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ABSTRACT **We investigated the developmental regulation of nuclear RNA sequences in tobacco vegetative (leaf, root, stem) and floral (petal, ovary, anther) organ systems using RNA-excess·single-copy DNA hybridization reactions. We found that 18% of the single-copy DNA, equivalent to 1.1 × 10^5 kilobases (kb) of diverse transcripts, is represented in the nuclear RNA of each organ. Each nuclear RNA population has both shared and organ-specific sequences. Depending upon the nuclear RNA, 10–40% of the complexity, or 1.1–4.4 × 10^4 kb of diverse sequence, is organ-specific. Collectively, at least 45% of the single-copy DNA, or 3 × 10^5 kb, is represented in the nuclear RNA of the entire plant. Hybridization experiments with polysomal RNA showed that organ-specific mRNAs are present in both the unique and shared nuclear RNA subsets. Together, our results show that tobacco nuclear RNA sequences are under striking developmental control and that both transcriptional and post-transcriptional processes play a role in regulating plant gene expression.**

The differentiated state of higher plant cells is characterized by the expression of specific gene sets. Comparisons of the sequence content of tobacco organ system mRNAs showed that each population has at least 6,000–11,000 mRNAs that cannot be detected in the polysomes of other organs (1). These organ-specific mRNAs represent 25–40% of the sequence complexity of each mRNA set, or approximately 0.8–1.4 × 10^4 kilobases (kb) of diverse message (1). Clearly, cellular processes must exist that enable thousands of plant genes to be specifically expressed in unique developmental situations.

Hybridization experiments with nuclear RNA suggested that post-transcriptional selection processes play an important role in establishing the sequence composition of tobacco mRNA populations (1). For example, most leaf-specific mRNAs were found to be present in stem nuclear RNA at concentrations equivalent to those of stem coding sequences (1). This observation raised the possibility that developmentally regulated tobacco structural genes are transcribed constitutively, as has been shown to be the case in sea urchin (2–4). Since tobacco leaf nuclear RNA has a complexity of 1.2 × 10^5 kb (5) and the total complexity of vegetative and floral organ system mRNAs is 8 × 10^4 kb (1), enough sequence information is present in the nuclear RNA to allow this possibility.

Constitutive transcription of most tobacco structural genes predicts that the sequence content of different nuclear RNA populations is similar. To test this, we compared the nuclear RNA sequence sets of all tobacco organ systems. Our results show that tobacco nuclear RNA populations are developmentally regulated and that each organ system has a set of organ-specific nuclear RNAs. Some of these organ-specific nuclear RNAs give rise to organ-specific mRNAs, indicating that both transcriptional and post-transcriptional events play a role in regulating plant gene expression.

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MATERIALS AND METHODS

RNA Preparations. The developmental characteristics of tobacco organ systems and procedures for isolating nuclear RNA and EDTA-released polysomal mRNA have been described (1, 5).

Isolation of Leaf HnDNA and Null HnDNA. Single-copy DNA sequences were isolated from tobacco DNA as described (5) and labeled *in vitro* with [^{3}H]dTTP by gap-translation with DNA polymerase I (2). The single-copy [^{3}H]DNA was 0.25 kb in length and had a specific activity of 8 × 10^6 cpm/μg. Approximately 91% of the single-copy [^{3}H]DNA bound to hydroxyapatite at DNA C_0t 100,000 in the presence of total DNA, as compared to 96% for the unlabeled driver DNA. The reassociation kinetics of the single-copy DNA were as described and did not reveal any contaminating repeated sequences (5).

Leaf HnDNA and null HnDNA were isolated as described (1). Briefly, single-copy [^{3}H]DNA was hybridized with a 300-fold sequence excess of leaf nuclear RNA to RNA C_0t 50,000 and then fractionated over hydroxyapatite. The hydroxyapatite-bound DNA, containing leaf nuclear RNA·DNA hybrids, was designated HnDNA. The unhybridized single-copy DNA was designated null HnDNA. To minimize cross-contamination between the labeled HnDNA and null HnDNA fractions, the preparations were subjected to a second round of hybridization and hydroxyapatite fractionation as described above. The sizes, reassociation kinetics, and reactivities of the purified HnDNA and null HnDNA tracers were similar to those of the parental single-copy DNA.

DNA·RNA Hybridization Reactions. RNA-excess hybridization reactions were carried out as described (1, 5). RNA C_0t values were corrected to those in 0.18 M Na^+ (6, 7). The fraction of single-copy [^{3}H]DNA in DNA·RNA hybrids was determined by hydroxyapatite chromatography (1). All terminal hybridization values were normalized to 100% tracer reactivity by dividing the observed terminal value by the most recently measured fraction of [^{3}H]HnDNA or null [^{3}H]HnDNA, which bound to hydroxyapatite at DNA C_0t 100,000 (1). The range of reactivities was 75–92%. Least-squares fitting procedures used to analyze the DNA·RNA hybridization kinetics were those of Pearson *et al.* (8).

RESULTS

Sequence Complexities of Tobacco Nuclear RNAs. We compared the sequence complexities of tobacco vegetative and floral organ system nuclear RNAs by hybridizing an excess

Abbreviations: kb, kilobase; HnDNA, hydroxyapatite-bound DNA containing leaf nuclear RNA·DNA hybrids; null HnDNA, unhybridized single-copy DNA.

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of each RNA with single-copy [^{3}H]DNA to RNA $C_0t >$ 50,000 and then measuring the extent of hybridization by hydroxyapatite chromatography. We included leaf nuclear RNA as a control because we previously had determined that the sequence complexity of leaf nuclear RNA is 1.2×10^5 kb and that 9–10% of the reactive single-copy [^{3}H]DNA should hybridize (5). Since the rate constant for the leaf nuclear RNA·single-copy DNA hybridization reaction is 1.5×10^{-4} M^{-1}·sec^{-1} (5), virtually all complementary sequences will have hybridized by RNA C_0t 50,000. A similar pseudo-first-order rate constant was obtained with stem nuclear RNA (data not shown).

Within experimental error, the complexities of all nuclear RNAs were similar (Table 1). Approximately 8–9% of the reactive single-copy [^{3}H]DNA hybridized with each nuclear RNA. This amount of hybridization is equivalent to 16–18% of the single-copy sequence, or about $1–1.2 \times 10^5$ kb of diverse transcript. Kamalay and Goldberg have shown (1) that the sequence complexities of tobacco organ system mRNAs are also similar (approximately 3.2×10^4 kb, see Table 1). We conclude from these findings that each organ system has a set of nuclear RNA sequences that are 3.5-fold more complex than are their corresponding mRNAs.

Characteristics of Leaf HnDNA and Null HnDNA. We isolated two single-copy [^{3}H]DNA fractions by hybridization with excess nuclear RNA in order to compare the sequence content of the different nuclear RNAs. One fraction, designated leaf HnDNA, measured the sequence overlap between leaf nuclear RNA and the nuclear RNAs of other organ systems. The other fraction, termed leaf null HnDNA, identified sequences undetectable in leaf nuclei but present in the nuclear RNA of other organs.

Hybridization of HnDNA and null HnDNA with excess leaf nuclear RNA is shown in Fig. 1 *A* and *B*. Approximately 81% of the reactive HnDNA hybridized with leaf nuclear RNA (Fig. 1*A*). This represents a 9-fold enrichment of single-copy sequences complementary to leaf nuclear RNA (81/9.3), or about 82% of the maximum possible enrichment (100/9.3). The HnDNA that failed to react probably represents random single-copy [^{3}H]DNA contaminants and could contribute a maximum of 2% hybridization to other nuclear RNAs (19% × 0.09). The rate of the HnDNA·leaf nuclear RNA hybridization reaction (1.5×10^{-4} M^{-1}·sec^{-1}) was identical to that obtained with total single-copy [^{3}H]DNA (5), demonstrating that the HnDNA represents complex class leaf nuclear RNA sequences rather than a minor subpopulation.

Table 1. Tobacco nuclear RNA complexities

HnRNA	% hybridization*	Complexity, kb × 10^{-4} HnRNA†	mRNA‡
Leaf	9.3 ± 0.6 (5)	12	3.3
Stem	8.9 ± 0.7 (10)	11	3.2
Root	8.8 ± 0.4 (2)	11	3.0
Petal	9.1 ± 0.3 (2)	12	3.3
Anther	7.8 ± 1.3 (4)	10	3.2
Ovary	9.2 ± 1.9 (4)	12	3.1

Single-copy [^{3}H]DNA was mixed with a >500-fold mass excess of nuclear RNA and hybridized to RNA $C_0t > 50{,}000$. At this C_0t, reactions go to completion (1, 5). The extent of hybridization was measured by hydroxyapatite chromatography.

*Extent of hybridization after correcting for tracer reactivity with tobacco genomic DNA. The number of independent hybridization reactions used to obtain the percent hybridization is given in parentheses.

†The nuclear RNA complexities (C_{RNA}) were calculated from the following relationship: C_{RNA} = (% hybridization) × (6.4×10^5 kb) × 2 × 0.01, where 6.4×10^5 kb is the complexity of tobacco single-copy DNA (9).

‡Taken from data of Goldberg *et al.* (5) and Kamalay and Goldberg (1).

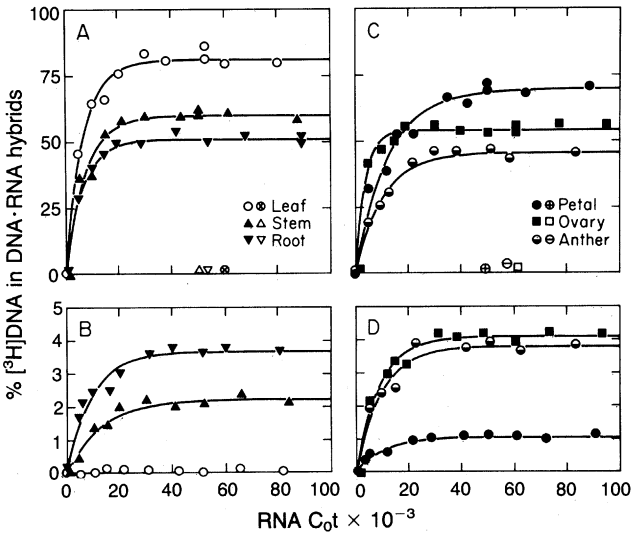

FIG. 1. Hybridization of leaf HnDNA and null HnDNA with tobacco nuclear RNAs. Trace amounts of labeled HnDNA or null HnDNA were hybridized with >2,500-fold sequence excess of nuclear RNA, and the percent hybridization was determined by hydroxyapatite chromatography. RNA C_0t values were corrected to conditions equivalent to those in 0.18 M Na^+ (6, 7). Curves through the data points represent best least-squares solutions for one pseudo-first-order component. Terminal hybridization values obtained from these solutions are given in Table 2. Data have been normalized to 100% tracer reactivity at DNA $C_0t \geq 100{,}000$. (*A*) Hybridization of HnDNA to vegetative organ system nuclear RNAs. The observed terminal hybridization value, rate constant, [^{3}H]HnDNA reactivity, and root-mean-square error, respectively, for each curve were: leaf (○) 68.0%, 1.5×10^{-4} M^{-1}·sec^{-1}, 83.9%, and 2.3%; stem (▲) 54.1%, 1.3×10^{-4} M^{-1}·sec^{-1}, 90.4%, and 2.7%; root (▼) 46.2%, 1.5×10^{-4} M^{-1}·sec^{-1}, 90.4%, and 1.2%. The data points ⊛, △, and ▽ represent the percent of [^{3}H]HnDNA bound to hydroxyapatite after treatment with RNase under conditions that destroy DNA/RNA hybrids (1). (*B*) Hybridization of null HnDNA with vegetative organ system nuclear RNAs. The observed terminal hybridization value, rate constant, null [^{3}H]HnDNA reactivity, and root-mean-square error, respectively, were: leaf (○) <0.02%, 79.9%; stem (▲) 1.8%, 7.7 × 10^{-5} M^{-1}·sec^{-1}, 76.9%, and 1.5%; root (▼) 2.79%, 1.0×10^{-4} M^{-1}·sec^{-1}, 76.9%, and 0.2%. (*C*) Hybridization of HnDNA with reproductive organ system nuclear RNAs. The observed terminal hybridization value, rate constant, [^{3}H]HnDNA reactivity, and root-mean-square error, respectively, were: petal (●) 62.2%, 8.0×10^{-5} M^{-1}·sec^{-1}, 90.4%, and 3.4%; anther (⊖) 41.3%, 9.6×10^{-5} M^{-1}·sec^{-1}, 90.4%, and 1.7%; ovary (■) 49.1%, 2.7×10^{-4} M^{-1}·sec^{-1}, 90.4%, and 1.8%. The data points ⊕, □, and ⊖ represent the fraction of [^{3}H]HnDNA bound to hydroxyapatite after destruction of DNA·RNA hybrids by RNase. (*D*) Hybridization of null HnDNA with reproductive organ system nuclear RNAs. The terminal hybridization value, rate constant, null [^{3}H]HnDNA reactivity, and root-mean-square error, respectively, were: petal (●) 0.78%, 1.0 × 10^{-4} M^{-1}·sec^{-1}, 74.1%, and 0.06%; anther (⊖), 2.92%, 1.1×10^{-4} M^{-1}·sec^{-1}, 76.9%, and 0.2%; ovary (■), 3.02%, 1.2×10^{-4} M^{-1}·sec^{-1}, 74.1%, and 0.2%.

Fig. 1*B* shows that labeled single-copy sequences complementary to leaf nuclear RNA were effectively eliminated from the null HnDNA. We estimated that <0.02% of the null HnDNA reacted with leaf nuclear RNA. This is equivalent to only 125 kb of diverse sequence and represents the amount of nonleaf sequence that would not be reliably measured by our procedures.

Hybridization of Leaf HnDNA and Null HnDNA with Heterologous Nuclear RNAs. Hybridization reactions between leaf HnDNA and null HnDNA, and heterologous organ system nuclear RNAs are shown in Fig. 1 and are summarized in Table 2. Each nuclear RNA hybridized with less HnDNA

Table 2. Summary of HnDNA and null HnDNA hybridization reactions*

HnRNA	HnDNA reactions: % hybridization†	HnDNA reactions: Complexity (C_L)‡ kb × 10^{-4}	HnDNA reactions: % of total HnRNA complexity§	Null HnDNA reactions: % hybridization†	Null HnDNA reactions: Complexity (C_{NL})¶ kb × 10^{-4}	Null HnDNA reactions: % of total HnRNA complexity§	Total complexity, kb × 10^{-4}: $C_L + C_{NL}$	Total complexity, kb × 10^{-4}: From single-copy DNA hybridization‖
Leaf	81.0 ± 2.4	12	100	No detectable reaction		0	12	12
Stem	59.8 ± 2.5	8.8	79	2.27 ± 0.14	2.4	21	11	11
Root	51.1 ± 1.7	7.5	66	3.63 ± 0.27	3.8	34	11	11
Petal	68.8 ± 2.8	10	90	1.05 ± 0.04	1.1	10	11	12
Anther	45.7 ± 1.4	6.7	63	3.80 ± 0.11	4.0	37	11	10
Ovary	54.3 ± 1.4	8.0	65	4.08 ± 0.11	4.3	35	12	12

*Taken from the data presented in Fig. 1.
†Terminal values obtained from the least-squares solutions to the hybridization data presented in Fig. 1. These values were normalized to 100% tracer reactivity as described. Standard deviations were calculated from the hybridization values at RNA $C_0t > 30{,}000$.
‡The complexity of leaf nuclear RNA is 1.2×10^5 kb (Table 1 and ref. 5). Since 81% of the reactive [^{3}H]HnDNA hybridized to leaf nuclear RNA, the complexity of nuclear RNA shared with leaf (C_L) was calculated from the relationship: C_L = [(% HnDNA hybridized) × (1.2×10^5)]/81%.
§$(C_L)/[(C_L + C_{NL}) \times 100]$ or $(C_{NL})/[C_L + C_{NL}) \times 100]$.
¶The null HnDNA complexity equals that of total single-copy DNA (6.4×10^5 kb) minus the leaf HnRNA complexity (1.2×10^5 kb), or 5.2×10^5 kb. The HnRNA complexity, which is unique to each nuclear RNA population and not shared with leaf (C_{NL}), was calculated from the relationship: C_{NL} = (% null HnDNA hybridized) × (5.2×10^5 kb) × 2 × 0.01. The collective HnRNA complexity of the entire plant equals the sum of the leaf HnDNA complexity (1.2×10^5 kb) and the unique or nonleaf HnRNA complexities of the other organ systems (1.6×10^5 kb). This value is 2.8×10^5 kb or 44% of the single-copy DNA.
‖Taken from the data presented in Table 1.

than did the leaf nuclear RNA (Fig. 1 *A* and *C*). The range of hybridization values was approximately 55% (anther) to 85% (petal) of the leaf RNA reaction (Table 2), indicating that although a large fraction of leaf sequences is present in heterologous nuclear RNAs, the entire leaf set is not represented. In addition, leaf nuclei must have a set of sequences that are undetectable in other nuclear RNAs because 15% of the reactive HnDNA did not hybridize with any heterologous nuclear RNA.

Fig. 1 *B* and *D* show that the nuclear RNAs of other organs hybridized with the null HnDNA. This finding demonstrates that each heterologous nuclear RNA has sequences undetectable in leaf nuclear RNA because single-copy sequences complementary to leaf nuclear RNA were removed from the null HnDNA (Fig. 1*B*). These nonleaf sequences comprise 15% (petal) to 45% (anther) of the heterologous nuclear RNA sequence complexity (Table 2). Together, these findings show that each organ system has a set of leaf and nonleaf nuclear RNA sequences. The additive complexity of these sequences equals that obtained by hybridization of nuclear RNAs with total single-copy DNA (Table 2).

Hybridization of Leaf Null HnDNA with Nuclear RNA Mixtures. We determined that each nuclear RNA has a set of organ-specific sequences by hybridizing leaf null HnDNA with nuclear RNA mixtures. Table 3 shows that the extent of hybridization obtained with each RNA mixture is similar to that predicted from the sum of the individual hybridization values. For example, 6% of the reactive null HnDNA hybridized with the root-plus-stem RNA mixture, as compared with 5.9% predicted from the separate reactions shown in Fig. 1*A*. Similarly 13.5% of the reactive null HnDNA hybridized with a mixture of all heterologous nuclear RNAs as compared with 14.8% predicted from the individual reactions. The small but significant difference between observed and expected hybridization values is due to sequences that are shared by anther and ovary nuclear RNAs (Table 3). We conclude from these findings that each nuclear RNA has a set of sequences that are undetectable in other nuclear RNA populations.

Table 3. Hybridization of null HnDNA to nuclear RNA mixtures

HnRNAs in mixture	% hybridization: Expected*	% hybridization: Observed†
Root/stem	5.90	6.02 ± 0.11
Anther/ovary	7.88	6.88 ± 0.20
Anther/ovary/petal	8.93	7.83 ± 0.37
Root/stem/anther/ovary/petal	14.83	13.51 ± 0.32

Null [^{3}H]HnDNA was hybridized with equimolar mixtures of nuclear RNAs to RNA $C_0t > 60{,}000$, and the extent of hybridization was determined by hydroxyapatite chromatography as described. The RNA/DNA sequence ratios were >2500.
*Predicted hybridization value if HnRNAs not present in leaf nuclei are different in each organ system, calculated from the sum of null HnDNA hybridization values in Table 2.
†Average of three independent reactions corrected to 100% null [^{3}H]HnDNA reactivity.

Hybridization of Leaf Null HnDNA with Polysomal mRNAs. Null HnDNA was hybridized with stem, root, and anther

Table 4. Null HnDNA hybridization with polysomal mRNAs

mRNA	% hybridization*	Complexity (C_M)† kb × 10^{-4}	Number of diverse organ-specific mRNAs‡: Observed	Number of diverse organ-specific mRNAs‡: Expected§
Stem	<0.02	<0.02¶	<200¶	6,000
Root	0.54 ± 0.20	0.56	4,500	6,500
Anther	1.24 ± 0.13	1.3	11,000	10,000

EDTA-released polysomal mRNAs were isolated from each organ system (1, 5), hybridized with labeled null HnDNA to RNA $C_0t > 75{,}000$, and the fraction of DNA·RNA hybrids was measured by hydroxyapatite chromatography. In each reaction there was at least a 2000-fold sequence excess of RNA.
*Average of three independent reactions corrected for labeled null HnDNA reactivity.
†The mRNA complexity (C_M) was calculated from the relationship: C_M = (% hybridization) × (5.2×10^5 kb) × 2 × 0.01, where 5.2×10^5 kb is the null HnDNA complexity (see Table 2).
‡Number of mRNAs = C_M/1.25 kb, where 1.25 kb is the number average mRNA size (1, 5).
§The number of diverse mRNAs undetectable in the polysomes of other organ systems, taken from the data of Kamalay and Goldberg (1). This represents the maximum number of mRNA species that could be absent from the nuclear and cytoplasmic RNAs of other organs.
¶Value represents the upper limit of stem mRNAs that could be represented in the null HnDNA population (i.e., are stem-specific). This number of sequences would not be reliably detected by the procedures used here.

EDTA-released polysomal mRNAs to determine whether the organ-specific nuclear RNAs contained structural gene transcripts. We showed earlier that EDTA-released mRNA is free of detectable nuclear RNA contaminants and represents structural gene transcripts associated with polysomes (1, 5).

Table 4 indicates that stem mRNA did not react detectably with the null HnDNA, confirming our previous observation that organ-specific mRNAs are represented in heterologous nuclear RNAs (1). It follows that the stem-specific nuclear RNA set, or 2.4×10^4 kb of diverse sequence (Table 2), is probably not derived from structural genes, at least not those expressed in vegetative and floral organ systems. In contrast, both root and anther mRNAs hybridized with null HnDNA, indicating that structural gene transcripts are represented in their organ-specific nuclear RNA sets. Based on our previous mRNA experiments (1), we estimated that 70% of the root-specific mRNAs, and 100% of the anther-specific mRNAs, are represented in their respective organ-specific nuclear RNA populations (Table 4). Although these mRNAs cannot account for all root- and anther-specific nuclear RNAs (compare Tables 2 and 4), our findings show that there are organ-specific mRNAs which are undetectable in the nuclear RNAs of heterologous organs.

DISCUSSION

Tobacco Organ Systems Have Similar Nuclear RNA Complexities. Fig. 2 summarizes the leaf HnDNA and null HnDNA hybridization reactions. These data and those obtained with total single-copy DNA (Table 1) indicate that approximately 1.2×10^5 kb of diverse transcript, or 18% of the genomic single-copy sequence, is represented in each nuclear RNA. This similarity in nuclear RNA complexities correlates with our earlier finding that 5% of the single-copy DNA, or approximately 3.3×10^4 kb of transcript, is present on floral and vegetative organ polysomes (1). Thus, only 25% of the nuclear RNA sequence diversity is represented in the cytoplasm of each organ.

Nuclear RNA Sequence Sets Are Developmentally Regulated. A major conclusion of the present study is that each nuclear RNA population has a set of transcripts that are undetectable in the nuclear RNA of heterologous organs (Fig. 2 and Table 3). Depending upon the organ system, organ-specific nuclear RNAs constitute 10–40% of the nuclear RNA sequence complexity, or $1–4 \times 10^4$ kb of diverse sequence. In fact, the root, anther, and ovary organ-specific nuclear RNA sets are more complex than their corresponding mRNA populations (compare Tables 2 and 3).

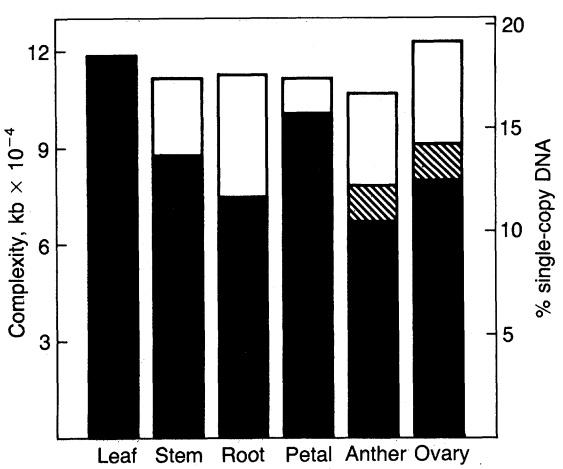

FIG. 2. Nuclear RNA sequences in tobacco organ systems. Columns represent the total nuclear RNA complexity of each organ system expressed in kb and percent single-copy DNA. Dark areas summarize the results of the HnDNA hybridization reactions and represent leaf HnRNAs that are present in the nuclear RNA of other organs. Open areas summarize the results of the null HnDNA hybridization reactions and represent HnRNAs that are unique to each organ system and absent from leaf nuclear RNA. Hatched areas represent HnRNA sequences that are shared by anther and ovary nuclear RNAs (Table 3).

A limitation to this conclusion derives from the assumption that organ-specific transcripts are not degraded instantaneously in the nuclei of other organs. Fig. 1 shows that both shared and organ-specific nuclear RNAs have similar prevalences (1–2 molecules per nucleus). Organ-specific transcripts must be below this level in heterologous nuclear RNAs because our measurements were sensitive enough to detect transcripts represented about once per 100 nuclei. This implies that they would have to turn over about 100 times faster than typical nuclear transcripts. Clearly, the simplest conclusion is that organ-specific nuclear RNAs derive from the restricted transcription of unique genomic regions.

Some Structural Genes Are Transcriptionally Regulated. The findings presented in Table 4 demonstrate that many tobacco structural genes are not expressed constitutively at the nuclear RNA level. Previously we showed that approximately 11,000 average-sized mRNAs are anther-specific (1). Hybridization between anther mRNA and leaf null HnDNA indicates that these anther-specific mRNAs are undetectable in heterologous nuclear RNAs, suggesting that their structural genes are under transcriptional control. A similar conclusion can be applied to about 70% of the root-specific mRNA set, or approximately 4,000 diverse messages (Table 4). Transcriptional control of complex class plant mRNAs differs significantly from that observed in sea urchin (3, 4, 10) but is similar to that found in mammals (11) and in *Dictyostelium* (12).

Some Structural Genes Are Post-Transcriptionally Regulated. Table 4 shows that virtually all stem-specific mRNAs, as well as about 2,000 root-specific messages, are present in leaf nuclear RNA. This result was predicted from our earlier finding that all leaf-specific and petal-specific mRNAs are represented in stem nuclei (1). Thus, developmentally regulated structural genes can be divided into two classes—those that are constitutively expressed in the nuclear RNA of heterologous organs and those that are not. The implication of this finding is that both transcriptional and post-transcriptional processes play a major role in establishing the sequence content of tobacco mRNA sequence sets.

Many Organ-Specific Nuclear RNAs Are Not Derived from Structural Genes. A surprising aspect of our results is that not all organ-specific nuclear RNAs can be accounted for by structural gene transcription. For example, we previously have shown that the petal and leaf mRNA sets are indistinguishable from each other (1), yet 10% of the petal nuclear RNA sequence diversity, or 1.1×10^4 kb of diverse sequence, is organ-specific (Table 2). Similarly, all stem mRNAs are represented in leaf nuclear RNA (Table 4), and all leaf mRNAs are present in stem nuclei (1), yet each of these organs has a large set of organ-specific nuclear RNAs (Table 2). Although the functional significance, if any, of these developmentally regulated nuclear RNA species is unknown, our results suggest that they are not transcribed from structural genes. This conclusion is similar to that of Ernst *et al.* (13) for sea urchin nuclear RNA.

At Least Half of Tobacco Single-Copy DNA Is Transcribed. The measurements presented here and elsewhere (1) enable us to estimate the extent to which single-copy DNA sequences are transcribed and expressed in an entire plant. Using the data listed in Table 2, we estimate that approximately 45% of the single-copy DNA, or 2.8×10^5 kb, is represented in the nuclear RNA of all vegetative and floral organ systems. The majority of these sequences are developmentally

regulated and appear to be under transcriptional control (Tables 2 and 3). Previously, we determined that 12% of the single-copy DNA, or 8×10^4 kb, is represented in the mRNA populations of all tobacco organ systems (1). Since 40% of the genome is single-copy DNA (9), at least 20% of the genome is transcribed and 5% is represented on polysomes in various developmental states. These results imply that a large amount of genetic information is required to enable a plant to achieve its developmental potential. The cellular processes and DNA sequences controlling the expression of this information remain to be determined.

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