Rise of the Terminator protein Tus: a versatile tool in the biotechnologist's toolbox

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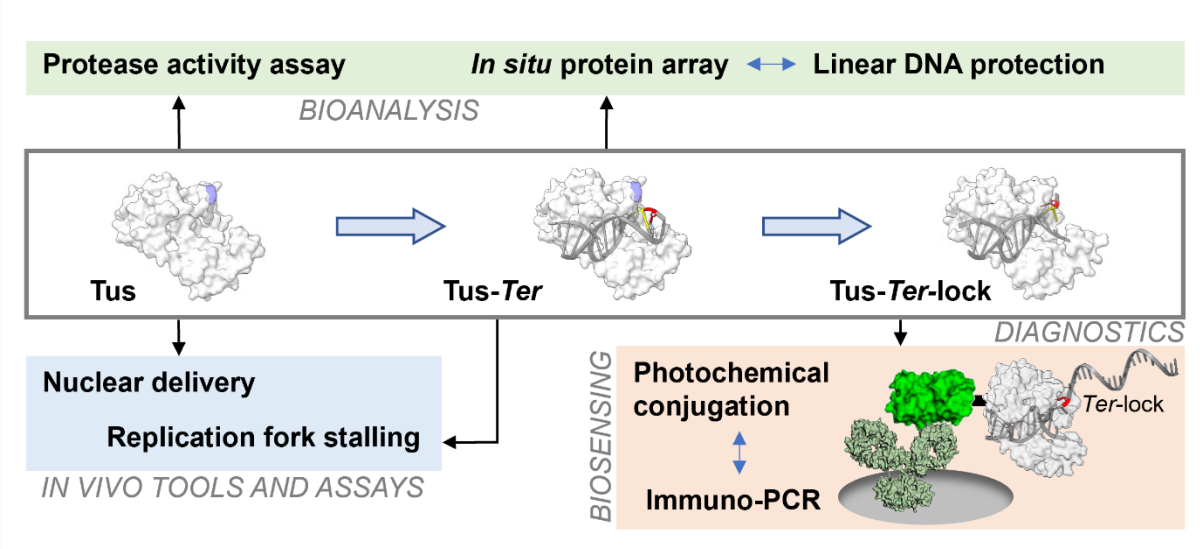
Abstract

Tus is a protein involved in DNA replication termination that binds specific DNA sequences (*Ter*) located around the terminus region of the chromosome in Enterobacterales. Tus and *Ter* form a unique monomeric protein-DNA complex which is one of strongest of its kind. A fascinating aspect of Tus-*Ter* is its ability to dramatically change conformation into a locked structure upon progression of a replication fork towards the non-permissive face of the complex. Over the last two decades, several new technologies have emerged harnessing the unique and interesting properties of this fascinating DNA-binding protein. This review highlights the important properties of the Tus-*Ter* complex and their exploitation for the development of diverse and novel ultrasensitive detection devices as well as innovative genomic and proteomic platform technologies. A variety of *ex vivo* and *in vivo* bioanalytical applications are discussed, including immuno-PCR diagnostic, bioassay and protein array technologies that are broadly relevant to the fields of cancer biology, microbiology and immunology. A perspective on future research and applications is provided.



Keywords

Tus-*Ter*, protease assay, immuno-PCR, bioassay, protein array, replication fork barrier

1. Introduction

The ultrastable complex formed between avidin, streptavidin or its derivatives and biotin has become a staple system used in a myriad of bioanalytical techniques [1-3]. In particular, biotinylation of oligonucleotides opened the doors to new and disruptive technology developments revolving around the principles of protein-DNA conjugation and display. One of the limitations of this system lies with its widespread success and applications, creating possible interferences and incompatibilities between methods and commercial kits that are based on this same interaction. Another limitation stems from the presence of variable concentrations of biotin in biological samples, a potential interference in clinical immunoassays that are based on the streptavidin-biotin interaction [4]. As such, proteins that form strong and specific interactions with ligands and nucleic acids offer interesting alternatives and application opportunities for biosensing and ultrasensitive detection technologies [5] that would also be compatible and even complementary with the streptavidin-biotin system.

Tus is a relatively small protein (36 kDa) involved in bacterial DNA replication termination where its function is to stop the giant replisome traveling along the chromosome at ~60 kb/min at specific *Ter* sites [6-8]. Over the years, the binding of Tus to *Ter* sequences as well as its ability to form a unique Tus-*Ter*-lock structure [9] have been dissected in minute detail (Figure 1). So much so, that in 2007, the *E. coli* Tus protein was used as a control protein to validate a sortase-mediated protein ligation assay for the attachment of proteins to surfaces [10] (Table 1). Various biotechnological applications making use of the intrinsic properties of *E. coli* Tus and *Ter* have been reported in the form of scientific literature (Table 2) and intellectual property (Table 3) since 2001. Interest in the applicability of the Tus-*Ter* interaction was initially sparked by its extreme stability and later by its ability to form a unique Tus-*Ter-lock* structure that is even more stable [9, 11, 12] (Figure 1).

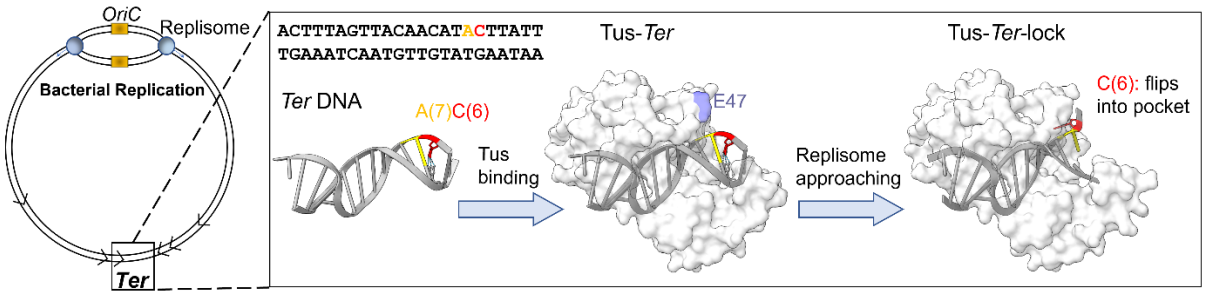


Figure 1: Bacterial DNA replication termination involving the Tus-*Ter* interaction. Tus binds to a *Ter* sequence with specific polarity. When the replisome approaches the complex from the C(6) nucleotide (red) end, the action of the helicase unwinds the DNA, liberating the C(6) base that can lodge into a specific binding pocket, yielding the Tus-*Ter*-lock structure. Specific bases and the E47 amino acid residue that are specific to biotechnological applications are indicated. Structures were generated with ChimeraX from the coordinates of PDB 2I05 (*Ter* and Tus-*Ter*) and 2I06 (Tus-*Ter*-lock) files.

A year after the use of Tus to validate a sortase-mediated protein ligation assay [10], the first example of a Tus-based technology was reported [13]. Indeed, taking advantage of the intrinsic DNA-binding property of Tus for the first time, Chatterjee et al. successfully developed an innovative protein microarray technology [13]. From that point onwards, the intrinsic properties of Tus and of its complexes with *Ter* were harnessed to develop new technologies and methods (Table 2). In parallel, and essentially due to its predictable nature, Tus was also chosen as a well-behaved reference protein to validate several innovative technologies (Table 1). These included protein-ligand assays such as GFP-based protein stability assay (GFP-Basta) and differential scanning fluorimetry of GFP-tagged proteins (DSF-GTP) [14, 15], a real-time PCR (qPCR) method to compare the binding of a protein to different DNA targets in polyplex [16], and the evaluation of active protein incorporation into a self-assembling peptide hydrogel [17].

Table 1: Technology validation with Tus

Year	Validation	Form	References
2007	Sortase protein ligation	Tus-LPETGG-His_6	[10]
2010	GFP-based protein stability assay	His_6-Tus-GFP	[14]
2012	DSF-GTP	His_6-Tus-GFP	[15]
2012	Polyplex qPCR-based Protein-DNA binding assay	His_6-Tus-GFP	[16]
2013	High-throughput DSF-GTP	His_6-Tus-GFP	[18]
2013	Self-assembling functionalized hydrogels	Tus-LPETGGFEFEFFKFKK	[17]
2015	In-gel detection of biotinylated proteins	His_6-Tus-AviTag-biotin	[3]

Similar to streptavidin, avidin and other high-affinity binders which are widely used in molecular sciences owing to their highly selective and stable interaction with biotin [2, 3], new developments of *E. coli* Tus-*Ter* into molecular tools will continue to emerge. Here, the small size and monomeric DNA-binding modality of Tus [6, 19] are desirable traits compared with the possible challenges of multimeric protein interactions such as the tetrameric streptavidin

[2]. This review presents for the first time a systematic and detailed historical account of the development of the Tus-*Ter* system and its translation into a variety of biotechnological applications.

Table 2: Biotechnological applications of Tus

Year	Target Application	Principle	References
2008	Protein array	Self-assembly of Tus fusion protein with *Ter*-containing plasmid	[13, 20]
2009	Protein-DNA conjugation	Photoactivatable protein-DNA conjugation	[21]
2010	Protein delivery into the nucleus	Mammalian-like nuclear localisation signal present in Tus	[22]
2010	Immuno-PCR	Tus-*Ter*-lock based protein-DNA conjugation	[23-28]
2011	Protease assay	Thermosensitivity of Tus-GFP containing a protease substrate	[29]
2014	DNA barriers and perturbations	Tus-*Ter* induced replication fork arrest in yeast and mammalian cells.	[30-35]
2021	Linear DNA protection	*Ter* added to DNA ends	[36]

Table 3: Intellectual property landscape

Year	Patent title	Patent number	Active country	References
2001	*Ter* sites and *Ter* binding proteins	PCT/US2002/003366	JP, NZ	-
2004	Method of selecting polypeptides	PCT/GB2005/004148	-	-
2005	Double-stranded oligonucleotides and uses therefor	PCT/AU2006/000136	-	[9]
2005	In situ assembly of protein microarrays	US12/105,636	US, DE	[13, 20]
2007	Diagnostics in a monoplex/multiplex format	PCT/AU2007/000798	-	[23-28]
2007	Polynucleotide backbones for complexing proteins	PCT/US2008/077887	JP	-
2008	Methods and compositions for protein delivery	PCT/US2009/059328	US	[22]
2011	Protease activity assay	PCT/AU2012/000343	-	[29]
2014	Compositions and methods for characterizing a DNA repair variant polypeptide	US14/941,769	US	[30-35]
2017	Method of replication or amplification of circular DNA	PCT/JP2018/007485	CA, SG, BR, RU, EP, JP, US, AU, CN, KR, IL	-
2021	Protection of Linear Deoxyribonucleic Acid from Exonucleolytic Degradation	Provisional US patent US63/142,097	-	[36]

2. Proteomic applications

2.1. Protein array

Typical protein array designs use strategies that immobilise purified proteins onto an array slide. They require large libraries of purified proteins and well-defined long-term storage conditions [13, 37]. Protein in situ array (PISA) and nucleic acid protein programmable array (NAPPA) technologies were developed to overcome these disadvantages [38-40]. However, these require the use of affinity tag-specific capture and display of proteins of interest, leading to potential issues with loss of avidity and off-target binding [41]. The Tus-*Ter* system was selected to develop a protein array strategy that would bypass most of these requirements [13, 20]. The array technology consists of a plasmid that is printed onto an array slide, including *TerB* and a *tus* gene cassette for *in situ* production of fusion proteins tethered to Tus. Here, the immobilised plasmid has a dual function for cell-free protein expression, and direct *in situ* capture of the Tus-fusion protein onto the array slide via complex formation with *TerB.* An N-terminal GFP-tagged Tus E47Q mutant (GFP-Tus) was used in the proof-of-concept stage of this protein array development. The E47Q mutation is known to further increase the affinity of Tus for *TerB* [42]. The array technology was evaluated further with a selection of known protein-protein interactions such as p53/MDM2, CDK4/p16, FRB/FKBP12 and Jun/Fos. The method led to the identification of p16 as a novel CDK2 binding partner [20]. While Tus can be successfully tagged, the expression of fusion proteins in functional form cannot be assessed in this array format. As such, care must be taken that protein expression conditions maximise the stability and functionality of fusion proteins, and reduce protein aggregation. In principle, identification of protein-DNA, enzyme-substrate, ligand-receptor and protein-drug interactions can be envisaged with this platform technology but are yet to be demonstrated [20].

2.2. Site-specific photoactivatable protein-DNA conjugation

Site-selective conjugation of native proteins with DNA enabled the development of innovative bioanalytical and imaging technologies such as immuno-PCR, proximity ligation, DNA-PAINT and Exchange-PAINT to name a few [43-47]. In 2009, a modified Tus-*Ter-lock* complex was reported as a very efficient protein-DNA photo-crosslinking system [21]. Here, the A(7) in the *TerB*-lock sequence (Figure 1) was replaced by a photoactivatable bromodeoxyuridine (BrdU) base. With this system, site-specific protein-DNA conjugation could be achieved in just a few minutes with yields up to 65%. The broad applications of this rapid conjugation system was evaluated with a C-terminal GFP-tagged Tus (Tus-GFP). This protein-DNA conjugation technique offers significant advantages, such as the synthesis of covalent protein-DNA conjugates with well-defined stoichiometry and regiospecificity, which can be performed in droplets on surfaces using low reagent concentrations. However, the presence of unreacted species could be a potential limitation when pure covalent conjugates are required. The procedure has obvious potential in protein array and display technologies, DNA-directed protein immobilization, proximity ligation and immuno-PCR, and has yet to find a suitable application in DNA nanotechnology [46, 48, 49] as an alternative to the biotin-streptavidin interaction.

2.3. Tus-based immuno-PCR diagnostics

Immuno-PCR is based on the high specificity and affinity of an antigen-antibody complex, and the extreme DNA detection sensitivity of PCR [50]. Real-time or quantitative Immuno-PCR (qIPCR) can improve the analytical detection sensitivity of a classic immunoassay such as the enzyme-linked immunosorbent assay (ELISA) by up to ~10^9 fold [51]. Several linking systems have been described for antibody-DNA conjugation that are useful for qIPCR [51]. Most of

these systems produce heterogenous antibody-DNA conjugates, and lack the high batch-to-batch reproducibility and simplicity necessary to translate qIPCR technologies into routine laboratory and diagnostic tools. The monomeric nature of Tus yields well-defined and predictable protein-DNA conjugates [23-26, 51]. As such, qIPCR is one of the most promising field of applications of the DNA binding property of Tus.

In its inception, a *Ter*-lock sequence extended with a short single-stranded template for PCR amplification was coupled with Tus-GFP and used as proof-of-principle detection device in the first Tus-based qIPCR assay [23]. This system allowed ultrasensitive detection of GFP-specific antibodies in the femtomolar range. The utility of the Tus-based qIPCR platform technology was evaluated further in various formats [23] and led to the development of a number of qIPCR assays for the sensitive detection of a variety of protein analytes [16, 23-28].

Tus fusions with various IgG binding proteins demonstrated the utility of the Tus-based qIPCR platform technology for the universal detection of mammalian IgG, with superior sensitivity compared to protein G-peroxidase-based ELISAs [24]. A Tus fusion with two IgG-binding domains from protein G (2G-Tus) was employed for the detection of antibodies to *Burkholderia pseudomallei* LPS in melioidosis patients [27]. Importantly, the 2G-Tus-based qIPCR was able to detect antibodies in the sera of culture-confirmed melioidosis patients that could not be detected using the widely used indirect hemagglutination assay (IHA).

In the same period, a proteomic application of the technology was also evaluated for quantifying dual affinity-tagged target proteins in a complex biological matrix such as serum, using a 'bridged' system [26]. In this format a Tus fusion with a short hemagglutinin epitope tag (Tus-HA) was used in combination with high affinity anti-HA antibodies to detect picomolar concentrations of HA-tagged proteins expressed or circulating in a variety of complex matrices, including serum, cell culture media and cell lysates. One of the most promising applications of this system was specifically designed to detect minute quantities of

tropomyosin-specific IgE antibodies [25, 28, 52]. The study compared the analytical sensitivity of a Tus-HA-anti-HA-Tropomyosin-HA 'bridged' system with a direct Tus-Tropomyosin fusion. The latter detection device performed best and was capable of detecting a reference tropomyosin-specific IgG present at a concentration of 1.2 pM, which is suitable for the detection of tropomyosin-specific IgE at the lower limit cutoff for allergy equalling 4.7 pM [25]. Using this format, tropomyosin-specific IgE could be detected using only 5 µl of shellfish allergic patient sera.

It seems likely, that the different protein-DNA conjugation systems that are already available will provide opportunities for multiplexing qIPCR assays in the future. With respect to the Tus-based qIPCR platform technology, additional avenues for multiplexing could arise from the discovery of Tus orthologs or reengineering of Tus with altered DNA sequence specificities.

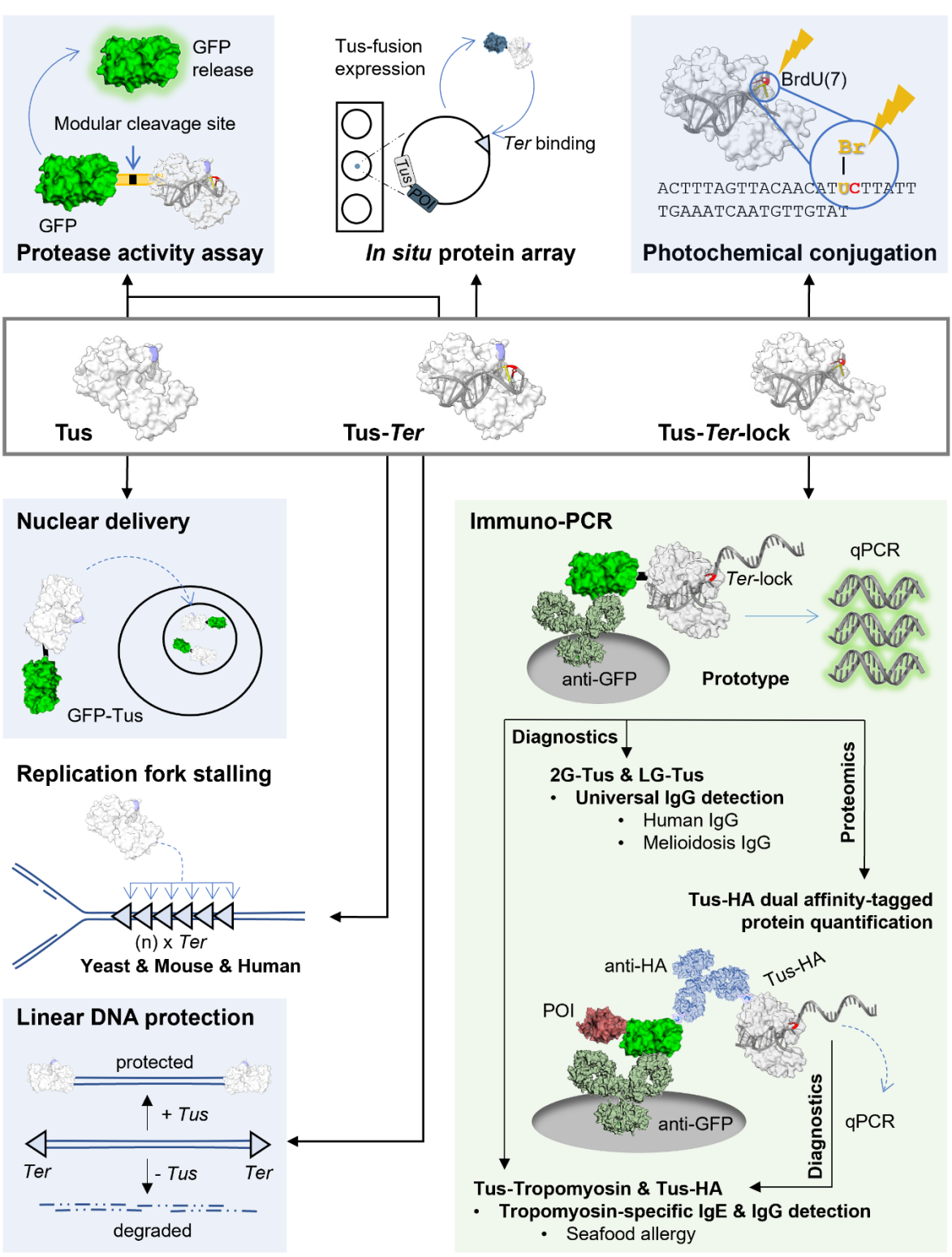


Figure 2: Tus-based platform technologies. Black arrows highlight the links between different platform technologies and the relevant forms of Tus as well as some of the important interlinks between applications. The Tus-GFP protease activity assay takes advantage of the large difference in thermal stability between Tus and GFP, which are linked by a modular

cleavage site. Proteolytic cleavage of free or *Ter*-bound Tus-GFP is followed by a thermal denaturation step. The GFP fluorescence is proportional to the protease activity. The *in situ* protein array uses plasmids including both a *TerB* and a *tus* cassette for cell-free production of Tus fusion proteins that are bound to the array slide *in situ*. Photochemical conjugation requires the A(7) of a *Ter*-lock sequence to be substituted with a photoactivatable BrdU to enable production of covalent Tus-DNA conjugates. Nuclear delivery of Tus and fusions thereof is possible due to the fortuitous presence of a nuclear localisation signal sequence. Replication fork stalling requires the site-specific insertion of a cluster of *Ter* sites with concomitant Tus expression. The linear DNA protection system requires the addition of a *Ter* sequence to each end of the DNA where Tus inhibits exonuclease activity. The immuno-PCR technology takes advantage of the superstable Tus-*Ter*-lock complex where a Tus-containing fusion is coupled with a *Ter*-lock-containing DNA template sequence to quantify the presence of proteins such as antibodies by qPCR.

2.4. Protease activity assay

In 2011, a protease activity assay was reported that is based on a Tus-GFP fusion cassette including a modular protease substrate sequence in its interdomain linker sequence [29]. The system takes advantage of the lower thermal stability of Tus compared to GFP. When Tus-GFP is incubated with a protease that cleaves the interdomain linker, the thermostable GFP is released. Following subsequent heat denaturation and centrifugation steps, non-proteolysed Tus-GFP aggregates are removed from the supernatant. As such, the increase in released GFP and fluorescence in the supernatant correlates directly with increased protease activity. This system was successfully applied to measure the protease activities of trypsin, caspase 3, and neutrophil elastase with analytical detection sensitivities in the nanomolar range. It was successfully validated with the human neutrophil elastase peptide chloromethyl ketone inhibitor (MSACK), demonstrating its suitability for protease inhibitor screening. An advantage of this system is that longer incubation times and higher reaction temperatures are possible when a *Ter* sequence is added to increase the thermostability of Tus, which in turn improves the analytical sensitivity of the assay. One of the major limitations of this system is that it cannot be performed in real-time and thus is not practical to monitor kinetics due to its endpoint assay format. Nevertheless, the assay should be useful in drug discovery programs for the screening and characterisation of protease inhibitors.

2.5. Linear DNA protection

One of the most recent developments of Tus could very well be its simplest, yet most valuable application and may become a staple in the biotechnologist's toolbox. Cell-free synthetic biology requires stable DNA templates for gene expression. The presence of exonucleases in bacterial cell-free systems undermines the potential for use of linear DNA template for protein expression, although modifications to the template can provide some nuclease protection specific to *E. coli*-based systems [53]. As such, circular plasmids with all their disadvantages

are the usual DNA format used in bacterial cell-free systems. However, the conception of terminal protection of linear template by DNA binding proteins [36, 54, 55] has been shown to significantly increase stability against exonuclease degradation in *E. coli* as well as non-model organisms. Initially the dimeric single-chain bacteriophage lambda Cro repressor and its operator recognition consensus sequence (half-life of 150 min [56]) was used [54]. In parallel, a similar method was developed using the mycobacterial Ku protein which binds non-specifically to exposed DNA ends [55]. More recently, Norouzi et al. [36] reported the successful use of the Tus-*Ter* interaction (half-life of 550 min [11]) as a means to protect the ends of linear DNA. They demonstrated the robustness of the Tus-*Ter* protection system with *E. coli* and *V. natriegens* cell-free extracts obtaining high levels of protein expression comparable to plasmid-based expression yields [36]. In future, incorporation of this protection strategy into a Tus-based protein microarray [13, 20] that would utilise linear DNA could be of high interest. In this scenario, Tus fusions binding to the DNA would also protect it.

3. Cell-based applications

3.1. Nuclear protein delivery

Unexpectedly, Tus contains an amino acid sequence resembling a mammalian nuclear localization signal that has significant nuclear targeting efficiency [22, 57]. Kaczmarczyk et al. reported that expression of GFP-Tus can be achieved in human cells with almost exclusive nuclear localization. Transduction of GFP-Tus from culture media also resulted in its accumulation in the nucleus [22]. Here, Tus was proposed as a possible protein delivery system into the nucleus of mammalian cells. Although the nuclear localisation of Tus was relatively moderate, and is obviously a fortuitous coincidence, it showed that Tus fusion proteins could be expressed in eukaryotic cells in a functional form hinting towards potential applications in higher organisms.

3.2. Tus-*Ter* induced replication fork arrest

A common bioanalytical method to track the fate and processing of a site-specific stalled eukaryotic fork *in vivo* utilises a naturally occurring polar replication fork barrier from *Schizosaccharomyces pombe,* which is based upon the replication termination site 1 (*RTS1*) and its binding protein (Rtf1) [58]. The system requires insertion of numerous bacterial *LacO* repeats to enable quantitative bioimaging of fluorescently labelled LacI foci and a DNA repair factor labelled with a different fluorescent tag. Unfortunately, the reporter LacI-*LacO* array also acts as a non-polar replication fork barrier, which must be differentiated, and the system is limited to fission yeast models.

In 2014, Larsen et al. reported the first application of Tus-*Ter* as a DNA replication perturbating system [31]. The system was applied to examine the impact of site-specific DNA replication fork barriers in the yeast *Saccharomyces cerevisiae* genome [34]. Larsen et al. reported that genomic insertion of *Ter* modules with concomitant expression of Tus, form site-specific replication fork barriers that can be resolved by RecQ helicase-dependent homologous recombination in the yeast. Willis et al. [30] designed a similar system that was applied to mouse cells. The study revealed that stalled replication forks contribute to genomic instability and specifically to breast and ovarian cancer predisposition in BRCA mutant cells, via aberrant long-tract homologous recombination. In 2018, the Tus-*Ter* system was used to examine whether homologous or non-homologous end joining recombination was triggered to repair chromosomal replication fork barriers in mammalian cells [32]. The authors found that the primary repair mechanism was homologous recombination via early recruitment of Rad51 at stalled replication fork sites. Interestingly, all these studies used a Tus with an NLS tag despite the fact that this may not be necessary [22] in contrast with what would be an inherent requirement for the LacI-*LacO* and TetR-*TetO* artificial replication fork barrier systems [59].

The use of Tus-*Ter* as a physical barrier on DNA has proven to be a very useful tool to induce recombination events and will no doubt be applied to other studies examining the impact of

artificial barriers on DNA in eukaryotes and prokaryotes. Of note, Ramachandran et al. [60] applied Tus-*Ter* to conditionally block the replication of chromosome 1 (Chr1) in *Vibrio cholera*. *Ter* sequences were inserted on each flank of the origin of replication *(ori1)* and Tus expression was controlled by an arabinose-inducible promoter. The experiment was performed in a microfluidic chamber, which allowed controlled addition as well as removal of the inducer. As such, the effects of blockage and release of replication forks could be observed and analysed in real-time with this advantageous and unique polar fork arrest system.

4. Conclusion and perspective

The Tus protein had been studied in great detail over several decades, eventually culminating in 2006 with the Tus-*Ter*-lock structure that enabled delineation of the currently accepted mechanism of polar fork arrest in *E. coli* [9]. Tus had finally reached the status of a 'model' DNA-binding protein with useful and predictable attributes. Not surprisingly, the first biotechnological applications of Tus emerged soon after. While its utility as a protein-DNA conjugation system was somewhat predictable, given its extremely strong DNA-binding affinity, some of its other applications in protease assays and nuclear protein delivery were not. Of note, its more recent deployment as a site-specific heterologous DNA replication perturbation system has the potential to become a very useful bioanalytical tool to examine DNA replication, repair and recombination [30-35, 58].

Moving forward, with the recent characterisation of different types of replication fork traps in Enterobacterales [8], there is obvious scope for the characterisation and development of interesting orthologous Tus-*Ter* systems with different DNA binding properties. There is also potential for reengineering the DNA-binding specificity and properties of Tus, offering the possibility of multiplexing immuno-PCR and protein array technologies in combination with other stable systems such as the streptavidin-biotin interaction [3]. Here, the biotinylated Tus

[3] and 2G-Tus [24] would have obvious potential to be translated into valuable commercial immunodetection devices.

One of the major advantages of the Tus-*Ter* interaction is that it is reversible, and its strength, on-rate and off-rate are easily tuneable properties [12, 14-16, 18] that have not yet been harnessed. It can also be turned into a rapid and site-specific photoactivatable protein-DNA conjugation system [21]. We expect future applications of Tus-*Ter* to take advantage of these aspects for the development of new predictable biomaterials and biosensors. Increasing the thermal stability of Tus would also be an obvious and desirable outcome with major benefits. The thermostabilization of Tus upon binding to *Ter* [14, 15] is already making its long-term storage at room temperature a possibility, and could be used as a thermo-controlled DNA protection or release system.

Of course, it would be somewhat one-sided to only mention the advantages of the Tus-*Ter* system. While the protein can be tagged in many ways, its expression and solubility are not the best, requiring slow and low-temperature expression conditions [21, 61]. As such, care must be taken to control for these drawbacks, long-term stability and functionality of Tus. Another issue stems from its unique binding mechanism leading to significant non-specific binding to DNA in low ionic strength buffers [12, 16, 18], currently limiting the possibility of multiplexing in qIPCR applications. However, with plenty of room for further protein engineering, characterisation of novel Tus proteins, and flexible tuning of the Tus-*Ter* interaction, we hope that the eclectic applications and insights presented in this review will provide the impetus needed to develop new ideas into innovative biotechnologies with bioanalytical applications across various disciplines and industries.

CRediT Authorship Contribution Statement

Casey J. Toft: Writing - Original Draft, Writing - Review & Editing, Visualization. Alanna E. Sorenson: Writing - Review & Editing. Patrick M. Schaeffer: Conceptualization, Writing - Review & Editing, Visualization.

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References

[1] M. Wilchek, E.A. Bayer, The Avidin Biotin Complex in Bioanalytical Applications, Analytical Biochemistry, 171 (1988) 1-32.

[2] C.M. Dundas, D. Demonte, S. Park, Streptavidin-biotin technology: improvements and innovations in chemical and biological applications, Appl Microbiol Biotechnol, 97 (2013) 9343-9353.

[3] A.E. Sorenson, S.P. Askin, P.M. Schaeffer, In-gel detection of biotin-protein conjugates with a green fluorescent streptavidin probe, Anal Methods-Uk, 7 (2015) 2087-2092.

[4] J.H.T. Luong, S.K. Vashist, Chemistry of Biotin-Streptavidin and the Growing Concern of an Emerging Biotin Interference in Clinical Immunoassays, Acs Omega, 5 (2020) 10-18.

[5] D. Zhao, Y.H. Kong, S.S. Zhao, H. Xing, Engineering Functional DNA-Protein Conjugates for Biosensing, Biomedical, and Nanoassembly Applications, Topics Curr Chem, 378 (2020).

[6] C. Neylon, A.V. Kralicek, T.M. Hill, N.E. Dixon, Replication termination in Escherichia coli: structure and antihelicase activity of the Tus-Ter complex, Microbiol Mol Biol Rev, 69 (2005) 501-526.

[7] P.M. Schaeffer, M.J. Headlam, N.E. Dixon, Protein--protein interactions in the eubacterial replisome, IUBMB Life, 57 (2005) 5-12.

[8] C.J. Toft, M.J.J. Moreau, J. Perutka, S. Mandapati, P. Enyeart, A.E. Sorenson, A.D. Ellington, P.M. Schaeffer, Delineation of the Ancestral Tus-Dependent Replication Fork Trap, International Journal of Molecular Sciences, 22 (2021).

[9] M.D. Mulcair, P.M. Schaeffer, A.J. Oakley, H.F. Cross, C. Neylon, T.M. Hill, N.E. Dixon, A molecular mousetrap determines polarity of termination of DNA replication in E. coli, Cell, 125 (2006) 1309-1319.

[10] L. Chan, H.F. Cross, J.K. She, G. Cavalli, H.F. Martins, C. Neylon, Covalent attachment of proteins to solid supports and surfaces via Sortase-mediated ligation, PLoS One, 2 (2007) e1164.

[11] P.A. Gottlieb, S. Wu, X.L. Zhang, M. Tecklenburg, P. Kuempel, T.M. Hill, Equilibrium, Kinetic, and Footprinting Studies of the Tus-Terprotein-DNA Interaction, Journal of Biological Chemistry, 267 (1992) 7434-7443.

[12] M.J. Moreau, P.M. Schaeffer, Differential Tus-Ter binding and lock formation: implications for DNA replication termination in Escherichia coli, Mol Biosyst, 8 (2012) 2783-2791.

[13] D.K. Chatterjee, K. Sitaraman, C. Baptista, J. Hartley, T.M. Hill, D.J. Munroe, Protein microarray on-demand: a novel protein microarray system, PLoS One, 3 (2008) e3265.

[14] M.J. Moreau, I. Morin, P.M. Schaeffer, Quantitative determination of protein stability and ligand binding using a green fluorescent protein reporter system, Mol Biosyst, 6 (2010) 1285-1292.

[15] M.J.J. Moreau, I. Morin, S.P. Askin, A. Cooper, N.J. Moreland, S.G. Vasudevan, P.M. Schaeffer, Rapid determination of protein stability and ligand binding by differential scanning fluorimetry of GFP-tagged proteins, RSC Advances, 2 (2012) 11892-11900.

[16] M.J. Moreau, P.M. Schaeffer, A polyplex qPCR-based binding assay for protein-DNA interactions, Analyst, 137 (2012) 4111-4113.

[17] S. Piluso, H.C. Cassell, J.L. Gibbons, T.E. Waller, N.J. Plant, A.F. Miller, G. Cavalli, Site-specific, covalent incorporation of Tus, a DNA-binding protein, on ionic-complementary self-assembling peptide hydrogels using transpeptidase Sortase A as a conjugation tool, Soft Matter, 9 (2013) 6752-6756.

[18] M.J. Moreau, P.M. Schaeffer, Dissecting the salt dependence of the Tus-Ter protein-DNA complexes by high-throughput differential scanning fluorimetry of a GFP-tagged Tus, Mol Biosyst, 9 (2013) 3146-3154.

[19] B.A. Berghuis, V.S. Raducanu, M.M. Elshenawy, S. Jergic, M. Depken, N.E. Dixon, S.M. Hamdan, N.H. Dekker, What is all this fuss about Tus? Comparison of recent findings from biophysical and biochemical experiments, Crit Rev Biochem Mol Biol, 53 (2018) 49-63.

[20] K. Sitaraman, D.K. Chatterjee, Protein-protein interactions: an application of Tus-Ter mediated protein microarray system, Methods Mol Biol, 723 (2011) 185-200.

[21] D.B. Dahdah, I. Morin, M.J. Moreau, N.E. Dixon, P.M. Schaeffer, Site-specific covalent attachment of DNA to proteins using a photoactivatable Tus-Ter complex, Chem Commun (Camb), (2009) 3050-3052.

[22] S.J. Kaczmarczyk, K. Sitaraman, T. Hill, J.L. Hartley, D.K. Chatterjee, Tus, an E. coli protein, contains mammalian nuclear targeting and exporting signals, PLoS One, 5 (2010) e8889.

[23] I. Morin, N.E. Dixon, P.M. Schaeffer, Ultrasensitive detection of antibodies using a new Tus-Ter-lock immunoPCR system, Mol Biosyst, 6 (2010) 1173-1175.

[24] I. Morin, S.P. Askin, P.M. Schaeffer, IgG-detection devices for the Tus-Ter-lock immuno-PCR diagnostic platform, Analyst, 136 (2011) 4815-4821.

[25] E.B. Johnston, S.D. Kamath, A.L. Lopata, P.M. Schaeffer, Tus-Ter-lock immuno-PCR assays for the sensitive detection of tropomyosin-specific IgE antibodies, Bioanalysis, 6 (2014) 465-476.

[26] S.P. Askin, P.M. Schaeffer, A universal immuno-PCR platform for comparative and ultrasensitive quantification of dual affinity-tagged proteins in complex matrices, Analyst, 137 (2012) 5193-5196.

[27] A. Cooper, N.L. Williams, J.L. Morris, R.E. Norton, N. Ketheesan, P.M. Schaeffer, ELISA and immuno-polymerase chain reaction assays for the sensitive detection of melioidosis, Diagn Micr Infec Dis, 75 (2013) 135-138.

[28] S.D. Kamath, E.B. Johnston, S. Iyer, P.M. Schaeffer, J. Koplin, K. Allen, A.L. Lopata, IgE reactivity to shrimp allergens in infants and their cross-reactivity to house dust mite, Pediat Allerg Imm-Uk, 28 (2017) 703-707.

[29] S.P. Askin, I. Morin, P.M. Schaeffer, Development of a protease activity assay using heat-sensitive Tus-GFP fusion protein substrates, Anal Biochem, 415 (2011) 126-133.

[30] N.A. Willis, G. Chandramouly, B. Huang, A. Kwok, C. Follonier, C. Deng, R. Scully, BRCA1 controls homologous recombination at Tus/Ter-stalled mammalian replication forks, Nature, 510 (2014) 556-559.

[31] N.B. Larsen, I.D. Hickson, H.W. Mankouri, Tus-Ter as a tool to study site-specific DNA replication perturbation in eukaryotes, Cell Cycle, 13 (2014) 2994-2998.

[32] N.A. Willis, A. Panday, E.E. Duffey, R. Scully, Rad51 recruitment and exclusion of non-homologous end joining during homologous recombination at a Tus/Ter mammalian replication fork barrier, PLoS Genet, 14 (2018) e1007486.

[33] L. Marie, L.S. Symington, Mechanism for inverted-repeat recombination induced by a replication fork barrier, Nat Commun, 13 (2022) 32.

[34] N.B. Larsen, E. Sass, C. Suski, H.W. Mankouri, I.D. Hickson, The Escherichia coli Tus-Ter replication fork barrier causes site-specific DNA replication perturbation in yeast, Nat Commun, 5 (2014) 3574.

[35] N.B. Larsen, S.E. Liberti, I. Vogel, S.W. Jorgensen, I.D. Hickson, H.W. Mankouri, Stalled replication forks generate a distinct mutational signature in yeast, P Natl Acad Sci USA, 114 (2017) 9665-9670.

[36] M. Norouzi, S. Panfilov, K. Pardee, High-Efficiency Protection of Linear DNA in Cell-Free Extracts from Escherichia coli and Vibrio natriegens, Acs Synth Biol, 10 (2021) 1615-1624.

[37] F.X. Sutandy, J. Qian, C.S. Chen, H. Zhu, Overview of protein microarrays, Curr Protoc Protein Sci, Chapter 27 (2013) Unit 27 21.

[38] J. Qiu, J. LaBaer, Nucleic acid programmable protein array a just-in-time multiplexed protein expression and purification platform, Methods Enzymol, 500 (2011) 151-163.

[39] M.Y. He, M.J. Taussig, Single step generation of protein arrays from DNA by cell-free expression and in situ immobilisation (PISA method), Nucleic Acids Research, 29 (2001).

[40] P. Diez, M. Gonzalez-Gonzalez, L. Lourido, R.M. Degano, N. Ibarrola, J. Casado-Vela, J. LaBaer, M. Fuentes, NAPPA as a Real New Method for Protein Microarray Generation, Microarrays (Basel), 4 (2015) 214-227.

[41] R. Manzano-Roman, M. Fuentes, A decade of Nucleic Acid Programmable Protein Arrays (NAPPA) availability: News, actors, progress, prospects and access, J Proteomics, 198 (2019) 27-35.

[42] T.A. Henderson, A.F. Nilles, M. Valjavec-Gratian, T.M. Hill, Site-directed mutagenesis and phylogenetic comparisons of the Escherichia coli Tus protein: DNA-protein interactions alone can not account for Tus activity, Mol Genet Genomics, 265 (2001) 941-953.

[43] P.M. Schaeffer, N.E. Dixon, Synthesis and Applications of Covalent Protein-DNA Conjugates, Aust J Chem, 62 (2009) 1328-1332.

[44] J.B. Trads, T. Torring, K.V. Gothelf, Site-Selective Conjugation of Native Proteins with DNA, Accounts Chem Res, 50 (2017) 1367-1374.

[45] B.Q. Liu, J.F. Chen, Q.H. Wei, B. Zhang, L. Zhang, D.P. Tang, Target-regulated proximity hybridization with three-way DNA junction for in situ enhanced electronic detection of marine biotoxin based on isothermal cycling signal amplification strategy, Biosens Bioelectron, 69 (2015) 241-248.

[46] S. Khan, B. Burciu, C.D.M. Filipe, Y.F. Li, K. Dellinger, T.F. Didar, DNAzyme-Based Biosensors: Immobilization Strategies, Applications, and Future Prospective, Acs Nano, 15 (2021) 13943-13969.

[47] A. Schipperges, Y. Hu, S. Moench, S. Weigel, J. Reith, D. Ordonez-Rueda, K.S. Rabe, C.M. Niemeyer, Formulation of DNA Nanocomposites: Towards Functional Materials for Protein Expression, Polymers-Basel, 13 (2021).

[48] Y. Hu, C.M. Niemeyer, From DNA Nanotechnology to Material Systems Engineering, Adv Mater, 31 (2019).

[49] L.M. van der Sleen, K.M. Tych, Bioconjugation Strategies for Connecting Proteins to DNA-Linkers for Single-Molecule Force-Based Experiments, Nanomaterials-Basel, 11 (2021).

[50] T. Sano, C.L. Smith, C.R. Cantor, Immuno-PCR: very sensitive antigen detection by means of specific antibody-DNA conjugates, Science, 258 (1992) 120-122.

[51] L. Chang, J. Li, L. Wang, Immuno-PCR: An ultrasensitive immunoassay for biomolecular detection, Anal Chim Acta, 910 (2016) 12-24.

[52] E.B. Johnston, S.D. Kamath, S.P. Iyer, K. Pratap, S. Karnaneedi, A.C. Taki, R. Nugraha, P.M. Schaeffer, J.M. Rolland, R.E. O'Hehir, A.L. Lopata, Defining specific allergens for improved component-resolved diagnosis of shrimp allergy in adults, Mol Immunol, 112 (2019) 330-337.

[53] M.A. McSweeney, M.P. Styczynski, Effective Use of Linear DNA in Cell-Free Expression Systems, Front Bioeng Biotech, 9 (2021).

[54] B. Zhu, R. Gan, M.D. Cabezas, T. Kojima, R. Nicol, M.C. Jewett, H. Nakano, Increasing cell-free gene expression yields from linear templates in Escherichia coli and Vibrio natriegens extracts by using DNA-binding proteins, Biotechnol Bioeng, 117 (2020) 3849-3857.

[55] S.S. Yim, N.I. Johns, V. Noireaux, H.H. Wang, Protecting Linear DNA Templates in Cell-Free Expression Systems from Diverse Bacteria, ACS Synth Biol, 9 (2020) 2851-2855.

[56] J.G. Kim, Y. Takeda, B.W. Matthews, W.F. Anderson, Kinetic-Studies on Cro Repressor Operator DNA Interaction, Journal of Molecular Biology, 196 (1987) 149-158.

[57] M. Ray, R. Tang, Z. Jiang, V.M. Rotello, Quantitative tracking of protein trafficking to the nucleus using cytosolic protein delivery by nanoparticle-stabilized nanocapsules, Bioconjug Chem, 26 (2015) 1004-1007.

[58] H.L. Klein, G. Bacinskaja, J. Che, A. Cheblal, R. Elango, A. Epshtein, D.M. Fitzgerald, B. Gomez-Gonzalez, S.R. Khan, S. Kumar, B.A. Leland, L. Marie, Q. Mei, J. Mine-Hattab, A. Piotrowska, E.J. Polleys, C.D. Putnam, E.A. Radchenko, A.A. Saada, C.J. Sakofsky, E.Y. Shim, M. Stracy, J. Xia, Z. Yan, Y. Yin, A. Aguilera, J.L. Argueso, C.H. Freudenreich, S.M. Gasser, D.A. Gordenin, J.E. Haber, G. Ira, S. Jinks-Robertson, M.C. King, R.D. Kolodner, A. Kuzminov, S.A. Lambert, S.E. Lee, K.M. Miller, S.M. Mirkin, T.D. Petes, S.M. Rosenberg, R. Rothstein, L.S. Symington, P. Zawadzki, N. Kim, M. Lisby, A. Malkova, Guidelines for DNA recombination and repair studies: Cellular assays of DNA repair pathways, Microb Cell, 6 (2019) 1-64.

[59] P. Beuzer, J.P. Quivy, G. Almouzni, Establishment of a replication fork barrier following induction of DNA binding in mammalian cells, Cell Cycle, 13 (2014) 1607-1616.

[60] R. Ramachandran, P.N. Ciacca, T.A. Filsuf, J.K. Jha, D.K. Chattoraj, Chromosome 1 licenses chromosome 2 replication in Vibrio cholerae by doubling the crtS gene dosage, Plos Genetics, 14 (2018).

[61] D.K. Chatterjee, K. Sitaraman, C. Baptista, J. Hartley, T.M. Hill, D.J. Munroe, Protein Microarray On-Demand: A Novel Protein Microarray System, Plos One, 3 (2008).