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学位の種類	博士（生命科学）
学位記番号	生博第３０８号
学位授与年月日	平成２７年９月２５日
学位授与の要件	学位規則第４条第１項該当
研究科，専攻	東北大学大学院生命科学研究科 （博士課程）分子生命科学専攻
論文題目	Studies on polyamines as inducers of the unfolded protein response in Arabidopsis （シロイヌナズナにおいて小胞体ストレス応答反応を誘導するポリアミンに関する研究）
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論文内容の要旨

Background

When *Nicotiana tabacum* carrying the resistance gene *N* was infected by Tobacco mosaic virus (TMV), host plant displayed a hypersensitive response (HR). Once HR occurs, it is known that the tissues surrounding the TMV infection sites were killed by a suicidal reaction to prevent the virus multiplication. In 1998, Yamakawa et al. reported that the polyamine spermine (Spm) enriched in the apoplastic space during HR triggered by *N. tabacum*-TMV pathosystem. Subsequently Takahashi et al. (2003, 2004) found that exogenously applied Spm stimulates the phosphorylation activities of two MAPKs, WIPK and SIPK which triggers the expression of downstream defense genes in *N. tabacum* and proposed the Spm-signaling pathway. Massive identification of the Spm-responsive genes using a SuperSAGE approach was performed in *Arabidopsis thaliana* (Mitsuya et al. 2009). The identified Spm-responsive genes behaved similarly during Cucumber mosaic virus-induced HR in *A. thaliana* (Mitsuya et al. 2009). One of the upregulated genes by Spm was a gene (*bZIP60*) encoding a basic region leucine zipper 60 protein. *bZIP60* was reported as a key transcription factor gene which is involved in the unfolded protein response (UPR) (Iwata and Koizumi 2005). UPR is induced, when unfolded or misfolded proteins are accumulated in endoplasmic reticulum (ER), to refold or degrade the corresponding proteins (Howell 2013). Two other bZIP genes, *bZIP17* and *bZIP28*, are also key transcription factor genes in UPR of Arabidopsis (Liu et al. 2007a, 2007b). Activation of bZIP60 is occurred by uncanonical splicing mediated by IRE1, whereas bZIP17 and bZIP28 activation is occurred by proteolytic processes (Liu and Howell 2010).

Aim of this study

Here I aimed to reveal the following points:

1. Does Spm only act on bZIP60 or does it induce the whole UPR in Arabidopsis?
2. What are the upstream components in Spm-induced UPR pathways?
3. Spm activated bZIP60 leads to the *BiP3* induction. Unlike the *BiP3* induction by a canonical UPR inducer, DTT, the size of *BiP3* transcript induced by Spm was longer. I examined to uncover its structure.

Results and discussion

1. I showed that Spm induces the expression of *bZIP17*, *bZIP28* and *bZIP60*, and their respective downstream target genes. I propose that Spm is a novel UPR inducer in plant. Spm treatment also activated the *bZIP60* splicing. In Arabidopsis, there are two *IRE1* genes, *IRE1a* and *IRE1b*. The encoded proteins sense unfolded proteins in the lumen of the ER by its N-terminal domain which leads to enzyme auto-activation. Then the active endoribonuclease domain splices *bZIP60* mRNA. In the double knock-out mutant, *ire1a ire1b*, Spm-induction of *bZIP60* splicing and expression of

Bip3, target gene of the active bZIP60, was strongly attenuated, indicating that Spm-induced *bZIP60* splicing is mediated by IRE1. I also showed that Spm recruits bZIP17, bZIP28 and bZIP60 proteins to nuclei in plant cells using GFP-protein fusion method.

2. In *N. tabacum*, NtMEK2 and two MAPKs, WIPK and SIPK, were identified as the MAPK cascade components in the Spm-signaling pathway. The upper components of this signaling pathway are Ca^{2+} channel activation and production of reactive oxygen species by polyamine oxidase action (Takahashi et al. 2004). In Arabidopsis, 10 *MKK* genes exist (MAPK group 2002). I tested the expression of *MKK1* to *MKK10* in Spm-treated Arabidopsis seedlings and found that *MKK9* expression is clearly enhanced in Spm-treated samples. *MPK3* (*WIPK* ortholog) and *MPK6* (*SIPK* ortholog) are identified as downstream kinases of MKK9 (Zhou et al. 2009). Actually Spm induced *MPK3* and *MPK6* expression. I tentatively concluded that MKK9 and MPK3/MPK6 are the MAPK cascade components in Arabidopsis. When I applied La^{3+}, a Ca^{2+} channel blocker, the expression of *bZIP17*, *bZIP28* and *bZIP60* by Spm treatment was abrogated, indicating that Ca^{2+} entry to the cell positioned upstream of these key bZIP genes' induction. In addition, the induction of *bZIP17*, *bZIP28* and *bZIP60* by Spm was totally compromised in the loss-of-function *mkk9* mutant. The result shows that Spm-induced UPR pathway is mediated by Ca^{2+}-influx into cytoplasm and by the MKK9-MPK3/MPK6 cascade.

3. I happened to find that the size of Spm-induced *BiP3* transcript is longer than that of DTT-induced one. Further analysis revealed that the transcript induced by Spm contained all the introns and the transcriptional start site differs from the one of the DDT-induced transcript. Currently I am investigating the physiological significance of this finding.

References

Chawla P et al. Spermine is a novel inducer of unfolded protein response in Arabidopsis. Submitted.

論文審査結果の要旨

Pratima Chawla は、病原菌感染に対する植物の抵抗性反応の際に、転写遺伝子 *bZIP60* が誘導される、また *bZIP60* の誘導は抵抗性反応の際に産生されるポリアミンのスペルミンによっても誘導される、との所属分野での知見に着目して研究を行った。モデル植物のシロイヌナズナでは、小胞体内に正しい高次構造をとれないタンパク質が蓄積した際に小胞体ストレス応答反応(Unfolded Protein Response, UPR)が引き起こされるが、この過程で鍵となる分子として *bZIP60*、*BZIP17* そして *bZIP28* の転写因子遺伝子が近年同定された。Pratima Chawla は外生のスペルミンが *bZIP17* および *bZIP28* の発現誘導活性があること、下流の標的遺伝子の発現を誘導すること、を示し、スペルミンが UPR を誘導する活性を持つことを明らかにした。*bZIP60* は、inositol-requiring enzyme1 (IRE1)がもつ RN ｱｰｾﾞによってスプライスされることが、他の UPR 誘導試薬を用いた実験から明らかにされている。彼女は、スペルミンによる *bZIP60* の発現誘導が IRE1 依存的に起こることも明らかにした。次に、彼女はスペルミンによる UPR 誘導には細胞内へのカルシウムの流入が要求されること、また MKK9 (Mitogen-activated kinase kinae9)-MPK3/MPK6 (Mitogen-activated protein kinase 3 および 6)からなるリン酸化カスケードを介すること、を明らかにした。更に、スペルミン処理した際に、シロイヌナズナに 3 種ある *BiP* 遺伝子のうちもっとも強く誘導される *BiP3* 遺伝子が、処理後 6 時間目に転写物サイズが大きくなること、シークエンスの結果、すべてのイントロンを含むこと、また転写開始点も変化することを明らかにした。このサイズの大きな転写物の生物学的意義については今後の研究の展開を待たねばならないが、彼女の見出した新規な現象である。

以上の諸点は、Pratima Chawla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，Pratima Chawla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。