Understanding bacteriophage T4 GoF protein, a putative RNA chaperone

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Abstract

Upon infection of *E. coli*, bacteriophage T4 relies on host RNA polymerase (RNAP) to propagate the phage's genetic material over that of the host. As phage genetic material is produced, infection viability is sustained through the protection of those phage products. Past investigations have shown that wild-type (WT) T4 does not grow on the host strain RhoO26. This strain has a mutation within *rho*, which encodes the main host factor for transcription termination. The inability of WT T4 to grow on RhoO26 arises from a decrease in the level of certain middle transcripts, including that of gene 41, the DNA helicase required for T4 DNA replication; a substitution within the T4 early gene *goF* restores growth and increases the level of gene 41 mRNA. This work initially suggested that *goF* might encode a transcription anti-termination factor and that the mutant *goF* is needed to allow growth on the 'super' *rho* mutant strain RhoO26. However, other studies revealed that *rho026* encodes a protein that is actually a poorer terminator because of a diminished ability to bind RNA. Consequently, the function of GoF during T4 infection and how the *goF* suppressor mutant promotes growth in RhoO26 has still not been elucidated. Using deep blast analyses and the structure predictor program Alphafold2, we have found that GoF belongs to a group of proteins conserved throughout the *Myoviridae* family. Within T4 there are three orthologs: GoF, MotB.1 and Frd.2. Interestingly, all 3 have predicted SM-fold domains at their N-termini. SM-fold domains have been observed in proteins that bind to RNA. Hfq, the major host RNA chaperone, is one such protein in *E. coli*. We postulate that GoF (and MotB.1 and Frd.2) may function as T4 RNA chaperones that work as a phage defense against host RNases. We are currently conducting experiments to test this hypothesis. We hope to leverage this mechanistic knowledge to further understand phage-host interactions.

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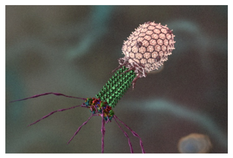
Background



T4 Bacteriophage
- Lytic (Myoviridae family) phage
- Early Gene Products: **GoF**, MotB.1, and Frd.2
- Products: involved in co-opting bacterial gene expression

Rho (Sen et al., J. Microbiol. 2006)
- Universal essential protein that binds to RNA and terminates mRNA synthesis
- Dissociates RNA polymerase from DNA template to release RNA
- Energy from hydrolyzing ATP

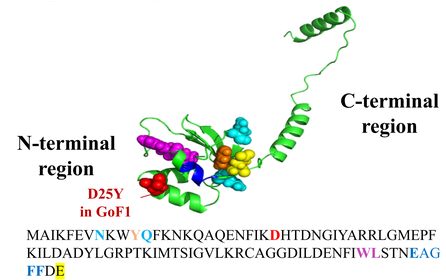
RhoO26 (Sozhamannan and Stitt, J. Mol. Biol. 1997)
- *E. coli* with a mutated Rho that has decreased RNA binding and prevents T4 bacteriophage growth

goF
- Mutations in *goF* such as *goF1* (D25Y) were shown to rescue T4 infections in RhoO26 mutants (Stitt and Mosig, J. Bacteriol. 1989)
- *goF* is either directly or indirectly involved in the expression of T4 gene 41 RNA
  - Gene 41: Essential DNA replication protein – required for lagging strand synthesis

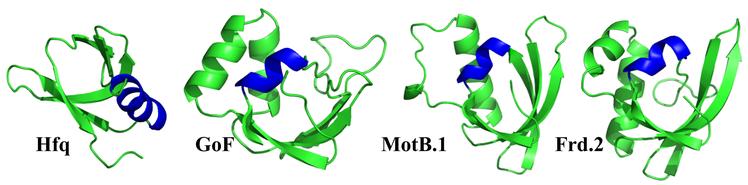
Table. Quantitation of RNA reading into *gene 41* versus RNA stopped downstream of *uvsX* just after a strong hairpin (Hinton, *J. Biol. Chem.* 1989)

Infection	% Readthrough RNA[a]	
	min	
T4$^+$/*rho*$^+$	4	74
	10	49
T4$^+$/*rho026*	4	39
	10	19
T4 *goF1*/*rho026*	4	69
	10	44
pDH428/DH1 (plasmid, no infection)		34

AlphaFold2 predicted structure of GoF



The N-terminal regions of GoF and 2 other T4 early proteins have predicted SM-fold domains.
SM-folds are found in RNA chaperones like *E. coli* Hfq



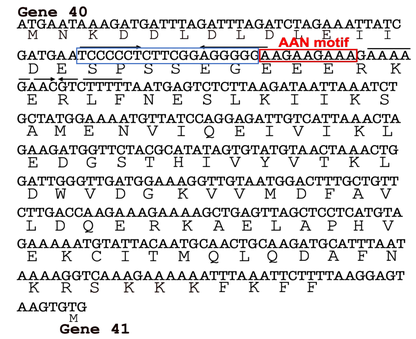
Hypothesis

Based on previous research, we hypothesize that the bacteriophage T4 early protein GoF might function as an RNA chaperone.

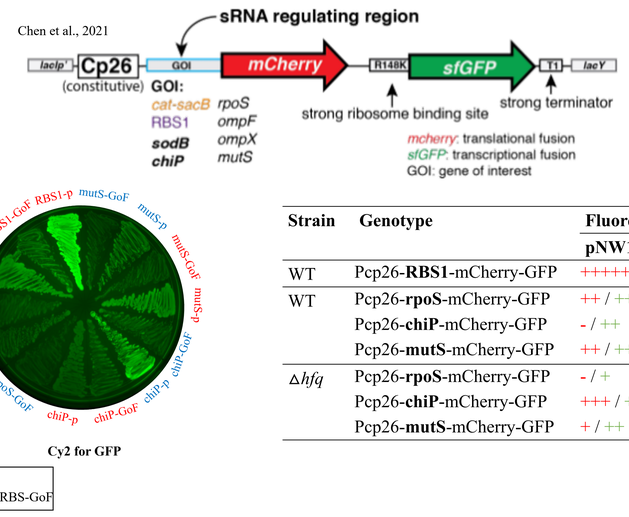
Results

GoF & GoF1 have been transformed into the following strains
Plasmid Background: pNW129 and pBAD33
SG13060
RhoO26
BL21
Top10
MG1655 (GoF only)

An AAN motif, the distal site motif of Hfq, is located upstream of gene 41 and just after a strong hairpin



Expression of *goF* affects the level of genes in a translation reporter system (Jinshil Kim)



Strain	Genotype	Fluorescence (mCherry / GFP)*	
		pNW129	pNW129-RBS-GoF
WT	Pcp26-**RBS1**-mCherry-GFP	+++++ / +++++	+ / ++
WT	Pcp26-**rpoS**-mCherry-GFP	++ / +++	- / +
	Pcp26-**chiP**-mCherry-GFP	- / ++	- / ++
	Pcp26-**mutS**-mCherry-GFP	++ / +++	- / +
Δ*hfq*	Pcp26-**rpoS**-mCherry-GFP	- / +	- / +
	Pcp26-**chiP**-mCherry-GFP	+++ / +++	+ / +
	Pcp26-**mutS**-mCherry-GFP	+ / ++	- / +

Future Experiments

- RNA-seq to understand the effect of *goF* overexpression on host strains (B and K12 strains) and T4 gene expression
- Pull-down assays to determine proteins and DNA/RNA that interact with GoF
- Phage spot tests with T4 to determine the effect of GoF and GoF1 production in Rho026
- Localization experiments to determine where the protein primarily resides within the cell

Localization

Express GoF within bacterial strains → Harvest and osmotically lyse Spheroplasts → Separate Total Cell, Soluble, and Insoluble Fractions through ultracentrifugation; determine protein localization through Western Blotting

Conclusions

- Predicted structures of GoF suggest that there may be some RNA-binding function and that the protein may act as a T4 RNA chaperone
- Expression of *goF* affects levels of genes in a translation reporter system, suggesting that GoF may also be involved in post-transcriptional regulation

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