Investigating the epigenetic mechanisms of trophoblast giant cells

Trophoblast giant cells (TGCs) are found in the placental walls of rodents and play a role in maintaining pregnancy. In contrast to most cell types which contain two copies of each chromosome (diploid), TGCs can contain more than 1000 copies of their genomic deoxyribonucleic acid (DNA), a condition known as polyploidy. In a recent paper published in the journal Scientific Reports, Assistant Professor Koji Hayakawa of the University of Tokyo Graduate School of Agricultural and Life Sciences unravelled the molecular mechanisms that allow so much DNA to be packaged into TGCs, a function accomplished by structures known as nucleosomes.

Nucleosome is a large molecule in the cell which is primarily made up of DNA and proteins. The major protein in nucleosome is called a histone, around which DNA wraps. These proteins are classified into canonical histones and non-canonical histones, which are also known as histone variants, based on the similarity of amino acid sequences and their cell cycle-dependent or independent expression. Nucleosome has a number of functions, including organising and condensing DNA, controlling its replication, and protecting DNA from damage. The array of nucleosome is called a chromatin. Chromatin is assembled and disassembled by usage of histone types and their post-translational modifications as the cell faces various metabolic demands and challenges.

During DNA replication and transcription (the beginning of protein synthesis), the DNA becomes more loosely wrapped around the histone, which allows crucial enzymes easier access to its structure. In general, histones tend to bind more strongly to inactive genes and more loosely to active ones.

Trophoblast giant cells, found in the placenta of rodents, are a unique cell type that replicate their DNA until the cell contains thousands of copies, unlike most cells which normally contain two sets of chromosomes (diploid cells). The reasons for this condition are not clear. However, it has been suggested that polyploidy (cells with multiple copies of genes) may increase a cell's capacity for protein synthesis, allowing for faster tissue growth than a diploid cell. Many polyploid cells identified in plants and animals appear to have a secretory or nutritive function.

Due to the enormous amount of genomic DNA packaged into its nucleus (the command centre of the cell), it is thought that the chromatin of TGCs may behave in a different manner than that of diploid cells. In a series of experiments recently published in the journal *Scientific Reports*, Dr Hayakawa and his collaborators set out to determine the dynamics of chromatin structure in TGCs and deduce the function and location of their histones during the differentiation of trophoblast stem cells (TSCs) (immature trophoblast cells which are still diploid) into TGCs.

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HISTONE PROFILE AND CHROMATIN STRUCTURE IN TGC

The researchers first stimulated trophoblast stem cells to cause them to mature (differentiate) and collected them every other day for 10 days. It was found that the cells were differentiated after six days, and that certain histone variants were associated with differentiated cells. Overall, there was much less variation in TGCs compared to the undifferentiated, diploid cells. They found the histone profile to be very similar in differentiated TSCs at day six of differentiation compared with *in vivo* TGCs isolated from a placenta. The scientists also compared the expression of histone variants in embryonic cells (diploid) with placental TGCs and found certain variants were much higher in placental TGCs than embryonic cells.

Chromatin is found in the nucleus, the organ in the cell that acts as a command centre. Dr Hayakawa's group stained the cells and were able to visualise chromatin dynamics by attaching one of the core histones, H4, to a green fluorescent protein (GFP) molecule and monitoring movement under a microscope during the differentiation process. They found that histones occupied different parts of the nucleus during differentiation. To test the mobility of histones, one half of the nucleus of cells that contained H4 attached to a green fluorescence molecule was bleached. Fluorescence intensity was then measured in the presence of cycloheximide, a drug that prevents protein synthesis. It was found that the histones in the TGCs did not move around as much as in the TSCs.

VARIANT HISTONES MAINTAIN 'LOOSE' NUCLEOSOMES IN MATURE TGC

In another experiment, the scientists digested genomic DNA at day six of differentiation using a special protein known as a DNase enzyme and showed that bands became more blurred. DNA from undifferentiated cells showed distinct bands when digested, demonstrating that DNA becomes more loosely bound to histones the more the cell differentiates. To confirm this, the scientists exposed TSCs expressing H4-GFPs to increasing concentrations of salt buffer to disrupt DNA-protein bonds. They then viewed them by single-cell imaging using a machine called a microfluidic device which manipulated individual cells using optical tweezers. As the TSCs

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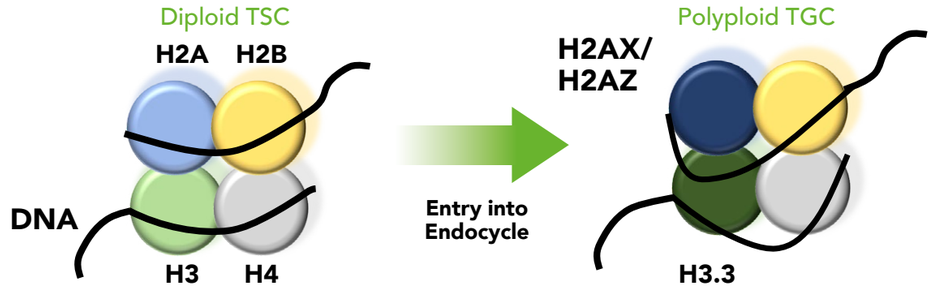
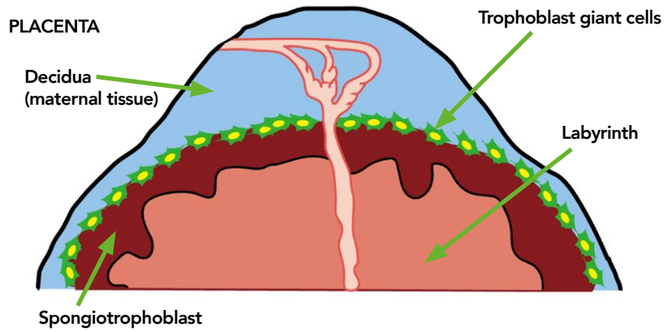
NUCLEOSOME STRUCTURE OF TROPHOBLAST GIANT CELL (TGC)

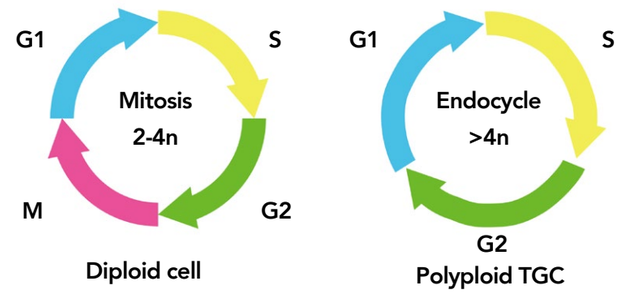


TGCs possess a loose chromatin structure owing to alterations in the histone composition of the nucleosomes, which involves the replacement of canonical histones with histone variants such as H2AX, H2AZ, and H3.3 during differentiation.

LOCATION OF TGC IN PLACENTA

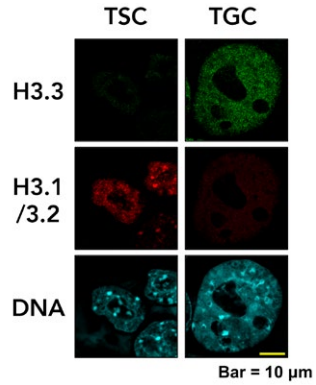


CELL CYCLE OF TGC



Rodent placenta consists of three layers, i.e. labyrinth-, spongiotrophoblast-, and TGC-layer. TGC can be polyploidized by non-typical cell cycles such as endoreduplication (endocycle), which is a system to replicate DNA without M-phase. Polyploidization results in an increase of the amount of DNA content accompanied by the enlargement of nuclei and the expansion of cell size.

INTERCELLULAR LOCALISATION OF HISTONE VARIANT H3.3 IN NUCLEUS OF TGC



Histone variant H3.3 was predominantly expressed in TGC, suggesting that this variant constitutes the unique chromatin structures in their nucleus by replacing canonical H3.1/3.2.

differentiated into TGCs, a decrease in binding DNA with histones was observed in the nucleus.

Based on Dr Hayakawa's research, it was found that histone variants H2AX, H2AZ, and H3.3 were potentially responsible for the formation of the loose nucleosome structure that was unique to TGCs. When differentiated TSCs were prevented from making H3.3, DNA remained tightly wound around histones but when H3.3 protein levels were raised again in the cells, the nucleosomes became more 'loose.' In contrast, the research showed the converse effect with canonical histone H3.1.

FUTURE DIRECTIONS

Dr Hayakawa believes that H3.3 may be a key player in maintaining the polyploid state of the cell. In future, his team hope to analyse H3.3 distribution in the genome of TGCs. In addition, the researchers hope to analyse the distribution of H2AZ and H2AX within the genome of TGCs and determine which histone variants make up the chromatin regions observed in the nucleus of TGCs.

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Research Objectives

Assistant Professor Koji Hayakawa of the University of Tokyo investigates cell-type specific epigenetic regulation.

Detail

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Bio
Koji Hayakawa earned a BS in animal reproduction from Hirosaki University and a MS from Hokkaido University. Eventually, he moved to the University of Tokyo and received his PhD. He is currently an Assistant Professor in the Department of Animal Resource Sciences at the University of Tokyo.

Funding
Lotte Shigemitsu Prize

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- Kanae Terada
- Prof. Satoshi Tanaka

Collaborators for nucleosome stability analysis using microfluidic device
- Tomohiro Takahashi (Department of Mechanical Engineering, The University of Tokyo)
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Personal Response

What do you find most satisfying about your work?

"From our study using TGCs, the nucleus of polyploid cells contains quite different chromatin structures, compared to those of well-studied diploid cells. These structures involve the replacement of canonical histones with histone variants such as H2AX, H2AZ, and H3.3. Therefore, other polyploid cells such as megakaryocytes and cancer giant cells may also have a unique chromatin structure to allow an enormous amount of genomic DNA to fit in a nucleus and to express their specific functions including high cellular invasive activity."