【13】

氏名	Gambe Arni Eguna（ガンベ アーニ エグナ）
博士の専攻分野の名称	博士（工学）
学位記番号	第 23355 号
学位授与年月日	平成21年9月25日
学位授与の要件	学位規則第4条第1項該当 工学研究科生命先端工学専攻
学位論文名	Functional analysis of human chromosomal proteins using digital image cytometry （細胞画像解析法によるヒト染色体タンパク質の機能解析）
論文審査委員	（主査） 教授 福井 希一 （副査） 教授 福崎英一郎　教授 野地 博行　教授 金谷 茂則 教授 原島 俊　教授 大竹 久夫　教授 渡邉 肇 教授 紀ノ岡正博　教授 仁平 卓也　教授 藤山 和仁 教授 清水 浩　教授 四方 哲也

論文内容の要旨

The rapid rate of discovery in cellular biology necessitates the need for more tools that not only render the research process more convenient but also provide means to improve the reproducibility of experiments. The purpose of this study is therefore to develop image analysis-based methods to facilitate the functional analysis of human proteins particularly those that may be or are involved in the maintenance of the metaphase chromosome structure.

To gain insights on the techniques and methodologies involved in determining the cellular roles of proteins, the functional analysis of the Regulator of Ribosome Synthesis 1 (RRS1) using biochemical and image analysis approaches was performed. Localization studies using a GFP-RRS1 over-expression cell line indicated that RRS1 is a nucleolar protein that relocates to the chromosome periphery during mitosis. Knockdown studies of RRS1 using RNA interference (RNAi) resulted in mitotic aberrations such as misaligned and non-aligned chromosomes. Furthermore, mitotic cells with prematurely-separated sister chromatids were observed after RRS1 knockdown. Interestingly, Aurora-B was mislocalized along the chromosome arms and the localization of Sgo1 in the centromeres was perturbed in RRS1-depleted cells. The results suggest that RRS1 is a nucleolar protein with an essential role in chromosome congression.

Based on insights obtained from the analysis of RRS1, the development of a statistical classifier using time-lapsed images of HeLa cells transfected with an EGFP-Histone-H1 fusion protein was undertaken. Features based on the shape and texture of the chromosomal regions in images of live HeLa cells were measured and analyzed. Linear discriminant functions were calculated for the eight cell-cycle phases namely interphase, prophase, prometaphase, metaphase, early anaphase, anaphase, telophase and cytokinesis. Several sets of linear discriminant

functions were formulated and combined to compose the multistage linear discriminant classifier with an average classification efficiency of 87.30%.

Since the aforementioned classification scheme cannot be used to discriminate between abnormal from the normal cell phenotypes, the development of classifiers that can be used for the purpose was performed. Cell images were collected and divided into the congressed and un-congressed chromosome groups, which were further subdivided into the normal or abnormal sub-groups. Four methods were employed for classifier development, namely, linear and quadratic discriminant analyses (LDA and QDA, respectively), MLP and LS-SVM. The LS-SVM classifiers had the best performance for both the congressed and un-congressed chromosome groups.

The classifiers proposed herein may be used in a high-throughput platform that automatically collects images and performs phenotype scoring and analysis. Although these classifiers were developed using adherent HeLa cells, the methods used in their development can be utilized for other cell lines.

論文審査の結果の要旨

本論文はヒト培養細胞の画像解析法を用いて、染色体タンパク質の機能解析を実施した論文である。染色体タンパク質 RRS1 が染色体構造形成に重要な役割を果たすことを見いだしている。さらに、蛍光画像を自動的に分類することで体細胞分裂期の各フェーズを分類することと染色体異常を検出することに成功している。

以上のように、本論文は自動画像解析によるバイオテクノロジーの発展に寄与する研究成果を納めたものである。よって本論文は博士論文として価値あるものと認める。