The Biological Functions of Short and Long Particles of Soil-borne Wheat Mosaic Virus

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ABSTRACT

Short and long virus particles of soil-borne wheat mosaic virus (SBWMV) were purified by three cycles of differential centrifugation using sucrose density gradients after preliminary partial purification from leaf extracts. It was shown by electron microscopy that the purified short-particle fraction contained no long particles, whereas the purified long-particle fraction was always contaminated with a few short particles. Infectivity assay on *Chenopodium quinoa* indicated no infectivity with short particles, and some infectivity with long particles, whereas the infectivity appreciably increased when they were mixed. The infectivity dilution curve of a mixture of purified long and short particles was of the double-hit type. Particles of both short and long types were always found in individual local lesions examined by electron microscopy when *C. quinoa* was inoculated with a purified long-particle fraction. It is likely that long particles and short particles are separately noninfectious, but they become infectious when they are mixed. Serological properties of the short and the long particles were closely related. Heterologous complementation tests using four isolates were performed with short- and long-particle fractions. Heterologous interaction could be observed in all of 12 possible combinations. When long particles of one strain were mixed with short particles of another, it was found that the short particles controlled particle length, serotype, and type of inclusion bodies. The long particles controlled the infectivity on tobacco and virus concentration in inoculated leaves of spinach. Both particles influenced symptom types in rye and *Tetragonia expansa,* although the effect of the long particles appeared to be much greater than the short particles. The parent virus systems were obtained again from these hybrids by repeating complementation in the opposite direction.

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Soil-borne wheat mosaic virus (SBWMV) has a diameter of 20 nm with two peaks of particle-length distribution at 110 to 160 nm and about 300 nm (1, 2, 3, 9). Gumpf (3) reported that the short particles were noninfectious, but that preparations of the long particles containing some short particles were infectious. The respective function or significance of the short and long particles of SBWMV have not been fully established. There are several strains of SBWMV, and these strains could be distinguished by their host ranges, particle

lengths, types of inclusion bodies, and serological relationships (9).

In this paper, we describe the biological functions of short and long particles of SBWMV in experiments using hybrids formed from two strains characterized by several markers.

MATERIALS AND METHODS.—Two strains (J-A and US-B) of SBWMV were mainly used in these experiments. Isolate J-A from Japan has two length-distribution peaks at about 110 and 300 nm, occasionally produces small inclusion bodies in epidermal cells of rye, and belongs to serotype I. It causes severe mosaic and stunt on rye, small distinct necrotic local lesions on *Tetragonia expansa,* necrotic or chlorotic local lesions on tobacco, and symptomless infection on inoculated leaves of spinach, in which the virus concentration is very low. Previously, we reported that SBWMV was not recovered from spinach leaves inoculated with Japanese isolates (J-A, J-B, and J-C) (9). In later experiments, however, it was found that the virus could be recovered, although the virus concentration was very low. Isolate US-B from America has two length-distribution peaks at about 160 and 300 nm, produces many large inclusion bodies bounded with a clear margin in epidermal cells of rye, and belongs to serotype II. It causes mild mosaic on rye, indistinct chlorotic ring local lesions on *T. expansa,* and symptomless infection on inoculated leaves of spinach in which the virus concentration is high. No infection occurs in tobacco. An additional two strains (J-E and US-C) of SBWMV were also used in some experiments. Isolate J-E from Japan and isolate US-C from America have two length-distribution peaks at about 160 nm and 300 nm, and about 120 nm and 290 nm, respectively. The characters of these isolates were reported previously (9).

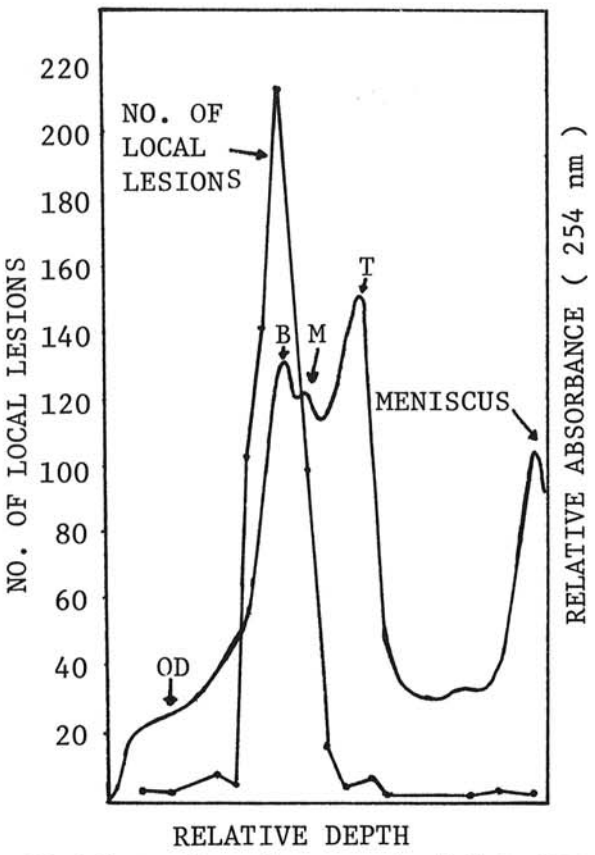

Fig. 1. Photometric scanning patterns of purified isolated J-A of soil-borne wheat mosaic virus after sedimentation in 10 to 40% sucrose density gradients for 3 hours at 60,000 *g,* and the average number of local lesions per leaf of *Chenopodium quinoa* inoculated with successive 1-ml fractions diluted 1:25 with 0.005 M phosphate buffer. T = Top zone; M = Middle zone; B = Bottom zone.

The isolates were maintained by successive mechanical transfers to young rye plants. Manual inoculations and recoveries of the virus were made as described previously (9).

Purified virus was prepared from infected leaves of rye plants which had been stored at −70 C. Leaf tissues were homogenized at 4 C in two volumes of 0.1 M phosphate buffer (pH 7.0) containing 0.01 M potassium cyanide. The extract was expressed through cheesecloth. After addition of carbon tetrachloride to the extract to 25% (v/v), the extract-solvent mixture was shaken for 15 minutes and the emulsion was broken by centrifuging at 8,000 *g* for 10 minutes. After addition of 5% polyethylene glycol 6,000 (PEG) to the supernatant fluid, it was stirred for 1-2 hours, and was centrifuged at 8,000 *g* for 15 minutes. The pellet was resuspended in 0.1 M phosphate buffer (pH 7.0) and clarified by centrifugation at 8,000 *g* for 10 minutes. This cycle of differential centrifugation using PEG was repeated three times, and the preparation was then centrifuged at 120,000 *g* for 60 minutes. The pellet was resuspended in distilled water containing 0.1% "Lipon" (alkylbenzensulfonate), and clarified by low-speed centrifugation as before. The resulting supernatant fluid was centrifuged at 60,000 *g* for 3 hours in linear density gradient columns of 10 to 40% sucrose in distilled water containing 0.1% "Lipon". Following centrifugation, the gradient columns were scanned at 254 nm and fractionated with an ISCO density-gradient fractionator. Two ultraviolet-absorbing zones were observed. Each zone was collected respectively and centrifuged at 120,000 *g* for 60 minutes. The virus pellets were resuspended in 0.005 M phosphate buffer (pH 7.0). Futher purification was achieved by two cycles of centrifugation in sucrose density gradients. Purified top and bottom components were resuspended in 0.005 M phosphate buffer to a final optical density of 0.05 at 260 nm (1 cm light path), and stored in 0.2-ml portions at −70 C until use.

Infectivities of purified top and bottom components of SBWMV were assayed by manual inoculation on *Chenopodium quinoa* using the half-leaf method. The numbers of lesions were counted 2 weeks after inoculation.

For electron microscopic examination of particle length of the virus, preparations were mounted on carbon-coated grids and negatively stained in 1% phosphotungstic acid at pH 7.0.

Purified top and bottom components of J-A and US-B were cross-mixed, and inoculated to rye plants. Formations of hybrids were examined by measuring the length of short particles using the leaf-dip method, and by

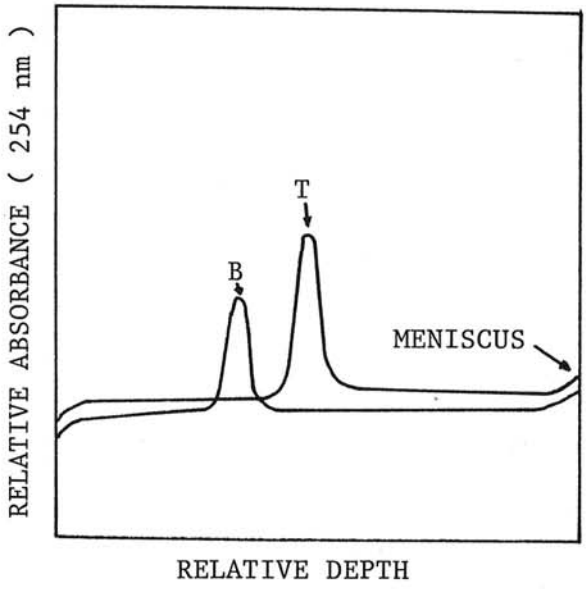

Fig. 2. Absorbancy profiles for the third density-gradient centrifugation of top (T) and bottom (B) components of isolate J-A of soil-borne wheat mosaic virus.

observation of inclusion bodies in leaf epidermal cells of infected rye plants, because it was suggested in a previous report (7) that the short particles may play some important role in the formation of inclusion bodies. The hybrid produced from mixed inoculation with top component from J-A and bottom component from US-B was designated H-1, and the hybrid produced from mixed inoculation with top component from US-B and bottom component from J-A was designated H-2. An infected plant selected from H-1 or H-2 was used as an inoculum in the next transfer. After several such successive transfers, the characteristics of the two hybrids were examined.

Antisera against short or long particles of isolate J-A were prepared in rabbits given two intravenous and two intramuscular injections of purified preparation of either short or long virus particles. The antisera titers were 1/320 in complement-fixation tests. Antisera against J-A and US-B prepared in previous tests (7) were also used in this experiment. The unit of each antigen was determined by "block" titration, and each antigen adjusted to four units was used for complement-fixation tests.

Light- and electron-microscopic examinations of inclusion bodies in infected leaves were made as described previously (4, 9).

RESULTS.—*Infectivity and optical density of fractions of isolate J-A centrifuged in a sucrose density gradient.*—Photometric scanning patterns of a preparation of J-A after centrifugation in a 10 to 40% sucrose gradient, and the infectivity of successive 1-ml fractions are shown in Fig. 1. There are three light-scattering bands, and virus particles were observed by electron microscopy in the corresponding fractions. The top band contained mostly short particles, of about 110 nm in length. The bottom band contained long particles, of about 300 nm in length, as well as some short particles. The middle indistinct light-scattering band contained particles of intermediate length (about 220 nm long), short particles, and a few long particles. The particles in the middle band may be aggregates of the short particles, because this band was occasionally missing. Isolate J-A showed no peak in length distribution around 220 nm when examined in dipped preparation by electron microscopy (Fig. 5).

Counts of the number of lesions on *C. quinoa* indicated that infectivity of SBWMV was related to the amount of long particles.

Infectivity of short and long particles of SBWMV.—Further purification of material collected from either the top or the bottom band after the first density-gradient centrifugation was made by two additional cycles of sucrose density-gradient centrifugation. Fig. 2 shows absorbancy profiles for the third density-gradient centrifugation of the top and bottom components of J-A. The purified top component contained only short particles (Fig. 3-A). On the other hand, the purified bottom component contained mainly long particles, but was still contaminated with a few short particles (Fig. 3-B).

Purified top and bottom components of four isolates (J-A, J-E, US-B, and US-C) and their homologous mixtures were inoculated to *C. quinoa* (Table 1). In these

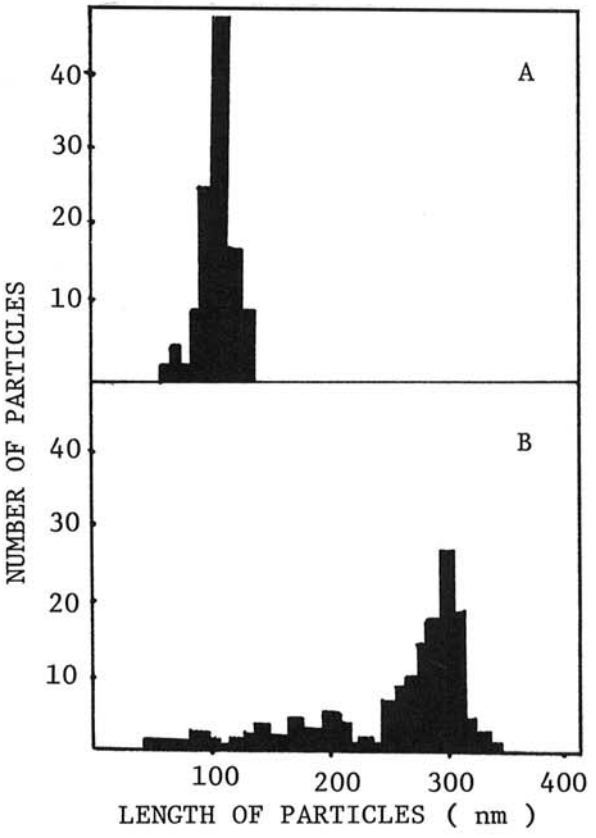

Fig. 3-(A, B). Length distribution of isolate J-A of soil-borne wheat mosaic virus in top and bottom components purified by three cycles of density-gradient centrifugation. **A)** Top component, **B)** Bottom component.

TABLE 1. Infectivity of top and bottom components of four isolates of soil-borne wheat mosaic virus (purified by three cycles of sucrose density-gradient centrifugation) on *Chenopodium quinoa*

Sample[a]	Infectivity			
	J-A	J-E	US-B	US-C
Top component	0[b]	0	0	0
Bottom component and buffer combined (1:1, v/v)	190[c]	46	27	64
Bottom and top components combined (1:1, v/v)	462[c]	196	61	149

[a]The concentrations of samples were adjusted to 6.25×10^{-4} (OD_{260}) for J-A and US-B, and to 12.5×10^{-4} for J-E and US-C, respectively.

[b]Total numbers of local lesions produced on 25 leaves.

[c]Total numbers of local lesions produced on 50 half-leaves.

experiments, equal volumes of top and bottom components at equal concentrations were mixed and inoculated to half-leaves of *C. quinoa*. The opposite half-leaves were inoculated with bottom component diluted (1:1, v/v) with 0.005 M phosphate buffer. The top components were noninfectious. The infectivities of bottom components mixed with the top component were higher than bottom component diluted with buffer in all cases. These results show that the addition of top component enhances the infectivity of bottom component.

Both long and short particles were always found by electron microscopy in dipped preparations made from individual local lesions on *C. quinoa* inoculated with purified bottom component containing mostly long particles.

Infectivity dilution curve of isolate J-A.—In experiments carried out to analyse the infectivity dilution curve of J-A, equal volumes of purified top and bottom component at the same concentration (0.05, OD_{260}) were mixed. The mixture was diluted with 0.005 M phosphate buffer (pH 7.0). One hundred leaves of *C. quinoa* were used for each. The results of the dilution experiments with the mixture of top and bottom components of J-A are shown in Fig. 4. The infectivity dilution curve of J-A followed the theoretical two-hit curve.

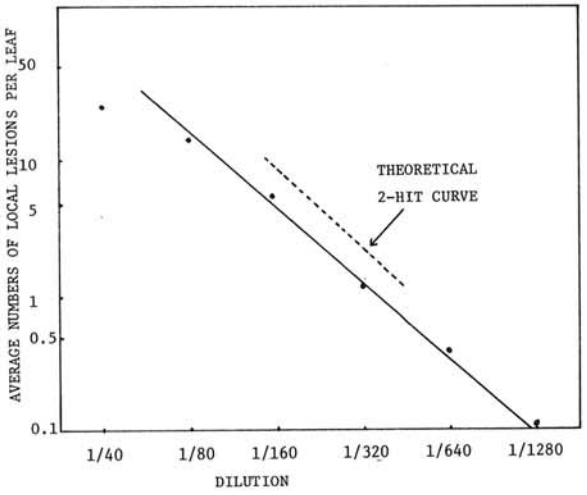

Fig. 4. Dilution curve of an artificial mixture of purified top and bottom components of isolate J-A of soil-borne wheat mosaic virus.

Serological relationship between short and long particles of isolate J-A.—Purified short and long particles of J-A reacted in complement-fixation tests with antiserum against either short or long particles of J-A to equal dilution end points. In absorption tests, neither the short nor the long particles reacted with antisera absorbed with the heterologous antigen. These results indicate that there is no serological difference between the short and long particles of isolate J-A.

Heterologous complementation among four isolates of SBWMV.—Top and bottom components of four isolates (J-A, J-E, US-B, and US-C) purified by three cycles of density-gradient centrifugation were used for heterologous complementation tests. Equal volumes of heterologous top and bottom components at the same concentration were mixed and inoculated to *C. quinoa*. The top components of the four isolates were noninfectious. The mixtures of heterologous components were inoculated on half-leaves of *C. quinoa*, and the opposite half-leaves were inoculated with bottom component diluted with 0.005 M phosphate buffer. The results of a series of such experiments are shown in Table 2. When bottom componet of four isolates was mixed with heterologous top component, the infectivities of mixtures were higher than those of corresponding bottom components diluted with buffer.

The length of short particles in randomly selected local lesions produced by mixtures was examined by electron microscopy using the leaf-dip method. The lengths of short particles were 110 and 120 nm for J-A and US-C, respectively, and 160 nm for both J-E and US-B, and therefore parental isolates of the virus in the local lesions were inferable in some cases by measuring the length of the short particles. Some local lesions contained short particles derived from the parent top component, while others contained short particles derived from the parent bottom component. On the other hand, all local lesions produced on plants inoculated only with bottom component contained short particles derived from the parent bottom component.

These results suggest that complementation between heterologous top and bottom components occurs in all of the 12 possible combinations tested.

Formation of hybrids.—Isolates J-A and US-B were used for tests of hybrid formation, because these two isolates had suitable genetic markers. Equal volumes of purified top and bottom components at equal concentration were heterologously mixed and immediately inoculated to rye plants. Top components of both isolates used for these tests were noninfectious on rye plants. On the other hand, bottom components of both isolates used for these tests were infectious on rye plants, and the infectivity of bottom component of J-A was higher than that of US-B at the same concentration. The results of the complementation tests are shown in Table 3.

Lengths of short particles of infected plants were

TABLE 2. Complementation between heterologous top and bottom components of soil-borne wheat mosaic virus isolated by sucrose density-gradient centrifugation

Inoculum (v/v)		Result of complementation			
Source of bottom component	Source of top component[a]	Total no. of local lesions[b]	No. of local lesions examined	No. of local lesions containing short particles of indicated length	
				110 or 120 nm	160 nm
J-A (*)[c]	Buffer	174	5	5	0
	J-E (*)	580	15	4	11
J-A (*)	Buffer	67			
	US-B (*)	169	15	6	9
J-A (*)	Buffer	54			
	US-C (*)	100			
J-E (**)	Buffer	54	7	0	7
	J-A (**)	210	15	11	4
J-E (**)	Buffer	34			
	US-B (**)	89			
J-E (**)	Buffer	25	5	0	5
	US-C (**)	146	15	11	4
US-B (*)	Buffer	72	5	0	5
	J-A (*)	203	15	11	4
US-B (*)	Buffer	45			
	J-E (*)	84			
US-B (*)	Buffer	21			
	US-C (*)	50	15	8	7
US-C (**)	Buffer	67			
	J-A (**)	100			
US-C (**)	Buffer	22			
	J-E (**)	54	10	5	5
US-C (**)	Buffer	55	5	5	0
	US-B (**)	107	15	9	6

[a] All top components used were noninfectious at these concentrations.
[b] Total numbers of local lesions from 50 half-leaves of *C. quinoa*.
[c] The concentrations of samples: The symbol (*) shows concentration of 6.25×10^{-4} (OD_{260}), and the symbol (**) shows that of 12.5×10^{-4}.

TABLE 3. Complementation between heterologous top and bottom components of two isolates of soil-borne wheat mosaic virus

Inoculum (v/v)		No. of rye plants systemically infected/No. of plants inoculated	Total no. of plants tested individually	No. of plants containing short particles of indicated length		
Source of bottom component	Source of top component[a]			110 nm	160 nm	110 and 160 nm
J-A (0.01)[b]	US-B (0.01)	38/254	15	8	4	3
US-B (0.01)	J-A (0.01)	13/443	13	5	4	4

[a] Both top components were noninfectious at this concentration on rye plants.
[b] Optical density at 260 nm.

examined to check kinds of infected virus. Three kinds of infected plants were found: those which contained short particles derived from the top component; those which contained short particles derived from the bottom component; and those which contained short particles derived from both the top and bottom components. It was supposed that all of these infected plants contained long particles derived from the bottom components, because the top components of both isolates were not contaminated with long particles. In these infected plants, plants which contained short particles derived from the top component were hybrids (H-1 or H-2). A single infected plant, which was selected by checking the kinds of short particles from infected plants inoculated with H-1 or H-2, was used as the inoculum source for subsequent transfers. These transfers were repeated several times.

Particle length distributions and sedimentation profiles of hybrids.—Isolates J-A, US-B, and their hybrids (H-1 and H-2) had long particles about 300 nm in length, and short particles of different lengths (Fig. 5). The short particles in J-A and H-1 were about 110 nm in length, and those in US-B and H-2 were 150 to 160 nm in length.

Sedimentation profiles of parental isolates (J-A and US-B) and their hybrids (H-1 and H-2) were examined in

TABLE 4. Comparative symptomatology of parental viruses (J-A and US-B) and their hybrids (H-1 and H-2) of soil-borne wheat mosaic virus

Hosts	Symptoms[a]			
	J-A	US-B	H-1	H-2
Secale cereale 'Petkuser'	S:Severe stunt, mosaic, and necrosis	S:Mild mosaic	S:Mild stunt, mosaic, and necrosis	S:Stunt, mosaic, and necrosis
Tetragonia expansa	L:Small necrotic spot S:Chlorotic spot	L:Indistinct chlorotic ring spot S:Chlorotic ring spot	L:Faint necrotic ring spot S:Chlorotic ring spot	L:Faint small necrotic spot S:Chlorotic ring spot
Nicotiana tabacum 'Ky 57'	L:Chlorotic or necrotic spot	...	...	L:Chlorotic or necrotic spot
Spinacia oleracea 'Nippon'	L:Symptomless	L:Symptomless	L:Symptomless	L:Symptomless

[a]S = Systemic infection; L = Local infection; ... = No infection.

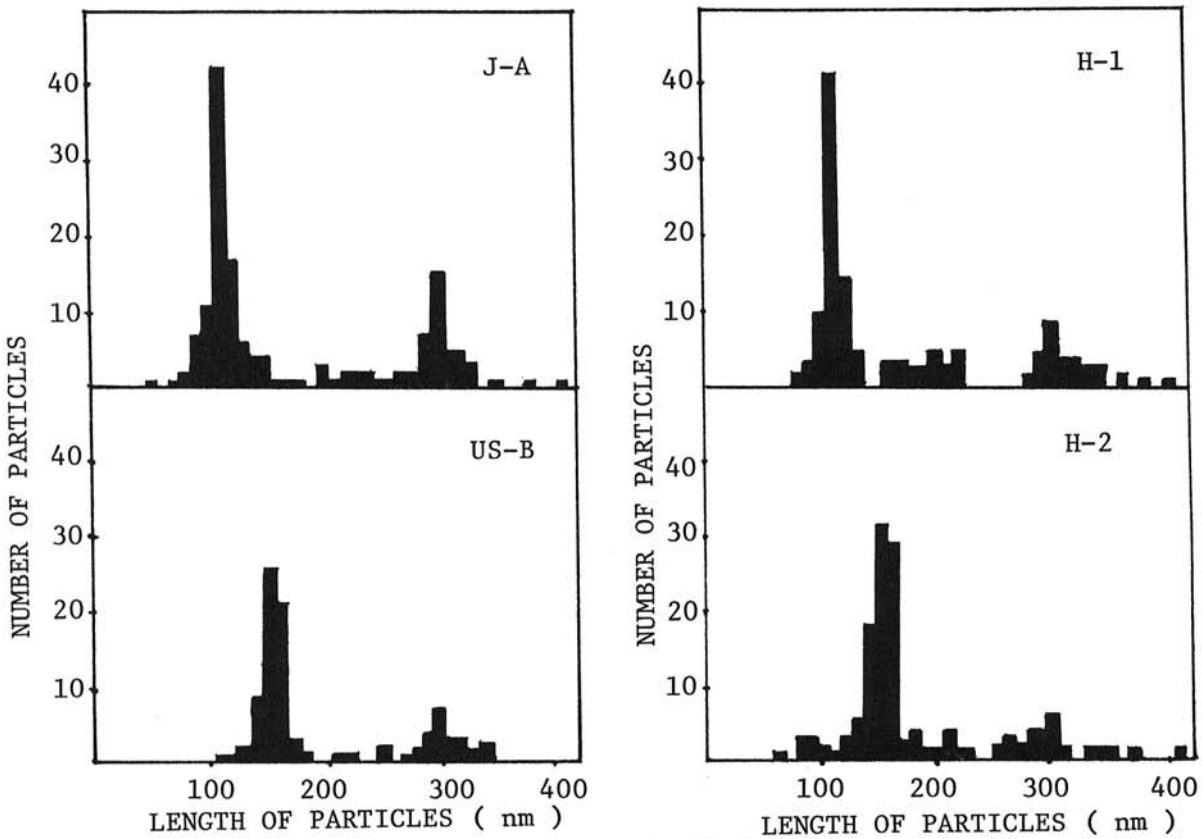

Fig. 5. Particle length distributions of parental isolates (J-A and US-B) and their hybrids (H-1 and H-2) of soil-borne wheat mosaic virus.

a 10 to 40% sucrose gradient in 0.1% 'Lipon' after 180 minutes at 240,000 rpm in tubes of an SW 25 rotor. J-A and H-1 showed the same profiles, and US-B and H-2 showed the same ones.

Host reactions with the virus hybrids.—Table 4 shows the reactions of four plant species inoculated with two isolates of SBWMV (J-A and US-B) and their hybrids (H-1 and H-2). Isolate J-A caused severe mosaic and necrosis on rye, followed by severe stunting, while isolate US-B caused only mild mosaic. Hybrids H-1 and H-2 showed intermediate symptoms on rye, the symptoms being more severe in H-2 than in H-1. The virus concentrations in rye plants infected with each isolate were found to be almost equal according to inoculation tests on *C. quinoa*. On inoculated leaves of *Tetragonia expansa,* isolate J-A caused distinct small necrotic spots, and isolate US-B caused very indistinct chlorotic ring spots. On the other hand, hybrid H-1 caused faint small necrotic spots, and H-2 caused faint necrotic ring spots. Thus, both hybrids showed intermediate symptoms in *T. expansa.* Isolate J-

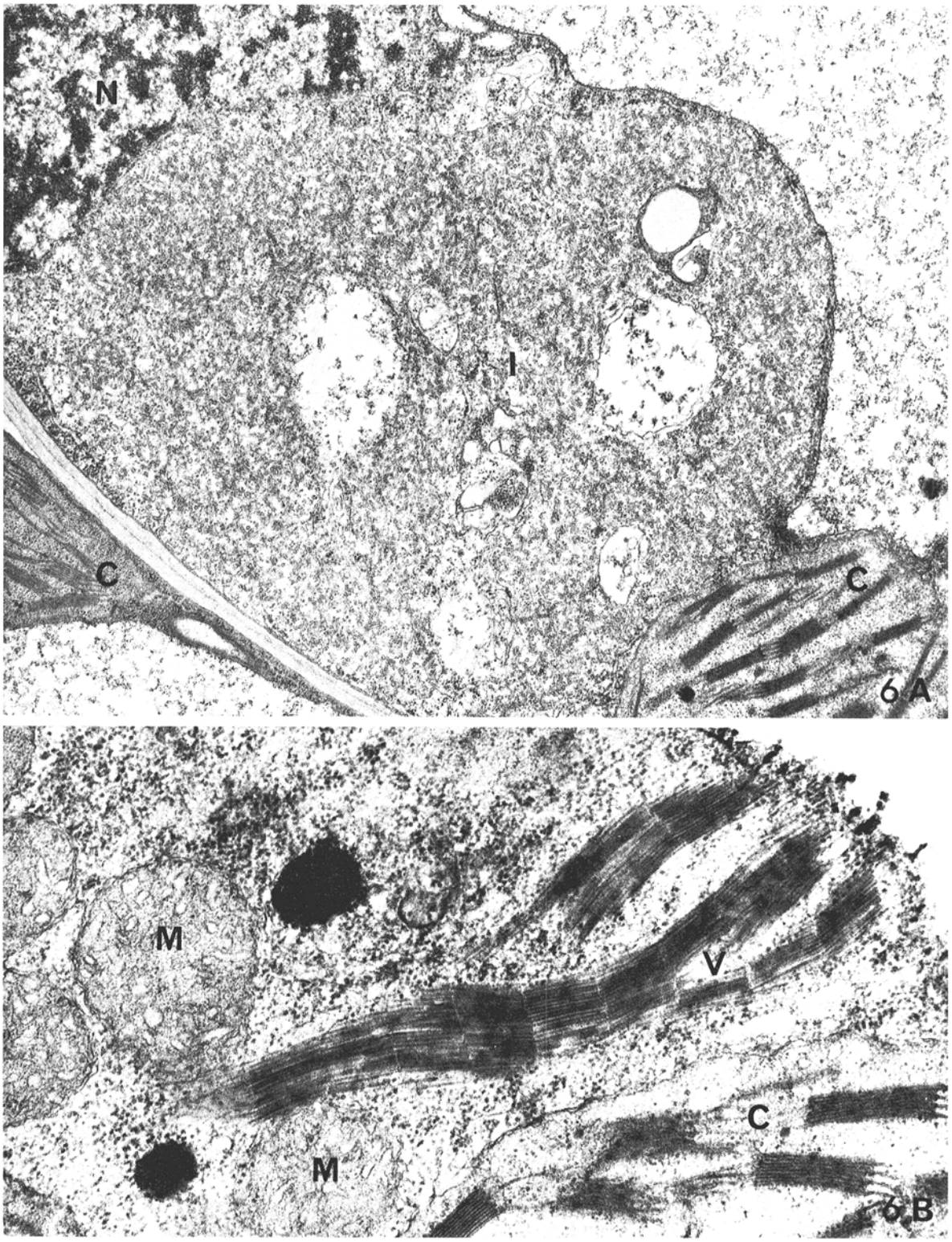

Fig. 6-(A, B). A) Part of a rye leaf cell infected with soil-borne wheat mosaic virus hybrid H-2 showing a mass of tangled tubules (I), a nucleus (N), and chloroplasts (C((× 18,000). **B)** Part of a rye leaf cell infected with soil-borne wheat mosaic virus hybrid H-1 showing aggregates of virus rods (V) in the cytoplasm, a chloroplast (C), and mitochondria (M). Note crystalline arrangement of the aggregates (× 40,000).

TABLE 5. Serological relationships among parental viruses (J-A and US-B) and their hybrids (H-1 and H-2) of soil-borne wheat mosaic virus

		Titre of antiserum[a]	
	Antiserum: →	J-A	US-B
Virus isolates	Absorbed with: →	US-B	J-A
Short particles of H-1		10	0
Long particles of H-1		20	0
Short particles of H-2		0	160
Long particles of H-2		0	320
J-A		20	0
US-B		0	320

[a]Figures are reciprocals of the dilution end points of the antisera in complement fixation tests. Zero means no reaction with antisera at ten times dilution.

TABLE 6. The functions of short and long particles of soil-borne wheat mosaic virus

Particles	Characters controlled by each particle type
Short	1. Own particle length 2. Type of inclusion bodies in cells of rye plants 3. Coat protein of both particles
Long	1. Infectivity to tobacco plants 2. Virus concentration in inoculated leaves of spinach plants
Both short and long	1. Production of local lesions on *Chenopodium quinoa* 2. Symptoms in rye plants and *Tetragonia expansa*

A and hybrid H-2 caused chlorotic or necrotic lesions in inoculated leaves of tobacco; whereas, no infection occurred on tobacco with US-B and H-1. Both the short and long particles were always found by electron microscopy in dipped preparations from any individual local lesions in tobacco inoculated with J-A or H-2. The length of the short particles was about 110 nm for J-A and 150 to 160 nm for H-2. Symptomless infection in inoculated leaves of spinach occurred with the four isolates. However, the virus concentration in the inoculated leaves was high in US-B and H-1, but low in J-A and H-2. In an experiment in which the virus was recovered on *C. quinoa* from inoculated leaves of spinach inoculated with four isolates, average numbers of local lesions per leaf of *C. quinoa* were 0.5, 1.8, 22.3, and 27.0 in J-A, H-2, H-1, and US-B, respectively. Thus, infection of tobacco and the virus concentration in inoculated leaves of spinach depended on the long particles. Symptoms on rye and *T. expansa* were influenced by both short and long particles, but symptom types appeared to depend much more on the long particles than on the short particles.

The observation of inclusion bodies of hybrids by light and electron microscopy.—Light microscopy of epidermal strips of rye plants infected with the two isolates and their hybrids revealed the presence of inclusion bodies. They could be grouped into two types. J-A and H-1 produced small inclusion bodies which occurred very rarely, while US-B and H-2 produced many large inclusion bodies bounded with clear margins.

The fine structure of US-B or J-A infected cells were described previously (4). Two types of cytoplasmic inclusions were found in rye leaf cells infected with hybrid H-2: convoluted masses of tubules (Fig. 6-A) and concentrically arrayed membranes with virus rods sandwiched between the membranes or attached to the outside of the membranes. Both types of inclusions were similar in morphology to those found in US-B-infected rye leaf cells. Virus rods were not aggregated in the crystal-like arrangement in the cytoplasm. Those inclusions were not found in rye leaf cells infected with hybrid H-1. Virus rods occurred in aggregates either in the cytoplasm, or in the vacuole of those cells. Some of those aggregates in the cytoplasm consisted of rods in a crystalline (Fig. 6-B) or spiral arrangement. Fine structure of J-A infected cells was the same as H-1. Thus, J-A and H-1 showed the same type of inclusion bodies, and US-B and H-2 showed the same ones.

These results show that the type of inclusion bodies is determined by the short particles.

Serological tests of hybrids.—Serological reactions with J-A and US-B antisera absorbed with heterologous antigens were made against J-A, US-B, and purified short and long particles of their hybrids (H-1 and H-2) (Table 5). They could be grouped into two serotypes. The reactions of J-A and both the short and long particles of H-1 were similar and these were designated serotype I (= low dilution end points in reaction with J-A antiserum absorbed with US-B, and no reaction with US-B antiserum absorbed with J-A). The reactions of US-B and both the short and long particles of H-2 were similar and these were designated serotype II (= high dilution end points in reaction with US-B antiserum absorbed with J-A, and no reaction with J-A antiserum absorbed with US-B). These results indicate that serotype is determined by the short particles.

Complementation in opposite direction.—It was tested whether complementation could also be repeated in the opposite direction by using hybrids H-1 and H-2. The top and bottom components of H-1 and H-2 were purified by three cycles of density-gradient centrifugation. Top H-1 and bottom H-2, and top H-2 and bottom H-1 were mixed and inoculated to rye plants, respectively. The selections of infected plants were made as described in the formation of hybrids. After several transfers, the characteristics of new isolates were tested. The isolate produced from short particles of H-1 and long particles of H-2 resembled J-A in all characters examined. Moreover, the isolate produced from short particles of H-2 and long particles of H-1 resembled US-B. These show that it is possible to obtain the parent virus systems again by repeating complementation in opposite direction.

Table 6 presents a summary of probable functions of short and long particles of SBWMV.

DISCUSSION.—Gumpf (3) reported that two virus zones were observed following centrifugation of purified SBWMV in sucrose density-gradient columns. The slower sedimenting zone, which contained virus particles of 148 nm in length, were noninfectious when inoculated to wheat, while the faster-sedimenting zone, which contained particles of 300 nm in length together with

some 148 nm particles, was infectious. Infectivity of the long particles alone could not be tested. In our experiments, purified SBWMV preparations showed a higher infectivity, and a better separation of the short particles from the long particles. *Chenopodium quinoa* was found to be much more sensitive to SBWMV than wheat, and more suitable for assay studies. We could confirm Gumpf's (3) finding that the short particles of SBWMV were noninfectious, but could not determine the infectivity of the long particles alone, because short particles could not be completely excluded from preparations of the long particles.

There was no serological difference between the short and long particles of SBWMV, whereas the short particles controlled the serotype in hybridization experiments. This may mean that the coat protein of SBWMV is genetically controlled by the short particles, as in the case of tobacco rattle virus (TRV) (5).

The infectivity of the bottom component was high, while the top component was noninfectious. When the bottom component was mixed with the top component, the number of local lesions remarkably increased. On the other hand, it was shown by electron microscopy that the purified top components contained no long particles, whereas purified bottom components were always contaminated with a few short particles. Furthermore, the shape of the infectivity dilution curve of SBWMV followed that of the theoretical two-hit curve. From this curve, it became evident that SBWMV infections were likely to result from some sort of cooperation between the short and long particles at the site of infection. Both long and short particles were always found by electron microscopy in leaf-dip preparations made from any individual local lesion on *C. quinoa* inoculated with purified bottom component which mostly contained the long particles. It was reported that TRV bottom fractions which contained mostly the long particles produced a few stable local lesions and many unstable local lesions which contained only RNA on tobacco (5). This may indicate that the long particles of SBWMV do not produce such local lesions containing only infective virus nucleic acid as in TRV. These results are well explained on the assumption that infection of SBWMV may occur only when particles of both types are contained in the inoculum.

Sänger (8) reported that heterologous interaction could be observed in only two out of 20 possible combinations when heterologous complementation tests of TRV were performed with long and short RNA, each derived from another isolate. In heterologous complementation tests using four isolates of SBWMV, heterologous interactions were observed in all of 12 possible combinations. The occurrence of heterologous interaction may be due to close relation among these four isolates of SBWMV.

When mixed inoculum of heterologous top and bottom components of J-A and US-B were inoculated to rye plants, some plants were infected with short and long particles from bottom component. These happened frequently in combination with bottom component of J-A and top component of US-B. In these experiments, a high concentration of virus was used as inoculum. Therefore, considerable numbers of short particles were contained in the bottom component fraction, and some of such short particles were probably aggregated with long particles in bottom component. The high frequency of the occurrence of plants infected with short and long particles derived from the bottom component may be due to such aggregation and high concentration of virus in the inoculum.

In the experiments on formation of hybrids, the top components used for formation were noninfectious. Therefore, it was supposed that hybrids were not contaminated with long particles from the top component. On the other hand, there was the possibility that hybrids were contaminated with a few short particles from the bottom component. However, it was demonstrated that there was no contamination in hybrids by observation of the particle length distributions and sedimentation profiles of hybrids after several careful transfers. These results show that the hybrids were actually formed.

Lister and Bracker (6) found that when short particles from one isolate of TRV were mixed with the long particles from another, symptom types in *N. clevelandii* were associated with the short particles. In similar experiments using other TRV isolates, Sänger (7) found that distinctive lesion types in tobacco depended rather on the long particles. In our experiments with SBWMV, symptom types in rye and *T. expansa* due to virus hybrids were intermediate between those caused by parent isolates and associated rather more with the long particles than the short particles. Infectivity in tobacco, and virus concentrations in inoculated leaves of spinach, depended on the long particles. The results indicate that the functions of the short and long particles of SBWMV in controlling symptom types, virus concentration, and infectivities in certain hosts do not always coincide with those of TRV. Moreover, the short particles of isolate US-B, which cannot infect tobacco, can infect tobacco when they are mixed with the long particles of isolate J-A, indicating that the long particles affect infectivity to tobacco.

Electron microscopic examination of rye leaf cells infected with two hybrids revealed that the information about the fine structure of inclusions and the location of virus rods in cells were apparently dependent on the short particles. Crystal-like arrangements of virus rods in the cytoplasm were found in cells infected with hybrid H-1 and not with hybrid H-2. The tendency of virus rods to aggregate in crystal-like arrangement is controlled by short rods.

In the experiment in which the short and long particles of two strains inciting different inclusion bodies were interchanged, it was indicated that the short particles controlled the formation of inclusion bodies, confirming the previous suggestion (9).

The length of the short particles in hybrids of two isolates having different lengths of the short particles remained unchanged after many successive transfers. This shows that the short particles of SBWMV also control their own particle length, the result corresponding with the case of TRV (7).

The complementations could also be repeated in the opposite direction by using the long and short particles of two hybrids of SBWMV. This probably shows that a direct genetic interaction evidently did not occur between short and long particles of SBWMV, as in the case of TRV (8).

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