CetZ1 in Cell Shape control of Haloarchaea:

Understanding the Functional Divergence of Tubulin Superfamily Proteins

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CERTIFICATE OF ORIGINAL AUTHORSHIP

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ABBREVIATIONS

AMP	Ampicillin
Å	Angstrom
BSA	Bovine Serum Albumin
BLAST	Basic local alignment search tool
DNA	deoxyribonucleic acid
g	Gram
GFP	Green fluorescent protein
GTP	Guanosine triphosphate
GDP	Guanosine diphosphate
h	Hour(s)
kb	Kilo base pair(s)
kDa	Kilodalton(s)
min	Minute(s)
ml	Millilitre(s)
MQW	Milli-Q water
OD	Optical Density
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
psi	Pounds per square inch
mRNA	Messenger Ribonucleic acid
M	Molar concentration
Mm	Millimolar concentration

MWCO	Molecular weight cut-off
n	nano
NCBI	National Centre for Biotechnology Information
RT	Room Temperature
rpm	Revolutions per minute
r.m.s.d	Root-mean-square deviation
SDS-PAGE	Sodium dodecyl sulfate polyacrylamide gel electrophoresis
sec	sec
TBS	Tris Buffered Saline
TEMED	N,N,N',N'-tetramethylethylenediamine
Tris	Tris(hydroxymethyl)methylamine
w	Weight
WT	Wild Type
v	Volume
µ	Micro

PUBLICATIONS AND CONFERENCE PROCEEDINGS

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- **de Silva R.T**. Ithurbide S.I., Duggin I.G. (2019) "CetZ1 in cell shape control of haloarchaea: Understanding the functional divergence of tubulin superfamily protein" at Motile and Contractile Systems Gordan Research Conference, USA (**oral and poster presentation**).
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ABSTRACT

Cell shape dynamics are important for cell survival. Eukaryotic cytoskeletal protein tubulin plays essential roles in internal structure organisation and cell shape. However, the prokaryotic tubulin homologue, FtsZ, controls the assembly and function of the division ring. The origin of this functional disparity is still unclear. A third group of the tubulin superfamily, CetZ, has been recently identified in archaea and shows characteristics in common with both the tubulin and FtsZ. A conserved member, CetZ1, is required for cell shape changes (from plate to rod) during the development of motile cells in the archaeon, *Haloferax volcanii* (Duggin *et al*., 2015).

The present study has defined additional culture conditions—metal nutrients depletion and early-log growth—that result in rod development, which has opened new ways of understanding cellular differentiation in archaea. A new Δ*cetZ1* strain, which can be complemented by resupply of CetZ1 on a plasmid, was also constructed. Using these culture conditions and Δ*cetZ1*, a functional CetZ1-mTurquoise2 fusion was identified after screening numerous fluorescent proteins and linker-peptide combinations. It displayed a patchy and dynamic localisation in discoid cells, then, during rod formation, displayed short dynamic filaments along the edges of the cell's long axis. During cell division, CetZ1 localises at the envelope around the division furrow; this differed significantly from FtsZ localisation pattern.

By using the CetZ1 and CetZ2 crystal structures as a guide, mutants were constructed to probe the functions and interactions of CetZ1 in *H. volcanii*. Mutation in the predicted CetZ1 membrane-interaction and self-association domains prevented rod development. The former displayed filament-like localisation detached from the cell edges, whereas the latter did not localise *in vivo*, consistent with the predictions. The GTPase mutants of CetZ1 prevented rod development but caused more intense and less dynamic localisation suggesting regulation of characteristic GTP-dependent dynamics is critical to CetZ1 function. CetZ1 *in vitro* studies revealed GTP-dependent polymerisation and these polymers were destabilised in predicted self-association mutant. Moreover, a mutation in the C-terminal tail displayed a decreased membrane localization.

These structure-function studies suggest CetZ1 forms polymers that has its longitudinal interactions controlled via the GTPase activity and the lateral interactions mediated by a

region similar to tubulin ‘M-loop’. The dynamic localisation of CetZ1 to the cell edges via the C-terminal tail region is essential to modulate the cell shape. Finally, these discoveries support the notion that cell shape control by tubulin superfamily proteins could have predated the emergence of eukaryotic tubulins.