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Virology 375 (2008) 383–390
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Phosphorylation of the integrase protein of coliphage HK022

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Received 10 October 2007; returned to author for revision 7 February 2008; accepted 13 February 2008
Available online 18 March 2008

Abstract

The integrase (Int) proteins of coliphages HK022 and λ, are phosphorylated in one or more of their tyrosine residues. In Int of HK022 the phosphorylated residue(s) belong to its core-binding/catalytic domains. Wzc, a protein tyrosine kinase of *Escherichia coli*, is not required for Int phosphorylation *in vivo*, however, it can transphosphorylate the conserved Tyr^{342} catalytic residue of Int *in vitro*. Int purified from cells that overexpress Wzc has a reduced activity *in vitro*. *In vivo*, the lysogenization of wild type HK022 as well as of λ is not affected by the overexpression of Wzc. However, the *nin5* mutant of λ, which lacks a protein-tyrosine phosphatase gene, shows a significantly reduced lysogenization. It is suggested that phosphorylation of Int by Wzc down regulates the activity of Int.
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Keywords: Integrase; Phage HK022; Protein phosphorylation; Wzc/Wzb

Introduction

The integrase (Int) protein of the temperate coliphage HK022 (Int-HK022) catalyses the site-specific integration and excision of the bacteriophage in the lysogenic cycle. The mechanism of the site-specific recombination system of phage HK022 is similar, if not identical, to its close relative coliphage λ. Their *int* genes share a 70 percent homology and each is preceded by an *int* promoter (Weisberg et al., 1999; Yagil et al., 1989). In λ the transcription of *int* is regulated by the CII and CIII proteins, CII is a transcriptional modulator that stimulates the transcription of Int-λ and of the CI repressor (Shimatake and Rosenberg, 1981). The CIII protein stabilizes CII thereby promoting the lysogenic pathway (Altuvia and Oppenheim, 1986). The many similarities between the regulatory elements of λ and HK022 indicate that the transcriptional regulation of the *int* gene of HK022 is identical to that of λ. These also include the promoters of Int, terminators and RNA processing sites *sib*/*tI* (Weisberg et al., 1999).

Post-translational phosphorylation and dephosphorylation of proteins by protein kinases and protein phosphatases play important roles in regulatory processes in the eukarya (Mukherji, 2005; Pawson and Scott, 2005). The hydroxyl amino acids serine, threonine and tyrosine are the substrates of phosphorylation and dephosphorylation. Protein kinases and phosphatases were later discovered in bacteria, however, their biological role is much less clear (Kennelly, 2002; Grangeasse et al., 2007). Wzc/Wzb is a pair of protein tyrosine kinase/phosphatase, respectively, that was characterized in *Escherichia coli* strain K12; they play a role in the production of colanic acid (Grangeasse et al., 2002; Doublet et al., 2002). Etk/Etp is homologous to Wzc/Wzb which was characterized in a pathogenic strain of *E. coli*; it is involved in the production of group 4 capsular exopolysaccharides (Ilan et al., 1999). Both kinases are autocatalytic enzymes, as they catalyze the phosphorylation of their own tyrosine residues. Few substrates that can be phosphorylated by these kinases are known. The autophosphorylated Wzc protein is able to transphosphorylate UDP-glucose dehydrogenase, an enzyme that is involved in the metabolism of acidic

Abbreviations: Int, integrase; IPTG, β-D-1-thiogalactopyranoside; anti-Ph-Tyr, monoclonal antibody against phospho-tyrosine.

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doi:10.1016/j.virol.2008.02.011

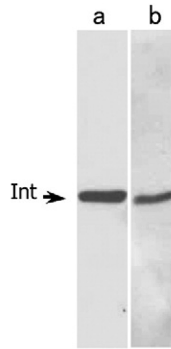

Fig. 1. Immunoblots of purified Int treated with anti-Int antibodies (lane a) and with anti-Ph-Tyr antibodies (lane b).

polysaccharides and colanic acid (Grangeasse et al., 2003). The autophosphorylated Etk can transphosphorylate RpoH and RseA, a sigma factor and an antisigma factor, respectively, that regulate the transcription of heat shock genes (Klein et al., 2003).

The presence of genes that encode protein kinases and phosphatases has also been reported in bacteriophages of the lambdoid family. Phage λ and some of its relatives encode a protein phosphatase (Cohen and Cohen, 1989), and phage 933W, which resides in a pathogenic *E. coli* strain, encodes an autophosphorylating tyrosine protein kinase (Stk) that can transphosphorylate tyrosine residues of artificial substrates (Tyler and Friedman, 2004). Coliphage T7 carries a serine/threonine protein kinase (Fp0.7) that can phosphorylate the β' subunit of the RNA polymerase of its host (Severinova and Severinov, 2006).

In the present work we show that the Int proteins of phages HK022 and λ are phosphorylated in tyrosine residues and that the active Tyr^{342} residue of Int-HK022 can be transphosphorylated by the Wzc kinase and dephosphorylated by the Wzb phosphatase. The effect of the transphosphorylation of Int by Wzc on site-specific recombination reactions has been examined.

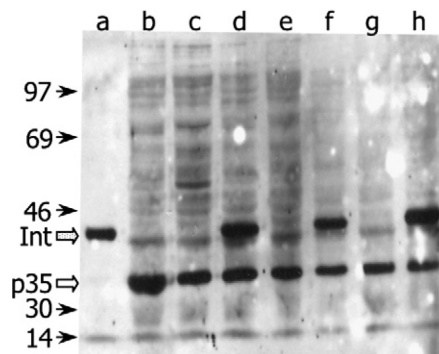

Fig. 2. Immunoblot of cell extracts of Int-overexpressing strains treated with anti-Ph-Tyr. Lane a — purified Int; lane b — induced cells with vector (pT7-3) alone; lanes c, d — uninduced and induced cells that over express the wild type Int-HK022; lane e, f — uninduced and induced cells that over express the Int-HK022 Y342F mutant; lanes g, h — uninduced and induced cells that over express Int-λ. Numbers show molecular weight markers in kDa.

Results

Int-HK022 and Int-λ are phosphorylated in vivo

Int protein, purified from cells that express it from a plasmid, was immunodetected by the antibody, raised against Int (anti-Int) (Fig. 1, lane a) as well as by monoclonal antibody against phospho-tyrosine (anti-Ph-Tyr) (Fig. 1, lane b). The immunodetection has indicated that Int is phosphorylated in one or more of its tyrosine residues. To gain further insight into this modification, extracts of *E. coli* cells that express different isopropyl β-D-1-thiogalactopyranoside (IPTG)-inducible plasmid-borne *int* genes were analyzed by an anti-Ph-Tyr immunoassay (Fig. 2). The induced Int-HK022 strain, shows a clear band that is identical to the purified Int (Fig. 2, lanes a and d, dotted arrow).

Int belongs to the tyrosine family of site specific recombinases and its Tyr^{342} residue is the active amino acid and is conserved within the entire family (Azaro and Landy, 2001). We have tested if this residue might be the only one that is phosphorylated. Lanes e and f in Fig. 2 show, respectively, extracts of uninduced and induced cells that overexpress an inactive Int-HK022 mutant whose Tyr^{342} residue has been mutated to Phe (the IntF mutant). The results indicated that both the wild type and Y342F mutant forms of Int-HK022 contained comparable levels of phosphorylated Tyr. Thus, in Int-HK022, Tyr residues other than Tyr^{342} were phosphorylated although the possibility that Tyr^{342} is also subject to phosphorylation was not excluded. Since the Int proteins of phages HK022 and λ are closely related, the induced wild type Int of λ is, as expected, likewise phosphorylated (Fig. 2 lanes g, h). In addition to Int, all cell extracts in Fig. 2 show a background of several other phosphorylated proteins that could include the 79.3 kDa *E. coli* Wzc protein tyrosine kinase. The identity of the prominent cellular

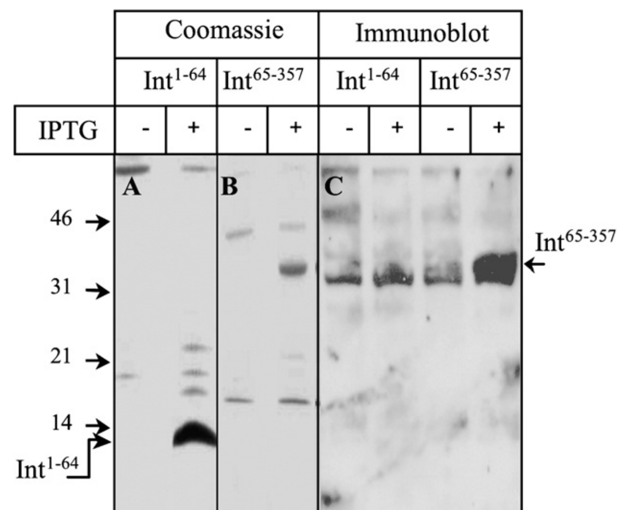

Fig. 3. A. Coomassie-stained gel of cell extracts that have overexpressed uninduced and IPTG-induced Int$^{1-64}$. B. Coomassie-stained gel of cell extracts that have overexpressed uninduced and induced Int$^{65-357}$. C. Immunoblot of the cell extracts shown in A and B treated with anti-Ph-Tyr. Numbers show molecular weight markers in kDa.

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