AMERICAN SOCIETY FOR MICROBIOLOGY | Journal of Bacteriology®

COMMENTARY

The Expanding Molecular Genetics Tool Kit in *Chlamydia*

Raphael H. Valdivia,[a] Robert J. Bastidas[a]

[a]Department of Molecular Genetics and Microbiology and Center for Host-Microbial Interactions, Duke University School of Medicine, Durham, North Carolina, USA

ABSTRACT *Chlamydia* has emerged as an important model system for the study of host pathogen interactions, in part due to a resurgence in the development of tools for its molecular genetic manipulation. An additional tool, published by Keb et al. (G. Keb, R. Hayman, and K. A. Fields, J. Bacteriol. 200:e00479-18, 2018, https://doi.org/10.1128/JB.00479-18), now allows for custom genetic engineering of genomic regions that were traditionally recalcitrant to genetic manipulation, such as genes within operons. This new method will be an essential instrument for the elucidation of *Chlamydia*-host interactions.

KEYWORDS *Chlamydia* genetics, *Chlamydia trachomatis*

For decades, *Chlamydia trachomatis*, the causative agent of ocular and venereal diseases, remained frustratingly intractable to any form of stable genetic manipulation, with only a few reports describing the successful transformation of *Chlamydia* species prior to 2007 (1, 2). However, the observation that *Chlamydia* readily exchanges DNA by lateral gene transfer (LGT) (3, 4) in the setting of coinfections paved the way for experimental system where mutations can be passed from one strain to another and thus enable genotype-phenotype associations (5, 6). Point mutations derived spontaneously or after exposure to mutagens provide hypo- and hypermorphic alleles (5) and temperature-sensitive mutations (7), further expanding the emerging genetic toolkit available to study *C. trachomatis*.

A turning point in *Chlamydia* genetics was the finding by Clarke and colleagues that *C. trachomatis* can be stably transformed with a chimeric plasmid derived from the *Chlamydia* "cryptic" plasmid and a ColE1 plasmid after $CaCl_2$ treatment and selection with penicillin G (8). The observation that *C. trachomatis* is transformable then led to the use of suicide vectors to deliver other tools for genome engineering, such as group II introns for targeted insertional disruption of *Chlamydia* genes (9, 10). This tool, known as TargeTron, is used broadly in many bacterial species but relies on gene-specific predictive algorithms for target design that vary in efficiency and specificity. Insertional mutagenesis with TargeTron-based vectors invariably will have polar effects if insertions occur at the 3′ end of operons, making it more difficult to assign specific function to individual genes within an operon.

Mueller and colleagues (12) developed an alternative strategy for targeted mutagenesis called fluorescence-reported allelic exchange mutagenesis (FRAEM). In this system, open reading frames are replaced by allelic exchange with a selectable marker (12). The FRAEM plasmid vector provides the substrate for allelic exchange in the form of a *gfp-blaM* cassette flanked by 3 kb of DNA homologous sequences upstream and downstream of the chromosomal region to be replaced. The elegance of this approach relies on making the FRAEM plasmid conditionally replicative by placing *pgp6*, which controls plasmid maintenance and inheritance (13, 14), under the control of an anhydrous tetracycline (aTet)-inducible genetic circuit. Allelic exchange occurs as a two-step process, where selection for penicillin resistance selects for transformed *Chlamydia* expressing green fluorescent protein (GFP) and mCherry, which is present in the FRAEM

Accepted manuscript posted online 8 October 2018

Citation Valdivia RH, Bastidas RJ. 2018. The expanding molecular genetics tool kit in *Chlamydia*. J Bacteriol 200:e00590-18. https://doi.org/10.1128/JB.00590-18.

Editor Thomas J. Silhavy, Princeton University

Address correspondence to Robert J. Bastidas, robert.bastidas@duke.edu.

For the article discussed, see https://doi.org/10.1128/JB.00479-18.

plasmid backbone. These clones are then screened in the absence of aTet for loss of the mCherry marker and maintenance of GFP, which selects for allelic exchange resulting from double-crossover events. In this issue, Keb and colleagues expand the FRAEM system by incorporating flanking *loxP* sites to the *gfp-blaM* cassette and adding a step where a second suicide plasmid encoding Cre recombinase is introduced by LGT (11). As a result, the floxed *gfp-blaM* cassette is rapidly excised from the chromosome, leaving a small *loxP* scar.

The utility of this new system is manyfold. An obvious one is the ability to generate markerless gene deletions, opening the door for generating *Chlamydia* strains with multiple gene deletions. This is especially advantageous for *C. trachomatis* given the small number of selectable markers that are currently available. Markerless strains also have the added benefit of limiting any impact the expression of the selectable marker may have on *Chlamydia* fitness. In addition, the broad application of the Cre-*loxP* system, either through FRAEM or TargeTron, opens the possibility for extensive genome editing in *Chlamydia*, allowing the excision of large regions of chromosomal DNA. Such a system might be useful for characterizing the function of chromosomal regions, such as the highly polymorphic plasticity zones, or the family of expanded homologous polymorphic membrane proteins (Pmp) found among various *Chlamydia* species and their contributions to both *Chlamydia* virulence and tissue tropisms (15, 16). The FRAEM-based Cre-*loxP* system should enable the deletion of *Chlamydia* noncoding RNAs and small RNAs, two gene regulatory elements that have remained largely uncharacterized in *Chlamydia* species. Furthermore, the Cre-*loxP* system can be adapted to generate conditional excision of essential genes by controlling the expression of Cre.

Bacterial genes are often organized in polycistronic operons so as to coregulate the synthesis of proteins that perform related functions. The FRAEM-based Cre-*loxP* now enables individualized disruption of genes within operons without polar effects on the transcription of genes in the 3′ direction from the targeted gene. Keb and colleagues demonstrate that such an analysis is feasible in *Chlamydia* spp. by utilizing their FRAEM Cre-*loxP* system to delete the gene encoding the secreted *Chlamydia* effector TmeA. Both *tmeA* (CTL0063) and *tmeB* (CTL0064) are transcribed as an operon (17). Excision of the *tmeA* locus by FRAEM revealed a role for TmeA in promoting *Chlamydia* invasion of cultured cells (18). However, the excision of *tmeA* also impacted *tmeB* transcription (12), confounding the role for TmeA in *Chlamydia* invasion. Using their FRAEM Cre-*loxP* system, Keb and colleagues excised *tmeA* without compromising TmeB production and observed the same phenotypes associated with a previous disruption of *tmeA*.

The application of the FRAEM Cre-*loxP* system will have an immediate impact on the field of *Chlamydia* molecular genetics. Together with new reports describing the feasibility of CRISPR/Cas in *Chlamydia* spp. (19), the current genetic toolkit now enables customized cleavage at essentially any locus in the genome, establishing *C. trachomatis* as a versatile model system for the study of host-pathogen interactions and pathogenesis.

REFERENCES

1. Tam JE, Davis CH, Wyrick PB. 1994. Expression of recombinant DNA introduced into *Chlamydia trachomatis* by electroporation. Can J Microbiol 40:583–591. https://doi.org/10.1139/m94-093.
2. Binet R, Maurelli AT. 2009. Transformation and isolation of allelic exchange mutants of Chlamydia psittaci using recombinant DNA introduced by electroporation. Proc Natl Acad Sci U S A 106:292–297. https://doi.org/10.1073/pnas.0806768106.
3. DeMars R, Weinfurter J, Guex E, Lin J, Potucek Y. 2007. Lateral gene transfer in vitro in the intracellular pathogen *Chlamydia trachomatis*. J Bacteriol 189:991–1003. https://doi.org/10.1128/JB.00845-06.
4. DeMars R, Weinfurter J. 2008. Interstrain gene transfer in *Chlamydia trachomatis* in vitro: mechanism and significance. J Bacteriol 190:1605–1614. https://doi.org/10.1128/JB.01592-07.
5. Nguyen BD, Valdivia RH. 2012. Virulence determinants in the obligate intracellular pathogen *Chlamydia trachomatis* revealed by forward genetic approaches. Proc Natl Acad Sci U S A 109:1263–1268. https://doi.org/10.1073/pnas.1117884109.
6. Kokes M, Dunn JD, Granek JA, Nguyen BD, Barker JR, Valdivia RH, Bastidas RJ. 2015. Integrating chemical mutagenesis and whole-genome sequencing as a platform for forward and reverse genetic analysis of *Chlamydia*. Cell Host Microbe 17:716–725. https://doi.org/10.1016/j.chom.2015.03.014.
7. Brothwell JA, Muramatsu MK, Toh E, Rockey DD, Putman TE, Barta ML, Hefty PS, Suchland RJ, Nelson DE. 2016. Interrogating genes that mediate Chlamydia trachomatis survival in cell culture using conditional mutants and recombination. J Bacteriol 198:2131–2139. https://doi.org/10.1128/JB.00161-16.
8. Wang Y, Kahane S, Cutcliffe LT, Skilton RJ, Lambden PR, Clarke IN. 2011.

Development of a transformation system for *Chlamydia trachomatis*: restoration of glycogen biosynthesis by acquisition of a plasmid shuttle vector. PLoS Pathog 7:e1002258. https://doi.org/10.1371/journal.ppat.1002258.
9. Johnson CM, Fisher DJ. 2013. Site-specific, insertional inactivation of *incA* in *Chlamydia trachomatis* using a group II intron. PLoS One 8:e83989. https://doi.org/10.1371/annotation/b7213da3-498c-43bf-b42c-1f22934e17dd.
10. Lowden NM, Yeruva L, Johnson CM, Bowlin AK, Fisher DJ. 2015. Use of aminoglycoside 3′ adenyltransferase as a selection marker for *Chlamydia trachomatis* intron-mutagenesis and in vivo intron stability. BMC Res Notes 8:570. https://doi.org/10.1186/s13104-015-1542-9.
11. Keb G, Hayman R, Fields KA. 2018. Floxed-cassette allelic exchange mutagenesis enables markerless gene deletion in Chlamydia trachomatis and can reverse cassette-induced polar effects. J Bacteriol 200:e00479-18. https://doi.org/10.1128/JB.00479-18.
12. Mueller KE, Wolf K, Fields KA. 2016. Gene deletion by fluorescence-reported allelic exchange mutagenesis in *Chlamydia trachomatis*. mBio 7:e01817-15. https://doi.org/10.1128/mBio.01817-15.
13. Song L, Carlson JH, Whitmire WM, Kari L, Virtaneva K, Sturdevant DE, Watkins H, ZhouB SGL, Porcella SF, McClarty G, Caldwell HD. 2013. Chlamydia trachomatis plasmid-encoded Pgp4 is a transcriptional regulator of virulence-associated genes. Infect Immun 81:636–644. https://doi.org/10.1128/IAI.01305-12.
14. Gong S, Yang Z, Lei L, Shen L, Zhong G. 2013. Characterization of *Chlamydia trachomatis* plasmid-encoded open reading frames. J Bacteriol 195:3819–3826. https://doi.org/10.1128/JB.00511-13.
15. Fehlner-Gardiner C, Roshick C, Carlson JH, Hughes S, Belland RJ, Caldwell HD, McClarty G. 2002. Molecular basis defining human *Chlamydia trachomatis* tissue tropism. A possible role for tryptophan synthase. J Biol Chem 277:26893–26903. https://doi.org/10.1074/jbc.M203937200.
16. Gomes JP, Nunes A, Bruno WJ, Borrego MJ, Florindo C, Dean D. 2006. Polymorphisms in the nine polymorphic membrane proteins of *Chlamydia trachomatis* across all serovars: evidence for serovar Da recombination and correlation with tissue tropism. J Bacteriol 188:275–286. https://doi.org/10.1128/JB.188.1.275-286.2006.
17. Mueller KE, Fields KA. 2015. Application of beta-lactamase reporter fusions as an indicator of effector protein secretion during infections with the obligate intracellular pathogen *Chlamydia trachomatis*. PLoS One 10:e0135295. https://doi.org/10.1371/journal.pone.0135295.
18. McKuen MJ, Mueller KE, Bae YS, Fields KA. 2017. Fluorescence-reported allelic exchange mutagenesis reveals a role for *Chlamydia trachomatis* TmeA in invasion that is independent of host AHNAK. Infect Immun 85:e00640-17. https://doi.org/10.1128/IAI.00640-17.
19. Ouellette SP. 2018. Feasibility of a conditional knockout system for *Chlamydia* based on CRISPR interference. Front Cell Infect Microbiol 8:59. https://doi.org/10.3389/fcimb.2018.00059.