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Appendix

Yield of Restriction Fragments from Yeast Mitochondrial DNA

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(a) *Preliminary considerations*

Yeast mitochondrial DNA can only be prepared, so far, in a more or less degraded form. DNA preparations having molecular weights of only 3·5 to $5{\cdot}2\times10^6$ (the genome unit size being about 50×10^6) were used in most of this work (Table A1). In spite of such extensive degradation, all the *Hae* and *Hpa* fragments could be

Table A1

Molecular weights of the mitochondrial DNAs before and after restriction enzyme degradation

Initial DNAs	Strains A†	A†	B	D	C
$s_{20,w}$	26	17·9	18·3	20·5	20·9
M_w (from sedimentation)‡	9	3·5	3·6	4·9	5·2
M_w (from eqn (A9))	9	3·4	2·2	4·3	4·2
Degraded DNA (*Hpa*)					
$s_{20,w}$		8·6	8·6	8·6	9·7
M_w (from sedimentation)§		0·48	0·48	0·48	0·71
M_w (from electrophoresis)‖		1·14	1·28	1·27	1·07
M_n (from electrophoresis)		0·47	0·52	0·50	0·53
M_w/M_n (from electrophoresis)		2·4	2·5	2·5	2·0
Degraded DNA (*Hae*)					
M_w (from electrophoresis)‖		1·18	1·35	1·20	1·53
M_n (from electrophoresis)		0·62	0·68	0·62	0·71
M_w/M_n (from electrophoresis)		1·9	2·0	1·9	2·2

† Values ($\times10^{-6}$) for 2 different preparations are given.

‡ M_w was calculated using the relationship of Richards & Bernardi (Bernardi & Sadron, 1964; Prunell & Bernardi, 1973). The relationship of Studier (1965) would lead to molecular weights higher by 50%.

§ M_w was calculated using the relationship of Prunell & Bernardi (1973).

‖ Using the well-known relationships:

$$M_w = \Sigma M_i^2/\Sigma M_i$$
$$M_n = \Sigma M_i/\Sigma_i.$$

M_i being the molecular weight of the fragment i as determined by gel electrophoresis.

detected since the size of the largest fragments was equal to only 4×10^6 in these digests, whereas for *Eco*RI and *Hin*dII + III higher molecular weight preparations had to be used to obtain satisfactory results (see Results, section (f) in the main text). Expectedly, however, the amount of DNA in the *Hae* and *Hpa* bands was not proportional to the molecular weights of the corresponding fragments, but showed a relative decrease with increasing molecular weight of the fragments. This effect, already evident upon inspection of Figures 2, 3 and 7 of the main text, is clearly demonstrated by a comparison of the molecular weights of *Hpa* digests, as obtained from their sedimentation coefficient with those calculated from the weight-average molecular weight of the fragments as determined from gel electrophoresis data (Table A1). Such a comparison indicates that the former always are lower than the latter by a factor of at least 2 for the *S. cerevisiae* DNAs. The higher yield of the highest molecular weight fragments in the *Hpa* digest of *S. carlsbergensis* DNA (see section (c), below) accounts for the relatively higher molecular weight (as estimated from sedimentation) of this digest.

The effect of the initial degradation of mitochondrial DNA on fragment yield was therefore studied using the approach outlined in the following section.

(b) *Restriction fragment yield from a randomly degraded DNA*

If the DNA degradation preceding the restriction enzyme digestion is random, the yield of the DNA fragments released by the restriction enzyme can be calculated from the molecular weights of the intact and of the degraded DNA. The probability R_i for a given restriction fragment of N_i nucleotides to be intact is given by:

$$R_i = (1 - p)^{N_i - 1}, \tag{A1}$$

where p is the probability for any bond to be broken; p can be expressed as the percentage of bonds broken in the intact DNA:

$$p = \rho/N_0, \tag{A2}$$

where N_0 is the number of nucleotides in the intact molecule. ρ, the number of random breaks, can be calculated according to Charlesby (1954):

$$M_0/M_w = \rho^2/2\ (e^{-\rho}+\rho-1), \tag{A3}$$

where M_w is the weight average molecular weight of the randomly degraded DNA and M_0 is the molecular weight of the intact DNA of N_0 nucleotides. Equation (A3) can be approximated by:

$$M_0/M_w = \rho^2/2\ (\rho-1)\ . \tag{A4}$$

Equation (1) can be approximated by:

$$R_i = e^{-\rho\times M_i/M_0}, \tag{A5}$$

where M_i is the molecular weight of a fragment containing N_i nucleotides.

R_i is the yield of fragment i and can be physically expressed as the ratio of the amount of DNA in the corresponding band to the amount that would be obtained if intact DNA had been used. Figure A1 shows the straight lines relating log R_i to M_i for different M_w values of the degraded DNA and for $M_0 = 50\times10^6$. It can be seen from Figure A1 that, for instance, the yield of a fragment having a molecular weight of 4×10^6 (corresponding to the largest fragments obtained by *Hae* or *Hpa* in this work) is equal to 20% if the molecular weight of the degraded DNA is 5×10^6. If Q_i is the

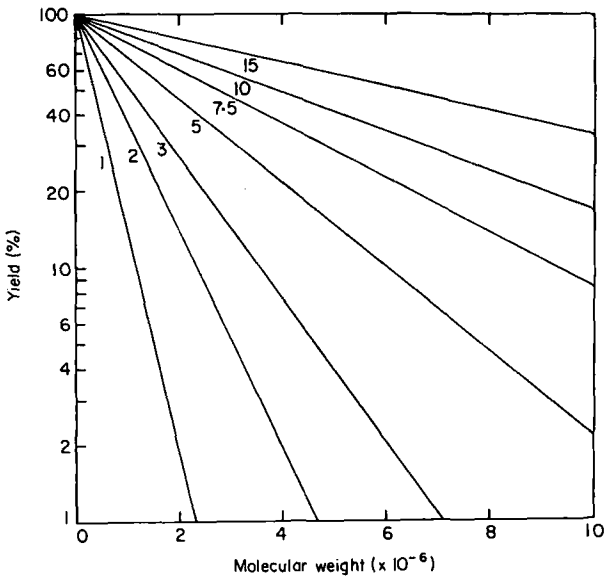

FIG. A1. Plots of the yield of the fragments, on a logarithmic scale, *versus* their molecular weight for different values of the molecular weight, M_w, of the randomly degraded starting DNA.

amount of DNA, per fragment, in band i, and M_i the corresponding molecular weight, the ratio Q_i/M_i is proportional to the yield, R_i, of the fragment, i.e.:

$$Q_i/M_i = \alpha R_i \,. \tag{A6}$$

Equation (A5) shows that the slope, β, of $\log Q_i/M_i$ *versus* M_i is:

$$\beta = -\rho/M_0 = -1/M_n \,, \tag{A7}$$

where M_n is the number average molecular weight of the DNA of weight average M_w, and that the ordinate at the origin, b, is:

$$b = \log_e \alpha \,. \tag{A8}$$

Equation (A8) allows us to derive α and therefore the absolute yield of the fragments from equation (A6).

It is possible to derive M_w from M_0; using equations (A4) and (A7) one obtains:

$$M_w = -\frac{2}{\beta 2}\left(\beta + \frac{1}{M_0}\right) \simeq 2\, M_n \tag{A9}$$

The M_w value calculated from this equation corresponds to the average size of the DNA molecules which are the enzyme target.

It is clear from equation (A9) that $1/M_0$, which is equal to 0·02, can be neglected compared to β whose absolute value is comprised here between 0·2 and 0·9 (see Results, section (d)). This prevents the use of equation (A9) to calculate M_0 from β and M_w.

(c) *Quantitative measurement of DNA amount in bands*

The treatment just described requires a precise knowledge of the amount of DNA present in the gel electrophoresis bands. A photographic procedure for this quantitation was therefore developed. This essentially requires the experimental set-up

described in Materials and Methods, section (d), of the main text, except that Kodak Ektapan (4 in × 5 in) films were used instead of Polaroid films. The blackening curve, i.e. the relationship between the intensity of the light hitting the film and the blackening of the film, was determined by illuminating a film through a step tablet having zones of different transmission. The resulting negative was scanned with a Joyce-Loebl microdensitometer and the blackening curve was obtained by plotting the pen deflections of the microdensitometer against the transmission of the zones of the step tablet. Densitometric tracings of DNA bands were analyzed by converting the pen deflections corresponding to the slices into which DNA peaks were cut into fluorescence intensities using the blackening curve. After baseline subtraction, the intensities corresponding to all slices of a peak were added together to give the total fluorescence of each band which was shown to be directly proportional to the amount of DNA. A detailed presentation of this method will be given elsewhere (Prunell *et al.*, unpublished data).

(d) Hae *and* Hpa *fragment yield from yeast mitochondrial DNAs*

Figure A2 shows plots of fragment yield *versus* the molecular weight of the fragments for the 15 to 20 bands of highest molecular weight. These results lead to a number of interesting conclusions.

(1) Loss of fragments having molecular weights higher than those corresponding to the top bands of Figures 2 and 7 of the main text can be ruled out since DNAs from strains A and B having higher molecular weights (9×10^6) did not show any additional bands of higher molecular weights, but simply higher yields of the top bands; data for DNA from strain A are shown in Fig. A2 (squares); a similar quantitative treatment was not done for the DNA from strain B.

(2) Multiple bands, as defined in Results, section (b) of the main text, fitted the straight line only after dividing the amount of DNA by the band multiplicity, confirming our assessment of the multiplicity.

(3) Some points showed a reproducible deviation from the straight line (Fig. 2). For instance, bands A1 and a3 (*Hpa*) appear to have yields approximately four times lower than expected. The yield became close to the expected value when the DNA preparation of higher molecular weight (9×10^6) was used (Fig. A2, squares), showing that a preferential breakdown of DNA is responsible for this phenomenon. Similar effects have been seen for bands A3 (*Hae*), and (not shown) for the faint band $A12_1$ (*Hae*).

(4) In the case of strains B and D, bands homologous to those just mentioned showed the same behavior, the deviation being stronger when the starting molecular weight was lower. This phenomenon provides an additional criterion of fragment homology between different strains. In contrast, similar deviations were not seen in the case of *S. carlsbergensis* DNA.

(5) The plots of Figure A2 permit us to calculate the target size of the DNAs for the restriction enzymes using equation (A9). The values so calculated (Table A1) are in general agreement with the molecular weight of the starting DNAs.

(6) The yields considered here were calculated assuming that the yield of the smallest bands is 100% (Fig. A2) and not on the basis of the amount of DNA loaded on the gel. As a consequence, the presence of contaminating DNA (like nuclear DNA and DNA from spontaneous "petite" and wild-type mutants (see Discussion, section

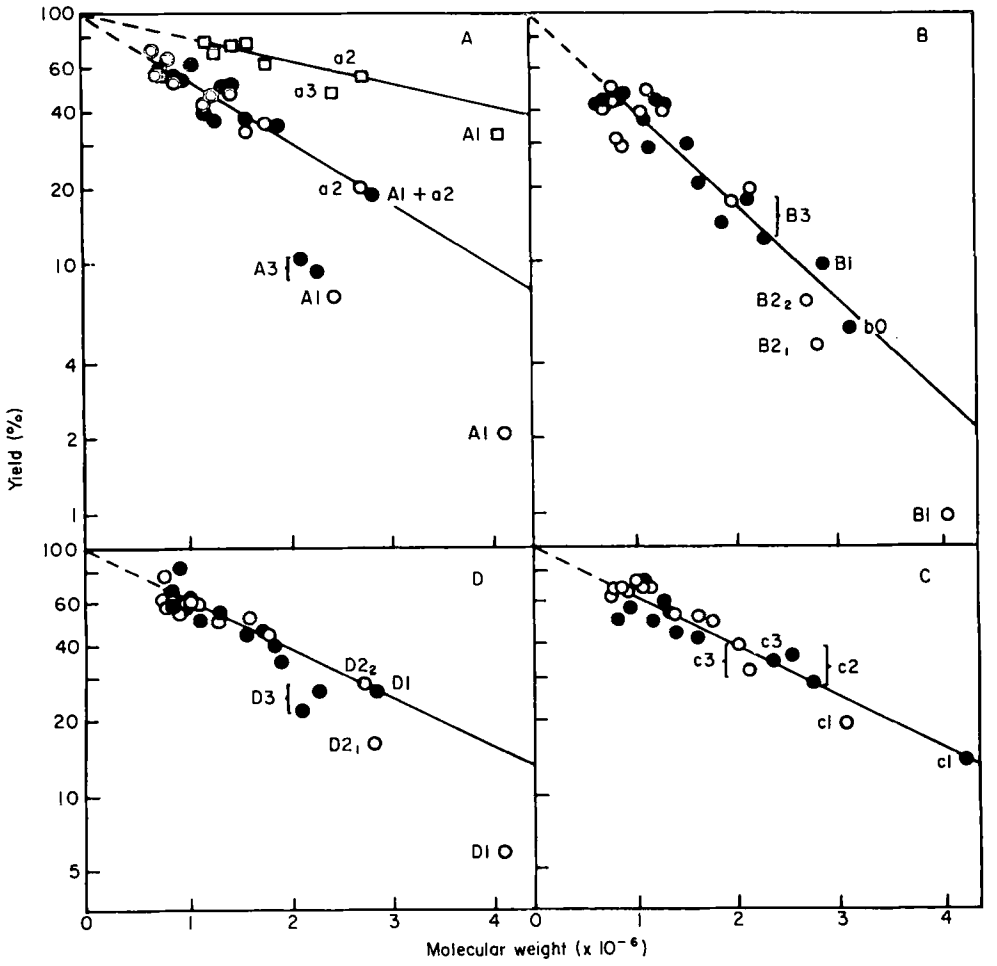

FIG. A2. Plot of the fragment yield, on a logarithmic scale, from the four mitochondrial DNAs degraded by *Hpa* (○) and *Hae* (●) restriction endonuclease, *versus* the molecular weight of the fragments. (□) Refer to the fragments obtained by *Hpa* degradation of mitochondrial DNA from strain A whose molecular weight was higher (9×10^6). The yield was calculated from equation (A6). In order to obtain the α values, Q_i/M_i was plotted *versus* M first, and α determined from equation (A8) using a least-squares procedure.

All Q_i/M_i values were then divided by α and replotted. Some points concern multiple bands or incompletely separated bands. In these cases the Q_i values were divided by the corresponding number of fragments. In the calculation of the α values, the points which deviate too strongly from the line were not taken into account, namely A1, a3 (*Hpa*) and A3 (*Hae*), B1 and $B2_1$ (*Hpa*), D1, $D2_1$ (*Hpa*) and D3 (*Hae*). The doublets in B3 (*Hae*), Fig. 8 of the main text, were considered as double bands.

(a) (ii), in the main text) contributing to the background smear does not affect the estimation of the fragment yield.

(e) *Endogenous degradation of mitochondrial DNA*

The fact that the target size is in general agreement with the molecular weight of the starting DNAs (Table A1) indicates that the overall degradation occurring during the preparation of the mitochondrial DNA can be considered as a random one.

Nevertheless, the observation of a specific endogenous breakage which is superimposed on the random breakage and which induces lower yields of some bands and the appearance of faint bands in the DNA from the *S. cerevisiae* strains, indicates the presence of a highly specific DNase in yeast. This activity, possibly localized in the mitochondria of *S. cerevisiae* strains, appears to be different from previously described ones, including the one recently reported (Zeman & Lusena, 1975) to attack preferentially the A+T-rich spacers. In *S. carlsbergensis* the specific activity is absent or much lower than in *S. cerevisiae*. This may be an explanation for the greater ease of preparation of high molecular weight DNA from *S. carlsbergensis*.

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