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·专题论坛·

植物细胞中蛋白质向叶绿体转运的研究进展

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摘要 植物细胞中叶绿体的功能主要依赖于叶绿体蛋白, 大部分叶绿体蛋白由核基因组编码, 在细胞质中合成并经过正确的分选后, 通过叶绿体外膜上的Toc复合体和/或内膜上的Tic复合体转运到叶绿体的不同部位。该文主要综述可能参与叶绿体蛋白分选的胞质因子以及Toc和Tic组分如何参与叶绿体蛋白转运的研究进展。

关键词 叶绿体蛋白, 转运, 易位子, 胞质因子, 分选

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叶绿体是高等植物以及藻类光合细胞中的重要细胞器, 其主要功能是通过光合作用制造碳水化合物, 另外, 氨基酸及脂肪酸的生物合成等也与叶绿体密切相关(Motohashi et al., 2001)。目前认为叶绿体起源于1种古老的光合细菌, 这种光合细菌是现今蓝藻的祖先(Reyes-Prieto et al., 2007)。植物叶绿体有其自身的基因组, 但只有大约100个叶绿体蛋白是由叶绿体基因组编码(Abdallah et al., 2000; Martin et al., 2002), 绝大部分叶绿体蛋白还是由核基因组编码, 在细胞质中合成后再转运到叶绿体。核基因组编码蛋白转运至叶绿体可为叶绿体的发育提供物质及功能基础, 同时也可以调控叶绿体基因的转录、加工及翻译等过程(李保珠等, 2014)。20世纪70年代, Kung等(1972)首次报道了核基因组编码的叶绿体蛋白。几年以后, 他们又报道了核基因组编码的Rubisco小亚基的合成及转运(Kung, 1976)。由于豌豆(*Pisum sativum*)和菠菜(*Spinacia oleracea*)的叶绿体相对容易提取, 因此早期主要以豌豆和菠菜作为模式植物进行叶绿体蛋白转运研究(Morgenthaler et al., 1975)。近几十年来, 随着分子生物学的快速发展及拟南芥(*Arabidopsis thaliana*)模式植物地位的确立, 研究者主要以拟南芥为材料逐步鉴定出叶绿体蛋白转运的十几种易位子(translocon) (Li and Chiu, 2010), 并对其编码基因、表达模式以及生物学功能等进行了全面研究。叶绿体蛋白转运研究的详细历史可见Bölter和Soll (2016)的综述。

易位子是叶绿体膜上负责叶绿体蛋白转运的蛋白复合体(Li and Chiu, 2010)。位于外膜上的易位子组分称为Toc (translocon of the outer membrane of chloroplasts)蛋白, 位于内膜上的易位子组分称为Tic (translocon of the inner membrane of chloroplasts)蛋白, Toc和Tic后面的数字表示其分子量(如Toc159) (Schnell et al., 1997)。大部分核基因组编码的叶绿体蛋白都是先在细胞质中合成1个N端带有转运肽(transit peptide)的前体蛋白, 然后叶绿体膜上的Toc/Tic复合体识别转运肽, 引导叶绿体前体蛋白进入或穿过叶绿体的双层膜。本文主要探讨细胞质中可能参与叶绿体蛋白分选的胞质因子, 同时对易位子的分子功能以及它们如何协同工作以完成后续的转运过程进行阐述。

1 引导蛋白转运和定位的信号——转运肽

大部分通过转运进入叶绿体蛋白质N端都含有1个可剪切的信号序列称为转运肽(Bruce, 2000; Lee et al., 2006, 2008; Li and Chiu, 2010)。一般而言, 转运肽对于蛋白转运到叶绿体是必需的。不同叶绿体蛋白转运肽的长度差异很大, 从13–146个氨基酸残基不等(Zhang and Glaser, 2002), 而且不具有共有序列(Bruce, 2001)。即使成熟蛋白高度相似, 其转运肽的

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差异也可能很大，这意味着在转运肽中可能蕴含着一些未知的功能基序(Teng et al., 2012)。研究表明，叶绿体前体蛋白可以分成3大类，第1类倾向于进入幼嫩的叶绿体；第2类没有特殊喜好；第3类则偏向于进入老化的叶绿体。每类蛋白的年龄选择信号都蕴含在转运肽中，而第3类转运肽中的2个连续的正电荷在蛋白靶向老化的叶绿体中发挥关键作用(Teng et al., 2012)，表明细胞内的蛋白质运输过程与细胞年龄密切相关且受到严格的调控。这一发现首次明确了叶绿体转运肽中蕴含的信息对于调控蛋白转运的重要性。

值得注意的是，并不是所有的叶绿体蛋白都具有转运肽。例如，除了Toc75 (Hofmann and Theg, 2005a; Li and Chiu, 2010)，叶绿体外膜蛋白一般不含有可剪切的转运肽。一些定位于内膜或类囊体腔等部位的叶绿体蛋白也没有可剪切的转运肽(Miras et al., 2002; Nada and Soll, 2004; Ueda et al., 2008)。此外，叶绿体蛋白质组研究表明，还有一些叶绿体蛋白在细胞质中合成时就是成熟蛋白(Kleffmann et al., 2004)。

2 协助叶绿体蛋白分选的胞质因子

蛋白质在核糖体合成后，一般都需要通过可以识别定位信号的胞质因子介导，将其定位到各个细胞器。目前已经在细胞质中发现一些可以和叶绿体前体蛋白互作的蛋白，这些蛋白可能负责识别叶绿体蛋白并将其运送到叶绿体表面(图1)。

第1种是14-3-3蛋白。14-3-3蛋白的识别和结合基序与一些前体蛋白转运肽的磷酸化位点类似(Waegemann and Soll, 1996; May and Soll, 2000)。14-3-3特异性介导叶绿体前体蛋白的定位，叶绿体前体蛋白可以通过磷酸化的转运肽和14-3-3及胞质Hsp70互作形成一种引导复合体(guidance complex)，从而更高效地将叶绿体蛋白转运至叶绿体(May and Soll, 2000)。

第2种是胞质Hsp90蛋白。Hsp90可以将前体蛋白定位到Toc64，Toc64的34肽重复序列结构域(tetratricopeptide repeat motif, TPR)识别Hsp90，随后前体蛋白从Toc64转移并穿过Toc75转运通道(Qbadou et al., 2006)。然而，在完全缺失叶绿体Toc64的突变体中，叶绿体蛋白的转运却没有受到影响(Hofmann and Theg, 2005b; Aronsson et al., 2007)，这暗示可能存在1条替代途径来弥补Toc64的缺失。

锚蛋白重复序列蛋白2A (ankyrin repeat protein 2A, AKR2A)也是一种负责叶绿体蛋白转运的胞质因子，AKR2A属于类伴侣蛋白(chaperone-like protein)，可以介导蛋白之间的互作(Li et al., 2006)，负责将N端含有跨膜结构域(transmembrane domain, TMD)的蛋白定位到叶绿体外膜(Bae et al., 2008)。AKR2A可以结合叶绿体外膜蛋白，但是不会与其它细胞器的膜蛋白结合。AKR2A的N端区域参与AKR2A蛋白与运输蛋白的结合(Bae et al., 2008)，然而在细胞质中AKR2A如何以及何时捕获这些运输蛋白仍不清楚。进一步研究发现，AKR2A和AKR2B都可以识别外膜蛋白的定位信号并把它们运送到叶绿体外膜(Bae et al., 2008)。研究发现小热激蛋白(small heat shock proteins, sHsps) Hsp17.8、Hsp17.4、Hsp18.1和Hsp17.6A都可以通过识别AKR2A的锚蛋白重复域与AKR2A互作，将外膜的脂质作为最初的停泊位点(Kim et al., 2014)。Hsp17.8可能同时与AKR2A及叶绿体结合，Hsp17.8和AKR2A的互作有助于其与叶绿体的结合(Kim et al., 2011)。Hsp17.8可以帮助AKR2A将外膜蛋白7 (OEP7)定位到叶绿体，但Hsp17.8却不会直接与叶绿体外膜蛋白结合，说明Hsp17.8是作为AKR2A的1个辅因子在蛋白定位到叶绿体外膜中发挥作用(Kim et al., 2011)。

3 蛋白穿过和插入到外膜

叶绿体外膜上的Toc复合体主要由Toc159、Toc34和Toc75组成，可以特异地识别和结合叶绿体前体蛋白，并帮助其穿过外膜进入膜间隙(Bauer et al., 2001)。体外实验表明，这3种蛋白确实可以组装成1个可以结合并帮助前体蛋白转膜的Toc复合体(Schleiff et al., 2003a)。

易位子Toc159和Toc34是位于叶绿体表面的2种同源GTP酶(Kessler et al., 1994)，它们通过直接与转运肽结合而识别叶绿体表面的前体蛋白(Sveshnikova et al., 2000; Schleiff et al., 2002; Smith et al., 2004)。Toc159包含3个区：1个大的N端酸性区(acidic domain, A-domain)、1个中部的GTP酶区(GTPase domain, G-domain)以及1个C端的膜锚定区(membrane-anchoring domain, M-domain)。研究表明，拟

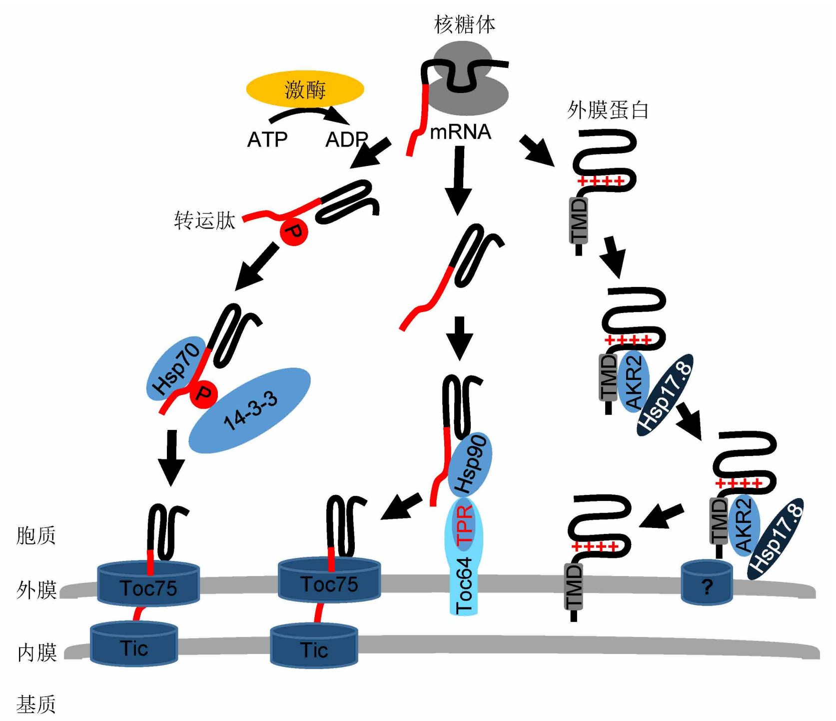

图1 胞质中叶绿体蛋白的分选与定位(改自Lee et al., 2013)

14-3-3和Hsp70识别磷酸化的转运肽，形成引导复合体将前体蛋白运输到叶绿体表面。Hsp90可以结合前体蛋白的转运肽及成熟N端区，与前体蛋白一起到达Toc64。AKR2结合到外膜蛋白的TMD区和C端正电荷区(C-terminal positively charged flanking region, CPR)，小热激蛋白Hsp17.8作为辅因子帮助AKR2将外膜蛋白定位到叶绿体表面，外膜蛋白的停泊位点仍不清楚。

Figure 1 Sorting and targeting of chloroplast proteins in the cytosol (modified from Lee et al., 2013)

14-3-3 and Hsp70 recognize phosphorylated transit peptide and form the guide complex to targeting precursor proteins to chloroplasts surface. Hsp90 binds to the transit peptide and the N-terminal region of the mature portion, and escorts preproteins to Toc64. AKR2 bind to the TMD and C-terminal positively charged flanking region of chloroplast outer envelope membrane proteins, small heat shock protein Hsp17.8 functions as a cofactor to facilitate chloroplast outer envelope membrane proteins targeting to chloroplasts surface, and the docking site for chloroplast outer envelope membrane proteins remains unknown.

南芥中的Toc159家族有atToc159、atToc132、atTo120和atToc90四个成员(Bauer et al., 2000; Kubis et al., 2004)。这4个成员的C端高度保守，而N端酸性区的保守性较差(Bauer et al., 2001)。其中，atToc159是拟南芥Toc159家族中最主要的成员。*atToc159*的突变植株表现出白化表型，植株在幼苗期就会死亡，光合蛋白的表达和转运都受到抑制，而非光合蛋白的表达和转运不受影响(Bauer et al., 2000)。但进一步的研究表明，Toc159可以转运更多的蛋白而不仅仅局限于光合蛋白(Köhler et al., 2015)。Toc159很可能是前体的初始受体，通过G区直接与前体结合并将其带往Toc复合体(Smith et al., 2004)，但转运肽如何与G区相互作用仍不清楚。

另1种重要的叶绿体外膜蛋白受体是Toc34，大约95%翻译后转运的叶绿体蛋白通过Toc34运送到叶绿体(Schleiff and Becker, 2011)。Toc34包含1个GTP酶区和1个短的C端膜锚定区。前体蛋白转运进叶绿体需要Toc159和/或Toc34介导的GTP水解，GTP水解除了可以为蛋白的跨膜转运提供能量外，还可以促进Toc159插入到外膜，与Toc75形成多聚复合体(Bauer et al., 2002; Smith et al., 2002; Wallas et al., 2003)，促进前体蛋白插入到易位子转运通道(Young et al., 1999; Schleiff et al., 2003a)。拟南芥Toc34家族有atToc33和atToc34两个成员，其蛋白的相似性很高(约80%)，其中atToc33是豌豆中Toc34的同源物。拟南芥atToc33的缺失会导致叶绿

素含量显著降低，蛋白的转运也受到显著影响(Jarvis et al., 1998; Gutensohn et al., 2004)。*atToc34*的突变仅对根的发育有影响(Constan et al., 2004)，表明atToc33可能更倾向于转运光合蛋白，而atToc34主要负责非光合蛋白的转运(Kubis et al., 2003)。

Toc75是1种镶嵌在叶绿体外膜的通道组分(Schnell et al., 1994)，预测其N端有3个可以结合转运肽的多肽转运相关(polypeptide-transport associated, POTRA)结构域，可能主要负责与Toc受体蛋白以及前体蛋白的结合(Ertel et al., 2005)。POTRA结构域后是1个β-桶状结构域，形成蛋白转运通道(Tranel et al., 1995; Hinnah et al., 1997)。一般认为, Toc75主要负责将蛋白转运穿过叶绿体外膜。拟南芥中的Toc75由*atTOC75-III*基因编码，其缺失导致胚胎致死，说明质体蛋白的转运对于植物的早期发育非常关键(Baldwin et al., 2005; Hust and Gutensohn, 2006)。

此外，还有1种在前体识别和搭建膜内空间易位子组分中发挥重要作用的Toc64。Toc64横穿外膜3次，形成1个C端TPR结构域和1个膜间隙结构域，膜间隙结构域可以与定位于膜间隙的易位子组分Hsp70、Toc12和Tic22形成1个复合体(Qbadou et al., 2007)。但随后的研究表明, Toc12定位于基质中(Chiu et al., 2010)，表明Toc64复合体在膜间隙的组分仍需要进一步研究。Toc64的C端TPR结构域暴露于胞质端，可以识别Hsp90 (Qbadou et al., 2006)，从而识别Hsp90和前体蛋白形成的复合体。因此, Toc64可以作为Hsp90与前体蛋白复合体的停泊蛋白(Qbadou et al., 2006, 2007)。在拟南芥中，有3个Toc64的同系物: atToc64-I、atToc64-III和atToc64-V。然而，只有atToc64-III是豌豆Toc64功能上的同源蛋白(Oreb et al., 2006)。在正常生长条件下, *atToc64-III*的T-DNA插入失活突变体没有明显的表型变化，叶绿体蛋白的转运也未受影响(Hofmann and Theg, 2005b; Aronsson et al., 2007)。但在逆境条件下，尤其是在盐胁迫下，突变体的生长受到明显抑制。进一步的研究表明，atToc64-III可以调控叶绿体蛋白的转运效率(Sommer et al., 2013)。

4 蛋白穿过和插入到内膜

叶绿体内膜的易位子主要包括Tic110、Hsp93、Tic40、Tic20和Tic21等。Tic110主要负责前体蛋白的识别; Hsp93可能水解ATP，为叶绿体蛋白的转运提供动力; Tic40可能作为辅助伴侣蛋白，协调Tic110和Hsp93的结合; Tic20和Tic21可能组成叶绿体内膜通道; 而3种氧化还原蛋白Tic55、Tic62和Tic32可能负责叶绿体蛋白的转运调控。

第1个获得的内膜易位子组分是从豌豆中得到的Tic110 (Kessler and Blobel, 1996)。Tic110的N端(大约9 kDa)含有2个短的跨膜螺旋结构(Jackson et al., 1998)，可以作为转运肽基质侧的受体。蛋白的其它部分(97.5 kDa)具有α-螺旋构型，含有1个转运肽的结合位点(Inaba et al., 2003)，定位于基质(Jackson et al., 1998; Inaba et al., 2003)。Tic110可能在Tic易位子的组装及转运通道的形成中发挥关键作用(Heins et al., 2002; Inaba et al., 2003)。研究表明, Tic110可以作为前体蛋白的停泊位点直接与前体蛋白接触(Inaba et al., 2003)，其C端部分或者整个基质域可能是作为基质伴侣蛋白Hsp93以及辅助伴侣蛋白Tic40等的支架(Inaba et al., 2005; Chou et al., 2006)。Tic110也可以与Hsp93 (Akita et al., 1997; Nielsen et al., 1997)以及cpn60互作(Kessler and Blobel, 1996)。因此，Tic110的主要功能是在蛋白转运的后期结合基质侧伴侣蛋白(Inaba et al., 2003)。在叶绿体内膜，Hsp93可以通过其N端直接与Hsp110结合(Chu and Li, 2012; Flores-Pérez et al., 2016)。在拟南芥中，atTic110表达降低导致植株生长受到抑制，叶色变黄，核编码的叶绿体蛋白含量显著降低。*atTic110*的T-DNA插入突变更是进一步表现出胚胎致死的表型(Inaba et al., 2005; Kovacheva et al., 2005)，说明Tic110在植物生长发育及叶绿体蛋白的转运中发挥关键作用。

Tic40最初是作为1个可以结合前体的蛋白被鉴定出来(Wu et al., 1994; Stahl et al., 1999)。Tic40的N端含有1个跨膜的α-螺旋，负责将Tic40锚定在叶绿体内膜，其后连着1个基质区，包含1个TPR结构域和1个C端Hip/Hop结构域(Stahl et al., 1999; Bédard et al., 2007)。TPR域可以结合Tic110，引起转运肽从Tic110上释放出来。拟南芥和豌豆中的Tic40均由单基因编码(Stahl et al., 1999; Chou et al., 2003)。缺失atTic40的植物突变体可以存活，但是叶色苍白，生长迟缓，蛋白的转运受到严重影响(Chou et al., 2003; Kovacheva et al., 2005)，说明Tic40在蛋白转运中不是必需的，但是可以提高转运效率。

Tic20和Tic21是2个位于相同复合体的小的疏水蛋白(Kikuchi et al., 2009)。预测这2种蛋白都有4个α-螺旋的跨膜结构(Li and Chiu, 2010)。这2种蛋白在蛋白跨膜转运通过内膜的过程中发挥重要作用(Chen et al., 2002; Teng et al., 2006)。拟南芥Tic20家族包含atTic20-I、atTic20-IV、atTic20-II和atTic20-V四个成员(Jarvis, 2008)。其中atTic20-I的表达量最高且与豌豆Tic20的相似性最高(Kouranov et al., 1998)，但只有atTic20-I这个主要的亚型在叶片和茎中表达，对植物的生长发育十分必要。

蛋白转运进入叶绿体需要基质中的ATP水解以提供能量(Theg et al., 1989)。Hsp93是1种基质ATP酶，可以与易位子稳定结合(Li and Chiu, 2010)。Hsp93属于Hsp100伴侣蛋白的亚族成员(subfamily)，该家族中的蛋白可以使用ATP水解产生的能量驱动蛋白转运进入基质(White and Lauring, 2007)。因此，Hsp93是理想的马达候选者。拟南芥中有2个Hsp93基因：*atHsp93-V*和*atHsp93-III* (Jackson-Constan and Keegstra, 2001)，其成熟蛋白氨基酸序列的相似性为91% (Kovacheva et al., 2005)。*atHsp93-V*的突变体叶色变白，同时叶绿体发育不全。*atHsp93-III*的突变体与野生型相比没有明显的表型差异。而*atHsp93-V*和*atHsp93-III*的双突变体则表现出胚胎致死表型(Kovacheva et al., 2007)，表明*atHsp93-III*与*atHsp93-V*的功能存在冗余(Constan et al., 2004; Kovacheva et al., 2005)。另外，基质Hsp70也被证明可以在叶绿体蛋白转运中行使功能(Shi and Theg, 2010)。真核生物中的Hsp70s是大肠杆菌伴侣蛋白DnaK的同源物，由多基因家族编码，参与蛋白运输，阻止蛋白的聚集以及帮助蛋白折叠等许多生理生化过程(De Los Rios et al., 2006)。Hsp70s有2个主要结构域，分别为N端的ATP酶结构域和底物结合域(Stevens et al., 2003)。研究表明Hsp70可以与转运肽互作(Rial et al., 2000)，说明基质Hsp70可能参与蛋白的转运(Vojta et al., 2007)。豌豆叶绿体中至少含有3个Hsp70s，1个与外膜结合，2个位于基质(Marshall et al., 1990)。小立碗藓(*Physcomitrella patens*)中的3个Hsp70分别为Hsp70-1、Hsp70-2和Hsp70-3，这3个蛋白都位于叶绿体基质。Hsp70-2的插入失活会引起致死，前体蛋白可以与Hsp70-2以及其它易位子组分结合，表明Hsp70-2在蛋白转运穿膜的过程中起关键作用(Shi and Theg, 2010)。因此叶绿体可能具有1个双马达系统，以负责蛋白转运至基质。最新的研究表明，Hsp93和Tic复合体的结合是在蛋白质量控制的蛋白水解中起作用而不是在蛋白转运中起作用(Sjögren et al., 2014; Flores-Pérez et al., 2016)。因此，基质Hsp70可能在水解ATP及将蛋白转运进基质的过程中发挥主要作用。

此外，光合作用是1个依赖于光的氧化还原反应，叶绿体内的氧化还原状态可能也会调控蛋白质的定位。3种氧化还原蛋白Tic55、Tic62和Tic32都可以与Tic110结合，其中Tic55和Tic62可以与Tic110形成1个稳定的230 kDa复合体(Küchler et al., 2002)。氧化条件下，Tic62可以锚定到内膜并与Tic110结合；而在还原条件下，Tic62则穿梭到基质(Stengel et al., 2008)。Tic32是1种依赖于NADPH的短链脱氢酶，可以结合钙调素(Chigri et al., 2006)，也可以在NADPH存在时从Tic110上分离下来。这表明Tic32也可能是氧化还原信号转换器的一部分(Hofmann and Theg, 2005b; Chigri et al., 2006)，而Ca^{2+}很可能通过Tic32调控蛋白的转运。

5 亚复合体的组装

叶绿体易位子组分需要组装成不同的亚复合体，在转运的不同阶段发挥作用。Toc159、Toc75和Toc34可以按照大约1:4:4–5 (Schleiff et al., 2003b)或者1:3:3的比例(Kikuchi et al., 2006)组装成1个稳定的复合体，从而介导前体蛋白转运通过叶绿体外膜(Akita et al., 1997; Schleiff et al., 2003b; Kikuchi et al., 2006; Chen and Li, 2007)。这种比例的不同可能是由于使用了不同的分析技术所致(Ling and Jarvis, 2015)。内膜上的Tic20和Tic21也可以组装成1个稳定的复合体(Kikuchi et al., 2009)。Toc复合体、Tic通道复合体和Tic110/基质组分复合体可以进一步组装成Tic/Toc超级复合体(Akita et al., 1997; Nielsen et al., 1997; Asatsuma et al., 2005)。

新鉴定出1种在蛋白转运进叶绿体的过程中，位于内膜且含有Tic20和Tic21的1 MDa (megadalton)的转运复合体，其中Tic20与转运中的前体蛋白紧密

结合，在内膜上形成1种稳定的1 MDa复合体，Tic21与这种复合体的结合较弱，与已发现的内膜易位子复合体不同，其它的Tic蛋白(如Tic110和Tic40)并没有在这种复合体中，这种1 MDa转运复合体可能是在外膜Toc复合体和Tic110/Tic40/Hsp93复合体之间发挥作用(Kikuchi et al., 2009)。进一步的研究表明，拟南芥中这种1 MDa的称为通用转运易位子的Tic复合体，包括Tic20-I、Tic56、Tic100以及叶绿体编码的Tic214(又称ycf1)，这种复合体是以Tic20-I为核心组装起来的(Kikuchi et al., 2013)。其中Tic20完全包埋在1 MDa复合体中，Tic214可暴露于内膜两侧，Tic100在复合体外围的膜间隙内，Tic56很可能是嵌入到复合体中(图2) (Kikuchi et al., 2013)。

研究表明，这种1 MDa的超级复合体可以在没有前体转运的情况下独立存在(Köhler et al., 2015)。其中，Tic56和Tic100的缺失会导致1 MDa的Tic易位子表达水平大大降低，与*Tic20-I*突变体一样表现出白化表型，且植株在很小的幼苗期就死亡，说明Tic56和Tic100在叶绿体蛋白输入方面发挥非常重要的作用(Kikuchi et al., 2013)。然而，蛋白质组数据表明，在*atTic56*缺失的叶绿体中仍然有很多叶绿体蛋白存在(Köhler et al., 2015)。因此，从某种程度上来说，*atTic56*缺失情况下蛋白还是可以输入到叶绿体，暗示在内膜上可能存在另外的转运机制。

外膜Toc复合体与内膜Tic20/Tic21复合体之间如何关联仍不清楚。Tic22可以在转运过程中直接与转运肽接触(Kouranov et al., 1998)，但却不是Tic20/Tic21通道复合体的组分(Akita et al., 1997)。Tic22也通过Toc复合体穿过叶绿体外膜，但Tic22是如何从Toc复合体或Toc/Tic易位子转移进入膜间空间仍不清楚(Li and Chiu, 2010)。拟南芥中的Tic22有2个成员: atTic22-III和atTic22-IV。*atTic22-IV*的表达量高于*atTic22-III*。*atTic22-IV*的突变体与野生型没有明显的表型差异；而*atTic22-III*的突变导致叶片轻微发黄；双突变体的表型更加明显，尤其是在发育的早期，其叶绿体较小，同时不能有效地转运叶绿体蛋白(Kasmati et al., 2013)。这2种基因的表达都可以回补双突变体的表型(Rudolf et al., 2013)，表明这2个Tic22成员在功能上具有一定的冗余性。在低光条件下，*atTic22-IV*和*atTic22-III*的双突变体与野生型的差异很小；但在高光条件下，双突变体的表型更加明显，蛋白转运效率降低，表明Tic22可能主要是在高光等需要蛋白高速转运的情况下发挥作用(Rudolf et al., 2013)。

目前已知的易位子组分总结如图2所示(其中不包括定位于叶绿体基质的Toc12)。目前已知的叶绿体蛋白转运相关易位子的转运肽、预测结构域及可能的功能见表1。

6　展望

叶绿体功能的重要性以及结构的复杂性吸引着诸多

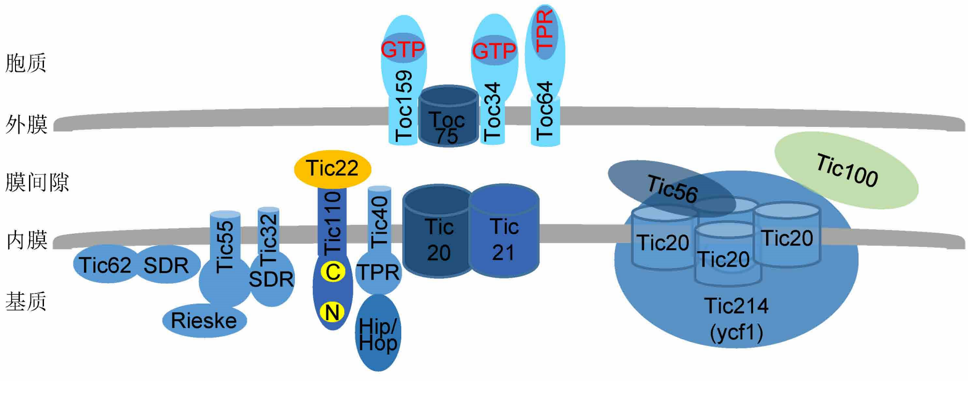

图2　转运易位子(改自Bölter and Soll, 2016)

外膜上的Toc复合体可以和内膜上Tic复合体进一步组装成Toc/Tic超级复合体，所以将Toc复合体放在中间位置。

Figure 2　Schematic presentation of the general translocation translocon(s) (modified from Bölter and Soll, 2016)

Both Tic complexes could interact with the Toc complex to assemble a Toc/Tic super complex, therefore the outer envelope translocation complex is set in the middle.

表1 易位子的转运肽、结构域及可能功能(Li and Chiu, 2010; Shi and Theg, 2011)

Table 1 The transit peptide, predicted domain(s) and proposed function(s) of translocon (Li and Chiu, 2010; Shi and Theg, 2011)

组分	转运肽	预测结构域	推测功能
Toc159	无	N端酸性结构域, 中部GTP酶结构域, C端膜锚定结构域	前体识别, 转运驱动马达
Toc34	无	N端GTP酶结构域, 1个跨膜结构域	前体识别
Toc75	N端双向转运肽	POTRA重复序列, β-桶状结构域	通道蛋白, 前体识别
Toc64	无	3个跨膜结构域, C端肽重复序列结构域, 酰胺酶结构域	受体蛋白
Toc12	N端转运肽	1个跨膜结构域, J结构域	辅助伴侣分子
Tic110	N端转运肽	模型1: 2个跨膜结构域, 伴侣蛋白结合域; 模型2: 6个跨膜结构域	通道蛋白, 前体蛋白及伴侣蛋白停泊
Tic22	N端转运肽	N/A	Toc和Tic支架
Tic40	N端转运肽	丝氨酸/脯氨酸丰富域, 1个跨膜结构域, 肽重复序列结构域, Hip/Hop结构域	输入马达伴侣蛋白
Tic32	无	NADP(H)结合域, 钙调素结合域	钙及氧化还原感应及调控
Tic55	N端转运肽	Rieske结构域, NADPH结合域, 2个跨膜结构域	氧化还原感应及调控
Tic62	N端转运肽	NADPH结合域, 疏水区, FNR结合域	氧化还原感应及调控
Tic21	N端转运肽	4个跨膜结构域	通道蛋白, 输入复合体组装, 离子转运
Tic214	无	6个跨膜结构域	蛋白转运
Tic20	N端转运肽	4个跨膜结构域	通道蛋白
Tic56	N端转运肽	N/A	输入复合体组装, 蛋白转运
Tic100	无	N/A	输入复合体组装

N/A: 不可用或未知。N/A: Not available or not known.

研究者投身其中, 正是由于他们的不懈努力, 叶绿体蛋白转运的各个方面才逐渐清晰地呈现在我们面前。在过去的几十年间, 随着分子生物学、全基因组测序以及蛋白质组学等技术的迅猛发展, 研究者在叶绿体蛋白转运方面已经取得了巨大的进步。利用先进的分子生物学技术以及遗传学工具, 人们可以得到并分析各种突变体植株, 从而对基因功能进行更深入的研究, 获得更全面的信息。但目前仍存在诸多问题需要进一步探索。例如, 虽已明确转运肽可以引导前体蛋白定向转运到叶绿体, 并调控不同的蛋白转运到不同发育阶段的叶绿体, 但转运肽中蕴含的信息仍需要进一步探索。再如, 我们在研究中发现水稻(*Oryza sativa*) Rubisco小亚基转运肽不能将所有外源蛋白转运至叶绿体, 说明叶绿体的成熟蛋白部分确实也参与前体蛋白的识别或转运过程(Rial et al., 2002; Ruprecht et al., 2010; Huang et al., 2016), 但目前对这一部分的研究尚未引起足够的重视。这也意味着在利用外源蛋白调控或改造叶绿体内代谢途径之前, 有必要先进行外源蛋白的叶绿体定位研究。此外, 联系内外膜的膜内空间组分是什么? 负责蛋白通过内膜的Tic通道究竟是哪一个? 这些问题都需要进一步深入研究, 才能使我们更深刻地理解叶绿体蛋白的转运。相信随着各种技术的发展, 特别是最近发展起来的更高效更易于操作的CRISP/Cas技术的不断完善与普及(Feng et al., 2013; Wang et al., 2017), 目前存在的叶绿体蛋白转运过程的诸多问题终将得以解决, 从而为我们呈现一幅更加清晰的各组分参与及协同调控叶绿体蛋白转运的图像。

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Research Advances in Protein Transport into Chloroplasts in Plant Cell

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Abstract Chloroplast function largely depends on chloroplast proteins in the plant cell; most chloroplast proteins are encoded by the nuclear genome and are transported to the chloroplasts after translation in the cytosol. The proteins needed to be correctly sorted in the cytosol first, then targeted to various compartments of the chloroplasts by the translocon at the outer envelope membrane of chloroplasts (Toc) complex and/or the translocon at the inner envelope membrane of chloroplasts (Tic) complex. This review focuses on cytosolic factors possibly involved in sorting chloroplast proteins and how Toc and Tic components translocate chloroplast proteins.

Key words chloroplast proteins, transport, translocon, cytosolic factors, sort

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