Example GO annotations

PMID:17698500
Protein p56 from the *Bacillus subtilis* phage φ29 inhibits DNA-binding ability of uracil-DNA glycosylase

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ABSTRACT

Protein p56 (56 amino acids) from the *Bacillus subtilis* phage φ29 inactivates the host uracil-DNA glycosylase (UDG), an enzyme involved in the base excision repair pathway. At present, p56 is the only known example of a UDG inhibitor encoded by a non-uracil containing viral DNA. Using analytical ultracentrifugation methods, we found that protein p56 formed dimers at physiological concentrations. In addition, circular dichroism spectroscopic analyses revealed that protein p56 had a high content of β-strands (around 40%). To understand the mechanism underlying UDG inhibition by p56, we carried out *in vitro* experiments using the *Escherichia coli* UDG enzyme. The highly acidic protein p56 was able to compete with DNA for binding to UDG. Moreover, the interaction between p56 and UDG blocked DNA binding by UDG. We also demonstrated that Ugi, a protein that interacts with the DNA-binding domain of UDG, was able to replace protein p56 previously bound to the UDG enzyme. These results suggest that protein p56 could be a novel naturally occurring DNA mimicry.
Protein p56 (56 amino acids) from the *Bacillus subtilis* phage φ29 inactivates the host uracil-DNA glycosylase (UDG), an enzyme involved in the base excision repair pathway.
Which experiments show function?

- Figure 1: analytical ultracentrifugation of p56 – NO
- Figure 2: CD spectroscopy of p56 – NO
- Figure 3: Temperature dependent CD of p56 - NO
Figure 4. Protein p56 inhibits *E. coli* UDG activity. The 5′-end $^{32}$P-labelled ssDNA-U$_{16}$ substrate (S) (1.3 nM) was incubated with UDG (5 nM) in the absence or presence of p56. After 8 min, the reaction mixtures were treated with NaOH. Formation of the cleavage
Materials and Methods:

To measure UDG activity, a 34-mer oligonucleotide containing a single uracil residue at position 16 (ssDNA-U16; from Isogen) was used as substrate. It was 5′-labelled with γ-32PATP (3000 Ci/mmol) (GE Healthcare) and T4 polynucleotide kinase (New England Biolabs). Reaction mixtures (20 μl) contained increasing amounts of the *E. coli* UDG preparation and the radiolabelled substrate in buffer B. After incubation at 37°C for 8 min, samples were treated with NaOH to a final concentration of 0.2 M, and heated at 90°C for 30 min. Samples were then dried in a Speed Vac, resuspended in 10 μl of formamide loading buffer (95% formamide, 20 mM EDTA, 0.05% xylene cyanol, 0.05% bromophenol blue), and subjected to electrophoresis in 8 M urea/20% polyacrylamide gels. The minimal UDG amount needed to obtain total cleavage of the substrate was used to examine UDG inhibition by p56.
Figure 4. Protein p56 inhibits *E. coli* UDG activity. The 5′-end \(^{32}P\)-labelled ssDNA-U\(^{16}\) substrate (S) (1.3 nM) was incubated with UDG (5 nM) in the absence or presence of p56. After 8 min, the reaction mixtures were treated with NaOH. Formation of the cleavage
Possible GO annotations

- *E. coli* UDG (control): Uracil DNA glycosylase activity
- Phi29 p56: inhibition of UDG activity
UDG activity

- Go to Uniprot.org
- Search for *E. coli* UDG
- Find the Uniprot record
- Follow link to complete GO annotation
- Is there a GO term?
- Is this paper (PMID: 17698500) used?
- If no, Make the annotation
Inhibition of UDG activity

- Go to Uniprot.org
- Search for phi29 p56
- Find the Uniprot record
- GO annotation?
- Is there a perfect GO term?
  - No!
Find the best existing GO term
Annotate to GO: 1902545 ! negative regulation of DNA N-glycosylase activity

Make new term request for the more specific term
Other annotations?

- Figures 6 and 7
- P56 prevents UDG from binding DNA
- GO:0043392 negative regulation of DNA binding

Figure 7. Protein p56 inhibits DNA-binding ability of UDG. Electrophoretic mobility shift assays were performed in the absence or in the presence of the indicated proteins. A radiolabelled dsDNA fragment (121 bp) containing uracil in place of thymine residues was used as substrate.
Follow the references:

“Our previous work showed that addition of purified protein p56 to B. subtilis cell extracts inhibited the endogenous UDG activity (23).

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Can we make transfer annotations?