



Syntrophy Volume 19

Issue 5 2018

THE AUSTRALIAN SOCIETY FOR MICROBIOLOGY NSW-ACT BRANCH (ABN 24 065 463 274)

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From the Editor

by Mitchell Brown

Dear all,

Welcome to another edition of Syntrophy members. Firstly, Congratulations to Kate Seib and the organising committee of ASM 2018 Brisbane. By all accounts the event was a great success and was certainly well attended. Kate and the team put together an appealing and diverse programme. Some highlights included the CSL sponsored Bazeley Oration by Dennis Burton 'Progress toward a neutralizing antibody-based HIV vaccine' and Paul Young's Rubbo Oration 'A Virologist in Wonderland: through the looking glass.' Hopefully many of you had the chance to attend.

Wonderful also to hear news from successful recent local events such as the clinical microbiology presentation at CSU in Wagga Wagga – it was great to have ~50 students in attendance to listen to real clinical case studies and hear about the latest career options for graduating microbiology students. Details page #4

Georgia Weaver has provided this edition's Focus article. Georgia was a very worthy finalist in this year's ASM NSW-ACT Student Awards and presents her work on DNA replication forks for us on page #3

Finally, please find in this edition details of the ASM NSW-ACT branch Annual General Meeting to be held on August the 15th at Harpoon Harry's. As well as formalities, we have confirmed Dr Jeremy Barr as a guest speaker, and will have a selection of canapés for you to enjoy. RSVP and payment details can be found on page #5. I encourage you all to come along, participate in the AGM and enjoy what promises to be a fun evening.

Enjoy the read

Processing of DNA replication forks at protein-DNA blocks

by Georgia Weaver

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SAVE THE DATE

ASM NSW-ACT Branch
58th Annual General Meeting
15th August 2018
Harpoon Harry's
Surry Hills

See details page #5

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NEXT SYNTROPHY

Deadline for submissions to next issue:
21st June 2018

CONTACT SYNTROPHY COORDINATOR
syntrophy@asmnsw.com.au

Upcoming Events

SAVE THE DATE

ASM NSW-ACT Branch
58th Annual General Meeting
15th August 2018
Harpoon Harry's
Surry Hills

See details page #5

Meeting Calendar



School of Life and Environmental Sciences James Vincent Scholarship:

Scholarship:

The James Vincent scholarship may take the form of either a travel grant to attend a relevant national or international conference, to obtain skills available only at another institution in Australia or overseas, or to satisfy other specific requirements of their higher degree research programme. The scholarship will be awarded by the School of Life and Environmental Sciences, The University of Sydney, on the recommendation of the NSW-ACT Branch of the Australian Society for Microbiology.

Eligibility:

- Honours and postgraduate research students at the University of Sydney or University of New South Wales.
- Students working in the area of symbiotic nitrogen fixation, the major research area of Professor Vincent, may receive preference.
- If not currently a student member of ASM, applicants must be eligible for membership and apply for membership at time of application for award.

Criteria:

1. Applicants should submit details of their academic record and two referee's reports
2. Applicants should briefly justify their proposal and suggested budget in terms of the object of the scholarship.
3. The value of the scholarship shall depend on the financial needs of the applicant subject to the availability of funding, but shall not exceed the previous year's net income to the fund. The amount offered each year will be limited to the earnings generated from the funds held in the Vincent award, less 10% which will be added to the capital to allow for growth. Generally this is around \$400.
4. The scholarship is tenable for 1 year.
5. 1 award per year. No award may be given in the event that the NSW-ACT branch committee in consultation with the J Vincent representatives at USyd and UNSW feel there is no suitable applicant.

Closing date: 30th July 2018

Send applications to:

Prof. Dee Carter
School of Life and Environmental Sciences
The University of Sydney
Email: dee.carter@sydney.edu.au
Ph: 9351 5383

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Focus

Processing of DNA replication forks at protein-DNA blocks

by Georgia Weaver

Despite DNA replication being a precise and rapid process, the replication machinery (replisome) responsible for chromosome duplication is often impeded, arresting the progression of replication. Replication fork arrest can arise from impediments such as proteins tightly bound to DNA or DNA damage (e.g.: UV damage) in the form of lesions, nicks or gaps in the DNA sequence. Nucleoprotein blockages (e.g.: RNA polymerase) are known to be the most common obstacle to replisome progression (1). Upon the stalling of a replisome at a protein-DNA block, the replisome may either partially or completely dissociate from the replication fork. Replisome dissociation occurs on average around five times during each round of replication even without collisions stalling the replisome (2). The failure to remove blockages prevents replication from completing, thus the cell will not survive. My research poses the question of what happens to the replication fork DNA following replisome collapse and how is the DNA manipulated to restart replication?

The DNA at a replication fork blocked by nucleoproteins can be processed by several potential pathways. Two currently proposed 'first action' responses at the collapsed fork involve nucleases; forked DNA may be cleaved by endonucleases to facilitate homologous recombination, or the nascent DNA strands could be degraded by exonucleases. The third possibility is replication fork reversal (RFR), whereby the newly synthesised DNA is unwound from the parental strands facilitating their annealing to each other as the parental DNA strands also reanneal, forming a Holliday junction (HJ) structure that is the substrate for homologous recombination pathways (Figure 1A). The regression of the replication fork away from the site of DNA damage or protein block allows repair proteins or accessory helicases to access the DNA and resolve the problem (3).

A site-specific roadblock to DNA replication can be induced at a known position in the chromosome using a transcriptional repressor (TetR) bound to an array of operator sites within the *E. coli* chromosome. Using a temperature sensitive allele of the replicative helicase DnaB, the synchronous inactivation of the stalled replisome can be triggered across a cell population leading to the collapse of the replication fork, which allows fork processing to begin (4). To ascertain what the most common DNA processing event is, I visualise the DNA structures using 2D agarose gel electrophoresis and Southern hybridisation (5). This site-specific replication blockage system was used to visualise the DNA structures at the block site and subsequent processing by RFR or nucleases into the upstream DNA region (Figure 1B), and the relative contributions of candidate proteins in these events. The results show the disappearance of the Y-shaped forked DNA with the arrival of HJ structures upstream of the block (Figure 1C). Some exonuclease activity is seen (potentially at the Y-shaped fork, or at the reversed HJ), however the movement of forks out of the TetR array suggests that RFR is the most frequent event with little or no evidence for endonuclease action at the fork. These results demonstrate that replication fork reversal (RFR) is the major pathway of replisome rescue after encountering a blockage.

RecG and RuvABC are helicases thought to conduct RFR as one of their functions. When genes encoding these helicases are deleted, the system displayed only mild hindrance to RFR/exonuclease action (Figure 1D & E) with the majority of DNA being processed as before. Strikingly, RecQ, a helicase not previously implicated in RFR in prokaryotes, had the greatest impact on fork reversal. The absence of *recQ* lead to 66% of forks remaining at the block site even after 1 hour, drastically decreasing the incidence of RFR and exonuclease action (Figure 1D & E). In conclusion, replication fork regression, involving

RFR and exonuclease action is the major pathway used in cells to deal with a persistent roadblock to replication and is promoted by RecQ.

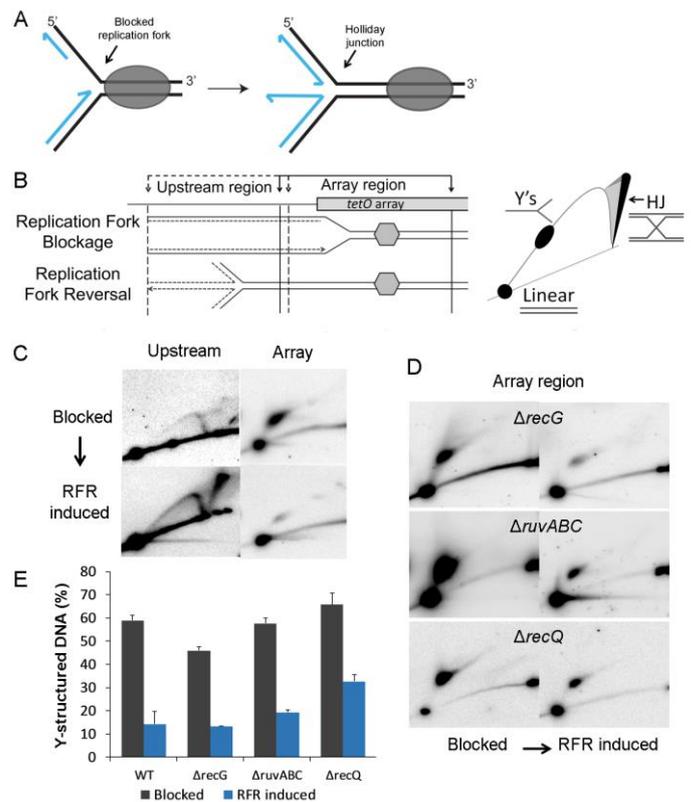


Figure 1: Replication fork reversal in *E. coli*. A) Diagram of replication fork reversal (RFR) at a nucleoprotein block. B) Schematics of the array and upstream regions analysed, and an illustration of the various DNA structures detected by 2D gels. C) DNA structures from indicated conditions in the WT strain D). DNA structures at the array region in various mutants. E) Percentage of Y-structured DNA within the array region.

ABOUT THE AUTHOR

Georgia Weaver is a PhD at the University of Newcastle in the School of Environmental and Life Sciences working the lab Dr Karla Mettrick and Assoc. Prof. Ian Grainge. Her research focuses on elucidating the roles of recombination proteins at replication forks arrested by nucleoprotein complexes.

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1. Gupta MK, Guy CP, Yeeles JT, Atkinson J, Bell H, Lloyd RG, et al. Protein-DNA complexes are the primary sources of replication fork pausing in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 2013;110(18):7252-7.
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3. De Septenville AL, Duigou S, Boubakri H, Michel B. Replication fork reversal after replication-transcription collision. *PLoS Genet*. 2012;8(4):e1002622.
4. Mettrick KA, Grainge I. Stability of blocked replication forks in vivo. *Nucleic Acids Res*. 2016;44(2):657-68.
5. Mettrick KA, Lawrence N, Mason C, Weaver GM, Corocher TA, Grainge I. Inducing a Site Specific Replication Blockage in *E. coli* Using a Fluorescent Repressor Operator System. *Journal of visualized experiments: JoVE*. 2016(114).

Clinical Microbiology presentation at Charles Sturt University, Wagga Wagga
 by Thiru Vanniasinkam

Tom Olma from the Clinical Microbiology Laboratory at Westmead Hospital, Sydney presented Case Studies in Clinical Microbiology at Charles Sturt University, Wagga Wagga on 18th May 2018 (sponsored by the NSW-ACT branch). In the presentation Tom also included information on career options in microbiology which the students found particularly useful. The presentation was also live streamed, so distance students could watch the presentation and ask questions. The audience comprised around 50 students and staff from both the university and some regional pathology laboratories.



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THE ASM NSW-ACT BRANCH 58th ANNUAL GENERAL MEETING

Wednesday 15th August
Harpoon Harry's
40-44 Wentworth Ave, Surry Hills NSW 2010
AGM: 6pm – 7pm
Speaker from 7pm

Cost: The AGM is free of charge
Post-AGM Prestige Canapés: \$15 members, \$30 non-
members
(includes drink voucher)

RSVP ESSENTIAL by August 8, 2018

jim.manos@sydney.edu.au

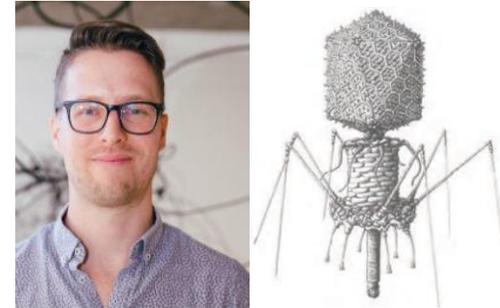
Preferred payment option is EFT

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Speaker: Dr Jeremy Barr

The Barr Research Group studies bacteriophage – viruses that infect bacteria – and specifically investigates symbioses between bacteriophage, their bacterial hosts and eukaryotic cells and surfaces. Bacteriophage (or phage for short) are the most abundant and diverse microbe found in the body. Phages control and manipulate bacterial populations, prevent infection and disease and have important roles in regulating the microbiome and body that have not yet been fully elucidated.





THE ASM NSW-ACT BRANCH COMMITTEE NOMINATION FORM

It's that time of the year again when we begin to prepare for the ASM NSW-ACT Branch Annual General Meeting. Nominations are required for the Chairperson Elect, the Treasurer (applicant must have previously served 12 months on the Committee for these executive positions) and Committee members. If you feel there is more you want to do for microbiology in NSW, now is a good time to fill in the Nomination form and become part of an energetic and motivated Committee. Don't hesitate - actions speak louder than words!

Please send all nominations to the Branch Secretary Tim Newsome:

EMAIL: tim.newsome@sydney.edu.au

**Please note that all nominations must be in writing.
Nominations WILL NOT be taken from the floor on the night of the meeting.
Only ASM members may nominate and be nominated.**

We, the undersigned, wish to nominate:

Print Name.....

For the position of (please tick)

- Chairperson-Elect (1-year term & Chair 2-year term & Chair Past 1-year term)
- Treasurer (2-year term)
- Committee member (1-year term)

Proposer:

Print Name.....

Signature.....

Secunder:

Print Name.....

Signature.....

I accept this nomination:

Print Name.....

Signature.....

Phone.....

Email.....

Date: / /2018

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Submissions and enquiries can be directed to the Syntrophy Coordinator, Susan Badman at syntrophy@asmnsw.com.au

Organisations with research opportunities or companies seeking to fill positions are welcome to place an advertisement in an upcoming issue of Syntrophy. Please contact the Syntrophy Coordinator with your details for inclusion.

Organisations interested in becoming a sponsor of ASM NSW-ACT Branch should contact the Sponsorship Coordinator, Bobby Dimitrijovski to obtain a copy of the current sponsorship prospectus: sponsorship@asmnsw.com.au.