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Greater Vulnerability of the Infecting Viral Strand of Replicative-Form Deoxyribonucleic Acid of Bacteriophage φX174

B. DATTA AND R. K. PODDAR[1]

Biophysics Laboratory, Saha Institute of Nuclear Physics, Calcutta 37, India

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Four types of φX-infected cells of *Escherichia coli* CR, a thymine-requiring strain of *E. coli* C, were prepared in which the parental replicative-form deoxyribonucleic acid (RF DNA) was labeled with same specific amounts of bromouracil in (i) both strands, (ii) only the infecting viral strand, (iii) only the complementary strand, and (iv) neither strand. The sensitivity of each type of infected cell toward irradiation by ultraviolet light, visible light, and X rays was measured. The results indicate that a certain amount of radiation damage in the infecting viral strand of the parental RF was more inhibitory to the production of progeny phage than when the damage was in the complementary strand. Similar conclusions were also drawn from "suicide" experiments of the phage-infected complexes containing ^{32}P of the same specific activity on either strand of the parental RF DNA. The results suggest that the beta decay occurring in the infecting viral strand was more effective in inactivating the plaque-forming ability of the complex.

In double-stranded deoxyribonucleic acid (DNA), one strand of which is complementary to the other, it may be expected that certain functions can be performed by one strand and not by the other. There is a good deal of evidence that the transcription of messenger ribonucleic acid (RNA) is made by copying only one of the two strands, but not always the same strand, of a double-stranded DNA molecule (5, 13). It has also been suggested that only one of the DNA strands transmits the genetic information to the progeny DNA in bacteriophage T4 (7, 15) and in *Escherichia coli* (4). From a study of photoinactivation of members of a population of bacteriophage λ having bromouracil (BU) in place of thymine in only one of their two DNA strands, Fox and Meselson (3) concluded that certain essential viral functions were carried out by one but not the other strand of λ DNA.

Though φX174 is a single-stranded DNA virus, upon infection its DNA becomes double-stranded by synthesizing its complementary strand; in the course of intracellular growth, single-stranded φX DNA molecules are produced which are ultimately surrounded by protein coats and come out as progeny phage (for review, see 10, 11). In our experiments, we selectively labeled in vivo the infecting viral and the complementary strands of the first intracellularly formed double-stranded replicative-form (RF) DNA of φX174 (hereinafter referred to as the "parental" RF) with BU and compared the effects of subsequent irradiation of the infected bacteria harboring such RFs with ultraviolet light, visible light, and X rays on the intracellular development of complete phage particles in the two cases. Also, the viral and complementary strands of the parental RF DNA were selectively labeled with ^{32}P of the same specific activity and the effects of the radioactive decay due to incorporated ^{32}P in both cases were compared. If only one of the two strands of the parental RF DNA were able to perform certain vital functions necessary for producing progeny phage, these comparative studies should reveal a differential vulnerability of one or the other strand of the parental RF.

MATERIALS AND METHODS

Phage and bacteria. Bacteriophage φX174 and *E. coli* C were obtained from the stock of I. Tessman of Purdue University. *E. coli* CR, a thymine-requiring strain of *E. coli* C, was a gift of R. L. Sinsheimer of the California Institute of Technology.

Media. Tris-glucose (TG) medium of Palchaudhury and Poddar (6) and the starvation buffer of Denhardt and Sinsheimer (1) were used. TGM (CR) was TG medium supplemented, per liter, with 10 g of Casa-

[1] Present address: Division of Biology, California Institute of Technology, Pasadena, Calif. 91109.

mino Acids, 3.2 ml of glycerol, and 4 ml of 50% glucose. UV-buffer contained: $MgSO_4$, 0.25 g; $CaCl_2$, 0.15 g; 1% gelatin, 1 ml; Na_2HPO_4, 7.6 g; KH_2PO_4, 1.5 g; NaCl, 4.0 g; potassium sulfate, 5.0 g; and water to make 1 liter; *p*H 7.0.

Preparation of BU-labeled φX174. A 10-ml amount of TGM (CR) containing (per ml) 10 μg of thymidine, 5 μg of 5-bromodeoxyuridine (BUdR), and 10 μg of uridine was inoculated with overnight-grown *E. coli* CR and was incubated with aeration until the bacterial concentration reached 2×10^8 to 3×10^8 cells/ml. The bacteria were centrifuged, washed with starvation buffer, and starved for 1 hr. The starved bacteria were infected with φX174 at a multiplicity of infection (MOI) of 2, allowed 15 min for adsorption, centrifuged, and then suspended in TGM (CR) containing 10 μg of BUdR per ml and 10 μg of uridine per ml. The complexes were incubated with aeration for 2 hr; after 2 hr, the culture was shaken with chloroform and subsequently frozen and thawed three times. After centrifugation, the supernatant, which contained BU-labeled φX174, was stored in the cold in darkness. The burst size was about 20, and the replacement of thymine by BU in phage DNA appeared to be complete; these phages banded with a single peak at a density of about 1.46, whereas normal, unsubstituted φX174 banded at a density of 1.43, after equilibrium sedimentation in a CsCl density gradient (cf. Denhardt, Ph.D. Thesis, California Institute of Technology, Pasadena, 1965).

Preparation of E. coli CR cells containing parental RF DNA differentially labeled with BU. A prestarved log-phase culture of *E. coli* CR was infected with BU-labeled φX174 at an MOI of 1 in starvation buffer. After 15 min of adsorption, the culture was centrifuged, and the pellet was resuspended in TGM (CR) supplemented (per ml) with 20 μg of thymidine, 10 μg of uridine, and 50 μg of chloramphenicol (CM). The infected cells were aerated for 30 min at 37 C, chilled to ice temperature, centrifuged in the cold, and resuspended in ice-cold UV-buffer. These cells are hereinafter referred to as (Th-CR + BU-φX) complexes. (As regards the number of RF molecules per cell of these and other complexes described below, please see Discussion).

(Th-CR + Th-φX) complexes containing parental RF DNA molecules with both strands unlabeled were obtained in exactly the same manner except that normal thymine-containing φX174 phage were used for infection.

(BU-CR + Th-φX) complexes containing BU label in the complementary strand of the parental RF were obtained under the same conditions used for the preparation of BU-labeled φX174: the bacteria initially grown in the presence of 5 μg of BU per ml were starved and then were infected with thymine-containing phage; the infected cells, freed from unadsorbed phage, were grown in the presence of 10 μg of BU per ml and 50 μg of CM per ml for 30 min and then were transferred to ice-cold UV-buffer. The (BU-CR + BU-φX) complexes, in which both strands of the parental RF DNA were labeled with BU, were prepared as above, except that the bacteria were infected with BU-labeled φX174 instead of normal phage.

Selective labeling of either strand of RF DNA with ^{32}P of the same specific activity. ^{32}P-labeled TG medium was prepared by evaporating the water from a desired amount of carrier-free orthophosphate (^{32}P) solution and then redissolving the phosphate in about 12 ml of TG medium. The specific activity of the medium was about 250 to 300 mc per mg of phosphorus, an activity sufficient to ensure incorporation of 4 to 5 ^{32}P atoms per labeled strand. This radioactive TG medium was used throughout. *E. coli* C cells were grown to log phase in about 4 ml of the radioactive TG medium; they were then washed and starved in the starvation buffer for 1 hr and were infected with nonradioactive φX174 at an MOI of 0.1. After 15 min of adsorption, infected bacteria were freed from unadsorbed phage by centrifugation and were resuspended in the ^{32}P-labeled TG medium and divided into two tubes. Infected cells of the first tube were allowed to grow at 37 C until lysis; the lysate was subjected to two cycles of low- and high-speed centrifugation; the sediment containing ^{32}P-labeled φX174 was finally suspended in 5 ml of tris(hydroxymethyl)aminomethane (Tris)-NaCl. After 6 min of growth, the contents of the second tube were diluted 50 times in storage buffer at ice temperature, and 1-ml volumes were distributed into a set of tubes, which were then quickly frozen in an ice-salt mixture and stored at −20 C. These were referred to as (^{32}P-C + φX) complexes, having ^{32}P in the complementary strand of the parental RF DNA.

Phage-bacteria complexes containing parental RF DNA with ^{32}P label in both strands were obtained as follows. A sample of the overnight bacteria was diluted into labeled TG medium, grown at 37 C to log phase, centrifuged, and starved in buffer for 1 hr. These bacteria were then infected with ^{32}P-labeled phage φX174 at an MOI of 0.1. Adsorption was allowed for 15 min, and unadsorbed labeled phage were discarded by centrifugation. The sediment, consisting of (^{32}P-C + ^{32}P-φX174) complexes, was resuspended in 2 ml of labeled TG medium. After 6 min of growth, samples were collected, diluted, and stored frozen at −20 C.

The (C + ^{32}P-φX) complexes, containing parental RF DNA labeled in the infecting viral strand, were obtained by infecting unlabeled bacteria with ^{32}P-labeled φX174 in unlabeled medium. The complexes were allowed to grow at 37 C for 6 min and then were stored as in the above method.

Sources of irradiation. The X-ray source was a continuously operated X-ray machine run at 80 kv, 9 ma. The samples were irradiated at a distance of 9 cm from the source. The ultraviolet source was a 15-w General Electric germicidal lamp. The samples were irradiated at a distance of 30 cm from the source. The source of visible light consisted of 2 × 40-w OSRAM fluorescent lamps. The samples were irradiated at a distance of 8 cm from the source.

Measurement of radiation sensitivity. Samples (2.5 ml) in UV-buffer were irradiated in a 5-cm petri dish at ice temperature. Radiation sensitivity was measured by plaque assay of free phage or of the infected

cells after irradiation with different doses. All experiments involving BU were performed in dim yellow light or in complete darkness.

RESULTS

Sensitivity of normal and BU-labeled free φX174 toward ultraviolet light, visible light, and X rays. Survival curves of BU-labeled φX174 and normal thymine-containing φX174 after ultraviolet light and X-ray irradiation are shown in Fig. 1a and b. It was observed that the sensitivity of BU-labeled phage to X rays and ultraviolet light was 3.15 and 1.60 times, respectively, that of the normal phage (Table 1). Labeling of φX174 with BU rendered the phage sensitive toward visible light, the slope of the inactivation curve being about $-0.10\ hr^{-1}$; normal thymine-containing phage was more or less stable in visible light (Fig. 1c).

Radiation sensitivity of φX-infected E. coli CR complexes containing BU in different strands of parental RF. Phage-infected complexes of *E. coli* CR in which the parental RF DNA was differentially labeled with BU as required were prepared and irradiated with ultraviolet visible light and X rays as described in Materials and Methods.

The logarithms of the surviving percentages of the plaque-forming units (Y) after various periods (X) of irradiation were plotted, and the regression lines (12) drawn through these points to fit the general equation of the form $Y = a + bX$ were taken as the survival curves in respective cases (Fig. 2–4). The regression coefficient, b, i.e., the slope of the survival curve, shown in Table 2, was assumed to be linearly related to radiation sensitivity. Experiments with ultraviolet light were repeated three to four times; those with X rays and visible light were performed twice. The slopes of the corresponding survival curves were quite reproducible, their range of variability being within the limits of standard deviations shown in Table 2.

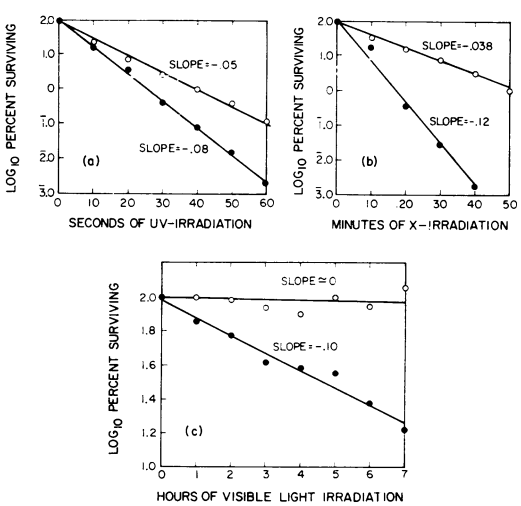

FIG. 1. *(a) Ultraviolet inactivation curves, (b) X-ray inactivation curves, and (c) survival curves after visible light irradiation of (●) BU-labeled φX174 and (○) nonlabeled φX174.*

TABLE 1. *Radiation sensitivity of normal thymine-containing φX174 (Th-φX) and bromouracil-labeled φX174 (BU-φX) to X rays, visible light, and ultraviolet (UV) light*

Phage	Slope of the survival curve		
	X ray	Visible light	UV light
	min^{-1}	hr^{-1}	sec^{-1}
(a) BU-φX	−0.12	−0.10	−0.08
(b) Th-φX	−0.038	No inactivation	−0.05
Relative increase in radiation sensitivity due to BU labeling (a:b)	3.15	—	1.60

The comparative analysis of the data of Table 2 and Fig. 2–4 on the relative radiation sensitivities of various types of φX-infected cells can be summarized as follows: (Th-CR + BU-φX) complexes, which were about equally as sensitive as (BU-CR + BU-φX) complexes, except towards X rays, were definitely less resistant to irradiation than (BU-CR + Th-φX) complexes in *all cases*; and (BU-CR + Th-φX) complexes were hardly any more sensitive than (Th-CR + Th-φX) complexes, except towards ultraviolet light. The causes of the two exceptions are not clear; they probably reflect some unknown interaction of BU-containing host cells with the respective irradiation. Nevertheless, it seems quite reasonable to conclude from our results that a certain amount of radiation damage on the infecting viral strand was more inhibitory to intracellular phage development than the occurrence of an equivalent amount of damage on the complementary strand.

Inactivation due to decay of radioactive phosphorus incorporated in (C + φX174) complexes. Phage-infected *E. coli* C cells, containing the parental RF DNA differentially labeled with ^{32}P, were stored, and survival curves due to decay of radioactive phosphorus incorporated in the DNA were obtained by assaying the infective centers of the complexes after different storage periods (Fig. 5). The infective centers were as-

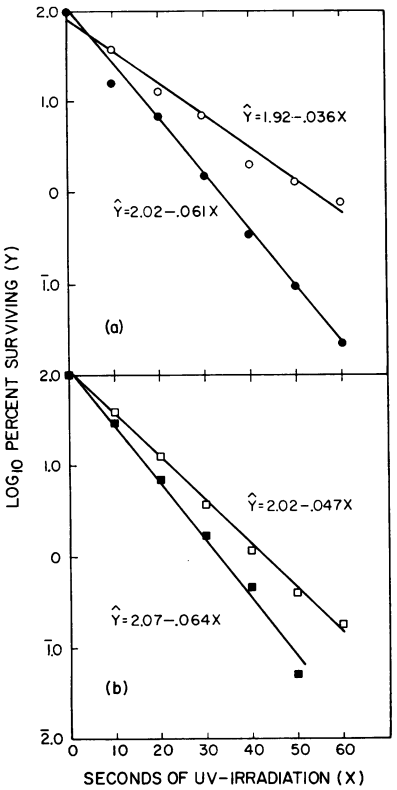

FIG. 2. *Survival curves of phage-infected complexes after ultraviolet irradiation. Symbols:* ●, *(Th-CR + BU-φX) complexes;* ○, *(Th-CR + Th-φX) complexes;* ■, *(BU-CR + BU-φX) complexes;* □, *(BU-CR + Th-φX) complexes.*

sayed by thawing the samples very slowly and then plating them immediately.

Complexes containing ^{32}P in the parental strand of RF DNA were about twice as sensitive toward inactivation due to incorporated ^{32}P decay as the complexes containing RF DNA labeled only in the complementary strand (Table 3). Complexes containing nonlabeled RF DNA were more or less stable during storage. Thus, the decay of an incorporated ^{32}P atom on the infecting viral strand of the parental RF molecule affected performance of certain vital functions necessary for production of progeny phage much more adversely than when such a decay occurred in the complementary strand.

The (^{32}P-C + φX) complexes contained about 10^4 times more radioactivity than the (C + ^{32}P-φX) complexes, assuming P content of *E. coli* cells to be approximately 3% of their dry weight. Since, in spite of this, (^{32}P-C + φX) complexes were found to be less sensitive, it is apparent that

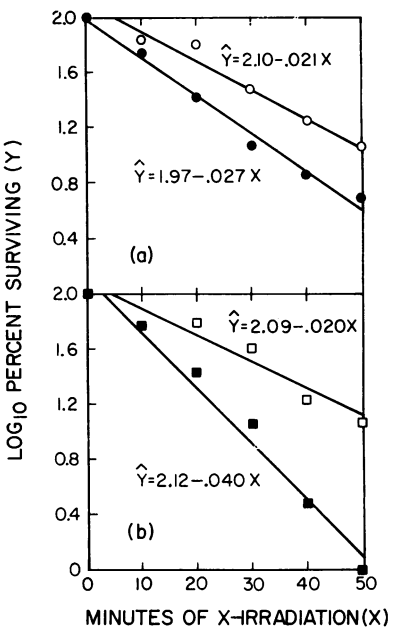

FIG. 3. *Survival curves of phage-infected complexes irradiated with X ray. Symbols:* ●, *(Th-CR + BU-φX) complexes;* ○, *(Th-CR + Th-φX) complexes;* ■, *(BU-CR + BU-φX) complexes;* □, *(BU-CR + Th-φX) complexes.*

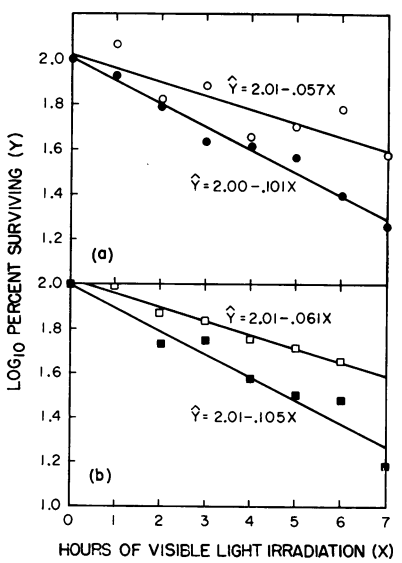

FIG. 4. *Survival curves of phage-infected complexes after visible light irradiation. Symbols:* ●, *(Th-CR + BU-φX) complexes;* ○, *(Th-CR + Th-φX) complexes;* ■, *(BU-CR + BU-φX) complexes;* □, *(BU-CR + Th-φX) complexes.*

TABLE 2. *Radiation sensitivity of φX-infected E. coli CR containing bromouracil in different strands of the parental RF, with X rays, visible light, and ultraviolet light*

Nature of the complex	Strand(s) labeled with BU	Slope of the survival curve ± SD		
		X ray	Visible light	UV light
		min^{-1}	hr^{-1}	sec^{-1}
(a) Th-CR + BU-φX	Parental	−.027 ± .001	−.101 ± .007	−.061 ± .001
(b) BU-CR + Th-φX	Complementary	−.020 ± .003	−.061 ± .005	−.047 ± .002
(c) BU-CR + BU-φX	Both	−.040 ± .003	−.105 ± .013	−.064 ± .003
(d) Th-CR + Th-φX	None	−.021 ± .002	−.057 ± .014	−.036 ± .002
Relative increase in radiation sensitivity				
a:b		1.35	1.64	1.30
a:d		1.29	1.75	1.70
a:c		0.65	0.95	0.99
c:b		2.00	1.72	1.36
b:d		0.95	1.07	1.30

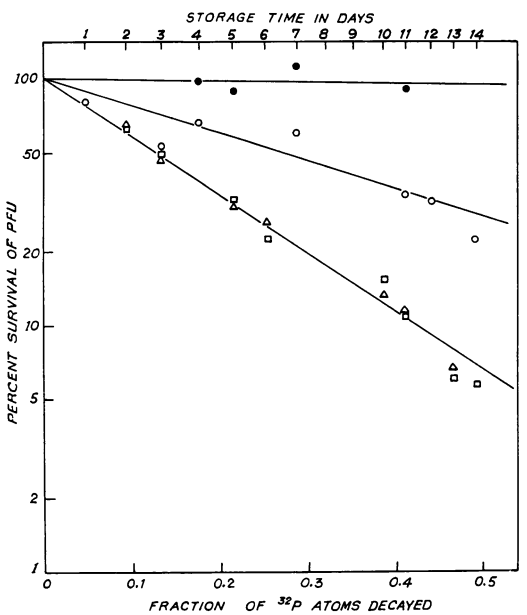

FIG. 5. *Inactivation curves due to decay of incorporated ^{32}P atoms in phage-bacteria complexes having RF DNA with strands differentially labeled with ^{32}P. Symbols: □, (^{32}P-C + ^{32}P-φX) complexes; △, (C + ^{32}P-φX) complexes; ○, (^{32}P-C + φX) complexes; ●, (C + φX) complexes.*

radioactivity external to RF DNA was practically ineffective and that the observed inactivation of the infective centers was essentially due to ^{32}P label in the parental RF molecule. We have, however, no good explanation of our finding that the parental RF molecule labeled in both strands was almost equally as sensitive as the RF molecule

TABLE 3. *Inactivation of φX-infected E. coli C due to decay of ^{32}P incorporated in different strands of RF*

Nature of the complex	Presence of ^{32}P in	Slope of the survival curve
(a) C + ^{32}P-φX	Parental strand	−2.5
(b) ^{32}P-C + ^{32}P-φX	Both strands	−2.5
(c) ^{32}P-C + φX	Complementary strand	−1.2
Relative increase in radiation sensitivity		
a:c		2.1
b:c		2.1
a:b		1.0

having ^{32}P label only on the infecting viral strand (Fig. 5). This is in conflict with what one would expect on the basis of the model of Stent and Fuerst (14) of the biological effect of decay of ^{32}P incorporated in a double-stranded DNA molecule.

DISCUSSION

On the basis of the findings of many workers [see review by Sinsheimer (10)], it was assumed that at least one RF DNA molecule was formed in each phage-infected bacterium which was allowed to grow for 30 min in the presence of CM or for 6 min in the absence of CM. Denhardt and Sinsheimer (2) showed that the material making up the complementary strand of the parental RF DNA molecule was derived from the medium. So it was also assumed that the complementary

strand of the parental RF DNA within the infected cells in our experiments was labeled with BU or ^{32}P whenever infection and subsequent growth were carried out in presence of BU or ^{32}P, respectively.

Single-stranded free ϕX DNA contains 25% adenine and 31% thymine (9). When phage-infected cells were grown with the same concentration of BU in the medium, labeling of the complementary strand might be only 6% less than that of the infecting viral strand. The specific activity of the ^{32}P label in either the parental strand or the complementary strand was, however, identical because the medium contained ^{32}P label at the same specific activity in all experiments described here. It is, therefore, concluded that the observed differences in radiation sensitivities of the infected cells were not due to unequal labeling of the two strands of parental RF DNA, if any.

In our experiments, the infected cells could possibly harbor a few progeny RF molecules besides the parental one. The progeny RF molecules in cells grown in labeled medium would obviously be labeled in both strands, whereas the progeny RF molecules would be unlabeled in the complexes growing in unlabeled medium. It was also very likely that a greater number of progeny RF molecules would be formed in the latter case because of a faster growth rate. If the progeny RF molecules were also radiation-sensitive "targets," each of which was to be hit for inactivation of the plaque-forming ability of the infected cell, the (BU-CR + ϕX) and (^{32}P-C + ϕX) complexes should have been more sensitive than the (Th-CR + BU-ϕX) and (C + ^{32}P-ϕX) complexes, because the former would harbor a smaller number of more sensitive targets. What we actually observed was, however, the opposite Hence, the effects we observed must be the outcome of irradiation damage or ^{32}P decay in the parental RF molecule containing single-stranded DNA of the infecting virus. Denhardt and Sinsheimer (2) have also obtained evidence that the intracellular RF molecule containing the parental single-stranded DNA of $\phi X174$ was "unique" in the sense that it contained the essential genetic information for the subsequent development of phage. Besides confirming their finding, we have concluded that the infecting viral strand of this "unique" parental RF molecule is biologically more important, because a certain amount of damage on this strand was more effective in interfering with the production of progeny phage.

The parental RF molecule conceivably carries out two biological functions, viz., by serving as the template for the replication of progeny RF molecules and for the transcription of messenger RNA molecules for some phage-specific proteins, such as those needed for single-stranded phage DNA synthesis. In view of the recent evidence of Russo, Stahl, and Stahl (8) that both strands of λ DNA transfer information from old to new chains of DNA duplexes, it seems very likely that only the transcriptional activity of the ϕX parental RF molecule is preferentially destroyed by the irradiation damage on the infecting viral strand. It has, however, to be borne in mind that modes of replication of λ DNA and ϕX RF DNA may not be identical.

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