Biological Functions of G-Quadruplexes

Telomere Maintenance and Transcriptional Regulation in Embryonic Stem Cells

Akademisk avhandling

som för avläggande av medicine doktorsexamen vid Sahlgrenska akademin, Göteborgs universitet, kommer att offentligen försvaras i hörsal Arvid Carlsson, Academicum, Medicinaregatan 3, Göteborg, tisdagen den 16 december 2014, kl. 9:00

av

Rikard Runnberg

Fakultetsopponent:
Docent Marita Cohn
Biologiska institutionen, Lunds universitet, Lund

Avhandlingen baseras på följande delarbeten:

I. **Runnberg, R**., Vizlin-Hodzic, D., Green, L.C., Funa, K., Simonsson, T. hnRNP U/SAF-A is a G-quadruplex binding protein that associates with telomeres in a cell cycle dependent manner.
Submitted manuscript, under revision.

II. Vizlin-Hodzic, D., **Runnberg, R.**, Ryme, J., Simonsson, S., Simonsson, T. SAF-A forms a complex with BRG1 and both components are required for RNA polymerase II mediated transcription.
PLoS ONE 6(12): e28049. doi:10.1371/journal.pone.0028049.

III. Johansson, H., Svensson, F., **Runnberg, R**., Simonsson, T., Simonsson, S. Phosphorylated nucleolin interacts with translationally controlled tumor protein during mitosis and with Oct4 during interphase in ES cells.
PLoS ONE 5(10): e13678. doi:10.1371/journal.pone.0013678.

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Telomere Maintenance and Transcriptional Regulation in Embryonic Stem Cells

Rikard Runnberg

Department of Medical Biochemistry and Cell Biology, Institute of Biomedicine
Sahlgrenska Academy at the University of Gothenburg, Gothenburg, Sweden

ABSTRACT

G-quadruplexes are four-stranded nucleic acid structures formed by quartets of Hoogsteen base paired guanine bases. They are known to form at telomeres and other genomic sites, and are predicted to do so at a large proportion of gene promoters. They may function in telomere maintenance and transcriptional regulation, but their biological functions remain largely unknown. The aim of this thesis was to study the association of proteins with telomeres and telomeric G-quadruplexes, and to study protein-protein interactions in embryonic stem cells (ESCs).

This thesis contains three papers. In the first paper the association of heterogenous ribonucleoprotein U/Scaffold Attachment Factor A (hnRNP U/SAF-A) with telomeres was identified by *in situ* Proximity Ligation Assay (PLA) and chromatin immunoprecipitation (ChIP). It was found that hnRNP U associates with telomeres in a cell cycle dependent manner. DNA pull-down and exonuclease protection assay showed that hnRNP U via its C-terminus binds and promotes the formation of telomeric DNA G-quadruplexes, and that in doing so it can prevent RPA in ESC extracts from binding telomeric single-stranded DNA. Immunofluorescence (IF) following shRNA mediated knock-down of *Hnrnpu*, showed that hnRNP U also has a role in preventing RPA association with telomeres in cells. In the second paper IF, PLA and co-immunoprecipitation (co-IP) were used to identify hnRNP U and BRG1 as interaction partners in ESCs. Using an ethynyl uridine incorporation assay it was shown that both components are important for global transcription by RNA polymerase II. In the third paper protein affinity purification, IF, PLA and co-IP were used to identify interactions between nucleolin (Ncl) and two proteins in ESCs. Phosphorylated Ncl interacts with Tpt1 during mitosis and with Oct4 during interphase.

In this thesis hnRNP U is identified as a novel telomere binding protein. The results presented here suggest G-quadruplex formation may be an important aspect of telomere maintenance. Three novel protein-protein interactions were identified in ESCs. The identified protein complexes may have roles in key aspects of ESC biology, such as transcription and cell cycle regulation.

Keywords: G-quadruplex, telomere, embryonic stem cells, transcription, hnRNP U, BRG1, Ncl, Tpt1, Oct4

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