Analysis of Cell–Cell Bridges in *Haloferax volcanii* Using Electron Cryo-Tomography Reveal a Continuous Cytoplasm and S-Layer

Shamphavi Sivabalasarma1,2, Hanna Wetzel1, Phillip Nußbaum1, Chris van der Does1, Morgan Beeby3 and Sonja-Verena Albers1,2*

1 Molecular Biology of Archaea, Institute of Biology II, Faculty of Biology, University of Freiburg, Freiburg, Germany, 2 Spemann Graduate School of Biology and Medicine, University of Freiburg, Freiburg, Germany, 3 Department of Life Sciences, Imperial College London, London, United Kingdom

Halophilic archaea have been proposed to exchange DNA and proteins using a fusion-based mating mechanism. Scanning electron microscopy previously suggested that mating involves an intermediate state, where cells are connected by an intercellular bridge. To better understand this process, we used electron cryo-tomography (cryoET) and fluorescence microscopy to visualize cells forming these intercellular bridges. CryoET showed that the observed bridges were enveloped by a surface layer (S-layer) and connected mating cells via a continuous cytoplasm. Macromolecular complexes like ribosomes and unknown thin filamentous helical structures were visualized in the cytoplasm inside the bridges, demonstrating that these bridges can facilitate exchange of cellular components. We followed formation of a cell–cell bridge by fluorescence time-lapse microscopy between cells at a distance of 1.5 µm. These results shed light on the process of haloarchaeal mating and highlight further mechanistic questions.

Keywords: *Haloferax volcanii*, electron cryo-tomography, archaea, horizontal gene transfer, cell fusion, cell fusion, fluorescence microscopy

INTRODUCTION

Horizontal gene transfer is fundamental to archaean and bacterial evolution. The diverse mechanisms of horizontal transfer, however, remain incompletely understood (Wagner et al., 2017). These mechanisms include uptake of DNA via natural transformation, transfer of conjugative plasmids, transduction, uptake of DNA via membrane vesicles and cell fusion hybrids (Wagner et al., 2017). Members of Euryarchaeota, *Pyrococcus furiosus*, and *Thermococcus kodakaraensis* are naturally competent taking up linear and circular DNA (Sato et al., 2005; Lipscomb et al., 2011). Transfer of conjugative plasmids was described first in *Sulfolobales* by the isolation of the first archaean conjugative plasmid in 1995 (Schleper et al., 1995; Prangishvili et al., 1998; Stedman et al., 2000). Interestingly, analysis of the genome of *Sulfolobales* revealed the insertion of proviral DNA from *Sulfolobus* spindle-shaped virus 1 (SSV1) (Schleper et al., 1992). SSV1 stays integrated in archaean genomes and produce viral particles budding from the cells for the transfer of viral DNA (Quemin et al., 2016). Also haloarchaeal viruses have been shown to drive the genetic variation...
of different haloarchaeal species (Cuadros-Orellana et al., 2007; Tschitschko et al., 2018; Mizuno et al., 2019).

*Methanococcus voltae* PS produces viral particles named “voltae transfer agent” (VTA) which can carry chromosomal fragments instead of viral DNA (Bertani, 1999; Eiserling et al., 1999; Lang et al., 2012). Similarly to VTA, *Thermococcales* release membrane vesicles packed with chromosomal and plasmid DNA for the exchange of genetic material (Soler et al., 2008). Members of *Sulfolobus* spp. can exchange DNA upon UV-induced DNA damage allowing for DNA repair using homologous recombination (Fröls et al., 2008; Ajon et al., 2011). Cell aggregates are formed mediated by UV-induced pili (Ups-pili) and the crenarchaeal exchange of DNA system (Ced-system) is activated (Fröls et al., 2008, 2009; Ajon et al., 2011). Using the Ups-pili cell–cell contact is established and DNA is exchanged (Van Wolferen et al., 2016). Remarkably the exchange is species-specific possibly being mediated by the degree of N-glycosylation of Ups-pili (van Wolferen et al., 2020). Finally bidirectional gene transfer occurs in haloarchaea via cell fusion (Mevarech and Werczberger, 1985; Rosenshine et al., 1989).

Here, the cell biological prerequisites for the previously observed DNA transfer through cell fusion in haloarchaea are elucidated. In the 1980s, it was described that mixing of two different auxotrophic strains of the halophilic euryarchaeon *Haloferax volcanii*, resulted in prototrophic recombinant cells. The mating frequency was determined in the presence of DNase to rule out natural transformation and was 10^-6 (Mevarech and Werczberger, 1985). It was proposed that transfer of genetic material occurred via an uncharacterized fusion-based mating mechanism (Mevarech and Werczberger, 1985). Remarkably, the transfer of DNA in *H. volcanii* is bidirectional without a specific donor or recipient and since mating and subsequent DNA exchange has been observed within the two species *H. volcanii* and *Haloferax mediterranei*, it is not necessarily species specific (Naor et al., 2012). It was observed that two *H. volcanii* cells can fuse to form a hybrid state (Naor et al., 2012; Naor and Gophna, 2013). In this state, large chromosomal DNA fragments are exchanged and after recombination followed by cell separation, this results in genetic hybrids of the parents (Naor et al., 2012; Naor and Gophna, 2013). CRISPR spacers matching chromosomal genes, including housekeeping genes, are also exchanged between species (Turgeman-Grott et al., 2019). Strikingly, mating frequency depends on factors that impact the cell surface such as external salt concentration and N-glycosylation of the surface layer (S-layer) (Shaley et al., 2017). Defects in the N-glycan of S-layer proteins significantly reduce mating frequencies, suggesting an important role for S-layer glycosylation in initiation of cell–cell interaction and cell fusion (Shaley et al., 2017). Early electron micrographs from 1975 when *H. volcanii* was isolated and characterized as well as other scanning electron micrographs of *H. volcanii* have suggested the formation of intermediate intercellular bridges prior to cell fusion (Mullakhbanbai and Larsen, 1975; Rosenshine et al., 1989). These cell–cell bridges might allow for an exchange of genetic material and drive cell fusion (Naor and Gophna, 2013). Exchange of genetic material has only been observed on solid media in previous studies, prompting questions about the mechanisms involved in mating. Formation of possible cell–cell bridges between cells has also been observed in other archaeal lineages, such as members of *Sulfolobales* (Schleper et al., 1995), *Thermococcales* (Kuwabara et al., 2005), and even between Nanoarchaea and Thermoplasmatales (Comolli and Banfield, 2014). Formation of cell–cell bridges was also reported in bacterial species. These nanotubes are enveloped by a membrane layer and build a bridge between two neighboring bacterial cells allowing an exchange of cytoplasm (Dubey and Ben-Yehuda, 2011; Baidya et al., 2018).

To better characterize the mechanism of horizontal gene transfer by fusion in *H. volcanii*, we used electron cryo-tomography (cryoET) to preserve whole cells in a frozen hydrated state. We identified and imaged cell–cell bridges connecting the cytoplasts of pairs of cells grown in liquid media. Tomograms revealed that two mating cells shared a continuous membrane, a continuous S-layer and had continuous connected cytosols. Strikingly, macromolecular structures were detected in the cell–cell bridges likely to be ribosomes. Fluorescence time-lapse microscopy of *H. volcanii* cells with fluorescently stained S-layers showed how cells established an intercellular bridge as an intermediate state prior to cell fusion.

**MATERIALS AND METHODS**

**Strains and Growth Conditions**

Growth of *H. volcanii* H26 and RE25 was performed as described previously (Allers et al., 2004; Esquivel and Pohlschroder, 2014; Duggin et al., 2015). The cells were grown in Hv-YPC medium containing 2.4 M NaCl, 0.17 M of MgSO₄ × 7 H₂O, 0.27 M MgCl₂ × 6 H₂O, 0.05 M KCl, 3 mM CaCl₂ and 12 mM TRIS, HCL (pH 7.5) with 0.5% (wt/vol) yeast extract (Difco), 0.1% (wt/vol) peptone (Oxoid), and 0.5% (wt/vol) casamino acids (Bacto). Selective Casamino acid medium (CA medium) was prepared as Hv-YPC medium but yeast extract and peptone were omitted. CAB medium was prepared as CA medium and contained a 100× fold diluted expanded trace element solution, containing 5 g/L ethylenediaminetetraacetic acid (EDTA), 0.8 g/L FeCl₃, 0.05 g/L ZnCl₂, 0.01 g/L CuCl₂, 0.01 g/L CoCl₂, 0.01 g/L H₂BO₃, 1.6 g/L MnCl₂, 0.01 g/L NiSO₄, and 0.01 g/L H₂MoO₄. The pH was adjusted to pH 7.0 with NaOH and the solution was filter sterilized.

**Electron Cryo-Tomography**

*Haloferax volcanii* RE 25 was inoculated in 5 mL CA medium supplemented with 1 g/L thiamine and 0.1 μg/L biotin, 10 μg/mL uracil and 50 μg/mL tryptophan that was incubated at 42°C overnight. A total of 5 and 15 μL of the pre-culture was inoculated in 20 mL CA medium and incubated again at 42°C overnight. At OD₆₀₀ of 0.05, the cells were harvested at 2,000 × g for 20 min at 40°C. The pellet was dissolved in 1 mL CA medium and again pelleted at 2,000 × g for 10 min at 40°C. The cell pellet was dissolved again in CA medium to a theoretical OD₆₀₀ of 3 or 5. The cells were mixed with BSA-coated 10 nm gold particles.
fiducial markers and 2.5 μL of cells were applied to a freshly
glow-discharged copper Quantifoil R2/2 grid (300 mesh). The
vitrification of the grid was done using the Vitrobot Mark IV
(Fei). The grid was blotted on the back using a repellent Teflon
membrane and subsequently plunge-frozen in liquid ethane.
Electron cryotomography was conducted with a 200 kV Twin FEI
F20 (FEG) equipped with a Falcon II direct electron detector.
The software package Leginon was used to record tilt series
(Carragher et al., 2000). A total cumulative electron dose of
120 e-/Å² was used per tilt series with −3 to −6 μm defocus.
The tomograms were collected bidirectionally in a tilt range of
±54° starting at +24° collecting through −54° and then the
remaining tilts with 3° increments and a pixel size of 8.28 Å.
Tomograms were reconstructed automatically using RAPTOR
software and IMOD
(Kremer et al., 1996; Mastronarde, 1997;
Amat et al., 2008).

S-Layer Staining

*Halofex volcanii* H26 was inoculated in 5 mL CAB medium
supplemented with 10 µg/mL uracil and grown overnight at
45°C. A total of 220 μL were inoculated in 20 mL of the main
culture which was grown overnight at 45°C. The cells were
harvested at OD₆₀₀ = 0.2 at 1,800 × g for 10 min at 25°C. The
pellet was resuspended in 2 mL of buffered media and washed
three times at 3,400 × g for 10 min at RT and resuspended in
500 μL buffered media. The pH was adjusted to pH 8–8.5 with 1
M NaHCO₃ and 50 μg of Alexa Fluor 488 NHS Ester (Thermo
Fisher Scientific) was added. The cells were incubated at RT for
1 h while rotating. To remove excess dye, the cells were washed
three times as above with 500 µL CAB medium.

Isolation of Stained S-Layer

Staining was checked by sonicating the cells for 10 min in an
ultrasonic bath. Afterwards, the cell debris was pelleted at
3,400 × g for 10 min at RT. SDS was added to a final
centration of 0.01% and the mixture was centrifuged again
at 3,400 × g for 10 min at RT. The supernatant was divided
into 2 × 500 μL and centrifuged at 190,000 × g for 1 h at
4°C. The resulting pellets were resuspended in 50 μL 1× loading
dye and in 5 μL 1× PBS, 0.1% Triton-X 100. Both pellets were
incubated at 6°C, in a light-protected manner for 48 h and
then mixed together. This was incubated for 2 1/2 h at 37°C
and 10 μL was used for an SDS-PAGE and detection of the
fluorescence signal.

S-Layer Isolation

For S-layer isolation, 400 mL of H26 was grown at 45°C to an
OD₆₀₀ of 1.37. The cells were pelleted at 6,200 × g for 25 min at
4°C. The pellet was resuspended in 200 mL CA medium
and 60 mL of 0.5 M EDTA (pH 6.7) was added. Subsequently,
the pellet was incubated at 37°C while shaking for 30 min.
The spheroplasts were removed via centrifugation in an iterative
manner at 3,000 × g for 15 min, 7,000 × g for 5 min and
13,000 × g for 10 min. The supernatant was concentrated
via Amicon (MWCO = 50 kDa, Merck Millipore) to 500 μL.
A total of 16 μL were used for an SDS-PAGE and sent for
Mass spectrometry.

Fluorescence Time-Lapse Microscopy

For microscopy, 3 μL of stained cells were pipetted on a
0.3% agarose pad consisting of agarose dissolved in CAB
medium supplemented with 10 μg/mL uracil. Phase-contrast
and fluorescence images were captured every 30 min for 16 h
at 100× magnification using a 100×/1.4 oil plan-apochromatic
objective lens Ph 3, in phase contrast and fluorescence mode,
using a Zeiss Axio Observer 2.1 microscope equipped with a
heated XL-5 2000 Incubator while running VisiVIEW® software.
Images were taken with the PCO Edge sCMOS Camera (PCO)
with 50 ms exposure time for the phase contrast images and
500 ms for the GFP images. Images were analyzed using ImageJ,
Fiji (Schindelin et al., 2012).

RESULTS

Whole Cell *in situ* Electron

Cryo-Tomography Captures Cell–Cell

Bridges Between Two *H. volcanii* Cells

To investigate the structure of the cell–cell bridges in *H. volcanii*,
whole-cell electron cryoET was used. CryoET offers the
possibility to image cells in their native environment in a
near-native frozen-hydrated state to macromolecular resolutions.
Cryomicrographs of vitrified *H. volcanii* cells grown in
liquid medium were acquired. The initial tomograms showed
archaellated cells with a possible storage granule as well as
ribosomes in the cytoplasm (Figure 1). All detected cells were
envolved by a continuous 2D crystalline and proteinaceous
S-layer over the membrane (Figure 1). In *H. volcanii*, the
S-layer consist of many copies of one highly glycosylated
protein that is secreted and lipid anchored to the membrane
(Kessel et al., 1988; Sumper et al., 1990). The S-layer protein
self-assembles to a 2D-lattice around the cell acting as a
molecular sieve, supposed to be involved in cell recognition
and cell shape maintenance (Sára and Sleytr, 2000; Sleytr
et al., 2014). Strikingly, S-layer proteins could be detected,
arranged in a hexagonal lattice around the cell similarly as
reported in an early study (Figure 1B; Kessel et al., 1988).
Upon closer investigation of a subtomogram slice, the dome
shape morphology formed by S-layer proteins can be identified
(Figure 1C; Kessel et al., 1988). The thickness of the S-layer was
determined by measuring the distance from the membrane to the
S-layer protein. The average thickness was determined to
20.4 ± 2.7 nm (Supplementary Table 1).

Interestingly, cryoET allowed the observation of several cells in
a hemifusion state connected via cell–cell bridges (Figure 2,
upper panels and Supplementary Figure 1). In total, out of 280
collected tilt series 20 tilt series of cell–cell bridges were acquired
with a magnification sufficient to focus on the intercellular
bridges (Figure 2, lower panels). As well as cell–cell bridges
between intact cells (Figure 2, left panels), we observed cell
bridges between intact and broken cells (Figure 2, middle panels)
and disrupted cell bridges. Probably, these bridges ruptured
during the cell isolation or during the blotting procedure.
A representative tilt series is shown in Supplementary Movie 1.
We measured the widths and lengths of cell–cell bridges. The width was measured over the length of the cell–cell bridge and average width of each cell–cell bridge was determined (Figure 3 and Supplementary Table 1). For determination of the length, only the cell–cell bridges were considered that connected two cells as shown in Figure 2 (left and middle panel). The width varied from 57 to 162 nm and the length varied from 253 to 2,144 nm (Figure 3 and Supplementary Table 1). The scatter plot and histogram (Figure 3) shows that the majority of cell–cell bridges have a width up to 100 nm with a length of 1–1.2 µm indicating that cells might need to be within ~1.2 µm for the formation of cell–cell bridges to occur. No relation between length and diameter could be detected.

Cell–Cell Bridges Are Surrounded by a Continuous S-Layer and Connect the Cytoplasms of Two Cells

Closer investigation of the cell–cell bridges showed that these bridges connected the cytoplasms of the two cells. The connected cytoplasm was surrounded by a continuous cytoplasmic membrane and a continuous S-layer (Figure 4). Since the cytoplasms of the two cells are connected, this would allow exchange of cytoplasmic materials between two cells. Indeed, we saw high molecular mass complexes consistent with the size, shape, and density of ribosomes within the tubular cell–cell bridges, suggesting that high molecular weight complexes are exchanged between mating cells (Figure 4 and Supplementary Movie 1). Next to the ribosomes also unknown thin filamentous helical structures of 199 ± 18 nm long and 9.0 ± 2.3 nm wide (Figure 4B and Supplementary Movie 1) were observed in the one of the analyzed cell–cell bridge. The function and the proteins that form the thin filamentous helical structures are unclear although we speculate that they may be cytomotive filaments to drive cytoplasmic exchange.

In vivo Observation of the Formation of an Intercellular H. volcanii Cell–Cell Bridge

The tomograms showed that intercellular bridges are encapsulated by an S-layer. To follow the formation of a cell–cell bridge using time-lapse fluorescence microscopy, the cells were incubated with the Alexa Fluor 488 NHS Ester and the cells were followed over a period of 16 h. As expected, the cells were mainly fluorescently labeled on the outside. Comparison
of the proteins which were labeled with the fluorescent probe in the total cell extract with isolated S-layers showed that, next to the S-layer protein, also several other proteins were labeled (Supplementary Figure 2). Several experiments were conducted where over 16 h every 30 min fluorescent and phase contrast images were acquired. In one of these experiments, the formation of a cell-bridge was observed. Notably, after 5 h of incubation, one thin fluorescent connection between two cells was detected that can be identified as a de novo formed cell–cell bridge (Figure 5 and Supplementary Movie 2). The time lapse movie shows fluorescent cells with increasing cell size due to an unknown cell division defect where a fluorescent septum is formed between two adjacent cells which do not separate. This is sometimes observed during these experiments and is most likely unrelated to the observed cell–cell bridge. Between the 2:30 and 3:00 h time points a full cell–cell bridge is formed. Remarkably, the cell–cell bridge is formed between two cells at 1.5 µm distance without any initial direct contact, suggesting that cell–cell bridge formation is an active process. The analysis of CryoET data showed that shortly after cell–cell bridge formation the cytoplasms are most likely connected providing a connection between two cells and possibly allowing an exchange between two cells. It was not possible to determine whether the cell–cell bridge is formed from one or from both cells. After 7.5 h, the length of the cell–cell bridge is decreasing indicating a contraction to bring both cells in close proximity (Figure 5). The cell–cell bridge was maximally 1.5 µm long, growing shorter and thicker over time. Unfortunately, the in vivo formation of only one cell–cell bridge could be observed. However, this is the first time that the formation of a cell–cell bridge was observed in real time.

**DISCUSSION**

Mechanisms of gene transfer are diverse in Archaea (Meile et al., 1990; Schleper et al., 1995; Prangishvili et al., 1998; Bertani, 1999;
**FIGURE 4** | Detection of macromolecular complexes in cell–cell bridges. (A) A micrograph of a targeted cell–cell bridge shows the presence of ribosomes in the cell–cell bridge (as indicated by red arrows). (B) The left panel shows the magnified tomographic slice of the cell–cell bridge and shows the ribosomes arranged in a chain-like manner (as indicated by red arrows). The right panel shows another slice of the selected cell–cell bridge showing a long filamentous structure inside the cell–cell bridge (as indicated by blue arrows). Scale bars are 100 nm.

**FIGURE 5** | Formation of a cell–cell bridge followed by fluorescence microscopy. Time-lapse fluorescence images of AlexaFluor488 labeled *H. volcanii* cells with the corresponding phase-contrast image. The fluorescence signal for the cell–cell bridge can be detected after 5 h and is indicated by a red arrow. In phase contrast, the cell–cell bridge can be detected after 7 h. Blue arrows indicate the septum formed between the cells due to a cell division defect. Scale bars are 4 μm.

Ajon et al., 2011; Allers, 2011; Naor et al., 2012; van Wolferen et al., 2013, 2020). In *H. volcanii* genetic transfer occurs in a bidirectional manner upon fusion (Mevarech and Werczberger, 1985; Rosenshine et al., 1989; Naor and Gophna, 2013) and it was proposed that the formation of cell–cell bridges may precede fusion as an intermediate state (Rosenshine et al., 1989; Naor and Gophna, 2013).

Whole-cell cryoET enabled preservation and the study of cells in this intermediate bridged state, while time-lapse fluorescence microscopy allowed for the observation of the formation of cell–cell bridges in real-time. Despite the high salinity of the cell cytoplasm and the medium, electron cryo-tomograms gave a high resolution snapshot of the features of the cell–cell bridges in *H. volcanii*. However, only a limited number of cell–cell bridges could be studied. In contrast to the cell–cell bridges observed by Mevarech and Rosenshine on solid media, bridge formation and fusion events were recorded in liquid media, but in liquid media, these events occur most likely much less frequently than
on solid media (Rosenshine et al., 1989). Furthermore, more cell–cell bridges would probably have been observed in a medium containing a higher salt concentration, as mating efficiency was shown to be higher at high salt concentrations (3.4 M NaCl), which is most likely caused by an altered S-layer glycosylation (Guan et al., 2012; Shalev et al., 2017). Since previous studies in *Halobacterium salinarum* showed that higher salt concentrations reduced the contrast of electron micrographs (Bollschweiler et al., 2017), images were obtained from cells grown in a medium containing 2.4 M NaCl where optimal growth was still observed.

Time-lapse fluorescence microscopy allowed for the observation of the *de novo* formation of a cell–cell bridge. This showed that cells are able to bridge the distance by the formation of a cell–cell bridge reaching to the adherent cell. The time-lapse fluorescence microscopy showed a cell–cell bridge that was formed over a distance of 1.5 µm whereas the cryoET showed that most cell-bridges were shorter than 1–1.2 µm, which demonstrates that cell-bridges with a length of ~1 µm can be easily formed. Full cell–cell bridge formation was observed within 0.5 h, suggesting it is a relatively fast process (see Supplementary Movie 2 between 2:30 and 3:00 h).

Indeed, a recent transcriptomic study showed that mating impacts genes involved in cell division and glycosylation. Strikingly, increased expression was detected for selfish genetic elements, restriction-modification system and CRISPR-Cas. These changes were detected in the first hours after transfer to a filter paper, indicating that mating efficiency is probably increased over a short period following contact. The formation of cell–cell bridges was observed within 0.5 h, suggesting it is a relatively fast process (see Supplementary Movie 2 between 2:30 and 3:00 h).

These observations raise several new questions that should be addressed in future studies: How are these cell–cell bridges formed? Do these cell–cell bridges grow from one or from both cells? How do the cell–cell bridges fuse, and which proteins are required for the formation of the cell–cell bridge? How are these cell–cell bridges formed? Do these cell–cell bridges grow from one or from both cells? How do the cell–cell bridges fuse, and which proteins are required for the formation of the cell–cell bridges?

**DATA AVAILABILITY STATEMENT**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**AUTHOR CONTRIBUTIONS**

SS, PN, MB, and S-VA designed the research. SS performed the cryoET with technical help from Florian Rossmann (FR). SS, S-VA, and MB analyzed the tomograms, designed the figures, and wrote the manuscript. HW and PN performed the fluorescence microscopy. HW, PN, SS, and S-VA analyzed the fluorescence microscopy data and designed the figures. All authors read and reviewed the manuscript.

**FUNDING**

The article processing charge was funded by the University of Freiburg in the funding program Open Access Publishing. SS was supported by the Deutsche Forschungsgemeinschaft (German Research Foundation) under project no. 40322702-SFB 1381 and from the European Union’s Horizon 2020 Research and
Innovation Program under grant agreement no. 686647. Mass spectrometry was performed with the help of the lab of Matthias Boll [supported by a grant from the German Research Foundation (INST 39/995-1 FUGG)].

ACKNOWLEDGMENTS

We thank Florian Rossmann (FR) and Paul Simpson for technical assistance with imaging H. volcanii for CryoET in the Imperial College Centre for Structural Biology Electron Microscopy Facility.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2020.612239/full#supplementary-material

REFERENCES

Ajón, M., Frös, S., van Wolferen, M., Stoecker, K., Teichmann, D., Driesen, A. J. M. M., et al. (2011). UV-inducible DNA exchange in hyperthermophilic archaea mediated by type IV pilis. Mol. Microbiol. 82, 807–817. doi: 10.1111/j.1365-2958.2011.07861.x

Allers, T. (2011). Swapping genes to survive - a new role for archaeal type IV pili. Curr. Biol. 21, 1444–1448. doi: 10.1016/j.cub.2012.05.056

Naor, A., Lapiere, M., Mevarech, M., Papke, R. T., and Gophna, U. (2012). Report low species barriers in halophilic Archaea and the formation of recombinant hybrids. Curr. Biol. 22, 1444–1448. doi: 10.1016/j.cub.2012.05.056

Näther, D. J., Rachel, R., Wanner, G., and Wirth, R. (2006). Flagella of Pyrococcus furiosus: multifunctional organelles, made for swimming, adhesion to various surfaces, and cell-cell contacts. J. Bacteriol. 188, 6915–6923. doi: 10.1128/JB.00527-06

Prangishvili, D., Albers, S. V., Holz, I., Arnold, H. P., Stedman, K., Klein, T., et al. (1998). Conjugation in archaea: frequent occurrence of conjugative plasmids in Sulfolobus. Plasmid 40, 190–202. doi: 10.1006/plas.1998.1363
Quemin, E. R. J., Chlada, P., Sachse, M., Forterre, P., Prangishvili, D., and Krupovic, M. (2016). Eukaryotic-like virus budding in archaea. mBio 7:e01439-16.

Rosenshine, I., Tchelet, R., and Mevarech, M. (1989). The mechanism of DNA transfer in the mating system of an archaeabacterium. Science 245, 1387–1389. doi: 10.1126/science.245.4927.1387

Sára, M., and Sleytr, U. B. (2000). S-layer proteins. Science 281, 1874–1876. doi: 10.1126/science.281.5386.1874

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Schleper, C., Kubo, K., and Zillig, W. (1992). The particle SSV1 from the extremely thermophilic archaeon Thermococcus kodakaraensis. Appl. Environ. Microbiol. 71, 3889–3899. doi: 10.1128/AEM.71.7.3889-3899.2005

Soler, N., Marguet, E., Verbavatz, J. M., and Forterre, P. (2008). Virus-like vesicles and extracellular DNA produced by hyperthermophilic archaea of the order Thermococcales. Res. Microbiol. 159, 390–399. doi: 10.1016/j.resmic.2008.04.015

Shalev, Y., Turgeman-Grott, I., Joseph, S., Marton, S., Eizenshtein, K., Naor, A., Soucy, S. M., et al. (2019). Pervasive acquisition of CRISPR memory driven by interspecies mating of archaea can limit gene transfer and influence speciation. Nat. Microbiol. 4, 177–186. doi: 10.1038/s41564-018-0302-8

van Wolferen, M., Ajon, M., Driessen, A. J. M., and Albers, S.-V. (2013). Molecular analysis of the UV-inducible pilus operon from Sulfolobus acidocaldarius. Microbiologynopen 2, 928–937. doi: 10.1002/mbo3.128

van Wolferen, M., Shajahan, A., Heinrich, K., Brenzinger, S., Black, I. M., Wagner, A., et al. (2020). Species-specific recognition of Sulfolobales mediated by UV-inducible pilus and S-Layer glycosylation patterns. mBio 11:e03014-19.

Wagner, A., Whitaker, R. J., Krause, D. J., Heilers, J.-H., van Wolferen, M., Wagner, A., et al. (2016). The archaeal Ced system imports DNA. Proc. Natl. Acad. Sci. U.S.A. 113, 2496–2501. doi: 10.1073/pnas.1513740113

Wagner, A., Whitaker, R. J., Krause, D. J., Heilers, J.-H., van Wolferen, M., and van der Does, C., et al. (2017). Mechanisms of gene flow in archaea. Nat. Rev. Microbiol. 15, 492–501. doi: 10.1038/nrmicro.2017.41

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2021 Sivabalasarma, Wetzel, Nagasak, van der Does, Beeby and Albers. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.