RecA-independent high-frequency deletion of recombinant cosmid DNA in *Escherichia coli*

MASAHIRO ISHIURA,^{1*} NOBUYOSHI HAZUMI,¹ HIDEO SHINAGAWA,² ATSUO NAKATA,² TSUYOSHI UCHIDA³ and YOSHIO OKADA^{1,3}

¹Division of Cell Fusion, Department of Cell Biology, National Institute for Basic Biology, Myodaiji-cho, Okazaki, Aichi 444, Japan

^{2,3}Research Institute for Microbial Diseases² and Institute for Molecular and Cellular Biology³, Osaka University, Yamada-oka, Suita, Osaka 565, Japan

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Segments of DNA were deleted from recombinant cosmid DNAs during propagation in *Escherichia coli* hosts in liquid culture. DNAs of more than 1000 cosmids propagated in various *E. coli* hosts were analysed by agarose gel electrophoresis (AGE). The effects of vectors, insert DNAs and host genetic characters on the formation of deletions were examined. The probability of deletion and the pattern of deletion bands observed by AGE differed from clone to clone, and after extensive culture the deletion band patterns remained almost constant during further culture. Most recombinant clones eventually showed deletion during prolonged liquid culture. Mutations in the *recA* gene of *E. coli* hosts, including a deletion mutation, did not prevent deletion. Most deletions occurred in the insert portions of cosmid DNAs. Nucleotide sequence analysis of six deletion junctions in test cosmid cMB15 demonstrated that deletions occurred between two short complete direct repeats of about 4–10 bp, irrespective of whether the cosmid was propagated in a *recA* host or a *rec*⁺ host. Some deletions occurred at the same sites either in a *recA* host or a *rec*⁺ host. These results suggest that the deletion events are mainly mediated by a *recA*-independent recombination system(s) of *E. coli* host cells.

Introduction

A cosmid vector is a plasmid vector carrying the phage λ *cos* sequence, which allows cloning of large DNA fragments of up to about 50 kb through *in vitro* packaging of DNA into phage particles (for a review, see Maniatis *et al.*, 1982). Due to its large capacity, such a vector is very useful for producing mammalian genomic libraries with a minimum number of clones and cloning genes of interest from the libraries, because many mammalian genes are more than 20 kb in size (for a review, see Ishiura *et al.*, 1989a), which is the cloning capacity of the λ vector (Maniatis *et al.*, 1982). Some genes can be cloned without interruption into a single cosmid. However, we have encountered several difficulties in cosmid cloning. One of the most serious problems is the occurrence of

deletions in cosmid DNAs during their amplification in *Escherichia coli* host cells: probably because of their large size, cosmids do not seem to be stably maintained in *E. coli* hosts. These deletions have been mentioned in several papers (Chia *et al.*, 1982; Steinmetz *et al.*, 1982; Yelton *et al.*, 1985), but they have not yet been extensively characterized, and the primary importance of *recA* mutations in *E. coli* hosts to prevent the cosmid deletion events has been presumed rather than investigated systematically (Ish-Horowicz & Burke, 1981; Chia *et al.*, 1982; Maniatis *et al.*, 1982). Several mechanisms have been proposed for the various deletion events that occur in *recA* *E. coli* hosts (Brutlag *et al.*, 1977; Collins, 1980; Lilley, 1981; Liu & Lark, 1982; Collins *et al.*, 1982; Boissy & Astell, 1985; Saing *et al.*, 1988). However, it has not been known whether such mechanisms are involved in the cosmid deletion events. Recently, we analysed

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*Author for correspondence (electronic mail address: Bitnet ISHIURA@JPNONRI).

Abbreviations: AGE, agarose gel electrophoresis; Ap, ampicillin; *bla*, β -lactamase gene; CHO cell, Chinese hamster ovary cell; LB, Luria-Bertani broth; LB-Ap medium, LB containing 30 μ g Ap ml⁻¹; LB-Tc medium, LB containing 10 μ g Tc ml⁻¹; *ori*, origin of replication; Tc, tetracycline hydrochloride.

cosmid deletion events using six specific cosmids and demonstrated that four of the cosmids showed deletions during propagation in *recA* hosts, whereas two did not show such deletions (Ishiura *et al.*, 1989b). We have also demonstrated that *recB recC sbcB recJ* hosts prevented the cosmid deletion events that occurred in *recA* hosts (Ishiura *et al.*, 1989b). However, it is uncertain whether all cosmid deletion events are *recA*-independent and prevented by *recB recC sbcB recJ* hosts. In addition, some authors have suggested the possible involvement of an insertion element in cosmid deletion events (Fernandez *et al.*, 1986), whereas others have suggested that the use of *ori* of phage λ in place of that of pBR322 as *ori* of the vector may reduce the frequency of the deletions (Little & Cross, 1985).

To address the problems described above, we have investigated the effects of various vectors, insert DNAs prepared from several organisms, and host genetic backgrounds related to recombination, especially the *recA* gene, on the generation of deletions in cosmids during propagation in *E. coli* hosts.

Methods

Bacterial strains, media, and manipulation of DNA. The bacterial strains used in this study are listed in Table 1. The derivatives of C600 *r⁻m⁻* and ED8654 carrying a deletion mutation in the *recA* gene were constructed by P1 transduction (Miller, 1972), using JC10289 and P1 *vir* as donor and transducing phage, respectively. Transductants were selected on LB agar plates (see below) containing 10 μ g Tc ml⁻¹. The *recA* mutation of the transductants was checked by a UV-sensitivity test (Maniatis *et al.*, 1982). Tc-sensitive clones of the *recA* deletion derivatives were selected on LB agar plates containing chlortetracycline hydrochloride, fusaric acid and ZnCl₂ (Maloy & Nunn, 1981; Bochner *et al.*, 1980). Selection of *recA⁺* revertants was avoided by confirming *recA* mutations of *E. coli* hosts for cosmid cloning just before their use by the UV-sensitivity test described above.

Appropriate UV-sensitive clones were used for cosmid cloning, or stored at -80 °C in the presence of 15% (v/v) glycerol or 7% (v/v) dimethylsulphoxide until used. LB (Maniatis *et al.*, 1982) was routinely used to grow bacterial cultures. LB agar plates contained 1.2% (w/v) Bactoagar in LB. Handling of bacteria, manipulation of DNA for molecular cloning, and restriction mapping of cosmids and deleted cosmids were carried out as described by Maniatis *et al.* (1982). DNA sequencing was done by the chain-termination method (Sanger *et al.*, 1977).

Cosmid libraries. We constructed cosmid libraries for mouse L cell genomic DNA with cosmid vectors pJB8 (Ish-Horowicz & Burke, 1981), pDC1 (Ishiura *et al.*, 1989a), pDC2 (Ishiura *et al.*, 1989a) and pDC λ 1 (Ishiura *et al.*, 1989a). We also constructed cosmid libraries for CHO cell genomic DNA and *Haemophilus influenzae* genomic DNA with the pDC1 vector and the pDC104 vector (Ishiura *et al.*, 1989a), respectively. Vector pDC λ 1 is a derivative of pDC1, which carries a 2.8 kb *NheI*-*XhoI* segment containing *ori* of phage λ derived from loric (Little & Cross, 1985) in place of that of pBR327 (M. Ishiura and co-workers, unpublished data). An equimolar mixture of the left and right vector arms of almost equal length was prepared from the vector DNA, simply by treating the DNA sequentially with three enzymes, *PvuII* (or *Clal* in the case of pDC λ 1), calf intestinal alkaline phosphatase, and *BamHI* (or *BglII* in the case of pDC2), as described previously (Ishiura *et al.*, 1989a). An equimolar mixture of the left and right arms of pJB8 was prepared as described by Ish-Horowicz & Burke (1981). Insert DNAs were prepared by the partial digestion of various genomic DNAs with *Sau3AI* and subsequent size-selection of the partial digests on a sucrose density gradient, as described previously (Ishiura *et al.*, 1989a). The equimolar mixture of the vector arms, which carried a single *cos* oriented from left to right, was ligated with insert DNA of 40–50 kb. The ligated recombinant molecules were packaged into λ phage particles by an *in vitro* packaging reaction using an Amersham packaging kit. Among the ligation products, only recombinant molecules which have a structure of 'left arm-insert-right arm' can be converted into transducing λ phage particles. Various *E. coli* strains were infected with λ phage particles of each cosmid library. To prevent double transduction of cosmids, about 2000 colony-forming units of packaged cosmids were infected into more than 2×10^8 host bacterial cells. The transductants carrying cosmids were selected on LB agar plates containing 30 μ g Ap ml⁻¹ or 10 μ g Tc ml⁻¹. To avoid the formation of double colonies, cells were plated at low density (about 50–150 drug-resistant colonies per 90 cm plate). We also constructed a

Table 1. *E. coli* strains

Strain*	Relevant genotype	Source (reference)
HB101	<i>recA13 hsdS20</i>	Our stock (Boyer & Roulland-Dussoix, 1969)
ED8767	<i>recA56 hsdS3</i>	A. R. Flavell, B. J. Bachmann (Murray <i>et al.</i> , 1977)
DH1	<i>recA1 hsdR17</i>	D. Hanahan (Hanahan, 1983)
RR1	As HB101 but <i>rec⁺</i>	Bethesda Research Laboratories (Maniatis <i>et al.</i> , 1982)
ED8654	As ED8767 but <i>rec⁺ hsdR514 trpR55</i>	B. J. Bachmann (Borck <i>et al.</i> , 1976)
MM294	As DH1 but <i>rec⁺ gyrA⁺</i>	B. J. Bachmann (Meselson & Yuan, 1968)
C600 <i>r⁻m⁻</i>	<i>rec⁺ hsdR hsdM</i>	K. Matsubara
M11	As C600 but <i>recA</i> (Δ)	This study
M12	As ED8654 but Δ (<i>recA-srIR</i>)306::Tn10	This study
SK1592	<i>sbcB15 hsdR4</i>	J. D. Diller (Kushner, 1978)
CES200	<i>recB21 recC22 sbcB15 hsdR</i>	C. Shurvinton & F. Stahl (Nader <i>et al.</i> , 1985)
CES201	As for CES200 but Δ (<i>recA-srIR</i>)306::Tn10	C. Shurvinton & F. Stahl
DB1161	<i>recB21 recC22 sbcB15 recA56 hsdR hsdM</i>	A. R. Wyman & D. Botstein
JC10289	Δ (<i>recA-srIR</i>)306::Tn10	B. J. Bachmann (Csonka & Clark, 1979)

*All strains except JC10289 were deficient in the major restriction activity. CES200, CES201, and DB1161 are derivatives of AB1157 (*rac*). Thus, these strains are also *rac*.

Fig. 1. Scheme for deletion assay. See text for details.

library for *L* cell genomic DNA with the pDC1 vector, and isolated three cosmids, cMB7, cMB15 and cMB30, each of which carried single *Bam*HI fragments of 40–50 kb (Ishiura *et al.*, 1989a). Essentially all cosmids constructed by our procedures (Ishiura *et al.*, 1989a) contained insert DNA and one copy of vector.

Assay of deletion in cosmid DNAs (see Fig. 1). Fifty independent Ap-resistant colonies (or Tc-resistant colonies for cosmids constructed with pDC2) were isolated from each cosmid library, separately inoculated into 2 ml LB-Ap medium (or LB-Tc medium) in small test tubes, and then incubated at 37 °C for 24 h with shaking at 175 r.p.m. These cultures are designated as '0th cultures'. Then 5 µl volumes of each 0th culture were inoculated into 1 ml fresh LB-Ap medium (or LB-Tc medium) and cultured at 37 °C for 24 h as described above to obtain the '1st cultures'. Serial cultures were continued to the '6th cultures'. For detection of possible deletions in the cosmids, cosmid DNAs were prepared by the boiling method (Holmes & Quigley, 1981) from 1 ml samples of each 0th to 6th culture and analysed by agarose gel electrophoresis (AGE) in 0.8% agarose gel in 40 mM-Tris/acetate buffer (pH 8.0) containing 2 mM-EDTA (Maniatis *et al.*, 1982).

Results

recA-independent deletion in cosmids

E. coli recA strains HB101, ED8767 and DH1 have been used as standard hosts and pJB8 as the standard vector for cosmid cloning. When we constructed cosmids using

the pJB8 vector and insert DNAs prepared from *L* cell genomic DNA and propagated the cosmids in these strains, only 0–4% clones showed deletion at the 0th culture, but the frequency increased greatly during serial cultures (Table 2): 34–50% clones showed deletions at the 2nd culture, 52–92% at the 4th culture, and 54–100% at the 6th culture. Although more than half the clones showed deletions after extensive serial cultures, clearly some clones did not show any deletion even after prolonged serial cultures. This finding suggests that the probability of deletions depends on the insert DNA. The frequencies of deletions in three *recA* strains, HB101, ED8767 and DH1, were essentially the same.

Fig. 2(a) shows AGE patterns of cosmids constructed with pJB8 and propagated in HB101. We could not detect any deletion in any of the 16 clones at the 0th culture, but detected deletions in all clones except no. 15 at the 6th culture. The pattern of deletion bands differed from clone to clone (see also Fig. 2b). Fig. 2(b) shows the changes in deletion band patterns during cultures of four typical clones propagated in three strains, HB101, ED8767 and DH1. Initially deletion bands were weak, if detected at all, but they became clearly evident at the 2nd or 4th culture and then the patterns did not change during further culture. The deletion band pattern seemed to be specific for each clone.

We determined whether the *recA* mutation of the *E. coli* host was maintained stably during the experiments described above by a UV-sensitivity test (Maniatis *et al.*, 1982). HB101 host cells carrying cosmid DNAs with deletions at the 6th culture were found to be as sensitive to UV-irradiation as those carrying the intact cosmid DNA at the 0th culture, suggesting that the *recA* mutation did not revert to the *RecA*⁺ phenotype during liquid culture.

To determine whether the *recA* gene is involved in the deletions in cosmids, we examined the deletions in cosmids during propagation in three *recA*⁺ strains, RR1 (as HB101 but *recA*⁺), ED8654 (as ED8767 but *recA*⁺ *hsdR514 trpR55*), and MM294 (as DH1 but *recA*⁺ *gyrA*⁺). With the exception of MM294, the frequencies of deletions in these *recA*⁺ strains were almost the same as or slightly higher than those obtained with the corresponding *recA* strains (see below). The frequencies observed with MM294 were rather lower than those with the corresponding *recA* strain DH1.

Effect of a recA deletion mutation in the E. coli host on the deletions in cosmids

The data described above showed that deletions in cosmids occurred irrespective of the *recA* mutations of the *E. coli* hosts. However, we could not completely rule

Table 2. Deletion of recombinant cosmid DNA during propagation in *E. coli* hosts grown in liquid cultures

Fifty independent cosmids, propagated in the indicated *E. coli* strains, from each cosmid library (constructed using the vectors and insert DNAs described below) were cultured, with daily serial passages, in LB-Ap medium at 37 °C with shaking. Cosmid DNA was prepared from the cells at the 0th to 6th culture and analysed by AGE to detect any deletion in the DNA. All strains were deficient in the major restriction activity.

Host	Relevant genotype	Vector	Insert DNA	Deletion frequency (no. of deleted clones per 50 clones)			
				0th	Culture: 2nd	4th	6th
HB101	<i>recA13</i>	pJB8	L cell	2	20	31	36
HB101	<i>recA13</i>	pDC1	L cell	0	16	47	49
HB101	<i>recA13</i>	pDC1	L cell	0	10	50	ND
ED8767	<i>recA56</i>	pJB8	L cell	0	17	26	27
ED8767	<i>recA56</i>	pDC1	L cell	0	23	33	37
DH1	<i>recA1</i>	pJB8	L cell	2	25	46	50
RR1	<i>rec⁺</i>	pJB8	L cell	0	24	31	41
ED8654	<i>rec⁺</i>	pJB8	L cell	2	35	41	45
C600 <i>r⁻ m⁻</i>	<i>rec⁺</i>	pJB8	L cell	0	45	45	46
MM294	<i>rec⁺</i>	pJB8	L cell	0	10	13	23
MI1	<i>recA(Δ)</i>	pJB8	L cell	3	27	41	46
MI1	<i>recA(Δ)</i>	pDC1	L cell	1	14	36	45
MI2	<i>recA(Δ)</i>	pJB8	L cell	0	37	48	50
MI2	<i>recA(Δ)</i>	pDC1	L cell	1	41	50	ND
HB101	<i>recA13</i>	pDC1	<i>H. influenzae</i>	1	29	47	47
HB101	<i>recA13</i>	pDC104	CHO cell	1	11	42	47
SK1592	<i>sbcB15</i>	pDC1	L cell	3	32	48	49
CES200	<i>recB21 recC22 sbcB15</i>	pDC1	L cell	3	20	33	37
CES201	<i>recB21 recC22 sbcB15 recA(Δ)</i>	pDC1	L cell	0*	20*	ND	ND
DB1161	<i>recB21 recC22 sbcB15 recA56</i>	pDC1	L cell	27	44	47	47

ND, Not determined.

*Numbers of clones with deletions per 20 clones. Only a very few transductant colonies of CES201 could be obtained.

out the possibility that the observed deletion was due to leaky function of the mutated *recA* genes of the *E. coli* hosts. To examine this possibility, we constructed two *E. coli* strains, MI1 and MI2, which carried a *recA* deletion mutation, and used them as hosts for propagation of cosmids. We also used the two *rec⁺* parental strains, C600 and ED8654, as control hosts (Table 2, Fig. 2c).

Neither MI1 nor MI2 prevented deletions, and the deletion frequencies obtained with the two strains did not differ much from those obtained with the three *recA* strains, HB101, ED8767 and DH1 (50–70% at the 2nd culture, 82–96% at the 4th, and 92–100% at the 6th) (Table 2). The deletion frequencies obtained with MI2

were also almost the same as those obtained with its *rec⁺* parental strain ED8654. The deletion frequencies obtained with MI1 were slightly lower than those obtained with its *rec⁺* parental strain C600. The deletion band patterns shown in Fig. 2(c) were also characteristic of each clone.

Effects of vectors on the deletion

There is a possibility that the vector DNA and *ori* of the vector might affect the deletion in cosmids. To test this possibility, we examined the effects of three other

Fig. 2. AGE patterns of cosmid DNAs propagated in *E. coli* hosts. Cosmids were constructed using the vectors specified and inserts prepared from L cell genomic DNA [except in (d)], and were propagated in *E. coli* hosts as indicated in parentheses. After serial cultures as indicated, cosmid DNA was analysed by AGE. (a) Sixteen cosmids constructed with the pJB8 vector and propagated in HB101. (b) Changes during serial culture in deletion bands of the typical cosmids propagated in HB101, ED8767 and DH1. Results for 16 independent clones are shown. Results for the 0th, 2nd, 4th, and 6th cultures of each clone are shown from left to right. (c) Deletion band patterns of the cosmids constructed with the pJB8 vector and propagated in two *recA(Δ)* strains, MI1 and MI2, and their parental *rec⁺* strains C600 and ED8654. Results for 16 independent clones are shown. Results for the 0th and 2nd cultures of each clone are shown from left to right. (d) Deletion band patterns of the cosmids derived from the cosmid libraries for the genomic DNAs of CHO cells and *Haemophilus influenzae*, which were constructed with the pDC104 vector and the pDC1 vector, respectively. Results for the 0th and 2nd cultures of eight independent clones are shown from left to right.

Fig. 3. AGE analysis of cosmids with single *Bam*HI inserts after extensive amplification in *E. coli* hosts. Three cosmids, cMB7, cMB15, and cMB30, which carried single *Bam*HI inserts, were propagated in HB101 until the 6th culture. #7, #15, and #30, DNAs of cMB7, cMB15, and cMB30 with deletions; + and –, with and without *Bam*HI digestion, respectively; M, size markers (λ *Hind*III digests); the arrowheads indicate the position of the vector band. (a) AGE patterns of cosmid DNAs with deletions prepared from the 6th culture. (b) Analysis of cosmid DNAs with deletions by retransformation. Cosmid DNAs with deletions obtained from the 6th cultures as described in (a) were reintroduced into HB101; three independent transformants of each cosmid were isolated and their cosmid-derived DNAs were analysed, directly or after *Bam*HI digestion, by AGE.

vectors, pDC1, pDC104 and pDC λ 1. pDC λ 1 is a derivative of loric that carries *ori* of phage λ (Little & Cross, 1985). The deletion frequencies with these three vectors did not differ much from those with the pJB8 vector (Table 2). Thus, at least with these vectors, the vector *per se* did not seem to have a large effect on the deletion frequency. The level of resistance to Ap is proportional to the number of copies of *bla*. Generally, the copy number of pMB-derived plasmids in *E. coli* host cells is inversely related to plasmid size. Therefore, although the concentration of Ap in the culture media used here was relatively low (30 μ g ml⁻¹), it is possible that the formation of deletions in cosmid DNA may provide a growth advantage for host cells carrying cosmids in medium containing Ap. However, the deletions did not seem to be due to *bla* carried on these four vectors, pJB8, pDC1, pDC104 and pDC λ 1, because use of the pDC2 vector, a derivative of pDC1 that carries the Tc-resistance gene of pBR322 in place of *bla*, did not prevent deletions (data not shown).

Effects of the sources of insert DNAs on the deletion

To determine whether cosmid deletion events are general phenomena that occur in any DNA, we used genomic DNAs of CHO cells and *Haemophilus influenzae* as inserts. The deletion frequencies of the cosmids with these inserts did not differ much from those of cosmids with L cell genomic DNA (Table 2). Fig. 2(d) shows the changes in deletion band patterns during cultures of eight clones. Initially deletion bands were not detected in most clones, but they became evident at the 2nd cultures or the 4th cultures and then the patterns did not change during further culture.

Effects of a *recB recC sbcB* host and related hosts on the deletion

In both λ and plasmid cloning, a *recB sbcB* host and a *recB recC sbcB* host have been reported to prevent the deletions of some kinds of recombinant DNAs that were not stably maintained in usual *rec*⁺ or *recA* hosts (Collins *et al.*, 1982; Leach & Stahl, 1983; Wyman *et al.*, 1985; Nader *et al.*, 1985). Therefore, we examined the effects of such *E. coli* hosts on the cosmid deletion events. However, the deletion frequency was not reduced by an *sbcB* host, a *recB recC sbcB* host, or a *recB recC sbcB recA* host (Table 2).

Approximate location of the deletions

We determined the approximate location of deletions in cosmids propagated in HB101, using three test cosmids, cMB7, cMB15 and cMB30, each of which carried single *Bam*HI inserts of 40–50 kb. Fig. 3(a) shows the AGE patterns of DNAs of these cosmids at the 6th culture. After *Bam*HI digestion, a clear vector band was seen, indicating that the vector portions were intact in most cosmid molecules with deletions and that the deletions were mainly in the insert DNAs.

For confirmation of these results, we reintroduced each DNA of these cosmids with deletions into HB101 cells, isolated the three independent transformants for the three cosmids, and analysed the deleted cosmid DNAs by AGE. Fig. 3(b) shows that in all nine clones except clone no. 15-3 each deletion band was composed of intact vector DNA and deleted insert DNA. In clone no. 15-3, the deletion end-points occurred in both the vector and the insert, eliminating one of the *Bam*HI junction sites between the vector and the insert.

Nucleotide sequences surrounding deletion junctions

We analysed the nucleotide sequences that surround deletion junctions in the cMB15 test cosmid. Restriction mapping experiments showed the existence of deletion hot-spots in the cMB15 DNA propagated in HB101 (*recA*) and RR1 (*rec*⁺). Some deleted cosmids had deletion junctions at almost identical sites irrespective of whether the cosmid had been propagated in HB101 or RR1 (Fig. 4a). All six deletions examined had occurred in the insert portions between two short complete direct repeats of about 4–10 bp which were separated from each other by about 40 kb in one direction and about 4 kb in the other direction, in both HB101 and RR1 (Fig. 4). Two deleted cosmids propagated in HB101, H33 and H35 had an identical deletion junction (D1). Another two deleted cosmids propagated in RR1, R9 and R23 also had an identical deletion junction (D2). The right break-point (DR3) of one deletion junction (D3) observed in the deleted cosmid R13 that had been propagated in RR1 was found within that (DR2) of the deletion junction (D2) observed in R9 and R23. Although R29 had been propagated in RR1, it had the same deletion junction (D1) as that observed in H33 and H35.

Various sequence motifs were observed around the deletion junctions (Fig. 4b). They were sequences related to the Chi sequence (5'-GCTGGTGG-3' or its complementary sequence 5'-CCACCAGC-3'), and sequences related to the specific 8 bp sequence 5'-GAGGC_G^TGG (or its complementary sequence 5'-C_A^CGCCTG-3'), T-stretches (or A-stretches), C-stretches (or G-stretches). One deletion junction (D1) contained the Chi-related sequence. The tandem repeats of 5'-CCT-3' (or its complementary sequence 5'-AGG-3') and 5'-CCA-3' (or its complementary sequence 5'-TGG-3') were observed in and near all six deletion junctions examined here (Fig. 4c). Inverted repeat sequences and symmetric sequences were also observed around deletion junctions. Among six break-points of the deletion junctions examined, three (DL2, DR2 and DR3) were found within such symmetric sequences, and two (DL3 and DR1) overlapped with such inverted repeat sequences. Tandem repeat sequences of more than 10 bp were also found in the flanking regions of the deletion junctions. One deletion junction (D1) was coincident with one such tandem repeat sequence.

Discussion

By analysing the DNAs of more than 1000 independent cosmids propagated in various *E. coli* hosts, we demonstrated that most cosmids were not stably maintained,

but suffered deletions, in *E. coli* hosts, including *recA* strains, such as HB101, ED8767 and DH1, which have generally been used as hosts for cosmid cloning. As observed previously (Ishiura *et al.*, 1989b), *recA* mutations in *E. coli* hosts do not reduce the frequency of cosmid deletion events. This was not due to back mutations of the host *recA* gene to the RecA⁺ phenotype (Chia *et al.*, 1982) since the deletion occurred similarly in *E. coli* hosts carrying a *recA* deletion mutation. Therefore, most of the cosmid deletion events described here were *recA* independent.

The deletion frequency and deletion band pattern of cosmid DNA differed from clone to clone. Furthermore, the deletion in cosmids occurred mainly in the insert DNA. These findings suggest that a specific sequence(s) or structure(s) that is often present in insert DNA was responsible for the deletions (see below). This specific sequence(s) or structure(s) of genomic DNA is present not only in mammalian cells but also in micro-organisms such as *Haemophilus influenzae*, because similar deletions in cosmids occurred irrespective of whether mammalian or *H. influenzae* genomic DNA was used as a source of the insert DNA.

Nucleotide sequence analysis demonstrated that all deletions in test cosmid cMB15 examined here occurred between two short complete direct repeats. Even deletions in the cosmid observed during propagation in a *rec*⁺ host occurred between two short direct repeats of about 4–10 bp. Homologous recombination mediated by the *recA* recombination system usually involves larger homologous sequences (Shen & Huang, 1986). Furthermore, some deletions occurred at the same sites irrespective of whether the test cosmid was propagated in a *recA* host or a *rec*⁺ host. These findings also suggest the *recA* independence of the cosmid deletion events. Similar *recA*-independent deletion events involving a short direct repeat have been also reported in plasmids (Albertini *et al.*, 1982; Jones *et al.*, 1982). However, such deletions occurred 25-fold more frequently in a *rec*⁺ host than in a *recA* host (Albertini *et al.*, 1982). We observed various sequence motifs, including Chi-related sequences, sequences related to the 8 bp sequence 5'-GAGGC_G^TGG (or 5'-C_A^CGCCTG-3'), T-stretches (or A-stretches), C-stretches (or G-stretches), and the tandem repeats of 5'-CCT-3' (or 5'-AGG-3') and 5'-CCA-3' (or 5'-TGG-3'), around the deletion junctions (Fig. 4b). Inverted repeat sequences and symmetric sequences were also observed around deletion junctions. Recently, we analysed the deletion junctions observed in human test cosmid cHEF2-12, which carries the human elongation factor 2 gene (Ishiura *et al.*, 1989b) that had been propagated in a *recA* *E. coli* host and found that deletions also occurred between two short direct repeats of 6–25 bp (M. Ishiura and co-workers, unpublished data). The

sequence motifs and DNA structures described here were also found around the deletion junctions observed in cHEF2-12 (M. Ishiura and co-workers, unpublished data).

Previously, we showed that *recB recC sbcB recJ* hosts prevent the cosmid deletion events which occur in *recA* hosts, using six test cosmids including cMB15 (Ishiura *et al.*, 1989b). Thus, it appears that a *recB recC sbcB recJ* quadruple combination of host mutations prevents deletions in cosmids which occur between short direct repeats. It has been reported that most of various *recA*-independent deletion events observed in *E. coli* involve short direct repeat sequences or short inverted repeat sequences (for a review, see Allgood & Silhavy, 1988), and two models have been proposed to explain such deletion events (Franklin, 1971), namely the slipped mispairing and the cutting and rejoining of DNA molecules (homologous recombination). Our present knowledge does not allow us to distinguish between these two mechanisms.

There are reports that a palindromic structure (Collins, 1980), inverted repeat sequences (Lilley, 1981), tandemly repeated sequences (Brutlag *et al.*, 1977), interspersed repeat sequences (Liu & Lark, 1982), and direct repeat sequences located in the long inverted repeats (Saing *et al.*, 1988), which are cloned in plasmid vectors, are unstable and induce deletions in recombinant plasmids in standard *recA* hosts. These deletions have been reported to be prevented in a *recB sbcB* host (Collins *et al.*, 1982), a *recB recC sbcB recF* host (Boissy & Astell, 1985), a *recE* host (Liu & Lark, 1982), or a host with a temperature-sensitive mutation in the *gyrA* gene (Saing *et al.*, 1988). However, we found that an *sbcB* host, a *recB recC sbcB* host, and *recB recC sbcB recA* hosts did not prevent deletions in cosmids. Our previous experiments suggested that *recB recC sbcB recF* hosts and a *recE* host also did not prevent the deletions (Ishiura *et al.*,

1989b). Preliminary experiments suggest that the temperature-sensitive mutant of the *gyrA* gene described above (Saing *et al.*, 1988) also does not prevent the deletions (M. Ishiura and co-workers, unpublished data). Plasmidic recombination, namely the multimerization and demultimerization of plasmids and interplasmidic and intraplasmidic recombination, occurs during propagation of plasmids in *E. coli* (Laban & Cohen, 1981; Fishel *et al.*, 1981; for a review, see Mahajan, 1988). However, the formation of deletions in cosmids is not likely to be directly related to plasmidic recombination because these two events are differently affected by various mutations involved in recombination in *E. coli* (Ishiura *et al.*, 1989b; Mahajan, 1988). Therefore, the cosmid deletion events of very high frequency, described here, depend mainly on a recombination system(s) other than those reported previously, although the latter recombination systems may be involved secondarily and in our system their involvement may have been masked by the very high frequency of cosmid deletion events.

Currently, it is not easy to analyse the cosmid deletion events of many cosmids using *recB recC sbcB recJ* strains as host because the efficiencies of transduction of cosmids obtained in such strains are very low (M. Ishiura and co-workers, unpublished data). Therefore, we have attempted to construct a restriction-deficient derivative from a *recB recC sbcB recJ* strain, JC12186. However, the candidates obtained so far show an efficiency of transduction only ten times higher than that obtained with JC12186 (unpublished data). It remains to be determined whether *recB recC sbcB recJ* hosts prevent all or most cosmid deletion events that occur in *recA* hosts.

Little & Cross (1985) suggested that use of a loric vector, which carries *ori* of phage λ , in place of the usual pBR322-derived vectors may reduce the frequency of cosmid deletions. Our results presented here and unpublished observations, however, demonstrate that *ori* of the

Fig. 4. Nucleotide sequences of deletion junctions in the cMB15 test cosmid propagated in a *recA* (HB101) or *rec⁺* (RR1) host. The cMB15 test cosmid DNA was packaged into phage particles *in vitro* and transduced into HB101 or RR1 cells. Each 20 clones of HB101 or RR1 cells that carried the cosmids were separately cultured, and cosmid DNAs with deletions obtained from the 6th cultures were reintroduced into HB101. Deleted cosmid DNAs obtained from separate cultures were analysed by restriction mapping and nucleotide sequencing. Deleted cosmids, H33 and H35, were obtained from separate HB101 cultures, whereas R9 and R23 were obtained from separate RR1 cultures. (a) Restriction mapping of deletion junctions in cMB15. Ba, *Bam*HI; E, *Eco*RI; Hp, *Hpa*I; K, *Kpn*I; Sm, *Sma*I. The thick, thin and dashed lines represent the insert portions, vector portions and deleted portions, respectively. (b) Partial nucleotide sequence of cMB15 which contains the break-points of deletion junctions. The nucleotide sequence is shown from the left end to the right end of test cosmid cMB15 and from the 5'-end to the 3'-end; the left end is defined as the end containing *bla* and *ori* (namely, the left arm of pDC1). The left end of the sequence is the left *Bam*HI site of the *Bam*HI insert of cMB15. The left (L) and right (R) sequences of deletion junctions are defined as sequences which contain, respectively, the left (DL1, DL2 and DL3) and right (DR1, DR2 and DR3) break-points of three deletion junctions (D1, D2 and D3) in cMB15. The sequences of the break-points are underlined with bold lines. Sequence motifs, such as sequences related to the Chi sequence (5'-GCTGGTGG-3' or its complementary sequence 5'-CCACCAGC-3'), T-stretches (or A-stretches), C-stretches (or G-stretches), are underlined with thinner lines. The sequences related to the 8 bp sequences 5'-GAGGC $\overline{\text{C}}$ GG (or its complementary sequence 5'-C $\overline{\text{C}}$ $\overline{\text{A}}$ GCCTG-3') are shown by the dashed underlines. The arrowed overlines indicate inverted repeat sequences ($\rightarrow\leftarrow$) and symmetric sequences ($\leftrightarrow$). Direct repeat sequences are shown by the numbered overlines. (c) Nucleotide sequences in the region of deletion junctions. Deletion junctions are boxed. The tandem repeats of 5'-CCT-3' (or its complementary sequence 5'-AGG-3') and 5'-CCA-3' (or its complementary sequence 5'-TGG-3') are underlined. Other conditions and symbols are the same as described in (b).

vectors examined does not seem to have a large effect on the frequency of cosmid deletions. However, there is a possibility that use of a low-copy-number vector may partially solve the deletion problem in cosmid cloning. The cosmid deletion events described here are likely to reflect two processes, the formation of deletions in cosmid DNA and the displacement of original cosmids by deleted forms. The number of copies of intact cosmids seems to be lower than that of the deleted forms. Therefore, the differential rate of replication of cosmids between the intact cosmids and the deleted forms may provide a bias in favour of deletions. In this connection, cosmids may be more stably propagated in *E. coli* host cells if the cosmids are constructed with a low-copy-number vector. To examine this possibility, we have constructed a low-copy-number vector with *ori* of pSC101 or that of F' and examined cosmid cloning with such a vector (unpublished data). However, we have not yet succeeded in increasing the stability of intact cosmids in *E. coli* cells, using a low-copy-number vector.

Fernandez *et al.* (1986) showed that spontaneous insertion of *E. coli* insertion element IS1 into two vectors, pNNL and pTCF, occurred during propagation of the vectors and suggested that use of a vector carrying IS1 for cosmid cloning caused a very high frequency of deletions in cosmids in standard *recA* hosts. However, this phenomenon could not account for our observations, because in contrast to the deletions observed in this study, IS1-mediated deletions always involve deletions in vector portions starting at the IS1 segment and eliminate one of the *Bam*HI junction sites between the vector and the insert. In addition, the vectors used here did not carry IS1.

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