高等植物花色苷的液泡摄取机制

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摘 要:综述了高等植物细胞中花色苷被液泡摄取的机制。花色苷通过细胞质中定位于粗糙内质网细胞质面的多酶复合体合成后被膜包裹形成囊泡。这些囊泡主要向液泡移动,在移动中相互融合形成更大囊泡,最终将花色苷带到液泡膜的表面。在大多数情况下,花色苷经过液泡膜上的各种载体被迅速运进液泡。另外两种较少的是:(1)囊泡直接与液泡融合;(2)液泡膜自主形成大的管状内陷,使囊泡在内陷处指向液泡内腔"发芽"。在上述种种可能的具体过程中,花色苷以非修饰或修饰两种形式被摄入液泡。花色苷跨液泡膜运送可能通过 4 种模型实现,即由 ATP 结合盒型的载体介导、由依赖 pH 梯度的载体介导、由 24-kD 液泡蛋白前体衍生的蛋白质介导和由多重药物和有毒化合物排出家族的载体介导。据推测,不同植物利用不同的摄取机制将花色苷积累在液泡中,而多重机制也可能被单个植物种同时使用。

关键词:高等植物;花色苷;液泡摄取;机制

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Vacuolar sequestration mechanisms of anthocyanins in higher plants

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Abstract: This review sumed up the mechanisms of anthocyanins being sequestered into vacuole in the cells of higher plants. After being synthesized by the multienzyme complexes locating at the cytoplasmic face of the rough endoplasmic reticulum in the cytoplasm, anthocyanins are enwrapped by membrane to form vesicles which migrate mainly toward the vacuole, coalesce each other to form larger vesicles in the migration, and ultimately bring the anthocyanins to the surface of the tonoplast. In most cases, anthocyanins are expeditiously transported into vacuole by various transporters locating on the tonoplast. Other two minor possibilities are that: (1) the vesicles fuse directly with the vacuole; (2) the tonoplast may independently form a large tubular invagination from which the vesicles "bud off" into the vacuole lumen. In the concrete courses of above possibilities, anthocyanins are sequestrated into vacuole in two forms, namely non-modified and modified. The transtonoplast transport of anthocyanins may be accomplished by four models, namely mediated by ATP-binding cassette (ABC) type transporter, by pH-dependent transporter, by the proteins derived from the 24-kD vacuolar protein (VP24) precursor and by multidrug and toxic compound extrusion (MATE) family transporter. It is speculated that different plant species utilize different sequestration mechanisms to accumulate anthocyanins in vacuole, and multiple mechanisms may be simultaneously used in individual plant species.

Key words: higher plants; anthocyanins; vacuolar sequestration; mechanisms

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Belonging to flavonoids, anthocyanins are the colored end product of the general phenylpropanoid pathway and consist of anthocyanidins and saccharides(Holton & Cornish,1995;Zhao *et al.*,2003).

Anthocyanins have been found distributing only in the particular parts of a specific range of plants (Andersen & Jordheim, 2006). They are commonly found in angiosperms but are replaced by betacyanins in all families of Centrospermae except Caryophyllaceae (Harborne & Hall,1964;Harborne,1965,1986). Moreover, they are typically detected in flower and fruit tissues and in the superficial cells of organs such as leaves and stems. The anthocyanin-pigmented cells are typically restricted to the epidermis and hypodermis (Harborne,1973;Pecket & Small,1980).

It has been revealed that anthocyanins are related not only to plant life but also to human health. In plant life, anthocyanins provide brilliant pigments in order to attract insects or animals for pollination and seed dispersal (Harborne, 1976; Gould *et al.*, 1995; Grotewold,2006). Nowadays, anthocyanins are regarded as one of the most important alternatives to a number of synthetic dyes which have been applied in foods, cosmetics and medicines, and found to be very harmful to human health(Mazza & Brouillard,1987).

A series of evidences have been found to support that the subcellular site of anthocyanins synthesis in plant cell is the cytoplasm and anthocyanins are synthesized on the cytoplasmic face of the rough endoplasmic reticulum(rER)(Hrazdina *et al.*,1980;Wagner & Hrazdina, 1984; Hrazdina & Wagner, 1985; Winkel-Shirley, 1999, 2001). Nevertheless, anthocyanins are uaually observed not to exist in the cytoplasm(Xu *et al.*,2001), and normally accumulate in the vacuole (Harborne, 1976; Saunders & Conn, 1978; Wagner, 1979; Hrazdina & Jensen, 1992; Gould *et al.*, 1995; Mol *et al.*,1998;Kitamura,2006). Therefore, it is obvious that, after being synthesized in the cytoplasm of plant cell, anthocyanins are transported into vacuole.

Up to now, no comprehensive explanation concerning how anthocyanins are transported from their synthesis site, namely cytoplasm, into vacuole has been published. Nowadays, anthocyanins have been one of the targets of plant metabolic engineering with the objective of creating new or altering the properties of existing, colored compounds (Winkel-Shirley, 2001). Knowing adequately the mechanisms of the vacuolar sequestration of anthocyanins in the cells of higher plants is of great significance to biochemists and molecular biologists who are interested in realizing the effective regulations on the biosynthesis and storage of anthocyanins.

As a result, this review attempts to sum up the possible multiple mechanisms of antocyanins being sequestered into vacuole.

1 From cytoplasm to tonoplast, anthocyanins are transported in vesicles

1.1 After being synthesized in the cytoplasm, anthocyanins are enwrapped by membrane to form vesicles

It is tempting to speculate that, after anthocyanins are synthesized by the multienzyme complexes locating at the cytoplasmic face of the rER, they are enwrapped by membrane to form vesicles just like the tannin vesicles, and are transported within the cytoplasm toward the tonoplast by means of various vesicle-mediated processes (Baur & Walkinshaw, 1974; Parham & Kaustinen, 1977; Pecket & Small, 1980; Zobel, 1986; Nozzolillo & Ishikura, 1988; Ibrahim, 1992; Grotewold, 2001; Grotewold, 2004). These vesicles can be defined as the pre-vacuolar compartment (PVC) of anthocyanins.

However, the formation mechanism of the vesicles has not been elucidated clearly. The vesiculation process of anthocyanins probably begins just after the anthocyanins are synthesized. The anthocyanin-containing vesicles may be concretely produced via the formation of the double layered structures and the cytoplasmic structures may underlie the forming process (Facchini, 2001). Initially, in the cytoplasm, the tiny vesicles enwrapping anthocyanins are likely to originate from the rER, and rER is thought to be the initial accumulation site of the vesicles (Grotewold *et al.*, 1998).

1.2 The moving behavior of the anthocyanin-containing vesicles in the cytoplasm

It is totally unknown about the impetus which is responsible for the motion of the anthocyanin- containing vesicles through the cytoplasm. Theoretically, despite the probable help provided by cytoplasmic structures(Facchini, 2001), it is not possible that the vesicles move in the cytoplasm only by simple diffusion.

The moving direction of the anthocyanin- containing vesicles in the cytoplasm is believed mainly to be the vacuole. When they migrate toward the vacuole, they coalesce each other to form larger vesicles(Grotewold *et al.*, 1998; Lin *et al.*, 2003). Ultimately, these vesicles bring anthocyanins to the surface of the tonoplast(Grotewold *et al.*, 1998). Then, three possibilities are proposed to account for the fate of the anthocyanins: (1) they are expeditiously transported into vacuole by various transporters locating on the tonoplast, which is thought not only to be a joint action of transporters and vesicles but also to be the most dominant possibility in plant cells(Marrs *et al.*, 1995; Grotewold *et al.*, 1998; Grotewold, 2004; Kitamura, 2006); (2) the anthocyanin-containing vesicles fuse directly with the vacuole(Scott *et al.*, 1997; Grotewold *et al.*, 1998); (3) the tonoplast form, maybe in an autonomous fashion, a large tubular invagination from which the vesicles"bud off"into the vacuole lumen. The vacuolar invagination's lumen is continuous with the cytoplasm, making this inverse budding reaction equivalent to microautophagocytosis(Müller *et al.*, 2000).

It is also observed that not all of the vesicles are transported to tonoplast, some stay in the cytoplasm, which makes anthocyanins be compartmented within the cytoplasm and the cytoplasm display special colors (Nozzolillo *et al.*, 1988; Calderon *et al.*, 1993; Lin *et al.*, 2003; Goodman *et al.*, 2004). A case in point is that, in maize(*Zea mays*), the cyanidin-3-glucoside appears violet when localized in the vacuole, but in *bz2* corn, anthocyanin remains in the cytoplasm where it undergoes oxidation and polymerization reactions, the oxidized products appear tan-bronze on the kernels (Marrs *et al.*, 1995; Alfenito *et al.*, 1998). The fact that the anthocyanins enwrapped in the vesicles are acylated glycosides appears to be the precondition for the stay of the vesicles in the cytoplasm(Markham *et al.*, 2001).

2 Mechanisms of anthocyanins being sequestrated into vacuole through various transporters on tonoplast

2.1 Anthocyanins are transported through tonoplast in two forms

It has been found that the anthocyanins can be transported through tonoplast in two forms. One is non-modified, namely the anthocyanins are directly transported into vacuole, e. g. barley anthocyanin-glucosides(Klein *et al.*, 1996). The other is modified, namely the modification of anthocyanins is the prerequisite for their effective vacuolar uptake, and glycosylation or acylation of anthocyanins appear usually to be the precondition for the vacuolar uptake of anthocyanins (Matern *et al.*, 1986; Hopp & Seitz, 1987; Wink, 1997; Bartholomew *et al.*, 2002; Springob *et al.*, 2003). It was further found that the glucose residue attached to the molecules is not sufficient to act as a signal of the vacuolar sequestration of anthicyanins(Frangne *et al.*, 2002). However, nothing is known regarding the mechanisms by which different plant species selects different anthocyanin forms to finish the vacuolar uptake of the anthocyanins.

2.2 Possible models of anthocyanins being transported through tonoplast

2.2.1 Transport mediated by ATP-binding cassette(ABC) type transporter　A growing body of evidence has demonstrated that glutathione S-transferase(GST) proteins may be involved in the vacuolar sequestration of anthocyanins (Marrs et al, 1995). However, not all GSTs are responsible for the vacuolar sequestration of anthocyanins, e. g. in parsley, GST1 appears to act in the early steps of a UV light-dependent signal transduction pathway leading to chalcone synthase gene(*CHS*) expression(Loyall *et al.*, 2000).

Different functions have been described about

GST in the anthocyanin transport course mediated by ABC-type transporter. Previously, GSTs were thought to form glutathione-conjugates of anthocyanidin 3-glucosides (Marrs *et al.*, 1995), because GSTs can catalyze the addition of a glutathione (GSH) molecule to a heterocyclic organic anion (Edwards & Dixon, 2000). The corresponding GSH conjugate serves the dual purpose of increasing hydrophilicity and marking the molecule for transport by the multidrug resistance-associated proteins(MRP)(Ishikawa *et al.*, 1997). It was ever believed that covalent glutathionation is a prerequisite for sequestration through a glutathione pump (GS-X pump) in the tonoplast membrane (Martinoia *et al.*, 1993; Li *et al.*, 1995). But presently, GSTs are believed to function as cytoplasmic "escort" proteins without actually catalyzing GSH conjugate production in the cytoplasm, because they may bind anthocyanins through hydrophobic interactions and the binding reaction occurs near the tryptophan residues. Afterward, they escort the bound anthocyanins toward the tonoplast (Marrs *et al.*, 1995; Müeller *et al.*, 2000; Müeller & Walbot, 2001).

After recognized and glutathionated by GST, anthocyanins are transported through tonoplast and into vacuole by ABC transporter. The glutathione"tag"served as a marker for vacuolar sequestration of anthicyanins(Müeller *et al.*, 2000). The GST- or glutathione-GST- bound anthocyanins are then taken up into vacuoles through a membrane-localized specific transporter, namely a Mg^{2+}-ATP-energized glutathione-specific pump (GS-X pump) (Martinoia *et al.*, 1993; Ishikawa *et al.*, 1997; Edwards *et al.*, 2000), which is classified as belonging to the MRP subfamily and the superfamily of ABC transporters(Martinoia *et al.*, 1993; Marrs *et al.*, 1995; Lu *et al.*, 1997; Borst *et al.*, 1999; Rea *et al.*, 1998; Rea, 1999; Goodman et al, 2004; Grotewold, 2004). In general, the substrate recognition of ABC transporters involves not only the glutathione or glycosyl moieties but also the basic C_{15} core of anthocyanin(Klein *et al.*, 2000), and the amount of GSTs binding anthocyanins available in the cell maybe modulate the activities of GS-X pump, and the modulation could be via allosteric activation by intermediates, phosphorylation, or protein- transporter interactions(Frangne *et al.*, 2002). Finally, the vacuolar deposition of anthocyanins is accomplished by a cotransport mechanism with reduced GSH, analogous to the transport of vincristine in the liver(Loe *et al.*, 1998; Müeller *et al.*, 2000).

2.2.2 Transport mediated by pH-dependent transporter A specific transporter depending on a pH gradient across the tonoplast has been supposed for the transport of anthocyanins acylated with sinapic acid into vacuole(Hopp & Seitz, 1987). The H^+-electrochemical potential difference is established by the vacuolar H^+-ATPase(V-ATPase)and vacuolar H^+-pyrophosphatase (Rea & Sanders, 1987; Zhen *et al.*, 1997; Sze *et al.*, 1999). Both pumps catalyze electrogenic H^+-translocation from the cytosol into the vacuole to establish an inside- acid pH gradient(ΔpH)and an inside-positive electrical potential difference ($\Delta\psi$) (Bartholomew *et al.*, 2002). However, it still remains to be determined whether anthocyanin transport and vacuolar acidification are joined directly(Spelt *et al.*, 2002). Moreover, ABC transporter-driven sequestration of anthocyanins should theoretically result in much higher accumulation of the anthocyanins within the vacuole as compared with the antiport or potential-driven mechanisms, respectively (Rea & Sanders, 1987; Kreuz *et al.*, 1996; Frangne *et al.*, 2002).

2.2.3 Transport mediated by the proteins derived from the 24-kD vacuolar protein(VP24) precursor

The proteins derived from the VP24 precursor is believed to mediate the transtonoplast transport of anthocyanins into vacuole because the specific localization, accumulation of VP24 and the expression property of *VP24* in plant cells seem to be closely related with the vacuolar ingestion of anthocyanins. In the anthocyanin-containing vacuoles, VP24 was found to be localized in anthocyanoplasts(ACPs) and accumulate as one of the major vacuolar proteins(Pecket & Small, 1980; Nozue *et*

al. ,1995;Nozue *et al.* ,1997). *VP*24 expression is intimately correlated with the accumulation of anthocyanins in vacuoles, but no transient increase of phenylalanine ammonialyase (PAL) or chalcone synthase (CHS) was determined (Lawton *et al.*, 1983; Chappell & Hahlbrock, 1984; Xu *et al.*, 2001). Therefore, it can be thought that the proteins derived from the VP24 precursor is probably involved in the transport or steady accumulation of anthocyanins in vacuoles (Xu *et al.* ,2001).

2.2.4 Transport mediated by multidrug and toxic compound extrusion (MATE) family transporter　At present, MATE family transporter is believed to mediate the transtonoplast transport of anthocyanins into vacuole mainly because, in *Arabidopsis*, *tt*12 is proved to encode a member of the MATE family transporters which is involved in the sequestration of flavonoids, maybe including anthocyanins, into vacuole (Brown *et al.* ,1999; Debeaujon *et al.* ,2001). But the universality of MATE family transporter in the transtonoplast transfer course of anthocyanins into vacuole still needs to be further corroborated.

Taken together, above four models are virtually difficult to reconcile. Now, it should be reasonably hypothesized that different plant species make use of different sequestration mechanisms to congregate anthocyanins in vacuole, and manifold mechanisms may simultaneously be used in individual species (Martinoia *et al.* ,2000; Müeller & Walbot, 2001).

Understanding the molecular mechanisms involved in the transtonoplast transport of anthocyanins into vacuole stand for a radical, yet weakly illuminated, problem in botany. Establishing the real pathways involved in the sub-cellular trafficking of anthocyanins is apparently an essential for the fruitful engineering of anthocyanin metabolism in higher plants.

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