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Gibberellin-induced changes in the translatable mRNA populations of stamens and shoots of gibberellin-deficient tomato

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Abstract. The *gib*1 mutant of tomato (*Lycopersicon esculentum* Mill.) is deficient in endogenous gibberellins and exhibits phenotypes including extreme dwarfism, reduced germination, and abnormal flower development, which are reversed by the application of gibberellic acid (GA_3). Previous work has demonstrated that, in stamens of the *gib*1 mutant, pollen mother-cell development arrests at the premeiotic G1 stage (Jacobsen and Olszewski 1991, Plant Physiol. **97**, 409–414). Following GA_3 treatment of developmentally arrested flowers, pollen mother-cell development resumes and is synchronous. The present study examines gibberellin-induced changes in the translatable mRNA populations of developmentally arrested stamens and of vegetative shoots of the *gib*1 mutant. Following rescue of developmentally arrested stamens by treatment with GA_3, we consistently detected increases and decreases in the abundance of 14 and 20 in-vitro translation products, respectively. Some of these changes were first detected 8 h post treatment and therefore represent the first changes observed in stamens whose development has been rescued by GA_3 treatment. In vegetative *gib*1 shoots, the abundance of 13 in-vitro translation products decreased within 6–24 h after GA_3 treatment. However, no in-vitro translation products that increased in abundance after GA_3 treatment were detected.

Key words: Gibberellin – *Lycopersicon* – Mutant (*gib*1) – Stamen development – Translatable mRNA populations

Introduction

Gibberellins (GAs) are endogenous plant growth regulators that are involved in many aspects of plant growth and development (for reviews, see Jones 1973; Pharis and King 1985; Graebe 1987). The *gib*1 mutant of tomato is deficient in GAs because its ability to convert geranylgeranyl pyrophosphate to copalyl pyrophosphate is reduced (Bensen and Zeevaart 1990). The phenotypes of this mutant, which include dwarfism, failure to germinate and failure to flower normally, are reversed by exogenously applied GAs (Koornneef et al. 1981; Nester and Zeevaart 1988; Jacobsen and Olszewski 1991).

Anther development in the *gib*1 mutant arrests shortly before the initiation of meiosis (Nester and Zeevaart 1988; Jacobsen and Olszewski 1991). Developmentally arrested anthers contain pollen mother cells which are at the G1 phase of premeiotic interphase, and outer and inner tapetum cells which are at the uninucleate and binucleate stages, respectively (Jacobsen and Olszewski 1991). Anthers become developmentally arrested when the flower bud is 2.5 mm in length and remain developmentally arrested, but responsive to treatment with gibberellic acid (GA_3) until the bud reaches 3.7 mm in length. Treatment of developmentally arrested flower buds with GA_3 restores normal anther development and produces fertile flowers. After GA_3 treatment of developmentally arrested flowers, anther development is synchronous; pollen mother cells complete premeiotic DNA synthesis and begin callose accumulation by 48 h post treatment, and prophase I of meiosis is initiated within 66 h post treatment. Flower buds that are longer than 3.7 mm are generally senescent and unresponsive to treatment with GA_3.

Gibberellins are known to cause specific changes in gene expression. Most studies of GA regulation of gene expression have focused on the aleurone system of barley and wheat, where, during germination, GA produced in the embryo stimulates the synthesis and secretion of hydrolytic enzymes located in the aleurone layer (for reviews, see Hammerton and Ho 1986; Jacobsen and Chandler 1987). The expression of α-amylase, the enzyme most extensively studied in this system, is regulated at the level of transcription (Jacobsen and Beach 1985). Accumulation of the α-amylase mRNA can be detected within 6 h after addition of GA (Jacobsen and Chandler 1987).

Abbreviations: 2-D PAGE = two-dimensional PAGE; GA = gibberellin; ID = identification number of a specific in-vitro translation product; IEF = isoelectric focusing

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Although cDNA clones for other GA-induced and GA-suppressed mRNAs in germinating seeds have been isolated, it is not known how GA causes their induction (Cejudo et al. 1992, and references therein).

Gibberellin is also known to regulate gene expression in shoot tissue (Shi et al. 1992; Weiss et al. 1992). The transcription of one gene from tomato, *GAST1*, is known to be stimulated within 2 h of GA_3 treatment of mature leaves and stems (Shi et al. 1992). The function of the *GAST1* gene is unknown. In addition, the expression of the chalcone-synthase gene (Weiss et al. 1992) and other genes involved in flavonoid biosynthesis (Weiss et al. 1990) have recently been shown to be regulated by GA in the corolla of petunia flowers.

A study of GA-induced changes in the populations of translatable mRNAs has been performed with seedling tissues of GA-deficient maize (*d5*) and pea (Progress No. 9; Chory et al. 1987). Following GA_3 treatment of etiolated *d5* shoot sections, at least 14 mRNAs increased in abundance while four decreased in abundance. Treatment of light-grown maize shoots and pea stem sections with GA_3 caused a number of mRNAs to become more abundant, but did not cause any detectable decreases in mRNA abundance. All of these changes in the abundance of specific mRNAs were detectable 0.5 h after treatment (Chory et al. 1987).

The present study extends the analysis of the role of GAs in anther development by examining the effect of GA_3 treatment on populations of translatable mRNAs from *gib1* stamens. In order to determine whether observed changes in the level of individual translatable mRNAs are specific to stamen tissue, we have also examined the effect of GA_3 treatment on populations of translatable mRNAs from mature *gib1* shoots. The results from this study provide the first description of the effect of GA on stamen translatable mRNA populations, and describe a previously unreported class of mRNAs which are present in shoot tissue and become less abundant following GA_3 treatment. Interestingly, and in contrast to the results of Chory et al. (1987), we did not detect significant GA-induced increases in the abundance of any translatable mRNAs in shoot tissue.

Materials and methods

Plant culture and gibberellin treatments. Seeds of the *gib1* mutant (which was derived from *Lycopersicon esculentum* Mill. cv. Moneymaker) were obtained from M. Koornneef, Department of Genetics, Agricultural University, Wageningen, The Netherlands. Plants were grown in a greenhouse or in growth chambers as described previously (Jacobsen and Olszewski 1991). Leaves and stems of six- to eight-week old *gib1* plants were treated by spraying whole plants to run-off with a GA_3 solution [$5 \cdot 10^{-5}$ M GA_3 (Sigma Chemical Company, St. Louis, Mo., USA), 0.05% (v/v) Tween-20 (polyoxyethylene-sorbitan monolaurate)] or with a control solution (0.05% Tween-20). The entire shoot above the fourth internode (except for inflorescences) was frozen in liquid nitrogen and stored at −80°C. Developmentally arrested *gib1* flower buds (2.5–3.7 mm in length; Jacobsen and Olszewski 1991) were treated with 2.5 µL of a GA_3 solution (50% ethanol, 0.025% Tween-20, and 20 $\mu g \cdot mL^{-1}$ GA_3) or a control solution (50% ethanol and 0.025% Tween-20). Stamens from these flower buds were harvested, frozen in liquid nitrogen, and stored at −80°C.

In-vitro translation and analysis by two-dimensional (2-D) PAGE. Polyadenylated RNA was isolated from frozen tissue as previously described (Shi et al. 1992). In-vitro translation, using rabbit-reticulocyte lysate, and 2-D PAGE were performed as described by Xin and Li (1993). For each sample, equal amounts of poly(A)$^+$RNA were used for in-vitro translation. As indicated by the incorporation of [^{35}S]methionine, all poly(A)$^+$RNA samples translated with equivalent efficiencies (data not shown). An equal amount of in-vitro translation products (based on the amount of [^{35}S]methionine incorporated into protein) was loaded onto each first-dimension tube gel. Changes in the abundance of specific in-vitro translation products were only reported if they were detected in two or more independent experiments.

Results

Analysis of translatable mRNA populations in stamens. Polyadenylated RNA was isolated from stamens of developmentally arrested flower buds that had been harvested 8, 24, or 48 h after treatment with GA_3 or a control solution. Analysis of these RNA samples by in-vitro translation followed by 2-D PAGE indicated that there were a number of specific in-vitro translation products which reproducibly changed in abundance following GA_3 treatment (Figs. 1, 2). Relative to the majority of the translation products, GA_3 caused an increase in the abundance of 14 specific translation products which could be placed into two distinct classes with respect to the kinetics of their accumulation (Table 1). The first class consisted of six translation products that exhibited a small but detectable increase in abundance 8 h after GA_3 treatment (Table 1, IDs 1–6). These translation products were maximally abundant either 24 h or 48 h post treatment. An increase in the abundance of a second class of translation products (Table 1, IDs 7–14) was not detected until 48 h post treatment.

Gibberellic acid also caused a decrease in the abundance of 20 translation products (Fig. 2), which could be placed into three distinct classes with respect to the kinetics of the decrease in their abundance (Table 2). The first class consisted of nine translation products that exhibited a small but detectable decrease in abundance 8 h after GA_3 treatment (Table 2, IDs 1–9). These translation products exhibited a much greater decrease in abundance at 24 h and at 48 h post treatment. A decrease in the abundance of the second class of translation products was detected at both 24 h and 48 h post treatment (Table 2, IDs 10–19), but not at 8 h post treatment. The final class consisted of one translation product (Table 2, ID 20) that was reduced in abundance only at 48 h post treatment.

Polyadenylated RNA from both developmentally arrested *gib1* stamens and *gib1* shoots, harvested 48 h after treatment with either GA_3 or a control solution, was simultaneously translated and these translation products were simultaneously subjected to 2-D PAGE analysis. Fluorographs from this experiment were then aligned to determine if (i) each stamen translation product corresponded to a shoot translation product that had a similar mobility, and (ii) if the abundance of the corresponding shoot translation product was regulated by GA_3. These

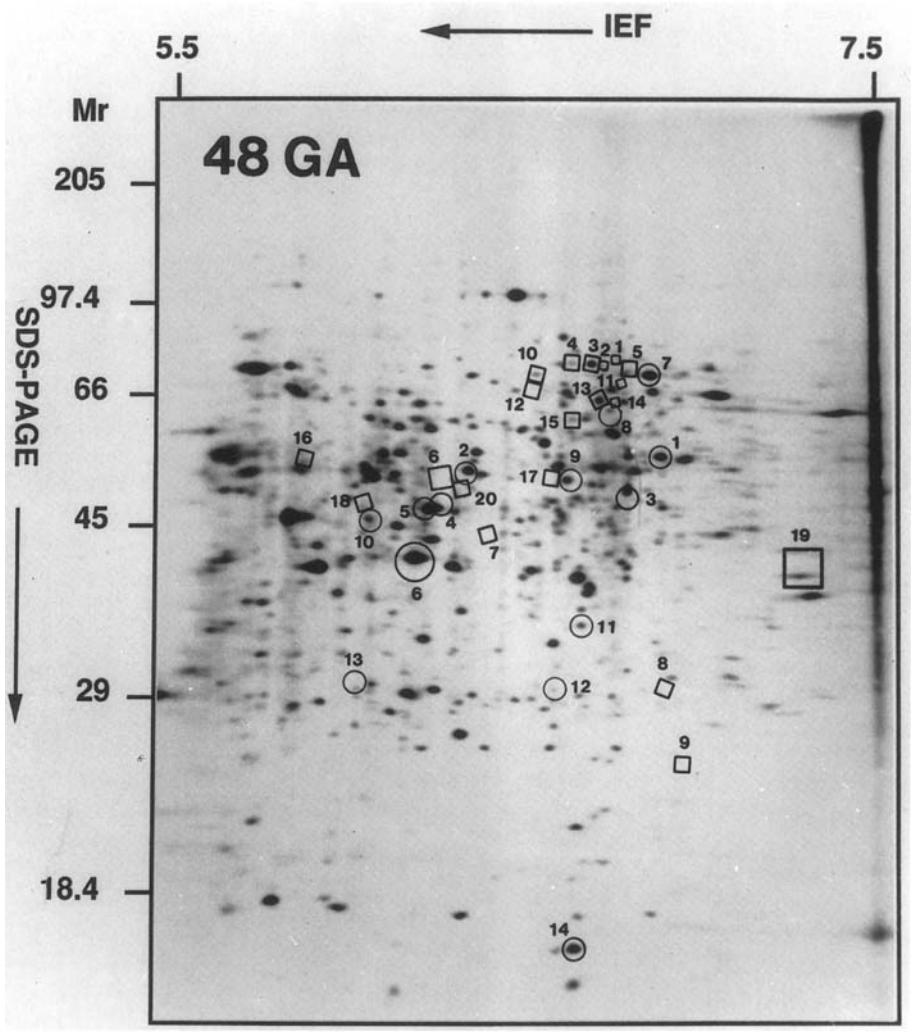

Fig. 1. Analysis of translatable mRNA populations from developmentally arrested stamens of the *gib*1 mutant of tomato that have been treated with GA_3 for 48 h. ○, in-vitro translation product that becomes more abundant following GA_3 treatment; the numeral adjacent to this symbol corresponds to the identification number used in Table 1. □, in-vitro translation product that becomes less abundant following GA_3 treatment; the numeral adjacent to this symbol corresponds to the identification number used in Table 2. The position and size of molecular-weight markers (expressed in kilodaltons) are shown on the left

comparisons indicated that 4 of the 14 translation products that became more abundant after treatment of stamens with GA_3 (Table 1) and 8 of the 20 translation products that became less abundant after treatment of stamens with GA_3 (Table 2) did not correspond to a similarly migrating translation product from shoot samples. The remaining stamen translation products each migrated similarly to a translation product that was present in shoot samples (Tables 1, 2). Interestingly, only three translation products that became less abundant after treatment of stamens with GA_3, migrated similarly to a translation product that also became less abundant after shoots were treated with GA_3, and none of the translation products that became more abundant after treatment of stamens, became more abundant after treating shoots with GA_3 (Tables 1, 2).

*Analysis of translatable mRNA populations in gib*1 *shoots.* In-vitro translation and 2-D PAGE analysis was also performed with poly(A)$^+$RNA isolated from *gib*1 shoots that had been harvested 0.5, 1, 2, 6, 12, 24, or 48 h after treatment with GA_3 or a control solution. These analyses detected no translation product that exhibited an appreciable GA-induced increase in abundance (Fig. 3, and data not shown). In contrast, GA_3 caused a decrease in the abundance of 13 translation products (Fig. 3). Based on the kinetics of the decrease in their abundance, these 13 translation products could be placed into three classes (Table 3). The first class contained three translation products that first became less abundant after 6 h of GA_3 treatment, and remained maximally suppressed for at least 48 h post treatment, (Table 3, IDs 1, 5, and 6). The second class contained six translation products that became detectably less abundant 6 h after GA_3 treatment, exhibited maximum suppression at 12 h post treatment but became more abundant again by 24 or 48 h post treatment (Table 3, IDs 2, 3, 4, 7, 8, and 9). The remaining four translation products did not decrease in abundance until 24 h post treatment (Table 3, IDs 10–13). We did not detect decreases in the abundance of any translation products prior to 6 h of GA_3 treatment.

Fluorographs from the experiments with *gib*1 shoots were aligned with those of the stamen experiments to estimate the relative abundance of the shoot translation product in stamen samples. Of the 13 shoot translation products shown in Table 3, the region encompassing 10 of these products could be unambiguously aligned with the corresponding region on the fluorograph from the stamen experiment. These comparisons indicated that two translation products (Table 3, IDs 6 and 7) that be-

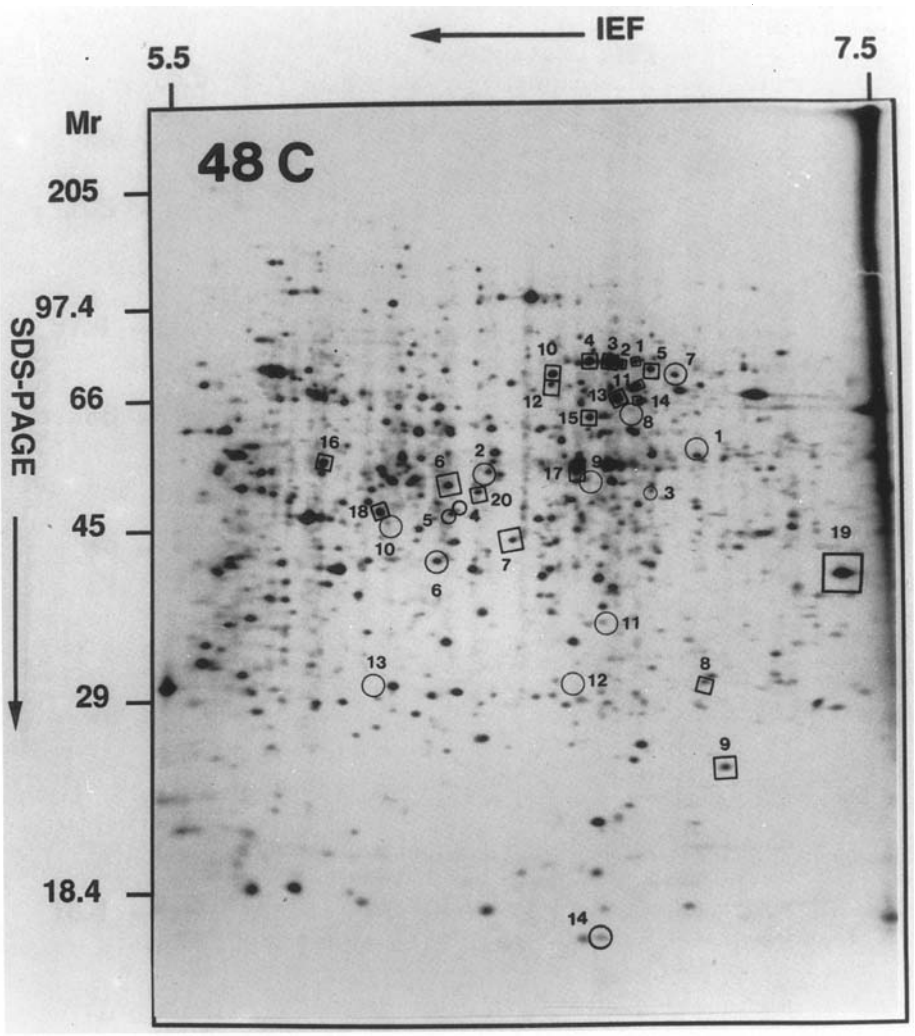

Fig. 2. Analysis of translatable mRNA populations from developmentally arrested stamens of the *gib*1 mutant of tomato that have been treated with a control solution for 48 h. Symbols are defined in the legend to Fig. 1

came less abundant in shoots following GA_3 treatment were not detectable in stamen samples. Eight products, however, each exhibited an electrophoretic mobility similar to a translation products from stamens. Three of these products (Table 3, IDs 10, 11, and 12) migrated similarly to a translation product from stamens that became less abundant after GA_3 treatment, while the remaining five translation products migrated similarly to a translation product whose abundance was not affected by GA_3.

Discussion

In the absence of exogenously applied GAs, stamen development of the *gib*1 mutant arrests at a premeiotic stage (Nester and Zeevaart 1988; Jacobsen and Olszewski 1991). Following treatment of arrested flowers with GA_3, the resumption of stamen development is correlated with changes in the abundance of a number of specific in-vitro translation products (Figs. 1, 2; Tables 1, 2) and hence of a number of specific mRNAs. Although it is not known whether any of these observed changes in mRNA abundance play a role in stamen development, the observation that some of these mRNAs are preferentially expressed in stamens and-or are regulated by GA_3 in stamens but not in shoots (Tables 1, 2) is consistent with this hypothesis. It is also interesting to note that many of these changes in gene expression occur prior to observable morphological changes; changes in the abundance of specific mRNAs were observed by 8 h post treatment (Tables 1, 2) while the morphological changes, S phase and callose deposition, are not detected until 48 h post treatment (Jacobsen and Olszewski 1991). Analysis of stamens 24 h after GA_3 treatment revealed no changes in DNA synthesis, callose accumulation, chromosome morphology, nuclei number, or any other aspect of cell morphology (Jacobsen and Olszewski 1991, and data not shown). Thus, it is possible that some of the mRNAs that change in abundance 8 to 24 h after GA_3 treatment play some causal role in GA_3-mediated stamen development. Because of the apparent lag in the time required to observe these changes in mRNA abundance, however, it seems unlikely that they represent a primary response to GA_3 treatment.

Changes in stamen mRNA abundance that are not detectable until 48 h post GA_3 treatment correlate with the onset of DNA synthesis in pollen mother cells and outer tapetum cells, and with callose deposition in pollen mother cells. These mRNAs may, therefore, be involved

Table 1. Kinetics of changes in abundance of in-vitro translation products that become more abundant in developmentally arrested stamens of the *gib*1 mutant of tomato following treatment with GA_3

ID[a]	M_r	pI	Relative abundance					Regulation by GA_3 in shoots
			Control	8 h	24 h	48 h	Shoots[c]	
1	56	7.1	+[b]	++	+++	++++	++	no[d]
2	53	6.5	+	++	++++	++++	+	no
3	51	7.0	+	++	+++	+++	++++	no
4	48	6.4	+	++	+++	++++	+++	no
5	48	6.4	+	++	+++	++++	+++	no
6	42	6.4	+	++	+++	++++	+	no
7	72	7.0	+	+	+	++++	+	no
8	63	6.9	+	+	+	+++	+	no
9	52	6.8	+	+	+	++++	−	−
10	46	6.3	+	+	+	+++	+	no
11	36	6.8	+	+	+	+++	+	no
12	30	6.7	+	+	+	+++	−	−
13	29	6.2	+	+	+	+++	−	−
14	16	6.8	+	+	+	+++	−	−

[a] Identification number for each in-vitro translation product
[b] "+" denotes the abundance of an in-vitro translation product in samples from control stamens and does not indicate the absolute abundance of the product. Increases in the number of +'s indicate increases in the level of individual in-vitro translation products
[c] Level of in-vitro translation product detected in RNA samples from *gib*1 shoots that had been treated with GA_3 for 48 h. "−" indicates that no in-vitro translation product was detected in shoot samples that migrated similarly to this product
[d] The abundance of the similarly migrating in-vitro translation product detected in shoot RNA samples did not exhibit GA_3 regulation

Table 2. Kinetics of changes in abundance of in-vitro translation products that become less abundant in developmentally arrested stamens of the *gib*1 mutant of tomato following treatment with GA_3

ID	M_r	pI	Relative abundance					Regulation by GA_3 in shoots
			Control	8 h	24 h	48 h	Shoots	
1	76	6.9	++++[a]	+++	++	+	−	−
2	76	6.9	++++	+++	++	+	++	no
3	76	6.9	++++	+++	++	+	++	yes, (11)[b]
4	76	6.8	++++	+++	++	+	+	yes, (10)
5	72	7.0	++++	+++	++	+	+	no
6	51	6.4	++++	+++	++	+	−	−
7	44	6.6	++++	+++	++	+	−	−
8	31	7.1	++++	+++	++	+	+	no
9	24	7.2	++++	+++	++	+	−	−
10	72	6.7	++++	++++	+	+	+	no
11	69	6.9	++++	++++	++	+	+	no
12	69	6.7	++++	++++	++	+	−	−
13	64	6.9	++++	++++	++	+	+	yes (12)
14	64	6.9	++++	++++	++	+	−	−
15	62	6.8	++++	++++	++	+	−	−
16	55	6.1	++++	++++	++	+	++	no
17	54	6.7	++++	++++	++	+	++	no
18	47	6.3	++++	++++	++	+	++	no
19	41	7.4	++++	++++	++	+	+	no
20	50	6.5	++++	++++	++++	+	−	−

[a] "++++" denotes the abundance of an in-vitro translation product in samples from control stamens and does not indicate the absolute abundance of the product. Decreases in the number of +'s indicate decreases in the level of individual in-vitro translation products
[b] The identification number of the corresponding in-vitro translation product from shoots (Table 3) is noted in parentheses. Other symbols are defined in Table 1

in these processes, or other processes associated with the onset of meiosis, such as pairing of homologous chromosomes.

Treatment of *gib*1 shoots with GA_3 reduced the abundance of at least 13 mRNAs, and changes in the abundance of 9 of these mRNAs were first detectable 6 h after treatment (Table 3). Consistent with these results, we have recently identified three unique cDNA clones to RNAs that decrease in abundance following GA_3 treatment of *gib*1 shoots (data not shown). These clones con-

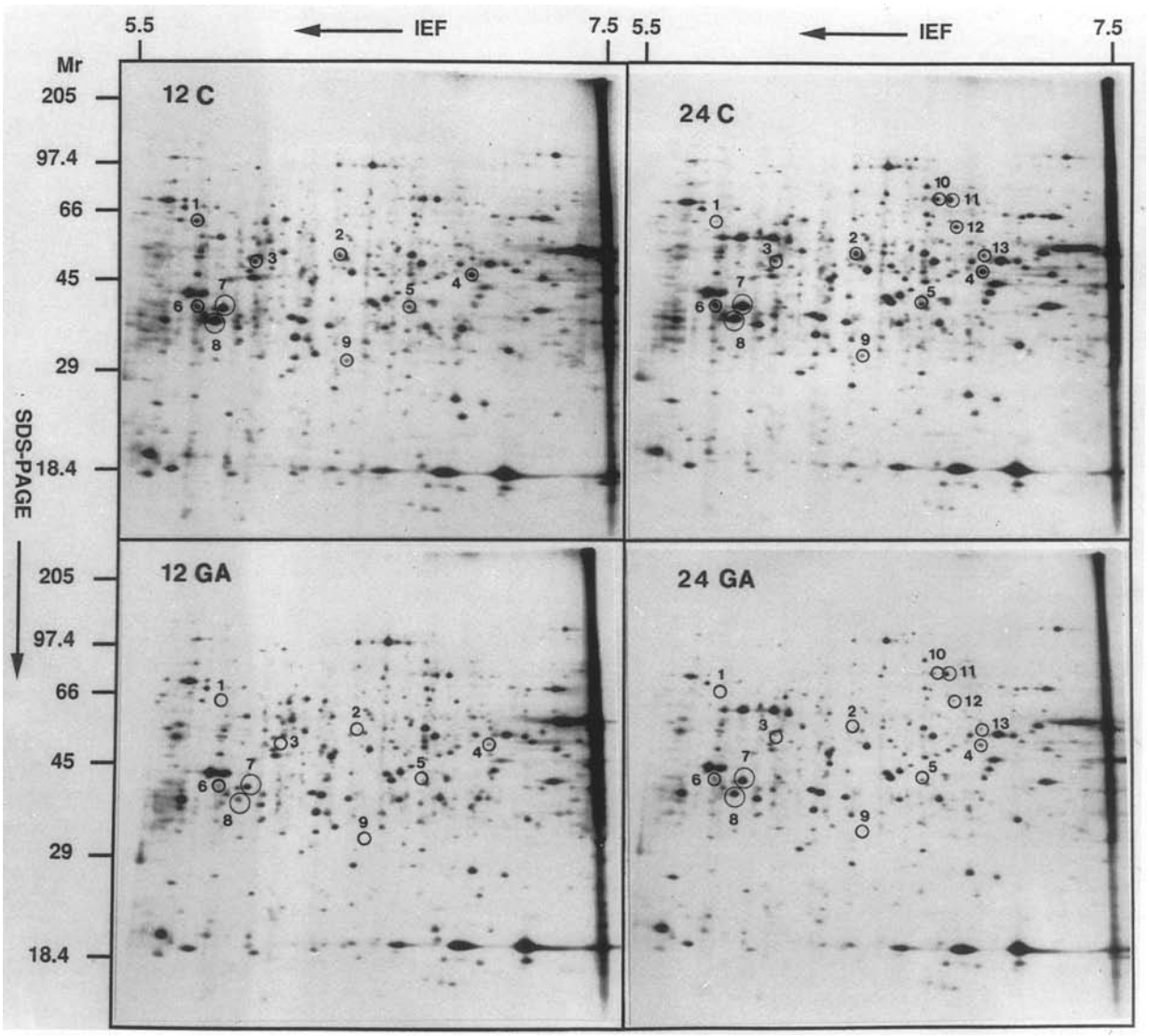

Fig. 3. Analysis of translatable mRNA populations from *gib*1 shoots that have been treated for 12 h with GA_3 (12 *GA*) or a control solution (12 *C*) or for 24 h with GA_3 (24 *GA*) or a control solution (24 *C*). ○, in-vitro translation product that becomes less abundant following GA_3 treatment; the numeral adjacent to this symbol corresponds to the identification number used in Table 3. The position and size of molecular weight markers (expressed in kilodaltons) are shown on the left

form to two of the kinetic classes of in-vitro translation products described here (Table 3). One clone corresponds to an RNA species that decreases in abundance 6 h post GA_3 treatment and remains maximally suppressed until 48 h post treatment. The other two clones correspond to RNA species which decrease in abundance by 6 h post GA_3 treatment but whose level returns to nearly the untreated level by 48 h.

The analysis described here detected no shoot mRNAs that became more abundant following treatment with GA_3. In addition, extensive screening of a number of cDNA libraries prepared to RNA isolated from mature *gib*1 shoots at various times following treatment with GA_3, has identified clones corresponding to only a single gene, *GAST*1, whose RNA increases in abundance following GA_3 treatment (Shi et al. 1992). GAST1 would not have been detected by the methods used in the present study because of its low predicted molecular weight (12.8 kDa), and its relatively high isoelectric point (9.7). Together, these results suggest that, in mature *gib*1 shoots, GA_3 induces the accumulation of few abundant mRNAs.

Gibberellin-induced changes in the levels of RNAs from mature *gib*1 shoots are strikingly different, both with respect to the kinetics and types of changes, from those previously reported for GA-deficient maize (*d5*) and pea (Progress No. 9) seedling shoots (Chory et al. 1987). Gibberellin treatment of maize and pea seedlings induced rapid (within 0.5 h of treatment) increases and decreases in the abundance of translatable mRNAs (Chory et al. 1987). In contrast, only GA-induced decreases in the levels of translatable mRNAs from *gib*1 shoots were detected (Fig. 3), and these changes did not become detectable until 6 h after treatment. Furthermore, GA-induced increases in *GAST*1 RNA are not observed until 2 h after treatment of *gib*1 shoots with GA_3 (Shi et al. 1992).

Although it is not known why GA-induced changes in

Table 3. Kinetics of changes in abundance of in-vitro translation products that become less abundant in shoots of the *gib*1 mutant of tomato following treatment with GA_3

ID	M_r	pI	Relative abundance						Regulation by GA_3 in stamen
			Control	6 h	12 h	24 h	48 h	Stamen[b]	
1	64	6.0	++++[a]	+++	+	+	+	++	no
5	44	6.8	++++	+++	++	++	++	++	no
6	43	6.0	++++	+++	++	++	++	–[c]	–
2	57	6.6	++++	+++	+	+	++	+	no
3	54	6.2	++++	+++	+	++	++++	N.D.[d]	N.D.
4	52	7.0	++++	+++	+	+	++	N.D.	N.D.
7	43	6.1	++++	+++	+	+	+++	–	–
8	40	6.1	++++	+++	+	++	++	++++	no
9	34	6.6	++++	+++	+	++	++	++	no
10	76	6.8	++++	++++	++++	++	++	+++++++	yes, (4)[e]
11	76	6.9	++++	++++	++++	+++	++	++++++	yes, (3)
12	64	6.9	++++	++++	++++	+	++	+++++++	yes, (13)
13	58	7.0	++++	++++	++++	++	++	N.D.	N.D.

[a] "++++" denotes the abundance of an in-vitro translation product in samples from water-treated shoots and does not indicate the absolute abundance of the product. Decreases in the number of +'s indicate decreases in the level of individual in-vitro translation products, relative to the water treated control

[b] The relative abundance of each in-vitro translation product in developmentally arrested stamens of the *gib*1 mutant

[c] An in-vitro translation product in stamen samples that migrated similarly to this product was not detected

[d] N.D. indicates that the abundance could not be determined because alignment of fluorographs from stamen and shoot experiments in the region encompassing this in-vitro translation product was ambiguous

[e] The identification number of the corresponding in-vitro translation product from stamens (Table 2) is noted in parentheses. Other symbols are defined in Table 1

gene expression in shoots of dwarf maize and pea seedlings are different from those observed in *gib*1 shoots, one possible explanation is the difference in the species of plants used in the different studies. This seems unlikely, however, since Chory et al. (1987) examined both monocotyledonous and dicotyledonous species and observed similar patterns of GA-regulated gene expression in both. A more likely explanation is that the difference in the developmental ages of the shoot tissues used accounts for the different results. Our study used shoot tissues of relatively mature plants (approximately six to eight weeks old) while Chory et al. (1987) used shoots from seedlings (5–11 d old).

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