Identifying biomarkers of old nuclear pore complexes by automated image analysis

Anton Melbye & Evgeny Onishchenko
University of Bergen, Department of Biological Sciences (BIO)

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UNIVERSITY OF BERGEN

1 Background

Nuclear pore complexes (NPCs) are massive protein assemblies that allow transport across the nuclear envelope (Figure 1). NPCs are composed of multiple nuclear pore proteins (NUPs). Some structures of the NPC survive for a long time, and can accumulate damage (Rempel et al., 2020). Nucleoporin-related damage is also known to result in many different diseases (Sakuma and D'Angelo, 2017). The aim of this project is to identify proteins accumulating in old nuclear pores of budding yeast by using recombination-induced fluorescence tag exchange (RITE) and NuRiM, an automated image analysis algorithm (Verzijlbergen et al., 2010)(Rajoo et al., 2018), to identify proteins accumulating in old nuclear pores of budding yeast. We benchmarked our approach using Mlp1 – a known biomarker of old NPCs (Onischenko et al., 2020) and used it to test predicted Mlp1-interating proteins, ESC1, PML39, PRE4, SAC3, STS1, ULP1, as potential biomarkers of old NPCs.

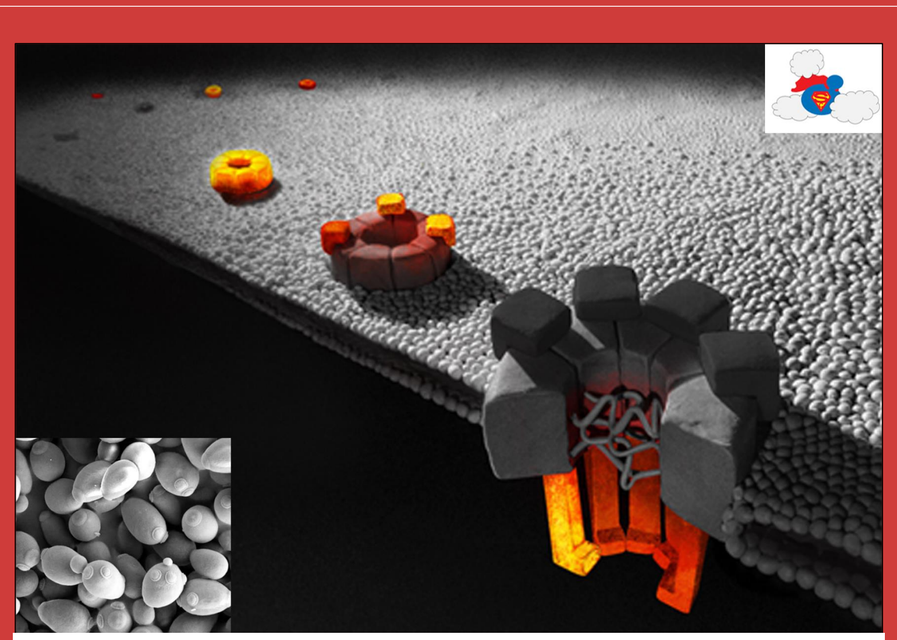

Figure 1: Biogenesis of Nuclear Pore Complexes in budding yeast (illustration).

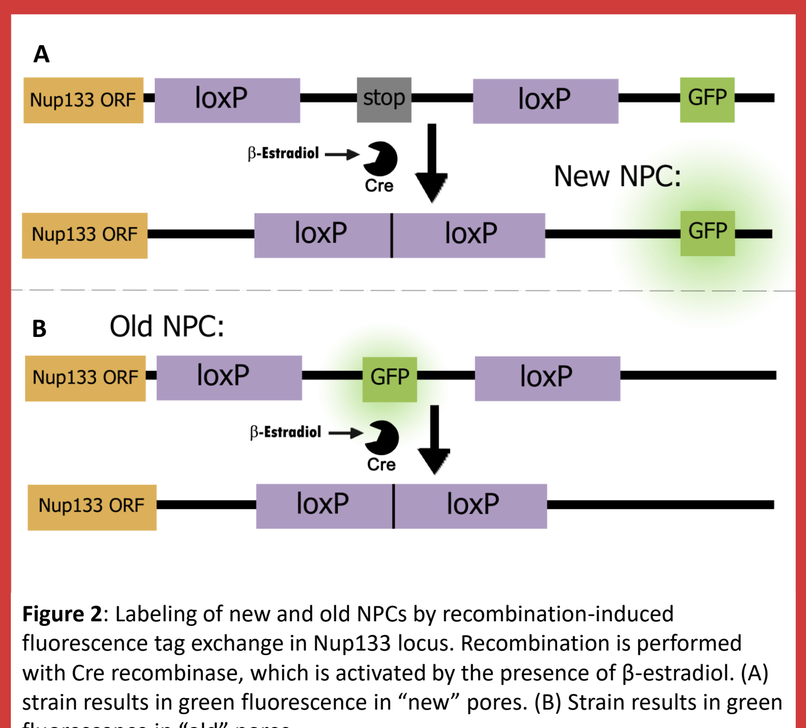

Figure 2: Labeling of new and old NPCs by recombination-induced fluorescence tag exchange in Nup133 locus. Recombination is performed with Cre recombinase, which is activated by the presence of β-estradiol. (A) strain results in green fluorescence in "new" pores. (B) Strain results in green fluorescence in "old" pores.

How it's done 2

The nucleoporin Nup133 is present both in old and new NPCs. We tag Nup133 with green fluorescent protein (GFP), which produces green fluorescence, and the tested proteins are tagged with mCherry, which produces red fluorescence. Nup133 can be tagged such that a stop-codon is flanked with two loxP-sites. A GFP cassette follows the flanked site, resulting in fluorescence in new pores when stop-codon is removed after recombination (Figure 2A). Alternatively, Nup133 can be tagged with a GFP cassette flanked with two loxP sites, which is removed and marks old NPCs. Old pores will continue to have fluorescence after recombination, but new pores will not (Figure 2B). By examining co-localization of GFP-tagged proteins and mCherry-tagged proteins with fluorescence microscopy (Figure 3), we get an idea if a protein prefers old pores. For the unbiased analysis, the microscopy images are then processed by NuRiM, an algorithm which detects nuclear envelope contours and takes light intensity from GFP and mCherry channels (Rajoo et al., 2018)(Figure 3). This data is used to analyze correlation between mCherry and GFP signal as the measure of test protein enrichment in the new and old NPCs.

3 Results

We used mCherry labeled Mlp1 – a known biomarker of old NPC – to benchmark our method. Figure 4 and 5A show analysis of correlation values, and GFP light intensity in the populations of old and new NPCs analyzed by NuRiM. Unlike Nup82, found in all NPCs (Figure 5B), Mlp1 shows strongly reduced localization with the new NPCs, which validates our strategy to identify the old NPC biomarkers. We will next apply this strategy to test the predicted Mlp1-binding proteins for localization with the old nuclear pore complexes (Figure 6). GFP light intensity range 400-700 is used for analysis. Except for Ulp1, where range 450-600 had to be used due to limited intensity values.

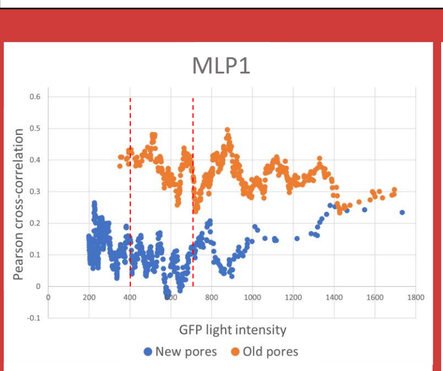

Figure 4: Pearson cross-correlation of new and old NPC signal (Nup133 and Mlp1). X-axis shows GFP intensity and Y-axis shows Pearson cross-correlation.

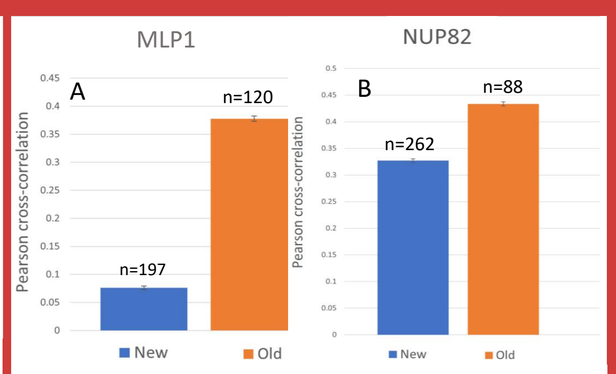

Figure 5: Average Pearson cross-correlation of **A**) Mlp1 and **B**) NUP82 in GFP light intensity range 400-700, with GFP-labeled old pores. n – number of analyzed contours.

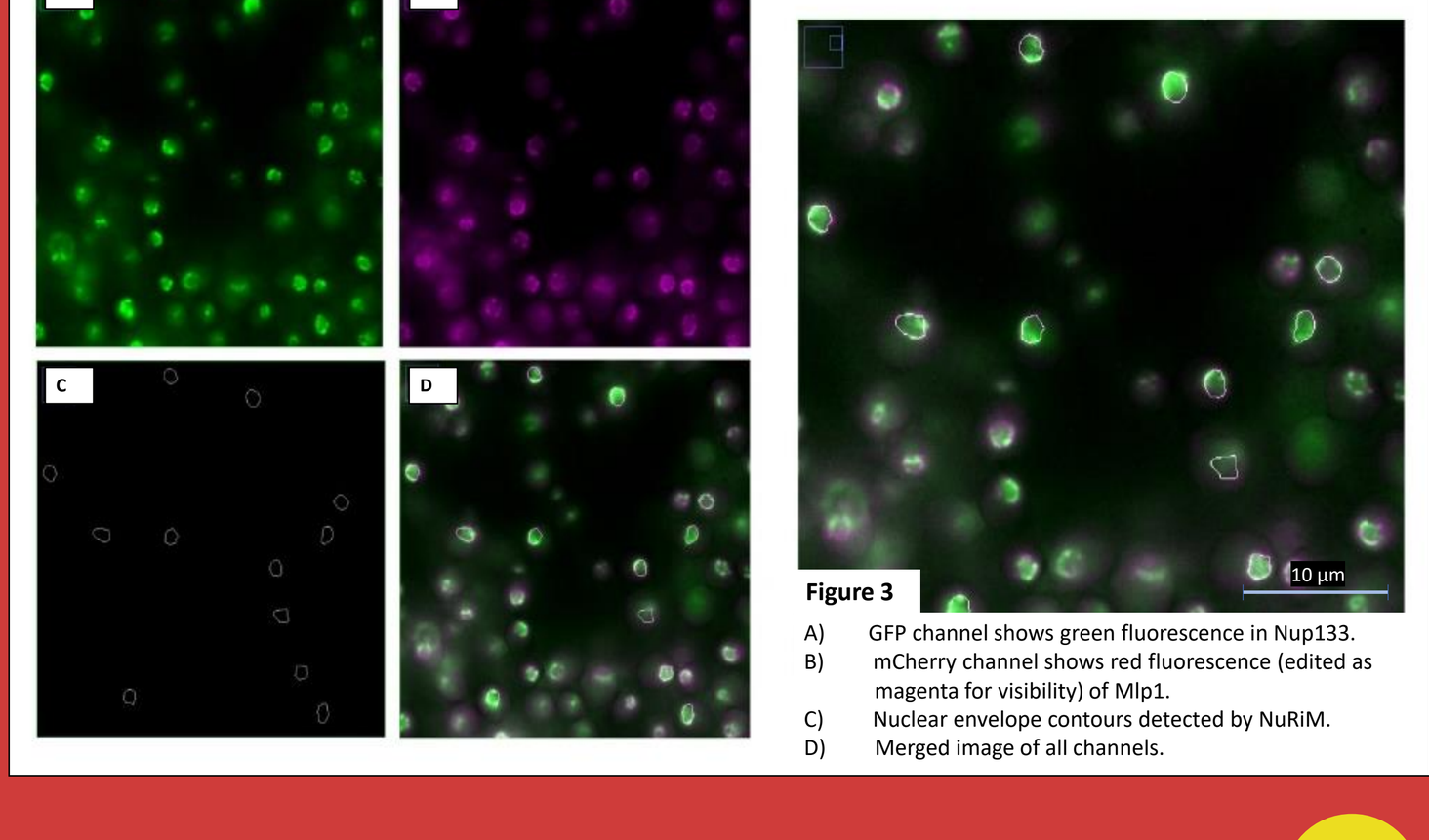

Figure 3

A) GFP channel shows green fluorescence in Nup133.
B) mCherry channel shows red fluorescence (edited as magenta for visibility) of Mlp1.
C) Nuclear envelope contours detected by NuRiM.
D) Merged image of all channels.

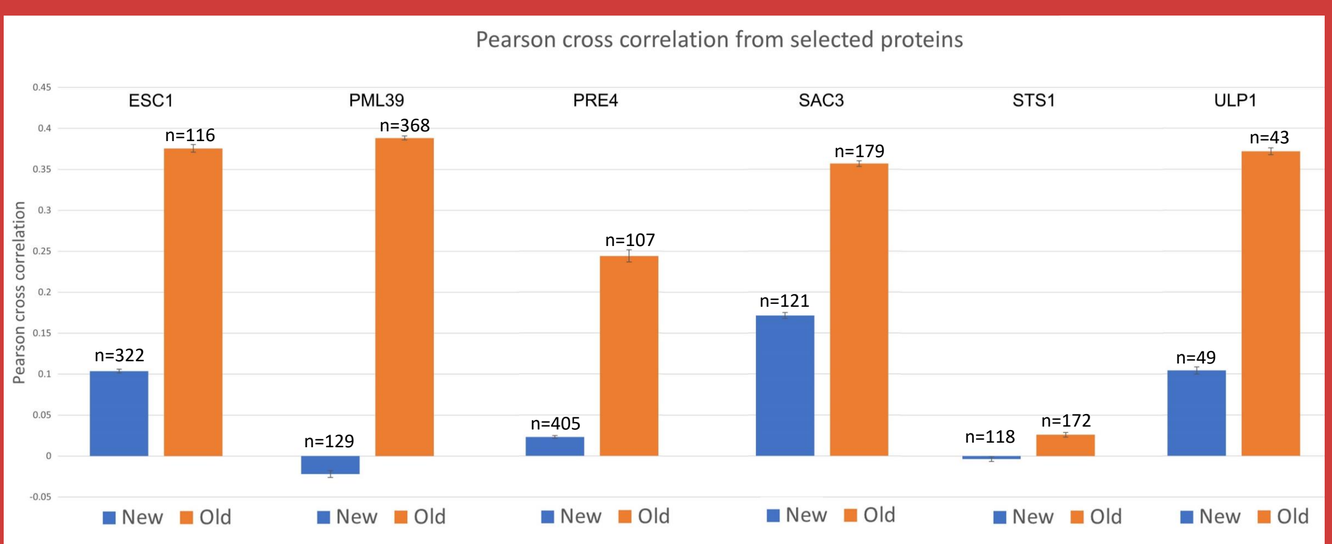

Figure 6: Pearson cross-correlation values for selected proteins. X axis shows the correlation value between mCherry intensity of the test proteins, and GFP signal from old and new pores in GFP light intensity range 400-700 in all except Ulp1, which is in the range 450-600.

Conclusion 4

Our NuRiM-based analysis shows that Esc1, Pml39, Pre4 and Ulp1 have strong localization preference for the old NPCs and therefore are promising biomarkers of old NPC structures. At the same time, Sac3 does not have a clear difference of cross-correlation between old and new pores. Sts1 has low correlation values, which make our analysis for this protein inconclusive. Altogether, our analysis suggests that old NPCs may accumulate factors associated with chromatin organization (Esc1), mRNA quality control (Pml39), protein degradation (Pre4) and cell cycle progression (Ulp1).

Sources:

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