Wash: Not Just For the Cytoplasm Anymore

April 20, 2015

GE Zentner

Organization of the cytoskeleton is critical for maintenance of cell shape and wound repair, among other processes. Key regulators of the cytoskeleton include Wiskott-Aldrich syndrome family (WAS) proteins, which regulate actin polymerization and are often misregulated in immune disorders and metastasis (Rottner *et al*., 2010). While studying the cytoplasmic functions of the *Drosophila* WAS protein Washout (Wash), research associate Jeffrey Verboon and colleagues in the laboratory of Dr. Susan Parkhurst (Basic Sciences Division) made the surprising finding that a substantial amount of Wash is present in the nucleus. "We found an unexpected interaction [of Wash] with B-type Lamin, which led us to collaborate with the Groudine lab, who have expertise in all things nuclear. Together with co-first-author Dr. Hector Rincon, we characterized Wash's nuclear defects finding global changes to the shape of the nucleus, components which mark nuclear compartments, PEV and chromatin accessibility," said Mr. Verboon.

Following up on their initial observation of significant localization of Wash to the nucleus, the authors found that Wash associates with several hundred sites on *Drosophila*polytene chromosomes, despite lacking a DNA binding domain. To address potential functions of nuclear Wash, the researchers knocked it down using RNA interference (RNAi) in cell culture. Wash depletion led to abnormal nuclear morphology, and this observation was confirmed in salivary glands.

The authors next asked if loss of Wash might affect nuclear organization. Microscopic examination of polytene chromosomes from *wash* mutants revealed a general lack of organization as well as increased susceptibility to breakage. Mutant chromosomes also tended to cluster at the nuclear periphery, in contrast to the well-defined chromosome territories observed in wild-type cells. It was also found that HP1, which marks heterochromatin, only weakly accumulated on chromosomes in *wash* mutants. The organization of the nucleolus and Cajal bodies, both nuclear organelles, was dispersed in *wash* mutants. Lastly, the authors found that histone modifications associated with both activation and repression of transcription were diminished in *wash* mutants. The requirement for nuclear localization of Wash in nuclear organization was confirmed by the observation of aberrant nuclear morphology in cells expressing a version of Wash lacking its consensus nuclear localization signal.

The authors identified a strong interaction between Wash and Lamin, a filament protein that forms a mesh that lines the inner nuclear membrane and has key functions in maintaining nuclear shape and

regulating nuclear organization and gene expression. Using the DamID method to identify Wash binding sites across the genome, the authors found a strong concordance between Wash-associated regions and Lamin-associated domains (LADs), suggesting a functional interaction between the two proteins on chromatin, particularly at regions of heterochromatin, which are often within LADs.

Given that *wash* mutants displayed general chromosomal disorganization and reductions in histone marks, the authors hypothesized that Wash impacts chromatin structure. Indeed, Wash knockdown in cell culture resulted in increased accessibility at the boundaries of heterochromatic regions. The increase in heterochromatin accessibility observed with Wash depletion was confirmed *in vivo* using position-effect variegation (PEV) reporter, which assays the ability of a protein to influence chromatin in different contexts. Consistent with expectations, *wash* mutants enhanced centromeric and heterochromatic PEV.

"What's really interesting, and the next step, is the mechanism by which these nuclear phenotypes are caused. Perhaps, the global defects are the consequence of a single process, for instance if Wash is a key player in the nucleoskeleton or in trafficking things inside of the nucleus, analogous to its roles with the cytoskeleton and endocytic trafficking in the cytoplasm. On the other hand, Wash may have many roles each leading to specific defects which overall read out as a global regulator," said Mr. Verboon. "Our next steps will be to use proteomics in tandem with cell and molecular approaches to tease out the mechanism(s) for the striking phenotypes we see."

Verboon JM, Rincon-Arano H, Werwie TR, Delrow JJ, Scalzo D, Nandakumar V, Groudine M, Parkhurst SM. 2015. Wash interacts with Lamin and affects global nuclear organization. *Curr Biol* 25(6):804-810.

See also: Rottner K, Hänisch J, Campellone KG. 2010. WASH, WHAMM and JMY: regulation of Arp2/3 complex and beyond. *Trends Cell Biol* 20(11):650-651.

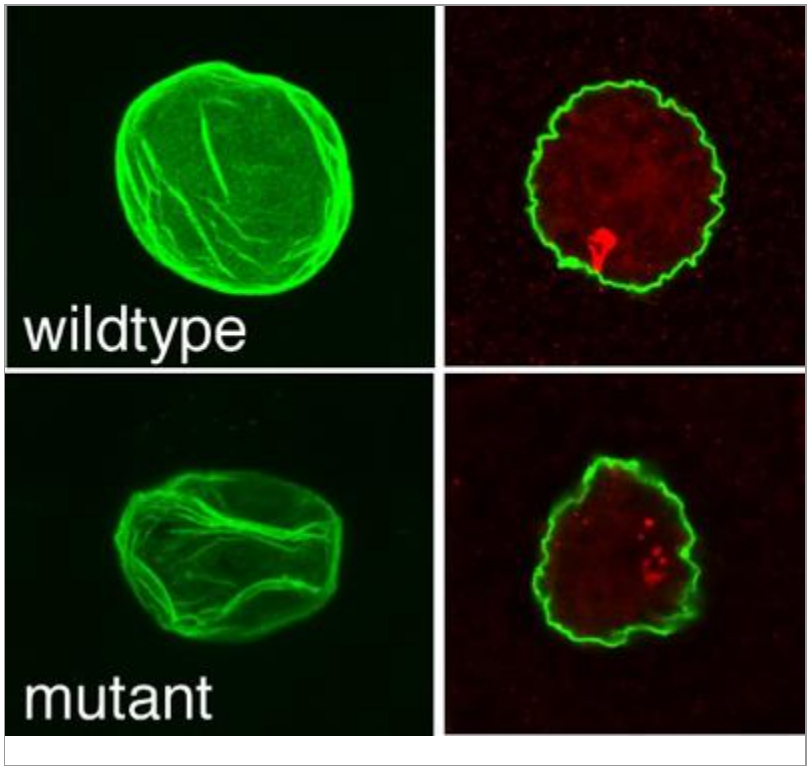

Image from the publication

(Top left) A cell from a wild-type salivary gland stained for Lamin, demonstrating normal nuclear architecture. (Bottom left) A cell from a wash mutant salivary gland stained for Lamin, demonstrating abnormal nuclear organization. (Top right) A cell from a salivary gland expressing wild-type Wash and stained for Lamin (green) and coilin, a marker for Cajal bodies (red), demonstrating normal Cajal body morphology. (Bottom right) A cell from a salivary gland expressing nuclear localization-deficient Wash and stained for Lamin (green) and coilin (red), demonstrating aberrant Cajal body morphology.