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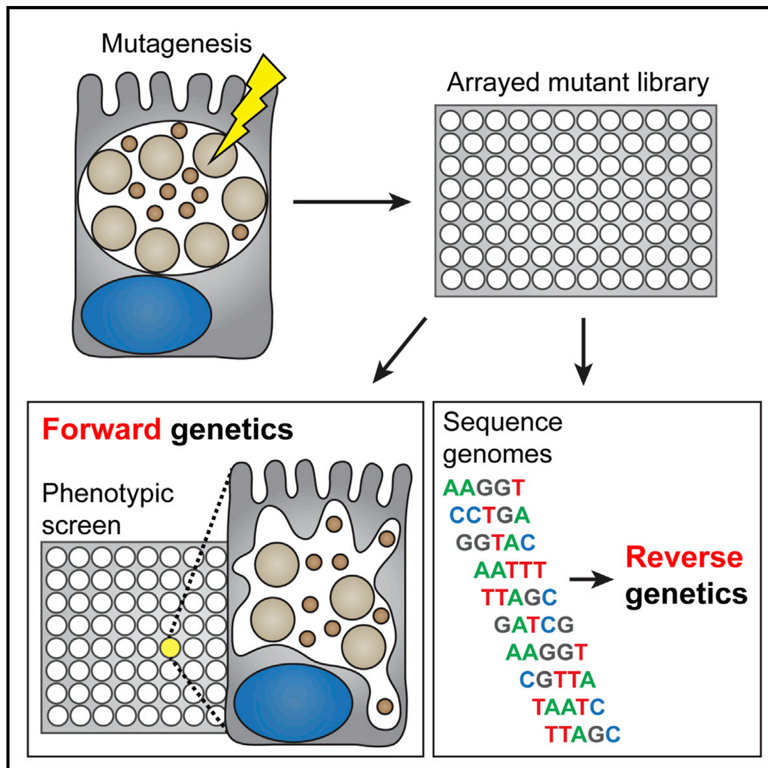
Integrating Chemical Mutagenesis and Whole-Genome Sequencing as a Platform for Forward and Reverse Genetic Analysis of *Chlamydia*

Graphical Abstract



Highlights

- A fully sequenced collection of chemically induced *C. trachomatis* mutants was generated
- Analysis of nonsense mutations reveals plasticity in *C. trachomatis* central metabolism
- A genetic screen found InaC modulates actin localization at the *Chlamydia* vacuole
- InaC modulates Golgi positioning and recruits host ARF GTPases and 14-3-3 proteins

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In Brief

Routine molecular genetic analysis of *Chlamydia* species is hampered by their limited genetic tractability. Kokes et al. describe a defined library of chemically mutagenized and sequenced *C. trachomatis* strains and its application for genetic screens.

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Integrating Chemical Mutagenesis and Whole-Genome Sequencing as a Platform for Forward and Reverse Genetic Analysis of *Chlamydia*

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SUMMARY

Gene inactivation by transposon insertion or allelic exchange is a powerful approach to probe gene function. Unfortunately, many microbes, including *Chlamydia*, are not amenable to routine molecular genetic manipulations. Here we describe an arrayed library of chemically induced mutants of the genetically intransigent pathogen *Chlamydia trachomatis*, in which all mutations have been identified by whole-genome sequencing, providing a platform for reverse genetic applications. An analysis of possible loss-of-function mutations in the collection uncovered plasticity in the central metabolic properties of this obligate intracellular pathogen. We also describe the use of the library in a forward genetic screen that identified InaC as a bacterial factor that binds host ARF and 14-3-3 proteins and modulates F-actin assembly and Golgi redistribution around the pathogenic vacuole. This work provides a robust platform for reverse and forward genetic approaches in *Chlamydia* and should serve as a valuable resource to the community.

INTRODUCTION

Advances in genome sequencing technologies enable the cost-effective sequencing of microbial genomes (Markowitz et al., 2012), many of which are recalcitrant to genetic analysis. As a result, the function of individual genes in these microbes is often inferred based on their homology to genes in model organisms where molecular genetic approaches, such as insertional mutagenesis or gene replacement, are standard. However, the development of a robust system for DNA transformation and the molecular tools to perform targeted mutagenesis in many microbes can be a lengthy process or may be unattainable. For instance, *Chlamydia trachomatis*, an intracellular bacterial pathogen responsible for blinding trachoma and sexually transmitted infections (Haggerty et al., 2010), was only recently reported to be amenable to DNA transformation and limited molecular genetic manipulation (Heuer et al., 2007; Kari et al., 2011; Wang et al., 2011; Nguyen and Valdivia., 2012; Johnson and Fisher., 2013), even though the organism was first cultured more than half a century ago.

The *Chlamydia* life cycle (Figure S1) alternates between an infectious elementary body (EB) and an intracellular replicative reticulate body (RB). Upon internalization, the EB modifies its membrane-bound vacuole to generate a compartment termed the inclusion (Hatch, 1999). Within the inclusion, EBs differentiate into RBs, replicate, and eventually differentiate back into EBs, which are released to initiate new rounds of infections (Dautry-Varsat et al., 2005). From within the inclusion, *Chlamydia* manipulates host cellular pathways to ensure its proliferation and survival (Bastidas et al., 2013), including changes to the organization of the host cell's internal architecture such as the redistribution of organelles and cytoskeletal elements around the inclusion (Kokes and Valdivia, 2012). Given the lack of robust tools for molecular genetic manipulation in *C. trachomatis*, the bacterial genes underlying these cellular changes, their contribution to *Chlamydia* pathogenesis, and the ensuing metabolic adaptations to the intracellular environment remain poorly understood.

In this work we generated and characterized a collection of chemically mutagenized *C. trachomatis* strains in which all induced gene variants were identified by whole-genome sequencing (WGS). In addition to providing a robust framework for reverse genetic applications, an analysis of variant alleles led to insights into the metabolic requirements of *Chlamydia* during infection of mammalian cells. Finally, we implemented a microscopy-based forward genetic screen and identified a bacterial factor important for regulating cytoskeletal rearrangements at the periphery of the inclusion. We find that this ARF and 14-3-3-recruiting factor also mediates Golgi reorganization, yet is dispensable for trafficking of Golgi-derived sphingolipids to the inclusion. Overall, our work illustrates the value of combining standard chemical mutagenesis and WGS as a platform for reverse and forward genetics applications.

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