Reorganization of the nuclear architecture in the *Drosophila melanogaster* Lamin B mutant lacking the CaaX box

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Introduction

Lamin B is a major component of nuclear lamina. It promotes the proper organization of chromosomes in the interphase nucleus by tethering the transcriptionally-silent chromatin to the nuclear periphery [1,2]. However, the precise mechanisms of interactions among Lamin B, nuclear proteins, nuclear envelope (NE), and chromatin are poorly understood.

Like humans, fruit flies have two types of lamins, B-type and A-type lamins [1,2]. Hydrophobic CaaX-motif of Lamin B promotes attachments of the lamin to the inner nuclear membrane, while the rest of the protein is indirectly connected to the chromatin [3]. Here, we tested whether the lack of the CaaX motif in Lamin B disrupts interaction of the protein with the nuclear periphery and affects chromatin distribution inside the nucleus. Understanding the role of lamins in functional organization of the genome may offer new avenues for the development of new treatments of human diseases and genetic controls of insect pests.

Research questions

1) Is the direct hydrophobic interaction of CaaX box with the nuclear membrane required for the peripheral localization of Lamin B?
2) Does the lack of the CaaX motif in Lamin B lead to spatial repositioning of the peripheral chromatin?
3) Does the lack of the CaaX box in Lamin B affect the localization of modified histones and nuclear proteins?

Methods

We used Lam[A25] mutants (Bloomington #25092) as a model of CaaX-deficient organisms [4]. Lam[A25] homozygous mutants that lack the CaaX box (**Fig. 1**). Canton S flies were used as a wild type (wt) control. The series of immunostaining experiments were performed on whole-mount salivary glands and proventriculus nuclei by using a combination of specific antibodies (Lamin B, Lamin C, HP1, H3K9me2, H3K27me3, JIL-1) with DAPI staining. The distribution of radial density of the DAPI-stained chromatin was compared between Lam[A25] and wt by using high-resolution confocal microscopy and radial profile plot plugin for ImageJ and custom Python script.

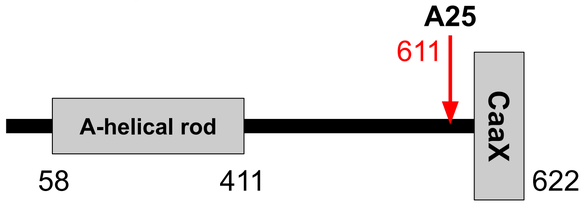

Fig. 1 – The position of the A25 mutation on the Lamin B protein.

References

[1] Briand, N., & Collas, P. (2020). Lamina-associated domains: peripheral matters and internal affairs. Gen. Bio., 21(1), 1-25. **[2]** Pałka, M. et al. (2018). Laminopathies: what can humans learn from fruit flies. Cel. & mol. bio. letters, 23(1), 32. **[3]** Goldberg, M. et al. (1999). The nuclear lamina: molecular organization and interaction with chromatin. Crit. Rev. in Eukar. G. Exp., 9(3-4). **[4]** Patterson K, Molofsky AB, Robinson C, Acosta S, Cater C, Fischer JA. The functions of Klarsicht and nuclear lamin in developmentally regulated nuclear migrations of photoreceptor cells in the Drosophila eye. Mol Biol Cell 2004; 15:600–10. **[5]** Filion, G. J. et al. (2010). Systematic protein location mapping reveals five principal chromatin types in Drosophila cells. Cell, 143(2), 212-224.

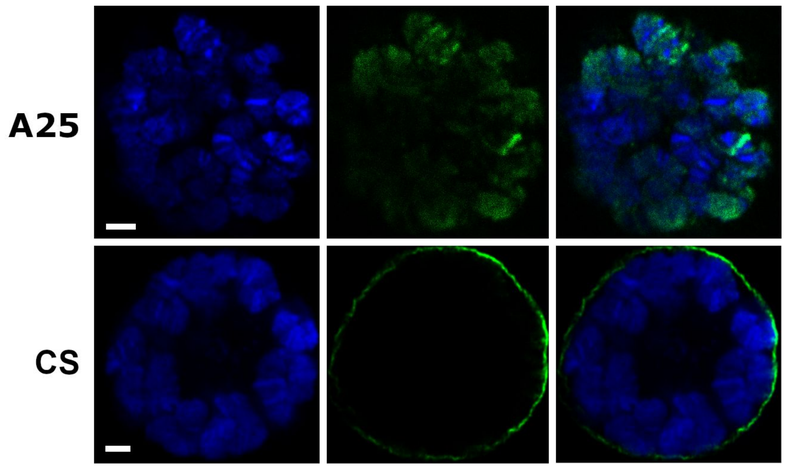

Fig. 2 – The immunostaining of Lam[A25] mutant and Canton S (CS) salivary gland nuclei (DAPI - blue, Lamin B - green). Scale bar = 2 μm.

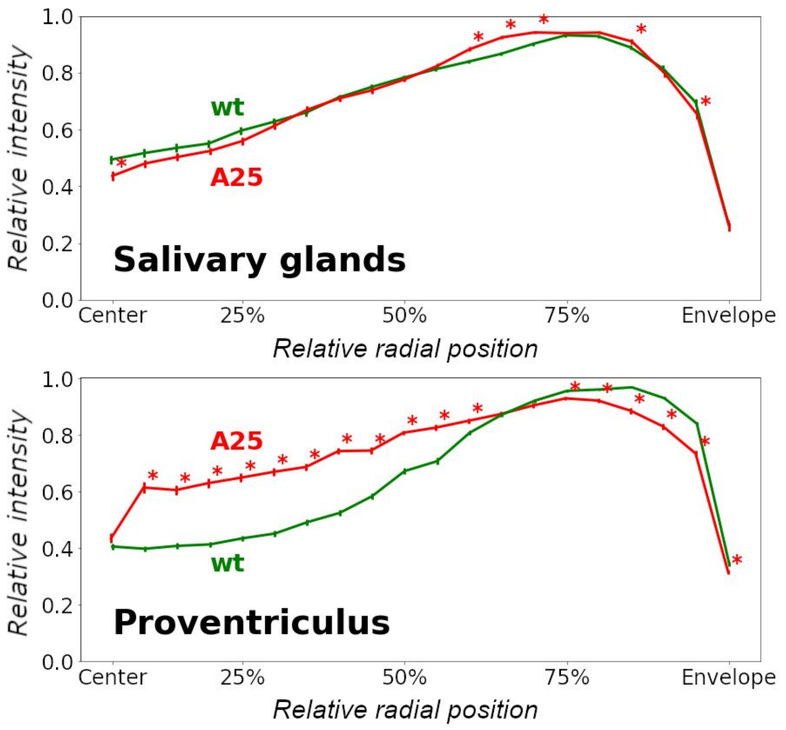

Fig. 3 – The radial density of chromatin in Lam[A25] mutant and Canton S (wt) nuclei.

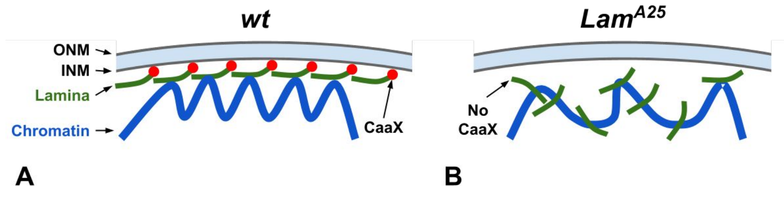

Fig. 4 – Scheme demonstrating the role of the CaaX box of Lamin B in nuclear organization. (**A**) Organization of the nuclear periphery in the wild-type (*wt*) nucleus. (**B**) The effect of CaaX box removal on nuclear architecture in the Lam^{A25} mutant.

Results

We found that the A25 mutant Lamin B is not confined to the nuclear periphery but is distributed throughout the nuclear interior colocalizing with chromosomes in both salivary gland and proventriculus nuclei (**Fig. 2**).

The fluorescence intensity of the peripheral chromatin significantly decreases, but that of the central chromatin significantly increases in the proventriculus nuclei of Lam[A25] flies compared to wt. However, the mutation had little effect on chromatin density distribution inside the salivary gland nuclei (**Fig. 3**). Taken together, our results demonstrate that the removal of the CaaX box by the LamA25 mutation disrupts the interaction of Lamin B with the nuclear membrane but not with the chromatin (**Fig. 4**).

The nuclear distribution of H3K9me2 and H3K27me3 histone modifications, which mark inactive chromatin [5], doesn't show obvious difference between the mutant and wt. The lack of CaaX-box in the A25 Lamin B doesn't affect the location of Lamin C (**Fig. 5**), which is reasonable, since the Lamin C and Lamin B meshworks don't directly physically interact [1]. Also, the chromocentral localization of HP1, which is associated with heterochromatin, is not affected in the mutant nuclei. Future studies of interactions between Lamin B domains and nuclear compartments (chromatin, nuclear membrane, nucleolus) will increase our understanding of the interplay between the spatial genome organization and the orchestration of gene activity in different cell types.

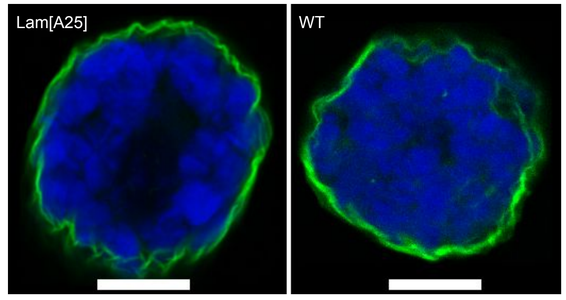

Fig. 5 – The immunostaining of Lam[A25] (left) mutant and Canton S (right) salivary gland nuclei (DAPI-white, Lamin C - green). Scale bar = 10 μm.

Conclusions

1) The direct hydrophobic interaction of Lamin B with the nuclear envelope is required and promoted by the CaaX box located at the end of the Lamin B molecule.
2) The lack of the CaaX box causes detachment of Lamin B from the nuclear periphery that significantly shifts the peripheral chromatin toward the center of the proventriculus nuclei. However, the radial density of chromatin in the higher-polytenized mutant nuclei of salivary glands does not change.
3) The lack of the CaaX box in Lamin B does not affect the localization of H3K9me2 and H3K27me3 histone modifications, HP1, or Lamin C.

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