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研究課題名（和文）Development of a methodology to map and quantify in the genome DNA single strand breaks.

研究課題名（英文）Development of a methodology to map and quantify in the genome DNA single strand breaks.

研究代表者
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研究成果の概要（和文）：本研究の主な目的は、ゲノム上のDNA一本鎖切断（SSB）を定量化し、局在化する方法の開発である。一本鎖切断は、DNA二重らせんの片方の鎖に生じる不連続な損傷である。紫外線や放射線、遺伝毒性物質への曝露によって生じることもありますが、主に内因性によって生じるDNAの最も頻繁な損傷の1つです。私は、ゲノム上のDNA一本鎖切断（SSB）を定量化し、局在化する方法を開発しました。In vitroおよびIn vivoで誘導されたSSBを検出する感度を確認した後、この方法を、触媒サイクルの一部としてSSBを生成するTopoisomerase Iのゲノムの活性を.

研究成果の学術的意義や社会的意義
DNAの一本鎖切断（SSB）は、DNAにおける最も一般的な損傷の一つである。一本鎖切断の蓄積は、癌、神経変性疾患、心不全などの病態に関与している。ゲノム中のSSBsの位置をマッピングする方法を開発することは、この種のDNA損傷の起源と修復、そしてそれらがどのように神経細胞の機能障害や早期老化につながるのか、また癌細胞におけるPARP阻害剤やトポイソメラーゼ阻害剤の作用機序の特徴をよりよく理解するのに役立つであろう。SSBがどのように認識され、修復されるかをよりよく理解することは、将来、がん治療のための改善された治療薬の設計に役立つ可能性がある。

研究成果の概要（英文）：The main objective of this research is the development of a method to quantify and localize DNA single-strand breaks (SSBs) in the genome. SSBs are discontinuities in one strand of the DNA double helix. It is one of the most frequent damage in the DNA and its mainly produced by endogenous sources, although it can be also the consequence of exposure to UV, irradiation or to genotoxic agents. I have developed a method to quantify and localize DNA single-strand breaks (SSBs) in the genome. After checking the sensitivity detecting induced SSBs in vitro and in vivo I applied it to map the activity of Topoisomerase I in the genome, which creates a SSB as part of its catalytic cycle.

研究分野：DNA repair

キーワード：DNA一本鎖切断　トポイソメラーゼI

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景 (Background at the beginning of the research)

The main objective of this research is the development of a method to quantify and localize DNA single-strand breaks (SSBs) in the genome. SSBs are discontinuities in one strand of the DNA double helix. It is one of the most frequent damage in the DNA and its mainly produced by endogenous sources, although it can be also the consequence of exposure to UV, irradiation or to genotoxic agents. The most common sources of SSBs are: (1) oxidative attack on bases or nucleotides, (2) intermediate products of the Base Excision Repair (BER) pathway, (3) aborted activity of DNA topoisomerases (4) base modifications by APOBEC and TET proteins and (5) intermediates of DNA replication, recombination. Unrepaired SSBs can result in genome instability by causing DNA replication stress, transcriptional stalling, and excessive PARP activation leading to DNA double-strand breaks (DSBs). Accumulation of SSBs is implicated in pathologies of cancer, neurodegenerative diseases, and heart failure. SSBs have received much attention recently as defects in its repair are associated with neurological disorders. In order to understand better the origin and repair of this type of DNA damage is important to develop a quantitative method that map the location, persistence, structure, and rate of generation.

２．研究の目的 (Purpose of research)

Although DNA single-strand breaks (SSBs) is one of the most common damage in the DNA, how SSBs are generated, sensed, repaired, and signaled remains still incomplete, largely because of the lack of efficient methods to detect them. Only in the recent years, Next-generation sequencing technologies opened the development of genome-wide mapping methods for mapping DNA damage. Here, I propose the development of a method to quantify and localize DNA single-strand breaks (SSBs) in the genome.

３．研究の方法 (method of research)

I have proposed the following research plan:

-1- Development of a methodology to map in the genome DNA single strand breaks. I tried 2 different approaches to label, purify and sequence SSB:

(i) Direct ligation of sequencing adapter to the SSB, based on the ligation of a biotin-labelled DNA sequencing adapter to the 3'-OH end of the SSB.

(ii) SSB labeling by nick translation. Nick translation incorporation of biotynilated nucleotides to the SSB.

In both cases, after labeling in each approach, genomic DNA is is sheared and fragments containing the SSB captured with streptavidin beads, ligated to sequencing adapters, PCR amplified and sequenced.

To test he specificity and sensitivity of the approaches, I used a nicking endonuclease that cleaves only one strand of DNA in vitro, and also CRISPR/CAS9-nickase, in vivo.

-2- Characterization of SSBs produced by Topoisomerase I (TOP1). During the TOP1 normal catalytic cycle, the enzyme creates a SSB and forms a covalent intermediate that can be used stabilized using drugs and used to map the activity of TOP1 in the genome.

４．研究成果 (research results)

I developed a method based on the direct ligation of a sequencing adapter to the 3' end of the SSB. After checking the sensitivity detecting induced SSBs in vitro and in vivo I applied it to map the activity of Topoisomerase I in the genome, which creates a SSB as part of its catalytic cycle.

-1- Development of a methodology to map in the genome DNA single strand breaks.

To avoid artificial generation of SSBs as a result of mechanical shearing during sample preparation, I embed live cells in agarose and after digestion of proteins and RNA I performed the enzymatic reactions inside of agarose plugs, based on a previous method that I developed to map DNA double-strand breaks in the genome (END-seq). I assayed 2 different approaches to label and capture SSB (Figure 1):

(i) Direct ligation of a biotinylated sequencing adapter to the SSB. I used a biotin-labelled DNA sequencing adapter to ligated directly to the 3'-OH of the SSB. The sequencing adapter starts in the 5' with the sequence of the Illumina p5, in this way the first base sequenced corresponds to the first base of the SSB.

(ii) SSB labeling by nick translation. The 3'OH end of the SSB is used for nick translation incorporation of biotinylated nucleotides to the SSB followed by ligation of the two ends of the DNA.

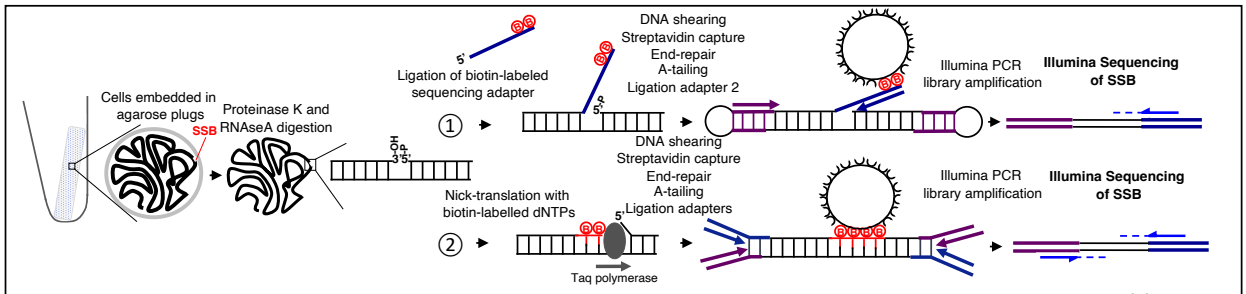

Figure 1. Detection of single-strand breaks. After RNAse and Proteinase K digestion of cells embedded in agarose plugs, (1) a biotin-labeled single stranded oligonucleotide sequencing adapter is ligated to the 3'-OH of the SSB. Other approach, (2) is labelling SSBs by nick translation using biotin-dNTPs. In both cases, after the DNA is recovered and sheared, fragments with SSB are captured with streptavidin beads and sequencing adapters ligated allowing PCR amplification and sequencing.

In both approaches, the DNA was recovered from the melted agarose plugs, sheared and the fragments containing the SSB labeled with biotin are captured with streptavidin beads, ligated to sequencing adapters, PCR amplified and sequenced. To setup and compare both approaches we used a nicking endonuclease Nt.BspQI to induce sequence specific SSBs in the DNA inside of the agarose plugs. Both approaches worked to detect Nt.BspQI SSB, but the direct ligation approach allowed to identify the position of the SSB at nucleotide resolution and the strand specificity. In contrast in the nick translation approach although it was very sensitive it lacks the information about SSB position and strand as the whole fragment is capture with streptavidin and sequencing adapters are ligated at the DNA ends of the fragment generated during the shearing. Next, I tried CRISPR Cas9 nickases D10A and H840A, that cleave the target and non-target strand respectively, both in vitro (inside of the agarose plug) and in vivo, inducing their expression in Abelson-immortalized mouse preB cells arrested in G1. The direct ligation approach was successfully able to detect both in vitro and in vivo the SSBs and the strand specificity.

-2- Characterization of SSBs produced by Topoisomerase I (TOP1).
Next, I used the direct ligation approach to map in the genome the activity of Topoisomerase I (TOP1). TOP1 relaxes supercoiling during transcription and replication by generating a transient SSB that we can use as a readout of its activity. During this process, TOP1 covalently binds to the 3' of the SSB forming a short-lived protein-DNA intermediate and the 5' can rotate around the unbroken strand releasing the torsional stress. TOP1 poisons such us camptothecin, can trap TOP1 in the covalent complex. I briefly treated with camptothecin G1 arrested Abelson-immortalized mouse preB cells and mapped TOP1-induced SSBs. After digestion of proteins and RNA, the 3'-tyrosine was removed by treatment by TDP1 followed by removal of the resulting 3'-phosphate by PNK, and SSBs were mapped using the direct ligation approach. I found that the activity of TOP1 is in actively transcribed genes both in the TSS and in the gene bodies, and the activity correlates with transcription.

５．主な発表論文等

〔雑誌論文〕　計3件（うち査読付論文　3件／うち国際共著　3件／うちオープンアクセス　1件）

１．著者名 Wu W, Hill SE, Nathan WJ, Paiano J, Callen E, Wang D, Shinoda K, van Wietmarschen N, Colon-Mercado JM, Zong D, De Pace R, Shih HY, Coon S, Parsadanian M, Pavani R, Hanzlikova H, Park S, Jung SK, McHugh PJ, Canela A, Chen C, Casellas R, Caldecott KW, Ward ME, Nussenzweig A	４．巻 593
２．論文標題 Neuronal enhancers are hotspots for DNA single-strand break repair	５．発行年 2021年
３．雑誌名 Nature	６．最初と最後の頁 440-444
掲載論文のDOI（デジタルオブジェクト識別子） 10.1038/s41586-021-03468-5	査読の有無 有
オープンアクセス オープンアクセスとしている（また、その予定である）	国際共著 該当する

１．著者名 Wong N, John S, Nussenzweig A, Canela A.	４．巻 2153
２．論文標題 END-seq: An Unbiased, High-Resolution, and Genome-Wide Approach to Map DNA Double-Strand Breaks and Resection in Human Cells	５．発行年 2021年
３．雑誌名 Methods in Molecular Biology	６．最初と最後の頁 9-31
掲載論文のDOI（デジタルオブジェクト識別子） 10.1007/978-1-0716-0644-5_2	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

１．著者名 Huang SN, Michaels SA, Mitchell BB, Majdalani N, Vanden Broeck A, Canela A, Tse-Dinh YC, Lamour V, Pommier Y.	４．巻 7
２．論文標題 Exonuclease VII repairs quinolone-induced damage by resolving DNA gyrase cleavage complexes	５．発行年 2021年
３．雑誌名 Science Advances	６．最初と最後の頁 not yet
掲載論文のDOI（デジタルオブジェクト識別子） 10.1126/sciadv.abe0384	査読の有無 有
オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 該当する

〔学会発表〕　計0件

〔図書〕　計0件

〔産業財産権〕

〔その他〕

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６．研究組織

	氏名 （ローマ字氏名） （研究者番号）	所属研究機関・部局・職 （機関番号）	備考

７．科研費を使用して開催した国際研究集会

〔国際研究集会〕　計0件

８．本研究に関連して実施した国際共同研究の実施状況

共同研究相手国	相手方研究機関