A Bacterial Survival Strategy Involving Degraded DNA

Researchers show that transformable bacteria can incorporate pieces of short DNA into their genomes, even when the DNA is centuries old.

Introduction

As one of earth's earliest inhabitants, bacteria have learned a thing or two about survival. Over billions of years, they have become ubiquitous in the environment and taken on various roles. Some wreak havoc on more complex organisms (*Mycobacterium tuberculosis*), while others benefit humanity by producing antibiotics (*Streptomyces*), enhancing the environment through oxygen production (cyanobacteria) and nitrogen fixation (Rhizobium), or feeding on crude oil spills (*Vibrio parahaemolyticus*).

Most bacteria are heterotrophs and act as scavengers, feeding off the leftover carbohydrates and proteins other organisms leave behind. For environmental bacteria, the DNA of decomposing plants and animals offers high nutritional value. There is also plenty of scientific evidence that bacteria absorb free DNA from the environment and integrate it into their genomes. The process of horizontal gene transfer by natural transformation is well-described for high molecular–weight DNA (fragments ≥ 10,000 bp), where it is mediated by RecA, a DNA repair protein. Until recently, there was no evidence that natural transformation occurred with the shorter, damaged DNA fragments (< 200 bp) that are more abundant in the environment.

Post-doctoral researcher Søren Overballe-Petersen, Ph.D., and teams at the University of Copenhagen and the University of Tromsø, decided to investigate whether bacterial transformation of shorter DNA fragments was possible. "DNA is all around us in the environment, especially in soils and sediments," states Dr. Overballe-Petersen. "It's degrading over time and existing alongside bacteria and other microorganisms. We wondered if any of this old, fragmented DNA could transform bacteria."

Søren Overballe-Petersen, Ph.D. is a post-doctoral researcher at the University of Copenhagen. He is holding the femur of a woolly mammoth that roamed the earth over 40,000 years ago.

Photo courtesy of Anders Fjeldberg

Their research showed that it was possible for bacteria to integrate pieces of DNA ≥ 20 bp, even the truly ancient DNA of a 43,000-year–old woolly mammoth[1]. iCommunity spoke with Dr. Overballe-Petersen about the team's research and its possible impact on our view of bacterial evolution.

Q: Why was it assumed that horizontal gene transfer could only occur with long DNA fragments?

Søren Overballe-Petersen (SOP): Up until recently, people couldn't even see extremely short DNA fragments, so they initially focused on long fragments. It turns out that bacterial natural transformation is highly efficient with kilobases of long DNA. When the DNA is shorter than a few kilobases, 1 to 2 kilobases roughly, the efficiency drops very fast. Even though it was shown to occur with fragments down to about 300 bp, there was such a steep decrease in efficiency that people didn't think that the process was relevant.

However, there's much more short and degraded DNA in the environment than long fragments. In fact, most free DNA fragments are < 100 bp. Despite continuous degradation, these short fragments persist for thousands of years. They're often released as the environment changes, such as when coastlines are worn away by storm tides, sediment is released by overflowing rivers, or as glaciers recede and expose land masses. With such a high amount of short DNA in the environment, we wanted to see if it could transform bacteria.

Q: Why did you choose Acinetobacter baylyi *for this study?*

SOP: *Acinetobacter baylyi* is a strain of soil bacteria that can be found in almost any environment and is part of the normal flora of many land and aquatic animals. *A. baylyi* doesn't have any special requirements to flourish and performs natural transformation under normal conditions. That's not true for all bacteria.

Q: What did you find when you exposed A. baylyi *to varying concentrations of modern, fragmented DNA?*

SOP: Previously, researchers had seen natural transformation occur with DNA fragments down to 250 bp. We wanted to take that further and find the lower limit of fragment size that could still be transformed in a bacterium. It surprised us that we could detect transformations down to 20 bp. It means that any remnant of DNA has a chance of transforming bacteria.

Q. Did the transformation frequencies change as you used smaller fragment sizes?

SOP: We saw a steep decrease in transformation frequencies as we moved from long sequences of several kilobases to shorter ones, until

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGACG
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA

we reached 200 bp. From 200 to 20 bp, the transformation frequency plateaued and stabilized.

We decided to test whether this plateau was due to the presence of RecA, the protein that initiates homologous recombination and integrates DNA into the genome. We performed a duplicate set of experiments with a RecA-deficient strain of *A. baylyi* and saw the same decrease in transformation frequencies and the plateau between 200 and 20 bp. This confirms that RecA is not responsible for the plateau and suggests that the transformations are the result of another, simpler variation of natural transformation that is not dependent on homologous recombination. It has been overlooked because classical natural transformation is so highly efficient with long-variant DNA, offering about 10,000-fold higher efficiency than the basal level of transformation we identified.

While basal transformation isn't as efficient as classical natural transformation, we showed that it happens in cells that are living and growing in an environment where they need DNA. It could be an original type of horizontal gene transfer.

Q. Did the transformation frequencies change when you used short, damaged fragments of DNA?

SOP: Surprisingly, damaging the short fragments with uracils, cross-links, base-loss, nicks, gaps, or tails in the DNA substrates had very little effect on transformation frequency. They were incorporated just as easily as undamaged fragments.

Q: Did the bacteria repair the damaged DNA fragments as they were incorporated?

SOP: For the most part, yes. Bacteria have repair systems that handle normal genome replication. It was clear that they repaired the atypical damage, but not at 100% efficiency. That opens up two possibilities. When damaged DNA fragments in the environment are incorporated into bacteria, they may introduce a new sequence into the bacterial genome. They may also introduce a variant of the damaged DNA sequence, generating a completely new diversity.

Q: What made you choose to use woolly mammoth DNA in your study?

SOP: We know that much of the free DNA in the environment is thousands of years old, potentially up to one million years old. We wanted to test if ancient DNA could be integrated into bacterial genomes in the same way as modern, damaged DNA.

Acquiring a large amount of authentic ancient DNA isn't easy, but we realized we needed as much true ancient DNA as possible. The more DNA we could feed into the experiment, the better chances of achieving a transformant. We also wanted to use the DNA of an extinct animal, because that eases the authentication of the sequences.

The ideal would have been using ancient bacterial DNA. Finding and analyzing ancient bacterial DNA, and authenticating it as true ancient and not a modern contamination would have been extremely difficult. So that was not practical.

Being an ancient-DNA research lab, we have access to large woolly mammoth bones. We thought that by using the DNA of an extinct animal it would be clear that there weren't any contamination issues. We can prove that we don't have a woolly mammoth walking around our laboratory.

Q: What did the results of the experiment with the woolly mammoth DNA show compared to what you found with the modern, fragmented DNA?

SOP: The 43,000-year–old ancient woolly mammoth DNA could still transform the bacteria, with the damaged DNA integrating successfully into *A. baylyi*. We observed the same transformation frequencies as we had with the modern, fragmented DNA.

The experiment confirmed, that at the molecular level, ancient DNA is nothing special. It's just short fragments of damaged DNA, no different than the modern, damaged DNA fragments we synthesized.

> "We think that bacteria feed on DNA, and through basal transformation sometimes integrate short DNA into their genomes."

Q: Were there any differences in the bacterial sequences that had been transformed with the short DNA fragments?

SOP: Our data showed that short DNA transformations increased the possibility of double nucleotides being incorporated over single nucleotides. The transformation frequencies for short DNA molecules increased 50-fold for DNA molecules containing two neighboring single nucleotide variants (SNVs) or what we call double nucleotide variations (DNVs). We found that adjacent nucleotide mismatches escape the DNA mismatch repair mechanism. DNVs therefore have a higher likelihood of successful recombination. If these mutations are neutral or advantageous, they may accumulate as double nucleotide polymorphisms (DNPs) in naturally transformable bacterial populations over time.

We decided to test this by investigating the prevalence of single and double polymorphisms in transformable versus nontransformable bacterial species. We accessed GenBank and acquired a data set of bacterial genomes. However, we were uncertain about the quality of these genomes and their consistency. They were published by different people, sequenced at different times with different sequencing technologies, and assembled using different algorithms. We were concerned that the sequence patterns could be affected if there were biases from the different methods, sequencing, and analytical tools used.

To test the quality and validate the GenBank data set, we obtained 91 GenBank genomes and resequenced 25 of the strains using the HiSeq® 2500 System.

After the DNA samples were prepared, it was about a month before we had the final results. It took longer to collect all the different DNA samples from around the world than it took to sequence the DNA.

Q: Why did you choose the HiSeq 2500 System to sequence the GenBank samples?

SOP: It offers the ability to multiplex 25 bacterial genomes in one lane. There were no differences in the way samples were handled or in the way they were sequenced. They were sequenced together in the same run and even in the same lane because of the high amount of data that Illumina sequencing generates. The downstream data analysis became an extension of one continuous process, using the same algorithms to analyze each sample.

Sequencing at 20× would have been sufficient, but we wanted to ensure that we had enough data for all strains in one run. For most of the samples, we obtained over 100× coverage. At that high coverage, we could confidently identify SNPs and indels.

The HiSeq 2500 data quality was wonderful. We confirmed that most of the sequences could be trusted. We had several samples where there was a complete match to the original sequence. We identified a few of the original genomes that had relatively high error rates and validated that they did not affect the result of the analysis.

> "The 43,000-year–old ancient woolly mammoth DNA could still transform the bacteria, with the damaged DNA integrating successfully into *A. baylyi*."

Q: What did the results of your comparison of transformable and nontransformable bacterial species show?

SOP: It confirmed our hypothesis that transformable bacterial species have an increased proportion of DNPs. The nontransformable bacterial species had a much lower proportion of DNVs to SNVs and a much higher proportion of multiples, defined as three to six adjacent polymorphisms, than DNPs.

Q: Why would bacteria develop this form of basal transformation?

SOP: Bacteria need to conserve energy. Unlike eukaryotic cells, bacteria don't have mitochondria to provide plentiful energy and fuel their processes. As a result, bacteria don't have an energy surplus that enables them to keep the DNA they aren't using. From an energy standpoint, it's expensive for bacteria to carry around extra DNA and keep synthesizing it. There's a distinct competitive advantage for bacteria to lose extra DNA.

This creates an environment where bacteria are in contact with a huge amount of genetic variation, from small DNA fragments released by once thriving dead bacteria. We think that bacteria feed on DNA, and through basal transformation sometimes integrate short DNA into their genomes. It's a simple process that is a consequence of living and competing in the microbial world.

Q: Could these genetic modifications be carried forward from one bacterial generation to the next?

SOP: It's possible, but we need to perform more experiments to determine this. The recombination of short DNAs, even highly degraded, ancient fragments, demonstrates just the first step along this path. We believe that this process occurs in all types of environments. How often it occurs, I can't tell you, but hope to be able to in the future.

It's possible that the frequency of transfers isn't as important as the effect of the transfer. It doesn't matter if a bacterium completes 1,000 transformations that turn into nothing. If one transfer gives it an advantage, that's the one that counts.

Q: What role could basal transformation play in bacterial evolution?

SOP: Our research changes the way we understand how bacteria behave and opens up some interesting possibilities concerning bacterial evolution. We've demonstrated that bacteria that are actively living and growing in an environment will ultimately die and release DNA that degrades into short fragments. Those pieces of DNA can be taken up by other bacteria and incorporated into their genomes, generating new diversity. It's possible that the higher the turnover, the more DNA will be released into the environment, generating even more diversity. There could be a positive feedback loop where high biological activity amplifies the generation of diversity. From a biological view, this concept is quite thought provoking.

Another possibility concerns genetic transfer. In recent years, there have been several studies looking into how cellular life could have evolved rapidly to begin with and then stabilized, turning into life as we know it today. These studies and simulations show that there must have been DNA transfer between early primitive cells, but up to this point we've been unclear as to how this could happen.

We believe basal transformation employing short DNA uptake is an inherent bacterial process and is the mechanism that explains how early cells exchanged genetic information.

> "The HiSeq 2500 System offered the ability to multiplex 25 bacterial genomes in one lane."

Q: Could basal transformation be responsible for what is often perceived as spontaneous mutations?

SOP: I think a fraction of what we call spontaneous mutations are actually the result of basal transformation. Certainly, the results look exactly the same.

There's also the possibility that this process occurs within a cell, where DNA is being repaired and small DNA pieces are being excised from the genome. If the DNA isn't degraded immediately, the fragment could move around in the cell and come in contact with a completely

unrelated part of the genome, changing the sequence there. That's an area of DNA dynamics and metabolism that we haven't given much consideration to before.

Q: Could the basal transformation process be responsible for microbial evolution in hospital settings?

SOP: Often the reason that a patient is in the hospital is that they have an antibiotic-resistant infection and the normal course of treatment has failed. If you keep bringing people in with the worst kind of infections, you're increasing the likelihood that the DNA fragments from dead, antibiotic-resistant bacteria contaminate surfaces. Certain types of antibiotic resistance are defined by only one or two nucleotide changes in a sequence. Basal transformation could explain how short DNA fragments are transferred into bacteria, imparting resistance.

This could change how we maintain hospital areas, such as patient rooms and surgical suites. We now focus on removing the bacteria, but if bacterial basal transformation is occurring, that won't be enough to sterilize the environment. We also need to destroy the DNA that could be imparting antibiotic resistance. The two best options for degrading DNA completely are to use UV light on any potentially infected areas or wash them in chlorine solution.

Q: What are the next steps in your research?

SOP: I would like to investigate whether basal transformation creates a positive feedback loop in environments where there's high bacterial turnover, increasing bacterial diversity.

I'd also like to investigate the role basal transformation may have in bacterial evolution. We have difficulty in understanding and modeling microbial evolution because complex things are happening in microbes that we don't understand. It's possible that basal transformation is introducing DNA from previous bacterial generations and that's what makes the models fail.

References

1. Overballe-Petersen S, Harms K, Orlando LA, Mayar JV, Rasmussen S, et al. (2013) Bacterial natural transformation by highly fragmented and damaged DNA. Proc Natl Acad Sci USA 110: 19860–19865.

Illumina, Inc. • 1.800.809.4566 toll-free (U.S.) • +1.858.202.4566 tel • techsupport@illumina.com • www.illumina.com

FOR RESEARCH USE ONLY

© 2014 Illumina, Inc. All rights reserved.
Illumina, HiSeq, and the pumpkin orange color are trademarks or registered trademarks of Illumina, Inc. in the U.S. and/or other countries. All other brands and names contained herein are the property of their respective owners.
Pub. No. 1270-2014-001 Current as of 17 March 2014

illumina®

AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
TCAACGTACCGTAACGAACGTATCATTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTT
CGACGAAAAGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACC
AACGTACCATTAAGAGCTACCGTGCAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGACGAAAAGAATGATAA
AGAATGATAACAGTAACACACTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAAC
GATTACTTGATCCACTGATTCAACGTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGCTTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAGCAACGAGC
CGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGTCTGTTAACCTTAAGATTACTTGATCCACTGATTCAACGTACCGTAACGAACGTATCAATTGAGACTAAATATTAACGTACCATTAAGAGCTACCGTGCAACGAAAAGAATGATAACAGTA