Présentation des projets financés au titre de l'édition du 2010
Programme « Blanc International SVSE 2 »

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Programme Blanc International SVSE 2

Edition 2010

Titre du projet	**DRHUEC** – Dynamic roles of H2B ubiquitylation in Eukaryotic cells
Résumé	Although histones were the first protein shown to be ubiquitinated, the enzymes responsible for the addition or the removal of a single ubiquitin on histones H2A and H2B were only recently identified. The characterization of these enzymes has linked monoubiquitination of histone H2A (H2Aub1) and H2B (H2Bub1) to the regulation of gene expression albeit with different outcomes. Whereas, H2A ubiquitination is usually considered as a repressive mark, H2B ubiquitination was shown to have roles both in gene activation and gene repression. Besides its function in transcription, histone ubiquitination was also shown to play a role in other processes such as mRNA export, gene gating, mitosis or DNA repair. Interestingly, a number of studies indicate that deregulation of histone ubiquitination may contribute to cancer development. In good agreement, we obtained preliminary results indicating that H2B ubiquitination is involved in the regulation of the cell cycle and in DNA replication in the budding yeast (Saccharomyces cerevisiae). However, the molecular mechanisms underlying these functions are poorly understood. The present project aims at determining the different functions mediated by H2B monoubiquitination and to decipher the mechanisms involved in these processes. We will also analyze how the factors that regulate histone ubiquitination are involved in carcinogenesis. Both groups have produced important contributions to the field of histone ubiquitination but with complementary expertises. The combination of expertise in yeast genetics and in transcription regulation in mammals in the two laboratories provides a unique set up to investigate novel aspects of the control of histone ubiquitination, a modification that plays key role in numerous processes and in pathological conditions. We will perform an unbiased biochemical approach to identify proteins that specifically

recognize H2Bub1using different mutant yeast strains. A synthetic genetic screen will identify the factors that are functionally redundant to H2Bub1 in yeast. We will also purify from mammalian cells chromatin associated proteins from cells in which the H2B ubiquitination is increased or reduced to identify functional partners, hence putative readers, of H2Bub1. This will also help elucidate the underlying mechanisms H2Bub1 may utilize for the exertion of downstream effects. The levels of H2Bub1 is tightly regulated by specific deubiquitnase such as Ubp8/USP22 a subunit of the transcriptional coactivator complex SAGA. We will characterize the regulation of USP22 catalytic activity by other SAGA subunits. This will allow us to produce a recombinant subcomplex that has a full DUB activity and that will be used to further characterize putative non-histone substrates. We will identify ubiquitinated non-histone proteins that accumulate in cells in which the SAGA deubiquitination activity is abrogated. This will shed light on the mechanisms by which SAGA DUB activity regulates transcription or other chromatin associated functions. Preliminary genetic analysis showing that H2Bub1 and two intra-S-phase checkpoint proteins, Sgs1p and Mrc1p, have overlapping functions in response to replicative stress provides clues to a role of H2Bub1 in DNA synthesis and S phase progression. Using a variety of biochemical and genetic analyses, we will further decipher the mechanisms by which H2Bub1 regulates DNA replication. To visualize the dynamics of SAGA complexes in respect to their gene regulatory, gene anchoring and mRNA export functions, we will create SAGA-dependent promoter-reporter systems. Using these models we will be able to determine upon activation, the relocation of SAGA-dependent gene loci.

Partenaires

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Aide de l'ANR **Début et durée**	193 000 € - 36 mois
Référence	ANR-10-INTB-1201