A three-dimensional view of the interface between nuclear envelope and chromatin

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Summary

The nucleus is an organelle characteristic of eukaryotic cells and its mechanical properties play an essential role in the behavior of the cell, in particular its motility, polarity and survival. It is surrounded by an envelope comprising an inner membrane and an outer membrane, as well as a large number of proteins. These proteins are either anchored at the nuclear membrane, as emerin, or form a filament meshwork lining the inner nuclear membrane, as lamins. My thesis objectives were to understand molecular mechanisms deficient in two types of genetic diseases caused by mutations in inner nuclear envelope proteins: Emery-Dreifuss muscular dystrophy, associated to mutations in emerin and A-type lamins, and progeroid syndromes caused by mutations in A-type lamins.First, we showed that the emerin protein self-assembles in vitro and in cells (Herrada, Samson et al., ACS Chem. Biol., 2015). I then studied the structure of emerin oligomers, determined the minimal protein fragment necessary for the formation of these oligomers, identify residues forming the structural core of these oligomers by solid-state NMR in collaboration with the group of Prof A. Lange (FMP Berlin), and described the impact of emerin mutations causing Emery-Dreifuss muscular dystrophy on emerin self-assembly (Samson et al., Biomol. NMR Assign. 2016, Samson et al., FEBS J. 2017). Then, I observed, mainly using solution-state NMR, that only the self-assembled form of emerin is able to interact with A-type lamin tail, and that mutants causing Emery-Dreifuss muscular dystrophy and unable to self-assemble are also defective in A-type lamin binding. I also obtained preliminary data showing that phosphorylation of emerin by the Src kinase, observed after a mechanical stress in purified nuclei, regulates the interaction between self-assembled emerin and A-type lamins.Finally, I showed that the monomeric form of emerin is able to form a ternary complex with A-type lamin tail through the chromatin-associated protein Barrier-to-Autointegration Factor (BAF). After having measured the protein-protein affinities within this complex, identified the minimal protein fragments involved in the complex and developed a robust protocol for purification of this complex, I was able to obtain crystals under several conditions. Subsequently, I solved the 3D structure of this complex by molecular replacement at a resolution of 2 Å. Finally, I showed that mutations in A-type lamins causing autosomal recessive progeroid syndromes impair interaction with BAF in vitro, and our collaborators at Univ. Paris Diderot, the team of Dr B. Buendia, showed that these same mutations induce a significant decrease in the proximity between lamin A and BAF in HeLa cells. An article with me as a first author is in preparation that reports all these new data.