**Archaeal horizontal gene transfers to Cyanobacteria constrain methanogenesis to a Paleoarchaean origin.**

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**Introduction:** The timing of the origin of biogenic methane production is a major question in early life and planetary evolution. Methane is a potent greenhouse gas, and its abundance in the Archaean atmosphere is a possible solution to the "faint young sun" paradox on the Early Earth. While generally assumed to be an extremely ancient pathway due to its phylogenetic distribution across most of Euryarchaeota, there is only limited geochemical data that has been interpreted as evidence for Archaean biogenic methane production, in the form of carbon isotopic compositions of kerogens ~2.7 Ga [1] and methane bearing fluid inclusions ~3.5 Ga [2]. No direct fossil or biomarker evidence of methanogens is known to exist from this time.

An alternative method for determining the age of biogenic methane production is to use molecular clocks to place absolute divergence times on archaeal phylogeny, including the ancestor node of known methanogenic lineages. However, to date such estimates have been highly uncertain, as deeper nodes remain uncalibrated, due to an absence of diagnostic fossil or biomarker evidence in the geological record.

Here, we attempt to surmount these obstacles by using horizontal gene transfer (HGT) events from methanogenic lineages to clades with established fossil records and more reliable calibration dates. This propagates these age constraints of recipient nodes to the ancestors of donor lineages within the methanogen phylogeny, improving the precision of molecular clock models. We present the results of this novel method, which we call TARDIS (Transfer Assisted Rate and Divergence time InferenceS).

Critical to this analysis is the HGT of three SMC complex proteins from methanogens to the ancestor lineage of Cyanobacteria. While cyanobacterial SMC proteins were previously detected as being of archaeal origin [3,4], in-depth phylogenetic analysis shows these transfers likely occurred from the clade containing group II methanogens, Halobacteriales, and Archaeoglobales (G2HA). Since previous molecular clock estimates using fossil and geochemical calibration have placed a high probability on the cyanobacterial ancestor predating 2.5 Ga [5], TARDIS constrains the G2HA donor group to be older still, diverging ~2.9 Ga. This calibration propagates to deeper nodes within the archaeal phylogeny, predicting that methanogenesis likely evolved between 3.6 and 3.3 Ga. This genome-based date estimate is consistent with previously proposed geochemical evidence, substantially strengthening the case for biogenic methane production as a major ecological component of the paleoarchaean world. As this time likely substantially predates the origin of oxygenic photosynthesis, biogenic methane oxidation may also have been limited, predicting relatively high atmospheric methane concentrations during the Paleoarchaean.

**References:** [1] K. Hinrichs. (2002) *Geochem Geophys Geosyst., 3*. [2] Y. Ueno et al. (2006) *Nature., 440,* 516-519. [3] J. Soppa (2001) *Gene., 278,* 253-64. [4] N. Cobbe & M.M. Heck (2004) *Mol Biol Evol., 21,* 332-47. [5] B.E. Schirrmeister et al. (2013) *Proc Natl Acad Sci USA. 110,* 1791-6.