Response to Questions Concerning the Science Article, “A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus”
-As of December 16, 2010-

A research article published December 2, 2010 by the journal Science provided several lines of evidence, collectively suggesting that a bacterium isolated from California’s Mono Lake can substitute arsenic for a small percentage of its phosphorus and sustain its growth.

This finding was surprising because six elements—carbon, oxygen, hydrogen, nitrogen, sulfur and phosphorus—make up most of the organic molecules in living matter, including nucleic acids, proteins and lipids. Scientists not affiliated with the research team have therefore asked appropriately challenging questions about the research.

A key purpose of scholarly publication is to advance science by presenting interesting data and proposing testable hypotheses. Understandably, the most surprising findings tend to generate the most intense response and scrutiny from the scientific community. Post-publication responses to original research, and efforts to test and replicate the results, especially in cases of unexpected findings, are an essential mechanism for advancing scientific knowledge.

Science editors have now received a number of technical comments and letters responding to the article, “A Bacterium That Can Grow by Using Arsenic Instead of Phosphorus,” by Felisa Wolfe-Simon and colleagues. The comments and responses will undergo review, and we will publish them in a future issue of Science.

Meanwhile, in an effort to promote public understanding of the work, the research article and a related news piece have been made freely available to the public via the Science Web site for the next month. These articles can be found online here:
Research article: http://www.sciencemag.org/content/early/2010/12/01/science.1197258.full.pdf
News article: http://www.sciencemag.org/content/330/6009/1302.full

The Wolfe-Simon team, theorizing that perhaps some bacteria might be able to use arsenic or tolerate some substitution for phosphorus in organic molecules, collected microbes from arsenic-rich Mono Lake and then gradually weaned them off phosphorus, feeding them arsenic instead. The team has reported that they took steps to rule out any phosphorus contamination. They concluded that their evidence suggested arsenic had replaced a small percentage of the phosphorus in their DNA.

Various types of evidence were described by the authors, including:

- Inductively coupled plasma mass spectrometry.
  The authors reported that these results revealed arsenic was inside bacterial cells, suggesting it was not merely a contaminant stuck to the exterior of the cells;
- Radioactive labeling of arsenic.
  Wolfe-Simon’s team said this evidence allowed them to spot the normally toxic substance within the protein, lipid, nucleic acid and metabolite fractions of the cells, suggesting it had been taken into molecules forming each fraction.
- High-resolution secondary ion mass spectrometry of the DNA after it had been separated from the bacteria.
  The authors reported that this evidence suggested the isolated DNA still contained arsenic.
- High-intensity (synchrotron) X-ray analysis.
  Based on this evidence, the authors concluded that arsenic in the bacteria did appear to be replacing phosphates in DNA and other molecules.
Questions about the findings have tended to focus on whether the bacteria had truly incorporated arsenic into the DNA and whether the microbes had completely stopped consuming phosphorus. While the team prefers to address questions through a peer-reviewed process, Felisa Wolfe-Simon and Ron Oremland have provided some additional information here as a public service, and to clarify their data and procedures. *Science* emphasizes that these responses have not been peer-reviewed; they are provided on behalf of the authors only as a public information service while more formal review of their responses to comments sent to *Science* continues.

**Preliminary Q&As**

*Question:*

*Some people have questioned whether the DNA was sufficiently cleaned by your technique using gel electrophoresis, to separate it from other molecules. Do you feel this is a valid concern?*

*Answer:*

Our DNA extraction and purification protocol begins with washed cells, pelleted from media. These are then subjected to a standard DNA extraction protocol, which included multiple phenol chloroform steps to remove impurities, including any unincorporated arsenate (As). After this, the DNA was electrophoresed, further separating the DNA from impurities. Any residual As from the media would have been removed by washing the cells prior to extraction and by partitioning into the aqueous phase during the 3 phenol:chloroform steps in the extraction. If As was incorporated into a lipid or protein it would have partitioned into the phenol, phenol:chloroform, or chloroform fractions. Additionally, DNA extracted in this manner on other samples was also successfully used in further analyses, including PCR, that require highly purified DNA.

The arsenic measured by NanoSIMS in the gel band is consistent with our other measurements and another line of evidence.

Our radiolabeled $^{73}\text{AsO}_4^{3-}$ experiment showed that of the total radiolabel associated with the cell pellet 11.0 % ± 0.1 % was associated with the DNA/RNA fraction. This indicated that we should expect some arsenate of the total pool associated with the nucleic acids. To interpret these data, we coupled our interpretation with our EXAFS evidence suggesting that intracellular arsenic was As(V) bound to C, and was not free in solution as an ion. This suggests the As is in, an organic molecule with bond distances consistent with a chemical environment analogous to phosphate (Figs. 3A, S3 “bond lengths” table). Further supporting our interpretation of the previous mentioned two analyses, we used a third line of evidence from NanoSIMS, a completely different technique from the other two. We find elemental arsenic (as measured by NanoSIMS) associated with the gel band that is more than two times the background in the gel. Based on the above discussion, we do not feel this is a valid concern.

*Question:*

*Others have argued that arsenate-linked DNA should have quickly fallen apart when exposed to water. Could you address this?*

*Answer:*

We are not aware of any studies that address arsenate bound in long chain polyesters or nucleotide di- or tri-esters of arsenate, which would be directly relevant to our study. Published studies have shown that simple arsenic esters have much higher hydrolysis rates than phosphate esters (1-3). The experiments published to date have specifically looked at the exchange or hydrolysis of alkyl tri-esters of arsenate [Eqn. 1] and alkyl di-esters of arsenite [Eqn. 2]:

$$\text{OAs(OR)}_3 + \text{H}_2\text{O} \rightarrow \text{OAs(OH)(OR)}_2 + \text{ROH} \quad [1]$$

$$\text{OAs(OH)(OR)}_2 + \text{H}_2\text{O} \rightarrow \text{OAs(OH)}_2\text{(OR)} + \text{ROH} \quad [2]$$

where R = methyl, ethyl, n-pentyl and isopropyl. Reference 2 demonstrated that the hydrolysis rates for these simple alkyl triesters of arsenate decreased with increasing carbon chain length (complexity) of the alkyl substituent (methyl > ethyl > n-pentyl > isopropyl). No work has been done on the hydrolysis rates of arsenate-linked nucleotides or other biologically relevant moieties.

If the hydrolytic rate trend reported in Ref. 2 continues to larger-weight organics, such as those found in biomolecules, it is conceivable that arsenate-linked biopolymers might be more resistant to hydrolysis than previously thought. The small model compounds investigated in Refs. 1-3 are relatively flexible and can easily adopt the ideal geometry for water to attack the arseno-ester bond. Arsenate esters of large, biomolecules, however, are likely to be more sterically hindered leading to slower rates of hydrolysis.

This type of steric constraint on reaction rate accounts for the wide range of rates seen in the behavior of some phosphate linked nucleotides. In small ribozymes, the phosphodiester linkages at the site of catalysis can be hydrolyzed on the order of tens of seconds (with a chemical rate of 1 s⁻¹). This rate enhancement is achieved by orienting the linkage for in-line attack by a nucleophile (an adjacent 2’ hydroxyl group). Moreover, the autodegradation patterns are consistent with specific base composition. On the other hand, the hydrolysis rates for phosphodiester bonds in A form duplexes of RNA are many orders of magnitude slower, because these linkages cannot access easily the geometry necessary for hydrolysis.

The rates in DNA may be much slower than model compounds because of the geometrical constraints imposed upon the backbone by the helix.

The kinetics of the hydrolysis of arsenate-linked biopolymers is clearly an area where more research is warranted.

**Question:**

Is it possible that salts in your growth media could have provided enough trace phosphorus to sustain the bacteria?

**Answer:**

The data and sample labeling in Table S1 has caused some confusion. To clarify, for every experiment, a single batch of artificial Mono Lake water was made with the following formulation: AML60 salts, no P, no As, no glucose, no vitamins. Table S1 shows examples of ICPMS measurements of elemental phosphorus (~3 µM) and arsenate made on this formulation prior to any further additions. Then we added glucose and vitamins for all three treatments and either As for the +As treatments or P for the +P treatments. The P measurements made on the medium after the addition of glucose and vitamins and after
addition of As were also ~3 µM in this batch. Therefore, it was clear that any P impurity that was measured (~3 µM, this was the high range) came in with the major salts, and that all experiments contain identical P background (including any P brought in with the culture inocula).

In the Science paper, we show data from one experiment of many replicated experiments that demonstrates no growth of cells in media without added arsenate or phosphate (Figure 1). These data clearly demonstrate that strain GFAJ-1 was unable to utilize the 3µM P to support further growth in the absence of arsenate. Moreover, the intracellular P content determined for the +As/-P grown cells was not enough to support the full requirement of P for cellular function.

Note on culturing: All experiments were initiated with inocula from sustained +As/-P conditions. Prior to the experiments, the cells had been grown long term, for multiple generations from a single colony grown on solid media with no added phosphate. Before this, they were grown as an enrichment for more than 10 transfers and always into new medium that was +As/-P. We therefore feel that there is not significant carry-over of P. We also argue that there would not have been enough cellular P to support additional growth based on an internal recycling pool of P.

Question: Is there anything else you’d like for the public to understand about your research, or about the scientific process?

Answer: For all of us, our entire team, what this was like was unimaginable. We are a group of scientists that came together to tackle a really interesting problem. We each used our talents, from technical prowess to intellectual discussion, to objectively determine what exactly was happening in our experiments. We freely admitted in the paper and in the press that there was much, much more work to do by us and a whole host of other scientists. The press conference even included a technical expert, Dr. Steven Benner, who voiced some of the concerns we responded to above. Part of our reason for bringing this work to the community was to make the intellectual and technical connections for more collaborations to answer many of the lingering questions. We were transparent with our data and showed every datum and interesting result. Our paper’s conclusions are based on what we felt was the most parsimonious way to interpret a series of experiments where no single experiment would be able to answer the big question. “Could a microbe use arsenic in place of phosphorus to sustain its growth?” The best science opens up new questions for us as a community and sparks the interest and imagination of the general public. As communicators and representative of science, we feel that support of new ideas with data is critical but also to generate new ideas for others to think about and bring their talents to bear on.

We look forward to working with other scientists, either directly or by making the cells freely available and providing DNA samples to appropriate experts for their analyses, in an effort to provide more insight into this intriguing finding.

References