Impaired Striatal Akt Signaling Disrupts Dopamine Homeostasis and Increases Feeding

The MIT Faculty has made this article openly available. Please share how this access benefits you. Your story matters.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>As Published</td>
<td><a href="http://dx.doi.org/10.1073/pnas.1107633108">http://dx.doi.org/10.1073/pnas.1107633108</a></td>
</tr>
<tr>
<td>Publisher</td>
<td>Proceedings of the National Academy of Sciences (PNAS)</td>
</tr>
<tr>
<td>Version</td>
<td>Final published version</td>
</tr>
<tr>
<td>Accessed</td>
<td>Sun Apr 07 20:11:51 EDT 2019</td>
</tr>
<tr>
<td>Citable Link</td>
<td><a href="http://hdl.handle.net/1721.1/70026">http://hdl.handle.net/1721.1/70026</a></td>
</tr>
<tr>
<td>Terms of Use</td>
<td>Article is made available in accordance with the publisher's policy and may be subject to US copyright law. Please refer to the publisher's site for terms of use.</td>
</tr>
<tr>
<td>Detailed Terms</td>
<td></td>
</tr>
</tbody>
</table>
A homing endonuclease and the 50-nt ribosomal bypass sequence of phage T4 constitute a mobile DNA cassette

Richard P. Bonocora1, Qinglu Zeng1,2, Ethan V. Abel1, and David A. Shub4

Department of Biological Sciences, University at Albany, State University of New York, Albany, NY 12222

Edited* by Marlene Belfort, Wadsworth Center, Albany, NY, and approved August 23, 2011 (received for review May 25, 2011)

Since its initial description more than two decades ago, the ribosome bypass (or “hop”) sequence of phage T4 stands out as a uniquely extreme example of programmed translational frameshifting. The gene for a DNA topoisomerase subunit of T4 has been split by a 1-kb insertion into two genes that retain topoisomerase function. A second 50-nt insertion, beginning with an in-phase stop codon, is inserted near the start of the newly created downstream gene 60. Instead of terminating at this stop codon, approximately half of the ribosomes skip 50 nucleotides and continue translation in a new reading frame. However, no functions, regulatory or otherwise, have been imputed for the truncated peptide that results from termination at codon 46 or for the bypass sequence itself. Moreover, how this unusual mRNA organization arose and why it is maintained have never been explained.

We show here that a homing endonuclease (MobA) is encoded in the insertion that created gene 60, and the mobA gene together with the bypass sequence constitute a mobile DNA cassette. The bypass sequence provides protection against self-cleavage by the nuclease, whereas the nuclease promotes horizontal spread of the entire cassette to related bacteriophages. Group I introns frequently provide protection against self-cleavage by associated homing endonucleases. We present a scenario by which the bypass sequence, which is otherwise a unique genetic element, might have been derived from a degenerate group I intron.

bacteriophage gene structure | group I intron | horizontal gene transfer | ribosomal frameshifting | mobile genetic element

In most of the phages of the T4 superfamily, the large subunit of DNA topoisomerase is a single polypeptide encoded by gene 39. However, in phage T4 this gene is disrupted by a 1-kb insertion, creating a truncated gene 39 plus a new gene (gene 60) that encodes the remainder of the topoisomerase subunit (1, 2) (Fig. 1). Furthermore, the C terminus of the truncated gene 39 and the N terminus of the newly created gene 60 each contain additional amino acid residues (43 and 30, respectively) that were not present in the original gene 39 homologs. These two independently translated proteins interact with the small subunit (encoded by gene 52) to create an enzymatically active topoisomerase (4). A second, 50-nt insertion beginning with an in-phase stop codon, is inserted into the newly created downstream gene 60 (2). Instead of terminating at this stop codon, approximately half of the ribosomes skip 50 nucleotides and continue translation in a new reading frame (5). Features involved in this remarkable process include: the stop codon, the codon at which translation resumes, a portion of the pre-hop nascent peptide, a stem-loop at the start of the bypassed sequence, a Shine/Dalgarno-like sequence within the bypassed RNA, and the structure of the bypassed mRNA (5–8).

The genome sequence of phage T4 (GenBank accession no. NC_000866) indicated that the insertion into gene 39 contains two ORFs: an apparent H-N-H homing endonuclease pseudogene (mobA), whose inferred translation product terminates after only 37 codons (Fig. 2), and gene 60.1, which overlaps the start of gene 60 and encodes a basic protein of 126 amino acids with no relatives in the databases. Interestingly, a screen of independently isolated phages related to T4 revealed several (5/37) having the same architecture in their large subunit topoisomerase genes. Although DNA sequences of the ~1-kb insertions were not provided, each newly created gene 60 contained a ribosome bypass sequence identical to that of T4 (9, 10).

Results and Discussion

MobA Is a Site-Specific Endonuclease That Nicks T2 DNA Close to the Insertion Site of the T4 Bypass Sequence. Thinking that one of the insertions initially identified by Repoila et al. (9) might encode an intact mobA gene, we sequenced the PCR products of the gene 39/60 regions of two of them (phages Pol and SKX, provided by Henry Krisch, Laboratory of Microbiology and Molecular Genetics, CNRS, Toulouse, France) and resequenced this region from phage T4. Surprisingly, the insertions in all three phages are almost identical, encoding a single ORF of 271 amino acids (including both the mobA H-N-H domain and g60.1) that is highly similar to the other phage T4 H-N-H family homing endonucleases1 (Fig. 2). The SKX and Pol mobA sequences are identical; the only differences from the T4 sequence are a synonymous G-to-A third position substitution in codon 156 and an A-to-G substitution at the second position of codon 169, changing threonine to alanine.

Free-standing homing endonucleases (i.e., those not encoded within a group I intron or intein) cleave the DNA of close relatives lacking the endonuclease gene, usually in a gene adjacent to the site of endonuclease gene insertion (12–14), and are copied into the recipient genome via recombination-mediated DNA repair, a process that has been called “homing” (for recent reviews, see refs. 14 and 15). Therefore, we tested the protein product of the mobA gene for endonuclease activity on DNA from phage T2, which has an interrupted gene 39.

The mobA gene of phage T4 was expressed in vitro using cell-free extracts and also in vivo by induction of plasmid expression vector pMobA. Incubation of either protein preparation with a

*Author contributions: R.P.B., Q.Z., and D.A.S. designed research; R.P.B. and Q.Z. performed research; E.V.A. contributed new reagents/analytic tools; R.P.B., Q.Z., and D.A.S. analyzed data; and R.P.B., Q.Z., and D.A.S. wrote the paper.

The authors declare no conflict of interest.

†This Direct Submission article had a prearranged editor.

‡R.P.B. and Q.Z. contributed equally to this work.

§Present address: Civil and Environmental Engineering, Massachusetts Institute of Technology, 15 Vassar Street, Cambridge, MA 02139.

¶Present address: Thomas Jefferson University, 233 South 10th St., Philadelphia, PA 19107.

*To whom correspondence should be addressed. E-mail: shub@albany.edu.

This article contains supporting information online at www.pnas.org/lookup/suppl/doi:10.1073/pnas.1107633108/-/DCSupplemental.

1After this work was completed, the phage T4 genomic sequence was redetermined (accession no. HM_137666) with mobA sequence identical to that presented here (11). The inaccuracies in accession NC_000866 were undoubtedly due to the common practice at that time of cloning genes in unregulated plasmids before sequencing. Genes like mobA, whose products are highly toxic to E. coli, were likely to have suffered inactivating mutations at the cloning step.
PCR-amplified region of T2 gene 39 generated a specific nick on the template (bottom) strand (Figs. 3 and 4B), but the proteins were inactive on the homologous T4 sequence (Fig. 4B). Introduction of a UAG stop codon at Tyr195 (Fig. 2) abolished activity (Fig. 3). To confirm that this activity was indeed encoded in T4 (and not in some other phage contaminant), we used DNA from a mutant T4 phage in our collection that has a deletion (sacΔ9) (16) located distantly from the g39/60 locus as template for in vitro protein synthesis. This protein product displayed similar nuclease activity on T2 DNA as when our wild-type T4 DNA was used to program the reaction (Fig. 3). Nucleotide resolution mapping of the cleavage position showed that MobA cleaves T2 DNA 19 bp upstream of the insertion site of the bypass sequence in T4 gene 60 (Fig. 4A and C).

**Function of the Bypass Sequence Is Prevention of Self-Cleavage by MobA.** The close association of the cleavage site and the bypass insertion site suggested that the bypass sequence might play...
and the gene from the donor plasmid (Table 1 and Fig. S1), a frequency consistent with homologous recombination between the gene 39/60 sequences on the phage and the donor plasmid.

Although models for homing invoke unidirectional gene conversion (with coconversion of flanking DNA) resulting from repair of double-strand breaks in DNA, the homing activity of nicking endonuclease MobA is not surprising. Although the mechanism remains to be elucidated, other nicking endonucleases of the H-N-H family have been shown to trigger highly efficient homing events (19).

**Origin of the Bypass Sequence.** The results presented here provide an explanation for the function (prevention of self-cleavage by MobA) and persistence (spread by horizontal gene transfer) of the ribosome bypass sequence. However, we still have no convincing evidence for how this remarkable element came to be inserted into gene 60 in the first place. Although programmed translational framshifts of one or a few nucleotides have been repeatedly encountered in bacteria and eukaryotes, nothing remotely close to the 50-nt ribosomal hop in T4 gene 60 has been substantiated in any other biological system (20). One possible scenario, consistent with the rarity of bypassing, is suggested by similarities of this system to the recently described phenomenon of “collaborative homing” of homing endonucleases and group I introns (13).

Intron-encoded homing endonucleases are protected from cleaving their own genomes by disruption of the DNA-binding/cleavage site by the intron (15) (Fig. 5A), whereas most intronless (or free-standing) homing enzymes have sequence polymorphisms at their target sites that prevent cleavage (17) (Fig. 5B). In collaborative homing, an intron (lacking its own homing endonuclease gene) is inserted into the target sequence of a free-standing homing endonuclease, thereby preventing self-cleavage by the enzyme (13) (Fig. 5C). When a related sensitive DNA sequence is cleaved, both the intron and endonuclease gene are incorporated into the recipient during recombination-dependent DNA repair. The relationship between mobA and the bypass sequence appears to be a variation of the collaborative homing theme (Fig. 5D), which raises the interesting possibility that the bypass may have once been a functional group I intron.

Despite the lack of sequence relatedness between the bypass sequence and any known intron, there are suggestive similarities in their structures and insertion sites. Like the bypass sequence, the 5′ ends of many bacteriophage introns begin with an in-frame stop codon, which is part of the base-paired stem (P1) with which the 5′-terminal part of the 5′-flanking DNA (anking DNA) resulting from recombination is inserted into a highly conserved sequence of an essential protein, the Mg2+-binding region at the active site of type II topoisomerases (26) (Fig. 1B).

These considerations, together with the results presented here, lead us to propose the following hypothesis as a plausible scenario for origin of the bypass. It is possible that the bypass sequence was derived from a group I intron that engaged in collaborative homing with free-standing endonuclease MobA. The intron may have suffered a large deletion that prevented splicing but, adventitiously, the conformation of the remaining RNA (8) may have permitted ribosomal bypassing. Unlike most other “essential” T4 genes, conditional lethal mutations in gene 39 still allow a significant yield of progeny phage under non-permissive conditions (27). So, despite the negative selection...
implied by reduced translation efficiency, the bypass could have been maintained because of its essential relationship with mobA, with continued selection favoring increased efficiency of ribosome hopping. In this context, it is interesting to note that a portion of the nascent gp60 peptide chain upstream of the bypass sequence, which is required for efficient ribosome hopping (6, 7), is not homologous to a segment of the original gene 39, but is contributed as part of the 1-kb insertion that includes mobA (Fig. 1A).

.mobA is one example of a homing endonuclease-like gene that disrupts the coding sequence of an essential gene without destroying its function (1), and two other examples have recently been described in phages belonging to the T4 superfamily. In one case, a GIY-YIG family endonuclease gene disrupts DNA polymerase (28, 29); in the other case, a H-N-H family endonuclease gene disrupts the large subunit of ribonucleotide reductase (30). In all three cases, the products of the split genes reunite to form enzymatically active proteins. In the ribonucleotide reductase case, this requires peptide sequences that had been added to the ends of the split proteins at the site of the endonuclease gene insertion (31). The C terminus of T4 gp39 and the N terminus of gp60 also have extensive amino acid sequences (43 and 30 residues, respectively) that are absent in the intact homologs (Fig. 1B), and it has been suggested (31) that these may also play a role in assembly of the split proteins into an active enzyme.

<table>
<thead>
<tr>
<th>Table 1. Homing frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Expression plasmid</td>
</tr>
<tr>
<td>pMobA</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>pF-CphI</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>pBAD</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>

*Cells also contain the donor plasmid pT4 (39-60ΔmobA).
We have shown here that mobA can be spread by horizontal transmission, and it is likely that other similar constructs will also be shown to practice homing. Recent metagenomic analysis of environmental DNA samples has yielded additional examples of genes split by insertion of putative homing endonucleases (32), indicating that the infectious splitting of essential proteins may be a more common phenomenon than previously thought.

Materials and Methods

Oligonucleotides. Restriction sites are underlined in the sequences listed below.

<table>
<thead>
<tr>
<th>Oligonucleotide</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>T2hop1</td>
<td>TCGGACGCTTATGCTTTCTCGCTATGTAAGAGATTTATTCGGG</td>
</tr>
<tr>
<td>T4hop1</td>
<td>TTCTGGCTCATTGCTTTCTCGCTATGTAAGAGATTTATTCGGG</td>
</tr>
<tr>
<td>T4hop2</td>
<td>TCCGAGCAGAGAAGGATAAATAGAACCTAATCCATCGTGATCTG-</td>
</tr>
<tr>
<td>T2T4-3</td>
<td>AAAATTAATACGACTCACTATAGGGATCCAAGCCATAGGGAGGACA-</td>
</tr>
</tbody>
</table>
| T2hop2 | GCTATTATGACAGACGCAGATCACGATGGATTAGGTTCTA |}

Plasmid Construction. For expression of MobA, T4 mobA was amplified by PCR with KOD HiFi DNA polymerase (Novagen) using primers EVA7 and EVA8, digested with Pfl and EcoRI and ligated into similarly digested pBAD/Myc-HisA vector (Invitrogen) to create pMobA. The 5′-rbp bacterial bypass from phage T4 was inserted in a fragment of T2 gene 39 by the SOEing PCR method (33) with KOD HiFi DNA polymerase using primers T2hop4, T4dhop4, T2hop2, T2T4-3, T2hop4, and T4dhop4, and ligated into similarly digested pBAD/Myc-HisA vector.

Endonuclease Assays. Cleavage site mapping was performed as described by Quirk et al. (18). Briefly, E. coli LMG194 cells harboring plasmids mobA (donor) and either pMobA, pF-CphI (35) or pBAD/Myc-HisA (the empty expression plasmid) were induced with increasing concentrations of i-arabinose on H agar plates at 30 °C for 2 h. Serial dilutions of T2 phage were spotted onto the plates and incubated overnight at 30 °C. The dilution that had almost cleared was scraped off, mixed with 1 mL buffered saline, shaken at 37 °C for 1 h, and diluted for single plaques on H plates. Plaques were transferred to a gridded lawn, and homing products were detected by plaque hybridization (17) using oligo lattes. Cells were harvested by centrifugation at 6,000 × g for 20 min, resuspended in equal volume ice-cold lysis mixture (50 mM Tris HCl, pH 7.2, 1 mM EDTA, 1 mM PMSE, 2 μg/mL leupeptin, and 200 mM KCI), sonicated to complete lysis and directly used in endonuclease assays.

Generation of DNA Substrates. Individually 5′-end-labeled targets were generated by 5′ end labeling (with [γ-32P]ATP and T4 polynucleotide kinase) one of the oligonucleotides before use in PCR together with an unlabeled primer partner (34). PCR was performed with Taq DNA polymerase as described above.

Endonuclease Assays. Endonuclease assay reactions (10 μL) containing 2 μL crude cell extract and 4 μL DNA substrate were incubated at 30 °C for 30 min in 0.05 M NaCl, 0.05 M Tris (pH 7.5), and 0.5 μg poly(dC-dC). Reaction products were extracted with an equal volume of phenol before separation on a 4% denaturing polyacrylamide gel.

Clearing Site Mapping. The location of the MobA cleavage site was mapped, as previously described (34), on the bottom strand of the T2 gene 39 using substrate amplified from pMobA54 with labeled M13fwd primer and unlabeled M13rew primer. PCR amplification, 5′ end-labeling, and endonuclease conditions were the same as described above. Cleavage products were separated by electrophoresis on 4% denaturing polyacrylamide gel next to sequencing ladders generated using the same template DNA and labeled primers.

Plasmid to Phage Homing. Plasmid-to-phage homing assays were performed as described by Quirk et al. (18). Briefly, E. coli LMG194 cells harboring plasmids mobA (donor) and either pMobA, pF-CphI (35) or pBAD/Myc-HisA (the empty expression plasmid) were induced with increasing concentrations of i-arabinose on H agar plates at 30 °C for 2 h. Serial dilutions of T2 phage were spotted onto the plates and incubated overnight at 30 °C. The dilution that had almost cleared was scraped off, mixed with 1 mL buffered saline, shaken at 37 °C for 1 h, and diluted for single plaques on H plates. Plaques were transferred to a gridded lawn, and homing products were detected by plaque hybridization (17) using oligo lattes. Cells were harvested by centrifugation at 6,000 × g for 20 min, resuspended in 0.02% NaCl, 0.05 M Tris (pH 7.5), and 0.5 μg poly(dC-dC). Reaction products were extracted with an equal volume of phenol before separation on a 4% denaturing polyacrylamide gel.

Acknowledgments. This work was supported by the Northeast Biodefense Center through National Institutes of Health Grant U54-AI057158 and by a research development award from the College of Arts and Sciences, University at Albany.


Bonocora et al. & PNAS | September 27, 2011 | vol. 108 | no. 39 | 16355

PNAS