[6] Dahlberg, J. E., and R. Haselkorn, to be published.

[7] Nirenberg, M., and P. Leder, *Science*, **145**, 1399 (1964).

[8] Boedtker, H., *J. Mol. Biol.*, **2**, 171 (1960).

[9] DeRosier, D., Ph.D. thesis, University of Chicago.

[10] When ribosomes are limiting, we are concerned only with RNA fragments that contain a binding site. Let L_i/d = the fraction of fragments containing sites, where d is the distance between sites, and, n_i = number of fragments of fractional length L_i after all the sites have been separated from each other by hydrolysis (> 15 breaks per molecule). Then the total number of *nucleotides* associated with sites is $\Sigma\, n_iL_i \cdot L_i/d$; the total number of *fragments* containing sites is $\Sigma\, n_iL_i/d$. When RNA is in excess, the number of fragments bound is fixed; we therefore measure $(\Sigma\, n_iL_i^2/d)/(\Sigma\, n_iL_i/d) = (\Sigma\, n_iL_i^2/(\Sigma\, n_iL_i) = \bar{L}_w$, or the weight average length.

[11] When ribosomes are in excess (see footnote 10), *all* nucleotides associated with a ribosome-binding site are detected. The fraction of radioactivity bound is therefore simply $\Sigma\, n_iL_i^2/d$, or $m\, \Sigma\, n_iL_i^2$, where m = number of binding sites per intact molecule. Since $\Sigma\, n_iL_i = 1$, the fraction of radioactivity bound in ribosome excess can be written as $m\, (\Sigma\, n_iL_i^2/\Sigma\, n_iL_i)$, or $m\, \bar{L}_w$. The ratio of radioactivities bound in the two experiments gives m. This treatment depends upon the assumption that $m = 1/d$, which is the case when the sites are uniformly spaced. This is not quite the case, since the coat protein does not correspond to one fourth of the RNA on the basis of a coding ratio of three, and the strongest binding site appears to be on a smaller-than-average fragment (Fig. 7). Alternative analytical expressions for the fraction of nucleotides bound at ribosome excess and RNA excess as a function of the number of hits, the number of sites, and the distance between sites have been derived by P. Meier and R. Blough. These results will be the subject of a separate communication; we need only note here that for a large number of breaks (> 20) the ratio of nucleotides bound in the two experiments reduces to the number of sites.

[12] The extrapolation of the data for ribosome excess was performed on the assumption that the fraction of RNA bound is approximated by a simple exponential function of the number of hydrolytic breaks. This is not the case for a multisite model, which produces a shoulder whose extent increases with the number of sites. For ten sites equally spaced, this error causes us to underestimate the number of pre-existing breaks by a maximum of three; shifting the upper curve in Fig. 6 to the right by this amount does not significantly affect the calculated number of sites.

[13] Voorma, H. A., P. W. Gant, J. van Duin, B. W. Hoogendam, and L. Bosch, *Biochim. Biophys. Acta*, **95**, 446 (1965).

[14] Salas, M., M. A. Smith, W. M. Stanley, A. J. Wahba, and S. Ochoa, *J. Biol. Chem.*, **240**, 3988 (1965).

[15] Terzaghi, E., Y. Okada, G. Steisinger, A. Tsugita, M. Inouye, and J. Emrich, *Science*, **150**, 387 (1965).

[16] Thach, R. E., M. A. Cecere, T. A. Sundararajan, and P. Doty, these PROCEEDINGS, **54**, 1167 (1965).

[17] Montroll, E. W., and R. Simha, *J. Chem. Phys.*, **8**, 721 (1940).

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*LOCATION OF A LOCAL LESION GENE IN TOBACCO MOSAIC VIRUS RNA**

BY C. I. KADO AND C. A. KNIGHT

VIRUS LABORATORY, UNIVERSITY OF CALIFORNIA, BERKELEY

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Evidence for distinctive genes in the tobacco mosaic virus (TMV[1]) genome[2] was provided when it was shown that the masked strain (*M*), whose symptoms in Turkish tobacco are markedly different from those of wild type, nevertheless appeared to have an identical protein coat as shown by amino acid analysis and mapping of

tryptic peptides.[3, 4] This conclusion was greatly fortified by the results of amino acid analyses made on a total of 204 nitrous acid-induced mutants of TMV. These analyses, made in two different laboratories, indicated that 55 and 73 per cent, respectively, of the mutants obtained had proteins possessing the same amino acid content as the wild type.[5, 6] From such data the conclusion can be drawn that only a relatively small part of the TMV genome is necessary for determination of the virus coat protein. If a triplet code is assumed, it can be calculated that less than 10 per cent of the TMV genome (i.e., 474 nucleotides of the approximately 6400 total) is needed in the synthesis of the TMV coat protein. The rest of the RNA strand must be assumed to consist of genes concerned with other functions such as those resulting in different symptoms in a given host.

A question that arises from the above conception of the genetic functioning of TMV-RNA is: where along the TMV-RNA strand are the various genes located? Recombination is an indispensable tool in the mapping of genes on the bacterial virus genome, but thus far has been technically impractical with mutants of TMV. Yet, gene mapping of the simplest plant viruses has considerable appeal, owing to the small number of genes presumably present. For example, one would guess that there are about a dozen genes total in the TMV genome. A possible means for locating the TMV genes was suggested by a series of observations. First, experiments on degradation of TMV by detergents and alkali suggested that TMV protein subunits might be removed from one specific end of the RNA.[7, 8] This idea was substantiated in the case of degradation brought about by sodium dodecyl sulfate when it was shown that stripping of TMV by SDS occurs through the progressive removal of protein subunits always from the 3′-end of the TMV-RNA.[9] The next step, which is the subject of this report, is to use polar stripping in a scheme for determining the location of genes. The rationale of the procedure is to strip protein from TMV particles in a stepwise fashion and to subject the exposed RNA to the mutagenic action of nitrous acid. This treatment should be marked by a substantial increase in the number of mutants of a specific type when the region of the specific gene is reached (nitrous acid can also react with the RNA still ensheathed in protein but at a rate substantially lower than that of exposed RNA[10]). For example, infection of Sylvestris tobacco (*Nicotiana sylvestris* Spegaz. and Comes) with common TMV results in a systemic disease characterized by a green mosaic pattern, whereas nitrous acid-induced mutants of TMV are known which cause the production of local lesions on this host. Therefore, it should be possible to locate the local lesion gene in the TMV genome by stripping protein from the 3′-end of TMV-RNA, treating with nitrous acid, and correlating the degree of stripping with the number of mutants produced that cause local lesions rather than a systemic infection in Sylvestris tobacco.

Evidence obtained by the approach outlined above suggests that the viral gene that codes for local lesion response in Sylvestris tobacco is located at a position approximately one quarter of the length of the RNA starting from the 5′-end.

Materials and Methods.—Preparation of TMV: TMV was prepared from systemically infected Turkish (*Nicotiana tabacum* L. var. Turkish) tobacco leaves. The leaf material, previously made very crisp by holding overnight at about −25°C, was crushed in a stout paper bag by kneading. (It has been observed that this method tends to yield a higher proportion of 3000 Å rods than when the leaves are ground in a meat chopper.) The addition of phosphate and subsequent extraction

was according to the usual procedure. In all cases, the extracted virus was purified by differential centrifugation. In the final high-speed cycle, the virus was sedimented at 26,000 rev/min for 25 min in the no. 40 rotor of a Spinco model L centrifuge. The final pellets of virus were dissolved in 10^{-3} *M* EDTA, pH 7.5, to give a solution at 30–40 mg/ml which was stored at 4°C.

Infectious RNA was prepared by the phenol method[11] from a standard preparation of purified TMV.

Segregation of 3000 Å TMV particles: TMV particles approximately 3000 Å in length were segregated from particles of other lengths by means of the 1% agar column method.[12] Instead of conventional agar, however, 1% agarose (Marine Colloid, Inc.) was employed. The agarose gel was prepared in the pearl-condensed bead form as described by Bengtsson and Philipson.[13] A 1.8 × 90.0-cm column of 40–60 mesh, 1%, bead agarose gel was packed in a buffer-filled glass column by gravity and equilibrated with 10^{-2} *M* EDTA, pH 7.5, by percolation. A layer (*ca.* 1 cm) of 5% agarose gel was placed on top of the 1% agarose to minimize surface turbulence during loading operations. The column was loaded with 1.0 ml of TMV (30–40 mg/ml) and eluted with 10^{-2} *M* EDTA, pH 7.5, collecting 7.5-ml fractions on a fraction collector. The peak fractions (determined by optical density readings at 260 mμ) which were found to contain 87–92% (expressed as weight %) 3000 Å particles were combined and enriched with respect to 3000 Å particles by centrifugation for 25 min at 26,000 rev/min in the Spinco L, no. 40 rotor. The enriched, pelleted virus was rinsed lightly with 10^{-3} *M* EDTA, pH 7.5, and then covered with the buffer. This virus, termed "3000 Å TMV," was resuspended after standing overnight at 4°C, and was used in stripping experiments.

Polar stripping of TMV: The particles were stripped with 1% SDS according to the method described by May and Knight.[9] Although various times of stripping were employed, usually 1.5–2.0 mg of 3000 Å TMV were incubated at 37°C for 2.5–3.0 hr in the presence of 1% SDS and 0.4% purified bentonite and 10^{-5} *M* EDTA, pH 7.5. The bentonite was purified according to Fraenkel-Conrat *et al.*[14] Stripping was stopped by diluting with cold water and was immediately followed by ultracentrifugation (100,000 × *g*, 2 hr). The pellets thus obtained were found to contain unstripped virus, partially stripped virus (PSV), and bentonite. The pelleted material was resuspended after soaking overnight at 4°C in 10^{-3} *M* EDTA, pH 7.5. Such a treatment provided workable quantities of particles ranging in the degree to which they were stripped from 0 to about 80% with a peak in concentration of particles that were about 50–60% stripped. Such PSV was fractionated into specific classes as described below.

Stripping with 5 M urea: TMV particles were also stripped with 5 *M* urea.[15, 16] The virus (3.0 mg) was incubated in the presence of 0.4% bentonite and 5 *M* urea at 0° for 35 min. Stripping was stopped by diluting the reaction mixture 30-fold, and the PSV was collected by ultracentrifugation as above.

Fractionation of PSV: PSV, stripped to various degrees as described above, was fractionated in a sucrose density linear gradient (8–20% sucrose in 10^{-3} *M* EDTA, pH 7.5, containing 0.4% bentonite) by centrifugation for 2.5 hr at 25,000 rev/min (Spinco L, SW 25.1 rotor, without brake). Graded lengths of PSV, representing about 85% of the original, were found to occur in the fractions taken from the top of the gradient at various levels and located above the dense unstripped TMV zone. The bentonite was at the bottom of the tube as a pellet. PSV in each 1.0-ml fraction was precipitated in the cold with 2.5 vol of 95% ethanol in the presence of 1 drop of 3 *M* sodium acetate, pH 4.9. Precipitation was allowed to take place overnight at 4°C, and the precipitate was collected by centrifugation. The degree of stripping was determined by electron microscopy (see below). Similar measurements were also made on unprecipitated fractions of PSV.

Electron microscopy: In order to determine homogeneity and lengths of PSV preparations, specimens were applied by the spray-droplet method[17] to collodion films on 400-mesh copper grids. The film-bearing grids were sprayed with sample (either density gradient fractions of PSV or such fractions after precipitation by alcohol as described above) while floating on glass-distilled water in order to dialyze away the residual sucrose present in each preparation. After 2–4 days of dialysis, the specimens were air-dried and lightly shadowed with uranium. The rotary shadow-casting technique[18] and low-angle shadowing were used to enhance contrast of RNA "tails" of PSV particles. Polystyrene latex spheres measuring 259 ± 2.5 mμ were used as internal standards of measurements. The specimens were examined in either the RCA electron microscope (model 3C) or in the Siemens-Elmiskop I (pole piece no. 2, accelerating voltage of 60,000 v). The degree of

stripping of PSV was determined by measuring images projected from electron microscope plates at 10,000× magnification. (The projector used was the Omega type D II made by Simmon Bros., Inc., Long Island City, N. Y., and adapted for projecting plates from Siemens and RCA electron microscopes.) The total magnification after projection was about 70,000.

Treatment with nitrous acid: Nitrous acid was generated for each experiment by mixing equal volumes of 4 *M* $NaNO_2$ and 1 *M* acetate buffer, pH 4.1.[10, 19, 20] Virus and PSV (fractionated as described above) at 5–50 μg per ml were reacted with the nitrous acid by mixing with an equal volume of the nitrous acid mixture (pH of the final reaction mixture was 4.2) and holding at about 25°C. A standard reaction time of 20 min was used in the experiments relating production of local lesion mutants to degree of stripping of protein, whereas a series of times ranging from 0 to 20 min was employed in the experiments concerned with rate of mutagenesis with variously stripped virus. The reaction was stopped by diluting the reaction mixture 100-fold with cold 0.1 *M* sodium pyrophosphate, pH 7.3. Parallel control mixtures were made containing identical constitutents, with the exception that $NaNO_3$ was used in place of $NaNO_2$.

Reconstitution of PSV: Both PSV and RNA were reconstituted with TMV protein by the method described by Fraenkel-Conrat and Singer.[21]

Bioassay: Nitrous acid mutants of the specific type sought here were detected by the appearance of local lesions after inoculation of half-leaves of Sylvestris tobacco, a host which responds with systemic symptoms when infected with wild-type virus. A quantitative estimate of the total infectious population (wild type plus mutants) remaining after treatment with nitrous acid was obtained by inoculating half-leaves of Xanthi tobacco (*Nicotiana tabacum* L. var. Xanthi nc) at the same time that tests were made on Sylvestris plants. Control samples were inoculated on opposite half-leaves. Between 18 and 24 half-leaves were used per sample. All PSV was reconstituted as described above before testing for biological activity, and these and the unstripped virus preparations were diluted with 0.05 *M* phosphate buffer, pH 7.0, to give 10–150 lesions per half-leaf on Xanthi tobacco. The same preparations were applied undiluted for assay on half-leaves of Sylvestris tobacco. The control in each instance was a sample treated in the same manner, except that $NaNO_3$ was substituted for $NaNO_2$. Lesions on Xanthi and Sylvestris tobacco were counted 4 and 8 days after inoculation, respectively. The average number of lesions per half-leaf observed on Sylvestris tobacco (0–40) was related to the average number of lesions obtained on Xanthi tobacco (usually 10–150) in such a way as to give "relative mutagenesis," a quantity simply defined as number of lesions on Sylvestris tobacco per 1000 lesions on Xanthi tobacco. The inoculation procedures used were those previously described.[9]

Results and Discussion.—Degree of mutagenesis of PSV: After treating TMV, stripped to various degrees, with nitrous acid, the degree of mutagenesis with respect to local lesion character was determined by the ratio of the number of lesions observed on Sylvestris tobacco to the number obtained on Xanthi tobacco. As shown by the data in Table 1, few mutants causing local lesions on Sylvestris tobacco were produced by exposure of PSV to nitrous acid when less than 60 per cent of the RNA (measured from the 3′-end) was exposed. In contrast, a substantial increase was observed in the number of Sylvestris lesions relative to Xanthi lesions when more than 60 per cent of the RNA was exposed, the peak effect appearing when about 70–80 per cent of the RNA was exposed.

Similar results were observed in the case of PSV obtained by treating virus with urea, although only a few experiments have been done with urea and the relative mutagenesis values obtained were lower than those observed with PSV obtained by treatment of virus with SDS.

Kinetics of mutagenesis: The results shown in Figure 1 represent one of four such experiments, in all of which the outcome was similar although substantial variation in relative mutagenic values was seen in the several experiments. In general, the production of mutants that caused local lesions on Sylvestris tobacco was found to vary substantially with the extent of stripping of protein from the RNA and with

TABLE 1

RELATION OF MUTAGENESIS BY NITROUS ACID TO DEGREE OF STRIPPING OF PROTEIN FROM THE 3'-END OF TMV-RNA*

Degree of stripping (%)	No. of values	Average relative mutagenesis
0–20	19	0.2
21–50	25	1.1
51–60	6	1.2
61–70	17	3.3
71–80	20	7.2
81–85	2	6.3
100	18	10.0

* The results summarized here represent the pooled data from numerous experiments as indicated in column 2. Relative mutagenesis is the number of lesions observed on Sylvestris tobacco per 1000 lesions on Xanthi tobacco.

the time of exposure to nitrous acid. As shown in Figure 1, the number of local lesion mutants observed with half-stripped virus was only a little greater than for whole virus over the time interval tested. At about 70 per cent stripped, a rise in number of mutants was seen, and this number increased severalfold when the virus was about 75 per cent stripped. No further increase occurred even with 100 per cent stripped virus, that is, with free RNA. Thus it appears that the local lesion gene for Sylvestris tobacco is situated near the end of the first quarter of the TMV-RNA as measured from the 5'-end.

Electron microscopy: Measurements from electron micrographs of the particles obtained in various fractions of PSV (see *Materials and Methods*) provided an indication of the size distribution occurring in various classes of PSV. Some of the data are shown in Table 2. It will be seen from the table that the degree of stripping can be moderately well defined, the fractionation appearing to be somewhat sharper in the case of the heavily stripped particles than with those stripped to less than 50 per cent. A micrograph showing the particles in a 71 per cent stripped fraction of PSV is illustrated in Figure 2*a*. The field shown was initially in a spray droplet but the boundaries of the droplet were lost in the dialysis step (see *Materials and Methods*).

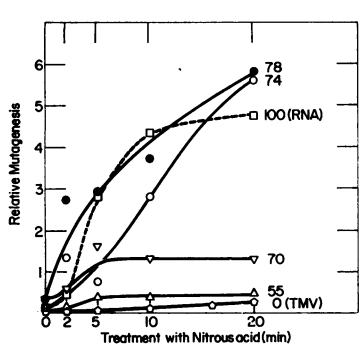


FIG. 1.—Kinetics of mutagenesis by nitrous acid. Relative mutagenesis is the number of lesions on Sylvestris tobacco per 1000 lesions on Xanthi nc tobacco. See text. Figures at ends of curves refer to per cent of stripping of protein from TMV particles prior to treatment with nitrous acid.

The RNA tails from PSV are not readily discerned on the usual micrographs, such as Figure 2*a*, and probably are broken off to a large extent during preparation of the specimens for electron microscopy. However, RNA tails and fragments of tails can be discerned under appropriate conditions (higher magnification, after rotary shadowing, etc.), and occasionally a long, intact strand of RNA such as illustrated in Figure 2*b* can be observed.

Gene location: It is assumed here that the observed local lesion response in Sylvestris tobacco represents the net result of the interaction between a nitrous acid-altered gene of the virus and the host cell genetic function. In the absence of mutation, this interaction results in a systemic mottle. Thus our view is that a specific segment

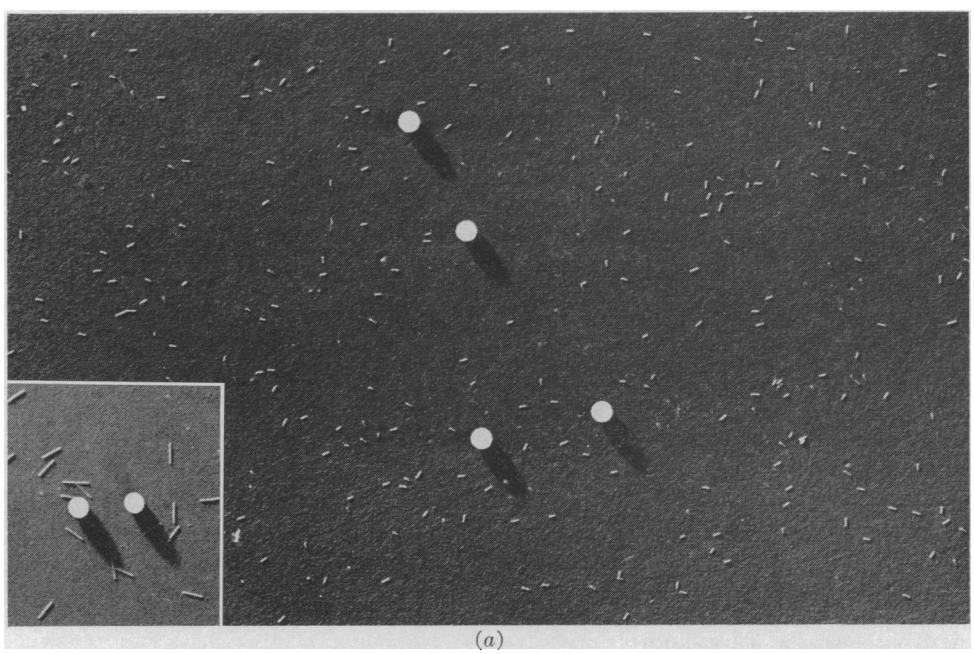
of the viral RNA strand determines whether the infecting virus will cause a local lesion or a systemic type of response in a given host, in this case, Sylvestris

TABLE 2

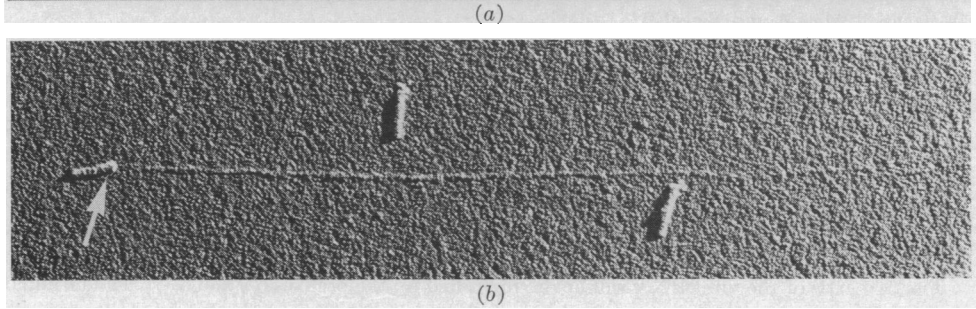
PARTICLE SIZE DISTRIBUTION OF PARTIALLY STRIPPED TOBACCO MOSAIC VIRUS*

No. of particles measured	Mean % stripped	% of Stripped Particles in		
		Mean class†	Less than mean class	Greater than mean class
211	27	59	20	21
106	35	60	18	22
145	49	74	11	15
156	64	64	23	13
786	67	65	21	14
1145	72	93	2	5
233	73	63	19	18
182	78	76	11	13

* Measurements taken from enlarged electron micrographs.
† Mean % stripped ±5%.



(a)



(b)

FIG. 2.—(a) A field of TMV particles from a class in which about 71% of the protein has been stripped. Exposed RNA is not observed for reasons given in the text. Whole TMV particles at the same magnification are illustrated in the *inset*. Polystyrene latex spheres are 259 ± 2.5 mμ in diameter. Shadowed with uranium. Magnification, 14,400×. (b) Partially stripped TMV, 67% stripped class, showing RNA tail. The postulated location of the local lesion gene is indicated by the *arrow*. Shadowed with uranium. Magnification, 57,000×.

tobacco. This segment of viral RNA conceivably constitutes one of a dozen or so genes comprising the total genome of the virus. The existence and location of such a gene are indicated by two sets of data presented here. Specifically, it was found that the mutagenic action of nitrous acid on PSV was rather small until the degree of stripping passed 60 per cent (Table 1), and a strong dependence of numbers of lesion mutants produced upon time of treatment with nitrous acid was observed only with virus that was approximately 74 per cent or more stripped (Fig. 1).

If the viral local lesion gene (LLS-gene) is about the same size as that calculated for the protein subunit on the assumption of a triplet code, it would consist of about 474 nucleotides. Such a polynucleotide chain would occupy an approximately 220 Å-long segment of the TMV particle based on the figure of 2.13 nucleotides per Å of length of the TMV particle.[22] This would make the LLS-gene about the second or third gene in the presumed direction of translation of the RNA, namely, from the 5′-end.[23]

There is some indication that the LLS-gene and the TMV coat protein-gene (CP-gene) may be closely linked, for Tsugita[5] reported that 59 of 60 strains that were found to have amino acid exchanges also caused local lesions on Sylvestris tobacco. Amino acid analysis of a series of the local lesion mutants obtained in the present experiments should shed some light on the location of the CP-gene and the suspected linkage.

Summary.—Protein subunits were stripped to various degrees from one specific end of tobacco mosaic virus particles by treating the virus with sodium dodecyl sulfate. Different classes of partially stripped virus were exposed to the mutagenic action of nitrous acid, and the resulting products were tested for total infectivity on Xanthi tobacco and for local lesion mutants on Sylvestris tobacco. A pronounced rise in the ratio of local lesion mutants to total infectivity occurred when about 75 per cent of the RNA starting from the 3′-end had been exposed. It is suggested that the local lesion gene is located in the segment of RNA exposed when the virus particle is more than 70 per cent stripped.

We are indebted to Mrs. June Kizu and Mrs. Helen Weaver for capable technical assistance. We also express our gratitude to Dr. D. S. May for technical advice, and to Dr. R. G. Milne for making confirmatory length measurements on the particles in various virus preparations. The idea of genetic mapping as developed experimentally here was proposed earlier by Schuster and Wittman,[24] May,[25] and Siegel.[26]

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[1] Abbreviations and terms used: TMV, tobacco mosaic virus; PSV, partially stripped virus; SDS, sodium dodecyl sulfate; EDTA, ethylenediaminetetraacetic acid.

[2] The term TMV genome will be used here to mean the single strand of RNA found in each TMV particle, while the term gene will be used to indicate any portion of the RNA strand that codes for a peptide chain or some specific function, such as the type of symptoms induced in an infected plant.

The two ends of the viral RNA will be distinguished by the prefixes 5′- and 3′-. The terminal nucleotide at the 5′-end is characterized by a free hydroxyl group on the 5′-carbon atom of its ribose component, whereas the terminal nucleotide at the 3′-end has a free hydroxyl group on the 3′-carbon of its ribose. It should be noted that the 5′-end has sometimes been designated the "3′-linked" end in order to indicate that the 3′-carbon is involved in the phosphodiester linkage of the first nucleotide to the next one in the chain. Similarly, the 3′-end has been termed the "5′-linked" end (for example, in May and Knight[9]).

[3] Knight, C. A., *J. Biol. Chem.*, **171**, 297 (1947).
[4] Woody, B. R., and C. A. Knight, *Virology*, **9**, 359 (1959).
[5] Tsugita, A., *J. Mol. Biol.*, **5**, 284 (1962).
[6] Wittmann, H. G., *Z. Vererbungslehre*, **95**, 333 (1964).
[7] Hart, R. G., these PROCEEDINGS, **41**, 261 (1955).
[8] Harrington, W. F., and H. K. Schachman, *Arch. Biochem. Biophys.*, **65**, 278 (1956).
[9] May, D. S., and C. A. Knight, *Virology*, **25**, 502 (1965).
[10] Mundry, K. W., and A. Gierer, *Z. Vererbungslehre*, **89**, 614 (1958).
[11] Gierer, A., and G. Schramm, *Nature*, **177**, 702 (1956).
[12] Steere, R. L., *Science*, **140**, 1089 (1963).
[13] Bengtsson, S., and L. Philipson, *Biochim. Biophys. Acta*, **79**, 399 (1964).
[14] Fraenkel-Conrat, H., B. Singer, and A. Tsugita, *Virology*, **14**, 54 (1961).
[15] Buzzell, Ann, *J. Am. Chem. Soc.*, **82**, 1636 (1960).
[16] Buzzell, Ann, *Biophys. J.*, **2**, 223 (1962).
[17] Williams, R. C., and R. C. Backus, *J. Am. Chem. Soc.*, **71**, 4052 (1949).
[18] Heinmets, F., *J. Appl. Physiol.* **20**, 384 (1949).
[19] Schuster, H., and G. Schramm, *Z. Naturforsch.*, **13b**, 697 (1958).
[20] Gierer, A., and K. W. Mundry, *Nature*, **182**, 1457 (1958).
[21] Fraenkel-Conrat, H., and B. Singer, *Virology*, **14**, 54 (1964).
[22] Caspar, D. L. D., *Advan. Protein Chem.*, **18**, 36 (1963).
[23] Smith, M. A., M. Salas, W. M. Stanley, Jr., A. J. Wahba, and S. Ochoa, these PROCEEDINGS, **55**, 141 (1966).
[24] Schuster, H., and H. Wittmann, *Virology*, **19**, 421 (1963).
[25] May, D. S., Ph.D. thesis, Univ. of California, Berkeley (1965).
[26] Siegel, A., *Advan. Virus Res.*, **11**, 25 (1965).

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*RELATION OF RIBONUCLEASE AND RIBONUCLEASE INHIBITOR TO THE ISOLATION OF POLYSOMES FROM RAT LIVER**

BY GÜNTER BLOBEL† AND VAN R. POTTER

MCARDLE LABORATORY FOR CANCER RESEARCH, MEDICAL SCHOOL, UNIVERSITY OF WISCONSIN, MADISON

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A currently favored hypothesis proposes that polyribosomes are aggregates of n ribosomes (where n may be any number from 2 to approximately 100) held together by a strand of mRNA.[1–8] In addition, it has recently been suggested that phospholipids are essential for maintaining the polysomal structure.[9] This latter suggestion was based on the observed breakdown of polysomes after incubation with high concentrations of phospholipase A, which, however, had not been demonstrated to be free of nuclease. Therefore, at present, mRNA is the only molecule for which there is strong evidence that it is essential in maintaining the integrity of the polysome. Two observations in particular have been made in a number of laboratories: (*a*) Electron micrographs show ribosomes linked by thin strands 10–15 Å in diameter.[1–5] (Actually, long before the proposal of the polysome concept, electron micrographs of thin slices of the ribosomal pellet of guinea pig liver had shown ribosomes in chains or clusters.[10]) (*b*) The polysomal structure is highly sensitive to small concentrations of ribonuclease.[4–8] The protein-protected ribosomal RNA is virtually unaffected, whereas the sterically exposed interribosomal segments of mRNA are easily available for attack. The hydrolysis of only one