1. Record Nr. TD18047909

Autore	GILISTRO, EUGENIA
Titolo	Regulated interactions of the SUMO ligase RANBP2 with the mitotic spindle and kinetochores during mitotic progression [Tesi di dottorato]
Lingua di pubblicazione	Inglese
Formato	Tesi di dottorato
Livello bibliografico	Monografia
Note	diritti: info:eu-repo/semantics/openAccess In relazione con info:eu-repo/semantics/altIdentifier/hdl/11573/937639
Sommario	RANBP2 is a large nucleoporin (NUP) residing at nuclear pore complexes (NPCs) in interphase and plays a role in nucleo-cytoplasmic transport of macromolecules across the NPC. In mitosis, when nuclear envelope (NE) breaks down and NPCs disassemble, RANBP2 localizes on mitotic structures. RANBP2 has SUMO (small ubiquitin-related modifier) E3 ligase and SUMO stabilizing activities and regulates protein SUMO conjugation, a post-translational modification of particular importance in dynamic processes such as the DNA damage response, stress response, various signaling pathways and mitosis. A characterized SUMOylated RANBP2 target is RANGAP1, the GTP-hydrolysis activating factor for theGTPase RAN. RANBP2 and RANGAP1, together with Ubc9 (a SUMO E2 enzyme), form a complex, called RRSU (RANBP2/RANGAP1-SUMO/UBC9), that has enhanced SUMO ligase activity and localizes to kinetochores (KTs) in metaphase with a mechanism that is not completely understood. The goal of my PhD project was to identify the molecular mechanisms regulating the RRSU complex localization in space and time during mitosis, particularly to KTs, given the importance of these structures as the connecting structures between chromosomes and the mitotic spindle and their crucial role in chromosome

	segregation. Both RANBP2 and RANGAP1 are known to interact with nuclear transport receptors, Importin beta and CRM1, during nuclear transport in interphase. In my project I have developed in situ proximity ligation assays (PLA) to visualize their interactions with these transport factors, follow their dynamics during cell division and assess whether nuclear transport receptors have themselves a functional role in the RSSU complex localization in mitotic cells. PLA results show that the RRSU complex engages in dynamic interactions with Importin beta and CRM1 during mitotic progression: it preferentially interacts with Importin beta in early mitotic stages along the spindle MTs. In metaphase, after MTs attach all KTs, this interaction decreases. Concomitantly, the RRSU complex also interacts with CRM1: this interaction becomes up-regulated in metaphase and becomes visible at MT attached-KTs. Thus, the RRSU complex appears to “switch partners” from prometaphase (prevalent engagement with Importin beta along the spindle) to metaphase (increased PLA signals with CRM1 at KTs), suggesting that protein SUMO conjugation takes place with a spatially and temporally regulated programme in mitosis. To validate the “switch partner” model I generated inducible cell lines, both for Importin beta and CRM1, to assess whether unbalancing one or the other would influence the RRSU complex localization in mitosis. Results from experiments with the inducible cell lines show that the mitotic localization of the RRSU complex depends on the antagonistic actions of Importin beta and CRM1: indeed, unbalancing each one of them impairs the RSSU complex localization and concomitantly generates segregation defects, suggesting that KT functions are defective. Overall, the results of my project highlight the importance of localized SUMOylation of proteins at the mitotic apparatus and KTs for balanced chromosome segregation, and indicate a role of nuclear transport receptors as upstream regulators in the process. It is of note that several cancer types overexpress these transport factors, which may contribute to the high level of genetic instability observed in these cancers.
Localizzazioni e accesso	http://memoria.depositolegale.it/*/http://hdl.handle.net/11573/937639