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Polyribosomes from Peas

VI. AUXIN-STIMULATED RECRUITMENT OF FREE MONOSOMES INTO MEMBRANE-BOUND POLYSOMES

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ABSTRACT

Auxin treatment of aged pea stems (*Pisum sativum* L. var. Alaska) caused a decrease in monosomes (especially free monosomes) and an increase in polysomes (especially membrane-bound polysomes). These effects were not duplicated by gibberellic acid or benzyladenine. These auxin-stimulated shifts in polysome distribution commenced at least 9 hours before significant growth took place. It is suggested that this auxin-stimulated incorporation of free monosomes into membrane-bound polysomes might involve increased utilization (through activation or synthesis) of messenger RNA(s) acting as template(s) for synthesis of secretable enzyme(s) involved in growth.

In an earlier paper (1), we showed that IAA elicited a 2-fold increase in the mRNA content of free polysomes within 6 hr of its application to aged pea epicotyls. We suggested that the hormone was acting to cause either synthesis or activation of mRNA to which existing ribosomes could attach (1). In a later paper (4), however, we showed that only a selected sample of polysomes had been analyzed for the data on polysome metabolism; the membrane-bound polysomes had been discarded entirely and only 30% of the FP[1] had been analyzed. This suggested an alternative explanation for the auxin-stimulated increase in free polysomes, *i.e.* pre-existing polysomes could have been released from membranes. To test this possibility, the experiments reported here were conducted to find out whether IAA had any effect on membrane-bound polysomes, and also to find out whether similar responses could be elicited by other hormones.

MATERIALS AND METHODS

Dark-grown seedlings of *Pisum sativum* L. var. Alaska were decapitated and treated with lanolin for 2 to 3 days in order to age the tissue (1). After this aging period, excess lanolin was wiped off, a mark was made 10 mm below the apex to delineate a "segment" of tissue, and the apex was coated with fresh lanolin with or without additives. The plants continued to grow in the dark until harvested for polyribosome isolation.

Polysomes were isolated as described earlier (4). Usually 50 segments were homogenized in 5 ml of buffer A (0.25 M sucrose; 0.2 M tris-HCl, pH 8.5; 60 mM KCl; 30 mM $MgCl_2$). The brei was squeezed through nylon cloth, and the liquid was centrifuged at 500*g* for 10 min. The pellet was discarded, and the supernatant was recentrifuged at 30,000*g* for 15 min to pellet the MBP. The supernatant, containing free polysomes, was layered directly on to gradients, the pellet containing MBP was resuspended in buffer A containing 2% (v/v) Triton X-100 prior to layering. The gradients, usually 125 to 500 mg/ml sucrose in buffer B (20 mM tris-HCl, pH 8.5; 20 mM KCl; 10 mM $MgCl_2$), were centrifuged in a Spinco SW-36 rotor at 34,000 rpm for 75 min (MBP) or 125 min (FP). All operations were conducted at 2 C.

The contents of the gradient tubes were monitored on an ISCO Model 640 gradient fractionator, and the A_{254} was monitored continuously. The areas under the regions: monosomes (M), small polysomes containing two to five ribosomes (SP), large polysomes containing more than five ribosomes (LP) were measured by planimetry.

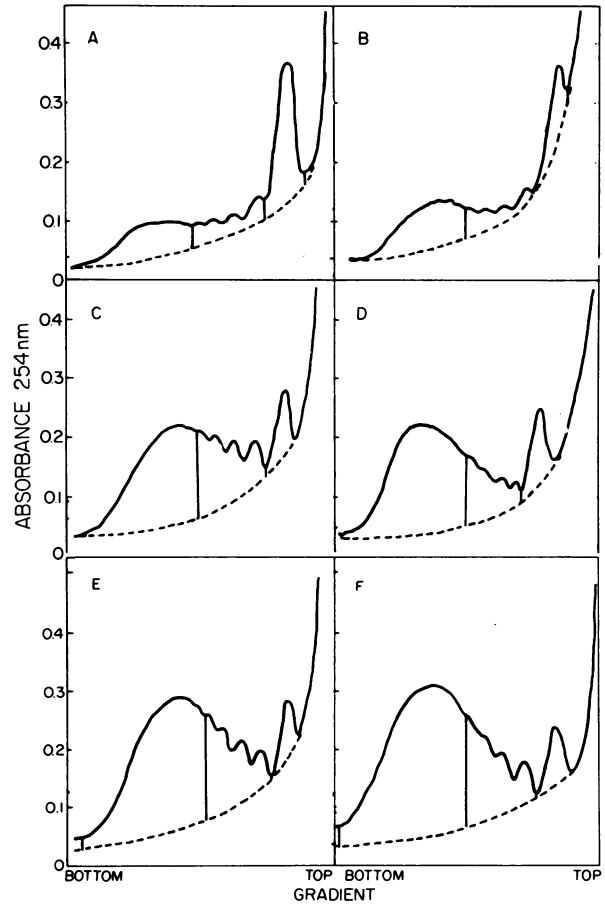

FIG. 1. Membrane-bound polyribosomes isolated at different times after auxin treatment of 2-day-aged pea epicotyls. Profiles represent material isolated from 20 epicotyls after auxin treatment of (hr): A: 0; B: 3; C: 6; D: 9; E: 12; and F: 15.

[1] Abbreviations: FP: free polysomes; MBP: membrane-bound polysomes; M: monosomes; SP: small polysomes (2- to 5-mers); LP: large polysomes (greater than 5-mers); NAA: naphthaleneacetic acid.

RESULTS AND DISCUSSION

The profiles depicted in Figure 1 and the analyses presented in Table I show the distribution of membrane-bound polyribosomes at different periods after treatment of aged epicotyls with 0.5% (w/w) IAA in lanolin. The hormone had little effect within 3 hr, a marked effect within 6 hr, and an increasingly dramatic effect after 9 hr on increasing the amount of membrane-bound polysomes. These changes in MBP were of far greater magnitude than growth which was not readily apparent until more than 15 hr after treatment (Table I). The initial response, within 3 hr, involved a reduction to 40% of the monosome fraction and a slight (20%) reduction in total MBP. From 6 hr on, the monosome peak remained constant while the SP, LP, and total MBP all showed a continuous increase, so that by 15 hr they were 2.8, 5.5, and 2.7 times the initial value, respectively.

The effects of other hormones were tested on their ability to stimulate formation of both membrane-bound and free polysomes. Polysome profiles obtained after treatment of aged tissue for 12 hr with lanolin (control); the nonauxin hormones, BA and GA; the auxins IAA, NAA, and the mixture of IAA plus BA were analyzed and the analyses presented in Table II. With the free polysomes, the monosome fraction was extremely large in nonauxin-treated tissues, whereas after all auxin treatments it declined to less than one-sixth of the control value (Table II). At the same time as auxins were causing a tremendous decrease in free monosomes, they caused a greater than 4-fold increase in large free polysomes, even though the total amount of material in the FP fraction declined after treatment with auxins. Changes in MBP were equally dramatic. Both small and large polysome fractions increased after auxin treatment, with IAA + BA causing a 6-fold increase in large polysomes. The decrease in total free ribosomal material after auxin treatment was almost exactly balanced by an increase in total membrane-bound material, so that the total amount of ribosomal material remained almost constant, regardless of treatment. The increase in free polysomes reported earlier (1) cannot be a result of the release of preexisting polysomes from membranes since the amount of membrane-bound polysomes also increases after auxin treatment (Fig. 1; Tables I and II).

This aged system has some advantages over the normal system for studying auxin effects since the former does not exhibit a fast growth response (Table I), whereas the latter does (3). The effects of the hormone on polyribosome metabolism in aged tissue precede growth and may be a prerequisite to it, whereas in fresh tissue, growth occurs before any measurable change in polysome metabolism occurs (1). However, it was shown in Table I that more than 3 hr elapsed before any increase in polysomes in the MBP fraction occurred. At least part of this lag may arise from the slow penetration of auxin into the tissue. In

Table I. *Time Course of Auxin-stimulated Formation of Membrane-bound Polyribosomes in Aged Pea Tissue*

Time after Treatment	Fresh Wt	Ribosome Distribution[1]			
		M	SP	LP	T
hr	*mg/segment*				
0	29.0	44	23	37	104
3	29.0	18	16	48	80
6	29.6	16	54	100	170
9	30.4	16	38	129	183
12	31.0	17	61	155	233
15	32.4	19	61	200	280

[1] Obtained from areas of regions depicted in Fig. 1.

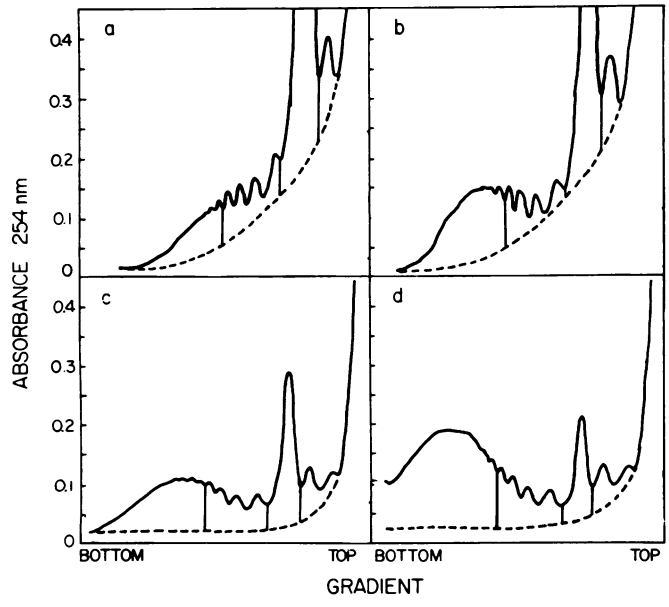

FIG. 2. Free and membrane-bound polyribosomes isolated from 3-mm tips of 3-day-aged pea epicotyls 3 hr after treatment. Profiles represent material isolated from 50 segments. Source and treatment were: a: FP, lanolin; b: FP, IAA + BA; c: MBP, lanolin; d: MBP, IAA + BA.

Table II. *Distribution of Ribosomes in Free and Membrane-bound Polyribosome Fractions from Hormone-treated Aged Pea Tissue*

Two-day-aged epicotyls were treated with lanolin paste containing hormone at 0.5% (w/w). Values correspond to extract from 20 segments obtained 12 hr after hormone treatment.

Treatment	Areas under regions of profiles								
	Free polysomes				Membrane-bound polysomes				Total
	M	SP	LP	T	M	SP	LP	T	(FP + MBP)
Lanolin	405	36	39	480	22	29	39	90	570
BA	375	36	30	441	0	33	70	103	544
GA	385	24	45	454	27	24	75	126	580
IAA	69	51	138	258	0	110	213	323	581
NAA	60	81	153	294	0	75	197	272	566
IAA + BA	48	69	147	264	0	108	241	349	613

Table III. *Early Hormone-induced Changes in Free and Membrane-bound Polysomes*

Data are from profiles depicted in Figure 2; Calculations as in Table I. Values correspond to extract from 50 segments (3 mm tips).

Treatment and Fraction	Ribosome Distribution			
	M	SP	LP	T
Lanolin				
FP	101	27	32	160
MBP	66	24	66	156
Total	167	51	98	316
IAA + BA				
FP	39	31	54	124
MBP	23	36	128	187
Total	62	67	182	311

order to find out how soon auxin effects on polysome metabolism could be observed, aged tissue was treated for 3 hr with IAA + BA and then only the terminal 3 mm of the epicotyl was excised. The profiles in Figure 2 and the analyses in Table III are from such 3-mm tips. In both the FP and MBP fractions, the monosomes decreased by about 60% after hormone treatment and the large polysome fraction increased almost 2-fold, even though the total amount of material did not change. The most noticeable effect of auxin treatment was to stimulate recruitment of free monosomes into membrane-bound polysomes. This suggests that the hormone has a specific effect on the synthesis of a particular group of proteins—those synthesized on MBP and possibly destined for secretion into the wall. Since this major shift in distribution of membrane-bound polysomes (Table III) preceded growth by at least 12 hr (Table I), it might be one of the initial responses to auxin. Since a potential wall-softening enzyme, cellulase, is synthesized primarily on membrane-bound polysomes (5) and since cellulase increases even more dramatically in aged than in fresh tissue after auxin treatment (2), the changes in polysome metabolism of aged tissue reported here might be related to auxin-induced synthesis on membrane-bound polysomes of secreted enzymes involved in growth.

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