CHROMOSOMAL LOCATIONS OF HIGHLY REPEATED DNA SEQUENCES IN WHEAT

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Received 17.ix.79

SUMMARY

C_0t 10^{-2} DNA was isolated from hexaploid wheat *Triticum aestivum* cv. Chinese Spring by hydroxyapatite chromatography (70°C in 0.12 M phosphate buffer). The higher T_m of the C_0t 10^{-2} DNA compared with total wheat DNA suggested that it was relatively GC rich and contained well matched hybrids. *In situ* hybridisation using wheat species of different ploidy levels located major sites of the C_0t 10^{-2} DNA on the B genome chromosomes. More than one particular highly repeated sequence is located in these sites. Other chromosomal locations could be visualised by heating *in situ* hybridisation reactions before renaturation. This was attributed to the availability for hybridisation both of chromosomal sequences additional to those normally available after acid denaturation of cytological preparations and to single stranded cRNA molecules which were otherwise present as double stranded structures.

1. INTRODUCTION

HIGHLY repeated DNA sequences, sometimes detected as rapidly reassociating DNA (Britten and Kohne, 1968), are a characteristic component of eukaryotic genomes. Rapidly renaturing fractions of hexaploid wheat DNA have been isolated (Mitra and Bhatia, 1973; Smith and Flavell, 1974, 1975; Dover, 1975; Flavell and Smith, 1976; Ranjekar *et al.*, 1976), up to 10 per cent of the genome being recovered as duplexes by hydroxyapatite chromatography after reassociation to C_0t values between 8×10^{-3} and 2×10^{-2} mol sec l^{-1} in 0.12 M phosphate buffer at 60°C. This DNA had thermal stabilities 8-10°C lower than that of native, total wheat DNA indicating that mismatched hybrids had been isolated (Dover, 1975; Smith and Flavell, 1975; Ranjekar *et al.*, 1976), and Ranjekar *et al.* (1976) found that the buoyant density profile was broad, with a mean density of 1.708 gm/cm³ compared with the density of 1.702 gm/cm³ of the total, native wheat DNA. The mismatching in the renatured molecules may be the result of sequence divergence during the evolution of the families of repeated DNAs (Flavell *et al.*, 1977).

The work described in this paper was undertaken to characterise the chromosomal locations of the rapidly renatured DNA C_0t 10^{-2} fractions which were isolated by hydroxyapatite chromatography under conditions which ensured that only well matched hybrids were formed. *In situ* cytological hybridisation located this DNA at sites on all chromosomes with distinct, major sites being present on the B genome chromosomes.

2. MATERIALS AND METHODS

(i) *Isolation of highly repeated DNA*

DNA was isolated from 3-day-old seedlings of hexaploid wheat *Triticum aestivum* cv. Chinese Spring grown at 27°C from surface sterilised seeds. Approximately 1000 seedlings were frozen in liquid nitrogen and homogenised to powder in a Waring Blender at high speed for 4 min. Following the addition of 250 ml buffer (0.15 M NaCl, 0.1 M EDTA, pH = 8.0), the mixture was homogenised for a further 2 min. at high speed; 2 g sodium lauryl sulphate was added and the suspension heated to 60°C for 20 min. before addition of 100 mg Pronase (Sigma) and further incubation for 1 hour at 45°C. The suspension was made 0.55 M with NaCl, centrifuged to remove particulate material and then extracted with an equal volume of phenol. The phenol was washed twice with water and nucleic acid was precipitated by addition of 2 volumes of ethanol (−20°C) to the pooled aqueous fractions. RNase A (Sigma, 10 µg/ml, 37°C, 30 min.) treatment followed resuspension of the DNA pellet in 2 × SSC (SSC is 0.15 M NaCl, 0.015 M sodium citrate). The DNA was again precipitated with two volumes of ethanol and purified by centrifugation in a CsCl buoyant density gradient. DNA was stored in TE (10 mM Tris-HCl, 1 mM EDTA, pH = 8.4) at 4°C.

For isolation of a rapidly renaturing DNA fraction the total DNA was first sheared by three passages through an Aminco French Pressure Cell (16,000 p.s.i.). Average single stranded DNA fragment length, calculated from the sedimentation velocity in 0.9 M NaCl, 0.1 M NaOH (Studier, 1965), was 700 bases. In two separate experiments the sheared DNA (15.8 and 15.2 µg/ml respectively) in 0.12 M PB (Sorensen's phosphate buffer, pH = 6.8) was denatured by heating to 100°C and aliquots incubated at 70°C to allow renaturation. The rapidly renaturing fraction was isolated by hydroxyapatite chromatography using a jacketed column maintained at 70°C. Flow rate was adjusted so that the average C_0t value of reassociated DNA aliquots applied to the column was C_0t 10^{-2} mol sec. l^{-1} (range = C_0t 0.5×10^{-2} to 1.5×10^{-2}). DNA bound to the column was eluted with 0.5 M PB at 70°C.

(ii) *Thermal dissociation profiles*

DNA samples were co-dialysed with *Escherichia coli* DNA (Sigma) against TE. The *E. coli* DNA was included in each experiment as an internal control; the melting temperature of the sample was adjusted relative to a standard melting temperature for *E. coli* DNA of 65.8°C in TE. Hyperchromicity was expressed as the percent increase in optical density (260 nm) compared with the initial optical density of the sample. Profiles were obtained with a Gilford recording spectrophotometer, with temperature programmed to increase at either 45 or 60°C/hour.

(iii) *Preparation of cRNA*

³H-cRNA (RNA complementary to a DNA template) was prepared using *E. coli* RNA polymerase. The reaction mixture (0.1 ml) contained 4 µg DNA template, 6.7×10^{-4} µmoles of ³H-GTP, CTP, UTP, 10^{-4} moles ATP and *E. coli* RNA polymerase (prepared according to Burgess, 1969)

in 0.04 M Tris-HCl pH = 7.9, 0.01 M MgCl_2 , 0.16 M KCl and 2×10^{-4} M dithiothreitol at 37°C. After 2-3 hours the template was removed by DNase treatment (Worthington DPFF, 100 $\mu\text{g}/\text{ml}$, 30 min.) and the cRNA isolated by phenol extraction, G-75 Sephadex chromatography and ethanol precipitation. cRNA preparations were stored in $6 \times \text{SSC}$ at -20°C .

(iv) *In situ hybridisation*

To accumulate root tip metaphases, 3-day-old seedlings grown at 27°C were treated with 0.25 per cent colchicine for 3 hours prior to fixation in 1 : 3 acetic ethanol. Root tip squashes were prepared in 45 per cent acetic acid, dehydrated in absolute alcohol and air dried before chromosomal DNA was denatured in 0.2 N HCl (37°C, 30 min.). Slides were then dehydrated through an alcohol series and air dried. 5 μl of ^3H -cRNA in $3 \times \text{SSC}$, 50 per cent formamide (10^4 - 10^5 cpm/ μl) was placed on each slide, covered with an 18 mm² cover slip, sealed with rubber gum. Slides were then incubated for 3 hours at 40°C to effect hybridisation. Cover slips were removed and slides were washed twice in $3 \times \text{SSC}$, 50 per cent formamide (10 min. each wash, 40°C), twice in $2 \times \text{SSC}$ (10 min. each, 40°C) before RNase treatment (10 $\mu\text{g}/\text{ml}$ Sigma pancreatic RNase, 1.25 units/ml Calbiochem T₁ RNase in $2 \times \text{SSC}$, 30 min. at 37°C). Following six further washes in $2 \times \text{SSC}$ the slides were ethanol dehydrated, air dried and coated with Ilford K2 emulsion (50 per cent dilution). Exposures were left for 1-3 weeks. Developed slides were stained with 1 per cent Gurr's Giemsa in 0.2 M PB, pH = 6.8.

In "pre-heated" hybridisation, slides complete with sealed reaction mixture, were placed at 75°C for 30 sec. prior to incubation at 40°C.

Species used in *in situ* hybridisation experiments were: hexaploid wheat, *Triticum aestivum* cv. Chinese Spring, $2n = 42$; tetraploid wheat, *T. dicoccoides* W1043, $2n = 28$; diploid wheat, *T. monococcum* W292, $2n = 14$; and *Aegilops squarrosa* G46, $2n = 14$. Hexaploid wheat was donated by Dr K. Shepherd, University of Adelaide, and the other species were a gift from Mr R. Dunstone, CSIRO, Division of Plant Industry.

(v) *Filter hybridisation*

Sartorius Membrane Filters (47 mm diameter) were washed for 30 min. in $4 \times \text{SSC}$, placed on a Millipore filtration apparatus and washed with 40 ml $4 \times \text{SSC}$. 120 μg total wheat DNA (estimated from OD_{260}) was denatured in 40 ml $4 \times \text{SSC}$ and 5 ml 1 N KOH at room temperature and cooled in ice after 5 min. The DNA was neutralised by adding 5 ml 1 N HCl and loaded onto the filters by vacuum filtration. The effective area of the filter loaded with DNA was 35 mm diameter. Small filters (7.75 mm diameter) were cut from the large filter and dried overnight in a vacuum oven at 60°C. Blank filters were prepared by omitting the DNA in the loading solution.

For hybridisation, the filters were first washed for 10 min. in $3 \times \text{SSC}$, 50 per cent formamide at 40°C. One DNA-filter and one blank filter was added to each of a series of vials containing 0.5 ml $3 \times \text{SSC}$, 50 per cent formamide, 2 μl ^3H -cRNA (2.9×10^5 cpm/ μl) and 5 μl unlabelled yeast RNA. If preheating of the hybridisation mixture was required the vials were heated to 70°C for 3 min. at this stage. The hybridisation was carried

out with gentle shaking at 40°C. Both blank and DNA-filters were removed at given times, rinsed in 3 × SSC, 50 per cent formamide and placed in fresh 3 × SSC, 50 per cent formamide until the end of the experiment. At the end of the experiment they were washed *en masse* with 3 × SSC (3 × 50 ml washes, 5 min. each) and treated with RNase (20 µg Sigma pancreatic RNase A, 50 units Calbiochem T₁ RNase in 2.5 ml 3 × SSC). Following RNase treatment the filters were washed with 3 × SSC for 5 min., dried at 60°C and counted. Radioactive label bound to each filter containing DNA was corrected for background as measured by the blank filter (this was always 60-75 cpm).

3. RESULTS AND DISCUSSION

(i) *Rapidly renaturing DNA*

C₀t 10⁻² DNA accounted for 7.2 per cent and 13.0 per cent of the DNA recovered from the hydroxyapatite columns in the two experiments. The total proportion of applied DNA recovered from the hydroxyapatite was 90 per cent and 83 per cent respectively. The differences between the two isolations may be due to different flow rates and bed volumes of the columns used (Britten *et al.*, 1974). Thermal dissociation profiles for total wheat DNA and the C₀t 10⁻² isolates in TE (fig. 1) show total native wheat DNA with a *T_m* of 63.0°C, significantly lower than the *T_m*'s of the C₀t 10⁻² fractions (70.7°C and 69.1°C respectively). The lower *T_m* of the second

FIG. 1.—Thermal denaturation profiles of total native wheat DNA (T) and two different C₀t 10⁻² fractions (I and II) in 10 mM Tris, 1 mM EDTA, pH = 8.4.

FIG. 2.—Autoradiographs after *in situ* hybridisation at 40°C with ^3H -cRNA to $\text{C}_0\text{t } 10^{-2}$ DNA. Root tip metaphase cells of *Triticum aestivum* (a), *T. monococcum* (b) and *Aegilops squarrosa* (c). One pair of *T. monococcum* chromosomes, arrowed in 2b, show a major centromeric hybridisation site relative to the other locations of the cRNA.

FIG. 3.—Autoradiograph karyotypes of *T. aestivum* (a) and *T. dicoccoides* (b).

FIG. 4.—Autoradiograph showing the effect of preheating a *T. aestivum* *in situ* preparation to 70°C for 3 min. before hybridisation at 40°C.

C₀t 10⁻² fraction may be the result of additional sequences since the yield from the second isolation was greater. The hyperchromicity of the second C₀t 10⁻² isolate was 28.7 per cent compared to 31.5 per cent for native total wheat DNA melted during this experiment. These observations suggest that the C₀t 10⁻² samples contain well-matched hybrids which are relatively GC-rich compared with total wheat DNA.

It seems likely that most of the C₀t 10⁻² DNA corresponds to the sequences in the late melting broad portion of the melting profile of total wheat DNA; the T_m of the hybrids must be within a few degrees of their native T_m . This contrasts with other C₀t 10⁻² fractions isolated from hexaploid wheat at 60°C in 0.12 M PB which had T_m values = 10°C lower than native, total wheat DNA (Dover, 1975; Smith and Flavell, 1975; Ranjekar *et al.*, 1976). The differences presumably relate to the high stringency of the present experiments. Also, the hydroxyapatite binding ability of mismatched hybrids is probably lower at 70°C than at 60°C (Britten *et al.*, 1974).

A feature of the melting profile of the C₀t 10⁻² DNA is a single, late-melting transition which comprises about 50 per cent of the melt profile. If this represents one sequence it would constitute more than 2 per cent of the wheat genome.

(ii) Chromosomal locations

The C₀t 10⁻² cRNA hybridised to a number of distinctive sites on wheat chromosomes. Autoradiographs of a root tip mitotic metaphase preparation (fig. 2a) and karyotypes (fig. 3a) of *Triticum aestivum* (genome constitution = AABBDD, 2n = 42) and *T. dicoccoides* (AABB 2n = 28, fig. 3b) after *in situ* hybridisation with C₀t 10⁻² cRNA at 40°C show that a number of heavily labelled chromosomes are common to both *T. aestivum* and *T. dicoccoides*. In contrast, chromosomes of the diploid species *T. monococcum* (AA, 2n = 14, fig. 2b) and *A. squarrosa* (DD, 2n = 14, fig. 2c) show only light hybridisation after comparable exposures. From these results it can be concluded that most of the labelled chromosomes are B genome chromosomes.

Fig. 4 shows the effect of "pre-heating" *Triticum aestivum in situ* hybridisation preparations (i.e., both cRNA probe and chromosomal DNA) to 70°C for 3 min. before hybridisation at 40°C, the exposure time of the autoradiograph being the same as that for the preparation in fig. 2 which was not preheated. The overall extent of labelling is higher and all chromosomes including those from A and D genomes, show substantial label. Sites similar to those seen when hybridisation is performed at 40°C without preheating are evident as more heavily labelled areas.

The thermal stability of the hybrids formed at the major sites was compared with those observed at the more general sites. This was done by preparing *in situ* hybridisation reactions, preheating them to 70°C to ensure that all sites were available for hybridisation and then carrying out the hybridisation at 40°C. After hybridisation duplicate slides were heated to either 45°C, 50°C, 55°C, 60°C or 65°C in 3 × SSC, 50 per cent formamide for 10 min. before preparing autoradiographs. After both 45°C and 50°C treatments the slides still showed heavy labelling at major sites and more general labelling as shown in Fig. 4. At 55°C the slides showed a substantial decrease in the amount of label at both major and general sites, and at 60°C

and 65°C the slides showed no label. Thus, the average thermal stabilities of these hybrids were not markedly different, with average T_m 's between 50°C and 60°C.

(iii) *Effect of preheating cRNA and chromosomal DNA sequences separately*

Preheating of *in situ* preparations (described above) was originally carried out to determine whether any sequences were present in the cRNA as double-stranded RNA molecules unavailable for hybridisation. Filter hybridisation experiments were used to determine whether preheating does make an increased amount of cRNA available for hybridisation. DNA on nitrocellulose filters was hybridised with cRNA in $3 \times \text{SSC}$, 50 per cent formamide which had been either (1) heated to 70°C for 3 min. immediately prior to hybridisation at 40°C, or (2) maintained at room temperature prior to hybridisation at 40°C. Filters were removed at intervals and the amount of radioactive cRNA hybridised was determined. Fig. 5 shows that preheating makes additional cRNA sequences available for hybridisation.

FIG. 5.—Hybridisation of C_6t 10^{-2} cRNA to filters containing total wheat DNA with and without preheating of the cRNA to 70°C immediately prior to hybridisation. Counts per minute cRNA bound to the filter are plotted against time of incubation of the hybridisation mixture at 40°C.

The hybrids formed with or without the preheating treatment had melting temperatures within 1°C of each other in $3 \times \text{SSC}$, 50 per cent formamide (data not shown), in good agreement with the *in situ* data.

Since Singh *et al.* (1977) reported that hybridisation of satellite DNA may be markedly enhanced when heat (or heat + formamide) denaturation is used rather than acid denaturation, the effect of preheating the chromosomal preparations rather than the cRNA was also tested. Chromosome preparations which had been heated in $3 \times \text{SSC}$, 50 per cent formamide (70°C, 3 min.) before loading with hybridisation mixtures at 40°C showed labelling patterns similar to that in Fig. 4. Thus, preheating of the chromosomes in $3 \times \text{SSC}$, 50 per cent formamide made available additional, more generally distributed chromosomal sites.

(iv) *The major chromosomal sites contain more than one highly repeated sequence*

A highly repeated DNA sequence which can be isolated as a satellite in $\text{Ag}^+/\text{Cs}_2\text{SO}_4$ buoyant density gradients of total wheat DNA also hybridises to the major chromosomal sites seen in Figs. 2a and 3a (Dennis *et al.*, 1980). An *in situ* competition hybridisation experiment was carried out to determine whether the hybridisation pattern seen in Fig. 2a, was solely due to hybridisation by the satellite sequence. $\text{C}_0\text{t } 10^{-2}$ cRNA was hybridised to acid denatured chromosomes in the presence of sufficient unlabelled satellite DNA to eliminate hybridisation by the sequences of this satellite in the cRNA (as judged by control experiments). The presence of the unlabelled competitor did not markedly affect hybridisation of the $\text{C}_0\text{t } 10^{-2}$ cRNA indicating that more than one particular highly repeated sequence must be present at all the major chromosomal sites. An arrangement of groups of different highly repeated sequences at particular chromosomal locations has been described in *Drosophila* (Peacock *et al.*, 1977a, b), primates (Gosden *et al.*, 1977) and rye (Bedbrook *et al.*, 1980). It seems likely that wheat also has distinct chromosomal sites which each contain a set of different highly repeated sequences.

4. CONCLUDING REMARKS

The observation that the B genome contains almost all of the major sites for highly repeated DNA sequences in wheat was surprising. The three wheat genomes, A, B and D, are considered to have similar informational contents because "homoeologous" chromosomes from each genome can be assigned on the basis of their ability to compensate for each other in aneuploid strains (Sears, 1966) and their similar distribution of genes for detectable isozymes, (Hart and Langston, 1977), but the present study shows that they contain large differences in the distribution and organisation of highly repeated DNA sequences. Flavell *et al.* (1979) have recently reached the same conclusions in their filter hybridisation studies of rapidly renaturing cereal DNA.

These large differences do not play an essential role in plant viability or fertility since, in most possible combinations, homoeologues from the A, B and D genomes can substitute for each other in nullisomic-tetrasomic stocks (Sears, 1966). However, it is notable that compensation is not always complete in these stocks, reduced vigour and fertility being present in a number of compensating aneuploids. This may well result from slightly different genetic informational contents of the homoeologues or, possibly, the effect of the changed proportion of highly repeated DNA. That the highly repeated DNA blocks are maintained in the B genome of several wheat species (Gerlach *et al.*, 1978) suggests some selective maintenance of these sequences in wheat.

Because *in situ* hybridisation shows that some rapidly renaturing sequence DNA is present on all wheat chromosomes it is likely that specific highly repeated DNA sequences can be developed as molecular probes for the cytological identification of wheat chromosome regions. Some of these sequences will probably label only the major highly repeated sequence locations which are predominantly in the B genome (cf. Dennis *et al.*, 1979), but because there is a general labelling of all chromosomes when *in situ*

preparations are preheated, it should be possible to isolate others characteristic of A and D genome chromosomes as well.

Acknowledgments.—The authors are grateful to Dr R. Appels and Dr E. Dennis for helpful comments on the manuscript and to Dr Appels for experimental advice. W. L. Gerlach was supported by a Rothmans Fellowship and was a Visiting Fellow at the Australian National University.

5. REFERENCES

- BEDBROOK, J. R., JONES, J., O'DELL, M., THOMPSON, R. D., AND FLAVELL, R. B. 1980. A molecular description of telomeric heterochromatin in *Secale* species. *Cell* (in Press).
- BRITTEN, R. J., AND KOHNE, D. E. 1968. Repeated sequences in DNA. *Science*, 161, 529-540.
- BRITTEN, R. J., GRAHAM, D. E., AND NEUFELD, B. R. 1974. Analysis of repeating DNA sequences by reassociation. *Methods Enzymol.*, 29E, 363-418.
- BURGESS, R. R. 1969. A new method for the large scale purification of *Escherichia coli* DNA-dependent RNA polymerase. *J. Biol. Chem.*, 244, 6160-6167.
- DENNIS, E. S., GERLACH, W. L., AND PEACOCK, W. J. 1980. Identical polypyrimidine-polypurine satellite DNAs in wheat and barley. *Heredity* (in Press).
- DOVER, G. A. 1975. The heterogeneity of B chromosome DNA: no evidence for a B-chromosome specific repetitive DNA correlated with B-chromosome effects on meiotic pairing in the Triticinae. *Chromosoma*, 53, 153-173.
- FLAVELL, R. B., O'DELL, M., AND SMITH, D. B. 1979. Repeated sequence DNA comparisons between *Triticum* and *Aegilops* species. *Heredity*, 42, 309-322.
- FLAVELL, R. B., AND SMITH, D. B. 1976. Nucleotide sequence organisation in the wheat genome. *Heredity*, 37, 231-252.
- FLAVELL, R. B., RIMPAU, J., AND SMITH, D. B. 1977. Repeated sequence DNA relationships in four cereal genomes. *Chromosoma*, 63, 205-222.
- GERLACH, W. L., APPELS, R., DENNIS, E. S., AND PEACOCK, W. J. 1978. Evolution and analysis of wheat genomes using highly repeated DNA sequences. *Proc. 5th Int. Wheat Genet. Symp.* 1, 81-91.
- GOSDEN, J. R., MITCHELL, A. R., SEUANEZ, H. N., AND GOSDEN, C. M. 1977. The distribution of sequences complementary to human satellite DNAs I, II and IV in the chromosomes of Chimpanzee (*Pan troglodytes*), Gorilla (*Gorilla gorilla*) and Orang Utan (*Pongo pygmaeus*). *Chromosoma* 63, 253-271.
- HART, G. E., AND LANGSTON, P. J. 1977. Chromosomal location and evolution of isozyme structural genes in hexaploid wheat. *Heredity*, 39, 263-277.
- HUGUET, T., JOUANIN, L., AND BAZETOUX, S. 1975. Occurrence of palindromic sequences in wheat DNA. *Plant Sci. Lett.*, 5, 379-385.
- MITRA, R., AND BHATIA, C. R. 1973. Repeated and non-repeated polynucleotide sequences in diploid and polyploid wheat species. *Heredity*, 31, 251-262.
- PEACOCK, W. J., APPELS, R., DUNSMUIR, P., LOHE, A. R., AND GERLACH, W. L. 1977a. Highly repeated DNA sequences: chromosomal location and evolutionary conservation. In *International Cell Biology 1976-1977*, eds. B. R. Brinkley and K. R. Porter. Rockefeller Univ. Press, 1977, pp. 494-506.
- PEACOCK, W. J., LOHE, A. R., GERLACH, W. L., DUNSMUIR, P., DENNIS, E. S., AND APPELS, R. 1977b. Fine structure and evolution of DNA in heterochromatin. *Cold Spr. Harb. Symp. Quant. Biol.*, 42, 1121-1135.
- RANJEKAR, P. K., PALOTTA, D., AND LAFONTAINE, J. G. 1976. Analysis of the genome of plants. II. Characterization of repetitive DNA in barley and wheat. *Biochim. Biophys. Acta*, 425, 30-40.
- SEARS, E. R. 1966. Nullisomic-tetrasomic combinations in hexaploid wheat. In *Chromosome Manipulations in Plant Genetics*, eds. R. Riley and K. R. Lewis. *Heredity* (Suppl.), 20, 29-45.
- SINGH, L., PURDOM, I. F., AND JONES, K. W. 1977. Effect of different denaturing agents on the detectability of specific DNA sequences of various base compositions by *in situ* hybridization. *Chromosoma*, 60, 377-389.
- SMITH, D. B., AND FLAVELL, R. B. 1974. The relatedness and evolution of repeated nucleotide sequences in the genomes of some Gramineae species. *Biochem. Genet.*, 12, 243-256.
- SMITH, D. B., AND FLAVELL, R. B. 1975. Characterization of the wheat genome by renaturation kinetics. *Chromosoma*, 50, 223-242.
- STUDIER, F. W. 1965. Sedimentation studies of the size and shape of DNA. *J. Molec. Biol.*, 11, 373-390.