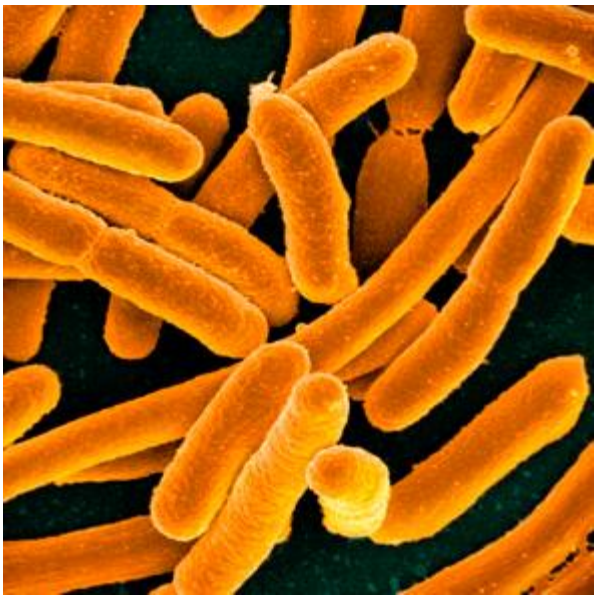
Highlights

- Modern to ancestral replacement of a proviral factor prevents virus propagation
- This result points to an approach to the engineering of virus resistance
- This approach could potentially be applied to the engineering of plant virus resistance
- Ancestral reconstruction may probe the evolution of biomolecular interactions

Summary

Proviral factors are host proteins hijacked by viruses for processes essential for virus propagation such as cellular entry and replication. Pathogens and their hosts co-evolve. It follows that replacing a proviral factor with a functional ancestral form of the same protein could prevent viral propagation without fatally compromising organismal fitness. Here, we provide proof of concept of this notion. Thioredoxins serve as general oxidoreductases in all known cells. We report that several laboratory resurrections of Precambrian thioredoxins display substantial levels of functionality within *Escherichia coli*. Unlike *E. coli* thioredoxin, however, these ancestral thioredoxins are not efficiently recruited by the bacteriophage T7 for its replisome and therefore prevent phage propagation in *E. coli*. These results suggest an approach to the engineering of virus resistance. Diseases caused by viruses may have a devastating effect in agriculture. We discuss how the suggested approach could be applied to the engineering of plant virus resistance.



Introduction

It has been estimated that direct losses caused by pathogens, animals, and weeds amount to 20%–40% of the global agricultural productivity (Savary et al., 2012). Plant viruses that affect economically important crops, in particular, often become a cause of severe hardship for large numbers of people (Strange and Scott, 2005, Rybicki, 2015). Several approaches to the engineering of plant viral resistance have been explored, and some crops with engineered resistance are grown commercially (Dasgupta et al., 2003, Prins et al., 2008, Collinge et al., 2010, Galvez et al., 2014, Whitham and Hajimorad, 2016). Briefly, introduction of viral genes into plants has been found in some cases to interfere with essential steps in the virus life cycle, thus giving rise to the so-called pathogen-derived resistance. Plant resistance genes encoding products that detect viral gene products and trigger resistance responses are known and have been shown to confer resistance to other plant species when used as transgenes. Finally, RNA-silencing systems in plants can be engineered to target viral genomes and transcripts.

Some host proteins play a permissive role for viral infection and some play a restrictive role ("proviral" and "antiviral" factors, respectively). There are abundant proviral factors as shown by recent studies (Friedel and Haas, 2011, Wang, 2015, Enard et al., 2016, Wilke and Sawyer, 2016, Sasvari and Nagy, 2016) thus pointing to many potential targets for resistance engineering. Very recent work has used gene-editing methodologies to suppress (knock out or render non-functional) proviral factors and achieve resistance (Chandrasekaran et al., 2016, Pyott et al., 2016). However, the applicability of this suppression approach is limited by the fact that proviral factors carry out "normal" functions in the host, and their suppression may therefore compromise host fitness and survival.

Pathogens and their hosts co-evolve. Proviral factors can be seen as proteins hijacked by viruses that are indeed losing the arms race (Daugherty and Malik, 2012, Demogines et al., 2013, Ng et al., 2015), as they have not yet adapted to evade viral hijacking. Therefore, it stands to reason that if a proviral factor in a host were exchanged for a functional analog, the fitness of the host might be only mildly affected, whereas the fitness of the virus could be substantially impaired. We hypothesize that a means to engineer this exchange is to take an ancestral version of the proviral host protein. Because the ancestral version would have co-evolved with a very different set of pathogens, it would be evolutionarily less simple for contemporary pathogens to swap to use the ancestral protein. That is, evasion and virus resistance can be achieved by using resurrected ancestral proviral proteins that are expected to maintain sufficient functionalities while being distant enough that they should represent a difficult challenge for the virus in terms of recruitment and adaptation. One can thus relatively preserve host fitness but impair viral fitness. Here, we present a test/demonstration of this approach using the infection of *Escherichia coli* by the bacteriophage T7 as a simple model. As explained below in some detail, the molecular features of the relevant intermolecular interactions make this system particularly attractive as a proof of concept of our proposal. Furthermore, in the Discussion section, we will speculate as to the greater relevance of the proposed approach in engineering viral resistance, and specifically, we will outline how it could be applied to engineering plant viral resistance.

Bacteriophage T7 recruits *E. coli* thioredoxin to be a part of its "minimalist" (four proteins) replisome (Hamdan and Richardson, 2009). When recruited, thioredoxin functions as a processivity factor for the gp5 DNA polymerase. It binds strongly to gp5 and increases its processivity from 1–15 to several hundred nucleotides per binding event, likely by suppressing hoping on and off the DNA (Etson et al., 2010). Binding to gp5 is mediated by specific interactions with a unique 76-residue fragment known as the thioredoxin-binding domain (for instance, see Figure 1 in Akabayov et al. [2010]]). Such specific interaction appears to reconfigure gp5 to enhance contact with DNA and create docking sites for the other proteins of the replisome (Hamdan and Richardson, 2009, Lee and Richardson, 2011). The thioredoxin-binding domain of the phage gp5 is not found in other members of this polymerase family and is likely the result of the evolutionary adaptation of bacteriophage T7 for efficient propagation in *E. coli*. In fact, with a value for the dissociation constant of 5 nM, the interaction between *E. coli* thioredoxin and the thioredoxin-binding domain in gp5 is actually one of the highest-affinity interactions involving replication proteins (Hamdan and Richardson, 2009). The

very high specificity of this interaction makes it plausible that at least some ancestral forms of thioredoxin may not be recruited by bacteriophage T7.

On the other hand, the “normal” role of thioredoxin in all known cells is to serve as a general oxido-reductase (Holmgren, 1995). As such, thioredoxin is involved in a diversity of cellular processes, and a large number of potential substrates for thioredoxin have been identified using a variety of approaches (Collet and Messens, 2010, Kumar et al., 2004). This wide substrate scope is likely achieved through a molecular mechanism that does not impose highly specific intermolecular interactions. In fact, the disulfide bridge of the conserved CGPC active site motif in thioredoxins is solvent-exposed and protrudes somewhat from the structure of the protein (thioredoxin has sometimes been referred to as a “male enzyme”) (Holmgren et al., 1975). This structural feature should promote the processing of a diversity of substrates. Certainly, any change in a complex system involving interacting parts or processes is likely to generate a less fit system (Kirschner and Gerhart, 2005) and, consequently, replacing a modern protein in a modern organism with a representation of one of its ancestors is expected to bring about a fitness cost in many instances (Hobbs et al., 2015). Still, given that the most common oxido-reductase function of thioredoxin involves molecular interactions that are not highly specific, it would not be unreasonable to expect ancestral thioredoxins to show some degree of functionality within an *E. coli* cell.

Overall, it seems plausible that at least some ancient thioredoxins could functionally replace the modern thioredoxin within *E. coli* and, in addition, make *E. coli* resistant to the propagation of bacteriophage T7. Certainly, an experimental test of this possibility would seem to face an insurmountable barrier, i.e., the fact that ancient proteins no longer exist. However, phylogenetic analyses of modern protein sequences can be used to derive plausible approximations to the sequences of proteins in extinct organisms (Pauling and Zuckerkandl, 1963). Indeed, proteins encoded by such reconstructed ancestral sequences (i.e., “resurrected” ancestral proteins, to use the common jargon of the field) have been extensively used in the last ∼20 years to address important problems in molecular evolution (for reviews, see Benner et al., 2007, Liberles, 2007, Harms and Thornton, 2010, Harms and Thornton, 2013, Risso et al., 2014, Merkl and Sterner, 2016). Here, we specifically use several resurrected Precambrian thioredoxins that actually span the ∼4 billion years of evolution of life on Earth (see Figure 1 for a description of the corresponding phylogenetic nodes and the values of the identity of the reconstructed sequences with the sequence of *E. coli* thioredoxin). These putative Precambrian thioredoxins have been previously prepared and exhaustively characterized (Perez-Jimenez et al., 2011, Ingles-Prieto et al., 2013, Romero-Romero et al., 2016). They are properly folded and share the thioredoxin fold. They are also stable, enzymatically active, and their in vitro properties define convincing evolutionary narratives as we have previously discussed in detail (Perez-Jimenez et al., 2011, Ingles-Prieto et al., 2013, Romero-Romero et al., 2016). For comparison, *E. coli* thioredoxin and human thioredoxin are also included in this study.

Links: http://www.cell.com/cell-reports/fulltext/S2211-1247(17)30531-4?_returnURL=http%3A%2F%2Flinkinghub.elsevier.com%2Fretrieve%2Fpii%2FS2211124717305314%3Fshowall%3Dtrue