平成 28 年 2 月 8 日

国立研究開発法人理化学研究所
理事　松本洋一郎

平成 27 年度実施 准主任研究員の中間レビューの結果について

准主任研究員制度設置規程(平成 25 年 3 月 28 日規程第 14 号)第 5 条に基づき准主任研究員の中間レビューを実施し、レビューアーから事務局に送られた評価結果を取りまとめ下記のとおり報告いたします。なお、評価委員の総意のもと、意見を取りまとめた報告書として提出いただいたこと、申し添えます。

１．評価対象：中川 RNA 生物学研究室　　中川 真一　准主任研究員

１）評価体制

実施日：平成 28 年 1 月 19 日（火曜日）
4 名の所外有識者を評価委員とするヒアリングレビューを実施。
評価者：
Masatoshi HAGIWARA, Professor
Graduate School of Medicine
Kyoto University Japan

Toshifumi INADA, Professor
Graduate School of PharmaceuticalSciences
Tohoku University, Japan

John MATTICK, Executive Director
Garvan Institute of Medical Research, Australia

Haruhiko SIOMI, Professor
School of Medicine Keio University, Japan

２）評価結果の概要等

General comments:

<Research objectives>

Dr Nakagawa's primary research objective is to understand the role of the abundant nuclear long noncoding RNAs (lncRNAs) in vertebrate and mammalian developmental biology and physiology.

This is a subject that is new, and an important subfield of what is perhaps the hottest area of contemporary research in molecular biology. Studies over the past decade, many led by RIKEN's Division of Genomic Technologies in Yokohama, have shown that, while less than 1.5% encodes proteins, the vast majority of the mammalian genome is transcribed, producing a surprisingly large number and diverse repertoire of non-protein-coding RNAs, including long non-coding RNAs (lncRNAs) and small

regulatory RNAs. These observations have led to a new concept: that the origin of the developmental complexity of higher organisms is due to the dramatic expansion of the non-protein-coding RNA inventory, including the absolute number of lncRNAs and small RNAs, rather than an increase in the protein-encoding inventory (Mattick 1994).

Many lncRNAs are species and cell-specific, indicating that they play important roles in adaptive radiation. A number of studies have shown that several nuclear lncRNAs direct specific chromatin modifications on particular chromosomes or particular regions of chromosomes, thereby regulating the activity of that chromosome or that region of the chromosome. This suggests that lncRNAs act as specificity determinants for chromatin modification enzyme complexes. Some lncRNAs also serve as architectural components of distinct nuclear bodies that are specific to higher vertebrates, which may be involved in organized compartmentalization of the nucleus to regulate the expression of specific genes and/or chromatin regions. In both respects, lncRNA-protein complexes are genetic elements that mediate and maintain epigenetic chromatin modifications of target loci and/or formation of specific nuclear structures. Therefore, lncRNAs add a great level of complexity to the way cells regulate the expression of genes.

However, the mechanisms underlying specific targeting of these nuclear lncRNAs to chromatin domains and the guiding of specific chromatin modifications by lncRNAs remain to be elucidated. It is also not yet clear how lncRNAs are involved in the formation of nuclear bodies and what the functions of these nuclear bodies might be. In addition, with the exception of Xist RNA involved in X chromosome inactivation, lack of these lncRNAs in individual animals often does not result in discernible morphological and/or behavioral defects.

The goal of the research in the laboratory of Dr. Nakagawa is to understand biological functions of abundant nuclear lncRNAs predicted on the very reasonable hypothesis that the emergence of lncRNAs enabled novel cellular processes that led to the diversification and increased developmental and cognitive complexity of animals, especially vertebrates. Dr. Nakagawa aims to exploit the combination of biochemical, genetic and cell biological approaches with state-of-the art super-resolution microscopic analyses to understand in detail how lncRNAs form nuclear bodies with their binding proteins and how they regulate the expression of specific genes, which leads to specific phenotypes.

His work has relevance for our understanding of both lncRNA-guided epigenetic modifications and basic chromatin processes that modulate gene expression. His work has also relevance in our understanding of lncRNA-mediated formation of specific nuclear domains. He is one of a very few researchers internationally who strategically and systematically seek to determine the fine structure of vertebrate-specific nuclear bodies in which lncRNAs act as architectural components. Understanding their function is almost certainly therefore of critical importance to understanding human development and cognition.

<Research results>

Dr. Nakagawa's laboratory is only one in the world looking strategically at nuclear lncRNAs involved in infrastructural processes. It is one of only a handful of to have produced knockout (KO) mice of the abundant nuclear lncRNAs Malat1 and Neat1, as

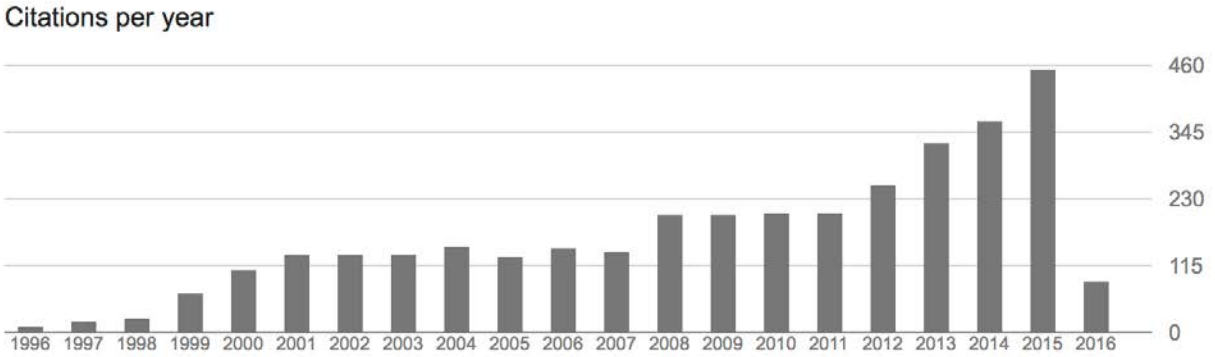
well as the only one to produce a knockout of Gomafu, which he discovered. By careful examination of their phenotypes, Dr Nakagawa has contributed greatly to the dissection and elucidation of the physiological functions of these lncRNAs. He has shown, surprisingly, that KO of these lncRNAs do not result in overt developmental defects, but rather the mice exhibit more subtle phenotypes in tissues where they are normally highly expressed, including reproductive problems (Neat1) and altered neurological behavior (Gomafu).

These are very important results for the lncRNA research field and open new ways of thinking about the roles of a class of lncRNAs that reside in the nucleus. He has also done a great deal of biochemistry, which has lifted the veil off previous puzzling findings of, for example, how Xist RNA stays on the inactivated X chromosome: hnRNP U tethers Xist RNA to the chromosome. Recently, he has examined fine structures of nuclear bodies such as paraspeckles, at the heart of which Neat1 lncRNA molecules reside. With the use of the Structured Illumination Microscope (SIM), he has found fine structural arrangements of both Neat1 and its associated proteins in the nuclear body. This is a very important finding that may lead to identification of specific domains of lncRNAs.

Dr. Nakagawa produces beautiful results constantly, and he is at the leading edge of the field. His overall career productivity to date is very good – a total of 70 publications that have been cited ~3600 times, with a h-index of 31 (Google Scholar).

The research productivity of his laboratory at RIKEN is also very good: 7 primary papers, 11 collaborative papers, 8 reviews / commentaries, and 4 methodological articles (total 30, plus being co-editor of one book and one special review issue of a journal) in the past 5 years, although none in very high impact journals.

There may be several reasons for this: The creation of knockout mice, systematic knockdown of RNA binding proteins, and detailed microscopic studies are difficult and time consuming. In addition, the lack of strong phenotypes makes the findings superficially less attractive to major journals, forcing publication in those of lower impact. On the other hand, there is a pleasing increase in his citation rate over recent years:



Moreover, the very enigma of Dr Nakagawa's findings – that widely expressed vertebrate-specific nuclear RNAs do not affect mammalian development – is a clue that

they play a more important role, almost certainly in the evolutionary adaptations that led to placental biology and cognitive advancement.

The review committee felt that Dr Nakagawa should maintain his primary focus on abundant nuclear RNAs and not be too distracted by work on more idiosyncratic clade-specific RNAs, especially those in non-mammalian species. A number of lncRNA genes are located in chromosomal regions where candidate genes for disease have been mapped, implicating defects in lncRNA functions in human disease. Thus his work may also have an impact on our understanding of human disease and the development of therapeutic strategies. For this reason too, Dr Nakagawa should maintain his primary focus on mammalian abundant nuclear RNAs.

<Management of the Laboratory>

Dr. Nakagawa interacts well with his colleagues at all levels of the research community. He is a core member of one of the JSPS Scientific Research on Innovative Areas ("ncRNA neo-taxonomy") and is also actively involved in the activity of the RNA Society of Japan as a Council Officer. He has also collaborated with the experts in the field, worldwide. He is interactive, helpful and widely read. He is a good teacher and well able to supervise students or postdoctoral fellows studying or working under him. Indeed, a graduate student, Yuko Hasegawa, in his laboratory was awarded the prestigious JSPS Ikushi Prize in 2011. He is well equipped to perform his aims.

Throughout the work in his laboratory, his postdoctoral fellows and other staff members demonstrate thoroughness and dedication. They spoke clearly about what they think, and we very much enjoyed the discussion with them. They interact well with each other in the lab and it was clear that Dr Nakagawa is respected and liked by them all.

<Future research plans>

The goals of this project are two-fold. First, this work will identify both functional modules of lncRNAs and RNA-binding proteins that interact with the modules, and characterize processes that underlie lncRNA-mediated regulation in the nucleus (aims 1~3). Second, the proposal will identify novel lncRNAs in non-traditional model organisms (aim 4).

Based on previous findings and some preliminary data, experiments will be carried out to identify and characterize modules on lncRNAs and factors required for lncRNA function and nuclear body formation in the nucleus. To facilitate these experiments, Dr. Nakagawa will use the SIM for fine structural analyses of nuclear bodies built on lncRNAs. This is an important and ambitious attempt. These experiments are feasible and will identify candidate modules on lncRNAs and RNA-binding proteins that form complexes with the lncRNAs and/or execute lncRNA-mediated regulation of gene expression, and will very likely produce many important results, which hail the prospect of fully deciphering the lncRNA pathway. We also recommend that Dr Nakagawa utilize high-resolution (even single cell) RNA sequencing to examine the transcriptional effects of knockdown of nuclear abundant lncRNAs, in response, among other things, to

reproductive hormones (Neat1), immune stimulation (Malat1) or neuronal activation (Gomafu).

The fourth specific aim will sample transcriptomes and identify species-specific lncRNAs, particularly in non-model organisms including marine invertebrates. To this end, RNA-seq analyses will be carried out to identify novel lncRNAs and elucidate the consequences of manipulating the expression of identified lncRNAs with the CRISPR/CAS9 system. As indicated above, we recommend that this not be a distraction to prosecuting the main agenda, to solve the enigma and functional mystery of vertebrate-specific nuclear RNAs, one of which (Malat1) is among the most highly expressed genes in the human genome.

In general, this is an innovative project that will address a 'hidden' layer of gene expression. It is directly relevant to mammalian development, including female fertility and the role of lncRNAs in cognitive adaptation. Given his research history and accomplishments, and his great collaborators, these experiments will likely produce many important results. The proposed work may have profound impact on our general understanding of the role of nuclear organization in the epigenetic control of human development and brain function.

以上