様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９　（共通）

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研究課題名（和文）Chemical biology of small RNA chromatin modifiers

研究課題名（英文）Chemical biology of small RNA chromatin modifiers

研究代表者
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研究成果の概要（和文）：Ｓｐｌｉｃｅｏｓｔａｔｉｎ　Ａ　は、世界初のmRNAスプライシング反応を阻害する化合物である。これまでにSSAを使用すると小さい　ＲＮＡ　によるヘテロクロマチンの増加が見られた。塩基配列を決めてもどんな　ＲＮＡ　がヘテロクロマチン
の増加に必要かについてまだ不明である。現在、データを引き続き解析しているところである。

研究成果の概要（英文）：Splicing inhibition with the small molecule SSA leads to a small RNA-dependent increase in heterochromatin. High throughput sequencing displayed an increase in histone methylation. However, a close association with novel small RNA sequences could not be confirmed. Data refinement and partial re-sequencing is ongoing.

研究分野：　Chemical Biology

キーワード：　Chemical Biology　Splicing

１．研究開始当初の背景

It has long been suspected that small RNA modifiers related to miRNA and siRNA species are involved in heterochromatin formation. We observed that inhibition of splicing with the small molecule inhibitor spliceostatin A (SSA) led to increased formation of heterochromatin.

２．研究の目的

Identification of small RNA chromatin modifiers.

３．研究の方法

High throughput sequencing of small RNA species and heterochromatin regions, using ChIP.

４．研究成果

Chemical inhibition of splicing leads to an increase in heterochromatin, exemplified by a global increase in the Histone H3 lysine 9 trimethylation marker H3K9me3). This increase is dependent on a working small RNA machinery as knock down of the nuclease dicer (dcr) abrogated the effect. Therefore we hypothesized that sequencing small RNAs upon inhibition of splicing using the small molecule spliceostatin A (SSA) and indentifying heterochromatinized regions with chromatin immunoprecipitation followed by high through put sequencing (ChIP Seq) would allow us to identify both small RNA modifiers and their target regions. To this end HeLa S3 cells were treated with SSA and their RNA content and heterochromatic landscape analyzed.

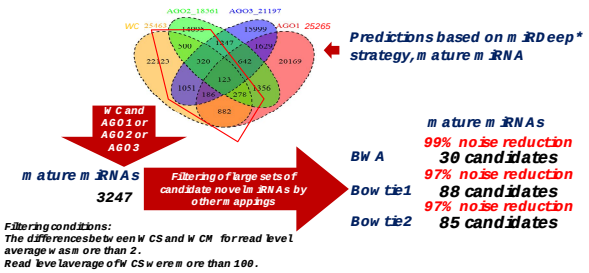

Figure 1: Identification of novel small RNA species from whole cell lysate and Ago associated RNAs.

We identified a number of new small RNA species, generated under splicing inhibition. (Fig 2) ChIP sequencing of heterchromatic regions proved more challenging than expected.

Small RNAs were isolate from whole cell lysate or via immuno-purification from the small RNA carrier proteins Ago 1, 2 and 3.

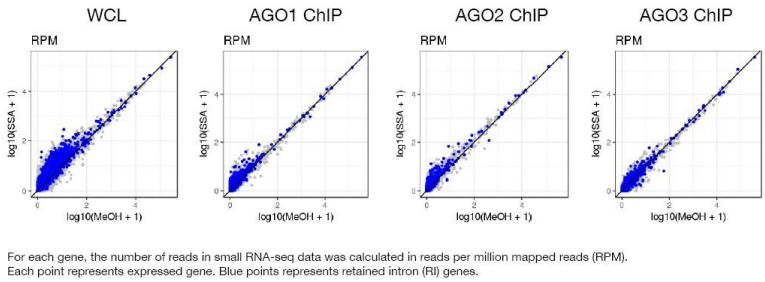

Figure 2: Changes in expression of small RNAs under SSA treatment. Expression changed especially in small RNAs associated with Ago 1 and 2, but not Ago 3.

It appeared that mainly the RNA content of Ago protein 1 and 2 but not 3 changed significantly under SSA. (Fig 2)while those RNAs were mainly derived from actively transcribed genes. (Fig 3)

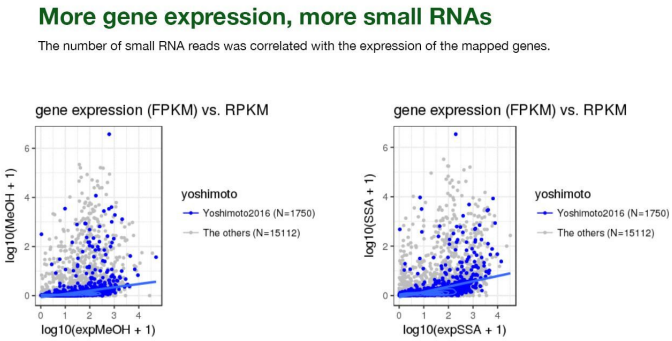

Figure 3: Increased expression of novel small RNAs from expressed genes.

While an overall increase in heterochromatin content was observed, it proved difficult to identify clear peaks. (Fig 4) Therefore matching areas of increased heterochromatin content to specific small RNAs proved harder than anticipated. The distribution of different chromatin markers also proved different, such as the splicing associated K36m3 mark. We re-evaluated our sequencing strategy and generated further data sets for in-detail analysis. Starting a collaboration with the Nikaido lab at RIKEN, we are currently refining our analysis and re-evaluating our small RNA

data.

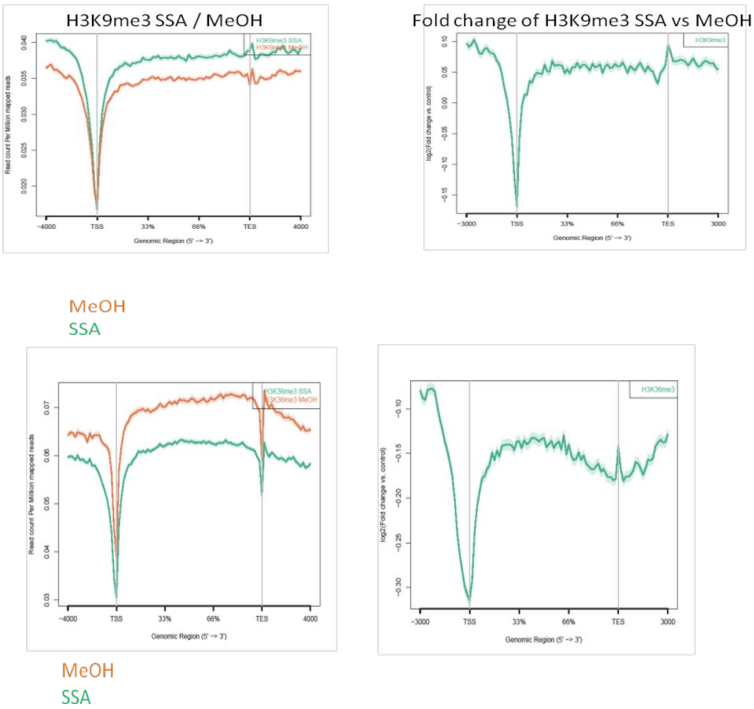

Figure4: distribution of heterochromatin marker histone H3 K9me3 across gene bodies. Under splicing inhibition overall heterochromatin increased, especially towards the 3' end of the gene. Meanwhile the distribution of the splicing associated K36me3 histone mark showed the opposite pattern (bottom).

At the moment, we observe very little overlap between novel small RNA species and regions of heterochromatin formation

WCL_MeOH	WCL_SSA	both	MeOH_only	SSA_only
FALSE	FALSE	3479	6145	10262
FALSE	TRUE	47	21	50
TRUE	FALSE	19	23	40
TRUE	TRUE	12	10	21

WCL_MeOH	WCL_SSA	both	MeOH_only	SSA_only
FALSE	FALSE	97.8071408	99.1288918	98.9299142
FALSE	TRUE	1.3213382	0.3387643	0.4820206
TRUE	FALSE	0.5341580	0.3710276	0.3856165
TRUE	TRUE	0.3373629	0.1613163	0.2024487

(Fig 5).

Figure 5: Poor overlap of small RNA species with detected heterochromatin peaks

We are continuing the project nevertheless, focusing on the expression of small RNA species and have repeated the generation of CHIP-Seq libraries for the generation of higher resolution maps. The peaks identified so far are being validated using quantitative PCR. We are further analyzing changes in gene expression under SSA treatment and how it relates to the expression of novel small RNA species.

(1) 研究代表者．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計　1　件）
Along the Central Dogma–Controlling Gene Expression with Small Molecules
Tilman Schneider-Poetsch Minoru Yoshida
Annual Review of Biochemistry 2018 87:1 , refereed

〔学会発表〕（計　　件）

〔図書〕（計　　件）

〔産業財産権〕

○出願状況（計　　件）

名称：
発明者：
権利者：
種類：
番号：
出願年月日：
国内外の別：

○取得状況（計　　件）

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種類：
番号：
取得年月日：
国内外の別：

〔その他〕
ホームページ等
http://www.csrs.riken.jp/en/labs/cgrg/index.html

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