Physiological roles of two tetraamines, spermine and thermospermine, in Arabidopsis thaliana

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論文題目	Physiological roles of two tetraamines, spermine and thermospermine, in *Arabidopsis thaliana*(2 種のテトラアミン、スペルミンとサーモスペルミン、のシロイヌナズナにおける生理的役割の解明)
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Polyamines (PA) are small organic polycations found in all living organisms. Putrescine (Put), spermidine (Spd), spermine (Spm) and thermospermine (T-Spm) are the most abundant PAs in plants. They are known to be involved in various processes such as cell proliferation, growth, morphogenesis, senescence and response to environmental stresses. In Arabidopsis, it had been believed that two genes, *ACL5* and *SPMS*, encode Spm synthase. Currently, however, it had been reported that the bacterially expressed recombinant ACL5 protein catalyzes the conversion of Spd to T-Spm, but not to Spm, indicating that *ACL5* encodes T-Spm synthase. As *SPMS* and *ACL5* encode different enzymes, firstly I examined the localization of *Spm synthase* (*SPMS*) gene promoter activity using the transgenic Arabidopsis plants expressing *SPMS* promoter-β-glucuronidase (*GUS*) gene and compared it with that of the transgenics expressing *ACL5* promoter-*GUS* gene. *SPMS* promoter activity was detected in almost all organs at all developmental growth stages, while *ACL5* promoter activity was only detected in the vascular systems. Upon high salt stress, *SPMS* promoter activity was highly enhanced in all organs except cotyledons whereas the *ACL5* promoter activity was reduced, being consistent with its endogenous *ACL5* transcript levels. The result indicates that *SPMS* expression is different from that of *ACL5* in respect to tissue specificity and stress response, supporting the idea that Spm and T-Spm have different function. The tetraamine-deficient mutant plants became hypersensitive to high salinity and drought stresses, suggesting that Spm and/or T-Spm play a protective role against those stresses. Thus, secondly, I examined the role of tetraamines, Spm and T-Spm, during heat shock (HS) stress. I found that *SPMS* and *S*-adenosylmethionine decarboxylase 2 genes are induced at the earliest stage upon HS. Correspondingly, Spm content increased linearly upon HS, and Put and Spd content also increased

but not thermospermine (T-Spm) content. Exogenously applied Spm had a potential to protect Arabidopsis plants from HS-induced damage. Such protection was also observed to the same extent with T-Spm and by Spd to a lesser extent but not by Put. Then I tested whether altered endogenous Spm content affects sensitivity to HS using both transgenic plants overexpressing *SPMS* and a Spm deficient (*spms*) mutant plant. The result revealed that the higher the Spm content the higher the thermotolerance. Even in the *spms* plant, representative genes encoding heat shock proteins (HSPs) and heat shock transcription factors were upregulated upon HS, while the expression of such genes was increased in a positively correlated manner with Spm content. Furthermore four kinds of HSPs (HSP101, HSP90, HSP70 and HSP17.6) were detected proportionally with the levels of their respective transcripts upon HS. Thereby I propose that Spm increases the HS response at transcriptional and translational levels and protects host plants from HS-induced damage. As exogenously applied T-spm can protect the plant from HS, thirdly, I checked whether T-Spm has a gene-inducing activity as similar as Spm. Expectedly T-Spm has an inducing activity on the expression of genes involving in the Spm-signal pathway and on the genes involving in CMV-triggered hypersensitive response (HR). In consistent with the inducing activity of a subset of the defence genes involved in HR, T-Spm represses the CMV multiplication at a similar extent as Spm does. Abiotic and biotic stresses cause the disruption of normal protein folding machinery in plants. Disturbance in protein folding in the endoplasmic reticulum (ER) induces an unfolded protein response (UPR) which plays a pivotal role to protect the plants in response to abiotic and biotic stresses. Our previous work revealed that Spm induces the expression of Arabidopsis *bZIP60* and its ortholog of *Nicotiana tabacum*. Fourthly, I examine whether Spm induces *bZIP60* specifically or rather induces the whole UPR process. Spm induced the expression of *bZIP17* and its predicted downstream target genes

such as *PP2C-like* gene and *RD20* gene, whereas a little effect of Spm on the expression of *bZIP28* was observed. Spm induced not only the expression of *bZIP60* but also activated its splicing mediated by inositol-requiring enzyme-1 (Ire1), resulting in an active, nuclear-targeted form of bZIP60. Likewise the other tetraamine, T-Spm, had such UPR-inducing activity at similar dose with Spm. Spm-induced expression of *bZIP17* and *bZIP60* was totally attenuated by Ca^{2+}-channel blocker and also *bZIP17* induction was blocked in specific mitogen-activated protein kinase cascade-mutants such as *mkk9* and *mpk3*, whereas *bZIP60* induction was not affected in those mutants, indicating that the respective signaling pathways were branched at downstream of cellular Ca^{2+} increment.

With the above work, I could say that two tetraamines, Spm and T-Spm, have distinct function in host plant while exogenously applied those tetraamines show similar activity in gene induction activity and in defensive activity against HS-induced damage.

論文審査結果の要旨

Sagor G.H.M.氏は、シロイヌナズナにおける 2 種のテトラアミン、スペルミンとサーモスペルミン、の生理的役割の解明を行った。まず、それぞれの合成遺伝子のプロモーターにレポーター遺伝子を連結させた形質転換植物を作成し、シロイヌナズナ内での時間的・空間的発現を調べた。スペルミン合成遺伝子が植物体のほぼ全域で高レベルで発現する一方、サーモスペルミン合成遺伝子は総じて発現が低く、維管束系に限定して発現することを明らかにした。次に、熱ショックストレスにおけるポリアミンン合成系の解析を行い、ポリアミン生合成系はスペルミン系が活性化されるが、サーモスペルミン合成遺伝子の発現の変化はなく、内在するポリアミン含量を測定してもプトレシンとスペルミン含量が増加するが、サーモスペルミン含量は変化しないことを明らかにした。熱ショックを与える前に低濃度の各種ポリアミンで処理すると、テトラアミンであるスペルミンとサーモスペルミンには熱ショックから宿主植物を保護する活性があることを見出した。さらにはシロイヌナズナ内のスペルミン含量を変えた植物体を作ることにより、スペルミン含量が高い植物体の方がより熱ショック耐性が高いことを明らかにした。事実、こうした植物体における熱ショック関連遺伝子の発現および代表的な熱ショックタンパク質の産生量がスペルミン含量と正の相関を持つことも明らかにした。サーモスペルミンにスペルミンと同程度の熱ショック耐性付与能力がある知見から、サーモスペルミンにはスペルミンと同様なシグナル誘導活性を持つとの作業仮説を立て、それを実証した。最後に、スペルミンはシロイヌナズナの小胞体ストレス応答に関与する３つの鍵転写因子遺伝子（*bZIP17*、*bZIP28*、*bZIP60*）の中、*bZIP17* と *bZIP60* の転写を活性化すること、前者の下流に位置する遺伝子群の発現誘導もスペルミンによっておこることから bZIP17 のプロセッシングもスペルミンが活性化すると予想されること、後者についてもスペルミンは RNase 活性をもつ IRE1 による *bZIP60* 転写物のプロセッシングを活性化すること、を明らかにした。スペルミンによる小胞体ストレス誘導活性は、スペルミンが示すストレスへの防御能力と密接に関連していることを提示した。以上の成果は、Sagor 氏が自立して研究活動を行うに必要な高度の研究能力と学識を有することを示している。したがって，Sagor G.H.M.氏提出の論文は，博士（生命科学）の博士論文として合格と認める。