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Special *Methods* collection on DNA helicases

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Abstract

In this special *Methods* collection on DNA helicases, I have solicited articles from leading experts in the field with a priority to gather a defined series of papers on highly relevant topics that encompass biological, biochemical, and biophysical aspects of helicase function. The experimental approaches described provide an opportunity for both new and more experienced scientists to use the information for the design of their own investigations. The reader will find detailed methods for single-molecule studies, novel biochemical experiments, genetic analyses, and cell biological assays in a variety of systems with an emphasis placed on state-of-the-art techniques to measure helicase function. Contributing authors were strongly encouraged to provide a carefully constructed description of the methods employed so that others might use this information in a manner that will be useful for their own particular application and helicase of interest. This special issue of *Methods* dedicated to DNA helicases offers readers a treasure chest of unique experimental approaches and protocols focused on rapidly developing techniques that are useful for studying both *in vivo* and *in vitro* aspects of helicase function.

DNA helicases have essential roles in virtually all aspects of nucleic acid metabolism involving the transient unwinding of the DNA double helix or other forms of DNA secondary structure. This special issue of *Methods* is comprised of a series of review articles by leaders in the field who deliver experimental procedures to study the molecular and cellular functions of DNA helicases and underlying mechanisms. We begin the special *Methods* collection with several distinct approaches and objectives for single-molecule studies of helicase proteins. This relatively new form of experimentation provides exciting approaches to derive insights into molecular aspects of helicase mechanism. The Seidel laboratory contributes a review article describing magnetic tweezer setups to study helicase-catalyzed DNA unwinding for different types of substrates and reaction conditions [1]. Techniques to modify the magnetic tweezer assay to acquire kinetic and thermodynamic information are also provided in the review by Kemmerich et al. [1]. The Spies laboratory has focused their review article on single-molecule sorting analyses that can be used to simultaneously quantify and distinguish the modulation of helicase-associated activities dependent on the post-translational modification of the helicase protein [2]. This paper by Bain et al. [2] provides detailed methodology to perform single-molecule sorting. The Kovacs laboratory delivers a review article for the *Methods* special collection that describes experimental approaches for highly mechanistic characterization of helicases that involves interactions with the nucleic acid, the nucleoside triphosphate hydrolysis cycle, and mechanochemical coupling of the two processes critical for duplex unwinding [3]. The experimental and theoretical aspects of fluorescence-based single-molecule studies are

described in exquisite detail by Gyimesi et al. [3]. To gain greater knowledge of how a DNA helicase facilitates the rescue of a stalled replication fork, Bianco describes a magnetic tweezer assay to examine the ability of *E. coli* RecG helicase to catalyze fork regression, even when a single-stranded DNA binding protein is bound to the forked duplex substrate [4]. It is anticipated that this article as well as the other single-molecule papers described above will be treasured resources for the scientific community.

Going beyond conventional DNA unwinding assays, the McGlynn lab has focused considerable effort to understand molecular functions and biological roles of DNA helicases to catalytically displace proteins bound to DNA in a nucleoside triphosphate hydrolysis-driven manner. In their contribution to this special issue of *Methods*, they describe detailed protocols to measure streptavidin displacement from various biotinylated DNA substrates, a system which serves as a useful and convenient model to characterize molecular and mechanistic aspects of nucleoprotein complex disruption by a helicase [5]. The paper by Bruning et al. [5] provides experimental design, execution, and data analysis to measure the cooperativity that exists between the bacterial DNA helicases DnaB and Rep to efficiently displace streptavidin bound to a biotinylated forked duplex DNA substrate.

Understanding how and under what conditions helicases interact with each other and other proteins in biological networks has captivated researchers. The Raney lab makes a unique contribution to this special *Methods* issue by describing a proteomics methods approach highlighted by affinity pull-downs and chemical cross-linking followed by mass spectrometry to seek out new helicase protein partners [6]. Zybailov et al. [6] also provide a useful assessment of helicase protein–protein interaction networks, informing the reader of their utility and limitations. In certain cases, the mass spectrometric/proteomics approach really helps to solidify the potential functional significance of protein–protein interaction networks studied by less direct measures.

At the heart of DNA replication is the efficient coupling between unwinding of the parental duplex and the nearly concomitant incorporation of nucleotides in the growing nascent DNA chain catalyzed by the leading-strand DNA polymerase. The Patel laboratory has emerged as a leader in this area, so I am delighted that they have contributed a very thorough and detail-oriented review article on experimental approaches to study functional coupling between helicase and leading strand DNA polymerase to this special issue of *Methods* devoted to DNA helicases [7]. Nandakumar and Patel [7] provide in their paper experimental protocols and issues that deal directly and effectively with presteady kinetic methods to measure single base pair DNA synthesis rate constants, an assay to measure energy consumption by the helicase-DNA polymerase complex, and the contributions of both enzymes to fork junction base pair separation.

The Trakselis lab has an active interest in the hexameric helicases responsible for unwinding of parental strands at the replication fork. Carney and Trakselis have contributed a comprehensive review in the *Methods* special collection on qualitative and quantitative methods to characterize the dynamic interactions of ringlike helicases with the excluded strand during duplex DNA unwinding [8]. Special emphasis is placed on experimental methodologies that help to define a steric exclusion and wrapping model for unwinding.

This review from the Trakselis group represents the first of its kind to describe in a highly approachable manner the systematic biochemical, biophysical and single-molecule approaches that can be used to study excluded strand contacts with the helicase hexamer, and their role in force production, rate of unwinding, and DNA damage sensing, a topic of considerable interest [9].

A highly valuable approach for studying *in vivo* aspects of DNA helicases in cellular replication is the DNA fibre technique. This technique for visualizing incorporated nucleotides at the level of single DNA molecules as opposed to bulk measurements in cells has been particularly informative for a growing number of investigators who aim to characterize the response to replication stress in which an expanding number of proteins, including DNA helicases, have been implicated. Although the DNA fibre technique has become quite popular, up to now there have been only a limited number of publications which describe in great experimental detail the method (and its permutations) as well as its applications so that those relatively new to the approach might benefit and harness the power of the technique. In the special issue of *Methods*, two papers from the Niedzwiedz [10] and Sidorova [11] labs provide detailed descriptions of the DNA fibre technique and its application to unique experimental setups. These detailed experimental procedures provide readily accessible insight and practical information for qualitative and quantitative analyses of cellular DNA replication at the single-molecule level which has been employed in human, mouse, frog, and chicken cells. In addition to characterizing fork kinetics and directionality, the techniques described in the two papers of this *Methods* special issue can be used to study more complex features of replication fork dynamics and the roles of helicases for origin firing, termination, and metabolism of stalled or collapsed forks, a topic of great interest in the field of genome stability and elaborated upon in terms of experimental methodology by the Nieminuszczy et al. paper [10]. A novel application of the microfluidic-assisted replication track analysis is presented in the paper by Welcsh et al. to measure DNA repair after cellular exposure to DNA damaging agents, which is compared to the more conventional techniques of pulse-field gel electrophoresis and comet assay [11].

Beyond replication or DNA repair, certain DNA helicases may play direct roles in gene expression by modulating the initiation or elongation phases of transcription. This aspect of helicase function has been less studied, but appears to be an emerging area of importance. To address the potential relevance of human RECQ1 in gene regulation, the Sharma lab examined genome-wide alterations in gene expression when RECQ1 was acutely depleted in HeLa cells by RNA interference [12]. Lu et al. [12] also examined by Chromatin Immunoprecipitation if RECQ1 binds preferentially to predicted G-quadruplex (G4) forming sequences, which are enriched in certain target gene promoters [13]. These studies provide a secure launchpad to systematically compare gene expression patterns of helicase-deficient cell lines. This is particularly relevant in the case of the RecQ helicases, in which five human homologs exist and there is an apparent complexity of partially overlapping roles [14].

Characterization of engineered site-directed mutations in DNA helicases has provided a wealth of information regarding their mechanisms of action as well as their genetic functions and pathways. Such designed amino acid substitutions may represent engineered mutations in conserved motifs or unique regions of the helicase protein that mediate binding to DNA,

nucleotide, or a protein partner. Alternatively, the amino acid substitution may represent a patient-derived mutation in which the biochemical and cellular characterization of the mutant allele may provide useful information of potential clinical significance. The Wu lab has contributed a comprehensive paper to this special issue of *Methods* on the molecular-genetic analysis of FANCD1 helicase in which mutations are linked to Fanconi Anemia or associated with breast cancer and ovarian cancer [15]. This paper by Guo et al. [15] provides new insights into the pathogenesis of FANCD1 disease-causing mutations.

To round out the *Methods* special collection on DNA helicases, the Brosh laboratory has contributed a review article that entails a series of experimental approaches and methodologies to identify and characterize DNA helicase inhibitors [16]. As a proof-of-principle, Banerjee et al. [16] describe their published studies of small molecules that modulate the unwinding activity catalyzed by the WRN helicase-nuclease that is implicated in the accelerated aging disorder known as Werner syndrome. A strong case is made that compounds which act as helicase modulators represent an alternative strategy for investigating the molecular and cellular functions of their targets, and in a broader scope, the sophisticated orchestration of overlapping and intersecting DNA metabolic pathways. Moreover, synthetic lethality paradigms are presented which suggest that helicases are good small molecule targets for emerging anti-cancer therapies.

In summary, readers of the *Methods* 2016 special collection on DNA helicases will find a wealth of accessible information on experimental approaches and methods to study helicases both *in vitro* and *in vivo*. I wish to thank *Methods* Editor Kenneth Adolph for inviting me to assemble this issue on DNA helicases, and *Methods* Journal Manager Ranjini Gopal, Editorial Assistant Tiffany Hicks, and *Elsevier* Content Development Manager Andy Deelen for their assistance. I am also very appreciative of the contributing authors from their respective laboratories for their fastidious efforts. I firmly believe this collection which offers a diverse and highly relevant set of helicase methodologies will be a valued resource for DNA scientists. I would especially like to acknowledge my PhD thesis advisor Steve Matson and PhD thesis committee members/2015 Nobel Laureates Aziz Sancar and Paul Modrich who first inspired me to pursue mechanistic aspects of helicase biochemistry and genetics during my graduate studies at UNC-Chapel Hill, a charge that has made a lasting impression on me. I hope that you will enjoy exploring the topics presented in each review article of this *Methods* special collection, as they were assembled with a focus on rapidly developing techniques, a guiding principle of the journal *Methods*.

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