جامعة غليزان
RELIZANE UNIVERSITY

COMMUNICATION

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Année universitaire
2022-2023

Chapitre 4.

Elargir le vocabulaire courant aux domaines techniques pour comprendre un article scientifique

Plan du cours

I. Introduction

II. Vocabulaire de la biologie

- Exemples de termes scientifiques utilisés en biologie

III. Lire et comprendre un article scientifique

- Exemple d'un article scientifique

I. Introduction

Le **vocabulaire scientifique** est un **ensemble des termes** propres à une science, à une technique, à un groupe, à un milieu, à un auteur (Dictionnaires Larousse);

La **terminologie** est une **discipline** qui a pour objet l'étude théorique des **dénominations** des objets ou des **concepts** utilisés par tel ou tel domaine du savoir, le fonctionnement dans la langue des unités terminologiques, ainsi que les **problèmes de traduction**, de **classement et de documentation** qui se posent à leur sujet (Dictionnaires Larousse).

I. Introduction

Un **article/publication scientifique** est un rapport écrit et publié qui recueille des informations décrivant les résultats d'une enquête menée (un **travail de recherche**) et les discute par rapport aux travaux déjà publiés.

Les **auteurs** de l'article scientifiques s'adressent à leurs **pairs scientifiques**.

Le **plan** de l'article est normalisé: il est structuré en six parties distinctes : le **titre** et le **résumé**, l'**introduction**, la **méthodologie**, les **résultats**, la **discussion** et la **conclusion**.

Une **revue scientifique** publie l'article selon certaines règles.

II. Vocabulaire de la biologie

Pour comprendre un article scientifique dans le **domaine de la biologie**, le lecteur doit élargir le vocabulaire scientifique utilisé par les **chercheurs en biologie**.

Exemples de termes scientifiques utilisés en biologie

Vocabulary	*definition*	*تعريف*	*مفردات*
Amino acids	are organic compounds that contain both amino and carboxylic acid functional groups.	الأحماض الأمينية هي لبنات البناء الرئيسية لبناء البروتين والببتيد. فالأحماض الأمينية هي مجموعة من المركبات العضوية متكونة على (NH2من مجموعة أمين (– الأقل مشتبكة مع مجموعة كربوكسيل (COOH–).	**الأحماض الأمينية**
Biodiversity	biodiversity or biological diversity is the variety and variability of life on Earth.	يقصد به التنوع البيولوجي وتنوع أشكال الحياة على الأرض.	**تنوع بيولوجي**
Deoxyribonucleic acid, DNA	is a polymer composed of two polynucleotide chains that coil around each other to form a double helix.	هو جزيء ضخم يتواجد داخل خلايا كل الكائنات الحية والعديد من الفيروسات ويحتوي الوراثية التي تسمح على المعلومات بعمل وتكاثر وتطور هذه الكائنات.	**حمض نووي ريبوزي ناقص الأكسيجين**
Enzymes	are proteins that act as biological catalysts by accelerating chemical reactions.	الإنزيمات هي محفِّزاتٌ بيولوجية جزيئة بروتينة. تسرّعُ التفاعلات الكيميائية.	**الإنزيمات**

Vocabulary	*definition*	*تعريف*	*مفردات*
Glycolipids	are lipids with a carbohydrate attached by a glycosidic (covalent) bond.	هو نوع من أنواع الليبيدات، الذي يرتبط به نوع من الكربوهيدرات (تُسمى أيضاً السكريات).	**دهنيات سكرية**
Hemolysis	is the rupturing (lysis) of red blood cells (erythrocytes) and the release of their contents (cytoplasm) into surrounding fluid (e.g. blood plasma).	هو تمزق كريات الدم الحمراء وإطلاق محتوياتها (السيتوبلازم) من بلازما الدم.	**إنحلال الدم**
Insects	are pancrustacean hexapod invertebrates of the class Insecta.	الحشرات طائفة (أو صنف) من الحيوانات اللافقارية في شعبة مفصليات الأرجل	**حشرات**
Virus	is a submicroscopic infectious agent that replicates only inside the living cells of an organism.	هو عامل ممرض صغير لا يمكنه التكاثر إلا داخل خلايا كائن حي آخر.	**فيروس**

III. Lire et comprendre un article scientifique

Les articles scientifiques sont généralement **rédiger en anglais** donc avoir un **bon niveau d'anglais** est important pour comprendre un article scientifique.

Exemple d'un article scientifique

PLOS ONE

RESEARCH ARTICLE

DNA damage induced during mitosis undergoes DNA repair synthesis

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Abstract

Understanding the mitotic DNA damage response (DDR) is critical to our comprehension of cancer, premature aging and developmental disorders which are marked by DNA repair deficiencies. In this study we use a micro-focused laser to induce DNA damage in selected mitotic chromosomes to study the subsequent repair response. Our findings demonstrate that (1) mitotic cells are capable of DNA repair as evidenced by DNA synthesis at damage sites, (2) Repair is attenuated when DNA-PKcs and ATM are simultaneously compromised, (3) Laser damage may permit the observation of previously undetected DDR proteins when damage is elicited by other methods in mitosis, and (4) Twenty five percent of mitotic DNA-damaged cells undergo a subsequent mitosis. Together these findings suggest that mitotic DDR is more complex than previously thought and may involve factors from multiple repair pathways that are better understood in interphase.

Introduction

DNA damage occurs naturally through various endogenous and exogenous processes. Unrepaired DNA can compromise genetic integrity leading to developmental disorders, cell death or cancer. Organisms have evolved a variety of pathways to respond to the damage. The vast majority of studies on DNA damage response have been done during interphase of the cell cycle. However, understanding the DNA damage response (DDR) during mitosis is also important since mutations accumulated during mitosis can lead to chromosomal alterations, genomic instability of daughter cells, senescence and eventual cell death [1–5].

Studies examining the extent of DDR activation and repair in mitosis have primarily assessed the cellular response to double strand breaks (DSBs). DSBs may be repaired by homologous recombination (HR) and non-homologous end joining (NHEJ). HR preserves

OPEN ACCESS

Citation: Gomez Godinez V, Kabbara S, Shannon A, Wu T, Cohen S, Kong X, et al. (2020) DNA damage induced during mitosis undergoes DNA repair synthesis. PLoS ONE 15(4): e0227949. https://doi.org/10.1371/journal.pone.0227949

Editor: Arthur J. Lustig, Tulane University Health Sciences Center, UNITED STATES

Received: December 27, 2019

Accepted: April 1, 2020

Published: April 29, 2020

Data Availability Statement: Raw images files are available through the UCSan Diego Library Digital Collections https://doi.org/10.6075/J0BV8R2K

Funding: This work is supported in part by JPSE Grant#P08220-09-1-0366 (MW & JM) Minority Health and Health Disparities International Research Training (IMHRT) Program NIH Grant MD-01449 (VGG), National Science Foundation MCB-1615701 (KY), and gift from the Beckman Laser Institute Foundation (MW&). The funders had no role in study design, data collection and

REVUE

TITRE

RÉSUMÉ

Les chercheurs synthétisent leur travail en exposant le but, les méthodes, les conclusions et les limites de celui-ci en quelques paragraphes.

INTRODUCTION

Les auteurs donnent du contexte sur ce qui a motivé leur démarche et expliquent la finalité de leur étude, c'est-à-dire à quelle(s) question(s) elle cherche à répondre.

whose function is better understood in interphase are capable of responding to mitotic DNA damage. Our results also indicate that DSBs generated on metaphase chromosomes lead to clustering of various proteins involved in NHEJ and HR. We show that DNA repair of mitotic DNA damage is ongoing and persists into G1.

Materials and methods

Reagents

See Supplemental S3 for a list of antibodies. Other reagents are listed under corresponding methods below.

Cell lines

Five human cell lines were utilized in this study. U-2 OS cells, referred to as U2OS in this paper, are an osteosarcoma cell line ATCC HTB 96 that was used for the majority of studies unless otherwise mentioned. CFPAC-1 ATCC CRL 1918, a line derived from cystic fibrosis pancreatic adenocarcinoma was also utilized. The isogenic cell lines, M059K ATCC 2365 and M059J ATCC 2366 were utilized to compare the mitotic DNA response when DNA-PKcs is absent (M059J). M059K contains the wild type form of DNA-PKcs making this line a good control for the DNA-PKcs mutant. Both cell lines come from a glioblastoma in the same patient. Rat kangaroo cells (PtK2) from the Potorous tridactylus were utilized due to their large chromosomes and strong adherence to the substrate that facilitate mitotic studies. These cells were grown in Advanced DMEM/F12 with 2% GlutaMax and 10% Fetal Bovine Serum. All Human cells were grown in Advanced DMEM supplemented with 1% GlutaMax and 10% Fetal Bovine Serum. All cells were maintained in a humidified 5% CO_2 incubator. Cells were plated onto glass bottom gridded dishes from MatTek to a confluency of 40% and used 1–2 days post subculture. Experiments were carried out in medium containing 40ng/mL roscovitine. For inhibitory experiments the following concentrations were utilized: ATM inhibitor (10μM KU55933), DNA PKcs inhibitor (5μM NU7441), and PARP inhibitor (100μM Nu1025).

Laser induced DNA damage and microscope image acquisition

Mitotic chromosomes in live cells were irradiated using diffraction-limited (0.5–1 μm diameter) focal spots with a Coherent Mira 76MHz 200 femtosecond micro-pulsed laser emitting at 780nm (Coherent Inc., Santa Clara CA). A series of beam expanders and mirrors coupled the beam into the right-side port of a Zeiss Axiovert 200M inverted microscope. An X-Y fast scanning mirror was positioned in the beam path prior to entry into the microscope port to facilitate moving the focused laser beam toward the desired target. The beam was focused through a 63x (1.4NA) Zeiss Plan-Apochromat oil objective. The irradiance of the laser was controlled through the use of a Glan-Thompson polarizer mounted on a motorized rotational stage. A Uniblitz mechanical shutter controlled the exposure time of the laser. A single chromosome within the cell was targeted by the laser unless otherwise noted. Chromosomes were exposed to the laser for a total of 10ms within the focal spot. This exposure resulted in 7.8×10^5 pulses of light to a spot measuring 0.68μm in diameter at the selected irradiance of $2.8–5.2 \times 10^{13}$ W/cm^2.

Images were collected using a Hamamatsu CCD Orca [35,40,41]. The polarizer, scanning mirror and shutter were controlled by software developed with LabView [42]. To determine the irradiance at the focal point, the transmission of the objective was measured using a modified dual objective method described previously [35]. The objective used in these experiments had a transmission of 0.50 at the laser wavelength used. Based upon the measured irradiance

MATÉRIEL ET MÉTHODES

On y trouve le détail du protocole de recherche utilisé : comment les chercheurs ont travaillé pour obtenir les réponses en limitant les biais, et pourquoi ils ont fait ces choix méthodologiques.

Pyrimidine dimer quantification

Mitotic U2OS were collected via mitotic shake off after synchronization with 9uM CDK1 inhibitor (Calbiochem; RO-3306). CDK1 inhibition results in arrest at G2; upon removal of inhibitor cells entered mitosis. Collected cells were exposed to 265nm UV light from a UV lamp UVG-11 (Science Company) for 20s in phenol red free medium. Cells were then separated into three aliquots and lysed at 30, 60 and 90 minutes after UV exposure. DNAzol Reagent (Life Technologies) was added to each sample for lysis and DNA isolation according to the manufacturer's protocol. Isolated DNA samples were quantified using a Nanodrop 2000c (Thermo Scientific) and diluted to a working concentration of 20ug/ml in cold PBS. DNA samples were further diluted to plate on 96-well DNA High-Binding Plates. An OxiSelect UV-induced DNA Damage ELISA Combo Kit was utilized to determine the concentration of pyrimidine dimers in each sample well (Cell Biolabs Inc). Plates were read with a Biotek Conquor ELX800 plate reader (Biotek Inc).

Statistical analysis

Prism 7 for MAC OS was utilized for all statistical analysis. A T test was performed for comparisons to controls unless otherwise noted. Values were considered significant if $P < 0.05$. Box plots show the 95% confidence intervals. Raw numbers and quantifications can be found in quantifications.xls under supplemental information. Images used for quantifications can be found at the following location: https://doi.org/10.6075/J08W58QK [44].

Results

The laser induces complex DNA damage on mitotic chromosomes

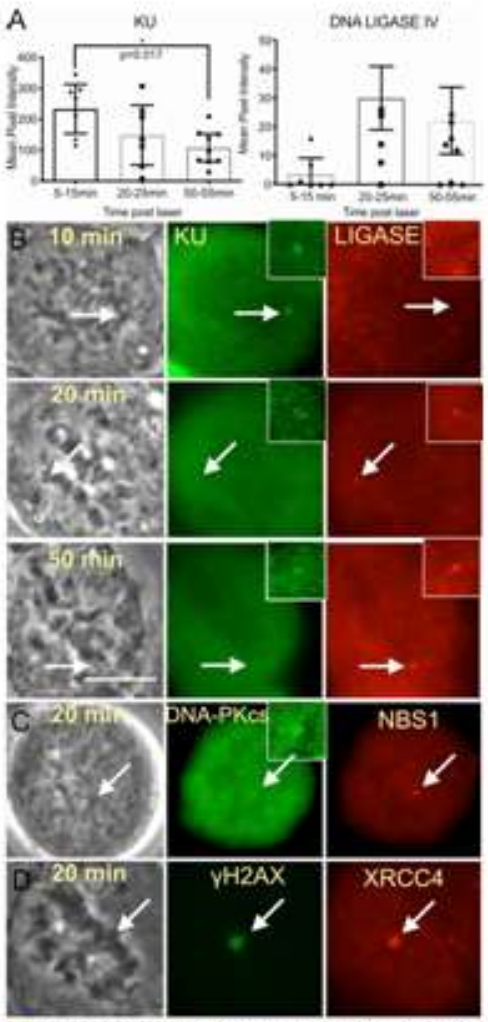
Optimal laser parameters for the detection and consistent production of DNA damage in mitotic chromosomes were obtained by (a) varying the irradiance and comparing phase contrast image changes, and, (b) assaying for Nbs1 accumulation and γH2AX production. For this study we utilized irradiances of $2.8–5.2 \times 10^{8}$ W/cm^2 unless otherwise noted. These irradiances allowed us to immediately determine that chromosomes were effectively damaged due to rapid phase contrast changes in the irradiated chromosome regions (Fig 2A, arrows at 4 and 6s). Dark material, which may reflect phase separation is visible at 16s post laser exposure. In a different example, of a cell fixed ~3s after the laser, we see that γH2AX surrounds an area targeted by the laser (Fig 2B). In a previous study we showed that dark material is a result of the accumulation of DDR proteins and that γH2AX may surround the proteins and/or overlap with them [34]. Additionally, at these irradiances γH2AX and Nbs1 were detected in early 100% of the time (48 of 48 cells) and (16 of 17 cells) respectively.

The type of DNA damage induced by the laser on mitotic cells was assessed by immunostaining for (1) DNA damage response proteins, (2) damaged bases, and (3) the TUNEL assay. Experiments were carried out in human U2OS cells unless otherwise mentioned. Positive TUNEL at laser induced DNA damage sites demonstrated the presence of END breaks, Fig 2C (magenta). TUNEL signal was higher in chromosomes fixed 10 minutes post laser when compared to those fixed 50 minutes post laser. These results indicate that factors may have bound broken ends.

Several DDR proteins were found at damaged chromosomes which provide information on the type of damage created by the laser. The consistent production of γH2AX demonstrates the lasers ability to induce DSBs. Pyrimidine dimers (CPD) as a result of the laser exposure were also detected and will be discussed further in this paper. XRCC1, which is involved in single strand break (SSB) repair, nucleotide excision repair (NER) and base excision repair (BER)

RÉSULTATS

Les auteurs exposent l'ensemble des résultats significatifs obtenus, accompagnés généralement de graphiques explicatifs et de détails statistiques (comme les marges d'erreur).



RÉSULTATS

Graphiques explicatifs et images.

The majority of mitotic cells damaged with the laser entered G1, 26 of 28 of cells damaged in metaphase and 8 of 10 cells damaged in anaphase (Fig 3A and 3B). One cell damaged in metaphase died. The daughters of cells that divided were followed and entry into mitosis i.e. completion of a cell cycle was assessed within an observation window up to 40–47 hours post DNA damage. A proportion (21%) of daughter cells with damage inflicted in pro-metaphase underwent a subsequent mitosis, compared to 57% for daughter cells carrying damage elicited in anaphase. Examples of time-lapse analysis of cells damaged in metaphase and anaphase are shown in which both daughter cells underwent subsequent mitosis (Fig 3DA and 3DC). Cells that did not divide and reverted are shown in Fig 3DB and 3DD. Daughter cells carrying the damaged chromatin took longer to divide than their counterparts without damaged chromatin (S5B Fig).

Damaged G1 cells were also followed to compare their ability to repair with that of mitotic cells. These cells were identified by following anaphase cells until completion of division and formation of two daughter cells. Of both daughter cells only one sister was damaged with the laser. However, both sisters were followed. Prior to fixation, all cells were incubated with EdU to check for S-phase status of cells that had not divided within the observation window. Fig 3 contains a summary of cell fates.

As expected, all undamaged G1 sisters entered mitosis within the observation window. Out of the nine damaged cells, five divided i.e. 55% divided. Our results suggest that damage in metaphase is more deleterious than damage induced in anaphase or G1 (Fig 3C). Notwithstanding, these results demonstrate that a percentage (25%) of cells laser damaged in mitosis (metaphase and/or anaphase) are capable of undergoing a subsequent mitosis.

Discussion

Using the highly focused NIR laser we have determined that the mitotic DNA damage response is more extensive than previously thought. We confirmed that under our conditions the NIR laser induces complex DNA damage, including DNA strand breaks and UV crosslinking damage, Fig 1A. Additionally, we demonstrated DDR-associated post-translational modifications at damage sites, including γH2AX, Ub and PAR, Fig 1B. Furthermore, we detected the recruitment of factors involved in different DNA repair pathways, including DSB and SSB repair, SSBR and NER, Fig 1B. Together with EdU incorporation at damage sites, our results strongly suggest that these repair pathways are activated. Our evidence also indicates, that while NHEJ may occur, the HR repair process is not completed during mitosis. Our results also suggest mitosis-specific effects of DDR signaling that dictates repair pathway choice, revealing intricate mechanisms of mitotic DNA damage response and repair.

The laser as a method to elucidate the DDR during mitosis

Previous studies that utilized ionising radiation and radiomimetic drugs to induce DSBs did not show the accumulation of ubiquitin (Ub), RNF8, RNF168, BRCA1, 53BP1 to mitotic chromosomes [2, 9–14]. However, this might have been due to the limited density of DNA damage, making these studies rely on ionising radiation induced foci (IRIF) formation of these factors. IRIF are distinct from initial recruitment of the DNA damage-recognition factors and entail further clustering of proteins as well as amplification signals that surround damage sites [50, 65]. Our ability to detect all of the factors is likely due to a higher density of DNA damage in a small submicron volume than these previous studies, thus enabling detection of a high concentration of damage factors. Indeed, Ub and KU have been detected at damage sites on mitotic chromosomes using similar laser systems [26, 55].

DISCUSSION

Cette partie importante explique les limites qu'ont rencontrées les chercheurs et la portée réelle de ces résultats (ce qu'ils signifient ou ne signifient pas) et en quoi ils peuvent être incertains. Elle donne aussi des interprétations possibles et des pistes à explorer ultérieurement.

S1 Data. Raw values and quantifications (quantifications.xls) that correspond to Figs 1C, 2C, 4A, 5A–5C, 6A, 6B, 7C, 7D, S2A, S2B, S2C and S2F.
(XLSX)

S1 Video. Movie of cells in 9A. Daughters of a DNA damaged metaphase cell undergo division.
(AVI)

S2 Video. Movie of cells in 9B. A DNA damaged metaphase cell undergoes furrow regression.
(AVI)

S3 Video. Movie of cells in 9C. Daughters of a DNA damaged anaphase cell undergo division.
(AVI)

S4 Video. Movie of cells in 9D. A DNA damaged anaphase cell undergoes furrow regression.
(AVI)

Acknowledgments

A special thanks to Arthur Forer, Ph.D. (York University, Toronto, ON) and Pheng-Lang Chan, Ph.D. (UC-Irvine, Irvine, Ca) for thought provoking conversations on mitotic response to DNA damage.

Author Contributions

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Supervision: Veronica Gomez Godinez, Kyoko Yokomori, Michael W. Berns.

Validation: Shirli Colton.

Visualization: Veronica Gomez Godinez.

Writing – original draft: Veronica Gomez Godinez.

Writing – review & editing: Kyoko Yokomori, Michael W. Berns.

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REMERCIEMENTS

Les auteurs remercient les institutions ou confrères / consœurs les ayant aidés.

CONTRIBUTIONS DES AUTEURS

Les auteurs décrivent la répartition des travaux.

BIBLIOGRAPHIE

Appelée « références », cette liste recense tous les articles scientifiques déjà publiés dans des revues à comité de lecture et cités dans la publication.