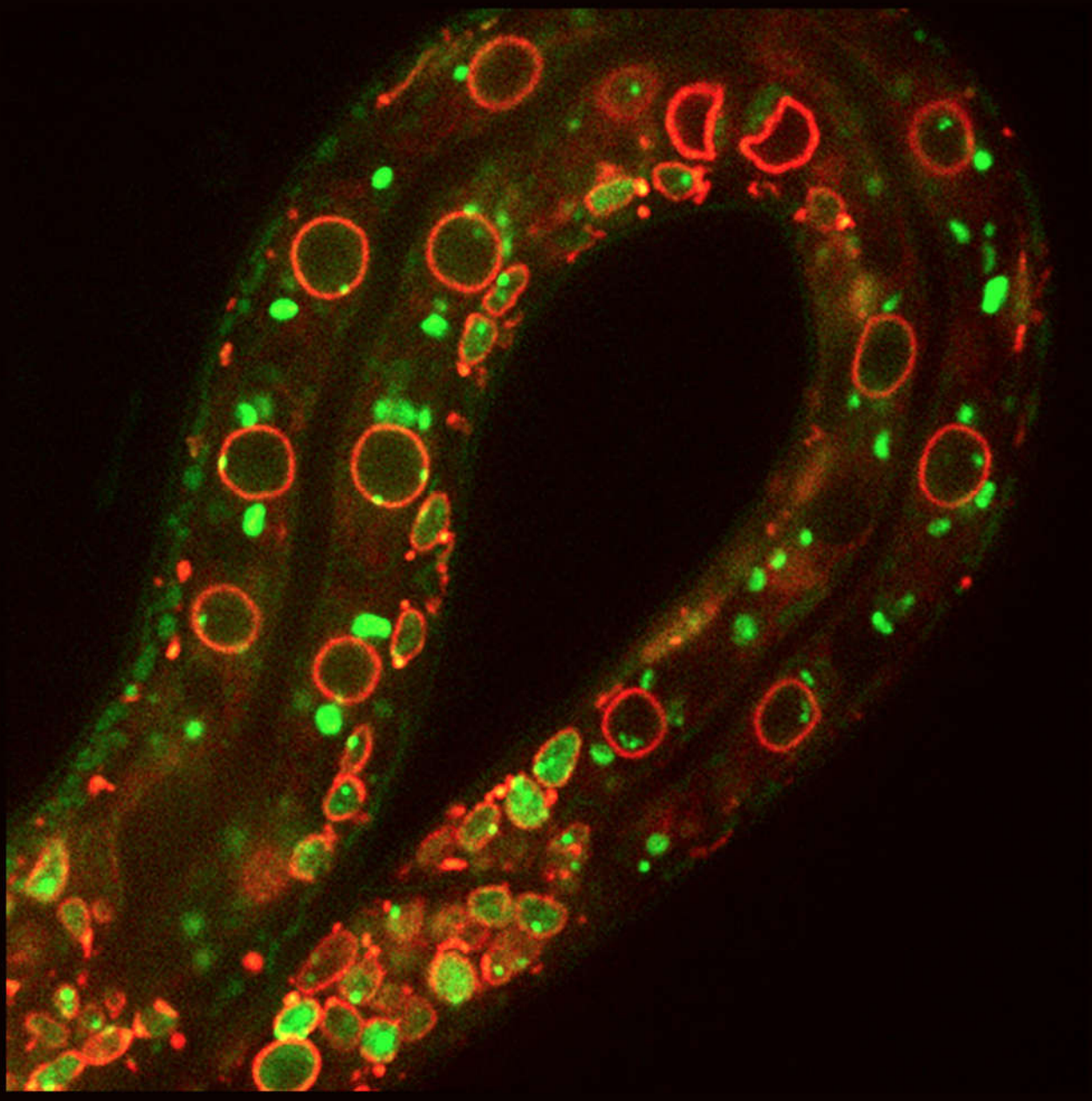
Abstracts of papers presented
at the 2020 *virtual* meeting on

EPIGENETICS & CHROMATIN

September 15–September 18, 2020



Cold Spring Harbor Laboratory
MEETINGS & COURSES PROGRAM

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EPIGENETICS & CHROMATIN

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Arranged by

Anja Groth, *CPR, University of Copenhagen, Denmark*
Kristian Helin, *Memorial Sloan Kettering Cancer Center*
Yang Shi, *Harvard Medical School*

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MEETINGS & COURSES PROGRAM

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Cover: The image shows a *C. elegans* stage 1 larva in which the nuclear envelope is labeled in red (Emerin-mCherry) and a heterochromatic array forms a GFP-labeled focus at the nuclear envelope. Large round intestinal nuclei are seen in the central area of the body. Courtesy of Daphne Cabianca, taken from Cabianca, Muñoz-Jiménez, Kalck, Gaidatzis, Padeken, Seeber, Askjaer & Gasser (2019) Active chromatin marks drive spatial sequestration of heterochromatin in *C. elegans* nuclei. Nature. 569(7758):734-739. *In recognition of the seminal contributions to the field of chromatin and epigenetics by Susan Gasser, who is retiring this year.*

PARP-1 AND PARYLATION INHIBIT TET1 DEMETHYLATION ACTIVITY

Anja Tolić[1], Jovana Rajić[1], Miloš Đorđević[1], Marija Đorđević[1], Aleksandra Uskoković[1], Nevena Grdović[1], Mirjana Mihailović[1], Jelena Arambašić-Jovanović[1], Svetlana Dinić[1], Nataša Nestorović[1], Tomasz Jurkowski[2,3], Melita Vidaković[1]

[1]Institute for Biological Research "Siniša Stanković", National Institute of Republic of Serbia, University of Belgrade, Belgrade, Serbia, [2]Institute of Biochemistry, University of Stuttgart, Stuttgart, Germany, [3]School of Biosciences, Cardiff University, Cardiff, United Kingdom

Since our previous work indicated interplay of Ten-eleven translocation (TET) and Poly (ADP-ribose) polymerase (PARP) proteins our aim was to further study the effects of their interaction.
The ability of recombinant PARP-1 to poly ADP-ribosylate (PARylate) catalytic domains of TET proteins was examined *in vitro*. It was observed that all TETs (TET1, TET2, TET3) readily undergo PARylation. To our knowledge, this is the first report of PARP-1 PARylation of TET2 and TET3 while TET1 PARylation has been previously documented. PARylation of TET proteins was evidenced by western blot signal stretching from the position of unmodified TET. This type of signal is characteristic for PARylation since proteins are modified to varying degrees by covalently attached negatively charged PAR polymers. This slows their movement during electrophoresis and individual molecules are expected to stop at different positions resulting in signal stretching upwards.
PARylation can introduce electrostatic and topological changes in modified proteins, resulting in altered enzymatic activity. Therefore we evaluated TET1 activity *in vitro* using an ELISA type in-house assay, which showed that PARP-1-dependent PARylation lowers the ability of TET1 catalytic domain to oxidize 5mC to 5hmC.
To corroborate these results *in cellulo*, we examined changes in DNA methylation in mouse embryonic fibroblasts (NIH3T3) compared to a PARP-1 knock out mouse embryonic fibroblast cell line (PARP-/-). Lower methylation was observed in PARP-/- cells by immunocytochemical staining. Next, we analyzed global DNA methylation by ELISA assay and we again detected lower methylation levels in PARP-/- and also in NIH3T3 cells treated by a PARP inhibitor niraparib. Finally, DNA hydroxymethylation was assessed by immunocytochemistry and stronger signal was observed in PARP-/- cells and NIH3T3 cells treated by niraparib, compared to control NIH3T3 cells.
In summary, inhibition of PARylation or absence of PARP-1 lead to decreased methylation and increased hydroxymethylation of DNA *in cellulo*. Together, our results point to the inhibitory influence that PARP-1 and PARylation exert on TET1 activity. Further studies are needed to evaluate the effects of PARylation of other TET proteins as well as to examine potential influence of other PARPs.