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Genetic Studies on Non-Lethal Recombination Gene Mutants of Bacteriophage T4

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Abstract. Mutants in the phage T4 recombination and repair genes *uvsX* and *uvsY* were newly isolated. Using these mutants we examined time course of the synthesis of the gene products by pulse-labeling of phage proteins with ^{3}H-leucine, followed by SDS-polyacrylamide gel electrophoresis and fluorography. In the wild type infection they were produced at an early period but not at the late. Their synthesis continued for a long period, when DNA synthesis was blocked. Mutations in *uvsX* were mapped consistently by genetic crosses and size of *am*-fragments, indicating that the gene is transcribed anticlockwise on T4 map. We have recently proven that gp*uvsX* (gene product of *uvsX*) catalyzes pairing of homologous DNA (m.s. in preparation). Host *recA* has an analogous function (Shibata *et al.*, 1979; McEntee *et al.*, 1979). Therefore we reexamined, by using newly isolated mutants, if *recA* could substitute for *uvsX in vivo*. UV-sensitivity and recombination frequency of two markers were not affected by the host gene, even when its product was overproduced.

Introduction

General recombination is an essential process for continuous DNA replication and for the repair of damaged DNA in T4 replication cycle (Broker and Doermann, 1975; Mosig *et al.*, 1978; Bernstein, 1981; Luder and Mosig, 1982). Extensive investigations have shown nearly 20 genes are involved in the recombination. Since many of the genes also participate in DNA replication and thus DNA replication and recombination are interwoven process, their mutation are lethal for phage growth. The process of the recombination comprises of several steps (Broker and Doermann, 1975; Radding, 1978). Among these the most crucial would be the pairing of complementary strands. Looking for the gene controling this step, we chose two genes, *uvsX* and *uvsY*. Their mutations are not lethal, although their viability is depressed. Their DNA synthesis starts at normal period with normal rate, but ceases about 20 min after infection (Cunningham and Berger, 1977; Dewey and Frankel, 1975). Furthermore vegetative DNA involves reduced concatemeric strands (Cunningham and Berger, 1977), which would be a consequence of recombination. These may suggest that in these mutant-infected cells recombination are suppressed independently of the replication. If so, these genes or either of these genes could be responsible for the crucial recombination step, pairing of homologous strands. Under these considerations we have studied the traits of *uvsX* and *uvsY* gene. We

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have identified gp*uvsX* and gp*uvsY* to be proteins of molecular weight of 39,000 and 16,000, respectively, purified nearly to homogeneity, and then shown that purified gp*uvsX* catalyzes homologous strand pairing (m. s. in preparation). Here we will describe isolation of the mutants and some properties of the mutations.

Materials and Methods

Bacterial strains, phages and plasmids. *Escherichia coli* CR63 and B40sul are permissive hosts for *am* phage mutants; CR63 (λh) was used as the selective host for *rII*$^+$ phage. All others, including BB and B^E, are non-permissive for *am* mutants. 594, 594*recA441* and 594 (pTM2) are K12 wild type, *recA*$^-$, and carrying plasmid pTM2, respectively.

T4 mutants were: gene *25*(*amS52*), gene *41*(*amN81*), gene *42*(*amN122*), gene *44*(*amN82*), gene *49* (*tsC9*), gene *62* (*amE1140*), gene *rII* (*rM36* and *r596*). Gene *40* (*amF12*) was provided by Dr. L. W. Black, and gene *uvsW* (*m22*), gene *uvsX* (x_m), and gene *uvsY* (y_m) by Dr. R. P. Cunningham. Mutants in *uvsX* (*amxa2, amxb, amxe2,* and *tsxcO*) and in *uvsY* (*amya1* and *amyd*) were isolated in this study. Multiple mutants were constructed in our laboratory by recombination.

Plasmid pTM2, provided by Drs. H. and T. Ogawa, is a derivative of pBR322 containing the *E. coli recA*$^+$ gene (Ogawa *et al.*, 1978).

Media and culture conditions. M9 and TG were described previously (Minagawa and Ryo, 1978). M9A and TCG were M9 and TG supplemented with 0.2% Casamino acids (Difco). PG (peptone-glucose) was used for agar plates (Miyazaki *et al.*, 1978). Cultures were grown in M9A by aeration at 37° unless otherwise stated.

Phage crosses. For genetic mapping, a culture of B40sul grown to 5×10^8/ml, was supplemented with 4 mM NaCN. After 5 min, the cells were coinfected with two mutant phages at multiplicities of 5 each. After 5 min, 0.1 volume of anti-T4 rabbit serum was added to k=2 and incubation was continued for 5 min. The infected culture was diluted 10^4-fold into 2 ml prewarmed medium and incubated stationary at 30° for 60 min. The cells were lysed with a few drops of chloroform and the lysate was plated on B40sul and B^E at 30°. For crosses with *ts* mutants, the B^E plates were incubated at 42°. Since *uvs* mutants made small plaques on B^E under these condition, normally sized plaques were counted as recombinants. Recombination frequencies were calculated as twice of the number of recombinants divided by the number of plaques on B40sul.

For *rII* recombination experiments, 594 and its derivatives were used as host strains at 37°. Progeny phages were plated on CR63 to count total numbers of progeny and on CR63 (λh) to count numbers of *rII*$^+$ recombinants. Burst sizes were calculated as the total number of progeny divided by the number of infected cells plated immediately after the treatment with antiserum.

Measurement of UV sensitivity of phages. 594 or 594*recA* cells at 5×10^8/ml were mixed with phages (previously UV-irradiated for 0, 20, 30, and 40 sec) at a multiplicity of 0.1 (monocomplexes) or 5 to 7 (multicomplexes), incubated for 5 min, and then treated with anti-T4 serum at k=1 for 5 min. The complexes were plated with B40sul after appropriate dilution. Since plots of log (surviving fraction) vs. UV dose were almost linear with both

monocomplexes and multicomplexes, UV sensitivity was expressed as a killing constant, k, from the equation surviving fraction$=e^{-kD}$, where D is the UV doses in sec.

UV irradiation. Five-ml suspensions of phage (2.5×10^{10}/ml in M9 without glucose) in a petri dish of 9 cm diameter were irradiated at a UV intensity of 0.8 J/m^2 sec for 20, 30, and 40 sec. For induction of *recA* protein a 2-ml suspension of 594(pTM2) at 5×10^8/ml in M9A was irradiated for 60 sec and aerated for 30 min before infection.

Electrophoresis of proteins. BB was grown in M9 to 5×10^8 cells/ml and infected with phage at a multiplicity of 5. For isotopic labeling, 0.2-ml cultures were sampled at desired times and incubated with ^{3}H-L-leucine (specific activity 64 C/mmol; 5 μC/ml for labeling gp*uvsX* and 10 μC/ml for labeling gp*uvsY*) for an appropriate period. Trichloroacetic acid was then added to 0.6 M and the mixture was centrifuged. The pellet was suspended in 0.2 ml γ-solution for gp*uvsX* and 0.1 ml gp*uvsY*, γ-solution being composed of 0.15 M Tris-HCl at pH 7.4, 0.02 M NaOH, 0.5% 2-mercaptoethanol, 1% SDS, 10% glycerol and a trace of bromphenol blue, heated together in a boiling water bath for 2 min. SDS polyacrylamide gel electrophoresis was performed using a 10% gel for gp*uvsX* and a 10% to 20% linear gradient of gel containing 6M urea for gp*uvsY*; the gels were fluorographed as described previously (Miyazaki *et al.*, 1978).

Results

Isolation of uvsX *and* uvsY *mutants.* Dewey and Frankel (1975) found that pseudorevertants of gene *49* mutants contained suppressor mutations located in *uvsX* and *uvsY*. We isolated spontaneous revertants of *tsC9* (*49*) which grew on BB (but not CR63) cells at 42°. All of these were more sensitive to UV irradiation than the wild type when plated on BB, and segregated *tsC9* when crossed with the wild type. We performed complementation and recombination tests with these mutants against x_m and y_m, scoring UV sensitivity as described previously (Minagawa *et al.*, 1983), and found 6 new mutations of an amber type and one temperature-sensitive. Three of the *am* mutations fell in *uvsX*, two in *uvsY* and one in *32*; the *ts* mutation fell in *uvsX*, (Minagawa *et al.*, 1983). Pseudorevertants were crossed with wild type to segregate *tsC9*, and single-mutation isolates in *uvsX* (*amxa2*, *amxb*, *amxe2* and *tsxcO*) and in *uvsY* (*amya1* and *amyd*) were recovered. Growth, UV sensitivity and recombination frequency of these *am* mutants were well suppressed by a suppressor, *Su1*$^+$.

*Time course of the appearance of gp*uvsX and *gp*uvsY. Gp*uvsX* has been identified to be protein of molecular weight of 39,000 by electrophoresis (Burke *et al.*, 1983; Yonesaki *et al.*, m. s. in preparation). We attempted to know synthetic time course of the protein. Figure 1A represents a result when cells were infected with wild type and protein were labeled with ^{3}H-leucine for 2 min at indicated times after infection. Labeled proteins were electrophoresed and fluorographed as described in Materials and Methods. The synthesis of gp*uvsX* was distinct until 10 min and was sharply reduced thereafter. Other experiments showed it to be detectable by labeling between 3 and 5 min. However, its synthesis continued for a long time when DNA synthesis was blocked by mutation (first column of Figure 1A), or by prior UV irradiation of the wild type phage (10^{-4} survival). (data not shown). These results indicate that gene *uvsX* is a typical early gene (Wiberg *et al.*, 1962; Young and Van Houwe, 1970; Rabussey and Geiduschek, 1977). We also identified gp*uvsY* by 1D gel as a band of molecular weight of

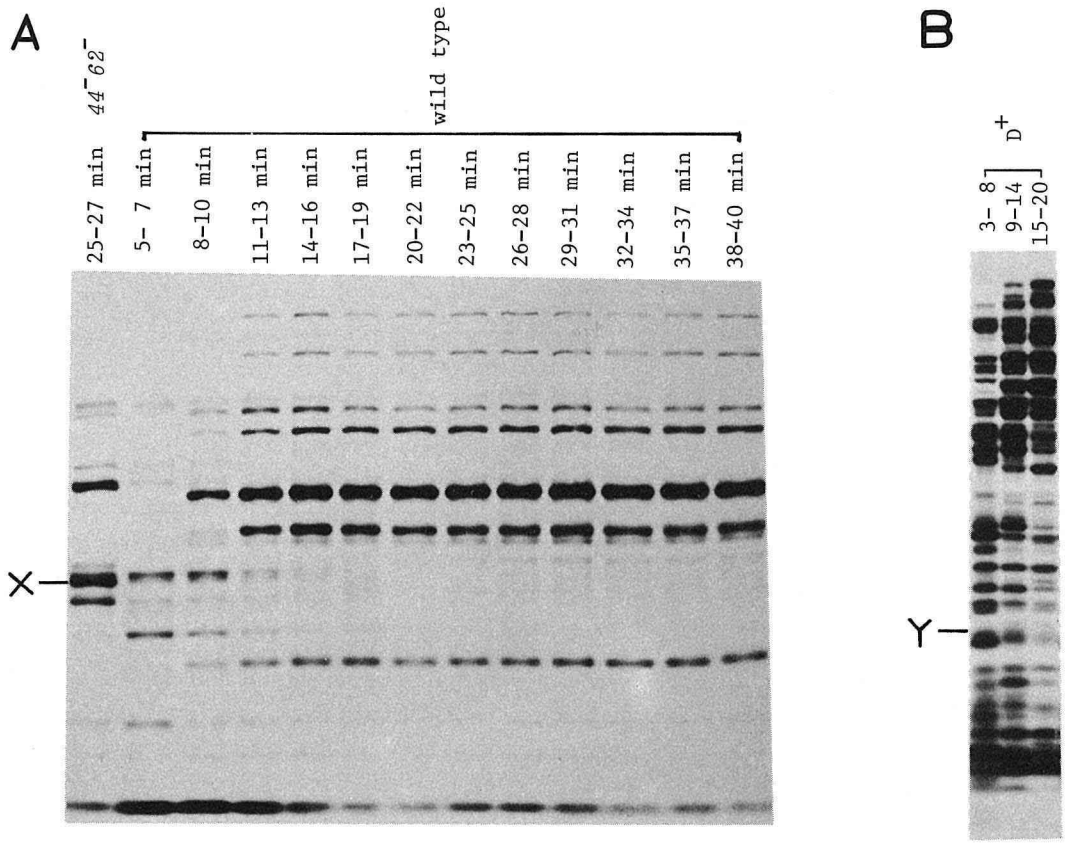

Fig. 1. Time course of synthesis of gp*uvsX* and gp*uvsY*. A. BB cells were infected with wild type or *44⁻62⁻* mutant (defective in DNA synthesis) and pulse-labeled with ^{3}H-leucine as indicated at the top. Infected cells were precipitated with 0.6 M trichloroacetic acid. The precipitate was dissolved and heat-treated, and was then electrophoresed in an SDS-10% polyacrylamide gel and fluorographed as described in Materials and Methods. X denotes gp*uvsX*.

B. BB cells were infected with wild type phage and pulse-labeled as indicated at the top, and processed as in A. Y denotes gp*uvsY*.

16,000 (Yonesaki *et al.*, m. s. in preparation). The protein was also detectable when cells were infected with wild type phage and were pulse-labeled at early periods but not at later periods as gp*uvsX* (Figure 1B), but its synthesis continued for a long time when the synthesis of DNA and late protein was blocked (data not shown), indicating that *uvsY* is also an early gene. Therefore cells infected with mutants defective in DNA synthesis are good source to isolate these gene products.

Genetic mapping of the mutants. Genetic crosses of *uvsX⁻*, *40⁻*, *41⁻* and *42⁻* mutants were performed to construct a genetic map. The results are summarized in Figure 2. The order of *am* mutations in *uvsX* is consistent to that expected from the size of the respective amber fragments, when they were assumed to be not degraded after synthesis. However, the order of *tsxcO* and *amxe2* is tentative, since every cross with the *ts* mutant gave lower recombination

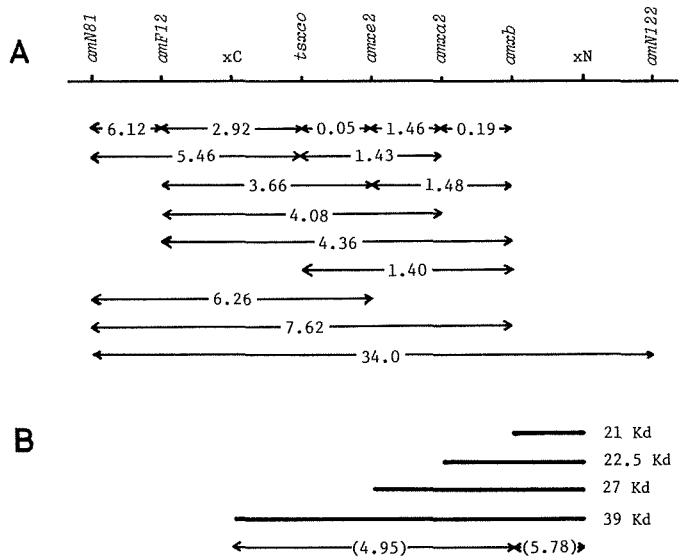

Fig. 2. The genetic map near *uvsX*. Two-factor crosses were performed and a genetic map was constructed (A). Gp*uvsX* and amber fragments of the mutants were arranged in B. The site for *tsxcO* is tentative, and xC and xN are presumptive sites for the C-terminus and N-terminus of gp*uvsX*. Numbers indicate recombination frequencies which are the average of more than three independent experiments. The distance is not drawn to be proportional to the recombination frequencies. Numbers in the parentheses indicate putative recombination frequencies.

frequencies when compared with *am* mutants, and a confirming value based on protein composition is unavailable. Genes *41*, *40*, *uvsX* and *42* are arranged in that order based both on our data and on the map of Shah and DeLorenzo (1977), who established the gene order *41-uvsX-42*. In the figure, *am* fragments are also drawn (B). If we accept the map distance between *amxb* and *amxe2* as 1.65% (1.46+0.19), and the molecular weight difference between these *am* fragments as 6,000, then 1% of map distance corresponds to 3,630 of polypeptide molecular weight, implying about 100 base pairs per centimorgan. This value is slightly larger than that of 70 estimated by Wood and Revel (1976), but smaller than the 164 for the lysozyme gene (Tsugita and Inoue, 1968; Stahl *et al.*, 1964). If we use the value of 100, the N-terminus and C-terminus of gp*uvsX* should be at 5.78% to the right and 4.95% to the left of *amxb*, respectively. Since the distance between *amxb* and *amF12* is 5.31 (1.65+3.66) and *amF12* is close to the right terminus of gene *40* (Hsiao and Black, 1978), *uvsX* appears to be adjacent to gene *40*. Three genes, *uvsX*, *β-gt* and *42*, fall between genes *40* and *43*, and the distance between *40* and *43* is about 3,600 bases (Kutter and Ruger, 1983). Since the molecular weights of the three gene products are 39,000, 46,000 (Huang and Buchanan, 1974) and 25,000 (O'Farrell *et al.*, 1973), respectively, these genes may fill the space between *40* and *43*, not inconsistent to the map constructed by Childs (1980). Figure 2 also shows that the direction of transcription is from right to left, the same with other many early genes (Wood and Revel, 1976).

Recombination frequencies between *amya1* and *amyd*, *amyd* and *amS52* (*25*), and *amS52* and *amya1*, were 1.1, 3.6 and 4.1, respectively, indicating that they are arranged clockwise in

the T4 map, *amya1-amyd-amS52*. Since *uvsY* is transcribed early and 25 late, it would be rational to assume that *amyd* is closer than *amya1* to a *uvsY* promotor.

Contribution of host gene to strand pairing in T4 recombination. As described we have recently found that gp*uvsX* catalyzed pairing of homologous DNA (m. s. in preparation). The host *recA* gene product also catalyzes the strand pairing (Shibata *et al.*, 1979; McEntee *et al.*, 1979). It is well documented that *recA* participates in recombination of phages, λ (Signer, 1971), P22 (Yamagami and Yamamoto, 1970; Botstein and Matz, 1970; Poteete and Fenton, 1983), and P1 (Rosner, 1972), in addition to phage gene products. Priemer and Chan (1978) reported that the *recA* product affected survival of UV-irradiated T4, raising survival especially when cells were multiply infected. Its effect strongly exceeded that of *uvsX* and *uvsY*. On the other hand, observations by Harm (1963) and Symonds *et al.* (1973) demonstrated the contribution of these two phage genes to multiplicity reactivation. However, phage mutants used by all these investigators carry additional unkown gene mutation(s) (Drake, 1973; Hamlett and Berger, 1975).

We reexamined the damage repair experiments using newly isolated mutants in the presence and the absence of the host *recA* function. Phages (wild type, *amxb* and *amyd*) exposed to various UV doses were adsorbed to 594 or 594*recA41* at a multiplicity of less than 0.1 (monocomplexes) or between 5 and 7 (multicomplexes), and plated on B40sul after inactivating unadsorbed phage with antiserum. As seen in Table 1, the survival of monocomplexes was clearly reduced by the *uvsX*$^-$ and *uvsY*$^-$ mutations, but was not affected by the *recA* mutation. The inactivation rate constant of wild-type multicomplexes was reduced to 10% of that of monocomplexes, and those of the *uvsX*$^-$ and *uvsY*$^-$ multicomplexes were reduced to 40% of the monocomplex value, irrespective of the mutation in *recA*. Plaques of the UV-irradiated mutants on B40sul, whether derived from monocomplexes or multicomplexes, were heterogeneous in size; the fraction of small plaques increased with UV dose. When 10 small plaques of both mutants were picked and replated on B40sul, they formed normally sized plaques. This implies that the mutants took longer to recover from damage. The difference between Priemer and Chan's results and ours might be due to different host strains and phage mutants. It

Table 1. Effect of host *recA* mutation on UV sensitivity of phages.

Host	Phage	Inactivation rate constant (k/sec)[a]	
		Monocomplexes[b]	Multicomplexes[c]
594 *recA*$^+$	Wild type	0.11±0.01	0.008±0.003
	amxb	0.20±0.02	0.078±0.004
	amyd	0.21±0.02	0.078±0.004
594 *recA41*	Wild type	0.11±0.01	0.010±0.003
	amxb	0.21±0.02	0.082±0.005
	amyd	0.21±0.02	0.079±0.004

a: Mean and standard deviation (±) of three or four independent experiments.
b: A multiplicity of infection less than 0.1.
c: A multiplicity of infection between 5 and 7.

should be added that *recA41* is not a leaky mutant (Ogawa *et al.*, 1978), and that the viable cell growth of 594*recA41* was comparable to that of 594.

We also examined the effects of *recA* and *uvsX* upon recombination between two *rII* markers, *rM36* and *r596*. Table 2 shows that recombination frequencies of *uvsX*$^-$ mutant are clearly lower than those of wild type and that they were not significantly different in the presence and the absence of *recA* gene. It was unexpected that the host gene affected the burst sizes; the burst size of wild type was markedly higher in *recA*$^-$ cells than in *recA*$^+$, while the burst size of *uvsX*$^-$ mutant was lower in *recA*$^-$ cells than in *recA*$^+$. In T-even phage recombination frequencies are known to correlate with burst sizes (Hershey and Chase, 1951; Levinthal and Visconti, 1953). Since the recombination frequencies were not affected by host *recA* gene, the differential effect of the mutation on the burst sizes could be due to unclarified physiological events. The intracellular concentration of the *recA* product is regulated and increased by agents such as UV irradiation (Witkin, 1976), or by increased copy numbers of the gene on plasmids (Ogawa *et al.*, 1978). The recombination frequencies were not affected even when the *recA* product was increased, as described in Materials and Methods, by prior treatment with UV, or by use of the pTM2 plasmid carrying the *recA*$^+$ gene (data not shown), implying that the above results did not stem from insufficient host gene product.

Table 2. Effect of *recA* on burst size and recombination frequency (*rM36*×*r596*).

Host	Phage	Burst size	Recombination frequency
594 *recA*$^+$	*am*$^+$	95.4± 6.4	2.73±0.25
594 *recA41*	*am*$^+$	208.3±26.5	3.85±0.54
594 *recA*$^+$	*amxb*	53.2± 1.9	1.09±0.14
594 *recA41*	*amxb*	26.1± 0.9	0.99±0.10

Mean and standard deviation (±) of three independent experiments.

Conclusion

We have isolated *am*-mutants in gene *uvsX* and *uvsY* to get a clue to understand the mechanism of these gene function. Upon infection of wild type the synthesis of these products started at an early period and ceased at the late (Figure 1). This is consistent to previous findings based on DNA synthesis in mutant-infected cells (Dewey and Frankel, 1975; Cunningham and Berger, 1977; Melamede and Wallace, 1977). When phage DNA synthesis was blocked, the protein synthesis continued for a long period and the products accumulated in mutant-infected cells. Thus, the infection gives a good source to isolate the gene products.

We have mapped isolated mutants by genetic crosses, and found that the arrangement of *am*-mutations in *uvsX* was in the same order with the size of corresponding *am*-fragments (Figure 2). This implies direction of the transcription is anticlockwise on T4 genetic map as other many early genes (Wood and Revel, 1976).

We have succeeded in purification of gp*uvsX* near to homogeneity, judging from SDS-polyacrylamide gel electrophoresis and staining with Coomassie brilliant blue, and found that homologous DNA strands pair in the presence of purified gp*uvsX* and ATP (m.s. in preparation). It was previously disclosed that host *recA* protein has an analogous function (Shibata *et al.*,

1979; McEntee *et al.*, 1979). We reexamined by using newly isolated mutants if gp*uvsX* can be replaced by *recA* product. *RecA* did not affect UV-sensitivity of monocomplex and multicomplex of wild type and mutant phages (Table 1), nor affected recombination frequencies of two rII-markers (Table 2), even under the conditions where *recA* protein was overproduced. *RecBC* DNase (exonuclease V) is the other essential protein for recombination and repair of host DNA, and its activity is drastically reduced after T4 infection (Behme *et al.*, 1976). *RecA* product might also lose its activity by the infection in unknown way, but such knowledge is not available at present time. The other explanation would be that *recA* protein does not catalyze the reaction when cytosine in DNA is modified as in T4 DNA; however this has not been proved. Finally *recA* protein can not substitute for gp*uvsX* in putative T4 recombination machinary, which is composed of replication and recombination proteins and functions by concerted mechanism of the components (Mosig *et al.*, 1978), or/and the T4 machinary is not replaceable by the host machinary.

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