The study of spatial relationship between Restrictor complex and RNA-Pol II through Expansion Microscopy

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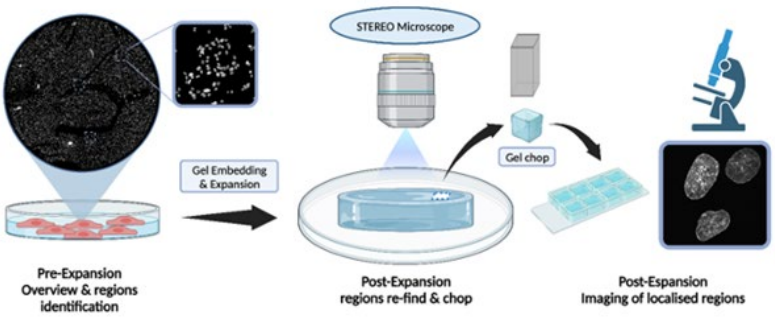
Background incl. aims
In recent years, there have been continuous research efforts to optimize the experimental Expansion Microscopy (ExM) protocol for studying spatial correlation among different proteins. Our focus has been on developing an effective method to relocate the same cells acquired in pre-expansion onto expanded gels, enabling precise calculation of expansion and deformation factors. We then applied ExM and colocalization analysis to uncover the spatial relationship between the Restrictor complex and RNA-Pol II. The Restrictor complex, comprising ZC3H4, a putative RNA-binding protein, and WDR82, an adapter protein that interacts with RNA-Pol II Ser5-p, terminates extragenic transcription arising from active enhancers and promoters, thereby mitigating the pervasive capacity of transcription of the genome, which may lead to genomic instability (1). However, the exact molecular mechanism of action of the Restrictor complex remains poorly understood.

Methods
HCT-116 WT and HA-WDR82 HCT-116 cells were seeded on 35 mm MatTek dishes before being processed for indirect immunofluorescence. ZC3H4, WDR82, and RNA-Pol II Ser5-p or RNA-Pol II Ser2-p were fluorescently labeled. A scratch with no symmetry for rotation and reflection on adhered cells, together with a pre-expansion overview of the sample, were used to identify pre-expansion imaging areas. Samples were then processed for ExM according to (2). After expansion, the same areas were identified under a stereo microscope based on their relative position with respect to the scratch (Fig.1). Both pre- and post-expansion images were acquired using a Yokogawa spinning disk confocal system (CSU-W1, Nikon Europe B.V.). Colocalization was assessed using Pearson's coefficient calculation and object-based colocalization analysis.

Results
Expansion factor, deformation, fluorescence intensity decrease upon expansion, and both 2D and 3D colocalization were evaluated. Across three experiments, an expansion factor up to 6 and an average deformation factor of 9% were obtained. Overall, the colocalization results obtained with ExM revealed a stronger spatial correlation between the Restrictor complex and RNA-Pol II Ser5-p compared to RNA-Pol II Ser2-p, consistent with molecular, genomic, STocastical Optical Reconstruction microscopy (3) and Structured Illumination Microscopy observations previously obtained in our laboratory.

Conclusion
Our ExM imaging workflow efficiently facilitated the acquisition of identical areas both before and after expansion, thus providing essential control over the expansion protocol. Considering that the expansion factor directly influences the ultimate resolution of any ExM experiment, it is crucial to consistently account for the precise expansion factor of each sample to ensure fair comparisons among different samples. The colocalization analysis of ExM images further supported the concept that Restrictor-mediated transcription termination primarily affects initiating and early-elongating RNA-Pol II complexes.

Graphic:



Keywords:

ExM, colocalization, extragenic transcription

Reference:

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