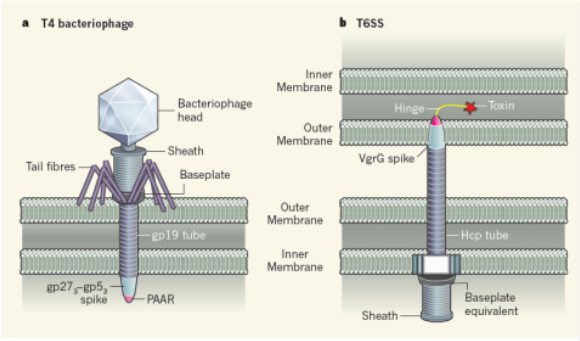
PAAR repeat proteins sharpen and diversify the type VI secretion system spike

Shneider et al. , 15th AUG 2013, Nature 500:350-353

Collaboration between J.J. Mekalanos' group (Harvard Med School, Boston) and P. Leiman's group (EPFL, Lausanne)



The type VI secretion system (T6SS):

- Present in a wide range of pathogenic bacteria
- Secretes effector proteins across bacterial Gram- cell envelope
- Targets and kills other bacterial species or eukaryotic organisms
- Genes usually encoded in a 15 gene operon
- Components share structural similarity with injection machinery of bacteriophages, but with inverted directionality (Fig. 1)

Fig.1 Similarity between bacteriophage and T6SS (A. Filloux, Nature 500, 284–285; 2013)

Structural similarity between T4 bacteriophage and T6SS:

- both complexes contain a tube of hexameric protein rings (gp19 in bacteriophage T4 and Hcp in T6SS) and a spike, made up of $gp27_3$-$gp5_3$ in bacteriophage and VgrG in T6SS (Fig. 1)
- both tubes are surrounded by a sheath that contracts to push the spike through bacterial cell membranes

What's new?

Shneider et al. extend the analysis of structural similarity between T4 bacteriophage and T6SS. The authors show:

- the presence of proteins of the PAAR (Pro-Ala-Ala-Arg) repeat superfamily bound to VgrG
- crystal structure of PAAR protein suggests a sharp canonical extension on the VgrG spike (Fig. 1)
- PAAR proteins are specific for their cognate VgrG spike protein
- T6SS effectors present as C-terminal extensions of VgrG or N- or C-terminal extension of PAAR protein (Fig. 3)

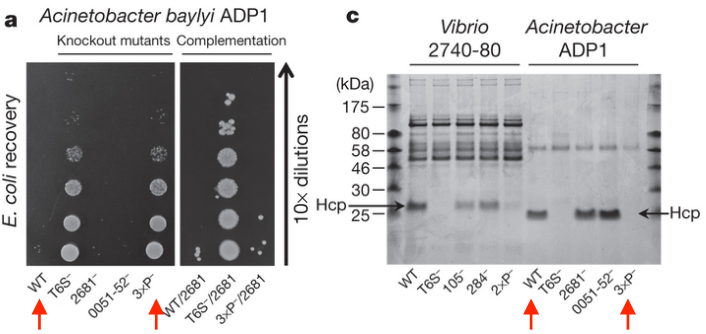


Fig. 2 PAAR proteins are required for killing activity (a) and assembly (c) of T6SS in *Acinetobacter baylyi*

PAAR proteins are essential for T6SS functionality:

- only simultaneous knock out of all 3 PAAR proteins (3xP-) of *Acinetobacter baylyi* abolishes killing of *E. coli* prey (Fig. 2a, compare WT vs 3xP-)
- this suggests that PAAR proteins are interchangeable within a species (Fig. 2a, see complementation)
- PAAR proteins participate in assembly of T6SS (Fig. 2c, compare Hcp expression WT vs 3xP- in *Acinetobacter*)

VgrG - PAAR complex translocates effectors:

-Interaction between VgrG and PAAR protein by a hydrophobic patch and 14-16 H-bonds (Fig. 3c, d)

-Effectors are predicted to be loaded onto the spike by 5 distinct mechanisms (Fig 3a):

1. C-terminal extensions of the VgrG spike
2. non-covalent binding to VgrG spike
3. N or C-terminal extensions of PAAR protein
4. non-covalent binding to PAAR protein
5. incorporation into cavity of VgrG

Mechanisms 1-3 are experimentally supported

Mechanisms 4-5 are hypothetical

C-terminal extensions (effectors) may have enzymatic activities (nuclease, peptidase, lipase, deiminase, etc)

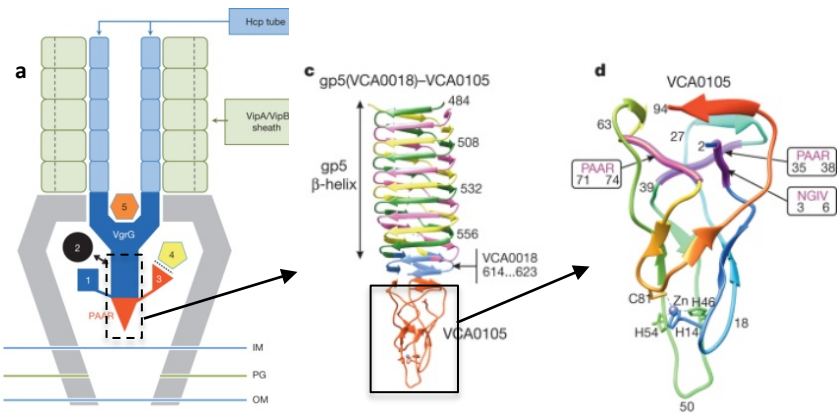


Fig.3 . Multiple effector translocation VgrG model (a) and crystal structure of gp5 (VgrG)- PAAR complex (c) and PAAR protein (d)

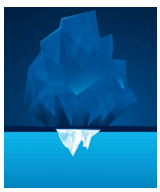
Open questions:

Do extensions affect puncturing activity of PAAR ?

What is the target specificity of T6SS ?

More adaptors and toxins associated with T6SS….

…only the tip of the iceberg ?



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