Taylor et al, Supplemental Data 1:

Urea Cycle Enzyme Subcellular Localisation in Plants

Much debate over several decades has surrounded the subcellular localisation of the enzymes of the Urea cycle in plants and resolving this puzzle is essential to determine what substrate transport between compartments is required. By combining recent data collected from both enzymatic studies and protein localisation studies (shown in Table 2 and outlined below), we propose that subcellular locations are now relatively clear (Figure 9B). Probably the least controversial localisation is that of carbamoyl-phosphate synthase (CPS1, EC 6.3.4.16), which is not to be confused with the glutamine dependant form (CPSII, EC 6.3.5.5) involved in pyrimidine synthesis. Early enzymatic studies in soybean, radish and pea indicated at plastid localisation for both these enzymes (Shargool et al., 1978; Taylor and Stewart, 1981; Shibata et al., 1986) and this observation is supported by mass spectrometry based localisation studies in Arabidopsis that show both proteins are located in plastids (Kleffmann et al., 2004; Peltier et al., 2006; Zybailov et al., 2008). Interestingly Taira et al. (2004) suggested using protein prediction software that both enzymes (CPSI & CPS II) were located in mitochondria. However the AGI number (At1g34320) supplied for CPSI and used in the prediction is not a carbamoyl-phosphate synthase and does not contain any of the Interpro domains (http://www.ebi.ac.uk/interpro/) (IPR005481, IPR005480, IPR011607, IPR005479) associated with CPSI functionality. The claim of mitochondrial localisation of CPS II was also based on only a single localisation prediction algorithm which has been shown to be of limited merit for accurate prediction (Heazlewood et al., 2004). Proteome analysis has not identified either CPSI or CPSII in Arabidopsis or rice mitochondria (Heazlewood et al., 2004; Huang et al., 2009). Given the weight of evidence it is most likely that these enzymes are solely located in the plastid in plants.

The carbamoyl phosphate produced by CPS1 is used by ornithine carbamoyltransferase (EC 2.1.3.3) (OCT) in the production of citrulline. Two early biochemical investigations suggest a mitochondrial location in mung bean and sugarcane (Bone, 1959; Glenn, 1977), but it was later fairly well established with enzymatic studies in soybean and pea that the actual localisation was to the plastid (Shargool et al., 1978; Taylor and Stewart, 1981). Recently proteomic studies have supported this finding in Arabidopsis and Rice (Peltier et al., 2006; Kleffmann et al.,

2007; Zybailov et al., 2008) and extensive surveys of the mitochondrial proteome in both rice and Arabidopsis have not identified this protein (Heazlewood et al. 2004, Huang et al. 2009). However, Taira et al. (2004) reported the measurement of the conversion of L-Orn to L-Citrulline in isolated Arabidopsis leaf mitochondria using a single percoll gradient isolation procedure. Two obvious explanations are that this protein is dual-localised in plants or that the activity measured by Taira et al. (2004) was in fact cause by chloroplastic contamination of their mitochondria samples. Given the current weight of evidence it seems most likely that this enzyme is solely located in plastids.

The next two enzymes in the Urea cycle are arginosuccinate synthase (EC 6.3.4.5) (ASS) and arginosuccinate ligase (EC 4.3.2.1) (ASL) which are responsible for the conversion of citrulline to arginosuccinate and the conversion of arginosuccinate to arginine and fumarate respectively. Recent organellar proteomics studies have suggested a plastidic localisation in Arabidopsis (Kleffmann et al., 2004; Peltier et al., 2006; Zybailov et al., 2008) for both of these enzymes.

The final enzyme of the urea cycle is arginase (EC 3.5.3.1) which is responsible for the breakdown of arginine releasing urea and ornithine. Evidence from both biochemical studies in pea and soybean (Goldraij and Polacco, 2000) and proteomic localisation studies in both Arabidopsis and rice suggest this enzyme is mitochondrial (Taylor and Stewart, 1981; Lee et al., 2008; Huang et al., 2009). However some contradictory evidence has been presented, for example in one proteomic localisation study of the Arabidopsis chloroplast proteome the presence of arginase was detected (Kleffmann et al., 2004) and Taira (2004) suggested using a single protein localisation prediction algorithm that it was also located in the plastid. However, the clear bulk of the evidence suggest a single localisation of arginine in mitochondria in plants.

EC Number	At	Name	MS	Os	Name	MS
6.3.4.16	At1g29900	Carbamoyl-phosphate synthase B	Plastid-Zybailov 2008, Peltier 2006, Kleffmann 2004	Os01g38970	Carbamoyl-phosphate synthase B	
2.1.3.3	At1g75330	Ornithine carbamoyltransferase	Plastid-Zybailov 2008, Peltier 2006	Os02g47590	Ornithine carbamoyltransferase	Plastid-Kleffmann 2007
6.3.4.5	At4g24830	Arginosuccinate synthase	Plastid-Zybailov 2008, Kleffmann 2004	Os11g19770	Arginosuccinate synthase	
4.3.2.1	At5g10920	Arginosuccinate ligase	Plastid-Zybailov 2008, Peltier 2006	Os03g19280	Arginosuccinate ligase	
3.5.3.1	At4g08870	Arginase	Mito – Lee 2008 Plastid- Kleffmann 2004	Os04g01590	Arginase	Mitochondrial-Huang 2009

Localization of components of the ornithine-urea cycle in Arabidopsis and rice. EC Number, Enzyme commission number; At, Arabidopsis AGI number; Name, protein description, MS, localisation by mass spectrometry; Os, Rice locus number.

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