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Intercellular communication in plants: Evidence for a rapidly generated, bidirectionally transmitted wound signal

[polyribosome formation/wheat germ system/poly(A) hybridization to poly(U)/protein synthesis]

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ABSTRACT Wounding (whether by excision, abrasion, or puncture) elicited rapid, massive, and enduring formation of polysomes in aged pea stems and other mature tissues. The response depended on temperature and severity of wounding but not on water uptake, and it occurred in tissues adjacent to, distant from, above, and below the site of injury. The kinetics of polysome formation were similar in tissues adjacent to or up to 150 mm distant from the point of injury. The wound-induced increases in protein-synthesizing capacity of the polysomes both *in vivo* and *in vitro* were much greater than the increases in rRNA and poly(A)⁺ RNA. The results indicate that wounding evokes the almost immediate production of a wound signal that travels rapidly both acropetally and basipetally to stimulate the recruitment of preexisting ribosomes onto primarily preexisting mRNA, thence forming polysomes with greatly enhanced protein-synthesizing capacity.

Plant tissues exhibit a variety of physiological responses to wounding, including the generation and transmission of action potentials (1), the formation of callose (2), changes in ion flux (3), enhanced production of ethylene (4), and synthesis of protease inhibitor-inducing factor (5). At least some of the responses can be linked to changes in the electrogenic or hydrostatic properties of membranes (6), and all of them have been demonstrated in nonstorage regions such as leaves, stems, and roots. Wounding also evokes very rapid polyribosome formation in many plant storage organs (7), but only one case of wound-induced polysome formation has been reported for nonstorage tissue (8).

The experiments reported here provide information concerning wound-induced polysome formation in a variety of plants and tissues and support the contentions that wound-induced signals are widespread among plants and that the ensuing wound signal-induced polysome formation is neither a transient nor an inconsequential response.

MATERIALS AND METHODS

Treatment of Plants. Seeds of peas (*Pisum sativum* L. cv. Alaska) were washed briefly in 10% (vol/vol) hypochlorite and rinsed and soaked overnight in tap water prior to being sown in vermiculite. After 8 days of growth in the dark at 20°C ± 2°C, the plumule and hook were excised from those plants with the third internode 2–6 cm long and the entire epicotyl 18–22 cm long. Lanolin was smeared over the cut surface to prevent the tissue from drying out. These decapitated plants were kept in the dark to age for 2.5–3.5 days. During this aging period, the apical 1- to 2-cm growing region of the third internode typically ceases to enlarge, and the ribosome population shifts from a polysome/monosome ratio of about 90%:10% to about 40–60%:60–40% (9).

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Aged pea epicotyls were wounded and treated as follows. (i) The 2-cm apical region was excised and incubated under various conditions prior to analysis. The excision cut was the wound. (ii) The entire epicotyl 17 cm below the apex was excised. The basal portion (adjacent to the excision or wound) and the apical portion (distant from the excision wound) were analyzed separately after incubation. (iii) Approximately 1 mm was trimmed from the apex (thereby inflicting a wound), and the adjacent (apical) tissue below and the distant (basal) tissue further below were both analyzed after incubation. (iv) The apical 2-cm portion was abraded with carborundum and incubated by inverting the plant in buffer prior to analysis of that region. (v) The epicotyl was punctured with a needle at a point 6 cm below the apex, incubated, and the three consecutive 2-cm regions above and the three below the puncture were analyzed.

Unaged (rapidly growing) pea epicotyls were wounded by excising the apical 1- to 2-cm growing region. After incubating the excised portion in buffer, it and a 2-cm region of basal (nongrowing) tissue were analyzed for polyribosomes.

Seeds of soybean (*Glycine max* cv. Amsoy) and corn (*Zea mays* cv. unknown) were washed and sown as pea seeds were. Soybeans were grown in the dark for 5–6 days until the hypocotyls were 10–15 cm long. They were wounded by excising immediately below the cotyledons or at the base and then were incubated with the cut surface immersed in buffer. Two-cm regions from the apex (growing tissue) and from the base (nongrowing tissue) were analyzed separately for polysomes. Corn seeds were grown in the dark for 4–5 days until the roots were 8–12 cm long. Apical (growing) 1-cm regions and basal (nongrowing) 2-cm regions were excised and incubated in buffer prior to analysis. Tobacco (*Nicotiana tabacum* cv. Havana) plants were grown in the greenhouse. Discs 2 cm in diameter were excised and incubated in buffer prior to analysis. Segments excised from dark-grown plants (peas, soybeans, and corn) were incubated in the dark. Tobacco leaf discs were incubated under fluorescent laboratory lighting.

Extraction and Analysis of Tissue Components. Total (free plus membrane-bound) polysomes were isolated by using the high pH, high ionic strength Tris buffer and analyzed as described (10). These analyses involved (i) subdividing the polysome profile into subunits plus monosomes, small polysomes bearing 2 to 5 ribosomes, and large polysomes bearing six or more ribosomes, and (ii) measuring the area under each subdivision. Total polysomes, P, refers to small plus large polysomes, and total ribosomal material, T, refers to subunits plus monosomes plus polysomes. Ratios of these values were used, rather than actual amounts measured from profiles, to eliminate slight variations in the total amount of material extracted.

Ribosomal RNA was extracted in glycine/phosphate/salt buffer, and rRNA was quantitated from gradients as described (10). Poly(A)⁺ RNA in whole tissue extracts or in polysomes was

assayed by hybridization to ^3H -labeled poly(U) as described (11).

Protein Synthesis *in Vivo* and *in Vitro*. Protein synthesis *in vitro* was measured by using purified polysomes to program the wheat germ system as described by Roberts and Paterson (12). Protein synthesis *in vivo* was measured by incubating 2- to 3-mm slices of control or previously excised tissue in phosphate buffer (250 mg of tissue per 0.6 ml) containing [^3H]leucine (100 $\mu\text{Ci}/\text{ml}$; 1 Ci = 3.7×10^{10} becquerels) for 30 min after vacuum infiltration at 33 kPa (0.33 atmosphere) for 2 min. Such infiltration techniques had little deleterious effect on polysome formation (see Table 2). Protein was extracted in 0.05 M Tris-HCl (pH 6.2) containing 0.7 M sucrose, 2 mM dithiothreitol, 0.1 M KCl, and 5 mM Na_2EDTA and was reextracted twice in an equal vol of phenol. The phenol extract was precipitated in 5 vol of 0.1 M NH_4OAc in methanol, washed three times with the same solution, and then dissolved in 60 mM Tris-HCl (pH 8.0), containing 0.15 M sucrose, 2 mM dithiothreitol, and 2% (wt/vol) sodium dodecyl sulfate. Radioactivity was measured on a Packard Tri-Carb B2450 scintillation counter optimized for tritium.

RESULTS

The profiles depicted in Fig. 1 show that excision of tissue from the apex of aged pea epicotyls resulted in a temperature-dependent formation of polyribosomes. The response was significant within 30 min, massive by 3 hr, and sustained for 24

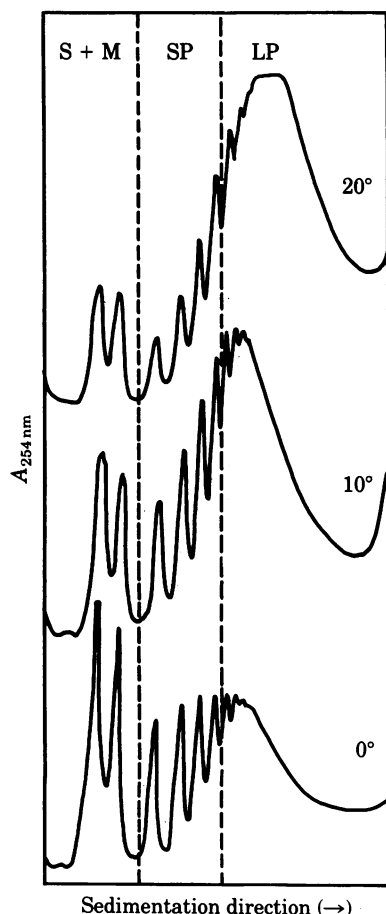


FIG. 1. Effect of temperature ($^{\circ}\text{C}$) on polysome formation after excision. Ten 20-mm segments were excised from the apex of 3-day aged pea epicotyls and incubated upright with their cut surfaces exposed to buffer. Incubation was for 3 hr at 0°C , 10°C , or 20°C . S+M, Subunits plus monosomes; SP, small polysomes; LP, large polysomes. Results from one typical experiment.

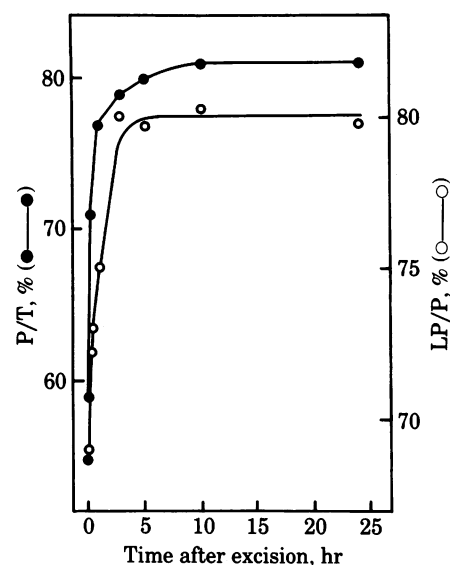


FIG. 2. Polysome formation after excision is rapid, massive, and enduring. Protocol is as in Fig. 1, except that incubation was at 25°C and periods varied from 30 min to 24 hr. Profiles were analyzed and the ratios of total polysomes:total ribosomal material (P/T) and of large polysomes:total polysomes (LP/P) were expressed as percentages. Typical results from one short-term and one long-term experiment were combined.

hr (Fig. 2). In some experiments the proportion of polysomes began to decline within 12 hr and always declined within 36 hr. Excision-induced polysome formation was not a response peculiar to aged pea epicotyls but occurred in other nongrowing tissues such as segments excised from basal soybean hypocotyls and fully elongated regions of corn roots and discs excised from fully expanded tobacco leaves. Little or no polysome formation was seen in segments excised from rapidly growing tissues such as unaged pea epicotyls, apical soybean hypocotyls, and corn-root tips (Table 1). However, upon wounding, such tissues did generate a signal and transmitted it basipetally to nongrowing tissues, which responded by forming polysomes (data not shown).

The formation of polysomes does not appear to be a consequence of enhanced water uptake through the cut surface be-

Table 1. Polysome formation in various tissues in response to excision

Tissue type and region	Polyribosome content after excision, %					
	P/T			LP/P		
	0*	30*	60*	0*	30*	60*
Tobacco leaf, mature	58	67	79	43	56	60
Corn root, mature	45	51	61	44	58	64
Corn root, tip	82	84	81	67	69	67
Soybean hypocotyl, basal	60	67	79	53	69	73
Soybean hypocotyl, apical	91	89	88	72	71	72
Pea epicotyl, aged	39	55	66	49	59	78
Pea epicotyl, fresh	91	86	89	78	77	79

Discs excised from tobacco leaves or segments excised from corn roots, soybean hypocotyls, and pea epicotyls were homogenized immediately or after incubation in phosphate buffer for 30 or 60 min. Results from one typical experiment. P/T, Ratio of total polysomes to total ribosomal material (subunits plus monosomes plus polysomes); LP/P, ratio of large polysomes bearing six or more ribosomes to total polysomes.

* Time in minutes after excision.

Table 2. Polyribosome formation in relation to water uptake

Incubation treatment	Polysome content, %	
	P/T	LP/P
None (zero time)	53	62
High humidity (no buffer)	87	76
Lanolin on base (no buffer)	84	82
Buffer (\pm mannitol)	78–81	71–79
None (zero time)	43	56
Pieces (3-mm) in buffer (0.5–1.0 bar)	68–71	72–78

Ten 20-mm segments excised from the apex of 2.5-day aged pea epicotyls were homogenized immediately (zero time) or after incubation for 1 hr (i) in a bell jar (high humidity), (ii) with lanolin smeared on the base, or (iii) in buffer or buffer containing 0.4, 0.6, or 0.8 M mannitol. Alternatively, five 20-mm segments from 3.5-day aged pea epicotyls were cut into approximately 3-mm pieces and incubated for 1 hr in buffer under normal atmospheric pressure or after being subjected to vacuum infiltration for 5 min at 0.83, 0.67, or 0.50 bar (1 bar = 10^5 Pa). Results from one typical experiment. P/T, Ratio of total polysomes to total ribosomal material (subunits plus monosomes plus polysomes); LP/P, ratio of large polysomes bearing six or more ribosomes to total polysomes.

cause tissues incubated in hypertonic solutions or in the absence of water exhibited the response and because vacuum-infiltrated tissue did not show enhanced response (Table 2). In fact, it does appear to be a direct consequence of wounding because abrading the cuticle, puncturing with a needle, and trimming the apex also evoked polysome formation (Table 3). However, rubbing the tissue (13) to wipe excess lanolin off the apex prior to reapplication of lanolin containing auxin was not sufficiently injurious to stimulate polysome formation, hence the auxin-induced polysome formation described earlier (9) is not an artifact of wounding. The effect of increasing the severity of wounding was tested by increasing the number of cut surfaces per 2-cm apical aged tissue. Some segments were excised 20 mm from the apex (to yield one cut surface), others were trimmed also 1 mm from the apex (two cut surfaces), and others were sliced in addition transversely into two (four cut surfaces) before being incubated in buffer for 1 hr. The proportion of ribosomes as polysomes (P/T), which was 51% in the control, increased similarly in all treatments to 87–88%. However, the proportion of large polysomes (LP/P) increased from 54% (control) to 58%

Table 3. Polyribosome formation in response to various forms of injury

Treatment	Polyribosome content, %	
	P/T	LP/P
None (zero time)	43	28
Excised	72	41
Apex trimmed	71	42
Cuticle abraded	65	41
Punctured	67	42
Intact plus lanolin	41	25
Intact plus lanolin and auxin	68	46

Ten 20-mm segments from the apex of 3.5-day aged epicotyls were homogenized immediately (zero time) or injured and incubated for 1 hr (i) upright in buffer (excised), (ii) upside down in buffer (trimmed or abraded, yet otherwise intact), or (iii) intact in air (punctured). Alternatively, 20 10-mm segments were excised from intact tissue after retreatment with lanolin \pm auxin for 12 hr. Results from one typical experiment. P/T, Ratio of total polysomes to total ribosomal material (subunits plus monosomes plus polysomes); LP/P, ratio of large polysomes bearing six or more ribosomes to total polysomes.

(one cut) to 67% (two cuts) to 72% (four cuts).

Two important conclusions can be derived from these experiments (Tables 1–3). First, the response is too massive for it to be restricted to the wounded cells alone because these comprise less than 1% of the files of 120–150 cells contained in 20-mm segments. This implies that some signal must be transmitted from the injured to the uninjured cells. Second, the signal can be transmitted both acropetally (segments excised or punctured at 20 mm; tissue above examined) and basipetally (apex trimmed; tissue below examined) (Table 3).

Such a wound-induced signal that elicits polysome formation has not to our knowledge been described previously, and so experiments were conducted to ascertain how far and how fast it could be transmitted and whether it declined in intensity at increasing distances from its site of generation (point of injury). The polysome content (P/T) of the six 20-mm regions (0–120 mm below the apex) of untreated aged pea epicotyls varied from 44% to 55%. However, the values for the same regions of similar epicotyls that had been punctured at a point 60 mm below the apex and “incubated” for 1 hr were 65–80%. The increase relative to the control was comparable (41–52%) in all regions. The signal must be transmitted simultaneously in both directions at a rate of at least 60 mm/hr without any apparent loss of intensity. However, these data do not differentiate between the time needed for generation of the signal (perception), its transmission (induction), and evocation of polysome formation (response).

Accordingly, an experiment was designed to provide partial separation of the time requirements of the perception, induction, and response mechanisms. Aged epicotyls were excised either 20 mm or 170 mm below the apex, and, at 5-min intervals thereafter, the 20-mm apical region was analyzed for polysome content. In both adjacent (excised at 20 mm) and distant (excised at 170 mm) tissue there was a lag of 10 min before polysome

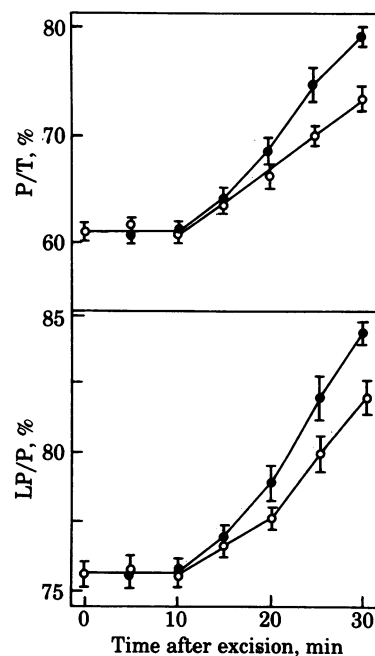


FIG. 3. Initial kinetics of polysome formation in tissue adjacent to and distant from the wounded site. Ten epicotyls were excised at 20 mm and 10 at 170 mm from the apex and were incubated upright in buffer for the times indicated. ●, Adjacent tissue; all of the tissue excised at 20 mm was homogenized. ○, Distant tissue; the 20-mm apical region was taken from epicotyls cut at 170 mm and immediately homogenized. Results are the mean of three experiments; vertical bars represent deviations.

Table 4. Changes in RNA and protein in tissues adjacent to and distant from the site of wounding

Excision site	Time after wounding, hr	rRNA content*	Poly(A) ⁺ RNA content [†]		Protein synthesis [‡]	
			Whole tissue	Polysomes	<i>In vivo</i>	<i>In vitro</i>
Adjacent tissue	0	10.1 ± 0.7	2480 ± 230	978 ± 32	1520 ± 110	3410 ± 190
	1	11.2 ± 0.9 (11%)	3170 ± 270 (28%)	1095 ± 76 (12%)	3390 ± 175 (123%)	7640 ± 320 (124%)
	3	12.2 ± 0.6 (21%)	3670 ± 210 (48%)	1340 ± 184 (37%)	3480 ± 130 (129%)	9210 ± 380 (170%)
Distant tissue	1	11.1 ± 0.3 (10%)	2730 ± 160 (10%)	1060 ± 122 (8%)	2675 ± 165 (76%)	5390 ± 270 (58%)
	3	11.3 ± 0.7 (12%)	3320 ± 210 (34%)	1270 ± 88 (30%)	2810 ± 185 (85%)	5730 ± 260 (68%)

Fifty 3-day aged pea epicotyls were excised at 20 mm (adjacent) or 170 mm (distant) from the apex. The apical 20 mm was homogenized immediately (zero time) or after incubation upright in buffer for 1 or 3 hr. Values are the means of three experiments with deviations. Values in parentheses represent the percentage increase over zero time.

* OD units per segment; derived from quantitation of peaks on gradients.

[†] ³H-Labeled poly(U) hybridized per segment.

[‡] ³H-Labeled leucine incorporated per segment.

formation became apparent at 15 min (Fig. 3). However, the magnitude of the response was somewhat greater in tissue adjacent to the cut. These results imply that the signal is generated in less than 15 min and transmitted at a rate in excess of 150 mm/5 min. They also suggest either that the signal declines in intensity at a distance from its site of generation or that a greater response is elicited in the wounded cells or in those immediately adjacent. Similar results were obtained in tissue trimmed at the apex and either the adjacent tissue (20 mm from the apex) or the distant tissue (150–170 mm from the apex) analyzed (data not shown). Therefore, the signal is generated and transmitted equally rapidly in both directions.

In an effort to determine which component(s) of the polyribosomes become(s) modified after wounding, numerous parameters were analyzed in tissue adjacent to or distant from the point of excision (Table 4). After 1 and 3 hr, in both adjacent and distant tissue there was little change in ribosomal RNA content, a slight increase in polysomal poly(A)⁺RNA, a somewhat larger increase in total tissue poly(A)⁺RNA, and a much more dramatic increase in protein-synthesizing capacity, both *in vivo* and by isolated polysomes *in vitro*. These results strongly infer that in both adjacent and distant tissues, the wound signal elicits the recruitment of preexisting monosomes onto primarily preexisting polysomal mRNA to cause the formation of polyribosomes with enhanced protein-synthesizing capacity.

DISCUSSION

It has been shown on numerous occasions that excision elicits polysome formation in a variety of plant storage tissues (7), although it is frequently considered to be a response to aging or dormancy-breaking (14) rather than to wounding. To our knowledge, there is only one report involving nonstorage tissues in which polysome formation occurs as a result of excision (8). Our data support the conclusions of Travis *et al.* (8) in that excised basal soybean hypocotyl tissue does form polysomes upon incubation, whereas apical tissue does not. However, the experiments reported here go much further and show that the response is due to wounding (Table 3) rather than to incubation in liquid media (Table 2), that a signal can be generated by the wounded cells and transmitted throughout the tissue (Tables 1 and 4; Fig. 3), and that apical tissue can generate and transmit this signal (data not shown) even though it does not appear, at least from polysome profiles (Table 1), to respond to that signal. These results also show that the signal can be transported very

rapidly (Fig. 3) both acropetally and basipetally (Table 3) and that the response is widespread amongst higher plant species and tissues (Table 1). Finally, these results show that the polysomes formed in response to wounding in both adjacent and distant tissues possess enhanced protein-synthesizing ability both *in vivo* and *in vitro* (Table 4).

The major questions that remain unanswered concerning wound-induced polysome formation and tentative answers based upon this and others' work are as follows.

(i) What is the signal and how is it transmitted? The signal may be a hormone such as ethylene (15), traumatin (16), Ricca's factor (17), or the protease inhibitor-inducing factor (5), which could be transported through the vascular system (17) or through the symplasm (18). Alternatively, the signal might be a reduction in hydrostatic pressure sensed and transmitted through the apoplast/symplast interface (6) or an action (variation) potential or ion flux transmitted via membranes (17). However, the rapidity with which the signal is generated, the bidirectional nature of its transmission, and the similarity between the responses induced by it and the pH/K⁺-induced polysome formation occurring during activation of sea urchin eggs (19) support a role for changes in membrane potential and ion fluxes as the signal.

(ii) Which component(s) of the protein-synthesizing machinery become(s) modified by the signal? The initial effect of the wound signal is to stimulate the recruitment of preexisting ribosomes onto preexisting polysomal mRNA (Table 4), and this argues strongly for an action at the site of initiation of translation. If the signal involves ion fluxes and changes in pH as is the case with sea urchin eggs (19), these same fluxes could have a direct effect on polyribosome formation and protein synthesis (i.e., the signal could directly elicit the response). This is not altogether unlikely because pH, K⁺, and Mg²⁺ all demonstrate optima for protein synthesis *in vitro*. A secondary effect of the wound signal might be to enhance transcriptional or posttranscriptional events (e.g., polyadenylation) because the amount of poly(A)⁺RNA does increase within 1 hr of wounding (Table 4).

(iii) Are novel proteins synthesized in response to wounding? Although the results presented here suggest that the primary event triggered by wounding is an increase in the rate of initiation of ribosomes onto preexisting polysomal mRNA and this would result in enhanced synthesis of existing proteins, results from other laboratories have demonstrated that novel proteins are synthesized in response to wounding. Such proteins in-

clude: 1-aminocyclopropane-1-carboxylic acid synthase, the regulatory enzyme in wound-induced ethylene biosynthesis (20); the proteinaceous protease inhibitors I and II (21) and carboxypeptidase (22) synthesized in leaves of wounded plants; wound-induced ribonuclease (23); and hydroxyproline-rich glycoproteins (24).

(iv) What advantages, if any, does this response confer upon the plant? It is impossible to answer this question yet, although it would appear highly unlikely that an organism would expend the energy needed to double its protein-synthesizing capacity within 1 hr of wounding (Table 4) and maintain an elevated level of polysomes for up to 24 hr (Fig. 2), unless the response conferred some advantage. However, it is premature to invoke this system as a defense mechanism against invading organisms or other environmental stresses.

(v) How do these findings relate to other work on excised or injured tissue? The finding here should generate caution on the part of physiologists, biochemists, pathologists, etc., who use excised, abraded, punctured, or otherwise damaged tissues to enhance uptake of exogenous materials such as precursors, hormones, antibiotics, or viruses because the properties of wounded tissues are markedly different from those of intact tissues. It is quite conceivable that the response to wounding could either magnify or diminish a response to the treatment (e.g., hormonal) under investigation. It appears as though plants can perceive that their integrity has been disrupted, and their compensatory responses may be deceiving to the unwary investigator.

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