Plasmid 76 (2014) 72–78

Contents lists available at ScienceDirect

Plasmid

journal homepage: www.elsevier.com/locate/yplas

Review

Two replication initiators – one mechanism for replication origin opening?

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ARTICLE INFO

Article history:
Received 23 June 2014
Accepted 10 October 2014
Available online 18 October 2014
Communicated by Saleem Khan

Keywords:
Rep proteins
DnaA
DNA replication initiation
Plasmid RK2

ABSTRACT

DNA replication initiation has been well-characterized; however, studies in the past few years have shown that there are still important discoveries to be made. Recent publications concerning the bacterial DnaA protein have revealed how this replication initiator, via interaction with specific sequences within the origin region, causes local destabilization of double stranded DNA. Observations made in the context of this bacterial initiator have also been converging with those recently made for plasmid Rep proteins. In this mini review we discuss the relevance of new findings for the RK2 plasmid replication initiator, TrfA, with regard to new data on the structure of complexes formed by the chromosomal replication initiator DnaA. We discuss structure–function relationships of replication initiation proteins.

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1. Introduction

Replication initiation of iteron containing plasmids depends on specific nucleoprotein complexes being formed in the origin by the plasmid encoded replication initiation protein (Rep) and the host encoded DnaA protein. RK2, an iteron containing plasmid that belongs to IncP-1 plasmid family, replicates in many Gram-negative bacteria. This plasmid has been extensively used for understanding DNA metabolism (i.e., replication, stable inheritance, horizontal gene transfer). Studies conducted on RK2 over the last few years have provided new data concerning origin structure, structure and function of the plasmid replication initiator TrfA, and host encoded chaperone and protease activities on the plasmid replication initiator. A study on nucleoprotein complex formation by the TrfA protein with single stranded DNA (ssDNA) has also been recently pub-

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http://dx.doi.org/10.1016/j.plasmid.2014.10.003
0147-619X/

lished. These findings have changed our understanding of the process of DNA replication initiation of iteron containing plasmids and also raise new questions.

2. Plasmid replication initiator – the TrfA protein

TrfA is the RK2 plasmid replication initiation protein. In the cell TrfA is present in two forms that differ in molecular mass. TrfA-33, with 33 kDa mass, and TrfA-44, with 44 kDa (Fig. 1), result from two alternative start codons in the same open reading frame in the *trfA* gene (Shingler and Thomas, 1989; Smith et al., 1984). Both forms of TrfA are functional; however utilization of a particular form depends on the host bacterium. Plasmid RK2 encodes only one other protein involved in DNA replication, SSB (Single Stranded Binding) protein, although the relevance for encoding its own SSB protein is not known. All other proteins required for replication of the plasmid are encoded by the host chromosome. It has been observed that in *E. coli* replication can be initiated by either TrfA-33 or TrfA-44, though TrfA-33 is sufficient in this process (Durland and Helinski, 1987; Shingler and Thomas, 1989). Both forms of TrfA are supported by replication proteins carried by the host bacterium (Durland and Helinski, 1987; Konieczny et al., 1997). In contrast, in *P. aeruginosa,* the TrfA-44 variant is essential for RK2 replication initiation and this process is independent of the host DnaA protein. While in *P. putida*, both forms act equally and also do not require DnaA (Caspi et al., 2001; Durland and Helinski, 1987). Recently, it has been reported that in *C. crescentus* two modes of replication initiation are possible. The first, similar to that exhibited in *Pseudomonas,* utilizes the longer version of TrfA, without the presence of DnaA. The second mode is DnaA-dependent and utilizes the shorter version of TrfA protein (Wegrzyn et al., 2013).

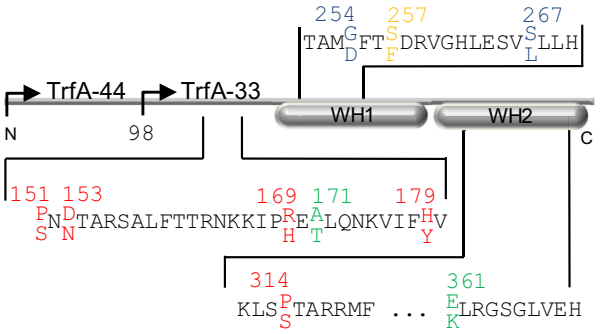

Fig. 1. Mutations affecting TrfA quaternary structure and the protein's interactions with DNA. Rounded rectangles correspond to the two WH (Winged Helix) domains; the disordered N-terminal region (aa 1–160) is simply represented as a line. Internal translation starts for the two protein variants: 44 kDa TrfA (TrfA-44) and 33 kDa TrfA (TrfA-33) are marked with arrows. The figure shows amino acid substitutions that affect dimerization of TrfA and TrfA-DNA binding. A combination of two substitutions G254D/S267L (colored blue) results in a monomeric form of the protein. Mutation S257F (colored orange) results in a super-dimer protein form. Substitutions P151S, D153N, R169H, H179Y, P314S (colored red) result in a DNA binding defective phenotype. Mutations A171T and E361K (colored green) result in proteins that interact with DNA more efficiently than wild type protein (based on Cereghino et al., 1994; Lin and Helinski, 1992; Pierechod et al., 2009).

It has been demonstrated that the quaternary structure of TrfA affects its stability and accessibility during the cell cycle. As with replication initiators from other plasmids, both TrfA variants, TrfA-33 and TrfA-44, exist in the cell as dimers but strongly bind to the origin of replication in the form of monomers (Lin and Helinski, 1992; Toukdarian et al., 1996). TrfA dimers are activated to the monomeric form by the ClpX chaperone (Konieczny and Helinski, 1997) or the cooperation of DnaK/ClpB (Konieczny and Liberek, 2002). The level of active TrfA protein within the bacterial cells is regulated not only by the monomerization process but also by degradation of unnecessary protein molecules. Proteolysis of TrfA protein depends on its oligomeric state. It has been shown that the ClpXP and ClpYQ proteases are able to degrade only TrfA dimers. For the ClpAP and Lon proteases the oligomeric state of the TrfA substrate is not crucial for proteolytic activity. Both of these proteases are able to process both monomers and dimers TrfA equally (Kubik et al., 2012; Pierechod et al., 2009). Interestingly, TrfA degradation by ClpAP or Lon is stimulated when the reaction mixtures also include plasmid DNA, which can be bound by both protease and the TrfA protein (Kubik et al., 2012).

The crystal structure of the TrfA protein has not been yet determined. However, structure prediction using fold-recognition homology modeling has been carried out (Pierechod et al., 2009). According to this structural prediction, the N-terminal part of TrfA (residues 1–160 of TrfA-44) does not show a unique three dimensional structure. The C-terminal part of protein (residues 195–382) has two Winged-Helix (WH) domains (Pierechod et al., 2009) (Fig. 2B). WH domains are typical of DNA Binding Domains (DBDs) that are present in both plasmids (e.g. RepE, RepA or π protein) and eukaryotes Origin Binding Proteins (OBPs) but are not present in bacterial chromosomal initiators (Giraldo, 2003; Giraldo and Fernandez-Tresguerres, 2004; Giraldo et al., 2003; Komori et al., 1999; Sharma et al., 2004; Swan et al., 2006). WH structures are composed of a bundle of three α-helices (Helix-Turn-Helix; HTH motif) with an antiparallel β-sheet. The helices of both of the TrfA WH structures interact with the major grooves of the DNA phosphate backbone (Pierechod et al., 2009). A number of mutations, located within the WH1 or WH2 domains, are known to affect TrfA–DNA binding (Fig. 1). Substitutions D198N, P314S, R338C result in TrfA variants defective in DNA binding while E361K results in an increased protein affinity for DNA as compared with wild-type TrfA (Cereghino et al., 1994; Lin and Helinski, 1992). Interestingly, outside the two WH domains in the N-terminal unstructured region, there are residues which when mutated affect TrfA–DNA interaction in both ways. Substitutions P151S, D153N and R169H decrease the affinity of TrfA for DNA, while A171T increases it (Cereghino et al., 1994; Lin and Helinski, 1992).

In the predicted WH1 domain of the TrfA protein, a dimerization interface (residues 234–290) is located on the extended antiparallel β-sheet (Pierechod et al., 2009). Specific substitutions (Fig. 1) in this interface affect the stability of the TrfA dimer. TrfA mutants G254D, S267L, and G254D/S267L are mainly monomeric proteins, while mutant S257F is a stable dimer which cannot be converted to monomers