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論文題目	Two types of polyamine oxidases catalyzing the back-conversion pathway or terminal catabolism pathway co-exist in *Oryza sativa*（イネには逆変換反応型と末端分解型の２つのタイプのポリアミン酸化酵素が共存している）
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論文内容の要旨

Two types of polyamine oxidases catalyzing the back-conversion pathway or terminal catabolism pathway co-exist in *Oryza sativa*

（イネには逆変換反応型と末端分解型の２つのタイプのポリアミン酸化酵素が共存している）

分子応答制御分野

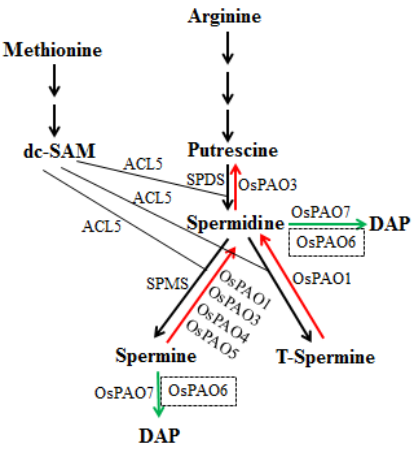
Taibo Liu

Major components of polyamines (PAs) in higher plants are diamine putrescine (Put), triamine spermidine (Spd), and tetraamines spermine (Spm) and thermospermine (T-Spm). Those amine compounds have fundamental roles in not only growth and development but also adaptive responses to various environmental stresses. The PA concentration is controlled by a dynamic balance between biosynthesis and catabolism. The latter process is governed by two enzymes, namely copper-contained amine oxidase and flavine adenine dinucleotide-associated polyamine oxidase (PAO). The best studied plant PAOs are maize PAO (ZmPAO) and two barley PAOs (HvPAO1 and HvPAO2). The analyses of reaction products revealed that those PAOs produce 4-aminobutanal from Spd and *N*-(3-aminopropyl)-4-aminobutanal from Spm, respectively, along with 1,3-diaminopropane (DAP) and H_2O_2. This type of catabolic pathway is named PA terminal catabolism (TC). On the other hand, mammalian PAOs convert Spm and Spd to Spd and Put, respectively, after the PA substrate is acetylated by Spm/Spd acetyltransferase. Mammals also have Spm oxidases (SMOs) which convert Spm to Spd without acetyl modification. Mammalian PAOs and SMOs are categorized into back conversion (BC)-type PAO.

The *Oryza sativa* genome contains seven PAO-encoded genes and they were termed *OsPAO1* to *OsPAO7*. One year ago, Ono et al (2012) characterized the expression of three *OsPAO*s, *OsPAO3, OsPAO4* and *OsPAO5*, and their genes' products. Their expression is rather abundant in entire growth stages. The recombinant proteins, OsPAO3, OsPAO4 and OsPAO5, catalyze BC-type reactions.

With the above background, in this study, I focus on two *OsPAO* genes, the one is *OsPAO1* and the other is *OsPAO7*. In rice plant, expression of *OsPAO1* seems to be quite low under physiological conditions, but is markedly induced by Spm or T-Spm treatment in a root-specific manner, suggesting that it is involved in tetraamine catabolism. In accord with this speculation, the recombinant OsPAO1 prefers T-Spm as a substrate at pH 6.0 and Spm at pH 8.5 and, in both cases, back-converts these tetraamines to Spd but not further to Put. OsPAO1 localizes to the cytoplasm of onion epidermal cells. The enzymatic behavior and subcellular localization of OsPAO1 are quite similar to those of AtPAO5 of *Arabidopsis thaliana*. Furthermore, the *Atpao5* mutant showed growth arrest of its aerial parts, but not roots, when

grown on MS agar medium containing low doses (5 or 10 μM) of T-Spm. This effect was specific to T-Spm because WT and *Atpao5* mutants showing almost comparable growth on 1 mM Put-, 1 mM Spd- or 300 μM Spm-contained media. Introduction of *OsPAO1* directed by a constitutively expressed promoter into the *Atpao5* mutant recovered the growth arrest of the host plant in the presence of low doses T-Spm. OsPAO3 did not have such activity. Taken all, I propose that OsPAO1 is an ortholog of Arabidopsis AtPAO5 and functions as a T-Spm oxidase in rice. Next *OsPAO7* and its product enzyme are studied. *In silico* data suggest that *OsPAO7* is specifically expressed in anther organ. In fact, I was able to isolate its cDNA from flower organ. OsPAO7 is localized in a peripheral layer of plant cells with the aid of its predicted signal peptide and transmembrane region, suggesting that OsPAO7 is an apoplastic enzyme. As expected from the high identity of OsPAO7 to ZmPAO, HvPAO1 and HvPAO2, the recombinant OsPAO7 produces DAP from both Spm and Spd in a time-dependent manner. It indicates that OsPAO7 is a first TC-type enzyme in *O. sativa*. Furthermore, *OsPAO7* is specifically expressed in anther organ with an expressional peak at the bicellular pollen stage.



In *Oryza sativa*, two-types of PAOs co-exist. Namely, of seven OsPAOs, OsPAO1 (this study), OsPAO3, OsPAO4 and OsPAO5 catalyze BC-type reactions whereas OsPAO7 (this study) catalyzes a TC-type reaction (see the left figure). While almost all the OsPAO proteins are consisting of 474-500 amino acids, OsPAO2 is a 351-amino acid-protein and lacks the N-terminal portion that harbors the part of catalytically essential residues. Therefore, I assumed that OsPAO2 might lack PAO enzyme activity. Meanwhile, OsPAO6 is expected to have TC-type PAO enzyme activity because it shows high identity to OsPAO7 and other TC-type PAO members in the entire protein region. More distinct function of each OsPAO member will be investigated in the future works.

Publication list

- **Taibo Liu** et al. (2014) Plant Cell Reports in press, doi:10.1007/s00299-013-1518-y.
- G.H.M. Sagor†, **Taibo Liu**† (2013) Plant Cell Reports 32:1477–1488.
  (†contributed equally to this work)
- **Taibo Liu** et al. Journal of Experimental Botany (under review)

論文審査結果の要旨

刘太波は，イネのポリアミン酸化酵素(polyamine oxidase、PAO と略)の解析を行い、２つの異なる反応様式を持つ PAO がイネには共存していることを実験的に証明した。イネには 7 種の *PAO* 遺伝子(*OsPAO1*～*OsPAO7* と命名)が存在する。従来の当分野の研究から、OsPAO3、OsPAO4 そして OsPAO5 がポリアミンに対する基質特異性は異なるものの生合成の逆反応である逆変換型の反応を触媒することが明らかにされていた。彼は、まずイネ幼植物をスペルミンとサーモスペルミン処理をした際に、根特異的に *OsPAO1* の発現が誘導されることを見出した。この現象はスペルミジンやプトレシン処理では起こらなかった。*OsPAO1* プロモーターに緑色蛍光タンパク質遺伝子を結合した遺伝子をイネに導入した形質転換イネを作出した。このイネをサーモスペルミンで処理すると根の分裂組織領域で見られていた緑色蛍光の範囲が広がることを明らかにした。スペルミンによっても同様の現象が見られたが、サーモスペルミンよりも弱い効果であった。根のテトラアミン濃度が上昇すると、*OsPAO1* の発現が誘導され、テトラミン濃度を一定範囲に維持すると考察した。また組換え OsPAO1 が逆変換型の酵素活性を持つことを明らかにした。次に、彼はイネ花器官由来の cDNA から *OsPAO7* を単離した。この遺伝子は、イネの花器官、特に葯で発現していること、葯の 4 段階の発達ステージの第 3 期目で最も高くなることを示した。*OsPAO7* は花粉でも発現することも確認した。さらに組換え OsPAO7 酵素は逆変換型ではなく、トウモロコシで見つかっていた末端分解型酵素活性を示すことを反応産物の解析から明らかにした。反応速度論的解析から、OsPAO7 は他の逆変換型の PAO に比べ数百倍高い触媒効率(k_{cat}/K_M)を持つことも明らかにした。

OsPAO1 そして *OsPAO7* について研究内容をとりまとめ、それぞれ国際誌へ公表している。

上記の内容は、刘太波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，刘太波提出の論文は，博士（生命科学）の博士論文として合格と認める。