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學號	493546091
研究生(中)	張凱超
研究生(英)	Chang, Kai-chao
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論文名稱(英)	Employing systems biology and RNA interference methods to dissect the physiological function of L-form starch phosphorylase in potato
其他題名	
指導教授(中)	陳翰民
指導教授(英)	Chen, Han-min
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關鍵字(中)	澱粉磷解酶 澱粉 蔗糖
關鍵字(英)	starch phosphorylase L-SP starch
摘要(中)	澱粉是植物中含量最高的生物聚合物，也是人類所攝取的最主要多醣來源，因此釐清澱粉代謝的機制，將有助於作物改良與栽培，其長遠目標是解決糧食問題。但是植物澱粉代謝的機制非常複雜，探討這些代謝相關酵素的功能，主要是利用篩選植物的突變株，利用功能消失 (function disability) 與表現型變化 (phenotype change)，來推論這些酵素在植物代謝上可能扮演之角色。以澱粉磷解? (starch phosphorylase，SP，EC 2.4.1.1) 為例，為高等植物中參與澱粉代謝的酵素之一，其催化反應如下：?-glucose 1-phosphate + Glucan(n) ⟷ Pi + Glucan(n+1)。但是由於突變株取得不易，至今對於 L-SP 的生理功能仍無法確定。植物澱粉的合成機制

	已經研究多年，一般認為主要參與合成澱粉的酵素是澱粉合成? (starch synthase，EC 2.4.1.11)，此酵素以 ADP-Glc 或是 UDP-Glc 為葡萄糖單元體之來源，在短鏈糖引子 (sugar primer) 的非還原端 (non-reducing end) 加成延長糖鏈。分支酵素 (branch enzyme，EC 2.4.1.18) 與去分支酵素 (debranching enzyme，EC 3.2.1.41)，則可對直鏈糖分子進一步修飾，產生各種不同分支度的葡聚醣。由上述可知，澱粉合成?必須在糖引子存在下才能進行延長糖鏈反應。因此，最初糖引子的生成反應，應是後續澱粉高分子形成不可或缺的步驟，而具有合成最初糖引子活性的酵素，應該參與澱粉合成的最初反應。根據本實驗室先前的結果顯示，L-SP 在試管反應 (in vitro) 中具有 primer independent phosphorylase (PIPh) 活性，也就是合成糖引子之能力，因此，L-SP 可能在植物澱粉生合成初期，具有關鍵之貢獻。在 1996 年時 Moreno 發現，馬鈴薯原生質體內的 L-SP 蛋白質含量可利用蔗糖來誘導，在本論文發現其誘導表現的階層從基因的層次開始，蔗糖可以誘導 L-SP mRNA 表現量增加，進而使 L-SP 蛋白質與活性增加。蔗糖除了誘導 L-SP 表現之外，也誘導葉綠體中澱粉的累積，暗示了 L-SP 與澱粉合成之高度相關性。原預計使用原生質體配合配合上 RNA 干擾術，在細胞內 (in vivo) 系統，更進一步確認 L-SP 在植物澱粉合成之角色。但是由於培養原生質體不如預期簡單，故在這部份無法繼續下去。另一方面，在探討蔗糖誘導後葉片中澱粉代謝相關的系統生物學中發現，在基因層次上發現除了 L-SP 外，H-SP (H-from starch phosphorylase)、granule-bound starch synthase (GBSS) 以及 ADP-glucose pyrophosphatase (AGPase) 也隨著誘導時間增加而增加。而在蛋白質的層次上發現，隨著蔗糖誘導時間增加，Leucine aminopeptidase 表現量增加，但 Rubiso 以及 oxygen evolving protein 表現量卻下降。
摘要 (英)	Starch is the most abundant biopolymer in plants, and the major source of carbohydrate in human diet. Therefore, elucidation of the metabolism of starch biosyntheis and degradation will be a great mileston in crop breeding. In the long run, understanding the detailed mechanism might solve the problem of food crisis. However, the mechanism of starch metabolism is more than complicated. The major way to study starch metabolism would be the utilization of mutant that lose the function or alter the phenotype. For example, starch phosphorylase (EC 2.4.1.1), which catalyzes the reversible phosphorolysis of starch in higher plants, is the enzyme with obscure function. Due to the inability to obtain a mutant, the physiological role of SP remains uncler. It is generally accepted that starch biosynthesis is predominantly exerted by starch synthase (EC 2.4.1.11) adding glucose unit to existing sugar primer by using ADG-Glc or UDP-Glc. The heterogeneity of starch is then complete with the assist of branching (EC 2.4.1.18) and debrancing enzyme (EC 3.2.1.41). Therefore, the formation of the sugar primer would be the key step for subsequent chain elongation is starch biosynthesis. On the other hand, enzymes that catalyze the formation of sugar primer should participate in the very initials stage in starch synthesis. Our previous study indicate that in vito L form SP (L-SP) catalyzed a primer independent amylase synthesizing activity, which means the ability to produce sugar primer. It implied that L-SP might have contribution at the early stage of

	starch biosynthesis. In 1996, it has been reported that the activity of L-SP in potato leaves increased when inducing by sucrose. In this study, it was also found that the induction by sucrose affected on transcriptional level. The mRNA, protein, and enzyme activity of L-SP all increased when sucrose was present. We also found that significant starch accumulation in the chloroplasts of potato leaves induced by sucrose. Many evidences support the idea that L-SP participate in starch biosynthesis. In order to validate this hypothesis, we have tried to use RNAi to knock down the expression of L-SP in the protoplast of potato leaves, and see if the loss of L-SP function in plant cells might hinder the starch accumulation by sucrose induction. Unfortunately, the culture and transfecton efficiency of protoplast was not as successful as expected. We also used 2-DE and realtime RT-PCR to evaluate the gene expression change in the potato leaves induced by sucrose. Interestingly, other than L-SP, the mRNA expression of H form starch phosphorylase (H-SP) and granule-bound starch synthase (GBSS) also increased in the sucrose induced leaves.On the level of protein expression, Leucine aminopeptidase was found increasing; Rubisco and oxygen evolving protein were found decreasing. The posssilbe mechanism is discussed.
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