

Alexei Budco

Epigenetic regulation of DNA replication studied by super resolution microscopy

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Ludwig-Maximilians-Universität München

- Department Biologie II -

Epigenetic regulation of DNA replication studied by super resolution microscopy

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vorgelegt von

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Abstract

DNA replication is a fundamental biological process responsible for accurate duplication of genetic information necessary for its faithful inheritance to the two daughter cells. Despite much effort, the underlying mechanisms controlling this process are not fully understood. In order to accommodate very large and complex genomes, replication dynamics in eukaryotes evolved to become controlled by major epigenetic mechanisms. Moreover, the spatio-temporal organization of S-phase progression changes throughout cell differentiation and development. The study of genome duplication has been largely hindered by the lack of appropriate monitoring techniques, and any comprehensive understanding ultimately requires quantitative approach.

In this master's thesis, we analyzed replication patterns in mouse somatic and embryonic stem cells (mESCs) with newly developed three-dimensional structured illumination microscopy (3D-SIM) to register the progression of S-phase in more detail than previously described. We successfully established an automated workflow to produce reliable and reproducible replication foci (RF) counts in C2C12 cells from 3DSIM data and TANGO (Tools for Analysis of Nuclear Genome Organization). Such an approach has not been described before, and could be used to evaluate further cell types and schemes.

Additionally, we observed significant differences in replication timing and progression between somatic (C2C12, C127) and mESCs (HI5). In this report we show that in mESCs S-phase lasts significantly longer (15 h), with a 'leaky' chromocenter replication profile compared to somatic cells. Furthermore, differentiated HI5 female mESCs into epiblast-like cells (EpiLCs) exhibit inactive X chromosome and differential replication timing of Xi within two distinct EpiLC populations, and a much shorter S-phase (10 h).

As a final aim of this work, we interfered with specific histone modifications with inhibitors and knockout cell lines. Inhibition of EZH2 methyltransferase resulted in global reduction of H3K27me3 levels in both somatic and mESCs, however replication dynamics were not affected. In contrast to somatic cells, viability of mESCs in presence of inhibitor was greatly reduced, suggesting a more important role of H3K27me3 in mESCs. Suv39H1/H2 double knockout mESCs had no observable effect on replication dynamics or proliferation. Moreover, differentiation of these cells into EpiLCs resulted in a distinct S-phase progression, with replication resembling HI5 EpiLCs.

Zusammenfassung

Replikation der DNA ist ein grundlegender biologischer Prozess, der für die genaue Duplikation genetischer Informationen verantwortlich ist und somit die zuverlässige Vererbung der DNA an die Tochterzellen gewährleistet. Trotz großer Mühe, wurden die zugrundeliegenden Mechanismen, die diesen Prozess steuern, noch nicht vollständig aufgeklärt. Um sehr große und komplexe Genome erfassen zu können, haben sich Replikationsdynamiken dahingehend evolviert, dass sie durch epigenetische Mechanismen reguliert werden. Dazu kommt, dass die räumliche und zeitliche Koordination des Fortschreitens der S-phase sich während der Entwicklung und Zelldifferenzierung verändert. Die Analyse der Genomreplikation war hauptsächlich dadurch gehindert, dass angemessene Beobachtungstechniken fehlten, und jegliches umfassende Verständnis braucht zuletzt quantitative Verfahren.

In dieser Masterarbeit wurden Replikationsmuster während des Fortschreitens der S-phase mittels der neu entwickelten, dreidimensional strukturierten Beleuchtungsmikroskopie (3D-SIM) in murinen somatischen und embryonalen Stammzellen (ESZ) detaillierter erfasst als zuvor. Wir haben einen automatischen Arbeitsprozess erstellt, der verlässliche und reproduzierbare Anzahlen an Replikationsfoci in C2C12 Zellen mittels Daten von 3DSIM und TANGO (Tools for Analysis of Nuclear Genome Organization) errechnet. Diese Herangehensweise wurde noch nicht beschrieben und kann für die Auswertung weiterer Zelltypen und Vorhaben verwendet werden.

Zusätzlich haben wir signifikante Unterschiede zwischen somatischen (C2C12, C127) und ESZ (HI5) bezüglich Replikationszeitpunkte und dem Fortschreiten der Replikation gefunden. In dieser Arbeit können wir zeigen, dass im Vergleich zu somatischen Zellen, die S-phase in mESZ signifikant länger ist (15h) und "durchlässige" Chromozentren Replikation zeigt. Des Weiteren können wir zeigen, dass zu EpiLC differenzierte weibliche HI5 mESZ ein inaktives X Chromosom besitzen, dass der Replikationszeitpunkt des Xi in zwei Zellpopulationen geteilt werden kann, und über eine stark verkürzte S-phase Dauer verfügt (10h).

Zuletzt haben wir auf spezifische Histonmodifikationen mittels Inhibitoren und Knockout-Zelllinien eingegriffen. Inhibierung der EZH2 Methyltransferase resultierte in globaler Verringerung der H3K27me3 Menge in somatischen und mESZ, jedoch waren Replikationsdynamiken nicht beeinflusst. Im Gegensatz zu somatischen Zellen, war das Überleben von mESZ bei Zugabe von Inhibitoren stark verringert. Suv39H1/2 dko mESZ hatten keinen erkennbaren Effekt auf Replikationsdynamiken. Darüber hinaus, resultierte die Differenzierung von Suv39H1/2 dko mESZ zu EpiLCs in einem distinkten Fortschreiten der S-phase, mit Änderungen, die denen der HI5 EpiLCs ähnelten.