RIBOSOME METABOLISM IN MILDEW-INFECTED BARLEY LEAVES

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1. Introduction

Nucleic acid metabolism is markedly altered in cereal leaves infected by obligate fungal parasites [1]. For example, in barley leaves infected with powdery mildew, total RNA increases immediately after the appearance of the first symptoms of disease and reaches a maximum relative to non-infected leaves at the time of sporulation. Subsequently, the RNA content decreases more rapidly in mildewed than in non-infected leaves [2–4].

In this paper we present results on ribosomal RNA (rRNA) metabolism in mildewed leaves which show that infection alters the metabolism of ribosomes from both the chloroplast and the cytoplasm.

2. Experimental

Barley (*Hordeum vulgare* L. var. *Manchuria*), highly susceptible to infection by powdery mildew (*Erysiphe graminis* var. *hordei* Marchal, race 3), was used as experimental plant material. Seeds were sown in pots of river sand in a green-house maintained at 22 ± 3°. Natural light was supplemented by fluorescent light between 6 a.m. and 10 p.m. each day, Seven days after sowing, some plants were inoculated with spores of powdery mildew. After harvesting, the surface of infected leaves was wiped with a damp tissue to remove the bulk of the fungus [2].

RNA was extracted from barley leaves (5 g) by the phenol–cresol procedure [5], fractionated by electrophoresis through 2.4% (w/v) polyacrylamide gels and made visible by staining with toluidine blue [6]. RNA was also extracted from leaves exposed to ^{3}H-orotic acid for 7 hr. The isotope was supplied to freshly excised leaves by transcription under white light (10,000 lux at 24°) [6]. The distribution of ^{3}H-RNA along the gel was determined by freezing the gel on dry ice, cutting it into slices 1 mm thick and washing the slices in ethanol and then toluene. Each slice was placed in a toluene-based liquid scintillant for radioactivity determinations [7].

3. Results

Ribosomal RNA was extracted from mildew-infected and non-infected barley leaves by the phenol–cresol procedure and fractionated into cytoplasmic rRNA and chloroplast rRNA by gel electrophoresis. Fig. 1 shows the profiles obtained when the rRNA bands were made visible by staining with toluidine blue. In infected leaves (fig. 1A), disease development was accompanied by pronounced changes in chloroplast rRNA. By 5 days after inoculation, the 1.1 M (million dalton molecular weight) chloroplast rRNA species had almost disappeared, probably by degradation to smaller fragments including those of approximately 0.7 M and 0.4 M [8]. By 9 days after inoculation, no chloroplast rRNA species was detected in infected leaves. In non-infected leaves of the same age (fig. 1B), only slight degradation of the 1.1 M species was observed.

The most straightforward interpretation of these results is that disease development is associated with a rapid decline in the number of chloroplast ribosomes in the infected leaf. However, to confirm this result, cytoplasmic and chloroplast ribosomes were ex-

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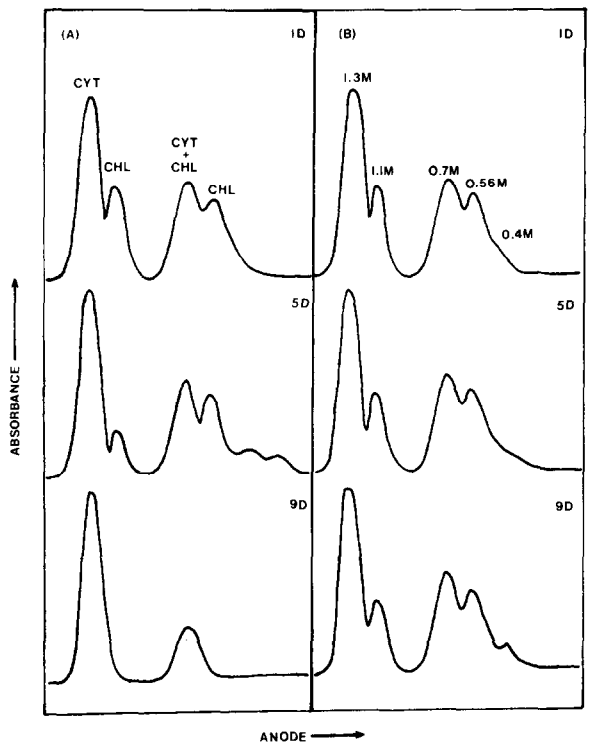

Fig. 1. Ribosomal RNA bands obtained by gel electrophoresis of RNA extracted from mildew-infected (A) and non-infected (B) primary leaves of barley. Leaves were inoculated 7 days after sowing and harvested 1, 5 and 9 days (1D, 5D and 9D) after inoculation. The bands were stained with toluidine blue and scanned at 630 nm using a Densicord microdensitometer. The cytoplasmic (CYT) and chloroplast (CHL) rRNA species have been identified by the notation of Dyer, Miller and Greenwood [8]. The profiles have been normalized so that the area under the 1.3 M (million dalton molecular weight) peak is constant.

tracted and the concentration of each species determined.

Ribosomes were extracted from non-infected and mildew-infected barley leaves 9 days after inoculation and analysed in the Spinco model E ultracentrifuge. The results are presented in table 1. Both cytoplasmic (80 S) and chloroplast (66 S) ribosomes were detected in extracts of non-infected barley leaves but only cytoplasmic ribosomes were detected in extracts of mildewed barley leaves. These results support the view that mildew infection is accompanied by a rapid decline in the number of chloroplast ribosomes.

The metabolism of cytoplasmic ribosomes was very different from that of chloroplast ribosomes.

Table 1

Ribosomes were extracted from barley leaves using the detergent Triton X-100. All operations were performed at 4°. Tissue (10 g) was ground with a little acid-washed sand in a mortar containing 20 ml of 0.4 M sucrose in 2 × TKM buffer[a]. After being squeezed through one layer of cheese cloth, the homogenate was made 1.0% (w/v) in Triton X-100 and was centrifuged at 20,000 g_{max} for 10 min. The supernatant was then layered over 4.5 ml of buffer (2 M sucrose in 1 × TKM) and centrifuged at 380,000 g_{max} for 10 hr in the MSE Superspeed 65 preparative ultracentrifuge. The pellets were carefully resuspended in 1 × TKM buffer, dialysed against the same buffer overnight and then examined in the Spinco model E analytical ultracentrifuge using the schlieren optics system. The operating conditions were 27,690 rpm and 20.0°. Sedimentation coefficients ($s_{20,w}$) were calculated and ribosome concentrations were determined from peak areas as described by Petermann [9], without correction for the Johnston-Ogston effect.

$s_{20,w}$	Ribosome concentration[b]		Mildewed
	Mildewed	Non-Infected	Non-Infected
80 S	630 ± 50	290 ± 30	2.1
66 S	< 30	230 ± 30	< 0.1
Total	630 ± 50	520 ± 60	1.2

[a] TKM buffer: 50 mM Tris, 25 mM KCl, 5 mM $MgCl_2$, adjusted to pH 8.0 with HCl.

[b] As μg per g fresh weight.

Table 1 shows that, on a fresh weight basis, there were twice as many cytoplasmic ribosomes in mildewed leaves 9 days after incubation as in non-infected leaves of the same age. As a result, the total ribosome concentration in mildewed leaves at this stage of infection was 20% higher than that in control leaves.

Another interesting aspect of cytoplasmic ribosome metabolism was revealed by studies on the incorpora-

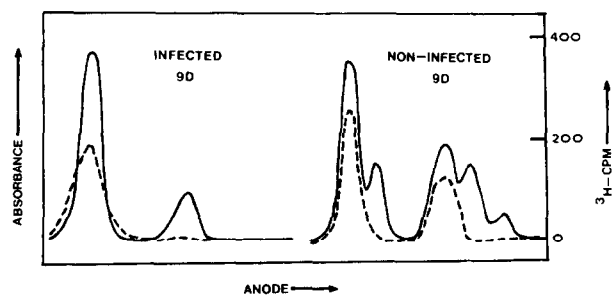

Fig. 2. Distribution of tritium amongst rRNA bands extracted from mildew-infected and non-infected barley leaves 9 days after inoculation. The leaves had been exposed to ^{3}H-orotic acid for 7 hr before harvesting.

tion of ^{3}H-orotic acid into rRNA. Mildew-infected and non-infected leaves (9 days after inoculation) were excised and allowed to take up ^{3}H-orotic acid. After 7 hr, RNA was extracted from the leaves and fractionated by gel electropheresis. The distribution of radioactivity amongst the various rRNA fractions was determined (fig. 2). In neither infected nor healthy leaves were the chloroplast rRNA bands labelled. The 1.3 M cytoplasmic rRNA band was labelled in both tissues while the 0.7 M rRNA band was labelled only in non-infected leaves. The absence of label from the 0.7 M band in mildewed leaves indicates that infection leads to an alteration in cytoplasmic ribosome metabolism.

4. Discussion

The results presented above show that rRNA metabolism is markedly altered in barley infected by the powdery mildew fungus. Within 9 days after inoculation, chloroplast ribosomes and ribosomal RNA were no longer detected in infected leaves. This may have been a consequence of chloroplast breakdown induced by infection. Although powdery mildew is an ectoparasite, invading only the epidermis of the leaf, the chloroplasts of mesophyll cells are also damaged by infection. Both chlorophyll concentration and photosynthetic activity decline in infected leaves [10] and chloroplast degeneration has been observed under the electron microscope (R.F. Sadler, personal communication).

Cytoplasmic rRNA metabolism was also changed by infection. By 9 days after inoculation, infected leaves contained twice as many cytoplasmic ribosomes as non-infected leaves. Quantitatively, this rise more than compensated for the disappearance of the chloroplast ribosomes. Infected leaves contained about 20% more rRNA than non-infected leaves, in agreement with earlier studies on total RNA [2–4].

The absence of radioisotope from the 0.7 M cytoplasmic rRNA band in barley leaves infected for 9 days is particularly interesting. In both animals and plants, the two major cytoplasmic rRNA species are synthesised together in the nucleolus as part of a larger precursor rRNA molecule (rpreRNA) [11, 12]. Preferential degradation of the 0.7 M rRNA can be induced by inhibition of the synthesis of ribosomal proteins, as in the case of cycloheximide-treated tobacco tissue [13] or lysine-deficient HeLa cells [12].

Mildew infection may interfere with the synthesis of ribosomal proteins in barley leaves. This suggestion is supported by three observations: (i) the absence of ^{3}H-orotic acid from the 0.7 M rRNA in infected leaves 9 days after inoculation, (ii) the rapid decline in total RNA in infected leaves after this time [2–4], and (iii) the fact that almost all of the RNA extracted from 19-day-old mildewed leaves was in the form of 1.3 M rRNA [14]. Further work is needed to establish the mechanism by which infection produces these changes in host ribosome metabolism.

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