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**Coping mechanisms of extremophiles to environmental disturbances**

Halophilic archaea can contend with extreme fluctuations in salinity

Nearly all extreme environments, from the frozen tundra of the Arctic to the hot springs of Yellowstone National Park, are inhabited by microorganisms called extremophiles. Extremophiles must contend with extreme heat, salinity, and acidity, as well as drastic variations in these conditions. These environments are worthy of study because they tell us both about the absolute limits of biology, as well as the conditions that may have given rise to early life on Earth. The most prolific extremophiles on Earth are the archaea, an ancient lineage of single-celled microbes. Particularly, the haloarchaea are found in habitats of extreme salinity like the Great Salt Lake, Utah, or the Dead Sea in Israel.

Evolution has supported development of haloarchaea resistant to extreme fluctuations of salt concentration that most other organisms could not deal with. Uncovering the mechanisms of adaptation through tRNA analysis is important to understanding how organisms respond to environmental disturbances.

Ben Farmer, a senior at the University of Kentucky and summer intern in the Rhodes lab at the College of Charleston, is using a haloarchaeal species called *Haloferax sulfurifontis* as a model for salt adaptation. *H. sulfurifontis* is capable of surviving in anything from 6% salinity to the saturation point of NaCl at 37% (more than ten times that of sea water). Drastic swings in salinity in a natural habitat such as a hypersaline lake is often due to rain and evaporation. Like other haloarchaea, *H. sulfurifontis* can survive extreme salt conditions because they have specially equipped proteins.

The protein surface of haloarchaea generally has a high amount of acidic amino acids, and a low amount of hydrophobic (water-fearing) amino acids, which keeps the proteins from unfolding. As salinity increases drastically, these amino acid trends are expected to become more pronounced. tRNAs, the molecules that code for amino acids, likely increase in abundance as their corresponding amino acids do so in the cell. Farmer uses tRNA as an indicator of how the haloarchaea are reacting in real time to the stress of high salinity.

According to Dr. Matthew Rhodes, “nobody has looked at tRNA profiles in the archaea and so the haloarchaea, due to their unique protein composition, offered an attractive place to start.” To look at these tRNA profiles in haloarchaea, Farmer set up a 96-well plate containing DNA sequences, or probes, for the 40+ tRNA found in *H. sulfurifontis* (Fig. 1). Farmer labelled the total tRNA by incorporating a radioactive phosphate (32P) into the living Haloferax cells at different salinities (10, 20, and 30%). The radioactively labelled tRNAs were then introduced to the probes in the microarray. Probes for each tRNA produced signals with different intensities depending on the frequency of that tRNA in the living cells. Farmer produced images of these signals in the form of spots, and the spot intensity was analyzed to determine tRNA abundance.

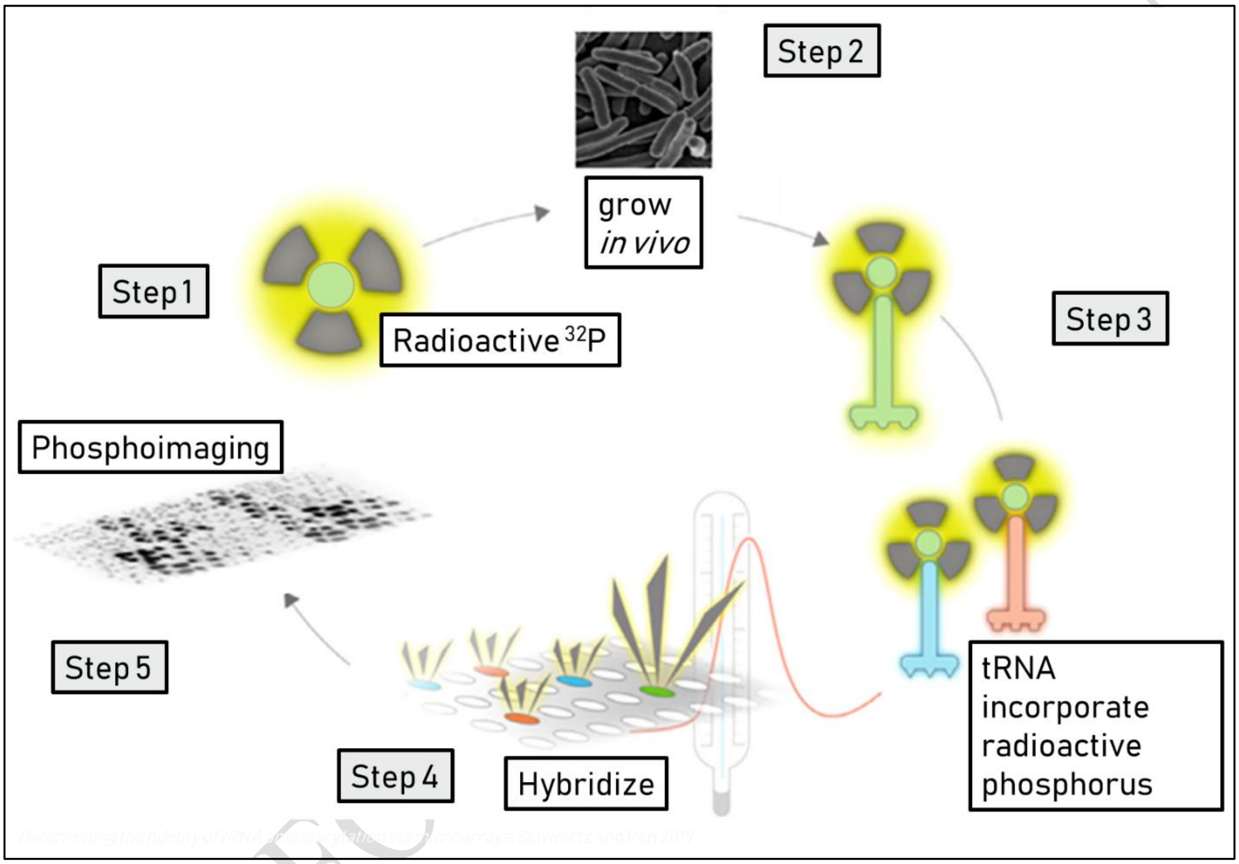


**Fig. 1** Radiolabeling procedure. Radioactive phosphate 32 Phosphorus is incorporated into the growing cells of *Haloferax sulfurifontis*, and all of the new tRNA are labeled. Labeled tRNA is hybridized (introduced) to a 96-well plate containing DNA complements for each tRNA. Phosphoimaging produces a microarray with spots that indicate the abundance of each tRNA in *H. sulfurifontis*.

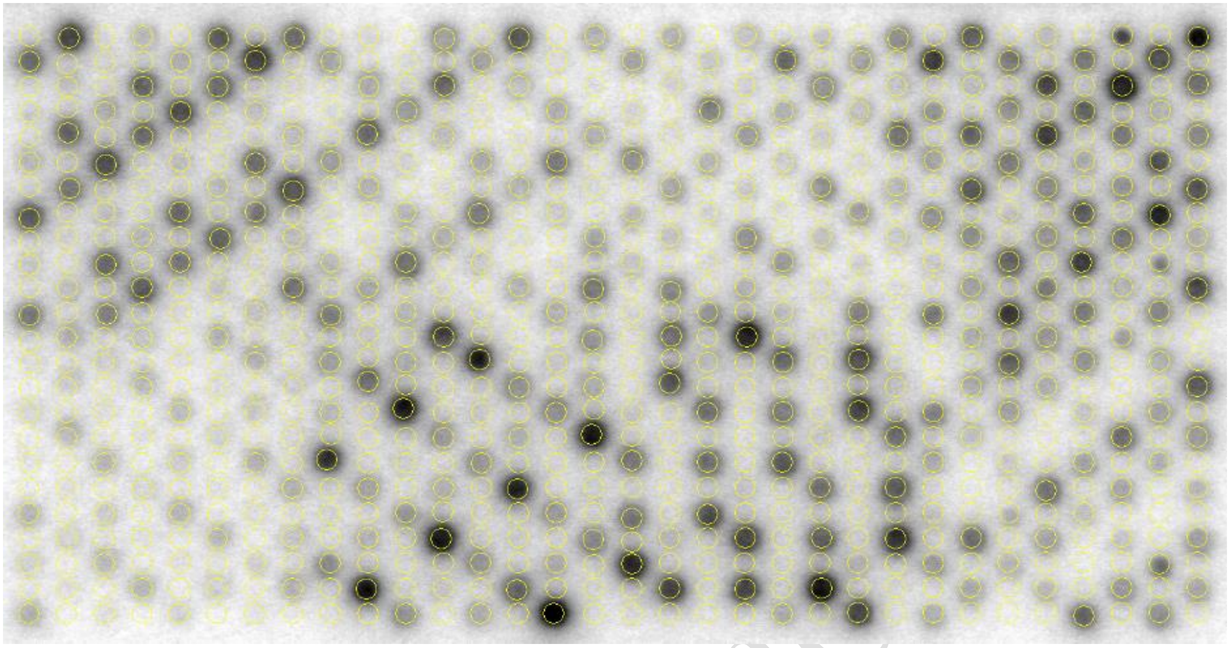

**Fig. 2** tRNA abundance analysis: an example of the spots produced by radioactivity. Each black spot indicates a specific tRNA, and the intensity of the spot tells us the abundance of that tRNA in Haloferax sulfurifontis.

Findings indicate that tRNA are significantly changing between salt conditions, in some cases representing a nearly fivefold change. The haloarchaea responded somewhat as predicted when comparing 20% to 30% salinity (an increase in tRNA coding for acidic amino acids), however the opposite was true from 10% to 20%. It is possible that this trend reversal between salinities indicates that the proteins' halophilicity resides on a sort of bell curve, and that protein acidity does not begin to increase until salinity begins to rise sufficiently. Nonpolar, or hydrophobic, amino acid tRNA also decreased as salinity became more extreme, potentially to allow proper protein folding in high salt content.

All organisms use tRNA, so results found here paint a clearer picture of how extremophilic life responds to environmental fluctuations. The Rhodes lab will continue to perform experiments on three other species of haloarchaea, and consistent results across species will solidify understanding of hypersaline adaptations.

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