Coopération entre les fonctions essentielles de la cellule telles que la transcription et la réparation de l'ADN et l'organisation de la chromatine

Cooperation between essential cellular functions such as transcription and DNA repair, and chromatin organization

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Résumé en français
Dans les cellules eucaryotes, l'ADN est compacté dans la chromatine et tous les processus essentiels liés à l'ADN y compris la transcription, la première étape d'expression des gènes, et la réparation de l'ADN se passent dans un contexte chromatinien. Le Médiateur de régulation de la transcription est un complexe multiprotéique essentiel et très conservé qui intègre les signaux de régulation des facteurs spécifiques à la machinerie de base de l'ARN polymérase II. Etant donné son rôle crucial dans l'activation de la transcription, des mutations dans les sous-unités du Médiateur sont impliquées dans des maladies comme les maladies de neurodéveloppement et le cancer. En plus, nous avons découvert que le Médiateur a un nouveau rôle liant la transcription et la réparation de l'ADN impliquée dans les maladies rares, Xeroderma Pigmentosum et syndrome de Cockayne. Même si le Médiateur a fait l'objet de nombreuses études, il reste en grande partie inconnu comment la fonction du Médiateur est coordonnée avec les régulateurs de la chromatine. Notre travail récent chez la levure a permis de montrer que le Médiateur interagit et coopère dans l'organisation des promoteurs et la transcription avec RSC (Remodèle la Structure de la Chromatine, PBAF chez l'homme), un complexe de remodelage de la chromatine multiprotéique, essentiel à la viabilité cellulaire et très abondant. L'importance de ce complexe est soulignée par le fait que des mutations dans l'homologue humain de RSC (complexe PBAF) sont associées au cancer et maladies de neurodéveloppement. En général, les complexes BAF et PBAF sont mutés dans 20% des cancers et représentent ainsi les complexes les plus fréquemment mutés dans les cancers. Dans ce projet interdisciplinaire, nous nous proposons d'étudier les mécanismes moléculaires d'action du Médiateur en relation avec le complexe de remodelage RSC/PBAF dans l'organisation de la chromatine (en nucléosomes et 3D), la transcription et la réparation de l'ADN. Nous allons caractériser comment le Médiateur et RSC sont impliqué dans la structure de la chromatine, la transcription et la réparation de l'ADN et adresser la question de la conservation de ces mécanismes chez les eucaryotes. Nous allons utiliser la levure et les cellules humaines comme nos modèles biologiques. Nous les étudierons expérimentalement par des approches de la génomique fonctionnelle avancée, la biologie moléculaire, la génétique, la biochimie ; et les résultats obtenus seront analysés par des méthodes computationnelles fondées sur l'apprentissage profond. En utilisant la modélisation numérique, nous dégagerons des concepts généraux qui permettront d'élucider la coopération entre les fonctions essentielles de la cellule et l'organisation de la chromatine. En conclusion, ce projet fournira des éléments importants pour la compréhension des mécanismes qui lient la transcription et la réparation de l'ADN avec l'organisation de la chromatine, et constituera un socle de biologie fondamentale, en particulier en biologie des chromosomes, supportant les recherches innovantes en radiobiologie. Notre approche computationnelle basée sur l'apprentissage profond (plus particulièrement l'adaptation de l'algorithme d'attention et les *transformers*) nous permettra en plus de concevoir un outil d'analyse numérique autonome et performant. Les résultats de nos travaux devraient avoir un impact important dans la compréhension des processus cellulaires impliquant la chromatine et les pathologies humaines sévères.

Résumé en anglais
In eukaryotic cells, the DNA is compacted in chromatin and all DNA-related processes including transcription, the first step in gene expression, and DNA repair occur in the crowded context of chromatin. Mediator of transcriptional regulation is essential and highly conserved multiprotein complex that integrates the regulatory signals from specific transcription factors to RNA polymerase II basal machinery. Given its crucial role in transcription activation, mutations in Mediator subunits are involved in pathologies such as neurodevelopmental disorders and cancers. Recently, we discovered that Mediator has a novel role linking transcription and DNA repair involved in rare diseases, Xeroderma Pigmentosum and Cockayne syndrome. Despite intensive studies, it remains largely unknown how Mediator function is coordinated with chromatin regulators. Our recent work in yeast has shown that Mediator interacts and cooperates in promoter organization and transcription with RSC (Remodels the Structure of Chromatin, PBAF in human), an essential and abundant multisubunit chromatin remodelling complex. Importance of this complex is underscored by the fact that mutations in human homolog of RSC (PBAF complex) are associated with cancer and neurodevelopmental diseases. In general, BAF and PBAF complexes mutated in 20% of cancers and thus represent the most frequently mutated complexes in cancers.
In this interdisciplinary project, we propose to study the molecular mechanisms of action of Mediator in relation to the RSC/PBAF remodelling complex in chromatin organization (in nucleosomes and 3D), transcription and DNA repair, and address the question of the conservation of these mechanisms in eukaryotes. We will use yeast and human cells as our biological models. We will study them experimentally by approaches of advanced functional genomics, molecular biology, genetics, biochemistry; and the obtained results will be analyzed by computational methods based on deep learning. Finally, using numerical modeling we will derive general concepts that will elucidate the cooperation between the essential functions of the cell and chromatin organization. In conclusion, this project will provide important elements for the understanding the mechanisms that link transcription and DNA repair with chromatin organization, and will constitute a foundation of fundamental biology, in particular in chromosome biology, supporting the innovative research in radiobiology. In addition, our computational approach based on deep learning (more particularly the adaptation of the attention algorithm and transformers) will allow us to design an autonomous and efficient numerical analysis tool. The results of our work should have an important impact in the understanding of cellular processes involving chromatin and severe human pathologies.

Description du sujet (8000c)
Gene expression is one of the fundamental biological functions of the cell. Mediator was identified as a regulator of transcription, the first step of gene expression (1). This large protein complex is essential for transcription regulation in all eukaryotes (2). In human, mutations that affect Mediator subunits lead to a number of pathologies including neurodevelopmental disorders and cancers (3). We significantly contributed to understanding of Mediator mechanisms by establishing its role in the recruitment of Pol II and TFIIH (4-6) and a functional interplay with TFIIB in relation to promoter architecture (7).

Moreover, we discovered that Mediator complex plays a novel role by connecting transcription with DNA repair (8). We have identified a functionally important contact between Mediator and Rad2 endonuclease, the yeast homolog of human XPG protein involved in nucleotide-excision DNA repair (9). Mutations in human XPG gene give rise to rare diseases including Xeroderma Pigmentosum (XP) associated with Cockayne syndrome (CS). Our results strongly suggest that Mediator is involved in transcription-coupled DNA repair by facilitating Rad2 recruitment to transcribed genes. We propose that Mediator can serve as an assembly platform

or a regulatory element connecting transcription with DNA repair (10). More recently, we provided a genomic view of functional dynamics between Mediator, Pol II and Rad2/XPG (11). Furthermore, physical and functional interactions of Mediator were also detected with other NER proteins, Rad26/CSB and Rad1-10/XPF-ERCC1 (12).

In eukaryotic cells, the DNA is compacted in chromatin and all DNA-related processes like transcription and DNA repair occur in chromatin context. The scientific challenge resides in understanding of the extremely complex DNA transactions operating on 3D-organized chromatin. It remains largely unknown how Mediator function is coordinated with chromatin regulators. Our recent work in yeast has shown that Mediator interacts and cooperates in promoter organization and transcription with RSC (Remodels the Structure of Chromatin, PBAF in human), an essential and abundant multisubunit chromatin remodelling complex (13). The yeast RSC complex belongs to the conserved SWI/SNF family of chromatin remodelers (14). In human, mutations of human homolog of RSC (PBAF complex) subunits have been linked to diseases. For example, BAF and PBAF complexes mutated in 20% of cancers, represent the most frequently mutated complexes in cancers. ATP-dependent chromatin complexes including RSC play a key role in the establishment of nucleosome-free regions in promoters and in nucleosome positioning (15). Previously, we have shown that RSC interacted with RNA polymerases (16) and that the loss of this interaction altered the chromatin structure in the promoter regions and impaired transcription. While the molecular mechanism of chromatin remodelling by RSC complex is well established, our recent work reveals, for the first time to our knowledge, the functional cooperation between RSC and Mediator (13) that opens important perspectives in fundamental chromatin-related and disease-relevant processes including transcription and DNA repair (17).

The general objectives of this project are to uncover a functional cooperation between Mediator and chromatin regulators in chromatin organization, transcription and DNA repair, focusing on RSC/PBAF remodelling complex based on our recent work (13). This project should give important insights in the mechanisms that link transcription and DNA repair with chromatin organization and will have a deep impact on our understanding of fundamental chromatin processes of the cell and severe human diseases.
We will use the budding yeast and human cells as our biological models and apply integrative approaches spanning molecular biology, genetics, biochemistry, functional genomics, bioinformatics and modelling, including a computational analysis of NGS data based on deep-learning with development of novel analytical and modelling tools.
To achieve this goal,

- we will determine how Mediator and RSC are involved in chromatin structure, transcription and DNA repair by investigating the effect of Mediator and RSC mutations on chromatin organization in nucleosomes and 3D, transcription and DNA repair genome-wide.
- we will address the question of the conservation of the mechanisms linking Mediator to chromatin remodelling in human cells.
- we will develop computational approaches for integrative analysis of experimental data and apply machine learning tools to model complex biological processes.

In conclusion, this interdisciplinary project will allow us to open new exciting perspectives for the molecular mechanisms that connect chromatin transactions including transcription and DNA repair to chromatin organization. The project aims to understand how a key coregulator of gene expression, the Mediator complex, cooperates with an essential regulator of DNA organization into chromatin, the RSC complex, to ensure genomic expression and integrity. The project will lead to particularly innovative concepts for understanding the mechanisms that link

gene expression and DNA repair with chromatin organization. We believe that uncovering the mechanisms at play represents an important issue in the chromatin field and will have a deep impact on our understanding of fundamental chromatin processes of the cell and of severe human pathologies including cancers and rare diseases. This project will constitute a foundation of fundamental biology, in particular in chromosome biology, supporting the innovative research in radiobiology. This work will also reinforce key CEA skills in data analysis (NGS, Big data) and modelling (machine learning). Our project is based on a solid publication list of our laboratory (Science 2011 (6), G&D 2013, 2016 (7,8); NAR 2019 (11), Genome Research 2022 (12), PLoS Comp Bio 2022 (18)) and recent work deposited on BioRxiv 2022 (13).

We will collaborate with M. Gérard, (CEA/Joliot), expert in chromatin remodelling in mammalian cells (19).

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