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Interaction of the Bacteriophage P2 Tin Protein and Bacteriophage T4 gp32 Protein Inhibits Growth of Bacteriophage T4

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Abstract The growth of bacteriophage T4 is inhibited by the presence of the *tin* gene product of bacteriophage P2. The interaction between purified Tin and gp32 proteins was observed using coimmunoprecipitation experiments. The *in vivo* interaction was confirmed by yeast two-hybrid experiments. A deletion analysis showed that the Asp 163 region of gp32 was needed for the interaction with Tin. The binding of gp32 to DNA substrates was not affected by the presence of Tin. Thus, it would appear that the inhibition of T4 growth by Tin was due to a protein-protein interaction rather than affecting the DNA-binding ability of gp32.

Key words: Bacteriophage P2, T4 exclusion, Tin, gp32, protein-protein interaction

The superinfection of a host cell already infected by another virus can be inhibited by various exclusion mechanisms [5, 9, 15, 16]. Bacteriophage P2 is a temperately virulent virus infecting *Escherichia coli* and inhibits the superinfection of T-even phages [2, 10]. The *tin* gene product expressed by the same promoter with *old* [7] in the P2 lysogen is known to be involved in the interference of T-even phage growth [12]. The expression of the *tin* gene product alone in a plasmid also inhibits the growth of T-even phages. The replication of DNA and late gene expression of T-even phages are also inhibited by the presence of Tin.

gp32 is a single-stranded DNA-binding protein, encoded by bacteriophage T4, and is involved in DNA replication, recombination, and repair by interacting with other phage and host proteins [1, 3, 4, 6, 8, 11]. Mutant T4 phages, named *asp* (*a*borts *s*ensitivity to *P2*), which can grow in the presence of Tin, were previously isolated and the sequence analysis revealed that the Asp163 of gp32 changed to either Asn or Gly [12]. These *asp* mutant T4 phages can grow regardless of the presence or absence of P2. The crystal structure of gp32 was reported [13, 14], in which Asp163 was found to be located away from the DNA-binding cleft and away from the N-terminal domain involved in the cooperative binding of gp32, and the C-terminal A box involved in the interaction with other enzymes for DNA replication, recombination, and repair.

The current study reports on the results of various protein-protein interaction experiments using Tin and gp32 and discusses a possible mechanism of inhibition.

The Tin and gp32 were both purified as N-terminally hexahistidine tagged forms. A 762 bp PCR product of *tin* was cloned in pQE30 (Qiagen, U.S.A) using the *Bam*HI and *Hin*dIII sites. A 909-bp gp32 PCR product was cloned in pQE30 using the *Bam*HI and *Hin*dIII sites. The two proteins were purified to near homogeneity using an Ni-NTA column. An electrophoretic mobility shift assay (EMSA) was performed on a synthetic 28-base-long, single-stranded DNA oligomer. The two proteins were incubated with the oligomer and subjected to electrophoresis in a TBE buffer in a 7% polyacrylamide gel. Mouse anti-gp32 antiserum was obtained by inoculating a mouse with the purified protein, 3 times, with or without the adjuvant. Coimmunoprecipitation was performed using Protein-A agarose (Roche). The identification of the precipitated proteins was established by Western blotting with an anti-hexahistidine antibody (Pharmacia, U.S.A.). The yeast two-hybrid assay was performed as follows. The PCR products of *tin*, *gp32*, and the mutant *gp32*s (Asp163 to Asn and Asp163 to Gly) were each cloned into both pGAD424 (activation domain, Clontech, U.S.A.) and pGBT9 (DNA-binding domain, Clontech). Each cotransformant was subjected to a colony lift filter β-galactosidase assay. Deletion mutants were generated by using exonuclease III digestion.

After coincubation of the purified Tin and gp32, the complex was immunoprecipitated with anti-gp32 antisera. The two proteins were coprecipitated and identified using an anti-hexahistidine antibody (lane 1, Fig. 1). Twenty-nine kDa

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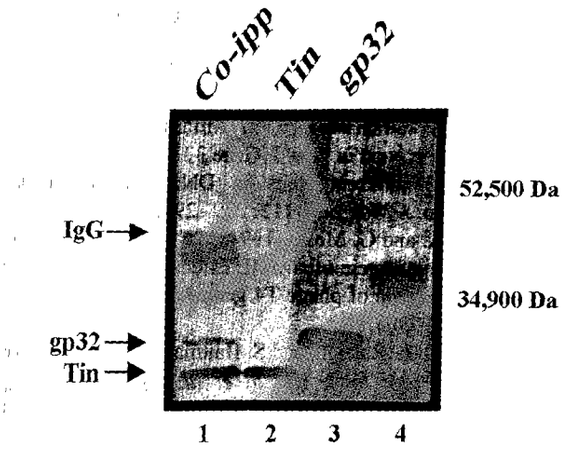

Fig. 1. Coimmunoprecipitation of Tin and gp32.
Lane 1, Tin and gp32 coimmunoprecipitated by anti-gp32 antisera; lane 2, purified Tin; lane 3, purified gp32; lane 4, protein molecular weight marker.

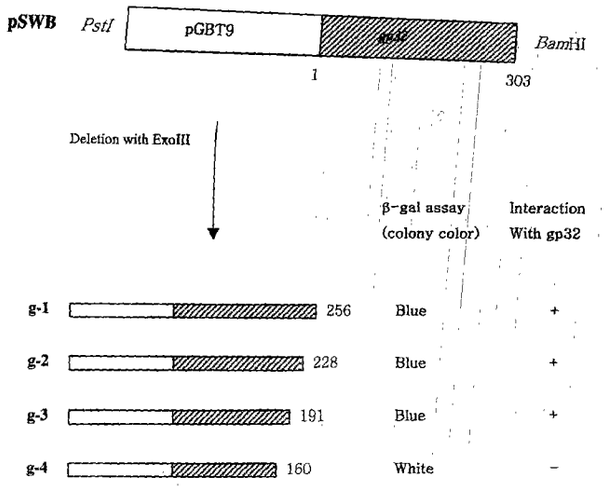

Fig. 2. Yeast two-hybrid analysis of interactions between Tin and a nested-deletion set of gp32 mutants.
Shaded areas show the remaining gp32 portion after C-terminal deletion by exonuclease III. The numbers indicate the amino acid positions at the C-terminus of each construct.

Tin and 34 kDa gp32 were found. The 40 kDa band was the IgG in the antisera, which was not detected by the anti-hexahistidine antibody, yet detected by the secondary antibody for the colorimetric measurement. The secondary antibody bound to the Fc region of the mouse IgG. The possibility of Tin binding to the anti-gp32 antisera was excluded by a Western blot analysis (data not shown). Thus, it was concluded that the two proteins bound to each other *in vitro*.

The *in vivo* binding assay results are shown in Table 1. The interaction of Tin and gp32 was observed when they were cloned in either the activation domain or the DNA-binding domain. However the mutant gp32s did not interact with Tin in either format. The deletion analysis showed that the N-terminal 191-amino-acid fragment of gp32 interacted with Tin, whereas the N-terminal 160-amino-acid fragment did not (Fig. 2). Accordingly, this region downstream of the 160th amino acid would appear to be important for the recognition of Tin. This region may form a conformational epitope for binding Tin. Based on the fact that Asp163 of gp32 was critical for Tin inhibition, the epitope could extend to Asp163.

The EMSA result is shown in Fig. 3. The gp32 bound to the DNA and retarded the migration (lane 2) of the single-stranded DNA, whereas Tin did not (lane 3). When both

Table 1. The interaction of Tin and gp32 (and mutant gp32s) using yeast two-hybrid analysis.

Vector insert		pGAD424 (Activation domain)	pGBT9 (DNA-binding domain)
gp32		pSWA	pSWB
Tin		pKAT	pKBT
Mutant gp32	163Asp→Asn	pSMA-7	pSMB-7
	163Asp→Gly	pSMA-8	pSMB-8

Plasmid 1 (DNA-binding domain)	Plasmid 2 (Activation domain)	β-gal assay (colony color)
pKBT	pSWA	Blue
pSWB	pKAT	Blue
pKBT	pGAD424	white
pSWB	pGAD424	white
pGBT9	pKAT	white
pGBT9	pSWA	white
pKBT	pSMA-7	white
pKBT	pSMA-8	white
pSMB-7	pKAT	white
pSMB-8	pKAT	white

The upper table indicates the construction of various plasmids for analysis. The lower table indicates the analysis results.

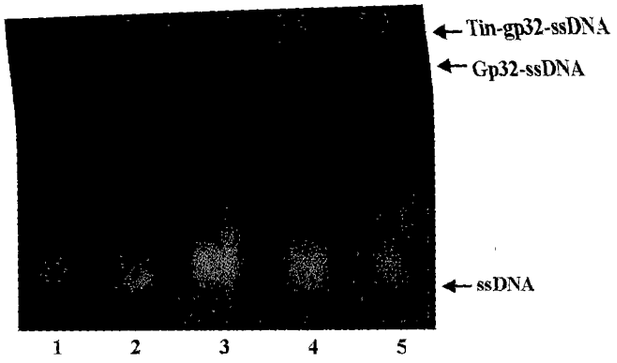

Fig. 3. The binding of gp32 to a single-stranded DNA in the presence or absence of Tin.
Lane 1, single-stranded DNA alone; lane 2, single-stranded DNA incubated with gp32; lane 3, single-stranded DNA incubated with Tin; lanes 4 and 5, single-stranded DNA incubated with both gp32 and Tin.

gp32 and Tin were incubated with the substrate DNA, the migration was further retarded, thereby indicating that the two proteins bound to the DNA as a complex (lanes 4 and 5). Thus, the gp32-Tin complex bound to the single-stranded DNA and gp32 bound to the single-stranded DNA in the presence of Tin.

It was shown that the poisoning of gp32 by Tin was due to a protein-protein interaction between the two proteins based on both *in vitro* and *in vivo* interaction analyses. It was also found that gp32 bound to single-stranded DNA even in the presence of Tin, whereas Tin seemed to piggyback on gp32. One possibility is that Tin distorted the gp32-DNA filament, thereby preventing other necessary protein recognition. As suggested by Mosig *et al.* [12], if T4 gp32 needs multiple conformations to function (possibly by interacting with other cellular and phage proteins), the binding of Tin may affect the transition of the conformational states of gp32. Based on its amino acid sequence, Tin has a cluster of basic amino acids, suggesting that it could possibly bind single-stranded DNA. However, the current study revealed that Tin did not bind single-stranded DNA by itself, thus it would not appear that the two proteins competed for the same DNA substrates. Yet certain *in vivo* conformations of DNA (e.g. DNA recombination intermediates) may still be a target for Tin binding. This possibility needs to be explored.

The exclusion mechanism of T4 superinfection by P2 was partly revealed in this study. As such, an exclusion mediated by a protein-protein interaction is a novel discovery [12], and the precise mechanism of this exclusion is currently under investigation.

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