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Research in Microbiology 159 (2008) 663–670

www.elsevier.com/locate/resmic

Molecular analysis of recombinase-mediated cassette exchange reactions catalyzed by integrase of coliphage HK022

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Received 31 July 2008; accepted 5 September 2008
Available online 24 September 2008

Abstract

The integrase (Int) protein of coliphage HK022 can catalyze in *Escherichia coli* as well as in in vitro integrative and excisive recombinase-mediated cassette exchange reactions between plasmids as substrates. Atomic force microscopy images have revealed that in the protein–DNA complexes that are formed, the plasmid substrates are connected via one and not two pairs of attachment sites. This observation, together with the elucidation of intermediate co-integrates between the two circular plasmids, suggest that a sequential mechanism of the RMCE reaction is possible.

Keywords: Site-specific recombination; Integrase; Bacteriophage HK022; Recombinase-mediated cassette exchange; Atomic force microscopy

1. Introduction

Site-specific recombination systems of lower organisms have become important tools for site-specific gene manipulations in eukaryotic organisms. These include insertions, deletions and activation of genes [3,6,19]. A recent important approach is recombinase-mediated cassette exchange (RMCE) technology in which a desired gene or any other chromosomal DNA fragment can replace a predefined chromosomal fragment. This is accomplished when the chromosomal fragment is flanked by two incompatible site-specific recombination sites and the replacing DNA is likewise flanked by the same recombining sites. The relevant site-specific recombinase can catalyze an exchange between the chromosomal fragment and the replacing DNA. The two incompatible pairs of sites can be substrates of the same recombinase [18] or substrates of two different recombinases [16,23]. In case of a single recombinase, one pair of compatible sites should be mutated in order to be incompatible with the wild-type sites. The use of mutated sites is less efficient and may still cause some undesired compatibility between the two flanking recombination sites [5,15].

Integrase (Int), the site-specific recombinase of coliphage HK022, catalyzes the reaction of the phage's lysogenic pathway in a mechanism that is very similar, if not identical, to that of well known coliphage λ. In both phages, Int catalyzes the integration and excision reactions by pairs of non-identical recombination sites (*att*) that differ in size and sequence, except for a 7-base pair (bp) core sequence that is the site of recombination and is identical in all four *att* sites. *attP* (246 bp) + *attB* (21 bp) are the sites of phage integration and their recombination products *attL* (101 bp) + *attR* (166 bp) are the substrates of phage excision. In *E. coli* and in the in vitro reactions, additional DNA-bending accessory proteins are required; integration requires the host-encoded integration host factor (IHF) protein and in addition, excision requires the phage-encoded excisionase protein (Xis). The latter protein can be partially compensated for by the host-encoded factor for inversion specificity (Fis) protein (reviewed in [1,9,27]).

The wild-type *int* gene of HK022 has been introduced and shown to be active in mammalian and plant cells [7,10]. Both types of reactions (*attB* × *attP* and *attR* × *attL*) can occur without the prokaryotic accessory proteins (IHF and Xis). In the case of Int-λ, only IHF-independent mutants are active in mammalian cells [4]. The Int system can therefore serve as a potential tool for gene manipulations in the eukarya.

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doi:10.1016/j.resmic.2008.09.005

Moreover, owing to its heterogeneous pairwise substrates, the Int system may operate in RMCE reactions without the need to use mutated sites.

A version of the λ Int system, known as Gateway cloning technology, has been developed by Life Technology Inc. as an in vitro gene cloning system that replaces the need for restriction and ligation in DNA cloning procedures. This system is based on an Int-λ catalyzed in vitro integrative RMCE reaction in which the gene to be cloned is flanked by two *attB* sites that are compatible with two *attP* sites on the vector. In the Gateway system all recombination reactions are carried out in vitro and the complete RMCE products are selected in *Escherichia coli* using a positive selection marker (antibiotic resistance) and a negative selection marker (*ccdB*). However, no data are available on the ability of Int to catalyze an RMCE reaction in vivo. Since our goal is to develop the Int-HK022 system for an in vivo RMCE gene replacement in the eukarya, it was essential to examine first how Int-HK022 catalyzes an RMCE in vivo in *E. coli* without a negative selection force. In the present paper, we show that the wild-type Int of phage HK022 can catalyze integrative and excisive RMCE reactions in *E. coli* as well as in vitro. An analysis of co-integrated intermediates and of protein–DNA complexes, using atomic force microscopy (AFM), suggests that the RMCE reaction may be sequential.

2. Materials and methods

2.1. Bacteria, growth conditions, plasmids and oligomers

E. coli K12 *recF* (strain DS941, [25]) served as the bacterial host. Cells were grown and plated on Luria–Bertani Rich medium with proper antibiotics. Plasmid transformations were performed by electroporation [22]. Plasmids used in this work and oligomers used as PCR primers are listed in Table 1.

2.2. Plasmid constructions

2.2.1. pMK155

The BglII–HindIII (*int*) fragment of plasmid pKH70 [13] was cloned between the same sites of the kanamycin-resistant (Km^R) vector pOK12.

2.2.2. pMK169

The EcoRI–HindIII (*int–xis*) fragment of pNK1773 [28] was cloned between the same sites of vector pOK12 [26].

2.2.3. pNA871

One *attL* site was extracted from plasmid pMK25 and inserted into the pBluescript Ap^R vector (Fermentas) between the KpnI + SalI sites. Next, a PCR fragment that carries the Tc^R gene was generated from vector pACYC184 (NEB Inc.) as template and oligomers oEY378 + oEY379 as primers and was cloned into the EcoRI site via the pGEM-T-Easy vector (Promega). The second *attL* fragment was generated by PCR from pMK25 with primers f + r and inserted between the PstI and NotI sites via vector pGEM-T-Easy.

2.2.4. pNA865

One *attR* site was extracted from plasmid pMK24 and cloned into the pBluescript vector between the KpnI and HindIII sites. A PCR fragment that carries the Cm^R gene was generated by PCR from vector pACYC184 using primers oEY376 + oEY385 and was cloned into the HindIII site. The

Table 1
List of plasmids and oligomers used as primers for PCR reactions

A. Plasmids

Plasmid name	Characteristics	Source or reference
pMK24	*attR* in pUC18, Ap^R	[8]
pMK25	*attL* in pUC18, Ap^R	[8]
pMK129	*intF* on pETI-1	[12]
pMK155	*int* in pOK12, Km^R	This work
pMK169	*int* + *xis* in pOK12, Km^R	This work
pNA865	*attR*- Cm^R-*attR*	This work
pNA871	*attL*-Tc^R-*attL*, Ap^R	This work
pNA890	*attP*-Cm^R-*attP*, Ap^R	This work
pNA929	*attB*-Tc^R-*attB*	This work

B. Oligomers

Primer	Sequence (5′→3′)[a]	Location
oEY135	AGGTCACTAATACTATCTAAGTAGTTGATTCATAGGACCTGG	P arm of *att*
oEY202	TAATACGACTCACTATAGGG	T7 promoter
oEY279	GGAATTAACCCTCACTAAAGGG	T3 promoter
oEY376	CCC**AAGCTT**CGGGAAGCCCTGGGCC	Cm^R gene
oEY385	CCC**AAGCTT**CAGGCGTAGCACCAGGCG	Cm^R gene
oEY378	GATAAGCTTTAATGCGGTAG	Tc^R gene
oEY379	CC**GAATT**CCACCCGTGGCCAGGACC	Tc^R gene
oEY451	GGG**AACCTTTTTCACCTAAAGTGC**CCACCCGTGGCCAGGACC	*attB*-Tc^R
oEY452	GGG**AACCTTTTTCACCTAAAGTGC**CGCACCTGAAGTCAGCCCC	*attB*-Tc^R
Forward (f)	GCCAGGGTTTTCCCAGTCACGA	Bluescript, pUC18
Reverse (r)	GAGCGGATAACAATTTCACACAGG	Bluescript, pUC18

[a] Restriction site and *attB* sequence are in boldface.