Translational Activity of Polysomes of Barley Leaves During Infection by *Erysiphe graminis* f. sp. *hordei*

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This work was supported by the Wheat Industry Research Council of Australia and the Australian Research Grants Committee.

We thank R. S. Simpson for help in making polysome extractions and B. J. Biggs for preparation of translation systems involving *Escherichia coli*.

Accepted for publication 19 April 1983.

ABSTRACT

Manners, J. M., and Scott, K. J. 1983. Translational activity of polysomes of barley leaves during infection by *Erysiphe graminis* f. sp. *hordei*. Phytopathology 73:1386-1392.

Polysomes were extracted from leaves of *Hordeum vulgare* infected by *Erysiphe graminis* f. sp. *hordei*, and their ability to direct protein synthesis in vitro was assessed. Polysome-directed protein synthesis with soluble factors (S147 fraction) from wheat germ was usually three- to fourfold greater than that with the S147 fractions of *Escherichia coli*. In each instance, insensitivity to aurintricarboxylic acid indicated that little or no reinitiation occurred in vitro. The inhibition of protein synthesis by chloramphenicol, lincomycin, and cycloheximide indicated that the S147 fractions of wheat germ and *E. coli* preferentially activated protein synthesis on cytoplasmic and chloroplast polysomes, respectively. When equal quantities of total polysomes were included in assays of protein synthesis, the amounts of protein synthesized with the S147 fraction of wheat germ by polysomes from susceptible barley cultivar Prior inoculated 1, 3, and 5 days previously with *E. graminis* f. sp. *hordei* race 3 differed only slightly from uninoculated controls. However, with the S147 fraction of *E. coli*, the activity of polysomes from inoculated leaves were all considerably reduced (36–51%) below that of the controls. Similarly, when polysomes from the near-isogenic cultivars M1622 (susceptible) and M1623 (resistant) were used, a decrease (36%) in translational activity was detected in polysomes from M1622 at 1 day after inoculation with the system involving *E. coli*, but no differences in the activity of polysomes from control and inoculated leaves of M1622 or M1623 were detected with the S147 fraction of wheat germ. All the above results were verified by SDS-polyacrylamide gel electrophoresis and fluorography, which indicated that the decreased activity affected most of the chloroplast polypeptides that were synthesized. These results are consistent with a reduction in chloroplast polysomes per unit of total polysomes in infected leaves. Heat shock (45 C for 5–80 min) of cultivar Prior plants had a different effect on infection, and caused a 40–60% decrease in the activity of both cytoplasmic and chloroplast polysomes. The translational activity of thylakoid-bound polysomes was also assessed with the system involving *E. coli*. In Prior, no differences in activity were observed per unit of chlorophyll 1 day after inoculation, while a decrease of 31% was observed 3 days after inoculation. This indicated a decrease in the translational activity of barley chloroplast polysomes at early stages of infection by the powdery mildew fungus in susceptible, but not in resistant, hosts.

Additional key words: chloroplast proteins, heat shock, *Hordeum vulgare*, induced susceptibility, leaf mRNA, leaf protein synthesis.

Powdery mildew fungi are obligate parasites known to have profound effects on the metabolism of their hosts (39). One of the earliest alterations in host metabolism induced by infection with these fungi is the reduction in chloroplast polysome content described in infected barley by Dyer and Scott (13). This decrease in the chloroplast polysomes was detected at 24 hr after inoculation of a susceptible cultivar of barley, but not in a near-isogenic resistant cultivar. Because resistance was not expressed until 24 hr after inoculation in these experiments, Dyer and Scott (13) suggested that the decrease in chloroplast polysomes may be a primary event determining the pathogenic compatibility of these fungi and their hosts. Furthermore, this change in the chloroplast polysome content is undoubtedly related to the disease symptoms detectable at later stages of infection, such as reduced rates of photosynthesis and losses of chlorophyll (39).

One prediction from the findings of Dyer and Scott (13) is that the rate of protein synthesis on chloroplast polysomes should decline relative to that on cytoplasmic polysomes upon infection. In this paper, we have tested this possibility and describe the activity of polysome populations from control and powdery mildew-infected barley leaves in directing in vitro protein synthesis. Previous studies of the synthesis of chloroplast proteins (2,30) by isolated polysomes of barley have indicated that soluble factors from *E. coli* and wheat germ will preferentially activate protein synthesis on chloroplast and cytoplasmic polysomes, respectively.

The purpose of our experiments was to confirm this specificity and to exploit it to examine the relative protein synthesis activity of chloroplast and cytoplasmic polysomes in a mixed polysome population.

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MATERIALS AND METHODS

Plant and fungal material. *Hordeum vulgare* 'Prior' was obtained from the Queensland Wheat Research Institute, Toowoomba, Queensland, Australia. This cultivar is susceptible to race 3 of the powdery mildew fungus, *Erysiphe graminis* f. sp. *hordei*. The near-isogenic barley cultivars M1622 (USDA Accession CI 16138) and M1623 (CI 16137) were obtained from the U.S. Department of Agriculture, Beltsville, MD. These are either susceptible (M1622) or resistant (M1623) to race 3 of *E. graminis* f. sp. *hordei*. Plants were maintained as described previously (9) and inoculated 8 hr into the light period of the 6th day after planting by shaking a well-infected plant over each pot. Uninoculated controls were maintained under similar conditions. Heat shock treatments of uninoculated plants were carried out at 45 C under normal lighting.

Isolation of leaf polysomes. The apical 6-cm of leaves from either inoculated, heat-treated, or control pots were excised, wiped with a damp tissue to remove surface fungal structures, and immediately frozen in liquid nitrogen. Polysomes and monosomes were isolated by described methods (34) and pelleted by centrifugation through 40% (w/v) sucrose in isolation buffer. From here on this final fraction is referred to as the polysome preparation.

Isolation of thylakoids. Thylakoids were isolated by the method of Alscher et al (1) except that 5 mM spermidine was included in all buffers, and the buffer used for breaking intact chloroplasts contained 40 mM tris-HCl (pH 8.5), 30 mM magnesium acetate (MgAc), 60 mM KCl, 5 mM spermidine, and 5 mM β-

mercaptoethanol. The chlorophyll content of isolated thylakoids was estimated according to Bruinsma (7).

Preparation of soluble factors from wheat germ and *E. coli*. Commercial wheat germ was obtained from Mungo Scott Flour Mills, New South Wales, Australia, and a cell-free translation system was prepared according to the method of Roberts and Paterson (36) and stored in liquid nitrogen. Prior to assays, 1 ml of this fraction was thawed and layered onto 7 ml of 40% (w/v) sucrose in 20 mM Hepes-KOH buffer (pH 7.6), 5 mM spermidine, 120 mM potassium acetate (KAc), 2 mM MgAc, 1 mM dithiothreitol (DTT), and centrifuged at 147,000 *g* for 2.5 hr at 4 C. The upper layer was removed and treated with micrococcal nuclease (P-L Biochemicals, Inc., Milwaukee, WI 53205) as described by Pelham and Jackson (33).

Cells of *E. coli* (strain PR7, RNase$^-$, polynucleotide phosphorylase$^-$) were grown at 37 C in 1% (w/v) Bacto tryptone, 0.5% (w/v) yeast extract, 0.5% (w/v) NaCl, 2 mM NaOH to an $A_{560\ nm}$ of 0.5. Cells were harvested, and a cell-free translation system was prepared according to the method of Bottomley and Whitfeld (5) and stored in liquid nitrogen. Prior to assays, this was layered onto 7 ml of 40% (w/v) sucrose in 100 mM tris-HCl buffer (pH 8.4), 50 mM KCl, 10 mM MgAc, 5 mM spermidine, 10 mM β-mercaptoethanol, and the soluble factors were isolated and treated with micrococcal nuclease as described above.

From here on the final fractions from micrococcal nuclease-treated wheat germ and *E. coli* are referred to as S147 fractions.

Translation of leaf polysome- and thylakoid-bound polysome-associated mRNA. Incubation mixtures for cell-free protein synthesis with leaf polysomes (1–5 $A_{260\ nm}$ units) and either wheat germ (a) or *E. coli* (b) systems were prepared in a final volume of 50 μl as follows: a) wheat germ S147 fraction, 20 μl; 2 mM DTT; 0.1 mM GTP; 1 mM ATP; 8 mM creatine phosphate, 36 mM Hepes-KOH (pH 7.0); 8 μg/ml creatine phosphokinase (Sigma Chemical Co., St. Louis, MO 63178); 0.2 mM spermidine; and 20 μM of the appropriate unlabeled amino acids. b) *E. coli* S147 fraction, 20 μl; 110 mM ammonium acetate; (1.25 mg/ml *E. coli* tRNA (Sigma); 2 mM DTT; 0.1 mM GTP; 1 mM ATP; 8 mM creatine phosphate; 8 μg/ml creatine phosphokinase; 36 mM Hepes-KOH (pH 7.0); 0.2 mM spermidine; and 20 μM unlabeled amino acids. Concentrations of MgAc and KAc in both of the above mixtures were varied for optimization of translational activity (Table 1), and 1–5 μCi of ^{35}S-methionine (800–1600 Ci/mmole, Radiochemical Centre, Amersham, U.K.) was included in each assay. Incubations with the S147 fractions from wheat germ and *E. coli* were carried out at 27 and 30 C, respectively.

Incubation mixtures for cell-free protein synthesis with thylakoid-bound polysomes were carried out in 200 μl, containing 80 μl of fraction S147 from *E. coli* and all other constituents as described for assays of leaf polysomes except those in which ^{35}S-methionine (10–15 μCi) were included; incubations were at 30 C.

Following the incubations, tubes were chilled, and samples were removed and added to 10 μl of 2 mM unlabeled methionine. For assays with total leaf polysomes, 3 μl of 40 mg/ml bovine serum albumin (BSA) were added as carrier, and the incorporation of isotope into acid-precipitable and alkali-soluble material was determined (34). Sodium dodecyl sulphate (SDS) at a concentration of 1% (w/v) was included in the alkali extraction of translation products from assays with thylakoid-bound polysomes.

SDS-polyacrylamide gel electrophoresis and fluorography. Proteins synthesized in assays with total leaf polysomes were precipitated and washed with acetone (5). After drying, they were resuspended in 50 mM tris-HCl (pH 7.5), made to 2% (w/v) with SDS, 10% (w/v) with sucrose, and 40 mM DTT, heated at 100 C for 2 min, and analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) in gradient polyacrylamide gels prepared according to Chua (12). Products of thylakoid-bound polysomes were precipitated and washed twice with 10% trichloroacetic acid (TCA) at 4 C, washed twice with ethanol and once with ether, dried, and analyzed by SDS-PAGE as above. The following molecular weight markers were used: BSA, Mr 67,000; ovalbumin, Mr 43,000; soybean trypsin inhibitor, Mr 20,000; and cytochrome C, Mr 12,400.

Gels were stained in 0.25% (w/v) Coomassie Blue G-250 (Sigma) in 10% (v/v) acetic acid, 40% (v/v) methanol, destained in 5% (v/v) acetic acid, and 25% (v/v) methanol. Radioactive areas on gels were detected by quantitative fluorography using the water soluble fluor sodium salicylate (11). Experiments were repeated at least twice and typical results are shown.

RESULTS

Characterization of the translational activity of leaf polysomes with the S147 fractions of *E. coli* and wheat germ. As previously reported (2,30), barley leaf polysomes were very active in protein synthesis in cell-free translation systems (Figs. 1 and 2). The final levels of ^{35}S-methionine incorporation into protein in assays with the same polysome concentration were consistently three- to fourfold greater with the S147 fraction of wheat germ over that obtained with the S147 fraction of *E. coli* (Figs. 1 and 2). Polysome-directed protein synthesis with the S147 fractions of both wheat germ and *E. coli* was not significantly affected by aurintricarboxylic acid (ATA) at 20 μM (Table 1). This concentration of ATA strongly inhibits the initiation of protein synthesis on both 70S and 80S ribosomes (18,20,27).

A previous investigation of in vitro protein synthesis directed by barley leaf polysomes had indicated that the soluble factors from *E. coli* will activate translation of mRNA associated with polysomes of the 70S type, whereas with wheat germ-soluble factors a preferential translation of the cytoplasmic (80S ribosome type) polysomal mRNA pool occurs (2). To test these findings, we studied the effects of the inhibitors chloramphenicol (CAM), lincomycin (LINC), and cycloheximide (CYC) on polysome-directed protein synthesis (Table 1). The translational activity of barley leaf polysomes with the S147 fraction of *E. coli* was much

TABLE 1. Characteristics of the translational activity of total leaf polysomes and thylakoid-bound polysomes from *Hordeum vulgare* 'Prior' with S147 fractions of wheat germ and *Escherichia coli*

	Optimal concentration (mM)		Inhibition (%)[a]			
	Mg^{++}	K^+	ATA (20 μM)	CAM (0.2 mM)	LINC (1.2 mM)	CYC (70 μM)
Total polysomes + the S147 fraction of wheat germ	2.8	152	5	22	24	75
Total polysomes + the S147 fraction of *E. coli*	13	60	7	73	69	18
Thylakoid-bound polysomes + the S147 fraction of *E. coli*	13	80	17	81	74	8

[a] Incubation for 1 hr at 27 C was used for S147 fractions of wheat germ and 0.5 hr at 30 C for S147 fractions of *E. coli*. These data summarize results from several experiments using 1–5 $A_{260\ nm}$ units of polysomes per assay. ATA = aurintricarboxylic acid, CAM = chloramphenicol, LINC = lincomycin, and CYC = cycloheximide.

more sensitive to CAM and LINC than that with the wheat germ S147 fraction. In contrast, polysome-directed protein synthesis with the S147 fraction of wheat germ was much more sensitive to CYC. As CAM and LINC are inhibitors of peptide elongation on 70S ribosomes and CYC is an inhibitor of both initiation and elongation on 80S ribosomes (42), these results indicate that the S147 fractions of *E. coli* and wheat germ preferentially activate translation of mRNA associated with chloroplast and cytoplasmic polysomes, respectively. The cation requirements for these translation systems (Table 1) are also consistent with this specificity; the results of previous studies involving mRNA-dependent, cell-free translation systems derived from prokaryotes and eukaryotes have shown that translation on 70S ribosomes requires higher Mg^{++} concentrations and lower K^{+} concentrations than translation systems based on 80S ribosomes (1,2,4,5, 20,27,33,36). A further indication that the S147 fractions of *E. coli* and wheat germ activate peptide elongation on separate subpopulations of the leaf polysome pool is that different size classes of polypeptides were synthesized with these systems (Figs. 3 and 4). It should be noted that a large proportion of the products of in vitro protein synthesis were of high molecular weight and were probably nearly or completely elongated.

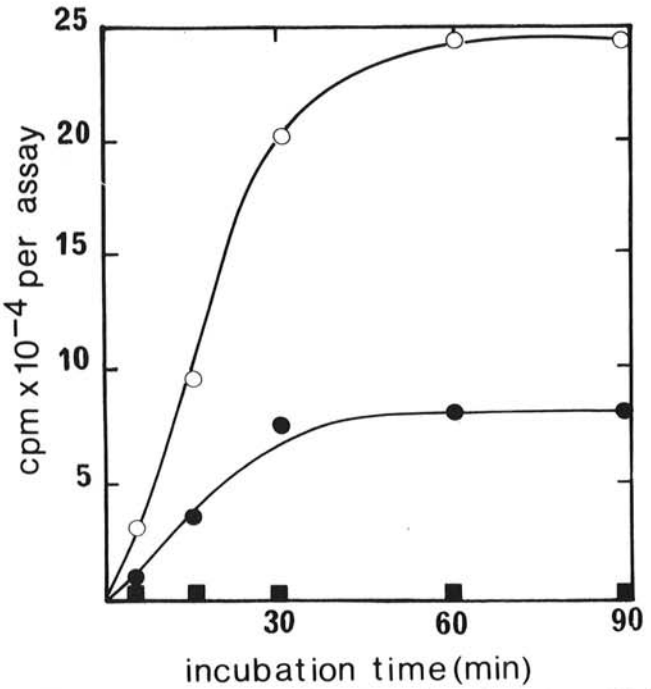

Fig. 1. Time course of polysome-directed protein synthesis. Assays (50 μl) were prepared as described in the methods section with one $A_{260\ nm}$ unit of polysomes from plants of *Hordeum vulgare* 'Prior' and 5 μCi of ^{35}S-methionine. Incubations were with either the S147 fraction of wheat germ (-o-) at 27 C or with the S147 fraction of *E. coli* (-•-) at 30 C. The activity without added polysomes (controls) for the S147 fraction of both the wheat germ and *E. coli* is indicated (-■-).

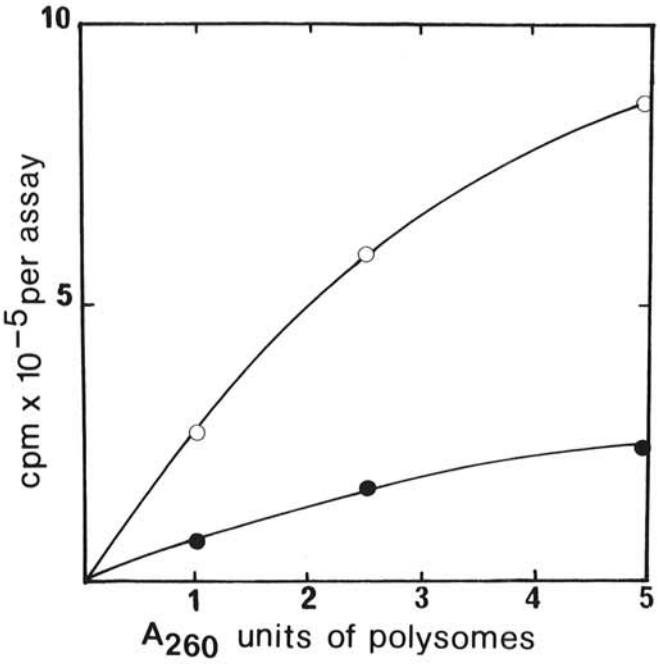

Fig. 2. Effect of increasing polysome concentration on polysome-directed protein synthesis. Assays (50 μl) were prepared as for Fig. 1 and incorporation of isotope into acid-precipitable material was estimated after 60 min of incubation with the S147 fraction of wheat germ (-o-) and 30 min with the S147 fraction of *E. coli* (-•-).

Translational activity of polysomes from control and inoculated leaves. A comparison was made of the translational activity of polysomes isolated from leaves of the susceptible barley variety Prior at 1, 3, and 5 days after inoculation with *E. graminis* f. sp. *hordei* and uninoculated controls. When equivalent $A_{260\ nm}$ units of polysomes from control and inoculated leaves were included in assays with the wheat germ S147 fraction very few differences were observed in their translational activities (Table 2). This was confirmed by SDS-PAGE and fluorography of the labeled products where the only differences observed were a slight reduction in incorporation into a few size classes of high molecular weight polypeptides (Fig. 5) synthesized in assays with polysome preparations extracted from leaves at 72 hr after inoculation when compared to controls. In contrast to results with the S147 fraction of wheat germ, when the translational activities of polysome preparations were assayed with the S147 fraction of *E. coli* a greatly reduced incorporation of isotope into acid-precipitable material was observed in assays containing polysome preparations from inoculated leaves compared to controls (Table 2). This effect of infection was evident as early as 1 day after inoculation and was still evident at 5 days when sporulation commenced. Analysis of the labeled products by SDS-PAGE and fluorography confirmed these results and indicated that all size classes of polypeptides synthesized by polysomes from inoculated leaves were decreased relative to controls (Fig. 3).

The effects of powdery mildew infection on the activities of polysomes from the near isogenic resistant and susceptible barley cultivars M1623 and M1622 were also investigated. The translational activities of polysome preparations from leaves of resistant and susceptible cultivars at 24 hr after inoculation were indistinguishable from uninoculated controls when the S147 fraction of wheat germ was used (Table 3). This was confirmed by analysis of the labeled polypeptides by SDS-PAGE followed by fluorography (Fig. 4). The similarity in the translational activities of polysome preparations from control and inoculated leaves of M1622 and M1623 was still apparent in the presence of CAM, which further indicated that the activity of the cytoplasmic pool of polysomes was unaffected by inoculation. When translations were carried out with the S147 fraction of *E. coli* there was a significant decrease of 36% in the translational activity of polysome preparations from the susceptible cultivar M1622 at 24 hr after inoculation when compared to uninoculated controls. This decrease in the translational activity of leaf polysomes was not observed in the resistant cultivar M1623. Results in the presence of cycloheximide were essentially similar, confirming that this represents an altered contribution of chloroplast polysomes to the activity of the total polysome preparations in inoculated leaves of susceptible barley cultivars only. Analysis of the polypeptides labeled in assays of these polysome preparations with the S147 fraction of *E. coli* by SDS-PAGE and fluorography indicated reduced incorporation of isotope into all size classes of polypeptide synthesized from polysome preparations of inoculated leaves of M1622. The products from assays of polysome preparations from control M1622 plants and both control and inoculated M1623 were very similar.

Translational activity of polysomes from heat-treated leaves. The translational activities of polysome preparations from barley leaves treated for various periods at 45 C were also assessed. Incorporation of ^{35}S-methionine by polysomes with the S147 fractions of both the wheat germ and *E. coli* was reduced to between 40 and 60% of controls by this heat treatment and as little as 5 min at 45 C was adequate to obtain this effect (Table 4). It should be noted that even after 80 min at 45 C there was still a large proportion (38–47%) of active chloroplast and cytoplasmic polysomes.

Translational activity of thylakoid-bound polysomes from control and inoculated leaves. The translational activity of thylakoid-bound polysomes from the cultivar Prior was assessed with the S147 fraction of *E. coli*. The concentrations of Mg^{++} and K^{+} that gave optimal incorporation of ^{35}S-methionine into acid precipitable material were similar to those obtained for total leaf polysomes with the S147 fraction of *E. coli* (Table 1). In addition,

TABLE 2. The effect of powdery mildew infection upon the translational activity[a] of polysomes isolated from *Hordeum vulgare* 'Prior' with the S147 fractions of either wheat germ or *Escherichia coli*

	S147 fractions					
	Wheat germ			*E. coli*		
Days after inoculation	Control (C)	Inoculated (I)	I/C	(C)	(I)	I/C
1	76.6	67.1	0.87	25.2	13.6	0.54
3	90.6	78.6	0.86	26.5	13.0	0.49
5	43.9	45.5	1.04	14.4	9.2	0.64

[a] Incubations were as described from Table 1. Assays (50 μl) contained 5 μCi of ^{35}S-methionine and 1.5 $A_{260\ nm}$ units of polysomes and results are expressed as cpm $\times 10^{-3}$/ 10 μl incorporated into acid-insoluble materials.

TABLE 3. Effect of powdery mildew infection upon the translational activity of polysomes isolated from susceptible (M1622) and resistant (M1623) near-isogenic lines of *Hordeum vulgare* with the S147 fractions of either wheat germ or *Escherichia coli*. Polysomes were isolated at 24 hr after inoculation

	S147 fractions					
	Wheat germ			*E. coli*		
Source of polysomes	Control (C)	Inoculated (I)	I/C	(C)	(I)	I/C
M1622	51.03	48.04	0.94	18.15	11.61	0.64
M1623	54.34	52.33	0.96	15.73	16.55	1.05
M1622 + CAM	37.01	39.14	1.05	ND	ND	ND
M1623 + CAM	41.06	40.30	0.98	ND	ND	ND
M1622 + CYC	ND	ND	ND	14.98	10.88	0.73
M1623 + CYC	ND	ND	ND	12.57	13.13	1.04

[a] Assays (50 μl) contained 2.4 $A_{260\ nm}$ units of polysomes and 5 μCi of ^{35}S-*methionine*. Incubations were as for Table 1 and results are shown as dpm $\times 10^{-3}$/10 μl incorporated into acid-insoluble material. ND = not determined.

TABLE 4. The effect of powdery mildew infection on the translational activity[a] of thylakoid-bound polysomes isolated from plants of *Hordeum vulgare* 'Prior'

Days after inoculation	Control (C)	Inoculated (I)	I/C
1	11.13	11.23	1.01
3	9.28	6.44	0.69

[a] Incubations were as for Table 1 and assays (200 μl) included 100 μg of chlorophyll and 12 μCi of ^{35}S-methionine. Results are expressed as dpm $\times 10^{-3}$/ 50 μl incorporated into acid-insoluble materials.

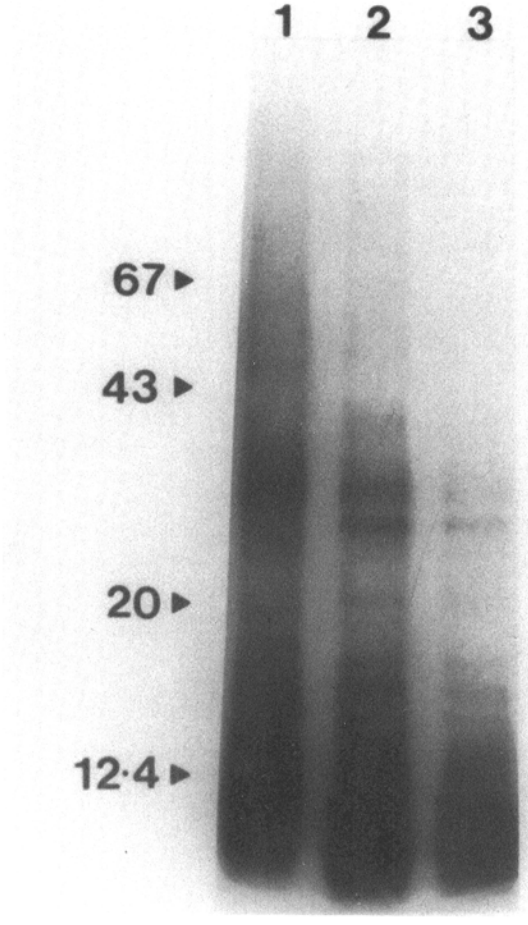

Fig. 3. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of the polypeptides synthesized by isolated leaf polysomes of control and powdery mildew-infected plants of *Hordeum vulgare* 'Prior' with the S147 fraction from *E. coli*. Lanes show products from the following: (lane 1) polysomes of 7-day-old plants plus the wheat germ S147 fraction, (lane 2) polysomes of 7-day-old plants plus the S147 fraction from *E. coli*, (lane 3) polysomes of 7-day-old plants 1 day after inoculation with *E. graminis* f. sp. *hordei* with the S147 fraction from *E. coli*. Assays were as for Table 2, and 20 μl was removed for analysis for lanes 2 and 3 and 7 μl for lane 1.

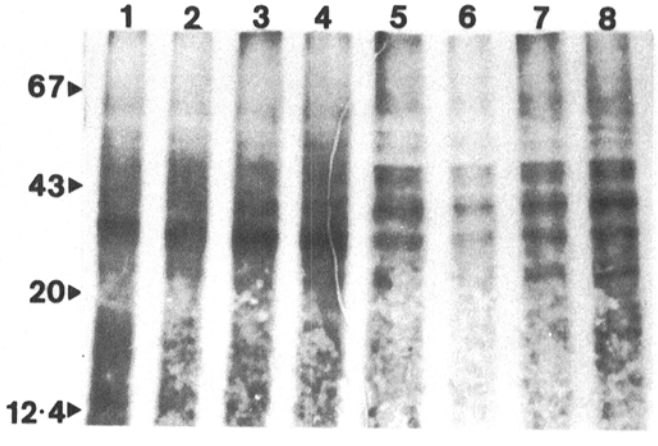

Fig. 4. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of the polypeptides synthesized by leaf polysomes isolated from control and powdery mildew infected susceptible (M1622) and resistant (M1623) near-isogenic lines of *Hordeum vulgare*. The S147 fractions from wheat germ (lanes 1–4) and from *E. coli* (lanes 5–8) were both used. Polypeptides synthesized from the following polysome preparations are shown: (lanes 1 and 5) 7-day-old M1622; (lanes 2 and 6) 7-day-old M1622 1 day after inoculation with *E. graminis* f. sp. *hordei*; (lanes 3 and 7) 7-day-old M1623; and (lanes 4 and 8) 7-day-old M1623 1 day after inoculation. Assays were prepared as for Table 3, and 7 μl was removed for analysis for lanes 1–4 while 20 μl was removed for lanes 5–8.

the time course of in vitro protein synthesis directed by thylakoid-bound polysomes from a 7-day-old plant was similar to that for total polysomes (Fig. 1) as incorporation ceased after 30 min at 30 C. After this period the incorporation exceeded that in zero time controls by 10- to 12-fold; incorporation in the absence of thylakoids was negligible. The optimal concentration of thylakoids in the in vitro assays was determined to be 0.5 mg/ml of chlorophyll (*unpublished*). The inhibitor ATA had only a slight effect on protein synthesis, indicating that very little reinitiation was occurring (Table 1). The effects of CAM, LINC, and CYC were similar to results described previously for total polysomes with the S147 fraction of *E. coli* and confirmed that protein synthesis was occurring on 70S ribosomes (Table 1).

When the translational activity of thylakoid-bound polysomes was assessed with equivalent chlorophyll concentrations in the assays, no differences were observed in the amount of protein synthesis from thylakoids of control and infected leaves at 24 hr after inoculation. However, a significant decrease (31%) was observed in the activity of polysomes from diseased leaves at 72 hr after inoculation compared to controls (Table 5).

Analysis of the products by SDS-PAGE and fluorography indicated more discrete products were synthesized on thylakoid-bound polysomes than those produced by total polysomes (Fig. 6). Again, no differences were observed in the various products at 24 hr after inoculation, but reduced incorporation was observed into most products at 72 hr after inoculation.

TABLE 5. The effect of heat treatment of plants of *Hordeum vulgare* 'Prior' on the translational activity[a] of isolated polysomes

Time (min) at 45 C	Incorporation (% of control) mediated by the S147 fraction	
	Wheat germ	*E. coli*
0	100	100
5	57	44
20	35	45
80	47	38

[a] Incubations were as for Table 1 and assays (50 μl) contained one $A_{260\ nm}$ unit of polysomes, 2.5 μCi of ^{35}S-methionine.

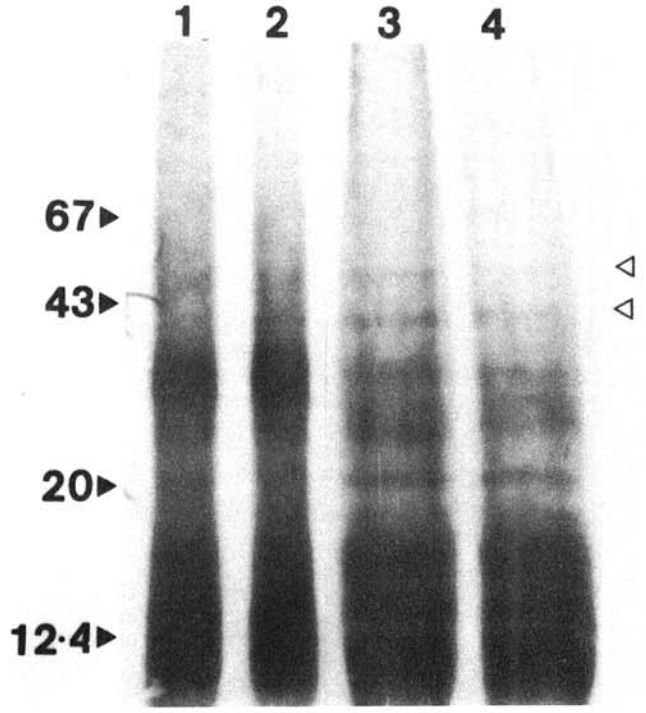

Fig. 5. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of the polypeptides synthesized by isolated leaf polysomes of control and powdery mildew-infected plants of *Hordeum vulgare* 'Prior' with the S147 fraction of wheat germ. Lanes represent products from the following polysome preparations: (lane 1) 7-day-old plants, (lane 2) 7-day-old plants 1 day after inoculation with *E. graminis* f. sp. *hordei*, (lane 3) 9-day-old plants, and (lane 4) 9-day-old plants 3 days after inoculation. Assays were prepared as for Table 2 and 20 μl removed for analysis as described in the Materials and Methods section. The positions and molecular weights ($\times 10^{-3}$) of marker proteins are indicated by solid arrows. Open arrows indicate polypeptides with obvious decreases in incorporation relative to controls.

DISCUSSION

The major finding of our investigation is that reduced amounts of in vitro protein synthesis by chloroplast polysomes are observed in total polysome fractions isolated from barley leaves following infection by *E. graminis* f. sp. *hordei*. In contrast to this, infection has little effect on the activity of cytoplasmic polysomes. The reduced activity of chloroplast polysomes per unit of total polysomes from infected leaves is detectable as early as 24 hr after inoculation and thus may be essential for the establishment of the compatible host-parasite interaction.

In the experiments reported here, protein synthesis with isolated polysomes, including both chloroplast and cytoplasmic species, was restricted to the in vitro elongation of polypeptides whose synthesis was initiated in vivo. Under these conditions, each polysome-associated ribosome will only produce a single polypeptide in vitro, and the relative amounts of protein synthesized from cytoplasmic and chloroplast polysomes is thus likely to reflect the proportions of 80S and 70S ribosomes present in the total polysome population. Thus, results imply a reduction in the number of chloroplast polysomes per unit of total polysomes, rather than a reduced rate of protein synthesis on each chloroplast polysome. This is consistent with the findings of Dyer and Scott (13) who demonstrated a reduction in the number of chloroplast polysomes using electrophoresis of RNA from purified polysomes.

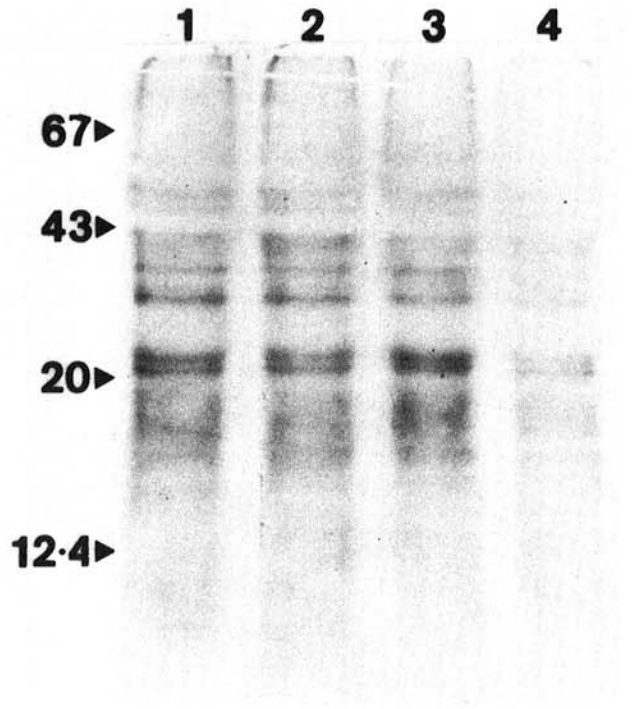

Fig. 6. Analysis by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and fluorography of the polypeptides synthesized by thylakoid-bound polysomes of plants of *Hordeum vulgare* 'Prior.' Lanes represent products from assays with the following thylakoid preparations: (lane 1) 7-day-old plants; (lane 2) 7-day-old plants 1 day after inoculation with *Erysiphe graminis* f. sp. *hordei*; (lane 3) 9-day-old plants; and (lane 4) 9-day-old plants 3 days after inoculation. Assays were prepared as for Table 4 and 50 μl removed for analysis.

By the same reasoning, the low incorporation of ^{35}S-methionine into protein by leaf polysomes with the S147 fraction of *E. coli* when compared to incorporation with the S147 fraction of wheat germ (Figs. 1 and 2) probably means that the chloroplast polysomes represent a smaller proportion of the total polysome population of barley leaves. This is also consistent with the data of Dyer and Scott (13) who demonstrated that cytoplasmic ribosomal RNA species predominated in the polysome population. The specificity of the translation systems is not absolute as some inhibition by CAM and LINC (22–24%) occurred even with the wheat germ system. It is possible that the inhibited fraction may correspond to the whole chloroplast population, but the overall contribution of chloroplast polysomes during translation with the wheat germ system is slight because of the preponderance of cytoplasmic polysomes.

It is unlikely that contamination by fungal haustoria affected our results, because changes in polysomes were detected at early stages of infection and then only in the activity of polysomes of the 70S ribosome type. These are unlikely to be major components of the fungus (24). In addition, we did not distinguish between the translational activities of chloroplasts and mitochondrial polysomes in this investigation. However, the effects of infection on the activity of polysomes of the 70S ribosome type can almost certainly be assigned to the chloroplast, as this is a major site of protein synthesis in green leaves (16). Because of the similarities of the mechanism of protein synthesis in chloroplasts and mitochondria it seems likely that mitochondrial polysomes may also be affected. This would be especially important in epidermal cells which lack fully developed chloroplasts.

Our ability to detect the decrease in chloroplast polysomes upon infection by monitoring the translational activity of the total polysome preparation clearly demonstrates that the fundamental metabolic processes of chloroplast protein synthesis are greatly affected by powdery mildew infection and at very early stages of disease development. The significance of these findings to the parasitism of these fungi is yet to be resolved, but it seems likely that an impairment of chloroplast protein synthesis in vitro would make larger quantities of nutrients and, in particular, amino acids available for transfer to the pathogen. It has been shown by double inoculation experiments that alterations in host metabolism are necessary for successful establishment of powdery mildew on barley (31). These changes in the host occur within 24 hr of inoculation; thus, one such change in host metabolism may possibly be a reduction in the translational activity of chloroplast polysomes.

The question arises as to how the powdery mildew fungus induces changes in chloroplast polysomes. As suggested by Dyer and Scott (13), the effect may be mediated by a diffusable product produced by the fungus. It is interesting that the mycotoxin, patulin, has been shown to induce polysome breakdown in mammalian systems (19). Although no information is available on the nature of such factors in mildewed barley, there is good evidence to indicate that polysome breakdown itself is mediated by an alteration in the catalytic properties of leaf ribonucleases during infection. A pH 5-insoluble RNase has been isolated from barley leaves and, unlike most of the leaf ribonuclease activity, this enzyme will preferentially degrade mRNA (41). Interestingly enough the pH 5-insoluble enzyme from inoculated leaves of susceptible barley hydrolyzes barley chloroplast polysomal mRNA to a greater extent than the enzyme from uninoculated controls (10,40).

A rapid breakdown of total polysomes to monosomes is a common reaction of plants and other organisms to stress conditions. These stresses include: heat shock (23,28), low temperatures (6), water stress (22,29), anaerobiosis (25), amino acid deficiency (37,38), and the application of metabolic inhibitors or toxins (19,25,26). In accordance with these observations, but in contrast to the effect of powdery mildew infection, we observed that treatment of barley leaves at 45 C for as little as 5 min substantially reduced the translational activity of both cytoplasmic and chloroplast polysomes. Similar heat treatment of barley leaves can render them susceptible to previously incompatible races of the barley powdery mildew fungus (32). Although both polysome types were affected by heat and only chloroplast polysomes by infection, these observations raise the possibility that impairment of leaf polysome function will suppress the resistance mechanisms of barley plants. In this respect, it is interesting that the change in activity of chloroplast polysomes in the susceptible barley cultivar M1622 at 24 hr after inoculation was not observed in the near-isogenic resistant cultivar M1623. Fungal development on leaves of M1622 and M1623 is very similar until 24 hr after inoculation (14) when reaction type is determined and the change in the activity of chloroplast polysomes is the earliest biochemical means of differentiating between these compatible and incompatible reactions. Models for the specificity of host-parasite interactions based on the suppression of host resistance reactions have recently been proposed (8) and our results indicate that pathogen-induced disfunction of chloroplast or mitochondrial polysomes requires consideration as a means for the suppression of resistance reactions in compatible powdery mildew interactions.

In some instances, heat shock and anaerobiosis of plants (3,17,23) results in the enhanced synthesis of a small array of stress related proteins in the cytoplasm. Similarly, stem rust infection of wheat has been reported to enhance the synthesis of some polypeptides using isolated polysomes of wheat (34,35). Our results with the wheat germ system and barley leaf polysomes indicate few changes in the polypeptides synthesized by polysomes of cytoplasmic origin in powdery mildew infected leaves. Thus, it would appear that these two obligate parasites have distinct effects on the metabolism of their hosts.

Although the role of chloroplast polysomes in compatibility remains unresolved, the reduced translational activity of these polysomes upon infection is almost certainly related to disease symptoms which are detected at later stages of infection. These include reduced photosynthetic activity and losses of chlorophyll, which are evident in mildewed leaves of barley from 3 days after inoculation. Unfortunately, it is not possible to project with certainty from our data obtained using in vitro polysome-directed protein synthesis to rates of protein synthesis operative in vivo. Overall rates of protein synthesis in vivo will necessitate consideration of the frequency of polypeptide initiation and rates of protein turnover. These factors are now being considered in our laboratory.

In view of the large change in the translational activity of total polysomes in infected leaves at 24 hr after inoculation with the system involving *E. coli*, it was surprising that changes in the activity of thylakoid-bound polysomes were not detected until 72 hr after inoculation (Table 3). This indicates that the decrease in chloroplast polysomes is initially restricted to polysomes of the stroma which predominate (15). Thylakoid-bound polysomes are thought to synthesize some proteins of the thylakoid membrane (1), and their binding to the membrane is probably mediated by the polypeptide undergoing elongation (1). Our results thus indicate that the synthesis of these proteins is only affected by disease at 3 days after inoculation on a chlorophyll basis. Recently it has been reported that no differences can be detected in electron transport rates and in the concentrations of P700, cytochrome *f* and cytochrome B559 in thylakoids from control and mildewed barley leaves up to 7 days after inoculation when expressed on a chlorophyll basis (21). However, a large decrease in the activity of these components occurred on a leaf fresh weight basis. Thus the decrease in translational activity of thylakoid-bound polysomes is the only detectable disfunction in thylakoid activity demonstrated on a chlorophyll basis so far in mildewed barley leaves.

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