Yeast Chromatin Subunit Structure

Abstract. *Micrococcal nuclease digestion of in situ (intranuclear) and in vitro yeast chromatin produces distributions of DNA molecules of discrete sizes. In both cases, these molecules appear to be integral multiples of the smallest size on polyacrylamide gel electrophoresis. This result implies a widespread generic occurrence of the periodic organization of chromatin seen in mammalian systems.*

Digestion of a number of mammalian chromatins [for example, rat liver (*1, 2*), Krebs II ascites cells (*2*), and calf thymus (*3*)] by in situ action of endogenous or exogenous nuclease yields DNA fragments of discrete sizes. Such results suggest that much of the nuclear DNA of these cells exhibits a periodic pattern of organization, presumably produced by chromosomal proteins. Is this pattern an invariant feature of chromatin structure?

Bakers' yeast, *Saccharomyces cerevisiae*, is a primitive eukaryote and contains a chromatin that differs fundamentally in several respects from that of higher organisms: yeast does not seem to show condensed metaphase chromosomes; it has a small genome size [about 7×10^8 daltons per chromosome (*4*)], with a very small fraction of repetitive DNA (*5*); and the histone complement of yeast is smaller than the histone complement of higher eukaryotes (*6*). Nevertheless, we shall show here that digestion of yeast chromatin also produces a series of discrete sizes of DNA molecules.

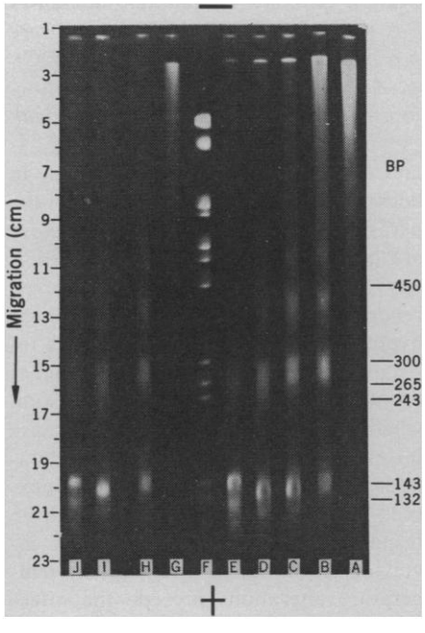

Fig. 1. Polyacrylamide gel electrophoresis of DNA from yeast chromatin digested in situ (A to E) and in vitro (G to J) by micrococcal nuclease (120 unit/ml at 37°C) for the times indicated. All gels were electrophoresed simultaneously. In situ: A, control (no nuclease, held at 37°C for 10 minutes); B, 1 minute; C, 3 minutes; D, 5 minutes; and E, 10 minutes. In vitro: G, control (no nuclease, held at 37°C for 4 minutes); H, 30 seconds; I, 1.5 minutes; and J, 4 minutes. F, PM2 DNA, cleaved by Hae III restriction endonuclease. Abbreviation: *BP*, base pairs.

Yeast nuclei were isolated from cells in the logarithmic phase of growth (*7*) and incubated in a solution of 1*M* sorbitol and 1 m*M* Ca^{2+} at 37°C, to which micrococcal nuclease (Worthington) was added (to 120 unit/ml). The reactions were stopped at various times by adjusting the solutions to 10 m*M* in ethylenediaminetetraacetate and chilling on ice. The digests were then treated with a mixture of bacteriophage T1 ribonuclease (100 unit/ml) and bovine pancreatic ribonuclease (100 μg/ml) for 20 minutes at 37°C to remove RNA, and the DNA was isolated by a modification of Marmur's procedure (*8*). The isolated DNA was electrophoresed on 2.5 percent polyacrylamide gels (*9*) and stained with ethidium bromide.

Several points are clear. The exogenous enzyme produces distinctive and rather sharp size classes of DNA whose distribution is time dependent (Fig. 1). One size class of DNA seems to be pivotal: short digestions produce fragments of this size class plus larger fragments, while longer digestions produce this class plus smaller, although less well resolved, fragments. Such a "basic" unit has been observed in the digestion patterns of chromatin from higher eukaryotes (*10*). Micrococcal nuclease acting on isolated yeast DNA produces a continuous smear of small DNA sizes (not shown) so that the enzyme is not recognizing and cleaving specific base sequences of yeast DNA to produce the discrete size classes.

As shown in Fig. 2, the bands plot as a straight line on a logarithmic plot of band number versus migration on the gel. This strongly suggests that the larger bands are multimers of the basic band (*11*). Tentative estimates of the number of base pairs in each band, given in Fig. 2, agree with the conclusion that the bands larger than the basic band are integral multiples of the basic or monomer band. Also, increasing times of digestion show an increase of the amount of DNA in the monomer band and the disappearance of the larger bands. This is shown by comparing the results of various times of digestion in Fig. 1 (gels B to E). The amount of DNA on each gel is comparable; the same amount of the nuclear suspension was extracted and run for each gel except E, which contained approximately 10 percent less than the others.

In vitro digestion of chromatin isolated from yeast nuclei yields a gel pattern similar to that found with in situ digestion. Bands are somewhat sharper, occur at approximately the same position, and the log plot of band number versus migration on the gel is again a straight line and is virtually superimposable on the plot for in situ digested chromatin (Fig. 2). Again, increasing digestion times produce increasing amounts of monomer, but there are significant differences. In all nuclear digestions there is some rather high-molecular-weight DNA present near the origin, which is not present

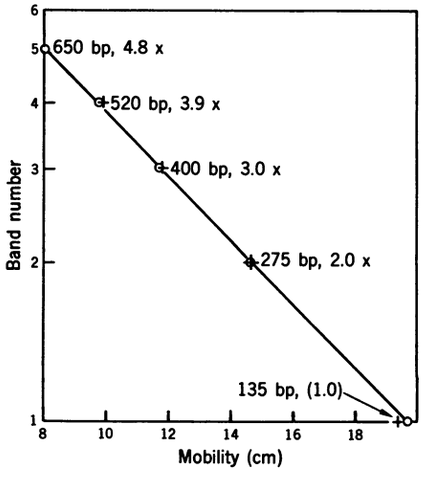

Fig. 2. Log plots of band number versus mobility on the gel, in arbitrary units, for gel B (Fig. 1; nuclear digestion; circles) and for gel H (Fig. 1; isolated chromatin digestion; crosses) calling the smallest band in Fig. 1 number 1. For each point in the in situ digestion the estimated number of base pairs (*bp*) is given. In addition, the ratio of the number of base pairs in each multimer to the number of base pairs in band number 1 is indicated by 2.0 ×, 3.0 ×, and so forth. These values for the DNA sizes in the digest bands were obtained by interpolation from a plot of ln(base pairs) versus migration for the PM2 bands of gel F in Fig. 1. The numbers of base pairs in the PM2 fragments were determined by simultaneous electrophoresis with fragments of DNA from SV40, produced by Hae III, whose size is known from an analysis of these fragments labeled with ^{32}P (*15*).

after even very short digestion times when isolated chromatin is used. Also, the time course of digestion is much more rapid in isolated chromatin. Both of these observations could be explained by the increase in accessibility of the nuclear chromatin in solution. However, it is significant that much of the same repeating structure still exists for the in vitro chromatin.

The width of the DNA monomer band in higher eukaryotes does not preclude microheterogeneity of these particles. The sharper yeast monomer bands, especially for the in vitro chromatin digests, suggest a much less polydisperse subunit.

As in higher eukaryotes, these size classes probably arise as a result of protection of sections of DNA by bound chromosomal proteins, rendering these sections inaccessible to nucleases. Evidence indicates that in mammalian systems these protected sections occur as repeating, globular particles in chromatin (*12*), and models for the structure of such particles have been proposed (*13, 14*). The presence of specific DNA size classes, and presumably a similar repeating subunit structure, in one of the most primitive eukaryotes indicates that this mode of organization must be a widespread characteristic of the structure of chromatin. Of course, the fine structure of such a subunit in yeast may very well differ from the subunit of higher eukaryotes, especially since histones F1 and probably F3 are lacking (*6*). If this is true, F3 cannot fulfill, in the yeast chromosomal subunit, the central role postulated for it by one model [see (*13*)] of the chromosomal subunit in higher eukaryotes. The presence of a periodic mode of chromatin organization in yeast, in which a relatively large proportion of the DNA corresponds to genetically expressed information, suggests that this mode of organization may involve structural genes as well as (or perhaps instead of) other types of nuclear DNA.

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6. Polyacrylamide gel electrophoresis of yeast histones shows three major bar→[U. Wintersberger, P. Smith, C. K. Letnansky, *Eur. J. Biochem.* **33**, 123 (197→ L. Franco, E. W. Johns, J. M. Navlet, *ibid.* **45**, 83 (1974)]; F1 is clearly absent nor has evidence for F3 been found.
7. The procedure used was essentially that of Wintersberger and co-workers (*6*) with minor modifications.
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10. On gels we used, size has been tentatively estimated relative to fragments of PM2 DNA produced by Hae III restriction endonuclease. These fragments were generously supplied by R. Kovacic. Figures 1 and 2 show that the basic band size centers around 135 base pairs. The (broader) calf thymus monomer band centers around 150 base pairs on similar gels (*3*). A monomer band size of 205 base pairs has been reported from rat liver (*2*). Exact comparison is difficult at this time because different standards have been used for size estimation. Furthermore, differences in size due to the different systems studied cannot be excluded.
11. Some workers have plotted log of band number versus the square root of gel mobility to show such molecular weight relatio→[R. Williamson, *J. Mol. Biol.* **51**, 157 (1970); D. R. Hewish and L. A. Burgoyne (*1*)], while log plots of molecular weight versus the first power of mobility are widely used in restriction endonuclease studies [see E. Huang, J. Newbold, J. S. Pagano, *J. Virol.* **11**, 508 (1973)]. Our results fit better the plot of log band number versus mobility; plots against the square root of mobility also give straight lines but the fit is not as good as for the first power plots. Since all such plots are empirical and lack firm theoretical justification, we have chosen to use the first power of mobility plots.
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16. We thank R. Kovacic for PM2 DNA fragments, information on the size calibrations of PM2, and helpful discussions; and J. Corden for stimulating and helpful advice. Supported in part by NSF grant GB 37307X.

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Fever and Survival

Abstract. *The significance of fever in response to a bacterial infection has been investigated using the lizard* Dipsosaurus dorsalis *as an animal model. These lizards develop a fever of about 2°C after injection with the bacterium* Aeromonas hydrophila. *To determine whether this elevation in body temperature increases the resistance of the host to this infection, as measured by survival, lizards were infected with the live bacteria and placed in a neutral (38°C), low (34° or 36°C), or high (40° or 42°C) ambient temperature. An elevation in temperature following experimental bacterial infection results in a significant increase in host survival.*

The significance of fever in disease is an enigma (*1–3*). Most mammals develop fever after becoming infected with bacteria; but it is unclear whether the fever is harmful or beneficial to the host. Bennett and Nicastri (*1*) reviewed arguments for and against the common notion that fever is advantageous to the host in combating infection. Studies with specific diseases (syphilis and gonorrhea) involved extraordinarily high and therefore unnatural elevations in body temperature, which do kill the parasites before the host and therefore are beneficial to the host ("fever therapy") (*1*). Other studies (*4*) involved rendering an animal hyperthermic or hypothermic and then measuring its resistance to infection. These approaches are not directly relevant to the question whether fever benefits the host because in fever therapy the temperature alterations, which are artificially induced, exceed the normal range of the febrile response, and in the experiments involving resistance to infection the temperature alterations precede the infection. A more direct test would involve determinations of LD_{50}'s (LD_{50} is the lethal dose to 50 percent of a population) in two groups of animals injected with suitable bacteria. In one group, the febrile response would be allowed to develop, and in the other group the fever would be suppressed. Fever could be suppressed by administering an antipyretic drug such as aspirin, by artificially elevating the hypothalamic temperature, by placing the animals in an ice bath, or by maintaining them in a

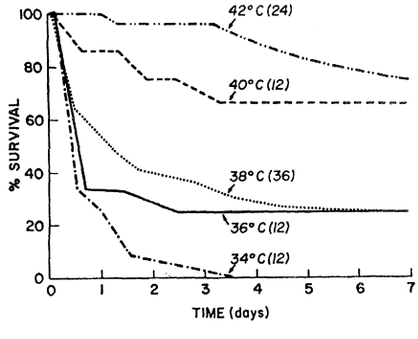

Fig. 1. Percentage survival of *D. dorsalis* injected with *A. hydrophila* and maintained at temperatures of 34° to 42°C. The number of lizards in each group is given in parentheses.