and the existence of a reversal potential that is invariant with light intensity, membrane potential, and time. It has been shown that reducing sodium concentration in the bathing solution is associated with a less positive reversal potential in this preparation (*10*). Therefore, the depolarizing receptor potential seems to be the result of a permeability increase to sodium ions.

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Short Fragments from Both Complementary Strands in the Newly Replicated DNA of Bacteriophage SPP-1

Abstract. Bacillus subtilis *bacteriophage SPP-1 has separable complementary DNA strands. Fragments of nascent DNA isolated a very short time after phage infection show that these short chains are complementary to both phage DNA strands, as observed by hybridization techniques.*

Okazaki *et al.* (*1*) have isolated newly synthesized DNA as short segments from both *Escherichia coli* and those infected with bacteriophages. These segments had a molecular weight of 1 to 2×10^6 daltons, as judged by sedimentation behavior in alkaline sucrose gradients. The nascent DNA was labeled by an incorporated radioactive tracer to which the cells were exposed for short intervals. As the duration of the labeling was increased, more of the labeled DNA was found in larger molecules. These results, together with the observations on the action of ligases (*2*), have prompted reexamination of discontinuous models of DNA replication (*3*).

In these experiments it is difficult to rule out the possibility that the fragments might have been the result of nuclease or shear action on the sensitive nascent DNA (*4*). Regardless of whether the fragments of nascent DNA are true intermediates in DNA biosynthesis or products of degradation, one can ask if they are complementary to one or both strands of the DNA template. The results of the following hybridization experiments with *Bacillus subtilis* bacteriophage SPP-1, whose complementary strands are readily separable, suggest that the fragments of nascent DNA are complementary to both strands of the phage DNA.

Bacteriophage SPP-1 was isolated by Riva *et al.* (*5*). Infection, growth, purification of phage DNA, and separation of the DNA strands were carried out as described (*5*). The phage has a double-stranded DNA molecule of 2.5×10^7 daltons, with a density of 1.703 g/cm^3 (in CsCl). The profile of separated strands in CsCl is shown in Fig. 1. Phage infection was carried out with an arginine-requiring *B. subtilis* (SB 1051) as host. Stationary phase cells were infected at 37°C and transferred to 30°C after 40 minutes. At specified intervals of phage growth, portions were taken and added to tubes containing H^3-thymidine. After exposure to tracer for the desired time (usually a few seconds), growth was stopped by the addition of ice-cold sodium azide until the final concentration was 0.01 mole/liter. The cells were washed after 10 minutes with 0.01*M* tris-HCl [tris-(hydroxymethyl)aminomethane] buffer, *p*H 7.5, containing 0.01*M* sodium azide; and they were then frozen. The killed frozen cells were thawed, and lysozyme was added to a final concentration of 100 μg/ml. After 2 minutes at 37°C, NaOH was added to a final concentration of 0.01*M*. The cleared lysate was subjected to zone centrifugation in an alkaline sucrose gradient (5 to 20 percent). Fractions were collected, and samples were neutralized and analyzed for radioactivity in the acid-precipitable portions.

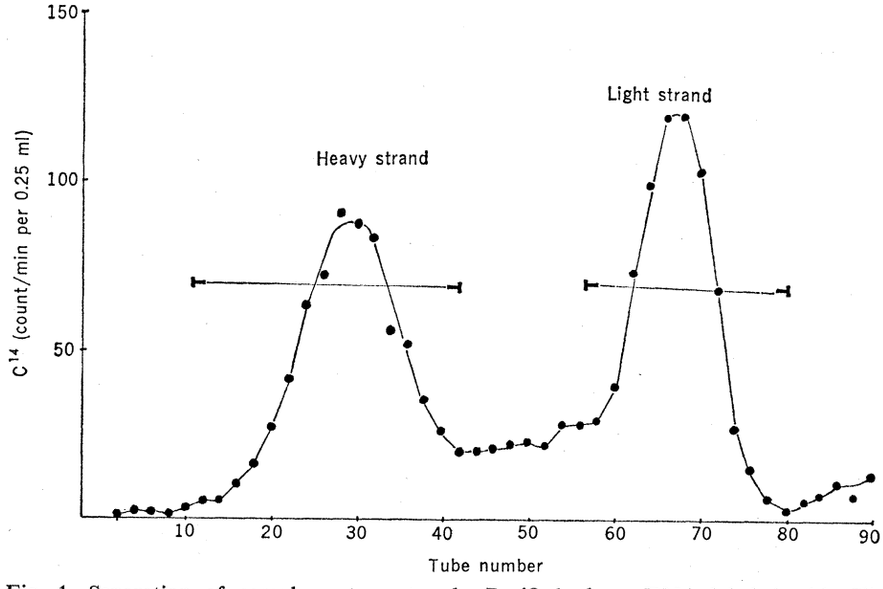

Fig. 1. Separation of complementary strands. Purified phage DNA labeled with C^{14}-thymidine (226 μg; 120 count min^{-1} μg^{-1}) in 0.8 ml of 0.2 × SSC and 50 μl of a solution containing 445 μg of *B. subtilis* ribosomal RNA were mixed with 12 ml of 0.2 × SSC in a 50-ml flask, heated for 10 minutes at 100°C, and chilled in ice. Solid CsCl was added to give a refractive index of 1.4030. Centrifugation was performed in a Spinco model-L centrifuge (rotor 50) at 32,000 rev/min for 64 hours at 20°C. From every other fraction of the first three gradients, 25 μl were taken and used for counting the radioactivity. The regions indicated on top of the heavy and light strand strata represent the pooled fractions from three gradients. SSC, standard saline-citrate solution (0.15*M* NaCl + 0.015*M* sodium citrate).

Table 1. Hybridization of nascent DNA fragments.

DNA		Incorporation (count/min)					
		2 second		5 second		10 second	
Species	Amount (μg)	Bound (count/min)	Input	Bound (count/min)	Input	Bound (count/min)	Input
Heavy strand	4.5	128	11(10.9)	132	12(12.2)	149	13(13.3)
Light strand	4.5	106	10 (9.6)	146	13(13.4)	134	12(11.9)
Unfractionated DNA SPP-1	9.0	284	24(24.3)	297	27(27.5)	311	28(27.8)
Bacillus subtilis DNA	3.3	4	1 (0.3)	2	1 (0.2)	10	1 (0.9)

These alkaline sucrose gradients, from which short segments were isolated, are shown, in Fig. 2, for 2, 5, and 15 seconds of synthesis in the presence of H^3-thymidine. The 15-second sample shows material sedimenting faster than the small fragments obtained with 2- and 5-second pulses. This was not included in hybridization experiments.

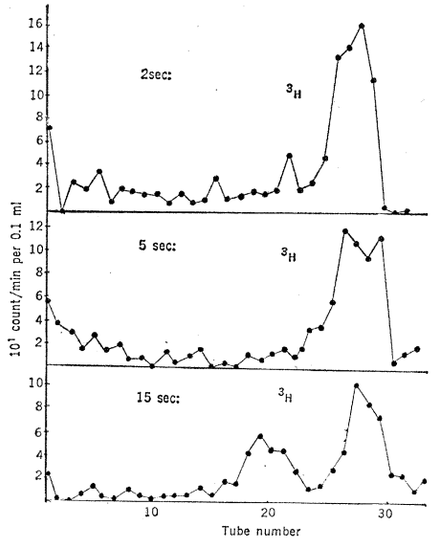

Fig. 2. Pulse-labeling of phage DNA during replication. Fifty milliliters of *Bacillus subtilis* SB 1051 were grown overnight at 37°C (*5*). The culture (~4×10^8 cell/ml) was centrifuged; all cells were then suspended in 40 ml of peptone (*5*) medium for 10 minutes at 37°C, 5 ml of phage suspension (approximately a multiplicity of five phage per bacterium) was added, and incubation was continued for 40 minutes at 37°C. Portions (10 ml) were distributed from this stock to each of four 50-ml flasks and incubated at 30°C to slow down the rate of phage DNA synthesis. After 10 minutes, each portion was exposed to 60 μc of H^3-thymidine (specific activity 15.9 c/mmole) for 2, 5, and 10 seconds and treated as stated in the text. Centrifugation was performed at 8°C (SW 25 rotor) at 21,000 rev/min for 21 hours.

Control gradients to determine the size of the fragments relative to *E. coli* transfer RNA (*6*) yield a value of 8 to 12*S*. The fractions containing 8 to 12*S* species of DNA fragments were pooled, deproteinized with phenol, and dialyzed extensively against 0.01*M* tris-HCl buffer, *p*H 7.5, containing 0.001*M* EDTA. The solution containing the fragments was concentrated by dialysis against a 50-percent solution of polyethylene glycol (Carbowax 6000) and finally dialyzed against 0.45*M* NaCl and 0.045*M* sodium citrate (3 × SSC) before hybridization with separated complementary strands.

To show that the labeled DNA segments were indeed part of the growing chains of phage DNA, the infected cells were exposed to H^3-thymidine for 5 seconds, and the tracer was diluted 2000-fold by the addition of unlabeled thymidine. The infection was continued for another 15 minutes. Phage DNA was isolated by neutral sucrose gradients after lysis of the infected cells with lysozyme. Fractions were collected and assayed for radioactivity and infectivity with competent cells of a tryptophan- and histidine-requiring *B. subtilis* (SB 25).

The pellet, containing bacterial DNA and completed phages, was not included in the infectivity assay. The fractions containing complete phage DNA molecules were identified by the plaque-forming ability and radioactivity. The profiles of infectivity and radioactivity coincided very well. The completed DNA molecules were susceptible to the action of deoxyribonuclease. A portion of the lysed cells was treated with NaOH, as described, and sedimented in an alkaline sucrose gradient. There was a substantial reduction in the amount of radioactivity at a position corresponding to the fragments. This result (Fig. 3) suggests that sequences labeled with H^3-thymidine during 5 seconds became part of large phage DNA molecules on subsequent growth.

Hybridization of DNA was carried out according to Denhardt (*7*). The purified nascent DNA fragments were hybridized separately to the "heavy" and "light" strands of phage DNA. Fractions from CsCl gradient corresponding to the separated strands of phage DNA (Fig. 1) were pooled and dialyzed against 0.05*M* NaOH and subsequently against 0.9*M* NaCl, 0.09*M* sodium citrate. Desired amounts of DNA (4.5 μg, labeled with C^{14}-thymidine; with a specific activity of 120 count/min per microgram of DNA) were placed on nitrocellulose membrane filters (13 mm in diameter) that had been soaked in 6 × SSC. When unfractionated DNA of phage or of *B. subtilis* was placed on the filters, the DNA was first denatured in alkali at a low salt concentration (0.01 × SSC), then the solution was neutralized with an equal amount of HCl in the cold, and the salt concentration was increased to 6 × SSC. The filters with the denatured DNA were first dried in a vacuum at room temperature for 1 hour and then for 2 hours at 80°C. Before hybridization of the DNA, the filters were incubated for 6 hours at 65°C in glass vials with 0.4 ml of a sterile solution containing 0.02 percent each of Ficoll (Pharmacia), polyvinyl pyrrolidone (Sigma), and bovine serum albumin (fraction V, Armour) in 3 ×

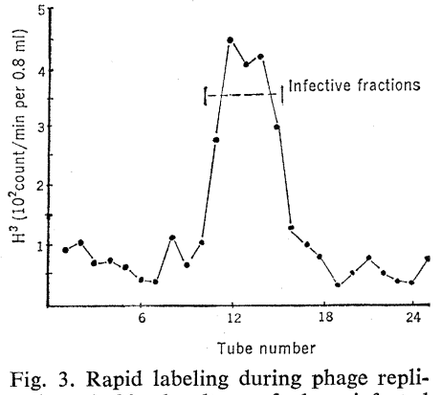

Fig. 3. Rapid labeling during phage replication. A 20-ml culture of phage-infected cells was exposed to H^3-thymidine, as described in the legend of Fig. 2, for 5 seconds. Unlabeled thymidine (2000-fold increase) was added, and the infected cells were allowed to grow for 15 minutes. Portions (10 ml) of cells were withdrawn and treated as described in Fig. 2. The rest of the culture was used for neutral sucrose gradients after lysis. Infectivity assays were performed with this sample.

SSC. This procedure reduces the background (as determined by the amount of DNA bound to a blank filter) to less than 1 percent of the input radioactivity. After this incubation, H^3-labeled nascent DNA fragments in 150 μl of 3 $\times$ SSC were added. Incubation was continued for 10 hours at 65°C; the temperature was lowered to 55°C, and the reaction mixture was kept at that temperature for another 4 hours. The filter was washed on both sides with 60 ml of SSC and dried, and the radioactivity was determined by counting the filters. Knowing the specific activity of C^{14}-thymidine, the labeled strands on the filter, and the relative number of thymidine in each strand of phage DNA (*5*), one can determine the exact amount of each DNA strand on the filter. These estimates always gave more than 96 percent of the input.

The results of hybridizing isolated DNA fragments to the separated strands of phage DNA, to unfractionated phage DNA, and to DNA from the host, *B. subtilis*, are presented in Table 1. Even after 2 seconds of exposure to the isotope, the labeled phage DNA fragments are complementary to both strands of phage DNA. There is no significant asymmetry in the proportion of fragments that bind either strand. Results from two other time points also agree with this observation. These results show that, within the limits of resolution of the experiments (a few seconds), short nucleotide sequences complementary to both strands of SPP-1 DNA are synthesized.

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Size Adaptation: A New Aftereffect

Abstract. *If, after prolonged observation of a striped pattern, one views a grating of the same orientation with somewhat narrower bars, then the bars seem even thinner than in fact they are. Broader bars seem broader still. This finding implies a system of size-detecting channels in human vision. The phenomenon may underlie many of the classical figural aftereffects.*

After observation of a high-contrast grating pattern, the intensity difference between light and dark bars necessary just to detect a grating is markedly elevated (*1*). This rise in contrast threshold occurs only for gratings similar to the adapting pattern in orientation and in spatial frequency (the number of cycles of the grating per degree of visual angle). This finding argues for the presence, in the human visual system, of neurons selectively sensitive to a limited range of spatial frequency. Such units have indeed been found in the visual pathway of cat and monkey (*2*). The optimal spatial frequency varies from neuron to neuron, and the sensitivity maxima are closely spaced along the spatial frequency continuum. It is possible that these neurons constitute a system of channels involved in the analysis of the size of retinal images.

A grating of some spatial frequency will arouse a distribution of activity in the population of size-detecting neurons. If the identity of the most active neuron or the shape of this distribution is used by the brain to recognize the size of the bars, then previous adaptation and depression of sensitivity at some other spatial frequency (which will shift the peak and skew the distribution of activity away from the adapting frequency) should make the grating seem different. In short, gratings with narrower bars than the adapting pattern should appear to be of even higher spatial frequency, and lower-frequency gratings should seem lower than in fact they are. We have found this to be the case and take it to be additional evidence for size-selective neurons in man.

You may observe this phenomenon for yourself by inspecting Fig. 1. First convince yourself that the two gratings on the right are identical in spatial frequency by looking from one to the other. Now place the illustration at a distance of about 2 m and look between the two gratings on the left for about a minute, allowing the gaze to wander back and forth along the horizontal bar. (This maneuver avoids the formation of a conventional after-

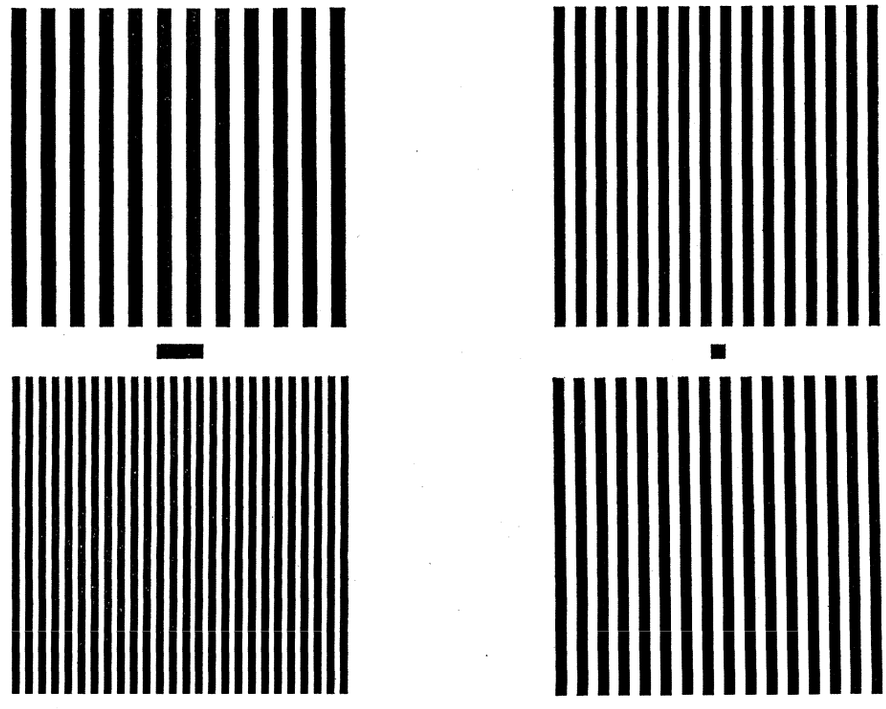

Fig. 1. Follow the instructions in the text to observe the aftereffect for yourself.