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論　文　名	*Trans*-species long-distance movement of small RNAs in the parasitic complexes （寄生複合体における低分子 RNA の種間長距離移行）	
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論文要旨

Stem holoparasitic plants *Cuscuta* attack domesticated and non-domesticated plants and cause serious damage to commercial crops. *Cuscuta* withdraws water and nutrients from hosts. In addition to water and nutrients, vascular connections allow *Cuscuta* to exchange larger molecules including various types of RNAs with the host. The exchange of mRNAs has been reported previously between *Cuscuta* and host plants. The movement of the endogenous mRNAs via the phloem within a single plant regulates developmental processes. The *trans*-species movement of mRNAs strongly suggests that RNA-based control is involved in the establishment of the parasitic complex. In addition to the mRNAs, the exchange of small RNAs (sRNAs) between pathogenic organisms and host plants has previously been found. The pathogenic fungus control host plant's immunity by exporting sRNAs. Conversely, host plants export microRNAs (miRNAs) to control the fungal hyphae, by silencing the genes expression essential for fungal virulence. In the *Cuscuta campestris-Arabidopsis thaliana* parasitic complex, *C. campestris* miRNAs were induced in the haustorial interface and repress the expression of *Arabidopsis* genes involved in the defense response and vascular development. These studies suggest that sRNA-based mechanisms are involved in the establishment of mutual regulation at the interfaces between host plants and other organisms. However, it has not yet been elucidated whether sRNAs derived from donor plants in parasitic complexes were translocated to distant tissues in the recipient plants or if they showed

effects after the establishment of a stable parasitic connection. In this study, we analyzed the accumulation of sRNAs of two host-parasitic plant complexes and tried to find out the common phenomenon involved in sRNA accumulation. To obtain the transcriptome of sRNA, RNAs were extracted from the parasitic stems of *C. campestris-A. thaliana* parasitic complex, and *Cuscuta japonica-Glycine max* parasitic complex. RNAs were also extracted from the non-parasitic stems. sRNA-seq reads obtained from a single sequencing library were mapped to the genome databases of both the parasite and host to identify mobile sRNAs. The analyses of sRNA accumulation of two parasitic complexes along with *trans*-species sRNA movement and biogenesis were described in four chapters.

Chapter 1: Analyses of small RNAs in *Cuscuta campestris-Arabidopsis thaliana* parasitic complex

The analyses of sRNA-seq data of different tissues of *C. campestris-A. thaliana* parasitic complex reveals the accumulation of sRNAs in parasitic conditions. In *A. thaliana*, sRNA accumulation was increased in 103 genes and decreased in 119 genes. Gene ontology (GO) analyses of the genes onto which sRNA accumulation were increased showed that majority of the genes were involved in 'glucosinolate biosynthesis', 'response to stress' and 'symplast'. The sRNA reads associated with genes, where reads per million (RPM) values increased more than 2-fold in parasitic condition, revealed that 22-nt reads were enriched. On the contrary, sRNA reads associated with genes that decreased more than 2-fold in parasitized stems of *A. thaliana* were enriched with 21-nt reads. In *C. campestris*, sRNA accumulation was increased in 458 genes and decreased in 445 genes in the parasitic condition. GO terms of genes onto which sRNA accumulation were increased were enriched with 'response to cadmium ion' and 'response to metal ion'. In the parasite, no significant enrichment was observed for any length of sRNA reads in *C. campestris*. Percentage of 21-nt reads significantly decreased that associated with genes that increased more than 2-fold in *C. campestris*.

Chapter 2: Analyses of small RNAs in *Cuscuta japonica-Glycine max* parasitic complex

In *C. japonica-G. max* parasitic complex, the genes which were responsible for the generation of sRNAs in parasitic condition were analyzed. In *G. max*, 666 genes of which sRNA accumulation was increased where 365 genes of which sRNA accumulation was decreased in parasitic condition. GO terms of genes onto which sRNA accumulation were increased suggest the involvement of two biological processes: hormone-mediated response such as 'auxin binding', 'regulation of gibberellic acid-mediated signaling pathway', 'cellular response to hormone stimulus' and hormone-mediated signaling pathway and associated with cellular connection such as 'phloem transport', 'plasmodesma', and 'cell-cell junction'. In *C. japonica*, sRNA accumulation was increased in 197 genes, whereas decreased in 367 genes, in the parasitic condition. GO analyses reveal that genes associated with lignin accumulation such as 'lignin

biosynthetic process', 'lignin metabolic process regulated' was controlled by sRNA accumulation in parasitic conditions. We mention that the whole loci analyses of the *C. japonica* genome have not to be done due to the unavailability of the full genome of *C. japonica*. In parasites (*C. campestris and C. japonica*), genes associated with 'response to cadmium ion' and 'response to metal ion' were commonly involved in sRNA accumulation in parasitic condition. In hosts, (*A. thaliana* and *G. max*), genes associated with 'stress response' were commonly involved in sRNA accumulation.

Chapter 3: *Trans*-species long-distance movement of small RNAs in the parasitic complexes

The screening of *trans*-species mobile sRNAs resulted in the selection of 20 sRNAs of *C. campestris*, 10 sRNAs of *A. thaliana* in *C. campestris-A. thaliana* parasitic complex, whereas 5 sRNAs of *C. japonica* and 20 sRNAs of *G. max* in *C. japonica-G. max* parasitic complex. From the predicted mobile sRNAs, we prioritized 7 of *C. campestris* sRNA (CcsRNA) and 6 of *A. thaliana* sRNA (AtsRNA) to confirm the long-distance movement. We confirmed 4 of the CcsRNA (CcsRNA0641, CcsRNA0897, CcsRNA4295 and CcsRNA5627) and 2 of the AtsRNAs (AtsRNA3031 and AtsRNA4348) were mobile to the apical region of the recipient plant. CcsRNA0641 was also detected in the distant oegans of *A. thaliana* such as leaf, stem and root in parasitic condition. The GO analysis of predicted target genes of the mobile sRNAs did not show any enrichment of specific GO terms. The decrease in the accumulation of predicted target genes (*AtNPC5* and *AtMo25* which were targets of CcsRNA4295, and *AtSKD1* which was a target of CcsRNA5672) strengthened the hypothesis of *trans*-species sRNA-mediated repression in the parasitic condition.

Chapter 4: Biogenesis of sRNA

To clarify the regulation of sRNA biogenesis in the parasitic complex, we measured the expression levels of sRNA biogenesis genes. Expression levels of *A. thaliana SUPPRESSOR OF GENE SILENCING 3* (*AtSGS3*) and *RNA-DEPENDENT RNA POLYMERASE 6* (*AtRDR6*), which play crucial roles in the tasiRNA production pathway, were significantly upregulated in the parasitized stems. The enrichment of 21-nt and 22-nt sRNAs of *A. thaliana* may correspond to the upregulation of *AtSGS3* and *AtRDR6*, which support the possibility of secondary siRNA production in the parasitic condition. In *C. campestris*, *DICER-LIKE 1* (*CcDCL1*), *CcDCL3*, and *CcRDR6* were significantly downregulated. The decrease in the proportion of 21-nt reads in the parasitic stems of *C. campestris* may be associated with the downregulation of *CcDCL1* and *CcRDR6*. Secondary siRNAs production triggered by *trans*-species mobile sRNAs was not detected in the parasitic condition. By using *A. thaliana sgs3* and *rdr6* mutants, *trans*-species mobile sRNAs from *C. campestris* moved long distances in the host plant, suggesting that long-distance movement of *trans*-species mobile sRNAs occurs without siRNA production.

In summary, the results of my study indicate that donor-derived sRNAs move long distances in recipient plants. In hosts, secondary siRNA producing pathway not only plays a crucial role in the accumulation of secondary sRNA but also control the mobility of parasite-derived sRNA. Comprehensive profiling of the *trans*-species sRNAs and their target genes will provide deeper insights into the RNA-mediated interactions between parasitic and host plants.

審査結果の要旨

低分子 RNA（small RNA、以下 sRNA）は、塩基配列特異的な mRNA の分解、翻訳抑制、ならびに転写抑制などを引き起こすことで、生体機能の調節に様々な役割を果たしている。sRNA は細胞から細胞へ移行することが知られており、移行による遺伝子機能の調節が植物と微生物の間で見られている。ある種の植物病原菌と宿主植物の間では、お互いに隣接する相手の細胞へ sRNA を送り込み、植物側の防御反応や病原菌側の病原性を弱めている。近年、sRNA の相互交換は多細胞性の異種植物個体間でも起こることが、寄生植物と宿主植物の研究から明らかになった。寄生植物は、寄生時に吸器とよばれる特殊な器官で sRNA を発現し、これらが吸器周辺の宿主植物細胞へ移行する。そして移行した sRNA は、宿主内で二次的な sRNA の生産を促し宿主植物の防御反応を弱める、という分子機構の存在が解明されている。一方で、寄生植物は吸器が形成される寄生部位から遠く離れた宿主植物の器官においても、特定の遺伝子群の発現レベルを低下させる。この現象に対しては、sRNA が器官間長距離移行することの関与が示唆されていた。しかしながら寄生植物と宿主植物という異種個体間で sRNA が移行するか、また移行した植物内で sRNA が長距離移行して機能する事が可能かどうかは未解明であった。

そこで申請者は本研究において、二つの異なる寄生植物‐宿主植物複合体を用いて、異種植物間で移行する sRNA の存在ならびに長距離移行性を検証し、それらの sRNA が寄生時にいかにして生成され、移行先でどのような機能を果たすのかを明確にするための解析を実施した。

第 1 章では、寄生植物 *Cuscuta campestris* と宿主植物 *Arabidopsis thaliana* を用いて、small RNA sequencing（sRNA-seq）解析での結果に基づいて、それぞれの植物内での sRNA プロフィールが明らかにされた。*A. thaliana* において sRNA が増加している座位では、22 塩基長の sRNA が主要な生成物となっていた。

第 2 章では、宿主植物 *Cuscuta japonica* と寄生植物 *Glycine max* を用いて、sRNA プロフィールが明らかにされた。*C. japonica* において sRNA が増加している座位では、22 塩基長の sRNA が主要な生成物となっていた。第 1 章と第 2 章での結果を比較し、寄生によって sRNA 量増加がおこる座位のうち、二つの寄生系で共通しているのは、寄生植物側、宿主植物側で共にストレス応答や細胞間連絡形成に関与する遺伝子群であった。

第 3 章では、*C. campestris*‐*A. thaliana* 寄生系を用いて異種間移行する sRNA の選抜と、

移行性の実証が行われた。sRNA-seq のデータ解析により候補化された sRNA の異種間移行性を、sRNA 配列特異的ステムループ RT-PCR 法で実験的に検証し、*C. campestris* から *A. thaliana* に移行する 4 つの sRNA、ならびに *A. thaliana* から *C. campestris* に移行する 2 つの sRNA が同定された。さらにこれらの sRNA の標的遺伝子を予測し qRT-PCR による発現量の測定を行ったところ、*A. thaliana* の 3 つの標的遺伝子が被寄生状態において抑制されていることが確認され、移行性 sRNA による抑制効果が示唆された。

第 4 章では、*C. campestris* - *A. thaliana* 寄生系における sRNA 生合成機構の研究がなされた。sRNA 生合成系遺伝子発現を解析したところ、*A. thaliana* では *SGS3* と *RDR6* の発現が被寄生時に上昇していた。このことは *A. thaliana* での 22 塩基長 sRNA の増加と整合し、二次的 siRNA 生合成の活性化が示唆された。そこで異種間移行性 sRNA が移行先で二次的 siRNA 生合成を誘導するかどうかを sRNA-seq データから解析したところ、この可能性は否定された。さらに、*A. thaliana* の *sgs3* ならびに *rdr6* 変異体を宿主として、*C. campestris* 由来の異種間移行性 sRNA が移行先植物内で長距離移行するために二次的 siRNA 生合成が必要かどうかを検証した。両変異体内で *C. campestris* 由来の sRNA は茎頂で検出され、移行先植物内での長距離移行に二次的 siRNA 生合成は必要ではないことが示された。

申請者は本研究で、寄生植物と宿主植物の複合体における異種個体間で sRNA の双方向移行があること、移行性 sRNA は移行先で長距離移行し、標的遺伝子の発現を抑制する事が可能であることを示した。ならびに移行先植物内での長距離移行に二次的 siRNA 生合成は必要がなく、異種間移行した sRNA が直接長距離移行することが示された。本研究は個体間での新規な RNA の機能様式を解明した独創性の高いもので、細胞分子生物学や植物生理学的な成果として高く評価できる。ならびに RNA を活用した植物保護や新育種技術への展開も期待される。よって本論文の審査ならびに最終試験の結果と併せて博士（応用生命科学）の学位を授与することを適当と認める。