Cloning of plastid division gene *GlFtsZ* from *Gentiana lutea* and its expression during petal development*

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Abstract A full-length cDNA of *GlFtsZ* was isolated by screening the cDNA library of *Gentiana lutea*. Analysis of the deduced amino acid sequence encoded by *GlFtsZ* indicated that GlFtsZ protein possesses the typical conservative motifs existed in all FtsZ proteins. The existence of putative plastid transit peptide in its N-terminus suggested that GlFtsZ might function inside of plastids. With the developmental process of petals of *Gentiana lutea*, the expression of plastid division gene *GlFtsZ* declined gradually, whereas the expression of carotenoids biosynthesis gene *Zds* increased obviously; meanwhile, in contrast to the increment of carotenoids, the content of chlorophyll in petals decreased sharply. The chloroplasts turned into chromoplasts, and the color of petals also turned from green to golden. All of these results suggested that the expression of *GlFtsZ* is accompanied with the development and differentiation of plastids.

Keywords: *FtsZ* gene, plastids, chromoplasts, *Gentiana lutea*.

Plastids are a group of specific organelles in plant cells. Under different conditions, proplastids can develop into distinct plastid types: chlorophyll-containing chloroplasts, cartenoid-containing chromoplasts, and colorless leucoplasts. Leucoplasts can also be distinguished by the compounds stored as energy reserves. For example, starch-storing plastids are called amyloplasts, protein-storing plastids are proteinoplasts and lipid-storing plastids are elaioplasts. In addition to the photosynthesis carried out by chloroplasts, portions of several major plant metabolism pathways, such as lipid biosynthesis and amino acid metabolism, occur in plastids. The development and growth of plastids are closely related to the accumulation of amino acid, protein and carotenoids, and also make great contribution to many important characteristics of plants[1]. The studies of plastid morphology have been carried out for virtually one hundred years; however, the mechanism of plastid division is still poorly understood. Some evidence supports the view that plastids originate from a cyanobacterium-like prokaryote[2~4].

The researches on the prokaryotic cell division have provided valuable clues to dissect the division mechanism of plastids. In 1980, several Fts (Filamenting temperature sensitive) genes were isolated from *E. coli fts* mutants. Because of the inhibition of cell division, these mutants displayed a long, undivided phenotype, and divided nucleus distributed along the filamentous cell regularly[5,6]. Given that plastids originating from cyanobacteria, it is expected that FtsZ, which is the key protein in prokaryotic cell division, may also play an important role in the division process of plastids in higher plants. In 1995, the homolog of prokaryotic cell division gene *ftsZ*, *AtFtsZ1-1*, was isolated firstly from *Arabidopsis thaliana*[7]. The function of plant FtsZ in the division of plastids was also confirmed by the antisense expression of *AtFtsZ1-1* and *AtFtsZ2-1* in *Arabidopsis thaliana*[8], and by the homologous gene knockout of *PpFtsZ1* in *Physcomitrella patens*[9]. All of these studies provide a good start point to further understanding the molecular regulation of plastid division.

Here we report the cloning of a full-length plastid division gene *FtsZ* cDNA from *Gentiana lutea*. Analysis of amino acid sequence indicated that GlFtsZ protein possessed a putative transit peptide and might function inside of plastids. The relationship between the expression patterns of plastid division gene *GlFt-*

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sZ and cartenoids synthesis gene *Zds*, and the development of chromoplasts, as well as the contents of chlorophyll and carotenoids were also investigated during the development of petals of *Gentiana lutea*.

1 Materials and methods

A *G. lutea* cDNA library was constructed with the flowers collected at different development stages. A pair of degenerate PCR primers corresponding to the known FtsZ conservative amino acid sequences was synthesized, they were F1: 5'-AA(T/C) GGI GTI AA (T/C) (C/A)GI ATG AT-3' and RF1: 5'-TC IAC IAC IGC ICC (G/A)AA (A/G/T) AT-3'. RT-PCR was performed by the TaKaRa RNA PCR kit (AMV), except that the RF1 primer was used as the reverse transcription primer. PCR reaction conditions were 30 cycles of 94℃ 20 s, 50℃ 30 s, 72℃ 1.5 min, and an additional extension at 72℃ for 15 min. The products of PCR were purified with Wizard® PCR preps DNA purification kit and cloned by TA cloning kit (Invitrogen). A 800 bp cDNA fragment was isolated from positive clones and used as the probe to screen the cDNA library. After three rounds of screening, 15 positive clones were identified; 4 out of these clones were sequenced and a full-length cDNA was obtained. The homology searches were performed with BLAST at http://www.ncbi.nlm.nih.gov/blast/, the search of protein motifs was preformed with PROSITE at http://www.tokyocenter.genome.ad.jp/SIT/MOTIF.html, and the prediction of subcellular localization of GlFtsZ was performed with PSORT at http://psort.ims.u-tokyo.ac.jp.

Total RNA extraction and Northern blot hybridization were performed as described in Ref.[10]. The contents ($\mu g/g \cdot FW$) of chlorophyll and carotenoids were determined by HPLC[11]. The standard samples of chlorophyll a, chlorophyll b and β-carotene used were products from Sigma. Images of the tissues and cells were obtained using a differential interference contrast (Nomarshi) optic. Microscopic observations and photography of flowers were preformed with an Olympus microscope.

2 Results and discussions

2.1 Identification and analysis of *GlFtsZ*

A 2037 bp cDNA, which contained a 1452 bp open reading frame encoding for 483 amino acids (Fig. 1), was isolated from the *Gentiana lutea* cDNA library. It was designated as *GlFtsZ* and deposited into GenBank under the accession number AF205859. Analysis of its deduced amino acid sequence indicated that the N-terminal extension of GlFtsZ had the typical characteristics of plastid transit peptide[1,7], suggesting that the GlFtsZ might function inside of plastids.

To further analyze the functional and conservative regions of GlFtsZ, an alignment including FtsZ sequences from *A. thaliana*, *P. patens*, *Pisum sativum*, *Nicotiana tabacum* and *E. coli* was performed with CLUSTRAL W1.7 software. The divergence of all FtsZ proteins existed mainly in the N- and C-terminal regions, whereas the functional region in the middle was highly conserved across phyla (Fig. 2). GlFtsZ contained two conservative motifs that had been found in FtsZs, namely, FTSZ-1: VIGVGGGGSNAVNRM (PROSITE; PS01134) and FTSZ-2; FATAGMGGGTGS/TGAAPV/IV/IA (PROSITE; PS01135). Moreover, a signature motif existing both in tubulin and FtsZ was also included in the FTSZ-2, say, GGGTGSG (PROSITE; PS00277). The function of FTSZ-1 was still kept unknown. Because of the similarity to tubulin, the function of FTSZ-2 was postulated to relate with the GTP binding capability and GTPase activity of FtsZ proteins. This postulation had been verified in the studies of prokaryotic FtsZ proteins[12,13]. Furthermore, all of the ever known plant FtsZs have an N-terminal extension, which also is an obvious feature to distinguish the eukaryotic FtsZs from prokaryotic FtsZs. After peforming the pairwise comparisons between GlFtsZ and other known plant FtsZs, it was found that the identity between GlFtsZ and AtFtsZ 1-1[7], PsFtsZ[14], NtFtsZ1-1, and NtFtsZ1-2 was above 80% at the amino acid level, whereas the identity between GlFtsZ and AtFtsZ2-1[8] or PpFtsZ1[9] was under 65% at the amino acid level. This finding suggested that the FtsZ proteins in plants could be classified into two different groups, with or without plastid transit peptide at their N-terminal extension, based on the sequence similarity. By the criteria, Osteryoung identified two small *FtsZ* gene families in plants[8], and found that the suppression of either of the two families could inhibit normal plastid division[8]. Thus, a model was put forward to describe the functional pattern of FtsZ proteins in the division of plastids. The FtsZ that possesses the plastid transit

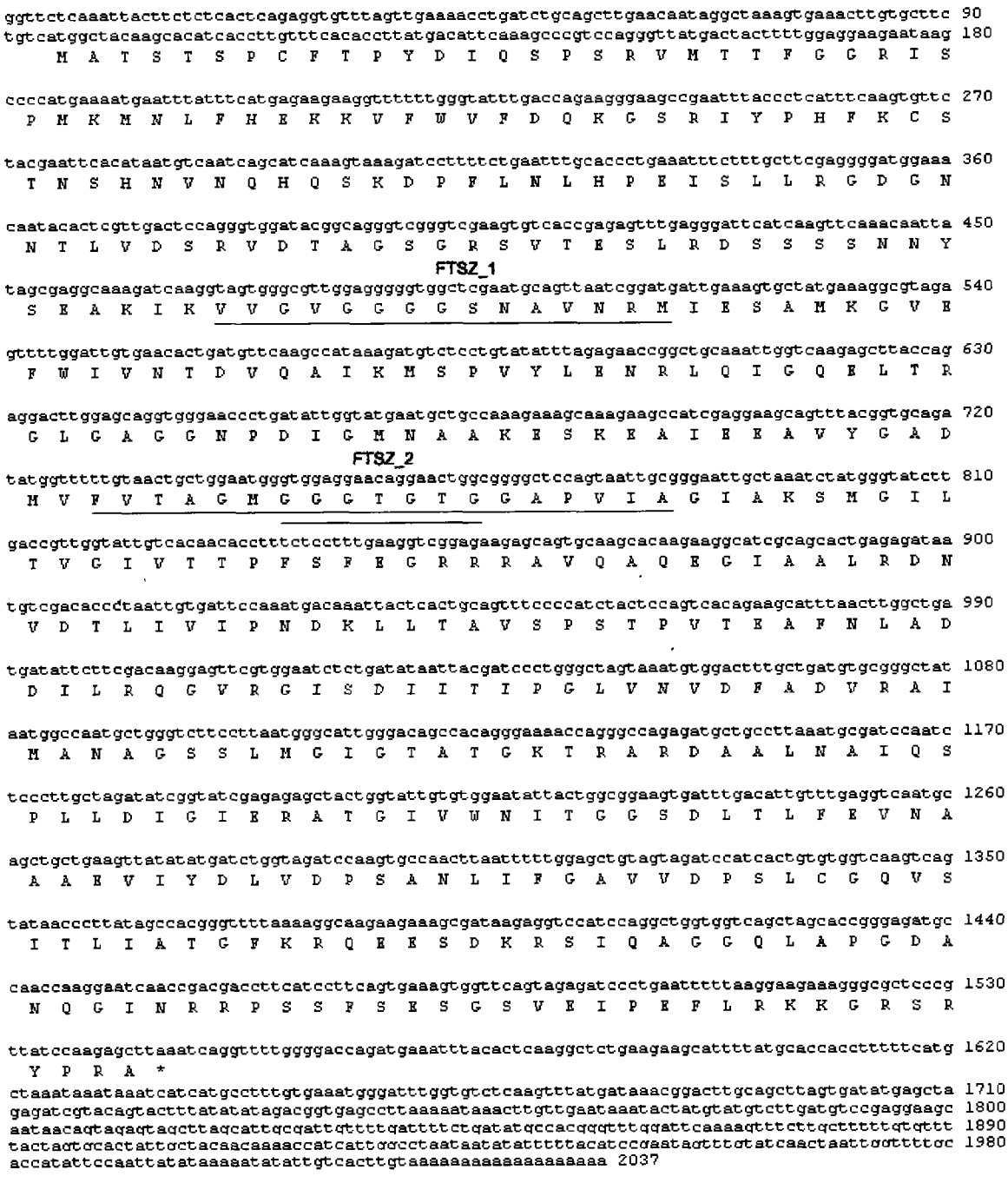

Fig. 1. Sequence of *GlFtsZ* cDNA and its deduced amino acid sequence. Underlined indicate two conservative motifs of FtsZ; double lined show the common GTP-binding sites of FtsZ and tubulin; * indicates a stop code.

peptide could be targeted into plastids, whereas the FtsZ without plastid transit peptide stayed in cytoplasm, the coincident constriction of two groups of FtsZs resulted in the division of plastids[8]. Because the N-terminus of GlFtsZ has some typical plastid transit peptide characteristics, we speculate that GlFtsZ might function inside of plastids. Furthermore, a cDNA fragment displaying 85% identity with *GlFtsZ* at the nucleotide level was also isolated from the *G. lutea* cDNA library, which implies that two groups of FtsZs might co-exist in *G. lutea*.

2.2 Expression patterns of *GlFtsZ* and *Zds* in the developmental process of petals

For the purpose of description, the development of *G. lutea* flower can be divided into five stages (Plate I (a)): (1) length of flower is <1.5 cm, the color of petals and sepals is deep-green; (2) length of

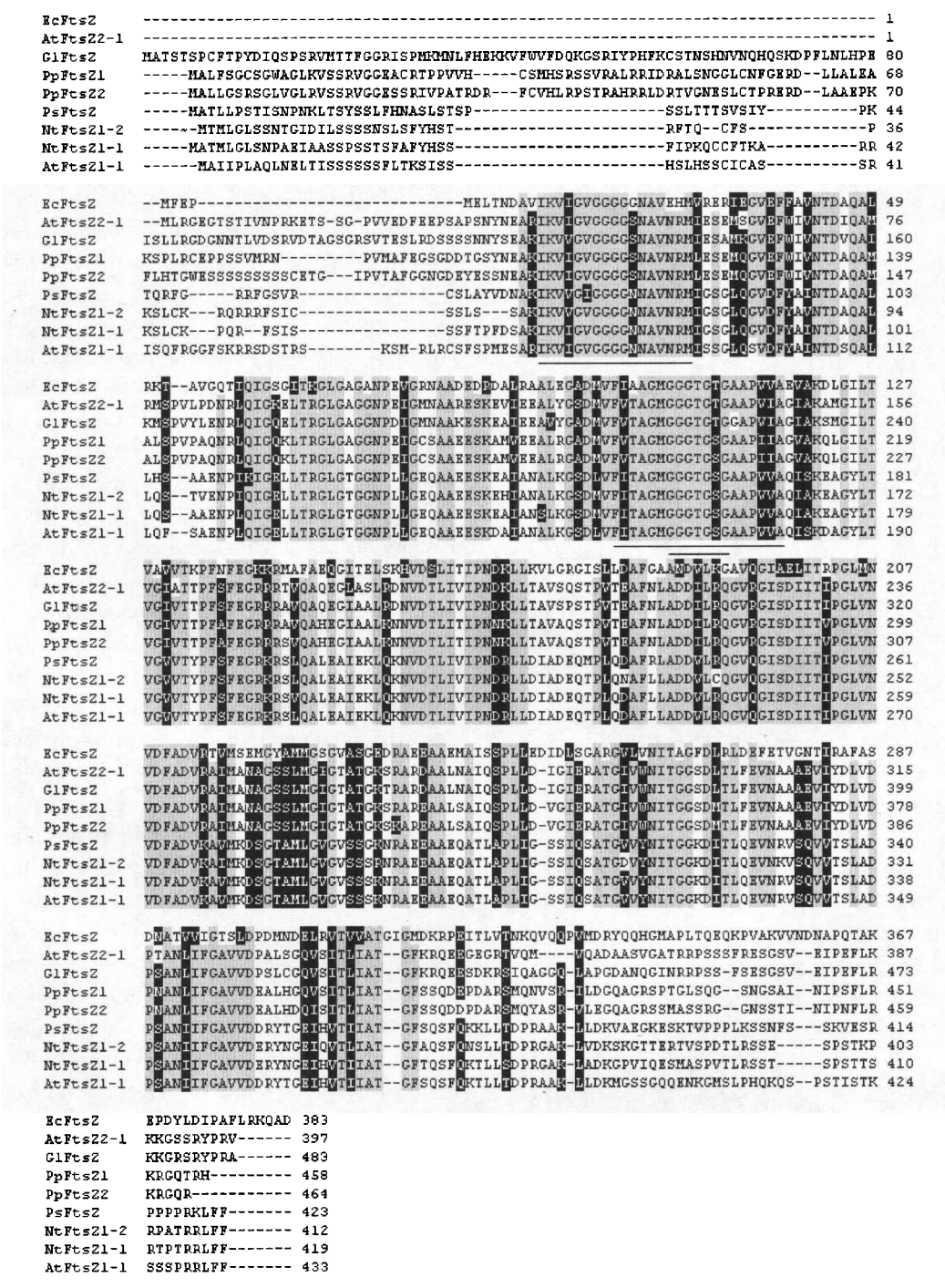

Fig. 2. Alignment of amino acid sequences of different FtsZs. GenBank accession numbers for the proteins in the alignment are: *E. coli* (EcFtsZ, 000119); *A. thaliana* (AtFtsZ1-1, U39877, AtFtsZ2-1, AF089738); *P. patens* (PpFtsZ1, AJ001586, PpFtsZ2, AJ249140); *P. sativum* (PsFtsZ Y15383); *N. tabacum* (NtFtsZ1-1, AJ133453, NtFtsZ1-2, AF205858); *G. lutea* (GlFtsZ, AF205859). Underlined sequences indicate the two conservative motifs of FtsZ; double underlined indicates the common GTP-binding sites of FtsZ and tubulin; gray colored indicate residues conserved among FtsZ proteins; black colored indicate residues similar among FtsZ proteins.

flower is about 1.5～2.5 cm, the color of petals and sepals is green; (3) length of flower is about 2.5～3.5 cm, the color of petals is green-yellow; (4) length of flower is >3.5 cm, the color of petals is yellow, the petals and sepals begin to loose; and (5) the petals are yellow and expand fully. At stages 1～2, the volume of petal cells is small and the cells are tightly arranged. There are numerous chloroplasts in

the cells, and they are distributed evenly on the inside surface of cytoplasmic membrane (Plate I (b)). At stages 3 ~ 5, the cells of petals become bigger and longer than those at stages 1 ~ 2. The green color of chloroplasts fades gradually. It is full of numerous golden chromoplasts inside petal cells (Plate I (c)). The content of chlorophyll declines sharply with the development of petals, whereas the content of carotenoids increases gradually (Fig.3).

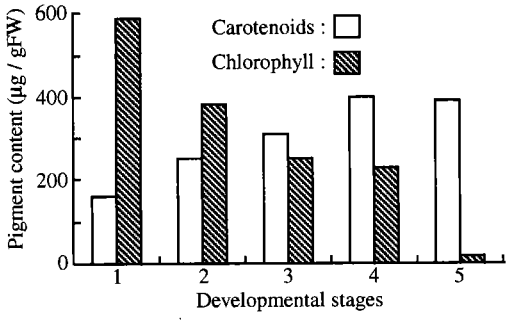

Fig.3. Variations of pigment contents during the development of *G. lutea* flowers. 1 ~ 5 represent different developmental stages as described in text.

The expression patterns of plastid division gene *GlFtsZ* and carotenoid synthesis gene *Zds* (which encoding ζ-carotene desaturase) associated with the development of petals were analyzed by Northern hybridization. The results showed that the expression of *GlFtsZ* was high at stages 1 ~ 2, it declined at stage 3 and became undetectable at stages 4 ~ 5 (Fig.4). In contrast, the expression of *Zds* increased with the developmental process of petals (Fig.4).

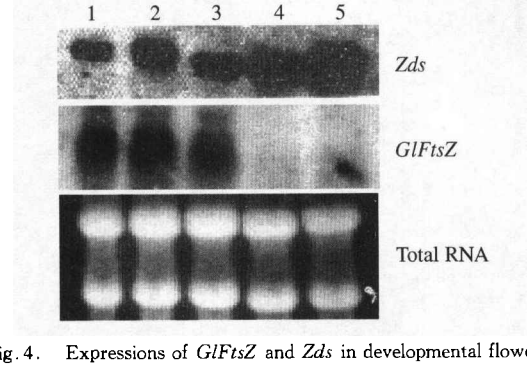

Fig.4. Expressions of *GlFtsZ* and *Zds* in developmental flowers. 1 ~ 5 represent different developmental stages as described in text.

Different genes expressed following specific spatial and temporal sequences control the growth and development of plants. Flower(s) is the sexual reproduction organ of plants; they become colorful with the growth and development. Carotenoids exist in all plant tissues, and the different kinds and contents of carotenoids accumulated in the plastids are the main reason for the different yellow colors of flowers. In fact, the change of different colors of flowers reflects the growth and development of plastids and the changes of pigment metabolisms.

The biosynthesis pathways of carotenoids mainly act within plastids. Carotenoids have well-known biological properties including light harvesting and protection against photo-oxidation[15]. The biosynthesis of carotenoids involves a series of complicated biochemical reactions, for instance, when the fruits of tomato enter into the period of color changing, the expressions of carotenoids biosynthesis genes such as *PSY*, *PDS*, and *GGPS* increase obviously. When the fruits enter into the mature period, the content of carotenoids in fruits can increase by 10 ~ 15 times. Among all carotenoids, the content of lycopene even increases by 300 times[16,17]. In the all carotenoids biosynthesis pathways, ζ-carotene desaturase encoded by *Zds* gene catalyzes in the reaction of the formation of neurosporene—a yellow color carotenoid molecule. With the growth and development of *G. lutea* flowers, the increment of *Zds* expression facilitates the synthesis of yellow carotenoids and promotes the transformation of flower color from green to brightly yellow. It is noteworthy that there are numerous uneven-sized yellow granules in the cells of mature petals (Plate I (c)). If these yellow granules came from the direct transformation from chloroplasts to chromoplasts by accumulation of carotenoids, why did these even-sized chloroplasts turn into uneven-sized chromoplasts during the differentiation? Furthermore, the decline of *GlFtsZ* expression should lead to the decrement of division events of chloroplasts, and then the numbers of chromoplasts in mature petals should not change so obviously. Two possible reasons might explain this phenomenon: first, the growth and development of plastids are asynchronous. In the young petal tissues, the strong expression of *GlFtsZ* indicates the active division of chloroplasts (Fig. 4). Along with the developmental process the decline of *GlFtsZ* expression will lead to the cessation of chloroplast division and the formation of uneven-sized chloroplasts. However, the small chloroplasts, which result from the asynchronous development with a little chlorophyll accumulation have to be observed under a microscope. Following the accumulation of carotenoids inside these small chloroplasts, they will become visible golden chromoplasts. Second, some yellow granules may not originate from chloroplasts.

There is some evidence showing that the yellow granules can also be formed by synthesis and accumulation of carotenoids within elaioplasts in cytoplasm of unicellular algae[15]. But now we cannot exclude the possibility that the yellow granules observed in the cells of *G. lutea* petals come from the elaioplasts accumulated with carotenoids in cytoplasm by our experimental results.

The changes of flower colors, in fact, are the reflection of development and maturation of petal cells. It has been proved that the division of plastids is active in the young petal tissues, but at rest in the highly differentiated and mature cells[18]. In *G. lutea*, the change of flower color from green to gold is also an indication of the differentiation and maturation of related cells. In this process, the expressions of *GlFtsZ* and *Zds* are highly coincident: the decline of *GlFtsZ* expression regulates the decrement of chloroplast division events and the maturation of cells. The increment of *Zds* expression is the essential reason for the increment of carotenoids synthesis and the change of flower color. Obviously, some other factors that regulate the synergic expression of these genes in plants should exist and exert their effects on this process. It is undoubted that finding and characterizing of these factors will help us to further understand the mechanism of gene expression and regulation in plants.

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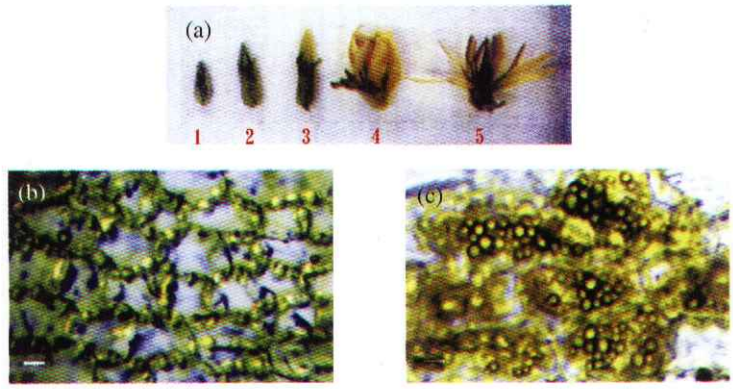

Plate Ⅰ. Transformations of plastids during the development of *G. lutea* flowers
(a) Flowers at different developmental stages; (b) the petal cells at the stages 1~2, chloroplasts distributed evenly along the inner cytoplasmic membrane; (c) the petal cells at the stages 3~5, chloroplasts have been turned into chromoplasts. Bars are 10 μm