

See discussions, stats, and author profiles for this publication at: <https://www.researchgate.net/publication/15163567>

# Structural Organization and Regulation of Transcription by RNA Polymerase I of Plant Nuclear Ribosomal RNA Genes

ARTICLE *in* RESULTS AND PROBLEMS IN CELL DIFFERENTIATION · FEBRUARY 1994

DOI: 10.1007/978-3-540-48037-2\_1 · Source: PubMed

---

CITATIONS

25

READS

23

---

## 2 AUTHORS:



Vera Hemleben

University of Tuebingen

145 PUBLICATIONS 2,483 CITATIONS

[SEE PROFILE](#)



Ulrike Zentgraf

University of Tuebingen

53 PUBLICATIONS 1,738 CITATIONS

[SEE PROFILE](#)

# **1 Structural Organization and Regulation of Transcription by RNA Polymerase I of Plant Nuclear Ribosomal RNA Genes**

Vera Hemleben and Ulrike Zentgraf

## **1 Introduction**

The RNA components of the plant cytoplasmic ribosomes consist of the 17/18S rRNA (ribosomal RNA) in the 40S ribosome subunit and the 5S, 5.8S, and 25S/26S rRNA in the 60S ribosome subunit. The corresponding genes for the 18S, 5.8S and 25S rRNA, encoded by the nuclear genome, are composed in transcription units which are located as rDNA (ribosomal DNA) repeats in the NOR (nucleolus organizing region) of the chromosome. As in higher animals, the genes for the 5S rRNA are localized separately at other regions in the genome (Hemleben and Grierson 1978). Coordinated regulation of the expression of the different components of the ribosomes can be expected since three RNA polymerases are involved to provide concomitantly the rRNA components and the mRNA for the ribosomal proteins (Sommerville 1986): RNA polymerase I (pol I) is responsible for the 18S–25S rRNA transcription, RNA polymerase III (pol III) produces the 5S rRNA, and the genes for the ribosomal proteins are transcribed by RNA polymerase II (pol II). The question how these different RNA polymerases are coordinately regulated is still a fascinating problem to solve for eukaryotic cells. Until recently, it was believed that the genes transcribed by pols I, II and III, respectively, utilize completely different sets of initiation factors; however, it has now become evident for animal cells that one common factor, the TATA-binding protein, plays a central role in transcription of all three RNA polymerases (for a review, see White and Jackson 1992).

Biosynthesis and processing of plant ribosomal RNA were extensively studied by pulse-chase *in vivo* labelling of the cell RNAs and gel electrophoresis of the RNA products (Rogers et al. 1970; Grierson and Loening 1972). Although the size of the rRNA precursor molecules is generally smaller (32S to 35S) than that determined for animals (45S), the processing steps resulting in the mature 18S, 5.8S, and 25S rRNA are assumed to occur principally in a similar manner (Rogers et al. 1970; Perry 1976; Rungger and Crippa 1977). The rate of rRNA synthesis can change enormously

---

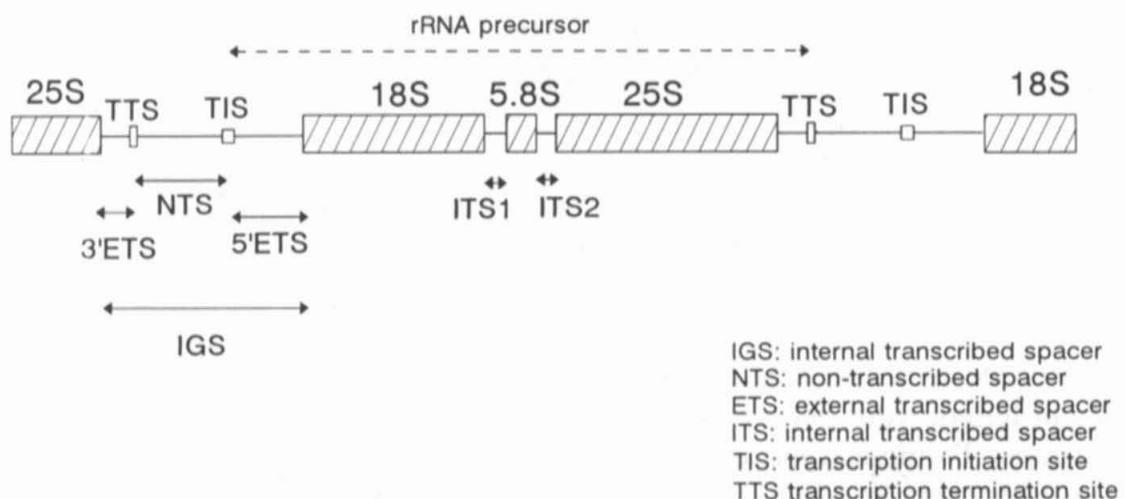
Department of Genetics, Biological Institute, University of Tübingen, Auf der Morgenstelle 28, D-72076 Tübingen, FRG

depending on the developmental and physiological stage of the cells or the environmental conditions. For example, stimulation of rRNA synthesis is observed after light treatment of dark grown plants (Grierson and Loening 1974; Tobin and Silverthorne 1985) or by treatment with 2,4-D, an artificial auxin (Guilfoyle et al. 1975). These changes in rRNA synthesis might reflect changes in the rate of transcription of a given set of genes, however, an increase in rRNA synthesis might also be possible by activating new genes of the ribosomal RNA multigene family, thus raising their accessibility for transcription (Thompson et al. 1988; Baerson and Kaufman 1990). The most detailed biochemical studies on plant pol I carried out in the 1970s were reviewed by Jendrisak (1980); however, these investigations have to be extended to the molecular level.

In higher plants often a large percentage of the nuclear genome represents ribosomal DNA. Variable numbers (between 1000 to more than 30 000) of ribosomal RNA genes forming multigene families arranged in long tandem arrays were reported for a variety of plant species (Ingle et al. 1975; Hemleben et al. 1988). Only recently, it was found that, in contrast to the general assumption of the occurrence of long tandem arrays, the rDNA repeats of flax, although located at a single locus, are frequently interrupted by non-rDNA sequences (Agarwal et al. 1992). In an excellent three-dimensional electron microscope investigation of ribosomal chromatin the spatial organization of rDNA within the fibrillar centers (FC) of the nucleolus was demonstrated using a combined cytochemical, immunocytochemical, and *in situ* hybridization approach (Motte et al. 1991).

The rDNA repeats containing the 18S–25S rRNA genes exhibit an enormous length and sequence heterogeneity (for reviews, see Rogers and Bendich 1987; Hemleben et al. 1988). Since the coding regions for the 18S, 5.8S, and 25S rRNAs are highly conserved with respect to length and nucleotide sequences, these length and sequence heterogeneities mostly affect the intergenic spacer (IGS) region (*per definitionem* located between the 25S and 18S rRNA coding sequences; Fig. 1).

Plant species of the same genus already differ in nucleotide sequence between 60 and 95%, depending on the IGS region analyzed, e.g., *Vicia faba*, *V. angustifolia* and *V. hirsuta* (Kato et al. 1990; Ueki et al. 1992; Yakura and Nishikawa 1992) or *Cucurbita maxima* and *C. pepo* (Kelly and Siegel 1989; King et al. 1993). Comparing species of different genera within a plant family considerable sequence divergency is observed, e.g., for radish (*Raphanus sativus*), *Sinapis alba* and *Arabidopsis thaliana* (Delcassotremousaygue et al. 1988; Rathgeber and Capesius 1990; Gruendler et al. 1991), *Lycopersicon esculentum* and *Solanum tuberosum* (Schmidt-Puchta et al. 1989; Perry and Palukatis 1990; Borisjuk and Hemleben 1993), or *Triticum aestivum* and *Zea mays* (Toloczyki and Feix 1986; Barker et al. 1988). Nearly no sequence similarity of the IGS (except for short motifs) among representatives of different plant families was found. Therefore, hybridization studies for restriction fragment length polymorphism (RFLP) analysis of closely related plants are possible using rDNA spacer fragments



**Fig. 1.** Schematic drawing of two ribosomal RNA genes arranged in tandem. The 18S, 5.8S and 25S rRNA coding regions internally separated by the internal transcribed spacers, ITS1 and 2, are boxed. The intergenic spacer (IGS) is divided into the 3' and 5' external transcribed spacer separated by the non-transcribed spacer region. Transcription initiation and termination of the rRNA precursor are indicated by arrows

(Torres et al. 1989; Torres and Hemleben 1991); more distantly related plants can be only distinguished by RFLPs hybridizing with rDNA probes coding for the mature rRNAs (Hemleben et al. 1992).

The length of an rDNA repeating unit varies from approx. 8 kbp (e.g., *Raphanus sativus*; Delseny et al. 1983) to 14–17 kbp (*Trillium*, *Paris*; Martini et al. 1982; Yakura et al. 1983). Length heterogeneity of the repeats can occur already within an individuum or in different representatives of a species. The high variability in length and sequence of the IGS in plant rDNA offers the opportunity to differentiate even between varieties or cultivars of a species by RFLP analysis as already applied on wheat or barley populations and wild species (Appels and Dvorak 1982; Shagai-Marof et al. 1984).

In the last decade nucleotide sequence data for the IGS of various higher plants from different plant families were collected and can be analyzed by computer analysis. These data together with recent studies on in vivo and in vitro transcription helped to elucidate the functional role of IGS sequences in the regulation of pol I transcription. Binding assays with IGS elements and crude nuclear or whole-cell extracts provided some evidence for the protein factors involved. In this chapter we will concentrate on the structural and functional organization of the 18S–25S rRNA genes and the role of rDNA methylation and chromatin structure; moreover, we will describe what is emerging from the literature and our own studies on *trans*-acting factors and *cis*-acting elements involved in the regulation of pol I transcription in plants.

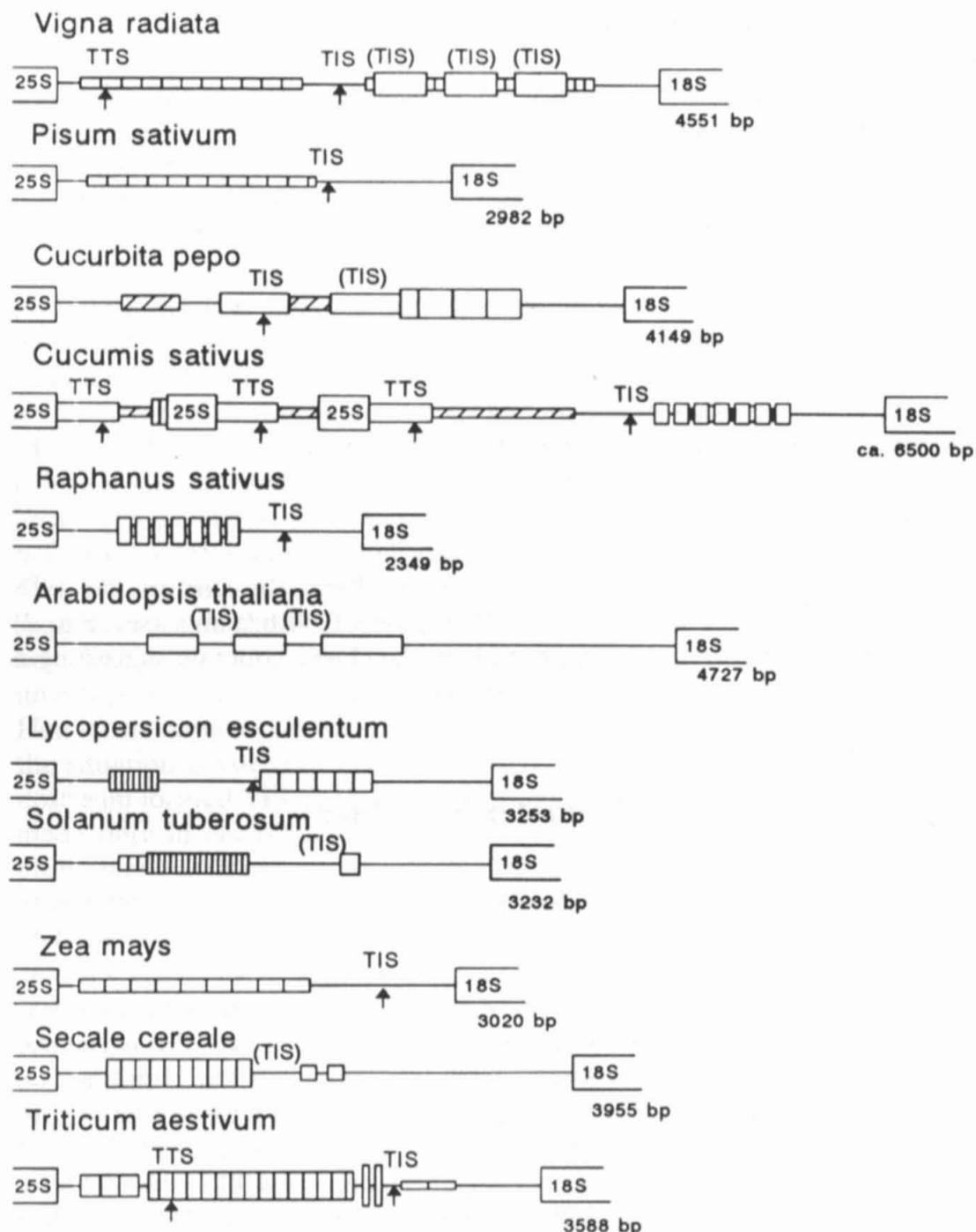
## 2 Structural Organization of the rRNA Genes Transcribed by RNA Polymerase I

The large 18–25S rRNA precursor transcribed by RNA polymerase I, starting at the transcription initiation site(s) (TIS), is encoded by the 5' external transcribed spacer (5'ETS) followed by the 18S rRNA coding region, the 5.8S rDNA sequences – 5' and 3' flanked by the internal transcribed spacer 1 and 2 (ITS 1 and 2) – and the 25S rRNA coding region with 3'ETS located downstream (Fig. 1). Non-transcribed spacer (NTS) regions of different length were determined for several plant species (Barker et al. 1988; Delcasso-Tremousaygue et al. 1988; Gerstner et al. 1988; Schiebel et al. 1989; Vincentz and Flavell 1989; Zentgraf et al. 1990).

Various regulatory elements suggested to be involved in pol I transcription are located within the IGS region (Fig. 1). Functional elements are the pol I promoter with the TIS (+1) which is at least in dicots often preceded by an AT-rich region (Zentgraf et al. 1990), the transcription termination site (TTS), putative enhancer sequences binding to regulatory proteins, and processing sites for the rRNA precursor. Replication origins are also assumed to be located within the rDNA IGS as shown for pea rDNA (Hernandez et al. 1988).

A general feature of the IGS is the often complex organization of repeated elements (Fig. 2). Principally, each region of the IGS can be affected by amplification processes and appear as a repeated element within the spacer. In some plants, sequences with similarity to a consensus TIS are duplicated (Gerstner et al. 1988; Kelly and Siegel 1989; Gruendler et al. 1991). Repeated elements characterized by a putative transcriptional terminator site (TTS) for pol I were found in mung bean (*Vigna radiata*; Schiebel et al. 1989); enhancer-like repeated elements are located upstream of the TIS in wheat (*Triticum aestivum*; Flavell et al. 1986; Barker et al. 1988), maize (*Zea mays*; Toloczyki and Feix 1986; Schmitz et al. 1989) and probably occur also in the rDNA of other plants (see Fig. 2). Obviously, even part of the coding regions can be amplified as shown for the cucumber (*Cucumis sativus*) IGS where the 3' end of the 25S rDNA and 3' flanking sequences, including the TTS, were duplicated (Ganal et al. 1988; Zentgraf et al. 1990). Mechanisms resulting in the amplification of sequences are considered to be mostly an unequal crossover during recombination occurring within a multigene family (Dover 1986; Rogers et al. 1986; Ganal et al. 1988).

Different numbers of repeated elements in the IGS result in heterogeneous repeat lengths frequently observed in various plants (data compiled in Rogers and Bendich 1987; Hemleben et al. 1988). Remarkably, in plant species with conserved rDNA repeat lengths, difference in length in one region of the IGS is compensated by a different number of subrepeats in the other region; e.g. this phenomenon was observed in a comparison of tomato and potato IGS, both with a repeat length of approx. 9 kbp (Fig. 2; Schmidt-



**Fig. 2.** Schematic drawing of the intergenic spacer (IGS) region of a representative of the rDNA repeats of several higher plants. The transcription initiation site (TIS) and the transcription termination site (TTS) are marked by an arrow if they are determined by S1 or mung bean nuclease or by primer extension mapping; shown in parentheses are TIS determined by a computer search of the consensus sequence. The size of the IGS is indicated by the numbers of base pairs. References: *Vigna radiata* (Gerstner et al. 1988; Schiebel et al. 1989), *Pisum sativum* (Kato et al. 1990), *Cucurbita pepo* (King et al. 1993), *Cucumis sativus* (Ganal et al. 1988; Zentgraf et al. 1990), *Raphanus sativus* (Delcasso-Tremousaygue et al. 1988), *Arabidopsis thaliana* (Gruendler et al. 1991), *Lycopersicon esculentum* (Schmidt-Puchta et al. 1989; Perry and Palukatis 1990), *Solanum tuberosum* (Borisjuk and Hemleben 1993), *Zea mays* (Toloczyki and Feix 1986), *Secale cereale* (Appels et al. 1986), *Triticum aestivum* (Barker et al. 1988; Vincentz and Flavell 1989)

Puchta et al. 1989; Perry and Palukatis 1990; Boriskjuk and Hemleben 1993).

Whereas the IGS can be highly heterogeneous in length, the size of the 18–25S RNA coding regions, including the ITS 1 and 2, appears relatively constant, mostly approx. 6 kbp. The lengths of the ITS 1 and 2 sequences flanking the 5.8S rRNA coding region were determined in various plant species to be approx. 180 to 250 bp (Torres et al. 1990). Sequence diversity is already observed between related species, however, there seem to be some constraints on these sequences, which are able to form secondary structures, probably with functional relevance for processing. Of particular interest is the observation that the GC content of ITS 1 and 2, compared with another, although variable among species of a given genus, is relatively equal. GC balance, therefore, appears to be a general feature of ITS 1 and 2 (Torres et al. 1990). Possibly, the 5' ETS singular region reflects a similar behaviour since in the region preceding the 18S rRNA coding sequences, the GC content resembles the percentage of G+C found in ITS 1 and 2 as shown for cucumber and zucchini (King et al. 1993). In contrast, the ETS repeats 5' upstream of the singular ETS region of both plants (see Fig. 2) are characterized by a rather high and similar T+G content, suggesting a functional role of these subrepeats (see Sect. 5.2).

### 3 Promoter Structure

For several plant rDNAs, the TIS of the rRNA precursor was recognized by S1 or mung bean nuclease or primer extension mapping (Toloczyki and Feix 1986; Delcasso-Tremousaygue et al. 1988; Gerstner et al. 1988; Vincentz and Flavell 1989; Kato et al. 1990; Zentgraf et al. 1990). The sequences directly surrounding the TIS are rather conserved; therefore, this region can now be detected in the IGS sequence by computer search. However, the promoter region located 5' upstream is only conserved among closely related species (Zentgraf et al. 1990; Gruendler et al. 1991; King et al. 1993); in fact, computer analysis of this region shows approx. 60 to 70% similarity among species of different families, but short common motifs occur which appear to be necessary for specific protein binding (Echeverria et al. 1992; Jackson and Flavell 1992; Nakajima et al. 1992; Zentgraf and Hemleben 1992).

In a newly developed in vitro transcription assay with a crude whole-cell extract from embryonic axes of *Vicia faba* and rDNA fragments of the putative promoter region already determined by S1 nuclease mapping (Kato et al. 1990), correct initiation of pol I transcription was demonstrated. Some longer transcripts were observed starting further upstream of the consensus TIS (Yamashita et al. 1993).

For rRNA genes transcribed by pol I in animal cells a common picture was obtained (reviewed by Sollner-Webb and Mougey 1991): transcription

of pol I is mediated by *trans*-acting protein factors binding at a core promoter (CP) preceding the TIS [approx. nucleotides (nts) -45 to +5] and reacting with an upstream control element (UCE) or upstream binding element (UBE; approx. -160 to -110; Sollner-Webb and Tower 1986). Although it was assumed for a long time that the transcription apparatus of pol I is species-specific (Grummt et al. 1982; Miesfeld and Arnheim 1984) and sequence divergency is observed in the promoter region, recent studies showed that the factors binding to UCE can substitute for each other in footprinting assays between human and *Xenopus* (Bell et al. 1989; Pikarrd et al. 1989). For plant cells it was of interest whether *trans*-acting proteins involved in the regulation of rRNA transcription form specific complexes with *cis* regions of the putative promoter (see Sect. 5.2).

As already mentioned, in different plants the transcription initiation site (TIS) previously determined by sequence comparisons with a consensus sequence, and later confirmed by functional analysis by S1 or mung bean nuclease mapping and in vitro transcription assays (Yamashita et al. 1993), is rather similar. Interestingly, some dicots contain several TIS or TIS-like sequences within the IGS (see Fig. 2). For *Cucurbita pepo* it was shown that actually the first (5' upstream) TIS is prominently used for transcription as investigated by mung bean nuclease mapping experiments (K. King and V. Hemleben unpubl. results). For the duplicated TIS in *Arabidopsis* rDNA the situation is not yet clear; Gruendler et al. (1991) assumed that the two upstream located TIS were "spacer promoters", however, this assumption is made only in analogy to animal rDNA for which the presence of spacer promoters was predominantly described (DeWinter and Moss 1987; Tautz et al. 1987).

Many IGS of dicotyledonous plants investigated so far possess a longer AT-rich sequence element in front of the pol I promoter (Zentgraf et al. 1990). Remarkably, in this region, sequence elements are located with similarity to ARS sequences (autonomous replicating sequences) characterized for years (Broad et al. 1983), which might indicate that replication origins are present in every rDNA repeat. Functional replication origins in the neighborhood of ARS-like sequences (consensus sequence: -A/TTTTATA/GTTTA/T-) have been demonstrated by studies on pea rDNA (Hernandez et al. 1988; Van't Hof and Lamm 1992).

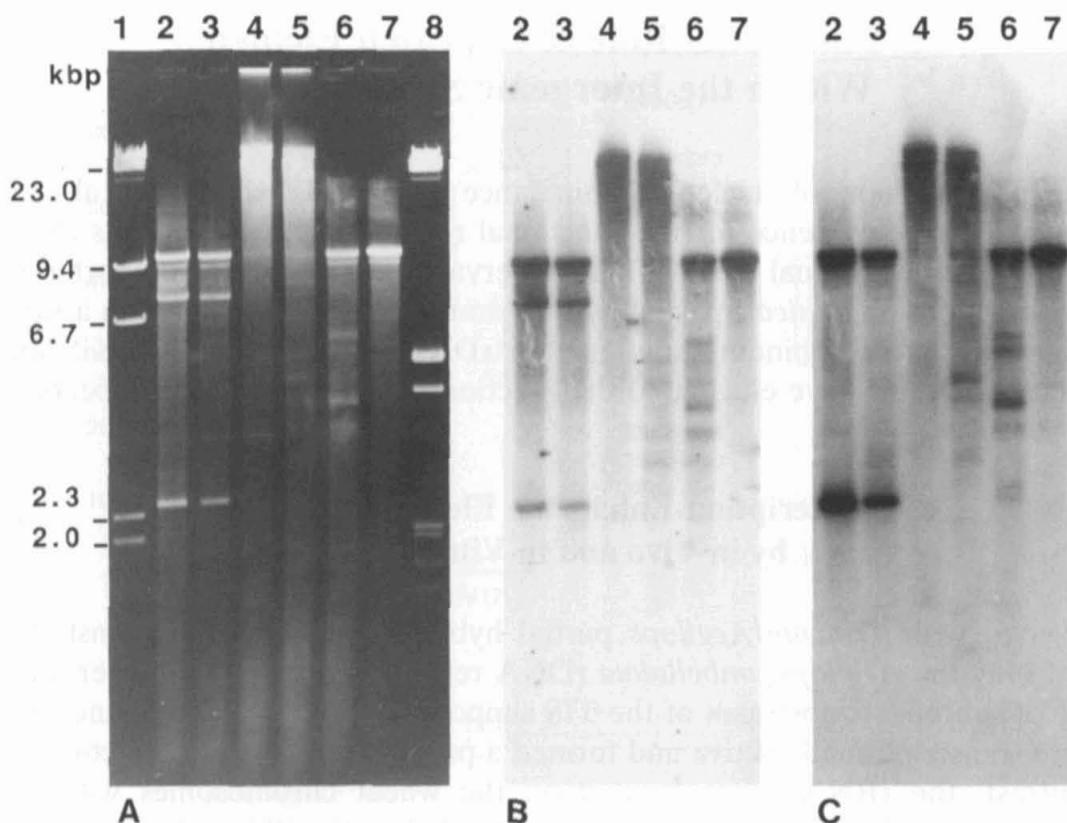
#### 4 Methylation and Chromatin Structure of rDNA

Methylation of DNA in plants at CpG and CpCpG or CpNpG motifs has been repeatedly considered to play a role in the regulation of transcription of nuclear ribosomal RNA genes (Hemleben et al. 1982; Leweke and Hemleben 1982; Flavell et al. 1983, 1988; Scott et al. 1984). In plants with a relatively large number of rDNA repeats a high percentage of the rDNA appears completely methylated at those sites and, in addition, these repeats

are probably packaged in a highly condensed and transcriptionally inactive heterochromatic stage. Most dramatically, this phenomenon was observed in various additional lines of wheat carrying a NOR-bearing chromosome of *Aegilops umbellulata*; here, the rDNA repeats in the NOR of the wheat genome were highly methylated and the nucleoli were much smaller than in the parent wheat line lacking the *Aegilops* chromosome (Martini et al. 1982); nucleolar dominance of the rDNA of *Aegilops* and suppression of the wheat rRNA genes were explained by a higher number of subrepeats with enhancer functions in the IGS of *Aegilops* (Flavell et al. 1986), but it might also be influenced by other factors. Moreover, Flavell et al. (1983, 1988) could show that in aneuploid lines of wheat, which include different doses of chromosomes carrying the NORs and which therefore vary in the number rRNA genes (between 3700 and 14 000), the proportion of DNA not digestible with the methylation-sensitive restriction enzyme HpaII increased with an increasing amount of rDNA.

In studies on wheat and pea (*Pisum sativum*) rDNA a correlation between DNA methylation and DNase I hypersensitivity as an indication for a differential chromatin structure along the rDNA stretches was proved (Thompson et al. 1988). Interestingly, a relationship between light stimulation of transcription and use of different rDNA length repeat classes could be shown (Kaufman et al. 1987). Length heterogeneity of the rDNA repeats of pea (*Pisum sativum* var. Alaska) originates from a different number approx. 180-bp subrepeats upstream of the TIS (Fig. 2). The longer rDNA repeats (L-variants 9 kbp in length) comprising 74% of rDNA are located on chromosome 7, the shorter repeats (S-variants 8.6 kbp in length) are localized on chromosome 4 (Ellis et al. 1984; Polans et al. 1986). L-variants are probably preferentially transcribed in the dark and in light-grown seedlings indicated by specific, hypersensitive DNase I sites near the promoter region; after light treatment the shorter repeats started to be transcribed and became sensitive to DNase I in this region, correlating with the occurrence of a demethylated HpaII site localized 31 bp downstream of the TIS (Piller et al. 1990).

Furthermore, detailed DNA methylation studies were carried out with members of the Cucurbitaceae generally equipped with a large number of ribosomal DNA (Ingle et al. 1975; Hemleben et al. 1988). *Cucurbita pepo* (zucchini) contains approx. 10 000 rDNA repeats per somatic cell; at least 70% of the repeats is completely methylated which was demonstrated after digestion of total DNA with the respective methylation-sensitive restriction endonucleases (Fig. 3). A fraction of approx. 3–5% exhibited only one accessible restriction site per repeat irrespective of the number of restriction sites actually present in the repeats and the methylation-sensitive enzymes used. Another small percentage of the repeats appeared completely unmethylated; these are proposed to represent the actively transcribed portion of rDNA (R.A. Torres and V. Hemleben, in prep.). A similar behaviour of the rDNA repeats was observed for cucumber (*Cucumis sativus*) with an



**Fig. 3.** Methylation analysis of the rDNA of *Cucurbita pepo* (zucchini). Enriched nuclear rDNA was restricted with restriction endonucleases in single or double digests. The DNA was separated on a 1% agarose gel (A), Southern-blotted and hybridized to <sup>32</sup>P-labelled 18S and 25S rRNA (B) or to a <sup>32</sup>P-labelled 18S rDNA fragment (C). Lane 1 lambda DNA, HindIII digested; lane 8 lambda DNA, EcoRI digested; lanes 2–7 zucchini rDNA digested with HindIII (lane 2), HindIII/HpaII (lane 3), HpaII (lane 4), CfoI (lane 5), CfoI/EcoRV (lane 6); EcoRV (lane 7). Note that a few distinct bands are visible cutting the DNA with the methylation-sensitive restriction enzymes HpaII (CCGG) and CfoI (GCGC); most of the rDNA remained undigested with HpaII and CfoI

even higher amount of rRNA genes (Ganal et al. 1988; Hemleben et al. 1988).

/ Previous reports that a specific demethylated HpaII site in front of the TIS is a general feature of all actively transcribed repeats (von Kalm et al. 1986; Flavell et al. 1988; Hemleben et al. 1988) are probably not valid for all plants since such a site is not always present in this region. However, studies on *C. pepo* rDNA showed that a specific fraction of rDNA repeats exhibits one randomly distributed demethylated restriction enzyme site irrespective of the methylation-sensitive enzyme tested. Thus, if an HpaII site is present in the promoter region, such a site will be randomly accessible for this restriction enzyme, possibly indicating that these repeats are prepared to be transcribed.

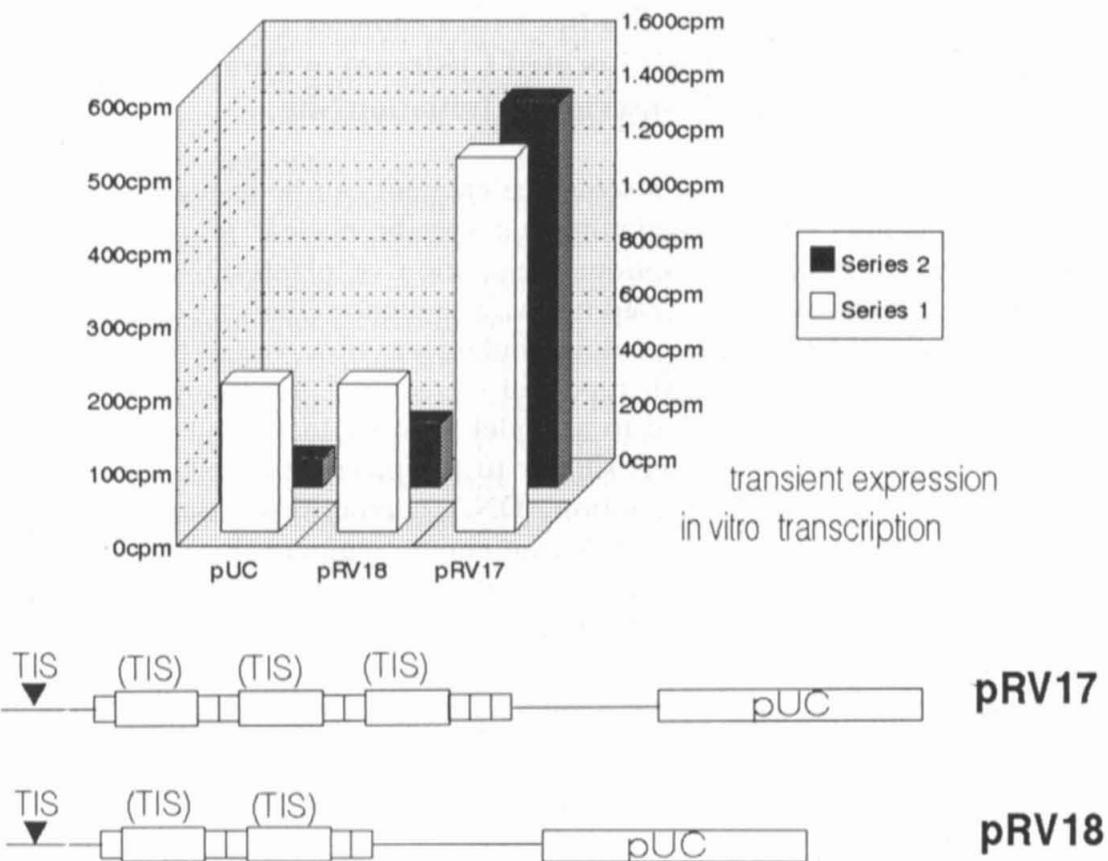
## 5 Functional Role of Repeated Elements Within the Intergenic Spacer (IGS)

The phenomenon of nucleolar dominance in partial hybrids has already provided some evidence for the functional role of repeated elements in the IGS of plant ribosomal DNA. These observations were further supported by investigations on *Triticale* partial hybrids and by in vitro transcription assays. Moreover, protein binding studies with rDNA fragments of the IGS and nuclear proteins have elucidated the functional role of rDNA subrepeats.

### 5.1 Transcription-Enhancing Elements Characterized by in Vivo and in Vitro Studies

In vivo, with *Triticum/Aegilops* partial hybrids, it could be demonstrated that only the *Aegilops umbellulata* rDNA repeats, containing a higher number of subrepeats upstream of the TIS supposed to fulfil enhancer functions, were transcriptionally active and formed a prominent nucleolus structure. In contrast, the rRNA genes located on the wheat chromosomes with less subrepeats within the IGS appeared relatively inactive (Flavell et al. 1986). Similar effects could also be observed with wheat-rye hybrids characterized by Appels et al. (1986): microscope observation indicated that the rye nucleolus was largely suppressed in the presence of a wheat NOR locus. In molecular studies it could be shown that the wheat rDNA repeats predominantly contributed to transcriptional activity, whereas the rye rDNA repeats appeared to be repressed (Capesius and Appels 1989). In fact, wheat and rye rDNA subrepeats in the IGS share regions of sequence similarity (Appels and Dvorak 1982; Appels et al. 1986), suggesting a functional role as protein binding sites.

In mung bean (*Vigna radiata*) length heterogenous rDNA repeats are observed differing mostly in the number of complex 5' ETS subrepeats containing a TIS-like sequence and in the different numbers of 174-bp subrepeats in the 3' IGS (Gerstner et al. 1988; Schiebel et al. 1989). Only cloned rDNA from longer repeats containing three ETS subrepeats subsequent to the first TIS (see Figs. 2 and 4) was transcribed using in vitro transcription assays with isolated nuclei, whereas with rDNA constructs containing the corresponding region of the shorter rDNA variants with two ETS subrepeats nearly no transcription could be observed (Fig. 4). This result was confirmed by transient expression experiments: the cloned rDNA sequences were introduced into protoplasts of mung bean by electroporation and newly transcribed RNA products of the template were measured. Again, only the rDNA constructs containing more ETS repeats were preferentially transcribed, indicating that subrepeats within the 5' ETS might have some stimulatory effect on transcription in vivo (K. Unfried and V. Hemleben unpubl. results).



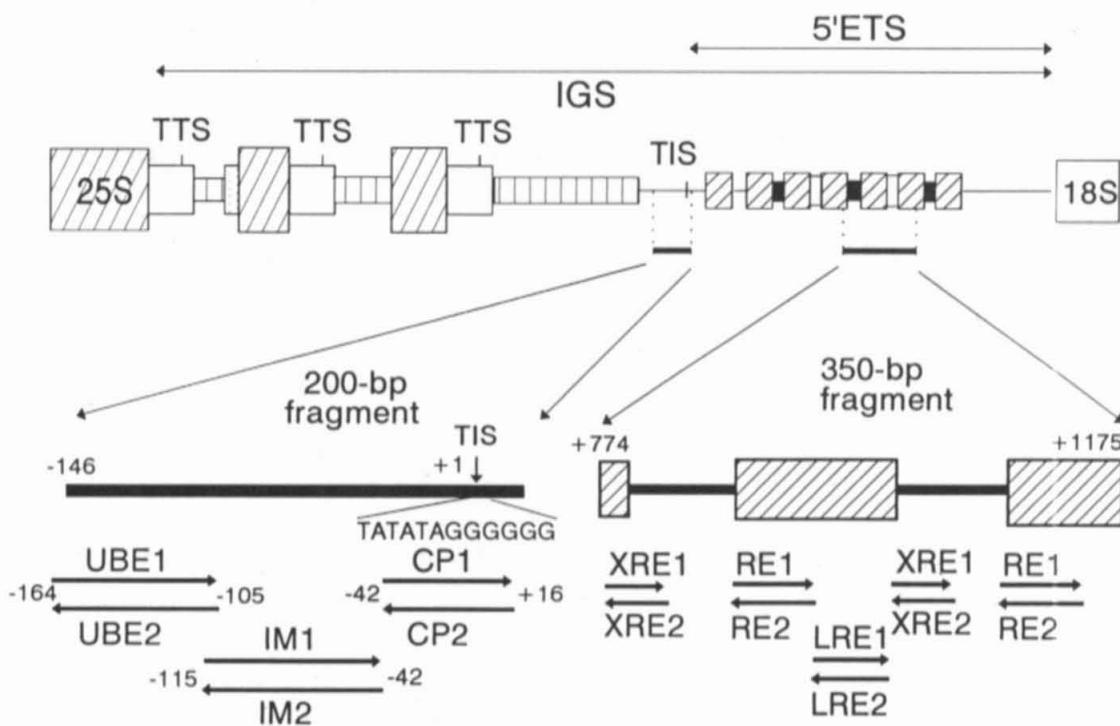
**Fig. 4.** In vitro transcription and transient expression assays with cloned rDNA fragments of mung bean (*Vigna radiata*). The clone *pRV17*, containing three complex ETS subrepeats, and the clone *pRV18*, containing two ETS subrepeats in addition to the main promoter region (Gerstner et al. 1988) cloned into a pUC vector, were applied to in vitro transcription with isolated nuclei (series 1, white boxes) or to transient expression assays in protoplasts of mung bean after electroporation (series 2, black boxes) in the presence of a low concentration of alpha-amanitin (100 µg/ml) which prevented pol II and pol III transcription. The transcription products were blotted onto nitrocellulose and hybridized with  $^{32}\text{P}$ -labelled pUC to estimate the amount of newly transcribed RNA after measuring the radioactivity (cpm). A pUC vector was used as control. (K. Unfried and V. Hemleben unpubl.)

The question arose whether subrepeats located upstream *or* downstream of the TIS can fulfil a similar enhancer-like function. For instance, as already mentioned, the length of the potato and tomato IGS is nearly the same (approx. 9 kbp); however, the location and the number of subrepeats in the IGS are different (see Fig. 2). The tomato IGS shows shorter and fewer subrepeats upstream of the TIS, but the difference in length is compensated by another type of subrepeat in the 5' ETS, where in potato only one of those sequences is present (Borisjuk and Hemleben 1993). Now it will be of interest to see whether a functional compensation of the different repeat types is possible.

## 5.2 Protein Binding Studies with Promoter Fragments and Repeated Elements Located Upstream or Downstream of the Transcription Initiation Site

The functional significance of a sequence element can be tested by studying DNA-protein interactions, assuming that specific nuclear *trans*-acting proteins are involved in transcriptional regulation of plant rDNA. The first studies reported have been carried out with sequence elements of maize IGS (Schmitz et al. 1989). Specific protein binding was observed at the suggested promoter region and also with repeated elements 5' upstream of the TIS (see Fig. 2). These findings led to a model showing that sequences in front of the promoter attract proteins similar to the promoter itself, thus having a stimulatory effect on transcription. DNA competition assays on wheat rDNA have shown that subrepeat A, containing fragments located upstream of the TIS, competes with specific promoter fragments for similar proteins (Jackson and Flavell 1992) confirming the enhancer-like function of these repeats as proposed earlier (Flavell et al. 1986).

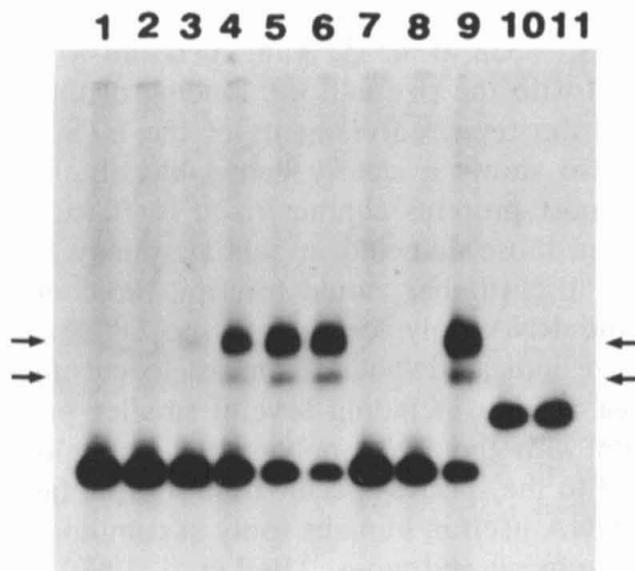
Echeverria et al. (1992) investigated the capacity of repeated elements upstream of the TIS in radish rDNA (see Fig. 2) to form specific complexes with nuclear proteins, assuming that these subrepeats play an enhancing role



**Fig. 5.** The intergenic spacer of a rDNA repeat of cucumber. Enlarged are the fragments (or oligonucleotides) used in DNA-protein binding assays: 200-bp promoter fragment containing the TIS and 350-bp fragment containing repeated elements of the 5' ETS. The arrows indicate the oligonucleotides used for protein binding and competition studies in the single- and double-stranded form. *UBE* upstream binding element; *CP* core promoter; *LRE* loop-forming element; *XRE* *Xenopus*-like repeated element; *RE* repeated element

in transcription. Actually, specific protein binding could be demonstrated with the dA/dT-rich regions between the subrepeats and with the dA/dT-rich sequences in the promoter region as proved by competition gel mobility shift and footprinting assays. A functional role of this DNA-protein interaction is as yet unknown.

More detailed information on nuclear proteins specifically recognizing functional rDNA spacer elements is now available for cucumber (*Cucumis sativus*) rDNA (Zentgraf and Hemleben 1992). Preliminary studies have revealed that sequences surrounding the TIS and repeated elements present in the 5' ETS (Fig. 5) attract similar proteins as shown by competition assays (Fig. 6). Dissecting the promoter region into smaller fragments, which were then applied to gel mobility shift assays with 0.42 M salt nuclear protein extracts from cucumber cotyledons, revealed that an upstream binding element (UBE) and a core promoter (CP) sequence bind the same proteins shown by competition assays. An intermediate sequence (IM) between these regions does not react with any nuclear protein. Remarkably, the DNA-protein interactions obtained with UBE or CP could compete with an LRE ("loop-forming" repeated element, since secondary structures can be formed; Zentgraf et al. 1990) of the 5' ETS subrepeats. Another part of the ETS subrepeats, called XRE ("Xenopus-like" repeated element, containing a sequence motif with high similarity to terminator sequences characterized



**Fig. 6.** Gel mobility shift assays with rDNA fragments and a crude nuclear protein extract of cucumber. A  $^{32}\text{P}$ -labelled putative promoter fragment spanning from -146 to +41 (see Fig. 5) including the TIS was incubated with increasing amounts of a 0.42 M salt nuclear extract: without nuclear proteins (lane 1), 5 µg (lane 2), 10 µg (lane 3), 20 µg (lane 4), 30 µg (lane 5), 50 µg (lane 6); lanes 7-9 200-bp promoter fragment with 50 µg nuclear proteins and a 100-fold excess of unlabelled 200-bp fragment (lane 7), with a 100-fold excess of a 350-bp ETS fragment (lane 8), and a 100-fold excess of an unspecific 123-bp ladder DNA (lane 9). As control a pUC fragment was incubated without (lane 10) or with 50 µg nuclear proteins (lane 11). The DNA-protein complexes formed are indicated by arrows. (Zentgraf and Hemleben 1992)

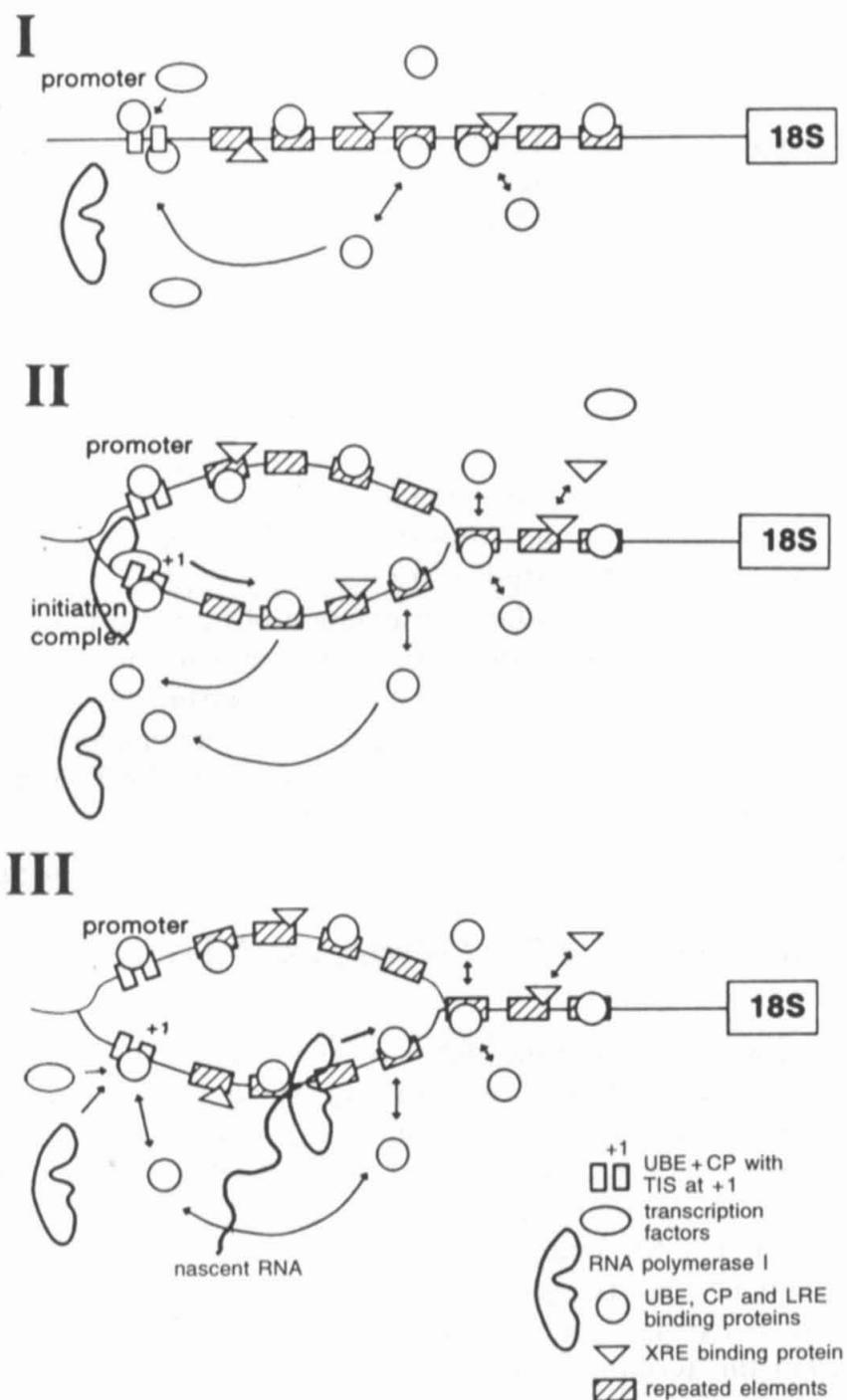
for *Xenopus* rDNA; Labhart and Reeder 1987; Schiebel et al. 1989), also reacts with nuclear proteins but does not interfere with these promoter complexes. Fragments used in these assays are shown in Fig. 5. The striking fact found in these studies was that for all sequence elements investigated the interactions with nuclear proteins occurred also with DNA fragments in the single-stranded form (Zentgraf and Hemleben 1993).

Possibly, higher order structures within the elements rather than a defined sequence play a role in these interactions, although short sequence motifs may be important (Jackson and Flavell 1992; Zentgraf and Hemleben 1992). For instance, the LRE of cucumber contains a short motif -TGTGGGT- which is present in a corresponding region of the ETS of several higher plants (Gerstner et al. 1988; Schmidt-Puchta et al. 1989).

The proteins reacting with rDNA elements of cucumber were characterized by affinity column chromatography, South-Western blots and after eluting the protein component of a DNA-protein complex in gel mobility shift assays (Zentgraf and Hemleben 1992): three proteins with the molecular masses of 16, 22 and 24 kDa are predominantly involved in the binding at the UBE and CP of the pol I promoter; only the 16-kDa protein binds directly to the LRE of the ETS subrepeats, whereas XRE reacts with a completely different protein of 70 kDa. Again, the function of these proteins can be only speculated.

The 16-kDa protein might be analogous to the p16 protein of rat which is a "HMG-like protein" binding the upstream activating sequences and a segment in the ETS (+352 to +545; Yang-Yen and Rothblum 1988). This correlates very well with the size and the binding of the 16-kDa protein of *Cucumis sativus* to the repeated elements of the ETS, but, in contrast to rat, this protein also shows a clearly detectable affinity to the promoter sequences. Since most proteins characterized for cucumber have a lower molecular mass than those detected in animal systems, smaller proteins in plants, particularly in cucumber, could transmit the contact of other transcription factors and RNA polymerase I to the DNA. South-Western experiments for maize indicated that in contrast to cucumber more proteins with DNA-binding affinity including several smaller ones are involved in complex formation with the RNA polymerase I promoter. This situation could be compared to the species-specific transcription factor SL1 which has no affinity to the DNA itself in humans (only in combination with UBF) but shows DNA binding in rat and mouse (Bell et al. 1990).

Nevertheless, these small proteins might be "HMG-like" proteins. HMG 1 and HMG 2 subgroups ( $M_r$  approximately 25 kDa) are comparable in size and also bind to double- and single-stranded DNA. In general, HMG proteins, including plant HMG proteins as well, are associated with transcriptional active chromatin (Spiker et al. 1983) and might play a role in transcriptional activation processes of RNA polymerase II in plants. Obviously, p16 of rat and the HMG homology boxes of different animal UBF reveal that "HMG-like" proteins seem to be involved in activating RNA polymerase I transcription.



**Fig. 7.** A model demonstrating how the initiation of RNA polymerase I transcription in cucumber might occur. **I** DNA-binding proteins interact with the promoter and the ETS repeats in a double-stranded stage and are enriched in this nuclear compartment. **II** The complementary strands are separated upstream of UBE, the DNA-binding proteins can bind to the single-stranded DNA form of the respective binding sites, additional transcription factors interact with the promoter region and mediate the contact of RNA polymerase I to the DNA to form the transcription initiation complex. **III** DNA-binding proteins can bind again to the single-stranded DNAs after the RNA polymerase I has passed into the elongation process; they can keep the transcription fork open, thus enhancing the next transcriptional round

The possible role of these DNA-protein interactions in transcriptional regulation has been shown in a model (Fig. 7), illustrating how specific proteins might interact and fulfil a stimulatory function in the transcription process: the complementary DNA strands are separated at an AT-rich region upstream of the promoter. Small proteins previously binding at the ETS subrepeats downstream of the TIS can now bind to the single-stranded UBE and CP region of the promoter, and interact with pol I and possibly with other transcription factors, directing the polymerase to the starting point. During the elongation process, these proteins could keep the DNA template accessible to transcription.

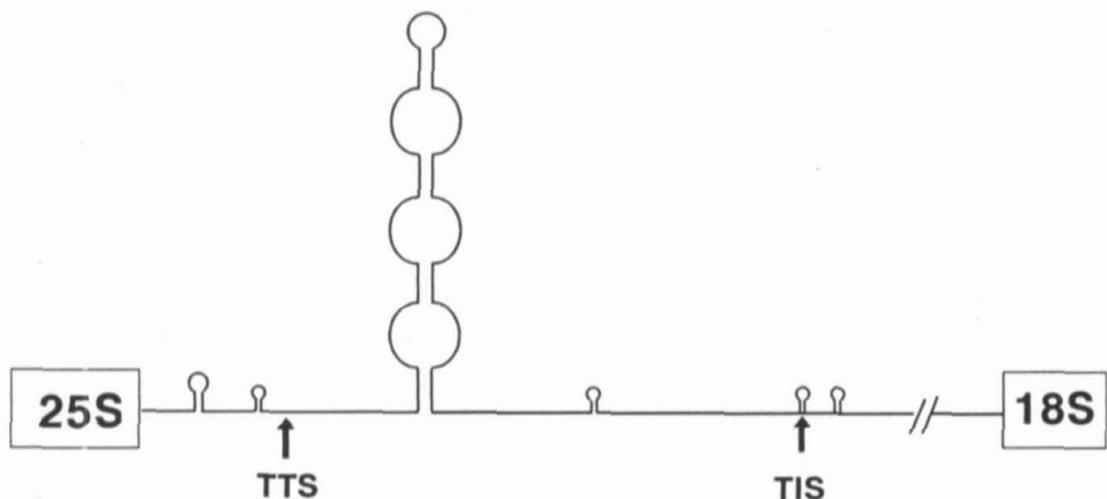
Summarizing the results on in vitro protein binding studies on maize, wheat, radish and cucumber rDNA, it can be concluded that repeated elements located upstream or downstream of the TIS could be involved in stimulating the transcription initiation process. However, more efficient in vivo and in vitro transcription studies are necessary in plant systems.

The questions of how and where termination of transcription occurs and whether termination of pol I transcription is coupled with the initiation process in a "read through enhancement" mechanism, as proposed for animal rDNA (Mitchelson and Moss 1987), are still not completely solved as yet. However, some evidence was obtained by localizing the TTS in the IGS of plant rDNA (see Fig. 2).

## 6 Termination of RNA Polymerase I Transcription

Transcriptional termination of the rRNA precursor has been determined for radish, mung bean, cucumber, zucchini and wheat by S1 or mung bean nuclease mapping (Delcasso-Tremousaygue et al. 1988; Schiebel et al. 1989; Vincentz and Flavell 1989; Zentgraf et al. 1990; K. King and V. Hemleben unpubl. results). A consensus sequence as observed for the TIS cannot be defined, although for mung bean, a region with 70% similarity to a terminator sequence in *Xenopus* rDNA was found near the termination signal present in each of the 174-bp repeats in the IGS (Schiebel et al. 1989). Whether this terminator acts concomitantly as enhancer of the transcription initiation process as it was shown for the T3-box (-GACTTGC-box) in *Xenopus* (Labhart and Reeder 1987) awaits further functional analysis. For mung bean a model of a higher order stem-loop structure that would support a "read through enhancement" was proposed (Fig. 8): the presence of inverted sequences within each of the 174-bp subrepeats allows the formation of a complex, higher order structure in the 3' ETS which brings the transcription termination site into closer proximity of the TIS, thus facilitating initiation of transcription of RNA polymerase I at the subsequent TIS.

Sequences with similarity to the -GACTTGC- box are found in the IGS of other plants, e.g., in the ETS repeats of cucumber (XRE; see Fig. 5),



**Fig. 8.** Possible secondary structure in the intergenic spacer of mung bean (*Vigna radiata*). The occurrence of inverted repeats in each of the 175-bp spacer subrepeats allows the formation of a complex stem-loop structure which brings the TTS into close proximity of the TIS. Small stem loops indicate a processing site and the secondary structures possibly formed near the TTS and TIS, respectively. (Schiebel et al. 1989)

which are suggested to take part in transcriptional regulation (Zentgraf and Hemleben 1992). In other *Vigna* species, however, the 174-bp repeated elements also present in the 3' IGS region do not contain a perfectly similar -GACTTGC- box, leaving only the possibility that the formation of a stem-loop structure might be of functional relevance for transcriptional termination (Unfried et al. 1991).

Several distinct termination signals were also detected in the 3' IGS of cucumber which could be correlated with the stem-loop secondary structures probably formed (Zentgraf et al. 1990), since in this region of the IGS duplications of the 3' end of the 25S rDNA and, subsequently, flanking sequences occur (Ganal et al. 1988). Obviously, in cucumber cells rRNA precursor molecules of different length are synthesized and later processed to the mature rRNAs. Different precursor lengths can be expected also for those plants which exhibit different numbers of 5'ETS subrepeats in their rDNA length variants as shown to occur in mung bean (Gerstner et al. 1988).

## 7 Conclusions

Detailed analysis of the nucleotide sequence, structure and organization of the ribosomal RNA genes of various higher plants indicated that, in higher plants, a remarkable high variability in length and sequence of the IGS appears. Among representatives of different plant families only the se-

quences around the TIS show some similarities; in addition, some common motifs occur.

The pol I promoter in higher plants apparently can be divided into a core promoter (CP; between approx. +5 to -64) and an upstream control element (UCE; between approx. -120 to -160), as shown by protein binding studies on cucumber and wheat which suggests that the pol I promoter is similarly organized as in animal cells (see Sollner-Webb and Mougey 1991). The results obtained with protein binding experiments on radish rDNA (Echeverria et al. 1992) demonstrate that specific protein binding is also obtained with a dA/dT-rich region of the promoter. Characterization of the proteins revealed that in cucumber mainly three small proteins, 16, 22 and 24 kDa in size, are involved in binding to the promoter region and to repeats located downstream of the ETS (Zentgraf and Hemleben 1992) and that in maize, several proteins, including some smaller ones, bind to the promoter region and to repeated elements located upstream (Schmitz et al. 1989).

Amplification and recombination processes can affect practically every region of the IGS, resulting in subrepeats which are differently organized; some of them are probably used as transcriptional control elements or as processing signals. The suggestion previously proposed that subrepeats in front of the TIS are used to attract protein factors responsible for the enhancement of transcription could now be extended. Functional analysis by *in vivo* and *in vitro* studies gave some evidence that subrepeats upstream and downstream of the TIS, respectively, can be involved in transcriptional regulation of pol I. These results led to models suggesting that repeated *cis*-elements can support transcription initiation by interaction with *trans*-factors independent of their localization with respect to the promoter region, therewith following the requirements of enhancer sequences.

More detailed analyses are necessary to prove the importance and functional relevance of short motifs occurring throughout the IGS with similarity to sequences known to be involved in tissue or stimulus specificity of pol II transcription (Weising and Kahl 1990). Transcription of rRNA by pol I can be developmentally or exogenously controlled by up- or down-regulation (during development, hormone control, light stimulation, starvation or heat shock conditions, to mention only some possibilities). Therefore, it can be expected that in some cases coregulation occurs between pol I and pol II (probably also pol III) transcription mediated by common *trans*-acting factors which are concomitantly interacting with *cis*-elements located in the rDNA IGS and in front of pol II-regulated structural genes. However, at this stage it is too early to present a complete picture of the regulation of pol I transcription in plants.

## References

- Agarwal ML, Aldrich J, Agarwal A, Cullis CA (1992) The flax ribosomal RNA-encoding genes are arranged in tandem at a single locus interspersed by "non-rDNA" sequences. *Gene* 120:151-156

- Appels R, Dvorak J (1982) The wheat ribosomal DNA spacer region: its structure and variation in populations and among species. *Theor Appl Genet* 63:337–348
- Appels R, Moran LB, Gustafson JP (1986) The structure of DNA from rye (*Secale cereale*) NOR R1 locus and its behaviour in wheat backgrounds. *Can J Genet Cytol* 28:673–685
- Baerson SR, Kaufman LS (1990) Increased rRNA gene activity during a specific window of early pea leaf development. *Mol Cell Biol* 10:842–845
- Barker RF, Harberd NP, Jarvis MG, Flavell RB (1988) Structure and evolution of the intergenic region in a ribosomal DNA repeat unit of wheat. *J Mol Biol* 201:1–17
- Bell SP, Pikaard CS, Reeder RH, Tjian R (1989) Molecular mechanisms governing species specific transcription of ribosomal RNA. *Cell* 59:489–497
- Bell SP, Jantzen H-M, Tjian R (1990) Assembly of alternative multiprotein complexes directs rRNA promoter selectivity. *Genes Dev* 4:943–954
- Borisjuk N, Hemleben V (1993) Nucleotide sequence of the potato rDNA intergenic spacer. *Plant Mol Biol* 21:381–384
- Broad JR, Li Y-Y, Feldman J, Jayaram M, Abraham J, Nasmyth KA, Hicks JB (1983) Localization and sequence analysis of yeast origins of DNA replication. *Cold Spring Harbor Symp Quant Biol* 47:1165–1173
- Capesius I, Appels R (1989) The direct measurement of ribosomal RNA gene activity in wheat-rye hybrids. *Genome* 32:343–346
- Delcasso-Tremousaygue D, Grellet F, Panabieres F, Ananiev ED, Delseney M (1988) Structural and transcriptional characterization of the external spacer of a ribosomal RNA nuclear gene from a higher plant. *Eur J Biochem* 172:767–776
- Delseney M, Cooke R, Penon P (1983) Sequence heterogeneity in radish nuclear ribosomal RNA genes. *Plant Sci Lett* 30:107–119
- DeWinter R, Moss T (1987) A complex array of sequences enhances ribosomal transcription in *Xenopus laevis*. *J Mol Biol* 196:813–827
- Dover GA (1986) Molecular drive in multigene families: how biological novelties arise, spread and are assimilated. *Trends Genet* 2:159–165
- Echeverria M, Delcasso-Tremousaygue D, Delseney M (1992) A nuclear protein fraction binding to dA/dT-rich sequences upstream from the radish rDNA promoter. *Plant J* 2:211–219
- Ellis THN, Davies DR, Castleton JA, Bedford ID (1984) The organization and genetics of rDNA length variants in peas. *Chromosoma* 91:74–81
- Flavell RB, O'Dell M, Thompson WF (1983) Cytosine methylation of ribosomal RNA genes and nucleolus organiser activity in wheat. In: Brandham PE, Bennett MD (eds) *Kew Chromosome Conference II*. George Allen and Unwin, Sydney, pp 11–17
- Flavell RB, O'Dell M, Vincentz M, Sardana R, Barker RF (1986) The differential expression of ribosomal RNA genes. *Philos Trans R Soc Lond B* 314:385–397
- Flavell RB, O'Dell M, Thompson WF (1988) Regulation of cytosine methylation in ribosomal DNA and nucleolus organizer expression in wheat. *J Mol Biol* 204:523–534
- Ganal M, Torres R, Hemleben V (1988) Complex structure of the ribosomal DNA spacer of *Cucumis sativus* (cucumber). *Mol Gen Genet* 212:548–554
- Gerstner J, Schiebel K, von Waldburg G, Hemleben V (1988) Complex organization of the length heterogeneous 5' external spacer of mung bean (*Vigna radiata*) ribosomal DNA. *Genome* 30:723–733
- Grierson D, Loening UE (1972) Distinct transcription products of ribosomal genes in two different tissues. *Nat New Biol* 235:80–82
- Grierson D, Loening UE (1974) Ribosomal RNA precursors and the synthesis of chloroplast and cytoplasmic ribosomal ribonucleic acids in leaves of *Phaseolus aureus*. *Eur J Biochem* 44:501–507
- Gruendler P, Unfried I, Pascher K, Schweizer D (1991) rDNA intergenic region from *Arabidopsis thaliana*. Structural analysis, intraspecific variation and functional implications. *J Mol Biol* 221:1209–1222
- Grummt I, Roth E, Paule MR (1982) Ribosomal RNA transcription in vitro is species specific. *Nature* 296:173–174

- Guilfoyle TJ, Lin CY, Chen YM, Nagao RT, Key JL (1975) Enhancement of soybean RNA polymerase I by auxin. *Proc Natl Acad Sci USA* 72:69–72
- Hemleben V, Grierson D (1978) Evidence that in higher plants the 25S and 18S genes are not interpersed with genes for 5S rRNA. *Chromosoma* 65:353–358
- Hemleben V, Leweke B, Roth A, Stadler J (1982) Organization of highly repetitive satellite DNA from two Cucurbitaceae species (*Cucumis melo* and *Cucumis sativus*). *Nucleic Acids Res* 10, 631–644
- Hemleben V, Ganal M, Gerstner J, Schiebel K, Torres RA (1988) Organization and length heterogeneity of plant ribosomal RNA genes. In: Kahl G (ed) *Architecture of eukaryotic genes*. VHC, Weinheim, pp 371–383
- Hemleben V, Zentgraf U, King K, Borisjuk N, Schweizer G (1992) Middle repetitive and highly repetitive sequences detect polymorphisms in plants. In: Kahl G, Appelhans H, Kömpf J, Driesel AJ (eds) *DNA-polymorphisms in eukaryotic genomes*. BTF 10, Adv. Mol Gen 5. Huethig, Heidelberg, pp 157–170
- Hernandez P, Bjerknes CA, Lamm SS, Van't Hof (1988) Proximity of an ARS consensus sequence to a replication origin of pea (*Pisum sativum*). *Plant Mol Biol* 10:413–422
- Ingle J, Timmis JN, Sinclair J (1975) The relationship between satellite DNA, ribosomal RNA gene redundancy, and genome size in plants. *Plant Physiol* 55:496–501
- Jackson SD, Flavell RB (1992) Protein-binding to reiterated motifs within the wheat rRNA gene promoter and upstream repeats. *Plant Mol Biol* 20:911–919
- Jendrisak J (1980) Purification, structures and functions of the nuclear RNA polymerases from higher plants. In: Leaver C (ed) *Genome organization and expression in plants*. NATO Adv Study Inst Ser: Series A, Life Sciences. Plenum Press, New York, pp 77–92
- Kato A, Nakajima T, Yamashita J, Yakura K, Tanifuji S (1990) The structure of the large spacer region of the rDNA in *Vicia faba* and *Pisum sativum*. *Plant Mol Biol* 14:983–993
- Kaufman LSJ, Watson JC, Thompson WF (1987) Light regulated changes in DNase I hypersensitive sites in the rDNA genes of *Pisum sativum*. *Proc Natl Acad Sci USA* 84:1550–1554
- Kelly RJ, Siegel A (1989) The *Cucurbita maxima* intergenic spacer has a complex structure. *Gene* 80:239–248
- King K, Torres RA, Zentgraf U, Hemleben V (1993) Molecular evolution of the intergenic spacer in the nuclear ribosomal RNA genes of Cucurbitaceae. *J Mol Evol* 36:144–152
- Labhart P, Reeder RH (1987) A 12-base pair sequence is an essential element of the ribosomal gene terminator in *Xenopus laevis*. *Mol Cell Biol* 7:1900–1905
- Leweke B, Hemleben V (1982) Organization of rDNA in chromatin: plants. In: Busch H, Rothblum L (eds) *The cell nucleus: rDNA*. Vol XI, part B. Academic Press, New York, pp 225–253
- Martini G, O'Dell M, Flavell RB (1982) Partial inactivation of wheat nucleolus organisers by the nucleolus organiser chromosomes from *Aegilops umbellulata*. *Chromosoma* 84:687–700
- Miesfeld R, Arnheim N (1984) Species-specific rDNA transcription is due to promoter-specific binding factors. *Mol Cell Biol* 4:221–227
- Mitchelson K, Moss T (1987) The enhancement of ribosomal transcription by recycling of RNA polymerase I. *Nucleic Acids Res* 15:9577–9596
- Motte PM, Loppes R, Menager M, Deltour R (1991) Three-dimensional electron microscopy of ribosomal chromatin in two higher plants: a cytochemical, immunocytochemical, and *in situ* hybridization approach. *J Histochem Cytochem* 39:1495–1506
- Nakajima T, Suzuki A, Tanifuji S, Kato A (1992) Characterization of nucleotide sequences that interact with a nuclear protein fraction in rRNA gene of *Vicia faba*. *Plant Mol Biol* 20:939–949
- Perry KL, Palukatis P (1990) Transcription of tomato ribosomal DNA and the organization of the intergenic spacer. *Mol Gen Genet* 221:102–112

- Perry RP (1976) Processing of RNA. *Annu Rev Biochem* 45:605–629
- Pikaard CS, McStay B, Schultz MC, Bell SP, Reeder RH (1989) The *Xenopus* ribosomal gene enhancers bind an essential polymerase I transcription factor, xUBF. *Genes Dev* 3:1779–1788
- Piller KJ, Baerson SR, Polans NO, Kaufman LS (1990) Structural analysis of the short length ribosomal DNA variant from *Pisum sativum* L. cv. Alaska. *Nucleic Acids Res* 18:3135–3145
- Polans NO, Weeden HF, Thompson WF (1986) Distribution, inheritance and linkage relationship of ribosomal DNA spacer length variants in pea. *Theor Appl Genet* 72:289–295
- Rathgeber J, Capesius I (1990) Nucleotide sequence of the intergenic spacer and the 18S ribosomal RNA gene from mustard (*Sinapis alba*). *Nucleic Acids Res* 18:1288
- Rogers MM, Loening UE, Fraser RSS (1970) Ribosomal RNA precursors in plants. *J Mol Biol* 49:681–692
- Rogers SO, Bendich AJ (1987) Ribosomal RNA genes in plants: variability in copy number and in the intergenic spacer. *Plant Mol Biol* 9:509–520
- Rogers SO, Honda S, Bendich AJ (1986) Variation in the ribosomal RNA genes among individuals of *Vicia faba*. *Plant Mol Biol* 6:339–345
- Rungger D, Crippa M (1977) The primary ribosomal DNA transcript in eukaryotes. *Proc Biophys Mol Biol* 31:247–269
- Schiebel K, von Waldburg G, Gerstner J, Hemleben V (1989) Termination of transcription of ribosomal RNA genes of mung bean occurs within a 175 bp repetitive element of the spacer region. *Mol Gen Genet* 218:302–307
- Schmidt-Puchta W, Günther I, Sänger HL (1989) Nucleotide sequence of the intergenic spacer (IGS) of the tomato ribosomal DNA. *Plant Mol Biol* 13:251–253
- Schmitz ML, Maier UG, Brown JWS, Feix G (1989) Specific binding of nuclear proteins to the promoter region of a maize nuclear rRNA gene unit. *J Biol Chem* 264:1467–1472
- Scott NS, Kavanagh TA, Timmis JN (1984) Methylation of rRNA genes in some higher plants. *Plant Sci Lett* 35:213–217
- Shagai-Marcoff MA, Soliman KM, Jorgensen RA, Allard RW (1984) Ribosomal DNA spacer length polymorphisms in barley: Mendelian inheritance, chromosomal location, and population dynamics. *Proc Natl Acad Sci USA* 81:8014–8018
- Sollner-Webb B, Mougey EB (1991) News from the nucleolus: rRNA gene expression. *Trends Biochem Sci* 16:58–62
- Sollner-Webb B, Tower J (1986) Transcription of cloned eukaryotic ribosomal RNA genes. *Annu Rev Biochem* 55:801–830
- Sommerville J (1986) Nucleolar structure and ribosome biosynthesis. *Trends Biochem Sci* 11:438–442
- Spiker S, Murray MG, Thompson WF (1983) DNase I sensitivity of transcriptionally active genes in intact nuclei and isolated chromatin of plants. *Proc Natl Acad Sci USA* 80:815–819
- Tautz D, Tautz C, Webb D, Dover GA (1987) Evolutionary divergence of promoters and spacers in the rDNA family of four *Drosophila* species. *J Mol Biol* 195:525–542
- Thompson WF, Flavell RB (1988) DNase I sensitivity of ribosomal RNA genes in chromatin and nucleolar dominance in wheat. *J Mol Biol* 204:535–548
- Thompson WF, Flavell RB, Watson JC, Kaufman LS (1988) Chromatin structure and expression of plant ribosomal RNA genes. In: Kahl G (ed) *Architecture of eukaryotic genes*. VCH, Weinheim, pp 385–396
- Tobin EM, Silverthorne (1985) Light regulation of gene expression in higher plants. *Annu Rev Plant Physiol* 36:569–593
- Toloczyki C, Feix G (1986) Occurrence of 9 homologous repeat units in the external spacer region of a nuclear maize rRNA gene unit. *Nucleic Acids Res* 14:4969–4986
- Torres RA, Hemleben V (1991) Use of ribosomal DNA spacer probes to distinguish cultivars of *Cucurbita pepo* L. and other Cucurbitaceae. *Euphytica* 53:11–17

- Torres RA, Zentgraf U, Hemleben V (1989) Species and genus specificity of the intergenic spacer (IGS) in the ribosomal RNA genes of Cucurbitaceae. *Z Naturforsch* 44c:1029–1034
- Torres RA, Ganal M, Hemleben V (1990) GC balance in the internal transcribed spacers ITS 1 and ITS 2 of nuclear ribosomal RNA genes. *J Mol Evol* 30:170–181
- Ueki M, Uchizawa E, Yakura K (1992) The nucleotide sequence of the rDNA intergenic spacer region in a wild species of the genus *Vicia*, *V. angustifolia*. *Plant Mol Biol* 18:175–178
- Unfried K, Schiebel K, Hemleben V (1991) Subrepeats of rDNA intergenic spacer present as prominent independent satellite DNA in *Vigna radiata* but not in *Vigna angularis*. *Gene* 99:63–68
- Van't Hof J, Lamm SS (1992) Site of initiation of replication of the ribosomal genes of pea (*Pisum sativum*) detected by two-dimensional gel electrophoresis. *Plant Mol Biol* 20:377–382
- Vincentz M, Flavell RB (1989) Mapping of ribosomal RNA transcripts in wheat. *Plant Cell* 1:579–589
- Von Kalm L, Vize PD, Smyth DR (1986) An under methylated region in the spacer of ribosomal RNA genes of *Lilium henryi*. *Plant Mol Biol* 6:33–39
- Weising K, Kahl G (1991) Towards an understanding of plant gene regulation: the action of nuclear factors. *Z Naturforsch* 46c:1–11
- White RJ, Jackson SP (1992) The TATA-binding protein: a central role in transcription by RNA polymerase I, II and III. *Trends Genet* 8:284–288
- Yakura K, Nishikawa K (1992) The nucleotide sequence of the rDNA spacer region between the 25S and 18S rRNA genes in a species of the genus *Vicia*, *V. hirsuta*. *Plant Mol Biol* 19:537–539
- Yakura K, Kato A, Tanifuji S (1983) Structural organization of ribosomal DNA in four *Trillium* species and *Paris verticillata*. *Plant Cell Physiol* 24:1231–1240
- Yamashita J, Nakajima T, Tanifuij S, Kato A (1993) Accurate transcription initiation of *Vicia faba* rDNA in a whole cell extract from embryonic axes. *Plant J* 3:187–190
- Yang-Yen H-F, Rothblum L (1988) Purification and characterization of a high-mobility-group-like DNA-binding protein that stimulates rRNA synthesis in vitro. *Mol Cell Biol* 8:3406–3414
- Zentgraf U, Hemleben V (1992) Complex formation of nuclear proteins with the RNA polymerase I promoter and repeated elements in the external transcribed spacer of *Cucumis sativus* ribosomal DNA. *Nucleic Acids Res* 20:3685–3691
- Zentgraf U, Hemleben V (1993) Nuclear Proteins interact with RNA polymerase I promoter and repeated elements of the 5' external transcribed spacer of the rDNA of cucumber in a single-stranded stage. *Plant Mol Biol* 22:1153–1156
- Zentgraf U, Ganal M, Hemleben V (1990) Length heterogeneity of the rRNA precursor in cucumber (*Cucumis sativus*). *Plant Mol Biol* 15:465–474