NUCLEOTIDE SEQUENCE ORGANISATION IN THE WHEAT GENOME

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SUMMARY

Chromosomal nucleotide sequence organisation has been studied in hexaploid wheat, *Triticum aestivum*, using methods based upon the different renaturation kinetics of repeated and non-repeated sequences. Approximately 25 per cent of the wheat genome consists of non-repeated sequences. Nearly two-thirds of these (15 per cent of the genome) are around 1000 base pairs long interspersed between repeated sequences. Approximately 7 per cent of the genome consists of non-repeated sequences several thousand base pairs long. Less than 5 per cent of the genome consists of very much longer non-repeated sequences.

Four per cent of the genome consists of extremely rapidly renaturing (foldback?) sequences. These sequences are distributed through at least 20 per cent of the genome.

Between 50 and 65 per cent of the genome consists of repeated sequence DNA which is cleaved to duplexes approximately 400 to 800 base pairs long by S_1 nuclease after renaturation *in vitro*. This implies that related repeated sequences frequently have different neighbouring sequences in the chromosomes. Repeated sequences (32 per cent of the genome) able to reanneal in very stringent incubation conditions are between approximately 350 and 650 base pairs long and are distributed through 85 per cent of the genome at intervals of less than 4000 base pairs. This distribution implies that short regions of essentially non-diverged repeated sequence DNA are interspersed with longer regions of diverged repeated sequence DNA in the chromosomes. Approximately 10 per cent of the genome appears to consist of very long repeated sequences or of long clusters of short, essentially identical repeated sequences.

1. INTRODUCTION

MOST hypotheses on the control of gene expression and chromosome behaviour in eucaryotes are currently based upon linear arrays of different kinds of nucleotide sequences (Britten and Davidson, 1969; Paul, 1972; Georgiev *et al.*, 1973; Gierer, 1973; Lewin, 1974). Using electron-microscopical and biochemical methods nucleotide sequence organisation has been investigated in the genomes of a number of organisms including *Xenopus* (Davidson *et al.*, 1973*a*, *b*), sea urchin (Graham *et al.*, 1974), *Drosophila* (Wu *et al.*, 1972; Kram *et al.*, 1972; Laird *et al.*, 1973; Manning *et al.*, 1975), rat (Bonner, J. *et al.*, 1973) and *Dictyostelium* (Lodish *et al.*, 1973; Firtel and Kindle, 1975). It appears to be a general finding that much of the DNA of these organisms with the exception of *Drosophila* (Manning *et al.*, 1975) is composed of non-repeated nucleotide sequences between 700 and 2500 nucleotides long interspersed by shorter repeated sequences (Davidson *et al.*, 1975). Chromosomes of higher organisms also have clusters of inverted duplications or palindrome sequences (Wilson and Thomas, 1974; Bonner, 1974) as well as long regions of tandemly repeated sequences (Walker, 1971; Yunis and Yasmineh, 1971). However, little information on genome organisation is currently available for higher plants.

In this paper we report on the chromosomal nucleotide sequence organisation of common bread wheat, *Triticum aestivum*. In earlier studies on the wheat genome (Smith and Flavell, 1975) three classes of DNA were distinguished: (1) a fraction that renatures extremely rapidly and may therefore include regions of the chromosomes containing inverted duplications (see Wilson and Thomas, 1974; Bonner, 1974); (2) a fraction which renatures slowly and appears to contain sequences present in about six copies per hexaploid cell; and (3) the remaining DNA, about 80 per cent of the genome, which reanneals at intermediate rates and consists of repeated sequences with repetition frequencies between 50 and 50,000 per haploid genome. The amounts, lengths and linear arrangements of these different kinds of sequences are investigated in this paper.

2. MATERIALS AND METHODS

(i) *Isolation of ³H-labelled and unlabelled DNAs*

Unlabelled wheat, variety Chinese Spring, DNA was isolated from leaves, as described by Smith and Flavell (1974). Tritium-labelled DNA was purified from 3-day-old seedlings germinated in the presence of ^{3}H-thymidine (CH_3-labelled, 41 Ci/m mol) as described previously (Smith and Flavell, 1974.) Specific activity of the DNA was 28430 cpm/μg. Purified *Xenopus* DNA was a gift from Professor H. C. MacGregor, University of Leicester. Calf thymus and *E. coli* DNAs were purchased from Sigma Chemical Co. 98 ± 1 per cent of the native DNAs were retained on hydroxyapatite at 60°C in 0·12-M PB (phosphate buffer).

(ii) *DNA fragmentation and estimation of average fragment sizes*

Different concentrations of DNA in ice-cold 0·12-M PB were sheared by different doses of sonication to a range of average fragment sizes as previously described (Smith and Flavell, 1974, 1975). Weight average single strand fragment lengths of unlabelled DNAs were determined by boundary velocity sedimentation in 0·9-M NaCl 0·1-M NaOH as described by Studier (1965) using an MSE " Centriscan " analytical ultracentrifuge. It is important to note that although only weight average fragment sizes are mostly quoted in this paper, all DNA preparations, especially those unlabelled DNAs with longer average fragment sizes, were heterogeneous. Distribution curves of the fragment sizes in unlabelled DNA preparations treated with similar doses of sonication have been presented previously (Smith and Flavell, 1975). Estimates of the size of double-stranded duplexes resistant to S_1 nuclease were determined by boundary sedimentation in 1-M NaCl (Studier, 1965). Tritium-labelled DNAs of different average fragment sizes were obtained by fractionating native DNA in 5 to 11 per cent w/w linear sucrose gradients made in 0·1-M NaOH after sedimentation for 19 hours at 24,000 r.p.m. at 20°C. Weight average single-stranded fragment lengths in each gradient fraction were determined as described by Burgi and Hershey (1963) after sedimentation for 16 to 19 hours in identical gradients. Approximately 1 μg of ³H-labelled DNA was sedimented together with 150 μg unlabelled marker DNA whose molecular weight was determined separately in the analytical ultracentrifuge. Size distribution

profiles of unlabelled DNAs were derived from the analytical ultracentrifuge curves by the method of Schumaker and Schachman (1957).

We recognise that upon boiling and incubating at 60°C some shearing of single-stranded DNA occurs, particularly with longer fragment lengths. In only some experiments (see fig. 3) did we make measurements of fragment size after denaturation and incubation. Thus the illustrated longer fragment lengths in many experiments may be overestimates of the sizes actually involved in duplex formation but this does not invalidate any of our general conclusions.

(iii) *Renaturation of denatured DNA*

DNAs, at selected concentrations in appropriate solvents, were denatured by heating for 5 to 10 minutes and immediately incubated to the required C_0t value to allow renaturation. The following incubation conditions were used (1) 0·12-M PB at 60°C, (2) 0·12-M PB 0·645-M NaCl at 65°C and (3) 62 per cent formamide 0·69-M NaCl at 42·5°C. After incubation under conditions 1 and 2 the DNA solutions were cooled, diluted with three volumes of ice-cold water and loaded on to hydroxyapatite equilibrated with 0·03-M PB at room temperature. After incubation under condition (3) the DNA solution was cooled and diluted with a 15-fold excess of 0·03-M PB before hydroxyapatite chromatography. The hydroxyapatite columns were washed with 0·03-M PB at room temperature before single-stranded DNA was eluted with 0·12-M PB at room temperature and 60°C and the renatured DNA was eluted with 0·5-M PB first at 60°C and then at 95°C. After incubation in formamide (condition 3) single-stranded DNA was usually eluted at 80°C with 0·12-M PB and the renatured DNA at 95°C with 0·12-M PB. However, elution of single-stranded DNA from hydroxyapatite at 60° or 70°C after incubation under condition 3 gives similar results.

(iv) *Estimations of DNA concentrations*

Unlabelled DNA in the 0·12-M PB and 0·5-M PB hydroxyapatite column eluates was estimated by OD_{260} measurements after low-speed centrifugation to sediment any hydroxyapatite. Recoveries of DNA from hydroxyapatite were approximately 100 per cent except when renatured DNAs of large initial average fragment sizes were chromatographed; in these cases a small proportion (less than 7 per cent) could not be removed with 0·5-M PB at 95°C. Reannealed DNA values were corrected for 20 per cent hypochromicity when recovered at 60°C (Smith and Flavell, 1975) and for the proportion of the DNA behaving as renatured DNA on hydroxyapatite immediately after boiling (see Davidson *et al.*, 1973*b* and fig. 5).

Percentages of reannealed DNA were also corrected for any OD_{260} or tritium in the native DNA preparations which did not adhere to hydroxyapatite at 60°C in 0·12-M PB.

Tritium-labelled DNA was estimated by precipitation with 5 per cent (final concentration) trichloroacetic acid (TCA) in the presence of 100 to 150 μg bovine serum albumin in the cold, collection on Whatman GF/B filters and counting in a scintillation mixture (Smith and Flavell, 1974, 1975). To investigate whether precipitation efficiency or collection efficiency on the filter varied with very different fragment sizes of native, denatured

or renatured DNAs as suggested by Schrier and Wilson (1973), a series of separate experiments were carried out. Two DNA preparations, one with an average fragment molecular weight of 860,000 daltons and a sheared preparation with an average fragment molecular weight of 82,500 daltons were used.   Where required, the DNAs were denatured by adding an equal volume of 1-M NaOH and neutralising after 15 minutes with four volumes of a solution containing 3-M NaCl, 1-M Tris-HCl pH 8·0 and 1-N HCl in the proportions of $2:1:1$. DNA precipitation was carried out as described above.   The precipitation and collection efficiencies were studied at DNA concentrations of 55 μg/ml, 17 μg/ml and 1·5 μg/ml.   The results in table 1 show that estimates of the recovered DNA were independent of the fragment size, concentration and DNA configuration over the ranges essential for many of the experiments described in this paper.   This method of estimating ^{3}H-labelled DNAs was therefore considered adequate.

TABLE 1

The effect of concentration, denaturation and shearing on the precipitation and recovery of ^{3}H-labelled DNA

	Final DNA concentrations[a]			Mean cpm ± standard error of means
	55 μg/ml	17 μg/ml	1·5 μg/ml	
Sonicated DNA[b]	7232	7848	7774	7618 ± 206
Unsonicated DNA	7508	8022	8164	7898 ± 190
Single-stranded DNA	7650	7908	7575	7711 ± 115
Double-stranded DNA	7390	7962	8363	7905 ± 261
Means cpm ± standard error of means	7423 ± 246	7935 ± 213	7969 ± 225	

[a] Concentrations of DNA before addition of 100 to 150 μg bovine serum albumin and TCA to a concentration of 5 per cent.

[b] Mean molecular weights of unsonicated and sonicated DNAs were 860,000 and 82,500 daltons respectively.

(v) *DNA melting curves*

Optical melting curves for DNA eluted from hydroxyapatite or after S_1 nuclease digestion were carried out in 0·12-M PB in a 1-cm light path cuvette heated in the spectrophotometer cell holder by circulating ethylene glycol.   The temperature was monitored by a thermistor probe in an identical adjacent cell.   All samples to be compared were dialysed into the same batch of 0·12-M PB before melting.

(vi) S_1 *nuclease digestion*

S_1 nuclease was prepared from " Takadiastase " (Koch Light Ltd) by the method of Sutton (1971) and stored in 50 per cent glycerol at $-20°$C. I microlitre of the solution contained 4·6 units of activity where 1 unit degrades 1 μg single-stranded DNA to acid soluble material at 25°C in 60 minutes (Shishido and Ando, 1972).

Treatment of samples of renatured DNA with S_1 nuclease was carried out as follows: after renaturation in 0·12-M PB at 60°C four volumes of cold 0·3-M NaCl and five volumes of assay buffer (Davidson *et al.*, 1973a) were added, the resultant pH being 4·5.   After equilibration to 37°C S_1 nuclease

was added and incubation continued for 90 minutes. The appropriate S_1 concentrations and incubation times were determined in preliminary experiments. The reaction was terminated by rapid cooling and the addition of 0·5 vol 0·12-M PB followed by gentle shaking with an equal volume of chloroform containing 1 per cent octanol. The aqueous layer was separated by centrifugation and applied to a hydroxyapatite column equilibrated with 0·03-M phosphate at room temperature. After washing the column with 0·03-M PB, 0·12-M PB at room temperature and 0·12-M PB at 60°C, the S_1 nuclease resistant DNA duplexes were recovered by elution with 0·5-M PB at 60°C. In control experiments 97 per cent of native DNA but only 4 per cent of denatured wheat DNA were recovered in the 0·5-M PB fraction after S_1 nuclease treatment. The 4 per cent S_1 nuclease-resistant DNA is believed to be the fraction of the genome which reanneals virtually instantaneously after denaturation (see fig. 5; Shishido and Ikeda, 1970; Smith and Flavell, 1975).

3. RESULTS

In our previous studies the renaturation kinetics of wheat DNA were investigated using hydroxyapatite chromatography to separate renatured and denatured DNA (Smith and Flavell, 1975). Estimates of the proportions of intermediate reannealing repeated sequence and non-repeated sequence DNAs using hydroxyapatite are dependent upon the linear sequence organisation in the chromosomes. If the non-repeated sequences are short and interspersed between repeated sequences, then even with DNA fragments around 400 nucleotides long, a significant proportion of the non-repeated sequence DNA will be present on duplex-forming fragments when only repeated sequences are allowed to reanneal (Davidson et al., 1973a, b; Graham et al., 1974). We therefore carried out experiments to investigate the inclusion of non-repeated sequence DNA in the renatured repeated sequence DNA fraction from which conclusions about the size and organisation of non-repeated sequences can be drawn.

(i) *The size and organisation of non-repeated sequences—the effect of fragment length on the proportion of DNA in the* in vitro *renatured repeated sequence DNA fraction*

Our studies on sequence interspersion are based upon the different renaturation kinetics of repeated and non-repeated sequences. Renaturation kinetics of denatured sheared wheat DNA indicate that almost all the repeated sequence DNA is in the duplex fraction upon incubation to C_0t 50 while most of the non-repeated sequences do not begin to reanneal until a C_0t of 500 (Smith and Flavell, 1975). To test whether single or few copy sequences are interspersed with repeated sequences in wheat chromosomes, DNA samples of average single-strand fragment sizes ranging from 200 to 6000 nucleotides were denatured and incubated to C_0t 50. The proportion of the DNA binding to hydroxyapatite in 0·12-M PB (phosphate buffer, 0·18-M Na+), *i.e.* the proportion of fragments containing renatured duplex DNA, is shown in fig. 1 for each average fragment size.

The dependence on fragment size of the proportion of DNA renaturing by C_0t 92 was also studied using ^{3}H-labelled DNA fragments with average sizes ranging from 150 to 8800 nucleotides. In these experiments the
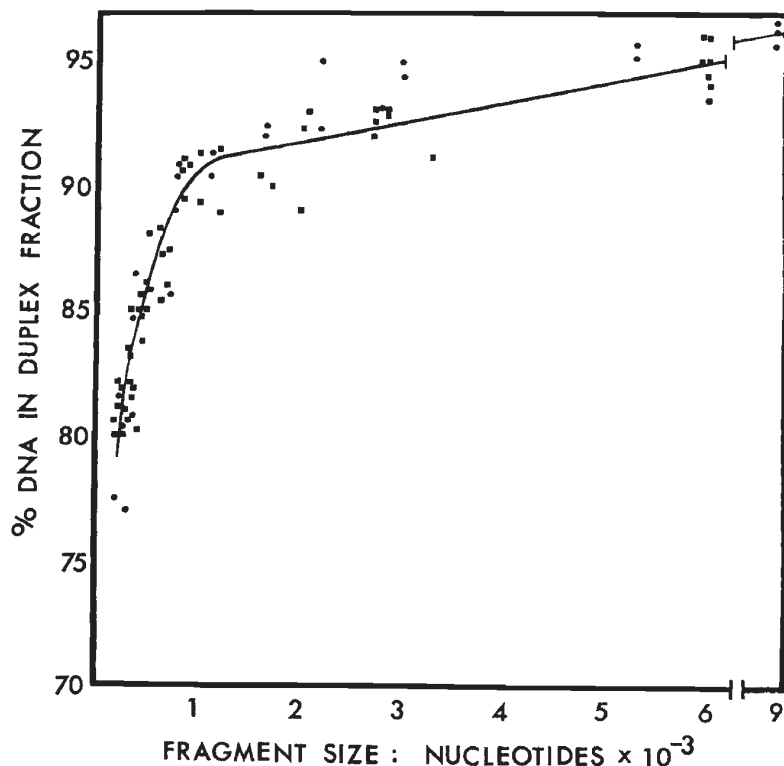
Fig. 1.—The effect of fragment length on the proportion of DNA in the *in vitro* renatured
repeated sequence DNA fraction.

(*a*) With unlabelled DNAs of different sizes (■). Aliquots of unlabelled DNA at 300 μg/
ml were sonicated in 0·12-M phosphate buffer to yield samples with average denatured
fragment sizes from 200 to 6000 nucleotides. Aliquots of each sample were denatured,
incubated at 60°C to C_0t 50 and the proportion in the renatured DNA fraction determined by
hydroxyapatite chromatography. In separate experiments (unpublished), the proportion of
DNA in the renatured DNA fraction between C_0t 20 and 200 was examined using various
sizes of fragments. For a given fragment size, the increase in the renatured DNA fraction
between C_0t 20 and 200 was less than 5 per cent. Thus even though different size fragments
renature at different rates (Wetmur and Davidson, 1968) and thus effectively to a different
C_0t value, this has little effect on the results presented in this figure.

(*b*) With labelled DNAs of different sizes (●). Tritium-labelled DNAs of average
fragment sizes from 150 to 8800 nucleotides were added to aliquots of unlabelled DNA
(400 μg/ml) in 0·12-M phosphate buffer, average fragment size 460 nucleotides. The ratios
of labelled to unlabelled DNA were between 1:750 and 1:3000. After melting and incuba-
ting to C_0t 92 at 60°C the proportions of ^{3}H-labelled DNA in the renatured DNA fractions
were determined by hydroxyapatite chromatography. The proportions of ^{3}H-labelled DNA
are corrected for the DNA binding to hydroxyapatite immediately after denaturation
(Davidson *et al.*, 1973*b*).

labelled DNA was incubated with a 750- to 3000-fold excess of unlabelled
" driver " DNA of average fragment size of approximately 460 nucleotides.
The rationale behind this experimental design is described by Davidson
et al. (1973*b*). The proportion of each size of labelled DNA involved in
duplex formation with unlabelled " driver " DNA was estimated by hydroxy-
apatite chromatography. These results are also shown in fig. 1. The results

of both kinds of experiments are essentially the same; the proportion of DNA in the renatured fraction increases considerably more rapidly with fragments shorter than approximately 1000 nucleotides than with fragments longer than 1000 nucleotides.

An increase in the proportion of renatured DNA, retained by hydroxy-apatite at 0·12-M phosphate, with increasing DNA fragment size is due to single-stranded non-repeated sequence DNA attached to reannealed repeated sequence DNA (Davidson *et al.*, 1973*b*). The lengths of these single-stranded DNA " tails " increase with starting fragment size. The relatively abrupt change in slope in fig. 1 around 1000 nucleotides indicates that approximately 15 per cent of the wheat genome (estimated from extrapolation to the *y* axis) consists of single or few copy sequences, around 1000 nucleotides long or less, interspersed between repeated sequences which form stable duplex DNA by C_0t 50/92 in 0·18-M Na$^+$ at 60°C. The 2 or 3 per cent of the DNA not retained on hydroxyapatite, even when relatively long fragments (>9000 nucleotides) are incubated to C_0t 50/92, must consist either of long non-repeated sequences distantly spaced from a repeated sequence or of denatured DNA unable to take part in stable duplex formation for some reason.

Davidson *et al.* (1973*b*) have reported that a small fraction (5-8 per cent) of the repeated sequence DNA of *Xenopus* fails to form a stable duplex after one incubation at 60°C in 0·18-M Na$^+$ (perhaps due to spurious involve-ment in a poor duplex structure which cannot bind to hydroxyapatite), but does form a stable duplex on a second incubation with more repeated sequence DNA. If this occurs with wheat DNA, all the DNA might be included in the duplex fraction on hydroxyapatite with fragments longer than 9000 nucleotides and we would have no evidence for very long non-repeated sequences in wheat. The 6 or 7 per cent of additional DNA included in the duplex fraction on hydroxyapatite with average fragment lengths between 1000 and 9000 nucleotides, probably consists of non-repeated sequences several thousand nucleotides long interspersed between repeated sequences.

Extrapolation of the curve in fig. 1 to very small fragments which would consist either of non-repeated or repeated sequences but not both, indicates that approximately 75 per cent of the wheat genome consists of repeated sequences able to form stable duplexes at 60°C in 0·18-M Na$^+$ by C_0t 50.

(ii) *The size and organisation of repeated sequences*

If repeated sequences in a genome are relatively short, their length can be estimated by determining the continuous lengths of duplex DNA that are present after renaturation of long fragments *in vitro* (Davidson *et al.*, 1973*a*). Discontinuities in duplex DNA arise when renatured repeated sequences, which are substantially shorter than the fragments, are adjacent to non-repeated or different repeated sequences in the genome. Such single-stranded discontinuities are illustrated in fig. 2A, C, D, E and F.

Single stranded DNA associated with renatured repeated sequence DNA fragments is degraded by the single-stranded DNA nuclease S$_1$ from *Aspergillus* (Ando, 1966; Sutton, 1971; Graham *et al.*, 1974). However, the degree to which this enzyme recognises mismatched bases in reannealed
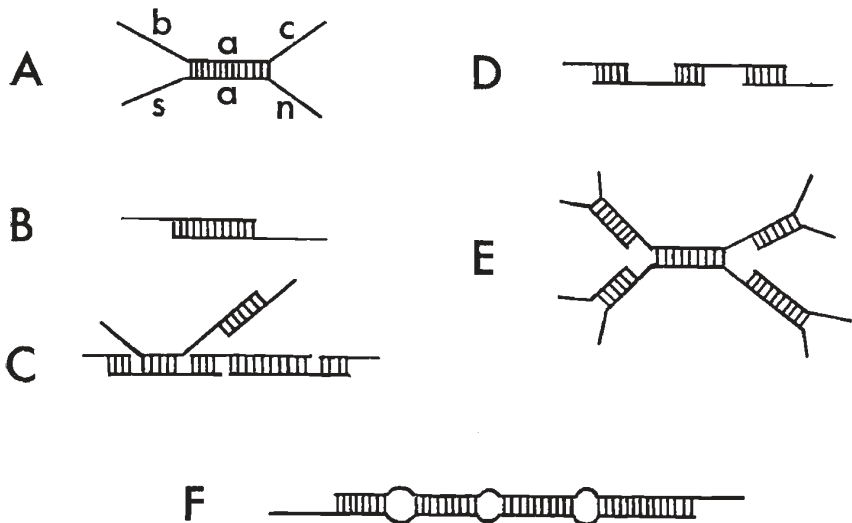
Fig. 2.—Different kinds of renatured DNA configurations. A, A duplex with four unrenatured tails which would form when renatured repeated sequences labelled *a* came from different places in the genome and had different neighbouring sequences; *b* and *c* on one fragment and *s* and *n* on the other fragment. B, A duplex with two unrenatured tails. This duplex would form when the fragments were portions of much longer homologous sequences. C, A multifragment duplex showing how branched structures can be formed in a predominantly linear arrangement. The hybridisations have occurred such that some of the single strand gaps are very small. D, A multifragment duplex where hybridisations have occurred leaving large single-stranded gaps. E, A multifragment duplex involving hybridisations between sequences on long fragments which are not homologous throughout their entire length. F, A duplex formed between long fragments which are only partially homologous. The non-homology could have arisen as a result of mutations within a long cluster of sequences which were originally identical.

repeated sequences, in addition to single stranded regions, is dependent upon the salt concentration and temperature of the assay mixture. We therefore chose 0·15-M Na$^+$ and 37°C as incubation conditions since in these conditions only single-stranded or grossly mismatched regions are degraded (Davidson *et al.*, 1973a). Most of the reannealed duplexes with a moderate degree of mismatching are left intact (Bonner, 1974).

DNA samples were sheared to several different average fragment sizes, denatured and incubated to C_0t 50 in 0·12-M PB at 60°C and then half of each sample was incubated with S_1 nuclease. Double-stranded duplexes were recovered from the S_1 nuclease treated and untreated samples by hydroxyapatite chromatography. Independent of the initial fragment size, the proportion of the genome that renatured to form S_1 nuclease resistant duplexes was 55 ± 2 per cent. This is consistent with the increasing proportion of DNA included in the renatured DNA fraction with increasing fragment size being single stranded DNA (see fig. 1). Optical melting curves of the S_1 resistant duplexes were indistinguishable, irrespective of the starting average fragment size of the DNA molecules. The hyperchromicities of the S_1 resistant duplexes were approximately 21 per cent of the final optical density after melting. Native DNA hyperchromicity was 25 per cent. Since the T_m of the duplexes was 10°C below that of native

DNA, the duplexes were mismatched by about 6 per cent on average (Ullman and McCarthy, 1973).

The results in fig. 1, as indicated earlier, show that some 75 per cent of wheat DNA is repeated sequence DNA. However, since only 55 per cent of the DNA is resistant to S_1 nuclease after renaturation to C_0t 50, approximately 20 per cent of the repeated sequence DNA must have been degraded by S_1 nuclease after renaturation to C_0t 50. Much of the degraded repeated sequence DNA was probably unrenatured " tails " of duplexes. When randomly sheared fragments collide and nucleation occurs *in vitro*, on average less than two-thirds of each fragment is base paired; the rest remains as denatured " tails ", as illustrated in fig. 2B, and is degraded by S_1 nuclease (Smith *et al.*, 1975). Where the renaturing repeated sequence length is considerably shorter than the average fragment length, as in experiments with many animal DNAs, the single-stranded S_1 susceptible DNA " tails " consist mainly of non-repeated sequence DNA which is interspersed with most of the repeated sequence DNA (Davidson *et al.*, 1973a; Graham *et al.*, 1974). Thus in these situations not much of the repeated sequence DNA is degraded by S_1 nuclease after repeated sequence DNA renaturation. However, with genomes where long regions of repeated sequence DNA occur, as in wheat, many of the " tails " of primary duplexes must consist of repeated as well as non-repeated sequence DNA. Repeated sequence DNA will thus be degraded by S_1 nuclease. Some secondary nucleations involving repeated sequence " tails " do occur with wheat DNA incubated to C_0t 50 (see later), protecting the " tails " from S_1 nuclease digestion, as illustrated in fig. 2E, but these are insufficient to involve all the repeated sequence " tails " of the primary duplexes unless very long fragments are used. Furthermore, all of each " tail " is not always protected from S_1 nuclease by secondary nucleations. This explanation of the digestion of repeated sequence DNA by S_1 nuclease is supported by the observation that 15 to 20 per cent of renatured *E. coli* DNA binding to hydroxyapatite at 0·12-M phosphate is degraded by S_1 nuclease under similar conditions.

Although much of the S_1 nuclease degraded wheat repeated sequence DNA was probably unrenatured " tails " of duplexes, some also consisted of very mismatched duplexes formed between diverged repeated sequences, since the melting curves of S_1 resistant duplexes show less DNA melting between 60° and 65°C.

The average size of the S_1 nuclease resistant wheat duplexes was determined by velocity sedimentation in a number of experiments. Aliquots of denatured DNA, sonicated to different lengths, were incubated to C_0t 50 at 60°C in 0·18-M Na^+ and treated with S_1 nuclease. The double-stranded DNA was dialysed to 1-M NaCl and run in the analytical ultracentrifuge. The size of the S_1 nuclease resistant duplexes of *Xenopus* and *E. coli* DNAs, treated in an identical way, were also studied in parallel experiments to serve as comparative controls. *Xenopus* DNA has previously been characterised by Davidson *el al.* (1973a, b). The weight average denatured fragment sizes of the incubated DNAs were determined after denaturation and incubation to C_0t 50, to take account of any single-strand breakage during denaturation and/or incubation at 60°C. The results for wheat, *E. coli* and *Xenopus* DNAs are shown in fig. 3.

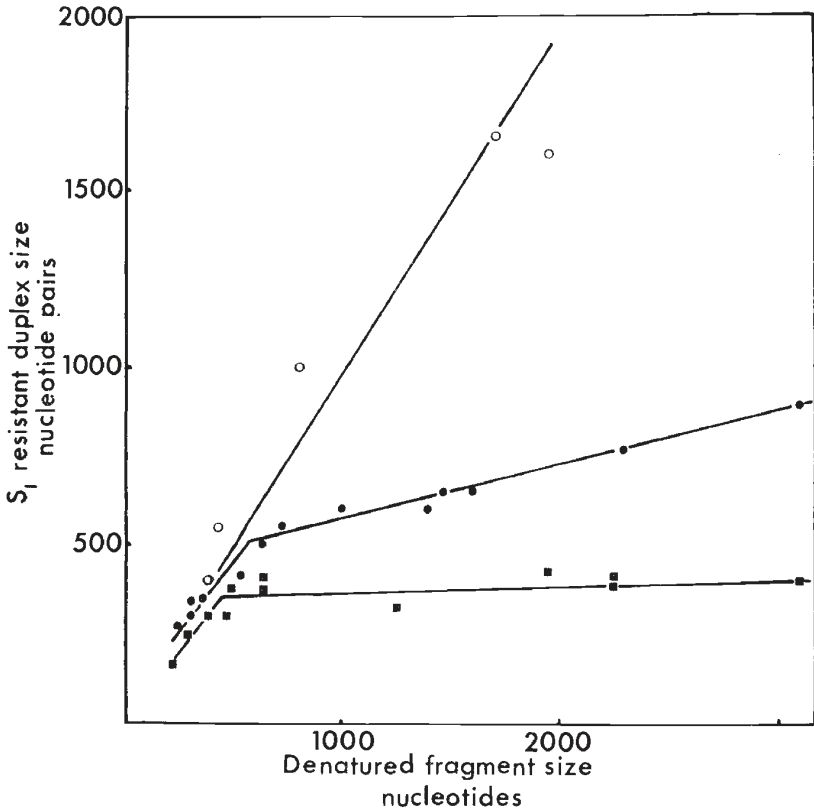With *E. coli* DNA, the average S_1 nuclease resistant duplex size after

FIG. 3.—The relationships between the weight average size of the S_1 nuclease resistant
duplexes and the weight average single-strand fragment size present during duplex
formation.   Samples of wheat, *Xenopus* and *E. coli* DNAs, 370 μg/ml in 0·12-M phosphate
buffer, were sonicated to various average fragment sizes.   After denaturation and
incubation at 60°C in 0·12-M phosphate buffer to C_0t 50 (wheat and *Xenopus*) or C_0t 119
(*E. coli*) half of each sample was used to gain the average single-stranded fragment sizes
present during duplex formation.   The other half was treated with S_1 nuclease, the
S_1 nuclease resistant duplexes recovered by hydroxyapatite chromatography, dialysed
extensively against 1-M NaCl and the average size of the duplexes determined in the
analytical ultracentrifuge.   ● Wheat.   ▦ *Xenopus*.   ○ *E. coli*.

renaturation of over 90 per cent of the DNA was similar to the starting
fragment size over the whole range of fragment sizes studied.   However,
on denaturation the S_1 nuclease resistant duplexes yielded fragments con-
siderably smaller than the starting fragment size.   For example, in an
experiment where fragments on average 2000 nucleotides long were in-
cubated to a C_0t of 119, the S_1 nuclease resistant duplexes had an average
length of 1625 nucleotide pairs.   After alkali denaturation these duplexes
yielded fragments with an average length of 880 nucleotides.   Thus many
of the S_1 nuclease resistant duplexes in the renatured DNA must be con-
structed of more than two single-stranded fragments and have some single-
strand gaps within the double helices that are too short to be recognised
by S_1 nuclease under our assay conditions.   Such a duplex structure is
illustrated in fig. 2c.   It is formed from multiple hybridisation events.

When larger single-strand gaps occur in multifragment duplex structures (see fig. 2D) or when there are only two fragments per duplex (see fig. 2A), S_1 nuclease would degrade the single-stranded regions leaving the duplexes shorter than the starting single-stranded fragments. Therefore, where the average S_1 nuclease resistant duplex length was found to be similar to or greater than the average starting fragment length in fig. 3, the reductions in duplex length caused by S_1 nuclease were presumably compensated by the presence of longer S_1 nuclease resistant duplexes involving more than two fragments, as illustrated in fig. 2c.

Whereas the average size of the S_1 nuclease resistant duplexes was similar to the starting fragment size over the whole range of fragment sizes studied in *E. coli*, with *Xenopus* DNA this was so only up to a weight average duplex size of around 350 nucleotide pairs. This is what could be expected from the known differences in sequence organisation between *E. coli* and *Xenopus*. Approximately 55 per cent of the *Xenopus* genome consists of short non-repeated sequences alternating with repeated sequences about 300 nucleotides long and a further 35 per cent consists of very long non-repeated sequences (Davidson *et al.*, 1973*a*, *b*). With this alternating sequence organisation most of the duplex regions would be expected to be only 300 nucleotide pairs long after renaturation of 2000 nucleotide long fragments to C_0t 50, which allows only repeated sequences to reanneal. The single copy sequences separating the short repeated sequences should remain denatured and therefore be digestible by S_1 nuclease.

The size distribution of *Xenopus* S_1 nuclease resistant duplexes after renaturation of 3100 and 2200 nucleotide long fragments is shown in fig. 4. This distribution was derived by the method of Schumaker and Schachman (1957) from the sedimentation behaviour of the duplexes in the analytical ultracentrifuge. As expected, most of the duplexes (20 per cent of the genome) were between 300 and 500 nucleotides long although some (about 6 per cent of the genome) were much longer. These results are in good agreement, therefore, with those expected from the chromosome model of Davidson *et al.* (1973*a*, *b*).

With wheat DNA the weight average S_1 nuclease resistant duplex size was similar to the weight average size of the starting denatured DNA fragments up to a length of 500 nucleotides, *i.e.* longer than in *Xenopus*. The size distribution of S_1 nuclease resistant duplexes after renaturation of wheat DNA fragments around 2900 nucleotides to C_0t 50, is shown in fig. 4. Most of the duplexes are between 400 and 800 nucleotides long. Together, the results for wheat DNA in figs. 3 and 4 imply that after renaturation of long single-stranded DNA molecules to C_0t 50, effectively single-stranded regions occur at each end of reasonably well-matched duplex regions, a large proportion of which are between 400 and 800 nucleotides long. This is what would be expected, based on the interpretation of the *Xenopus* results, if much of the S_1 nuclease resistant fraction of renatured wheat DNA consisted of repeated sequences between 400 and 800 nucleotides long. However, repeated sequences of this length could not always be separated by non-repeated sequence DNA as in *Xenopus*, since in wheat we have shown above that only 15 to 20 per cent non-repeated sequence DNA is interspersed in 75 per cent repeated sequence DNA. Furthermore, we have illustrated with *E. coli* DNA (and with small wheat and *Xenopus* DNA fragments) that where fragments consist entirely of
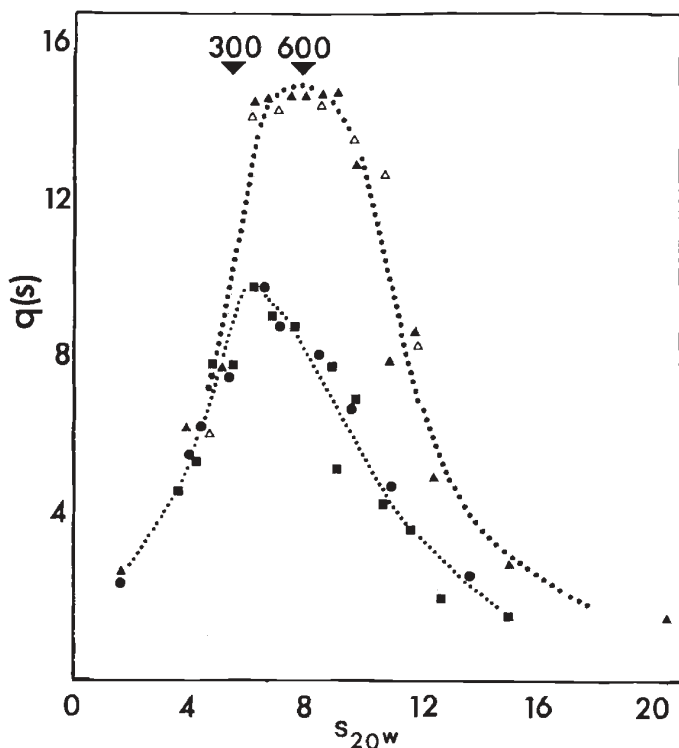
Fɪɢ. 4.—Distribution of sizes of S_1 nuclease resistant duplexes formed *in vitro* between repeated sequences. Results are from two different experiments. Distributions were calculated from the boundary sedimentation profiles in the analytical ultracentrifuge by the method of Schumaker and Schachman (1957). Average single-stranded fragment size before denaturation and incubation were 3100 (●) and 2200 (■) nucleotides for *Xenopus* and 3000 (△) and 2800 (▲) nucleotides for wheat.

$$q(s) = \frac{100}{C_{\text{obs}}} \times \frac{dC_{\text{obs}}}{dS_{20}W},$$

where C_{obs} = observed concentration (see Schumaker and Schachman, 1957). Arrows indicate duplex lengths in nucleotide pairs.

sequences able to renature under the incubation conditions, S_1 nuclease resistant duplexes, as long or longer than the starting fragments, form readily. Why then are nearly all wheat DNA duplexes cleaved by S_1 nuclease to give duplexes between 400 and 800 nucleotides long after incubation of fragments with an average starting size of around 2900 nucleotides, many of which must consist entirely of repeated sequence DNA?

We suggest that the high number of S_1 nuclease sensitive sites in re-annealed wheat duplexes is due to closely related repeated sequences having different neighbouring sequences at different places in the genome. This would lead on renaturation of long fragments either to duplexes with four " tails ", as illustrated in fig. 2, A and E, if the neighbouring sequences were entirely unrelated or, if the neighbouring sequences were partially related, to duplexes with regions of extensive base mismatching as illustrated in fig. 2, F. The " tails " in fig. 2, A, the branch nodes in fig. 2, E and the mismatched regions in fig. 2, F would be sensitive to S_1 nuclease.

The reduction in slope of the wheat DNA curve above 500 nucleotides to 15 per cent of the slope with starting fragments shorter than 500 nucleotides in fig. 3, implies that most of the DNA fragments are cleaved by S_1 nuclease after renaturation. This suggests that the interspersion of short non-repeated sequences and of different kinds of repeated sequences occurs throughout most of the chromosomes. However, the presence of a 15 per cent slope implies that some repeated sequences renature to form S_1 nuclease resistant duplexes much longer than 800 nucleotide pairs. We estimate from the size distribution curve of S_1 nuclease resistant wheat duplexes in fig. 4 that about 20 per cent of the duplex DNA is considerably longer than 800 nucleotide pairs. The DNA in this distribution curve represents 55 per cent of the genome. We therefore estimate that approximately 10 per cent of the wheat genome consists of repeated sequences which renature to form long S_1 nuclease resistant duplexes. (The 20 per cent repeated sequence DNA in the genome which is degraded by S_1 nuclease after *in vitro* duplex formation (see above), is more likely to consist of the short interspersed repeated sequences than of the long repeated sequences.) The long duplexes in fig. 4 presumably consist either of repeated sequences several thousand nucleotides long or of short repeated sequences, arranged in the chromosomes such that multifragment duplexes with few S_1 nuclease digestible regions can form *in vitro*.

(iii) *Binding of denatured wheat DNA to hydroxyapatite*

Wheat DNA, in common with DNA from many other higher organisms (Wilson and Thomas, 1974; Bonner, 1974; Cech and Hearst, 1975), includes a fraction which renatures immediately after denaturation, even at very dilute DNA concentrations. We have already published some experiments describing this wheat fraction (Smith and Flavell, 1975). Recently we have re-studied the proportion of ^{3}H-labelled wheat DNA of different average fragment lengths retained by hydroxyapatite as duplex DNA in the presence of 25 μg/ml calf thymus DNA after denaturation by boiling or by alkali treatment and immediately forcing through hydroxyapatite. The estimated C_0t value for the wheat DNAs was less than 2×10^{-7}. These experiments differ from those previously reported since the wheat DNA concentration was much lower in the experiments reported here.

The relationship between the proportion of DNA retained by hydroxyapatite as duplex DNA and the average fragment size is shown in fig. 5. The increase in DNA in the duplex fraction is approximately linear with increasing fragment size and the line intersects the y axis at about 4 per cent.

DNA binding to hydroxyapatite immediately after denaturing has been found in several other organisms to contain " hairpin " structures which form due to inverted duplications (palindromes) being present on the same fragment (Wilson and Thomas, 1974; Bonner, 1974). In wheat also, most of the 4 per cent instantly renatured fraction could consist of such hairpin structures (Huguet, Jouanin and Bazetoux, 1975). The additional DNA associated with these structures in long fragments probably exists as single stranded " tails ". This is supported by the very low hyperchromicity of such DNA and the fact that it is degraded by S_1 nuclease (Smith and Flavell, 1975).
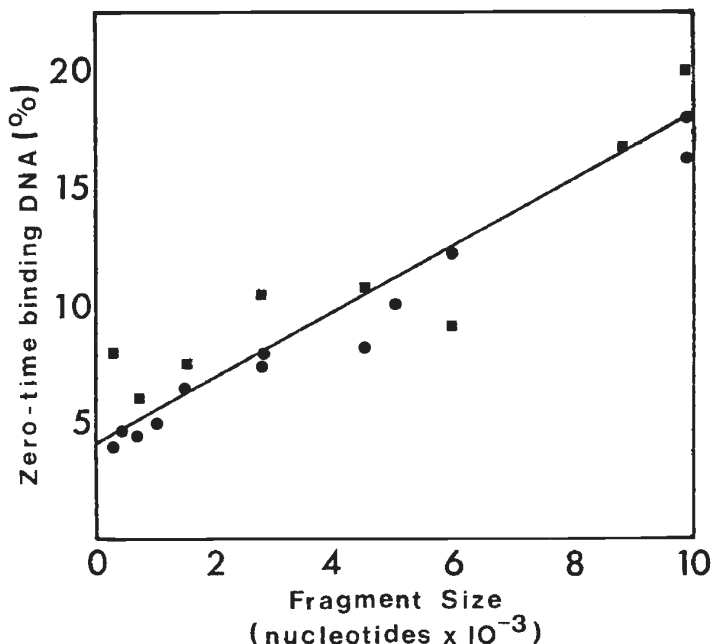
FIG. 5.—The proportion of denatured wheat DNAs of different average fragment sizes behaving on hydroxyapatite as duplex DNA. Two methods of DNA denaturation were used: ■ heat denaturation; ● alkali denaturation. In heat denaturation experiments, ^{3}H-labelled wheat DNAs of various average single stranded fragment sizes ranging from < 200 to > 10,000 nucleotides were mixed with calf thymus DNA (average single stranded fragment size = 300 nucleotides) in 0·12-M phosphate buffer such that the final concentration of labelled DNA was 0·025 µg/ml and of unlabelled DNA was 25 µg/ml. 2 ml aliquots were heated to 100°C for 3 minutes and immediately passed through hydroxyapatite equilibrated with 0·12-M phosphate buffer at 60°C. After rapid washes with 0·12-M phosphate buffer at 60°C the renatured DNA was recovered by elution with 0·5-M phosphate buffer. In alkali denaturation experiments ^{3}H-labelled wheat DNAs of various fragment sizes were mixed with calf thymus DNA to give final concentrations of labelled DNA of 0·02 µg/ml and of unlabelled DNA to 20 µg/ml. 2·5 ml aliquots were heated to 60°C and mixed with 0·11 vols of 1-M NaOH. After 10 minutes the solution was neutralised with 0·55 vols of 0·318-M NaH$_2$PO$_4$ at 60°C and immediately fractionated on hydroxyapatite as described above. The incubation times before fractionation in these experiments were estimated to produce C_0t values for the wheat DNA of less than 2×10^{-7}.

From the theory described by Hamer and Thomas (1974) the results in fig. 5 indicate that the instantly renaturing sequences in wheat appear to be clustered into groups, as in some other organisms (Wilson and Thomas, 1974), with the groups distributed throughout at least 20 per cent of the genome. However, we have no more direct evidence at present for clustering of the instantly renaturing sequences.

(iv) *The arrangement of diverged repeated and non-repeated sequences between non-diverged repeated sequences*

From the increasing proportion of renatured wheat DNA bound to hydroxyapatite with increasing fragment size after incubation to C_0t 50/92 (fig. 1) it was concluded that approximately 75 per cent of the genome

consists of repeated sequences. These repeated sequences form duplexes *in vitro* with a range of thermal stabilities (Smith and Flavell, 1974, 1975), indicating that some repeated sequences are identical, or nearly so, and therefore form very stable duplexes while other repeated sequences are less closely related and form less stable duplexes. For convenience we have called identical or nearly identical repeated sequences " non-diverged repeated sequences " and the less closely related repeated sequences " diverged repeated sequences ". These terms were chosen on the assumption that all sequences sufficiently related to form stable duplexes at 60°C in 0·18-M Na^+ have originated from the same ancestral sequence.

To investigate the size and arrangement of the diverged and non-diverged repeated sequences, denatured wheat DNA was incubated in 62 per cent formamide 0·69-M NaCl at 42·5°C. These incubation conditions are considerably more stringent than 0·18-M Na^+ at 60°C. They approximate to 80°C in 0·18-M Na^+ (McConaughty *et al.*, 1969; Bendich and McCarthy, 1970) and allow only identical or nearly identical sequences to reanneal. A 700- to 3000-fold excess of denatured, unlabelled DNA, of average fragment size 300 nucleotides, was incubated to C_0t 90 with ^{3}H-labelled DNA of average fragment sizes ranging from 180 to $> 12,000$ nucleotides. The renatured DNA fractions were separated on hydroxy-apatite in 0·12-M PB at 80°C although similar results are obtained if the fractionation is carried out at 60°C. The proportion of ^{3}H-labelled DNA included in the renatured DNA fraction with each fragment size is shown in fig. 6. The proportion increased from approximately 40 per cent with fragments 200 nucleotides long to 85 per cent with fragments 4000 nucleotides long. With fragments longer than 4000 nucleotides there was very little increase in the proportion of the genome included in the duplex fraction on hydroxyapatite.

Incubation of different sized *E. coli* DNA fragments under the high stringency conditions to a C_0t of 90 and fractionation on hydroxyapatite at 80°C produced quite different results (see fig. 6). There was very little decrease in the proportion of DNA in the duplex fraction on hydroxyapatite with short fragments. This is what would be expected with DNA which renatures to form duplexes with virtually no mismatched bases. Such duplexes are stable at 80°C in 0·18-M Na^+.

Thirty-two per cent of the wheat DNA was found to be resistant to S_1 nuclease after renaturation of long fragments (4000 nucleotides) in 62 per cent formamide 0·69-M NaCl at 42·5°C. This value agrees with the value gained from extrapolation of the curve in fig. 6 to the y axis indicating that the duplexes forming under these conditions are short compared with the starting fragment length of 4000 nucleotides (see earlier). Thus approximately 32 per cent of the wheat genome consists of repeated sequence regions able to form perfect or nearly perfect duplexes after reannealing *in vitro* and these regions are distributed throughout 85 per cent of the genome at intervals of less than 4000 nucleotides. That the additional 53 per cent associated with the reannealed DNA with long fragments consists of single-stranded tails is confirmed by the finding that it is completely digested by S_1 nuclease. Comparison of figs. 1 and 6 indicates that approximately 43 per cent (75-32) of the wheat genome consists of repeated sequence DNA sufficiently diverged to renature as non-repeated sequence DNA in 62 per cent formamide 0·69-M NaCl at 42·5°C.
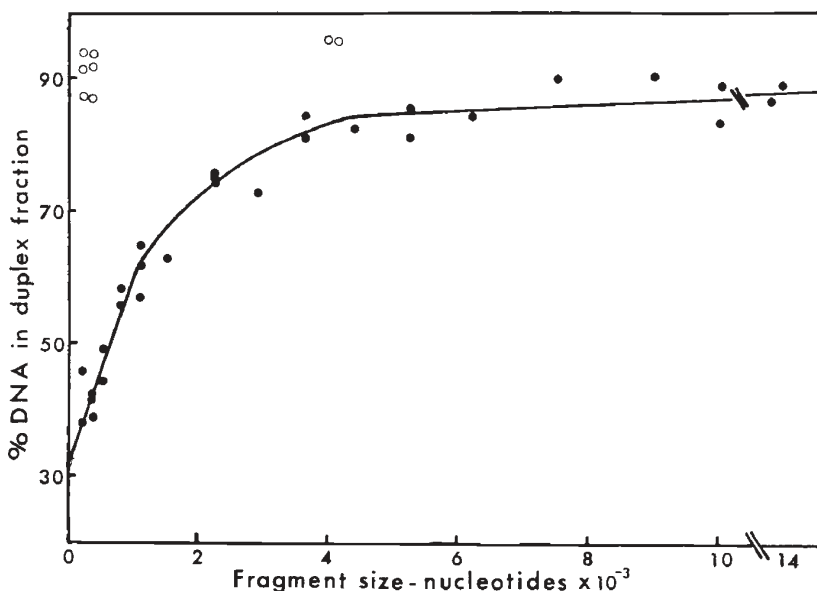
Fig. 6.—The dependence on fragment size of the proportion of DNA included in the re-
natured DNA fraction on hydroxyapatite after incubation to C_0t 90 in 62 per cent
formamide 0·69-M NaCl at 42·5°C. Tritium-labelled wheat DNAs of average single-
stranded sizes from 180 to > 12,000 nucleotides were added to aliquots of unlabelled
wheat DNAs (average single stranded fragment size = 300 nucleotides) to give solutions
containing 62 per cent formamide, 0·69-M NaCl and ratios of labelled to unlabelled
DNAs of 1:700 to 1:3000. Tritium-labelled *E. coli* DNAs of average fragment sizes of
220, 360, 4000 and 4150 nucleotides were also mixed with a 1000-fold excess of un-
labelled *E. coli* DNA (average single stranded fragment size = 220 nucleotides) to give
solutions containing 62 per cent formamide 0·69-M NaCl. After denaturation by heating
to 85°C for 3 minutes the DNA solutions were incubated at 42·5°C to a C_0t of 90. At
this C_0t value essentially all the *E. coli* DNA and all the wheat repeated sequence DNA
able to reanneal under these conditions is included in the hydroxyapatite duplex
fraction while wheat single copy DNA does not reanneal until a C_0t value in excess of
500 (unpublished results and Bendich and McCarthy, 1970). After incubation, the
samples were diluted with 0·03-M phosphate buffer at 3°C and fractionated on hydroxy-
apatite; renatured DNA was separated from single stranded DNA at 80°C in 0·12-M
phosphate buffer. ● Wheat. ○ *E. coli*. The point where the extrapolated wheat
curve reaches the *y* axis (32 per cent) is that fraction of the wheat DNA that is resistant
to S_1 nuclease after incubation in these conditions.

After renaturation of 4000 nucleotide long fragments at 60°C in 0·18-M
Na⁺, approximately 95 per cent of the genome was included in the duplex
fraction on hydroxyapatite (fig. 1), while after renaturation of similar size
fragments in 62 per cent formamide 0·69-M NaCl at 42·5°C approximately
85 per cent of the genome was included in the duplex fraction. The 10 per
cent of the genome not included after incubation and fractionation in the
more stringent conditions probably consists of sequences located thousands
of nucleotides from sequences able to form stable duplexes at 80°C in 0·18-M
Na⁺. Alternatively this 10 per cent could represent a fraction of repeated
sequences which did not form a stable duplex during the single incubation
but would on a second incubation with more repeated sequences (see
Davidson *et al.*, 1973*b*).

Linear extrapolation of the central portion of the curve in fig. 6 involving 1000 to 4000 nucleotide long fragments, gives an intercept on the y axis of about 50 per cent.   From this comes the estimate that about 18 per cent of the wheat genome (50-32 per cent) consists of sequences approximately 1000 nucleotides long which reside between repeated sequences able to form near-perfect duplexes *in vitro*.   These 1000 nucleotide long sequences must be either non-repeated sequences or diverged repeated sequences interspersed between repeated sequences (see fig. 1).   From other experiments, which we will describe in detail elsewhere, it appears that at least some of the non-repeated sequences, 1000 nucleotides long or less, lie adjacent to the repeated sequences able to form perfect duplexes *in vitro*.

The additional DNA (32 per cent) included in the duplex fraction with DNA fragments between 1000 and 4000 nucleotides long after incubation under the high stringency conditions (see fig. 6) must consist of sequences 2000 to 4000 nucleotides long which fail to form well-matched duplexes *in vitro*, but which reside between repeated sequences able to form near-perfect duplexes *in vitro*.

(v) *The sizes of the non-diverged repeated sequences*

Estimates of the sizes of the repeated sequences able to form near-perfect duplexes *in vitro* were gained by incubating denatured DNA, with an average fragment size of 2250 nucleotides in 62 per cent formamide 0·69-M NaCl at 42·5°C to a C_0t of 90, recovering the S_1 nuclease resistant duplexes and sedimenting the duplexes in the analytical ultracentrifuge. The weight average fragment size was 570 nucleotides and the distribution of sizes derived from an ultracentrifuge trace of the sedimenting duplexes is shown in fig. 7.   We have also included for comparison in fig. 7 a distribution curve of a preparation of rat liver DNA fragments all 380 ± 20 nucleotides long.   Nearly all the wheat S_1 nuclease resistant duplexes formed under the high stringency incubation conditions appear to be between 350 and 650 nucleotides long.   Thus the 30 per cent non-diverged repeated sequences in the wheat genome are nearly all between 350 and 650 nucleotides long. The broader distribution profile of the wheat DNA compared with that of the rat liver DNA indicates there is size heterogeneity in the wheat S_1 nuclease resistant fraction.   However, there does not appear to be a substantial proportion of non-diverged repeated sequence duplexes longer than 800 nucleotide pairs.   From the equivalent distribution curve of total repeated sequence duplexes (fig. 4) it was estimated that 20 per cent were considerably longer than 800 nucleotide pairs.   It therefore appears likely that most of the long-repeated sequences are from families with more sequence heterogeneity or which have a high A-T content and are thus unstable at 80°C in 0·18-M Na$^+$.

4. CONCLUSIONS

We have summarised our findings on the different kinds of sequences in the wheat genome in fig. 8.   The proportion of the wheat genome consisting of repeated sequence DNA is approximately 75 per cent from fig. 1. This is a considerably higher proportion than found in other genomes analysed in similar detail, *e.g. Xenopus* (Davidson *et al.*, 1973*a*, *b*), sea urchin
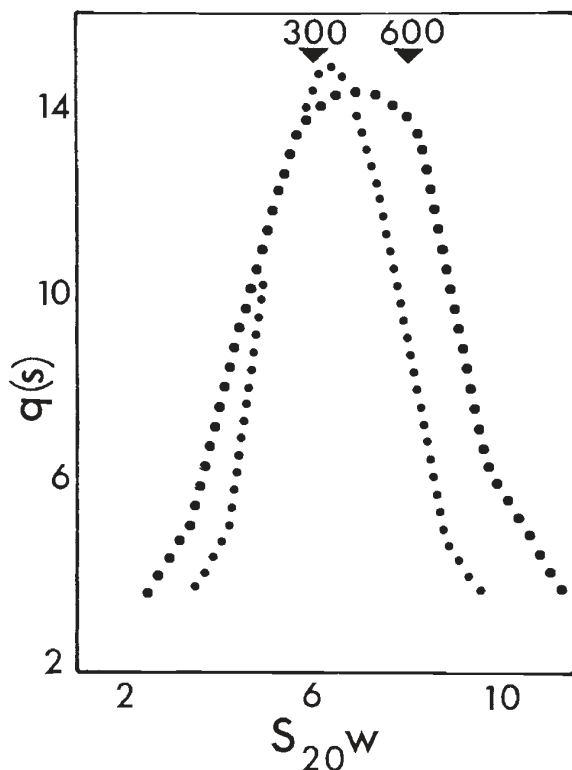
FIG. 7.—Distribution of sizes of S_1 nuclease resistant near-perfect duplexes formed *in vitro* between repeated sequences. ● Wheat S_1 nuclease resistant duplexes. ● Rat liver DNA control consisting of fragments 380 ± 20 nucleotides. This rat liver DNA preparation was a gift from Dr M. Noll, Medical Research Council's Laboratory of Molecular Biology, Cambridge. It was prepared by incubating rat liver chromatin with micrococcal nuclease and isolating the 380 nucleotide DNA fragments formed by preparative electrophoresis on polyacrylamide (see Noll, 1974). Wheat DNA, average fragment size 2250 nucleotides, was incubated in 62 per cent formamide, $0 \cdot 69$-M NaCl at $42 \cdot 5°C$ to a C_0t of 90, treated with S_1 nuclease and the renatured fraction recovered on hydroxy-apatite. The S_1 resistant duplexes were dialysed against 1-M NaCl before being sedimented in 1-M NaCl in the ultracentrifuge. The distributions were calculated from boundary sedimentation profiles of the analytical ultracentrifuge by the method of Schumaker and Schachman (1957). The average denatured fragment size of the wheat DNA after denaturation and incubation, but before S_1 nuclease treatment was 1900 nucleotides.

$$q(s) = \frac{100}{C_{obs}} \times \frac{dC_{obs}}{dS_{20}W},$$

where C_{obs} = observed concentration (see Schumaker and Schachman, 1957). Arrows indicate duplex lengths in nucleotide pairs.

(Graham *et al.*, 1974), *Drosophila melanogaster* (Laird *et al.*, 1973), rat (Bonner *et al.*, 1973) and *Dictyostelium* (Lodish *et al.*, 1973). However, the haploid nuclear DNA content of wheat (6 pg) is larger than those of all these other species. *Xenopus*, for example has a haploid DNA content of $3 \cdot 1$ pg. We have previously reported that large amounts of repeated sequence DNA are common in higher plant species, especially those with higher DNA

contents (Flavell *et al.*, 1974). Straus (1971) has reported large proportions of repeated sequence DNA in amphibians with high DNA contents. Thus large amounts of nuclear DNA in plants appear to have resulted in part from sequence amplification as well as from polyploidy and chromosome duplication (Stebbins, 1971; Flavell *et al.*, 1974).
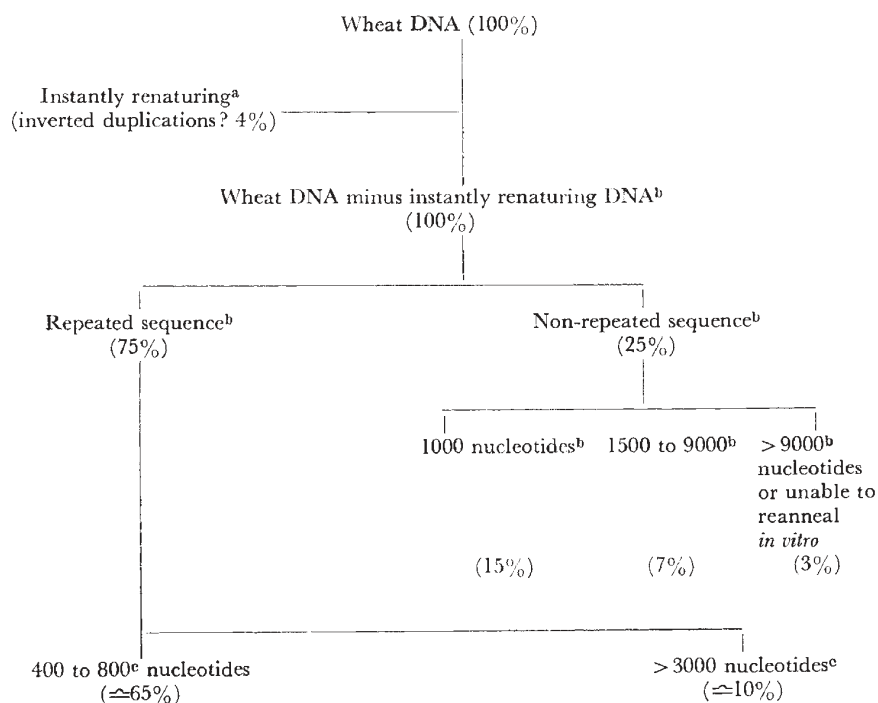
Wheat DNA (100%)

Instantly renaturing[a]
(inverted duplications? 4%)

Wheat DNA minus instantly renaturing DNA[b]
(100%)

Repeated sequence[b]   Non-repeated sequence[b]
(75%)   (25%)

1000 nucleotides[b]   1500 to 9000[b]   >9000[b]
nucleotides
or unable to
reanneal
in vitro
(15%)   (7%)   (3%)

400 to 800[c] nucleotides   >3000 nucleotides[c]
(≃65%)   (≃10%)

Fig. 8.—Summary of wheat genome fractionation. (*a*) Behaves as double-stranded DNA immediately after denaturing. (*b*) Amounts of repeated and non-repeated sequence DNAs are calculated from fig. 1 the results of which are corrected for instantly renaturing DNA. (*c*) These sizes are estimated from the S_1 nuclease resistant duplex size distribution curve in fig. 4. It was assumed that the 20 per cent repeated sequence DNA degraded by S_1 nuclease and therefore not included in the size distribution in fig. 4 consisted of the repeated sequences 400-800 nucleotides long. About half of the 400 to 800 nucleotide long sequences (32 per cent of genome) form essentially perfect duplexes in 62 per cent formamide 0·69-M NaCl at 42·5°C.

(i) *Non-repeated sequence DNA*

Approximately 20 to 25 per cent of the wheat genome behaved as non-repeated sequence DNA in renaturation at 60°C in 0·18-M Na$^+$. Much of this renatures with kinetics expected for sequences present in one or two copies per haploid genome (see Smith and Flavell, 1975). A small amount (3 per cent) may consist of non-repeated sequences located many thousands of nucleotides from a repeated sequence or this fraction may simply reflect repeated sequence DNA fragments unable to form stable duplexes (see Results). However, most of the non-repeated sequence DNA is present on fragments also containing repeated sequences when the average fragment size exceeds 1000 nucleotides. Thus most of the non-repeated sequences

of the wheat genome appear to be around 1000 nucleotides long and inter-
spersed between repeated sequences.

It is not possible to be precise about the size distribution of the inter-
spersed non-repeated sequences from these experiments. The considerable
change in slope in fig. 1 at around 1000 nucleotides certainly indicates a
population of non-repeated sequences with a restricted size distribution
around 1000 nucleotides; more data must be gained from other methods,
e.g. electron microscopy.

The shape of the curve in fig. 1 also suggests that 6 or 7 per cent of the
genome may consist of much larger interspersed non-repeated sequences
up to 9000 or so nucleotides long. In most other organisms examined in
similar detail so far, non-repeated sequences 800 to 2500 nucleotides long,
interspersed between short, *e.g.* 300 nucleotide, repeated sequences are also
common (Davidson *et al.*, 1975). Non-repeated sequences around 800 to
2500 nucleotides long would be the kinds of sequences expected to code for
proteins and this has led to the attractive hypothesis that the functional
unit of all eucaryotic chromosomes consists of a repeated sequence(s)
associated with a non-repeated coding sequence; the repeated sequence
having some role in the control of gene transcription (Britten and Davidson,
1969; Crick, 1971; Paul 1972; Georgiev *et al.*, 1973; Gierer, 1973). Such
a model may be appropriate for wheat, but it would be surprising to us if
most of the large proportion of repeated sequence DNA in the chromosomes
were directly involved in gene regulation.

Although perhaps only 15 per cent of the wheat genome consists of
interspersed non-repeated sequences around 1000 nucleotides long, this
amounts to about 1·0 pg of DNA per haploid genome which is many times
the haploid DNA content of *Drosophila melanogaster*. Thus there is presum-
ably ample DNA in the interspersed non-repeated sequence fraction of
wheat to programme a plant through its life-cycle.

(ii) *Repeated sequence DNA*

Of the 75 per cent repeated sequence DNA it is likely that only a small
proportion (e.g. 10 to 15 per cent) consists of very long repeated sequences or
long clusters of essentially identical repeated sequences, although we have
no good direct estimates of this. After renaturation of 3000 nucleotide long
fragments *in vitro* most of the repeated sequence DNA can be cleaved into
units mostly between 400 and 800 nucleotides long by S_1 nuclease acting
essentially as a single-stranded DNA specific nuclease. We interpret this
to imply interspersion of different repeated sequences in many parts of the
genome with closely related repeated sequences often having different
repeated sequences as neighbours. We cannot distinguish between the
alternatives (1) that the different neighbouring repeated sequences are
entirely unrelated, as implied in fig. 2, E, and (2) that the differing neigh-
bouring sequences are diverged members of the same family of repeated
sequences, as implied in fig. 2, F. However, it is clear that short regions
of non-diverged repeated sequences are interspersed in longer regions of
diverged repeated sequences. We plan to discuss the alternatives of repeated
sequence DNA arrangements more fully in another paper where more
results on the fine structure of the wheat genome gained from a different
approach will be presented (Rimpau, Smith and Flavell, in preparation).

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