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Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer

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Abstract

Ribosomal RNA genes in plants are highly variable both in copy number and in intergenic spacer (IGS) length. This variability exists not only between distantly related species, but among members of the same genus and also among members of the same population of a single species. Analysis of inheritance indicates that copy number change is rapid, occurring even among somatic cells of individual plants, and that up to 90% or more of the gene copies are superfluous. Subrepetitive sequences within the IGS appear to be changing rapidly as well. They are not only variable in sequence from one species to the next, but can vary in number between neighboring gene repeats on the chromosome. In all species examined in detail they are located in the same region of the IGS and contain sequences that can be folded into stem-loop structures flanked by a pyrimidine-rich region. It has been suggested that these subrepeats function in transcriptional enhancement, termination or processing, or in recombination events generating the high multiplicity of ribosomal genes.

Introduction

The nuclear RNA genes (rDNA) in higher plants are arranged in long tandem repeating units, much like those of other higher eukaryotes [5]. The 18S, 5.8S and 25S genes are clustered and are transcribed as one unit, while the 5S genes (which will not be discussed in this article) are located elsewhere in the genome. Between the 3′ end of the 25S gene and the 5′ end of the 18S gene is located a DNA spacer that is from 1 kb to over 12 kb in length for different species (Table 1). The spacer has been most frequently designated the "non-transcribed spacer". However, it is clear from several animal studies that transcription does occur within the spacer, but that the transcripts have been difficult to detect since they are short-lived [8, 22, 42, 53]. The terms "external spacer", "ribosomal gene spacer" and simply "spacer" have also been used, but they are somewhat ambiguous since other spacers exist at the 5′ end of the 18S gene, between the 18S and 5.8S genes and between the 5.8S and 25S genes [61]. The most appropriate term that has been used [83] is "intergenic spacer" (IGS), which is the one we will use in the article.

Plants generally have more rRNA genes than do other groups of organisms [5]. As discussed below, it is apparent for both plants and animals that most of these genes are superfluous. It is not known whether the "extra" rDNA is important to the organism in some as yet unappreciated fashion or is truly non-functional and simply tolerated. What is known is that in many plants heterogeneity exists in the 5′ portion of the IGS (Fig. 1). The IGS itself contains several different regions that control rRNA transcription and/or processing in animals [8]. Plants

Table 1. Ribosomal gene characteristics for various plant species.

Species	Loci	Individual variation	IGS length (kb)	Subrepeats (bp)	Gene copy number (haploid)	References
Aegilops umbellulata	$2^{C,N}$		2.9		2500	[67]
Allium cepa (onion)	$1-4^{N}$	L	6.4		2500 – 6950	[50, 63, 64, 87]
Arabidopsis thaliana	2^{N}	H	3.8 – 4.2	< 100	570	[74, 88]
Carduus nutans (thistle)			6.8			[96]
Citrus limon (lemon)			3.0 – 6.8			[34]
Clematis fremontii			4.0 – 6.8	200		[57]
Cucumis melo (muskmelon)			4.0 – 4.3		1000	[35]
Cucumis sativus (cucumber)			3.6 – 5.9		4500	[35, 45, 51, and [b]]
Cucurbita maxima (squash)			4.0 – 4.8	126	5000	[44 and [c]]
Cucurbita pepo (pumpkin)			2.1 – 4.0		5000	[35, 45, 90]
Cynara cardunculus (artichoke)			7.2			[96]
Daucus carota (carrot)			4.4 – 4.8			[49]
Ferula communis (fennel)			1.4 – 4.4		430 – 2000	[71]
Galactites tomentosa			4.2 – 5.9			[96]
Glycine canescens			2.3 – 3.8			[24]
Glycine clandestina			2.1 – 2.5			[24]
Glycine falcata			6.1			[24]
Glycine latifolia			2.4			[24]
Glycine latrobeana			2.6			[24]
Glycine max (soybean)			1.3		700 – 1600	[24, 43, 98]
Glycine soja (wild soybean)			1.3			[24]
Glycine tabacina			2.1 – 3.3			[24]
Glycine tomentella			1.9 – 3.8			[24]
Hordeum spontaneum (wild barley)	2^{M}		3.3 – 4.8	115		[83]
Hordeum vulgare (barley)	$2^{N,M}$	H	3.1 – 4.5	115	2900 – 4200	[4, 36, 83, 84, 92]
Lilium henryi (lily)			5.1			[99]
Linum usitatissimum (flax)			2.5		200 – 1300	[15, 16, 17, 39]
Lisianthius sp.			5.8 – 6.7			[93]
Lupinus luteus (yellow lupine)			1.3			[75]
Lycopersicon esculentum (tomato)	$1^{C,N,M}$		2.5			[97]
Lycopersicon pennellii (currant tomato)	$1^{C,N,M}$		4.5			[97]
Matthiola incana (stock)					1100	[40]
Onopordum acanthium (thistle)			4.2 – 4.6			[96]
Onopordum illyricum (thistle)			4.0			[96]
Oryza sativa (rice)			1.5 – 1.8		430	[72]
Paris verticillata			9.3 – 10.8			[107]
Petunia hybrida			3.0 – 4.9			[104]
Pisum sativum (pea)	$2^{N,M}$	H	2.8 – 9.1	180	500 – 3900	[20, 45, 47, 73]

Table 1. Continued.

Species	Loci	Individual variation	IGS length (kb)	Subrepeats (bp)	Gene copy number (haploid)	References
Raphanus sativus (radish)			2.3	100	3800	[22, and [d]]
Scilla peruviana (squill)			6.5 – 6.7		6900 – 8600	[14]
Secale cereale (rye)	1^{N}		3.0	134	2900	[4, 6, 45, 54]
Trillium apetalon			7.4 – 12.2			[107]
Trillium hagae			7.4 – 12.2			[107]
Trillium kamtschaticum			9.3 – 12.2			[107]
Trillium tschonoski			7.4 – 12.2			[107]
Triticum aestivum (wheat)	$2-4^{N,M}$	H	2.5 – 4.9	135	3000 – 7500	[2, 4, 31, 33, 56, 67]
Triticum dicoccoides (wild wheat)	$2^{N,M}$	H	3.5 – 4.9	135		[2, 32]
Vicia benghalensis (vetch)		H	1.9 – 3.2		950	[45, 55, 80]
Vicia dasycarpa (vetch)		H	1.9 – 3.1			[55, 80]
Vicia faba (broad bean)	$1^{C,N}$	H	3.0 – 13.5	325[a]	250 – 22000	[50, 52, 55, 66, 80, 82, 85, 86, 108, 109, 110]
Vicia melanops (vetch)		No	3.2 – 3.5			[55, 80]
Vicia pannonica (vetch)		H	2.0 – 3.0			[55, 80]
Vicia sativa (vetch)	1^{C}	No	3.3		1900	[55, 65, 66, 80]
Vicia tetrasperma (vetch)		No	1.1			[55, 80]
Vicia villosa (vetch)		H	1.9 – 10.0		1300	[55, 65, 80]
Vigna radiata (mung bean)			3.8 – 4.6	180	1500	[37, 43]
Zea mays (maize)	$1^{C,N,M}$	H	2.4 – 3.8	200	1700 – 12000	[13, 38, 68, 79, 95, and [e]]

Species names are followed by common names (in parentheses) when one exists. The number of loci have been determined by location of secondary constrictions on chromosomes (C), number of nucleoli (N), or genetic mapping (M). Individual variability means that individual members of a population of plants or tissues of the same plant have been assayed either for rDNA loci (L) or by restriction enzyme/Southern hybridization (H). Although individual variability was not found for *Vicia melanops, V. sativa* or *V. tetrasperma,* the sample size was only three individuals in each case [80].

[a] the 325-bp subrepeat of *V. faba* consists of two nearly identical 155-bp subrepeats and about 15 bp of unrelated sequences.

[b] M. Ganal, R. Torres, V. Hemleben, personal communication.

[c] R. Kelly, A. Siegel, personal communication.

[d] D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication.

[e] T. Rocheford, J. Osterman, C. O. Gardner, personal communication.

also contain different IGS regions similar, in form at least, to the animal IGS regions. These may also be controlling regions in plants and their variability offers an opportunity to investigate transcription and processing of rRNA genes.

In this review we will not consider all aspects of rDNA in plants, since some topics have been covered previously [5, 30, 43, 58, 55, 61], but instead will focus on two main topics that have not been thoroughly discussed in the literature: gene copy number variation and IGS length variation. Often the experimental data for plants simply do not exist, and in those cases it will be necessary to present animal data for comparison to the plant data that do exist, in order to interpret results from plant studies.

Ribosomal DNA copy number

Most of the RNA in a cell is ribosomal RNA. The enormous demand for rRNA is satisfied by various mechanisms in different organisms. In bacteria,

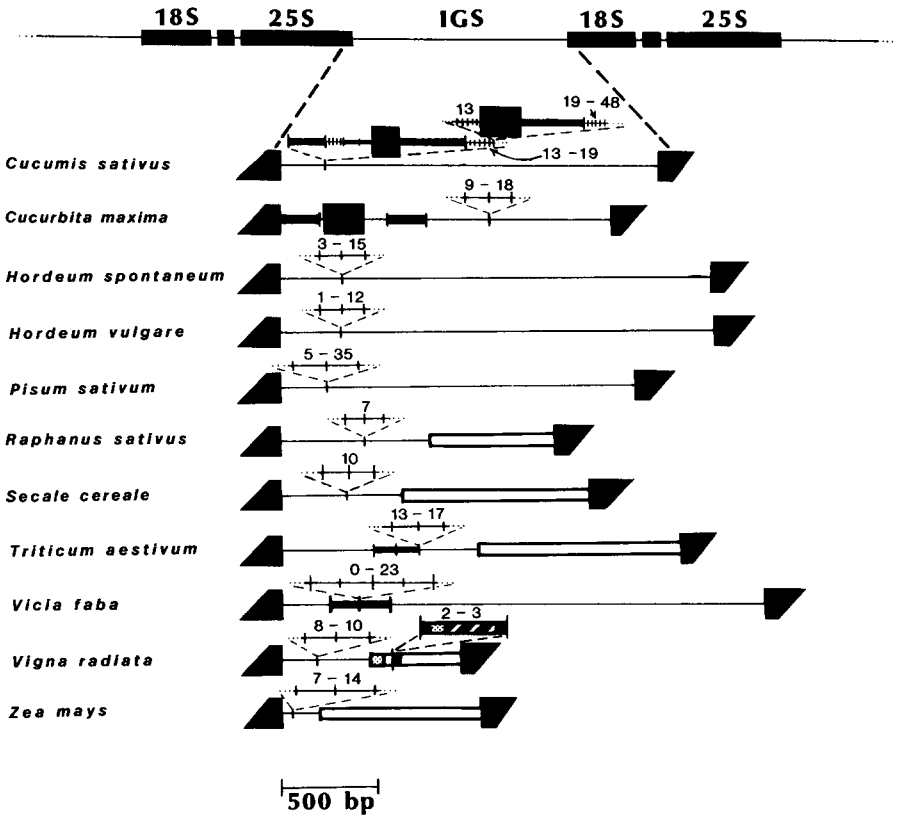

Fig. 1. Comparison of the intergenic spacers (IGS) for ribosomal RNA genes. The subrepetitive elements are probably all direct repeats for each species. The range in the number of subrepeats shown is the minimum and may be much greater, especially in those cases where individuals have not been examined (*Cucumis sativus, Cucurbita maxima, R. sativus, S. cereale* and *Vigna radiata*). The length of the external transcribed spacer (ETS; shown by an open box preceeding the 18S gene) is known only for the species indicated. The ETS was identified by sequence analysis for *R. sativus, S. cereale* and *T. aestivum,* by S1 nuclease mapping for *Z. mays* and inferred from size estimates on non-denaturing gels of the primary rRNA transcript for *Vigna radiata. Cucumis sativus* has at least three different types of repetitive elements represented by lines of different thickness (the shortest is 30 bp) in addition to one or two copies of the 3′ end of the 25S gene (indicated by the thickest boxes). *Cucurbita maxima* also has two types of repeats as well as a second copy of the 3′ end of the 25S gene (thickest box). *T. aestivum* has elements of 135 bp (thin line) and 150 bp (thick line). *T. dicoccoides* (a wild tetraploid wheat) has 10 to 20 of the 135-bp repeats [33]. *V. faba* has direct repeats of 150 bp each (thick line) that flank the 325-bp subrepeat region. Each 325-bp subrepeat consists of two nearly identical 155-bp elements and 15 bp of unrelated sequences. A wild relative of pea (*Pisum elatius*) contains from 7 to 12 of the 180-bp repeats [47]. *Vigna radiata* contains additional repeated sequences within the putative ETS: 3–4 copies of a 70-bp element (shaded regions), 2–3 copies of a 340-bp element (striped region), and 5–7 copies of a 52-bp element (filled region) that border the entire repeated region. Data are from the following sources: *Cucumis sativus* [35, 51, 43, and M. Ganal, R. Torres, V. Hemleben, personal communication], *Cucurbita maxima* [R. Kelly, A. Siegel, personal communication], *H. spontaneum* [83], *H. vulgare* [4, 36, 83], *P. sativum* [47, 73], *R. sativus* [21, and D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication], *S. cereale* [6], *T. aestivum* [2, 4, 33, 56], *Vicia faba* [50, 80, 82, 108, 109, 110], *Vigna radiata* [37, 43] and *Z. mays* [68, 95, and T. Rocheford, J. Osterman, C. O. Gardner, personal communication].

which have from one to ten rRNA genes [28], the transcription rate per gene can be varied so that rRNA production matches the growth rate of the cells [70]. Such variable transcription rates have not been documented in higher organisms, although it is possible that they do occur. A second mechanism, that of extrachromosomal amplification, exists in some protozoans [48], in slime molds [58, 61, 100, 101], and in some animal oocytes [48]. One to several copies of rDNA are used as templates to produce

hundreds to thousands of extrachromosomal copies. In amphibians, such as *Xenopus,* this amplification occurs only during oogenesis so that large numbers of ribosomes will be available at the time of fertilization. In *Tetrahymena thermophila, Physarum polycephalum* and *Dictyostelium discoideum* the amplification events also only occur at specific developmental stages. A third method for meeting the increased need for certain gene products is to replicate the entire genome in order to increase the number of the required genes. This can occur in the cell nucleus through added rounds of DNA replication without cell division to create polyploid or polytene chromosomes. The method is usually thought useful to increase copy number of nuclear genes other than rRNA genes, although it is possible that the additional rDNA is used to increase rRNA production as well. It has been proposed that the reason for multiple copies of the chloroplast and mitochondrial genomes is to increase the dosage of rRNA genes rather than protein-coding genes [11]. A fourth method is the one used by most eukaryotes so far examined, including plants. A large number of rRNA genes, most tandemly arranged, is maintained in the nucleus of all cells. Animals usually have from 100 to 1 000 copies per diploid cell [5], while plants generally carry from 500 to 40 000 copies per diploid cell [5, 45, 80] (Table 1).

The copy number of ribosomal genes has commonly been determined using DNA from pooled individuals. It has been tacitly assumed that the value thus obtained is characteristic of the species and representative of its individual members. This assumption may be incorrect for individuals of most species. For example, in a wild population of *Plethodon cinereus* (salamander) a 7.5-fold variation in copy number among individuals was measured and up to a 15-fold variation was thought to exist [62]. Similarly, copy number variation among *Triturus vulgaris* (newt) individuals was as great as 10-fold [1] and copy number differences were found even among tissues of the same individual [60]. Strains of *Drosophila melanogaster* containing an average of from 30 to 100 copies of rDNA survive, although their development is slowed. However, strains containing 100 copies or more are indistinguishable in growth characteristics [89, 94]. Thus, while a certain minimum copy number must be retained, an rDNA level beyond this appears to have no effect on phenotype. In maize, variation of the average copy number by five-fold does not appear to affect the amount of rRNA produced, the amount of protein produced or the general phenotype of the plants [13]. A six-fold range in copy number was found among 101 individuals of *Hordeum spontaneum* (wild barley) [Q. Zhang, M. A. Saghai-Maroof, R. W. Allard, personal communication]. Up to a four-fold range was reported in the F1 generation and a three-fold range in the F2 generation from crosses using various genotrophs of *Linum usitatissimum* (flax) [16]. Small changes (up to 25%) were also found within individual flax plants after cutting and culturing manipulations [19]. Nearly a 100-fold difference in copy number (from 500 to 44 000 per leaf cell) was found among 434 *Vicia faba* (broad bean) individuals [80]. In the same study the rDNA levels among the tissues of an individual were similar for twelve plants, although the tissues of three individuals differed by from 4- to 12-fold in rDNA amount. The changes were not tissue-specific, since the seed pod had the highest level in one individual but the lowest in another. Finally, individual copy numbers were determined for parental, F1 and F2 generations of *V. faba.* Parents with high or low copy number often gave rise to progeny with either low or high copy number both in crosses and even after self-pollination. In none of these cases were the changes in copy number correlated with any obvious phenotypic changes.

These between-individual and within-individual differences constitute the strongest evidence that superfluous rDNA copies exist in plants, that these extra copies may comprise up to 90% or more of all of the rDNA copies and that the additional copies are of no phenotypic consequence.

It has been suggested that the maintenance of repetitive DNA sequences, including rDNA, is important for plants because they cannot move to avoid unfavorable changes in their environment [102, 103]. Ribosomal DNA changes have been associated with stress in several reports [15, 17, 18, 32, 41]. While it is possible that maintaining a large rDNA pool is advantageous in times of stress, most wild and cultivated species maintain a high copy number under non-

stressful conditions. Furthermore, large changes among individuals have been found for *Vicia faba* under non-stressful conditions [80] and among mobile salamanders [62] and newts [1]. It is possible that copy number changes occur in many plant populations, some only in response to environmental alteration and others irrespective of such alterations. It is also possible that all populations undergo constant change and it is the detection of the change that is facilitated under certain environmental situations. For example, if a large change occurred in a few individuals, the change could go unnoticed unless many individuals were sampled. However, stressful conditions could select for certain genotypes (irrespective of rDNA) associated (by stimulated recombination, linkage or otherwise) with rDNA, thus enriching the population for individuals with altered rDNA levels. Even if the environmental perturbation plays a role in initiating the change, such as the appearance of a CsCl satellite band of rDNA after altering the auxin concentration in cultured *Cucumis melo* (melon) cells [12, 41], in no case has it been demonstrated that the change in rDNA has any adaptive significance. Finally, tissue-specific changes in rDNA copy number were observed in onion [7] and mustard [46] and other changes have been noted in several species, but their functional significance, if any, is not clear, especially since the reported changes were small [9, 23, 59].

It is possible that environmental factors play some role in selecting for rDNA copy number variants. The most likely explanation, however, is that the changes and variability are both caused by recombination events that lead to an adequately large rDNA pool, but most of the rDNA is in excess of that needed to produce rRNA and is merely tolerated. In this respect rDNA is probably not very different from most other DNA in plant nuclei [12], mitochondria [10] and chloroplasts [11] thought to be in excess of that needed to direct metabolism and heredity.

Intergenic spacer length variation

Another form of change is the heterogeneity in rDNA repeat length seen among members of the same genus [24, 25, 55, 80, 83] or species [5, 80, 83, and T. Rocheford, J. Osterman, C. O. Gardner, personal communication], attributable to variability in the IGS. In *V. faba,* which has only one chromosomal locus for rDNA [52, 66, 86], the heterogeneity is enormous and the arrangement of neighboring IGS lengths is highly variable [80]. In pea (*Pisum sativum*), two loci are present, one containing many IGS size classes and the other containing only one or two size classes [27, 73]. From these and other examples (Table 1) it is evident that the number of genetic loci is not correlated with length heterogeneity. In the cases where it has been thoroughly investigated, the variability is in the number of a subrepetitive element that is generally between 100 and 200 base pairs (bp) in length (Fig. 1) and is species-specific in sequence [3, 33, 56, 68, 95, 108, and D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication]. The variability in the number of these units from one IGS to another has been attributed to unequal crossing over between subrepetitive units either on the same chromosome or its homolog [29, 80, 106]. Crossing over between distant genetic loci does not appear to be common [26, 73], although in onion the rDNA loci have been inferred to "jump" from site to site [86]. The IGS length changes more slowly than does gene copy number in *V. faba* since the former was detected in F2 but not F1 generations in crosses, but copy changes were found in many parental/F1 comparisons both in crosses and after self-pollination. The exchanges are probably occurring during mitotic as well as meiotic cell cycles since different numbers of rRNA genes can be found among tissues of the same plant [7, 46, 80].

Subrepetitive elements have been found even among plants that do not always exhibit variation in IGS length [68, 95]. Inbred lines of maize had 10 tandem copies of a 200-bp subrepeat, with no detectable variation, although the sample sizes were small [68, 78, 95]. This may indicate that other species that show no variation may contain similar elements as well. It should be noted, however, that the only non-inbred cultivar of maize for which data are available (Hayes Golden open pollinated) does exhibit variability among individuals in the number of subrepetitive elements per IGS [T. Rocheford, J. Osterman, C. O. Gardner, personal communication]. So far, ev-

ery IGS that has been analyzed has contained subrepetitive elements at a similar location (Fig. 1). While these elements differ from one species to another, they are all from 100 to 200 bp in length, although in *Cucumis sativus* (cucumber) smaller and larger units are also found.

Suggestions have been made that these subrepetitive elements arose from transposition events since they show some similarities with transposons. For example, in *V. faba* the tandem array is surrounded by 150-bp direct repeats (Fig. 1) and the elements are also found elsewhere in the genome [50]. In onion, the rDNA loci may be found at different chromosomal locations in different cells of an individual [87]. While these similarities to transposons are intriguing, direct evidence of mobility will be needed to support the idea that these elements are transposons.

It appears that the subrepeats are "hot spots" for recombination and that by increasing exchanges in the rDNA regions they promote copy number change [80, 81]. While maintaining a multiplicity of rDNA may be part of their function, it is probably not their only function. For example, one of the several types of subrepetitive units in the IGS of *Xenopus laevis* appears to be involved in nucleolar dominance, a phenomenon in which one class of rRNA is transcribed while another is not [77]. Repetitive sequences near the 5′ end of the 18S gene may bind a factor (probably a protein) involved in RNA polymerase I attachment to the spacer region. These sequences have been termed "enhancers" since they can affect transcription in either a forward or reverse orientation and may sequester limiting transcription factors in nucleolar dominance.

The subrepeats in wheat have been considered as enhancers similarly involved in nucleolar dominance partly because of sequence homology (10 out of 16 matched bases) with a *Xenopus* promoter region [33]. There is no homology, however, for this region among vertebrates [91] and the part of the IGS containing the region diverges rapidly even among six *Triticum* species [3]. Another possibility is that the subrepeats in plants are either RNA processing sites or transcription termination sites as indicated in several animal studies [22, 42, 53]. Their effect is to allow the polymerase to be retained within the IGS instead of falling off of the DNA at the end of the 28S gene. Within the authentic promoter region in *Xenopus* the terminator sequence is repeated again in close proximity to the transcription start site. It has been found that this terminator is essential for transcription of the adjacent gene [8, 44, 53, 69]. In plants a similar arrangement may be present, but so far only sequence data are available and no functional tests have been reported. Sequences in the presumptive promoter region are also found in the subrepeats of rye, wheat, maize, radish and *Cucurbita maxima* (squash) [6, 33, 68, 95, D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication and R. Kelly, A. Siegel, personal communication].

These subrepeat sequences may be enhancers or promoters as suggested [33]. For the following reasons, however, it is possible that they are terminators and also appear within the promoter vicinity because they are required for promoter activity as they are in *Xenopus* and mouse [8]. First, the repeat units in plants are located a few hundred base pairs from the 3′ end of the 25S gene (Fig. 1), similar to the location of terminators in *Xenopus* and mouse [8, 22, 44]. Also, stem-loop structures bordered on one end by pyrimidine-rich regions have been inferred to be important in transcription termination [44]. Such structural motifs can be found in the subrepeats of rye [6], wheat [33], maize [68, 95], broad bean [108], radish [D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication] and squash [R. Kelly, A. Siegel, personal communication] and within the putative promoter regions of rye, wheat, maize, radish and squash (data are not available for broad bean; Fig. 2). Additionally, in *Cucumis sativus* and *Cucurbita maxima,* the region containing the 3′ end of the 25S gene and adjacent sequences is repeated in the IGS (Fig. 1). It has been suggested that this region includes transcription termination signals rather than sequences involved in transcription initiation [43]. Thus, positional and structural characteristics suggest that the subrepetitive units are transcription termination regions important for control of polymerase retention and movement, although any functional assignment will require experimental verification. It has been suggested [8] that terminators in the IGS of animals may

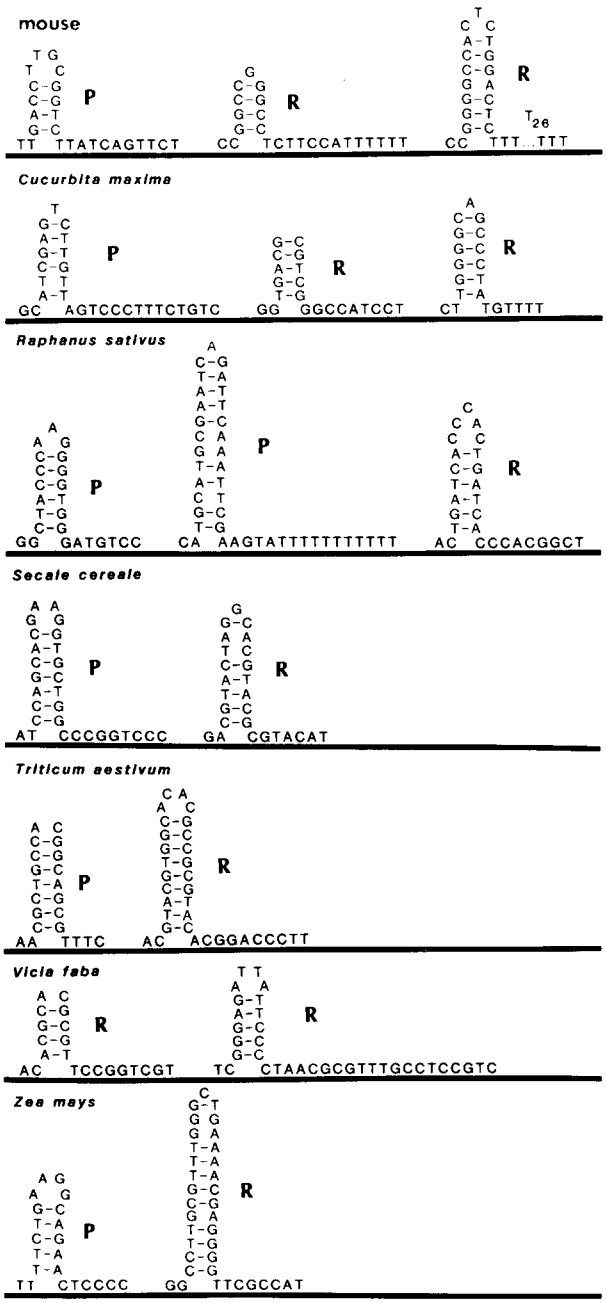

Fig. 2. Possible stem-loop structures in the subrepeat region and near the putative promoter regions of mouse and six plant species. The non-coding strand (RNA-like) is shown in the 5′ to 3′ direction. In mouse these structures, followed by pyrimidine-rich regions, have been identified as possible transcription termination signals [44]. Hyphens indicate base pairing (including G-T, as in [44]). A "P" signifies a promoter (mouse) or putative promoter (plants) region (within 100 to 200 bp of the transcription initiation site, although this site is not known for *C. maxima*) and "R" signifies a sequence repeated in a region upstream from the promoter. Two stem-loop structures are found in the "P" region of *R. sativus*. The only sequence data available for *V. faba* is for the "R" region. The short length of the pyrimidine tail for *T. aestivum* is due to limited data for that region. Data are from the following sources: mouse [44], *C. maxima* [R. Kelly, A. Siegel, personal comminication], *R. sativus* [D. Delcasso-Tremousaygne, F. Grellet, F. Panabieres, M. Delseny, personal communication], *S. cereale* [6], *T. aestivum* [33, 56], *V. faba* [108] and *Z. mays* [68, 95].

The position of the last nucleotide for each "P" region sequence with respect to the 5′ end of the 18S-rRNA is as follows: mouse −181; *C. maxima* −304; *R. sativus* −696 and −824, respectively; *S. cereale* −988; and *Z. mays* −849. Values for *V. faba* are not available and for *T. aestivum* the value is −73 with respect to the transcription initiation point. The corresponding values for each "R" region with respect to the 3′ end of the 25S (or 28S) rRNA are: mouse 6 and 177, respectively; *C. maxima* 3398 and 3417, respectively (the ones shown are within the eighteenth repeat, although similar or identical sequences are found in the other seventeen repeats); *R. sativus* 1177 (the one shown is in the seventh repeat, although nearly identical sequences appear in four of the other six repeats); *S. cereale* 367 (the one shown is in the first repeat, although identical sequences appear in all of the remaining ten repeats); *T. aestivum* 57 (of consensus sequence from [56]); *V. faba* 14 and 151/304 (the second repeat occurs twice per 325-bp repeat), respectively; and *Z. mays* 42 (of consensus sequence from [68]). The stem-loop structures found in the repeats of *S. cereale* and *T. aestivum* occur at the same location within the respective repeats.

modulate rRNA production in response to metabolic demand by providing stop/restart control of transcription at each ribosomal gene. If the subrepeats in the upstream region of the IGS do contain terminators, their high copy number in plants relative to animals may be useful in preventing unnecessary transcription of so many superfluous copies of rDNA.

Conclusions

It is perhaps surprising that the number and IGS length of ribosomal genes can be so variable among individuals. It should be recalled, however, that most previous analyses have employed DNA from pooled individuals, both for plants and animals. Thus we have no large body of data supporting the expectation that individuals within a species or population should be uniform in their rDNA. What is truly surprising is that for no eukaryote do we know whether

the intensity of transcription per nuclear rRNA gene may change in response to changing need for protein synthesis, as is the case for *E. coli* [70] but not for chloroplast and mitochondria according to one hypothesis [11]. Thus it is possible either that there is only one rate of transcription per gene and more genes are recruited as rRNA requirements increase, or that any gene may be transcribed at a rate set by metabolic need. In either case much of the rDNA is in excess of maximum requirements as inferred from copy number variation among individuals in a population. It could be argued that the best test of this inference would be to remove rDNA from an individual and assess the resulting effects. Nearly the equivalent experiment has been done, however, since there is a large variation (up to 18-fold) among F1 progeny of self-pollinated *V. faba* individuals that exhibit no obvious phenotypic difference [80].

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