receptors have been identified in the anterior pituitary by radiotracer binding technique and by immunocytochemical methods (*8*), their tissue specificity has not been demonstrated. Bernardo *et al.* (*9*) have reported putative GnRH binding sites in the mouse adrenal cells. It is possible that ovarian granulosa cells may have GnRH receptors and that the inhibitory effect of GnRH that we observed is mediated through hormone-receptor interactions.

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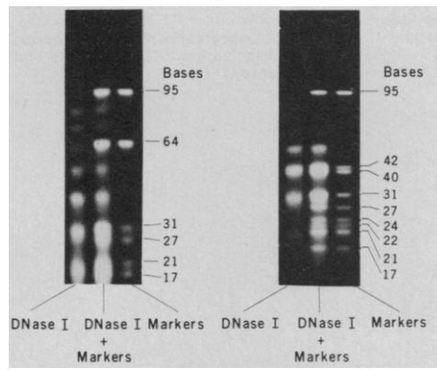
14 December 1978; revised 22 February 1979

Periodicity of Deoxyribonuclease I Digestion of Chromatin

Abstract. *Two methods have been used to measure the single-strand lengths of the DNA fragments produced by deoxyribonuclease I digestion of chromatin. The average lengths obtained are multiples of about 10.4 bases, significantly different from the value of 10 previously reported. This periodicity in fragment lengths is closely related to the periodicity of the DNA double helix in chromatin, but the two values need not be exactly the same.*

The first level of condensation of DNA in chromatin is brought about by its interaction with histones to form nucleosomes, the elementary subunits of the structure. For a detailed understanding of how the DNA is folded, it is necessary to know both the path of the double helix and its periodicity, or the number of base pairs per turn, in the nucleosome. X-ray crystallographic studies indicate a path in which the DNA is wrapped twice around the histones (*1*). This appears to conflict with measurements on closed, circular DNA extracted from SV 40 chromatin showing nearer one superhelical turn (*2*). The conflict can be resolved by postulating a change in the periodicity of the DNA as it is folded into a nucleosome (*1*). A decrease of only about 5 percent is required, and so before firm conclusions can be drawn it is necessary to know the relevant parameters as accurately as possible.

Noll (*3*) has suggested that the perio-



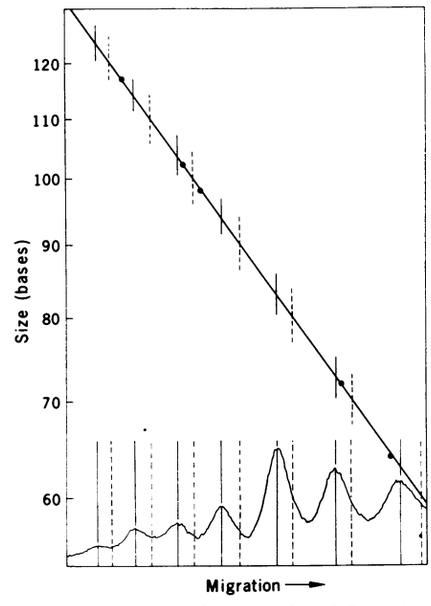


Fig. 1 (left). Comparison of DNase I and marker fragments in polyacrylamide gels. DNase I fragments were prepared by DNase I digestion of rat liver nuclei and extraction of the DNA (*3*). Marker fragments were prepared as described in Table 1. All fragments were labeled with ^{32}P as follows. DNA (40 μg/ml) in 50 m*M* sodium acetate (*p*H 4.6) and 1 m*M* EDTA was boiled for 1 minute and treated with spleen acid phosphomonoesterase B (*16*) (0.1 unit per milliliter) for 2 hours at 37°C. The *p*H was then raised to 8.0 by the addition of tris base to 85 m*M* and the mixture was boiled for 1 minute, supplemented with $MgCl_2$ (10 m*M*), 2-mercaptoethanol (15 m*M*), and γ-^{32}P-labeled ATP (tenfold excess over 5′-OH termini), and treated with polynucleotide kinase (20 unit/ml) (New England Biolabs) for 1 hour at 37°C. The labeled DNA was purified by filtration through Sephadex G-25 in a mixture of 0.1*M* NaCl, 10 m*M* tris-HCl (*p*H 7.5), and 1 m*M* EDTA and subjected to electrophoresis in 12 percent (left panel) and 20 percent (right panel) polyacrylamide–98 percent formamide gels (*17*). Negatives of autoradiograms of the gels are shown. Fig. 2 (right). Size determination of DNase I bands. Densitometer traces of lanes in left panel of Fig. 1 containing DNase I (bottom) and marker fragments were aligned. The sizes and distances of migration of the marker fragments (filled circles) were used to construct a calibration curve from which the sizes of the DNase I fragments were derived. The distances of migration of the DNase I fragments (solid vertical lines) are contrasted with those expected if the fragments were multiples of 10.0 bases (dashed vertical lines).

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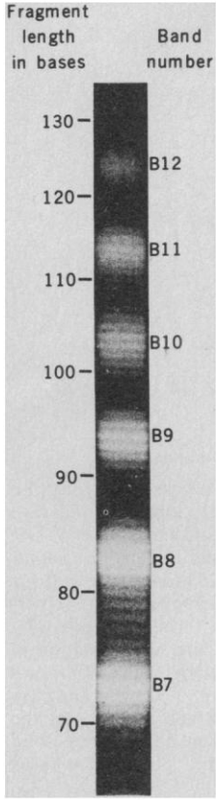
Table 1. Size determination of DNase I bands with the use of markers of known length. Each column lists the sizes of bands obtained from one gel and the markers used to construct the calibration curve from which the sizes were derived (see Fig. 2).

Sizes (bases) in acrylamide at						Average size	Periodicity (size/number)
8 percent	12 percent	12 percent	12 percent	12 percent	20 percent		
Band sizes							
123.9		123.9				123.9	10.33
113.5		113.9		112.8		113.4	10.31
102.9		103.4	103.5	102.3		103.0	10.30
93.1		93.8	93.6	93.1		93.4	10.38
82.2	83.0	82.8	83.2	82.2		82.7	10.34
72.0	72.6	72.6	73.1	72.4		72.5	10.36
		62.9	63.0	62.7		62.9	10.48
			52.2	51.6		51.9	10.38
			41.1		40.9	41.0	10.25
			30.1		30.3	30.2	10.07
			20.0		20.1	20.0	10.00
*Marker sizes**							
128	82	117	95	121	42		
95	76	102	64	119	40		
64	72	98	42	82	31		
		72	40	76	27		
		64	31	72	24		
			27	50	22		
			21	48	21		
			17		17		

*Markers were prepared from *E. coli lac* DNA-containing plasmids pOP203-1 (*10*) and pMCl (*11*). Purified pOP203-1 DNA was digested with Eco R1 to release a lac DNA fragment of 207 bp, which was digested with Alu I to give flush-ended fragments of 95 and 64 bp and two uneven-ended fragments, one consisting of strands of 31 and 27 bases and the other of strands of 21 and 17 bases. The 64-bp fragment was treated with T4 DNA ligase to give a further fragment of 128 bp; it was also digested with Hha I to give two further uneven-ended fragments, one consisting of strands of 42 and 40 bases, and the other of strands of 24 and 22 bases. Purified pMCl DNA was treated with Hinc II to release a lac DNA fragment of 789 bp, which was digested with Hae III to give flush-ended fragments of 203, 169, 117, 102, 98, and 72 bp. The 169-bp fragment was digested with Hha I to give two uneven-ended fragments, one consisting of strands of 121 and 119 bases and the other of strands of 50 and 48 bases. The 102- and 98-bp fragments were digested with Alu I to give further fragments of 82, 76, 22, and 20 bp. The sizes of all the fragments are known from DNA sequences (*12*). The sizes of the 95-bp fragment and 31-, 27-, 24-, and 22-base strands are also known from RNA sequences (*13*). The sizes of the 98-, 76-, and 72-bp fragments and 21- and 17-base strands were checked against the lac repressor amino acid sequence (*14*). The sizes of the 117-, 102-, and 82-bp fragments and 121-, 119-, 50- and 48-base strands were checked against the β-galactosidase amino acid sequence (*15*).

dicity of DNA in the nucleosome is revealed directly by the periodicity of digestion by deoxyribonuclease (DNase) I. He reported single-strand lengths for the products of digestion that are multiples of ten bases. We describe a redetermination of these fragment lengths by two different methods. The first is a comparison by gel electrophoresis of the products of DNase I digestion with a large number of markers of known size. In the second method, the DNase I fragments are analyzed in a gel system capable of resolving fragments differing in size by a single nucleotide up to a total of 160. The results of both methods are in close agreement and give an average periodicity of DNase I digestion of 10.4 bases, a number significantly larger than the previous value of 10 (*3*). As discussed below, the periodicities of DNase I digestion and of the DNA double helix in the nucleosome may differ, and the value of 10.4 bases is compatible with a structure of DNA in the nucleosome possessing as few as 10.0 bases per turn.

We have measured the lengths of the DNA fragments resulting from DNase I digestion of chromatin by electrophoresis in a formamide-containing polyacrylamide gel together with marker fragments of known size. The DNase I and marker fragments were run as a mixture in one lane of the gel and separately in adjacent lanes (Fig. 1). A densitometer



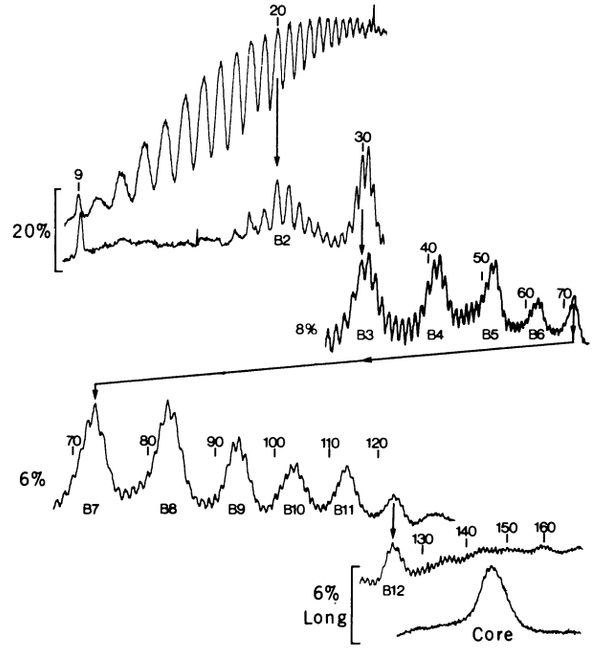


Fig. 3 (left). DNase I fragments analyzed by high-resolution polyacrylamide gel electrophoresis. DNase I fragments were prepared as in Fig. 1. Electrophoresis was carried out in polyacrylamide gels containing 6 percent acrylamide, 1 percent methylene-bis-acrylamid (MBA), and 7*M* urea (*7*). A photograph of an ethidium-stained gel is shown. Fig. 4 (right). Densitometer traces of high-resolution gels. To obtain high resolution of DNA fragments over a range of about 10 to 160 bases, electrophoresis was carried out in polyacrylamide gels of various densities. The acrylamide and MBA contents of these gels were 20 percent acrylamide plus 2.4 percent MBA, 8 percent acrylamide plus 1.25 percent MBA, and 6 percent acrylamide plus 1 percent MBA. Densitometer traces are labeled according to the acrylamide content of the gels. A bracket enclosing two traces indicates that they are from adjacent lanes of the same gel. The upper of the two traces from the 20 percent gel is from a lane to which was applied 0.9 μg of dpTTCTGTTGA (gift of Dr. Michael Gait; position marked by the number 9) and 35 μg of a DNase I digest of naked DNA. The lower of the two traces from the 20 percent gel is from a lane to which was applied 0.9 μg of dpTTCTGTTGA and the DNA from a DNase I digest of nuclei. The trace labeled 6 percent is from the gel in Fig. 3, and identical samples were used to produce the trace labeled 8 percent and the upper of the two traces labeled 6 percent long. The gel from which the two traces labeled 6 percent long were obtained was identical to that labeled 6 percent, except that a longer electrophoresis time was used. The lower of the two traces labeled 6 percent long is from a lane to which was applied 1 μg of nucleosome core DNA (*9*).

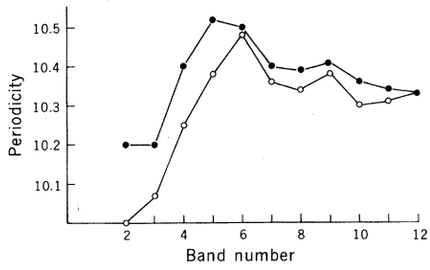


Fig. 5. Dependence of periodicity, that is, the ratio of band size to band number, upon band number. Results from size determination by the use of marker fragments (Table 1) (open circles) and size determination by high-resolution gel electrophoresis (Table 2) (filled circles) are shown.

trace of the mixture was used to align traces of the other lanes, from which the sizes of the DNase I fragments were determined. Strongly denaturing conditions of gel electrophoresis were used to avoid any nucleotide sequence dependence of the mobilities of the fragments. The absence of sequence effects is shown by the single symmetrical band formed by the two strands of all the marker fragments (Fig. 1), and by the absence of any deviation greater than 1 percent in plots of size as a function of mobility (Fig. 2). Twenty-one markers ranging in size from 17 to 128 base pairs (bp) were used. They were produced by restriction enzyme digestion of cloned segments of DNA from the lac region of *Escherichia coli*. Their sizes are known from both DNA and amino acid sequences (repressor and β-galactosidase gene regions) or both DNA and RNA sequences (promoter-operator region).

The sizes found for most of the DNase I fragments are multiples of between 10.3 and 10.4 bases (Table 1). The precision of the measurements is about ± 0.5 percent. This is true despite the breadth of the bands in the gels (approximately five bases at half-height, or about 5 percent of the size of even the longest fragments) since the midpoints of broad but symmetrical bands can be precisely determined. The midpoints of the bands are, for example, clearly distinguishable from those expected if the fragments were multiples of 10.0 bases (Fig. 2).

It could be imagined that the pattern of digestion by DNase I is determined, wholly or in part, by subtle features of the interaction of enzyme and histone-bound DNA. Digestion with other nucleases, however, gives similar results; DNase II and micrococcal nuclease cut the DNA in nucleosomes at sites different from, but identical in spacing to, those cleaved by DNase I (*4*); exonuclease III degrades the DNA in nucleosomes from the 3′ ends, pausing at points that are again identical in spacing to the DNase I sites (*5*). Apparently the periodicity of digestion of chromatin by nucleases depends not on the mechanism of the enzyme but rather on the structure and organization of the DNA.

The broad bands typically found on gel electrophoresis of a DNase I digest of chromatin (*3*) are resolved into subbands in more cross-linked gels containing 7*M* urea (Figs. 3 and 4). The 7*M* urea gel system (*6*) has intrinsically greater resolving power than the formamide system used in the work described above. Additional cross-linking appears to suppress effects of DNA sequence variation and allow the resolution of DNA fragments differing in size by a single base (*7*). The length of any fragment can be determined by simply counting up the bases (subbands) from the position of a fragment of known length as reference. A chemically synthesized fragment of nine bases was used as reference.

The peaks of the broad bands (numbered B7 to B12 in Figs. 3 and 4) fall between subbands and therefore correspond to nonintegral lengths of fragment. The lengths found are multiples of 10.2 to 10.5 bases (Table 2), with an error, due to uncertainty in locating centers of the peaks, ranging from ± 1 percent for the shorter fragments to ± 0.5 percent for the longer ones. [The average length of DNA contained in the "140 base pair" core particle of the nucleosome was also determined (Fig. 4) and found to be 146 base pairs, as expected for a multiple of 14 times 10.4 base pairs.] The data for all but the shortest DNase I fragments agree to within 0.5 percent with the results from the alternative method (Table 1). This close agreement increases our confidence that the fine variations in the data (Fig. 5) are not simply due to experimental scatter but rather reflect a true variation in the periodicity of digestion by DNase I.

Table 2. Size determination of DNase I bands by high-resolution gel electrophoresis. Sizes were determined by locating the centers of the broad bands in Fig. 4 with respect to the "ladder" of subbands.

Band	Size (bases)	Periodicity (size/number)
B12	124.0	10.33
B11	113.7	10.34
B10	103.6	10.36
B9	93.7	10.41
B8	83.1	10.39
B7	72.8	10.40
B6	63.0	10.50
B5	52.6	10.52
B4	41.6	10.40
B3	30.6	10.20
B2	20.4	10.20

The value of about 10.4 bases that we have obtained for the spacing of DNase I cleavage sites in chromatin coincides with the value of 10.4 base pairs per turn for the periodicity of DNA in solution (*8*). A possible interpretation is that the periodicities of DNA in chromatin and in solution are the same. This would argue against the two-turn model for the nucleosome (*1*) which, taken in conjunction with the measurement of the superhelicity of isolated DNA (*2*), requires a change from about 10.4 to 10.0 base pairs per turn on wrapping of the DNA around the histones. That the two-turn model is nonetheless compatible with the DNase I digestion results can be seen from the drawing in Fig. 6. We assume, for the purposes of exposition, that there are 10.0 base pairs per turn of the double helix and then consider how an enzyme would attack this DNA. In the case of a structure with many superhelical turns of DNA, attack would be expected from the direction perpendicular to the cylinder axis, indicated by the open arrows in

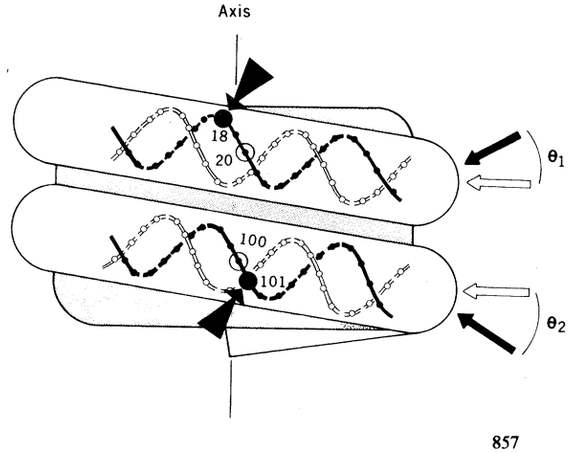


Fig. 6. Schematic drawing of a core particle of a nucleosome in which the DNA is wound in a left-handed superhelix around the histones. The arrows indicate the average directions of attack by an endonuclease. The white arrows (and open circles) represent the angle (and points) of attack at the positions that would be maximally exposed on an infinite superhelix. The black arrows (and circles) represent the angle (and points) of attack on a limited superhelix such as that on the nucleosome core.

Fig. 6. Cleavage would then occur at points spaced multiples of exactly 10.0 bases apart. In the case of a nucleosome, in contrast, with only two superhelical turns, the lower surface of the lower turn and the upper surface of the upper turn are also exposed to enzyme digestion, and attack should occur on the average at angles of θ_1 and θ_2 from the perpendicular, as is indicated by solid arrows in Fig. 6. If $\theta_1 + \theta_2$ were 108°, or three times the angle between adjacent base pairs, then fragments produced by cuts one turn of the superhelix apart would be increased from 80 to 83 bases. For $\theta_1 + \theta_2 = 144°$, the fragment length would become 84 bases. The lengths are increased rather than decreased compared to an exact multiple of 10.0 bases because the double helix is right-handed, while the superhelix is left-handed. This effect can be seen not only in Fig. 6, but also in a detailed drawing of a left-handed superhelix used to explain the relative frequencies of cutting by DNase I at various sites in the nucleosome (*9*). The arguments concerning fragment lengths and frequencies of cutting are of essentially the same type, invoking steric bias in the ease of access by an enzyme to the two portions of the same strand of DNA in the two turns of a nucleosome. In summary, the periodicity of digestion of DNA coiled in a two-turn nucleosome is expected to be greater than the periodicity of the double helix itself. Specifically, fragments that are multiples of about 10.4 bases could be produced from DNA with a helical repeat of 10.4 base pairs.

The distribution of fragment lengths determined in our work does not reflect the actual location of cleavage sites but only the distances between them. In further studies, the locations of cleavage sites within the core particle of the nucleosome have been established (*7*). The sites are spaced at multiples of about 10.4 bases, but the precise spacing is variable. The consistence of these further data with the results we report here has been checked by using the locations of sites along with the probability of cleavage at each site (*9*) to calculate a distribution of fragment lengths. This calculated distribution is in reasonable agreement with the measured distributions displayed in Fig. 5. The variation in the spacing of cleavage sites and in the periodicity of fragment lengths may reflect variation in the helical repeat of DNA in the nucleosome. Alternatively, the helical repeat may be constant while the angle of attack by the enzyme varies from one cleavage site to the next (rather than changing in a uniform manner as might be inferred from Fig. 6). The exact interpretation of any digestion data will be rather speculative until much more is learned about nucleosome structure.

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17 August 1978; revised 5 March 1979

"Transfer Connections": Specialized Pathways for Nutrient Translocation in a Red Alga?

Abstract. *"Transfer connections" are morphologically and developmentally distinct pit connections in* Polysiphonia *(Ceramiales). They are intracellular rather than extracellular and have been observed between all cells of the diploid carposporophyte plus those specialized cells of the gametophyte suspected of providing nutritive materials to it.*

Cytoplasmic channels between animal cells (for example, gap junctions) and between plant cells (for example, plasmodesmata) permit intercellular transport and communication which appear necessary for differentiation (*1*). In the complex tissues of some green and brown algae, plasmodesmata are likewise believed to permit transport (*3*). However, plasmodesmata per se are absent in the red algae, although pit connections link adjacent cells in most groups (*2*). In the red alga *Polysiphonia*, during development after fertilization, morphologically distinct pit connections, which I call "transfer connections," interconnect differentiating cell layers, and their role in enhancing cell-to-cell interactions would be consistent with developmental strategies observed in other multicellular organisms.

Red algal pit connections form between cells after incomplete cytokinesis, and their extracellular position and plug-like structure suggests that a role in intercellular transport is unlikely (*2, 3*). However, translocation of radioactively labeled compounds along files of cells has been reported (*4*), although there is no direct evidence that movement occurred through the pit connections. Structural differences in pit connections have been observed within different generations of a single organism (*3, 5*), and such modifications might effect intercellular transport, especially between cells of the reportedly parasitic carposporophyte.

After fertilization in *Polysiphonia*, a diploid generation (termed the carposporophyte) proliferates while attached to the female gametophyte (*6*). The carposporophyte consists of an outer, rapidly dividing layer of cells (the gonimoblast), of which some eventually develop into reproductive carpospores, and a central, irregularly shaped fusion cell which arises during early development and continues to expand outward by the gradual incorporation of adjacent gonimoblast cells. In addition, specific haploid cells of the female gametophyte, on which the diploid carposporophyte is borne, eventually establish cytoplasmic continuity with the fusion cell. It has frequently been suggested (*6, 7*) that the fusion cell itself, and those cells (both haploid and diploid) about to be incorporated into the fusion cell, provide nutritive material to the proliferating fringe of dividing goni-

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