

An *Arabidopsis* embryonic lethal mutant with reduced expression of alanyl-tRNA synthetase gene

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ABSTRACT

In present paper, one of the T-DNA insertional embryonic lethal mutant of *Arabidopsis* is identified and designated as *acd* mutant. The embryo development of this mutant is arrested in globular stage. The cell division pattern is abnormal during early embryogenesis and results in disturbed cellular differentiation. Most of mutant embryos are finally degenerated and aborted in globular stage. However, a few of them still can germinate in agar plate and produce seedlings with shorter hypocotyl and distorted shoot meristem. To understand the molecular basis of the phenotype of this mutant, the joint fragment of T-DNA/plant DNA is isolated by plasmid rescue and Dig-labeled as probe for cDNA library screening. According to the sequence analysis and similarity searching, a 936 bp cDNA sequence (EMBL accession#: Y12555) from selected positive clone shows a 99.8 % (923/925bp) sequence homology with Alanyl-tRNA Synthetase (*AlaRS*) gene of *Arabidopsis thaliana*. Furthermore, the data of in situ hybridization experiment indicate that the expression of *AlaRS* gene is weak in early embryogenesis and declines along with globular embryo 'development' in this mutant. Accordingly, the reduced expression of *AlaRS* gene may be closely related to the morphological changes in early embryogenesis of this lethal mutant.

Key words: *AlaRS* gene, embryonic mutant, *Arabidopsis*.

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INTRODUCTION

The embryogenesis of flowering plant *Arabidopsis* is beginning with polarity formation and asymmetric division of zygote. The early events of embryo development involved oriented cell division and cell type specialization which are critical for further tissue differentiation and primordia initiation. However, molecular approach for these early events has been difficult because of the small size of embryo. Recently, embryonic lethal mutants (or embryo-defective mutants) provide unique opportunity to study the essential functions and regulatory process in plant embryo development. The T-DNA and transposon insertional mutants further facilitate the cloning and subsequent molecular characterization of these genes involved in early development of embryo (Castle and Meinke 1993)[1].

By using embryonic mutants, the cell division pattern and establishment of pattern formation in early embryogenesis of *Arabidopsis* has been investigated. In *gnom* mutant, the zygote divided into two similar daughter cells, instead of unequal base cell and apical cell in wild type embryo. The near symmetric division then raised a disturbed cell arrangement and finally resulted in abnormal seedlings with both apical and basal structures being absent (Mayer et al. 1991, Mayer et al. 1993)[2, 3]. In the mutant of *monopteros*, the abnormal cell division pattern in central and basal regions of embryo led to seedlings that were lack of hypocotyl, root and root meristem (Berleth and Jurgens 1993)[4]. In *knolle* mutant, again owing to abnormal cell divisions and cell enlargement during early embryogenesis, the epidermis was not separated from the inner cell mass by periclinal division. In this case, the mutant seedlings lacked a well-formed epidermis layer (Mayer et al. 1991, Lukowitz et al. 1996)[2, 5]. These investigations provided valuable information on early embryogenesis and establishment of pattern formation.

To understand further the regulation of early development of plant embryo, several genes from embryonic mutants have been isolated and sequenced. The *EMB30/GNOM* gene was reported as yeast *Sec7P* secretory protein gene (Shevell et al. 1994)[6] and as yeast *YEC2* gene (Busch et al. 1996)[7] respectively according to partial sequence similarity. The *KNOLLE* gene product was reported as Syntaxins related protein which is involved in vesicular trafficking (Lukowitz et al. 1996)[5]. In this present paper, we will report the reduced expression of alanyl tRNA synthetase gene in an *Arabidopsis acd* mutant with abnormal cell division pattern in early development of embryo.

MATERIALS AND METHODS

Plant materials

The wild-type seeds of *Arabidopsis thaliana* ecotype Columbia and seeds of 3 embryonic lethal mutants were kindly provided by NASC (The Nottingham Arabidopsis Stock Center). These mutants were originally donated by Dr. Csaba Koncz (Max-Planck Institute) as T-DNA insertional embryonic lethal mutants. Among them, mutant of N4081, was further characterized in our laboratory and designated as *acd* mutant due to its abnormal cell division and differentiation in embryo

development. This mutant was used as material in this paper.

Plant growth conditions

Seeds were placed in 4 °C for 4 d to break dormancy and then sown on wet mixture of soil and vermiculite in plastic pots. The plants were maintained in phytotron at 22 °C under 16 h light/8h dark cycle. The seedlings were watered daily with nutrients as described by Heath et al. (1986)[8].

Light and electronic microscopy

Whole mount-clearing observation: The siliques in different development stages were opened with needles under dissecting microscope and the ovules were taken and transferred onto the surface of the drops of Hoyer's solution (gum Arabic 7.5 g; chloral hydrate 100 g; glycerol 5 ml; water 30 ml) on a slide. Then, the slide was covered with cover slip and observed after several hours.

Light microscopy: The samples were fixed in 3% glutaraldehyde with phosphate buffer (pH 7.3) at 4 °C overnight. The post fixation was carried out in 1% osmium tetroxide fixative for 2 h. Then, followed by dehydration and infiltration, the samples were embedding with EPON-812 resin and sliced into 3 μm thick sections using microtome (LKB, Bromma, Sweden). The sections were further stained with methylene blue and observed under Olympus microscope.

Electronic Microscopy: After screening under light microscope, the samples with correct orientation and appropriate developing stages were further sectioned using KCD-2 ultratome. The sections with thickness of about 60 nm were then observed and photographed under JEM-100 cx-2 transmission electron microscope.

Observation of seed germination

The seeds were surface sterilized by saturated bleaching powder solution and rinsed with 70 % ethanol. After successive wash with sterilized water, the wild type and mutant seeds were transferred into 0.3 % agar plate contained Murashige-Skoog basal medium (without growth regulator) for germination in growth chamber at 22 °C, under 16 h illumination.

Probe labeling and cDNA library screening

The joint fragment of T-DNA and Plant DNA from mutant plant has been cloned and sequenced in previous paper (Yao et al. 1996)[9]. The junction fragment of T-DNA and plant DNA was obtained by ClaI and XbaI digestion of above clone and further labeled by digoxigenin-UTP as probe using Dig DNA labeling and detecting kit (Boehringer Mannheim).

The cDNA library λ PRL-2 from *Arabidopsis* ecotype columbia was provided by *Arabidopsis* biological Resource Center, which was originally donated by Dr. T. Newman and associates. The cDNA library was screened by Dig labeled probe and the independent positive clones were isolated repeatedly.

The positive clones were checked with agarose electrophoresis and Southern blot, then, the insert fragment was cloned into pBluescript SK⁺ for sequence analysis by using Applied Biosystem 373A Sequencer.

Non radioactive whole-mount in situ DNA/RNA hybridization

The procedure of this technique was developed on the basis of whole-mount clearing (Zhu et al. 1990)[10] and in situ hybridization (Coen E. et al. 1990)[11] techniques, which included following steps:

1) Fixation. The unmaturing seeds were harvested and fixed in a solution of acetic acid/methanol (1:3) for several h and then kept in 70 % ethanol.

2) Ethanol series dehydration. The specimen in 70 % ethanol were passing through the following steps: 70 % ethanol/0.85 % NaCl, 1.5 h on ice; 85 % ethanol/ 0.85 % NaCl, 1.5 h on ice; 95 % ethanol, 1.5 h, 4 °C; 100 % ethanol, 1.5 h 4 °C; 100% ethanol, over night, 4 °C.

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3) Opposite hydration to 50 % ethanol/0.85 % NaCl.

4) Pretreatment of hybridization. The samples were further treated by acetone and acetic anhydride through following steps: 30 % ethanol/0.85 % NaCl, 1-2 h; 0.85 % NaCl, 0.5 h; acetone, 0.5 h; 1 × buffer (10 × buffer contains: 1.3 sl M NaCl; 0.07 M Na₂HPO₄; 0.03 M NaH₂PO₄), 0.5 h; acetic anhydride (100 μl acetic anhydride in 5 ml 0.1 M triethanolamine, pH 8.0), 0.5 h; 1 × buffer, 10 min; 1 × buffer, 10 min.

5) Hybridization and immuno-colour reaction. After the pretreatment as mentioned above, the sample can be put in hybridization buffer contained the probe and then followed the steps for hybridization and immuno-colour reaction in an eppendorf tube according to the instructions of Dig DNA labeling and detecting kit (Boehringer Mannheim). The digoxigenin labeled DNA probe used for cDNA library screening as mentioned above was denatured and applied for hybridization in 68 °C.

6) Clearing. The immuno-colour reaction was stopped by washing and dehydration (H₂O, 15 min; 70 % ethanol, 15 min; 95 % ethanol, 15 min; 100 % ethanol, 15 min). The samples were finally cleared by passing through: 50% ethanol / 50 % methyl salicylate (1:1) mixture, 2 h; 100 % methyl salicylate, 2 h or overnight, if necessary.

RESULTS

The embryonic lethality of acd mutant is caused by mutation in a single recessive gene

Three mutants were provided by NASC (The Nottingham Arabidopsis Stock Center) previously as T-DNA insertional embryonic lethal mutant. The segregation of hygromycin resistance was 3:1 for resistance and sensitive seeds according to NASC. In our previous paper (Yao et al. 1996)[9], the copy number of T-DNA insertion was further checked. The transgenic plant, DNA was extracted, EcoRI digested and then assayed by Southern blot using Dig-labeled PBR328. The mutant N4081 which had only a single hybridized band representing one T-DNA insertional site was chosen for further investigation.

In this paper, the segregation ratio of aborted and normal seed was investigated under dissecting microscope. The aborted and normal seeds in heterozygous siliques can be distinguished by about 9d after anthesis. At this time, the aborted seeds were smaller in size and milky white in color, while the normal seeds were larger than aborted seed and turned to green. For segregation ratio assay, 20619 seeds from 191 heterozygous plants were examined. The result showed a segregation ratio of aborted seeds to normal seed of 1:3.04 (5098:15521) on average. Accordingly, the mutant appeared to be segregated for a single recessive gene.

The acd mutant embryo development is arrested at globular stage

By means of whole mount-clearing technology, the arrested stage of mutant embryo development was observed. The seeds from single silique were observed in same slide and both wild type and heterozygous siliques at different developmental stage were compared.

In heterozygous plant, the developmental stages of both mutant and normal embryos were similar at the beginning in smaller and younger siliques, and then, when

the normal embryos (Aa, AA) were getting mature, the mutant embryos (aa) was still arrested in globular stage. In Fig 1, a, b, the normal embryos already developed into walking stick and mature cotyledon stage respectively, while the mutant

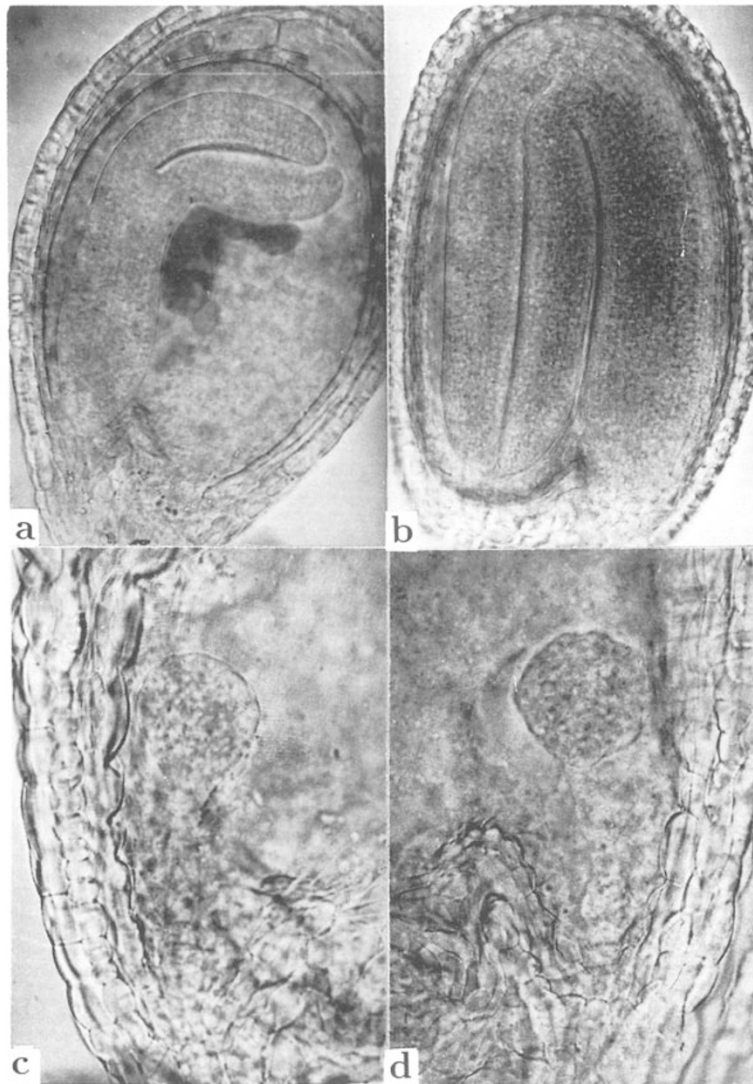


Fig 1. Arrested stage of development of acid mutant embryo

All of the seeds in single heterozygous silique were observed in same slide by means of whole mount-clearing technology. When the normal embryo (AA, Aa) already developed into walking stick stage (a) and mature stage (b), while the mutant embryo (aa) still arrested in globular stages (c), (d). Two globular stage mutant embryos and two normal embryos were in same silique. (a), (b) 250 ×; (c), (d) 520 ×.

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embryos were still kept in globular stage (Fig 1, c, d). The mutant globular embryos appeared slightly larger and more rough in surface than wild type embryos at this stage. However, there are no significant difference in appearance between them.

The abnormal cell division pattern in early development of acd mutant embryo

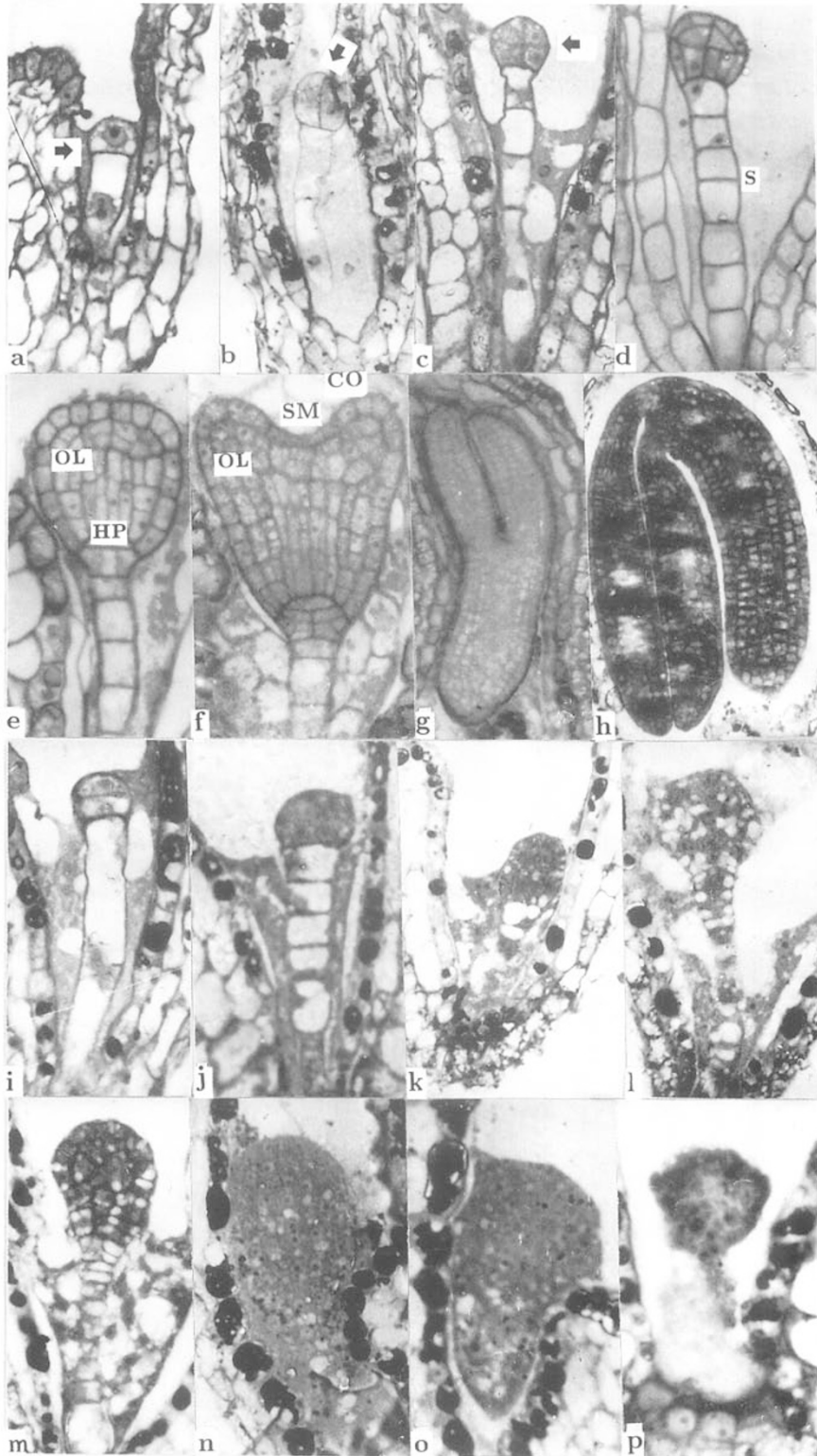
To trace the primary changes of early development of mutant embryo, the developmental course of wild type embryo and mutant embryo were compared by histological sections under light microscope.

In wild type embryo, the proembryo development was passing through typical asymmetric division of zygote (Fig 2a), vertical, horizontal cleavage divisions (Fig 2, b, c) and tangential division (Fig 2d) to form 8 cell proembryo. The abnormal cell division pattern could be observed early in development of mutant embryo. Instead of above typical cell division pattern, only irregular cell division can be observed (Fig 2, i, j, k), leading to abnormal cell arrangement and misoriented cell divisions. Therefore, further anticlinal and periclinal division were also seriously influenced (Fig 2, l, m). In fact, these cells were undergoing degeneration (see the further observation with electronic microscopy).

The misoriented cell division and abnormal cell arrangement also interfered the apical-basal axis pattern formation. Normally, in wild type embryo, O-line(ol) notably divides the developing embryo into two parts (Fig 2, e, f). The upper part of the embryo will form the cotyledon and shoot meristem and the basal part will develop into hypocotyl and most part of embryonic root (Jurgens 1995)[12]. While, in *acd* mutant embryo, the O-line and hypophysis were not formed due to disturbed cell division in globular stage (Fig 2, l, m).

▷ **Fig 2.** Development of wild type and *acd* mutant embryo

Resin sections (thickness) of wild type embryo (a-h) and *acd* mutant embryos (i-p) were observed under light microscope. (a) the apical and base cell produced by asymmetric division. (b) two cell stage, showing a vertical cleavage division. (c) four cell stage, showing a horizontal cleavage division. (d) eight cell stage, showing tangential division and epidermal precursors, (e) globular stage, the hypophysis (hp) has divided by asymmetric division and the O line (ol) is clearly in globular embryo, (f) heart stage, showing origin of cotyledon (CO) and shoot meristem (SM). (g) torpedo stage, showing developing cotyledons, (h) mature stage, showing cotyledon and embryonic axis. (i-k) proembryo of *acd* mutant, equivalent to stage of (b-d), showing irregular cell arrangement in proembryos. (k-m) globular stage of *acd* mutant, the differentiation of cell type was disturbed and no distinguished epidermal cell layer, and inner cell were clearly shown. The O-line and hypophysis could not be observed too. (n-p) The embryo in the state of degeneration. (n) The embryo was enlarged but no further differentiation of cotyledon. The cell wall was not clear in this stage. (o) degenerating embryo. The embryonic cells were destroyed. (p) The embryo was aborted. Arrows in c, b, indicate the division orientation, (OL): O-line, (CO): cotyledon, (SM): shoot meristem, (HP) hypophysis, (S) suspensor. (a)-(f), (i)-(p) 800 ×; (g)-(h) 250 ×



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Finally, the *acd* mutant embryos were aborted at globular stage (Fig 2, p), a fact which is different with some of other mutants that could complete the whole course of embryogenesis, even if their cell division was also disturbed in the early stage of embryo development, as in the cases of *knolle* (Lukowitz et al. 1996)[5] and *fass* mutant (Torres Ruiz and Jurgens 1994)[13].

The embryonic cells are degenerated in early globular stage of acd mutant embryo

From the observation of histological sections, it was clear that the embryonic cells began to degenerate in the early globular stage of mutant embryos (Fig 2, o). For

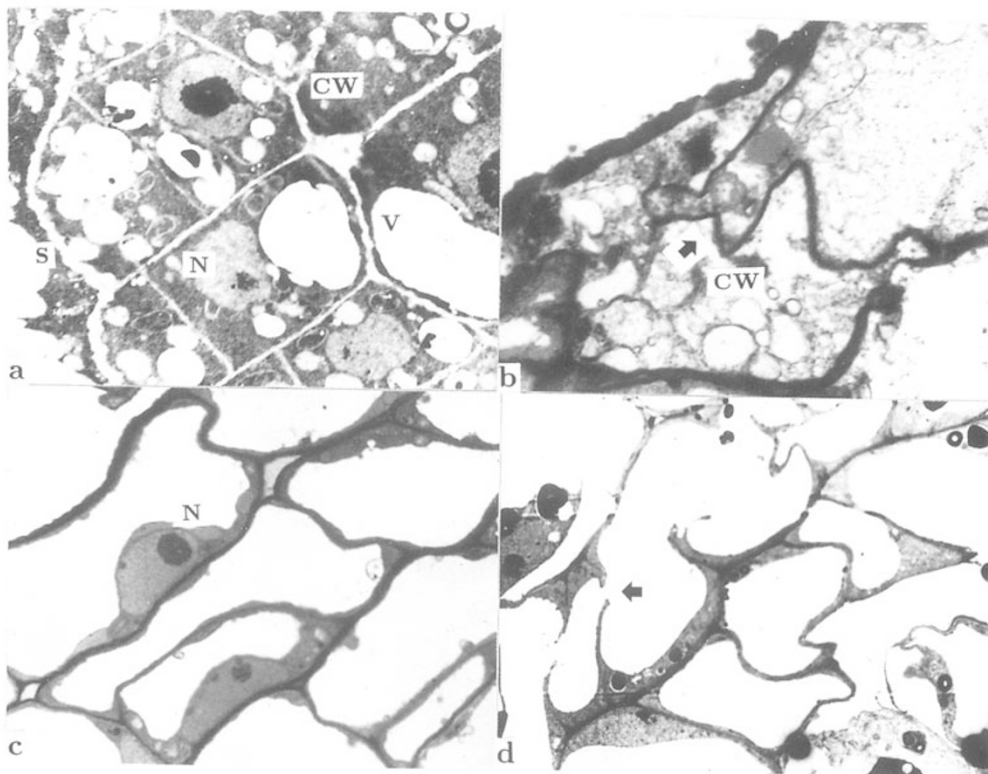


Fig 3. Electron micrographs of cellular defects in *acd* mutant embryos

(a) Wild type embryo at globular stage, showing normal cell structure and surface of a globular embryo, $\times 4320$ (b)-(d) *acd* mutant embryos at early globular stage. (b) The cells were in the state of degeneration, The cell wall became distorted and the cell vacuolization appeared, $\times 8640$ (c) The cells were fully vacuolized, cell nucleus appeared to the cell wall. $\times 3484$ (d) The organelles disappeared, nucleus degenerated and the incomplete cell wall was present in the out layer cells of embryo. The arrows indicated the distortion and rupture of cell wall. $\times 2280$

Abbreviations: CW, cell wall; S, surface of globular embryo; N, cell nucleus; V, vacuole.

further approaching in more details, the ultrathin sections from mutant embryos at early globular to globular stage were examined respectively. In comparison with wild type globular embryo (Fig 3a), both the epidermal and subepidermal cells of mutant embryos were found to be degenerated involving cell vacuolization, organelle degeneration and cell wall distortion (Fig 3b). Usually, the mutant embryonic cells were fully vacuolized and only cell nucleus was present along the cell wall (Fig 3, c), and their cell wall often ruptured (Fig 3 d).

The above results again indicated that the disturbed cell division pattern can directly influence embryo differentiation.

*The abnormal seedlings produced from uncomplete differentiated embryos of *acd* mutant*

The most of homozygous (*aa*) mutant seeds were not able to germinate, because their embryos would be aborted at globular stage as mentioned above. However, few of these seeds could germinate for a short time, if they are incubated in agar plate with basal MS medium without growth regulators. To study the developmental fate of these mutant embryos in which tissue differentiation were seriously influenced, their early germination were observed. In Fig 4, three mutant seedlings were shown. One of the common symptoms of them was the expanding and distorting shoot meristem as well as the smaller and etiolated cotyledons (Fig 4, b, d), although these seedlings were grown under light. These developmental changes might be related to the uncomplete differentiation of upper part of the globular embryo. Another morphological change was the shorter hypocotyl (Fig 4b, 4d), if compared with wild type seedlings (Fig 4a). In the case of mutant seedling in Fig 4c, even both embryonic root and hypocotyl were defective and this must be due to the disturbed cell division and lack of hypophysis in globular embryo. Taken together, the uncomplete cellular differentiation and defect of special early region can be correlated to the abnormal development of seedlings.

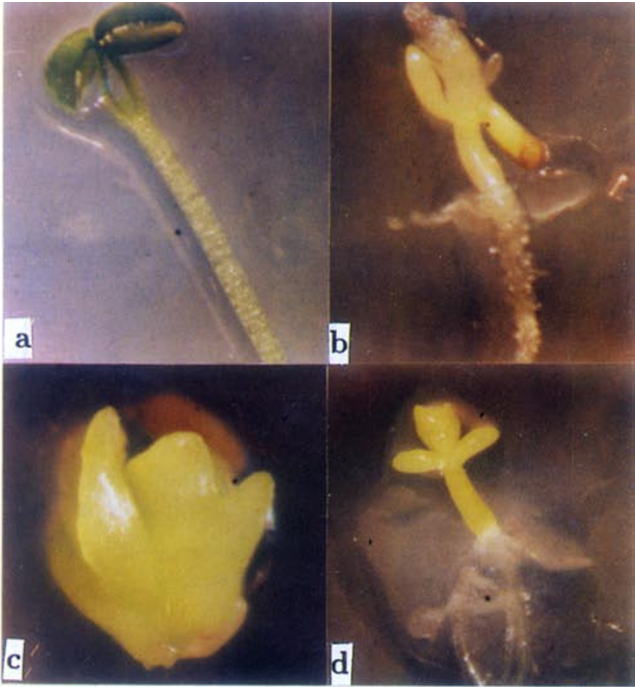
*The T-DNA flanking plant DNA from *acd* mutant is highly homologous to *alanyl-tRNA synthetase (AlaRS)* gene of *Arabidopsis**

To characterize the T-DNA flanking plant DNA of the mutant, the joint fragment of T-DNA and flanking plant DNA were isolated by plasmid rescue technique in our previous paper (Yao et al. 1996)[9]. To further characterize the sequence of plant DNA from this T-DNA/plant DNA junction, the junction fragment of T-DNA and flanking plant DNA was cut from plasmid DNA and labeled by digoxigenin-UTP as probe for cDNA library (PRL-2) screening. Several independent positive clones were recovered. The No.1 positive clone with 1Kb insertion was cut by restriction enzymes and ligated to pBluescript SK⁺ for sequence analysis.

The sequence was analyzed by Applied Biosystem 373A Sequencer from both strands. Finally, a 936 bp cDNA sequence (EMBL Accession #: Y12555) was

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Fig 4. The early germination of *acd* mutants. The wild type and *acd* mutant seeds were germinated in agar plates respectively for about one week , then observed under dissecting microscope . The photo were taken under same magnification.
 (a) A wild type seedlings .
 (b) An *acd* mutant seedling , showing etiolated cotyledon, distorted, swollen shoot meristem and shorter hypocotyl.
 (c) an *acd* seedling without embryonic root.
 (d) Another *acd* mutant seedling, showing smaller etiolated cotyledon, abnormal shoot meristem and shorter hypocotyl.



obtained (Fig 6). The similarity searching was performed by BlastN program using E-mail Server at the National Center for Biotechnology Information (blast@ncbi.nlm.nih.gov). The results indicated that its sequence is 99.8 % homologous (923/925bp) with the cDNA sequence of alanyl-tRNA Synthetase (*AlaRS*) of *Arabidopsis thaliana* (EMBL accession #: z22673) (Mireau 1996)[14]. Furthermore, according to the similarity searching, the T-DNA insertion site was located at the 3'-end of *AlaRS* gene, about 250 bp far from the transcription terminal.

The AlaRS gene is expressed weakly in embryogenesis of acd mutant

To demonstrate the relationship between the phenotypic effect and the expression of *AlaRS* gene, the whole-mount in situ DNA/RNA hybridization was conducted. The Dig-labeled ClaI-XbaI fragment of T-DNA flanking plant DNA was denatured by heating-quick cold and then used as probe. The results showed that the *AlaRS* gene is expressed through out the embryo development in wild type *Arabidopsis* embryo (Fig 5, a-e). However, there were only weak immunological response in early globular embryo of mutant (Fig 5f). The immuno-colour reaction declines along with globular embryo 'development' (Fig 5, g, h). In later developmental stage of mutant embryo, there was no colour reaction observed at all (Fig 5, i, j). On the basis of these results, it can be suggested that the weak expression of *AlaRS* gene may influence the development of mutant embryo. This consideration is also in line with the results of embryonic cell degeneration obtained from electronic microscopic observation.

DISCUSSION

Abnormal cell division pattern causes abnormal cellular differentiation

The cell division pattern is one of the important event in early embryogenesis, since the orientation of cell division (Tang 1953)[15] and the cell division cycle (Ferreria et al 1991; Hemley et al 1992)[16; 17] are both closely related to the initiation of early region which will give deep influence on the further development of an embryo.

In *acd* mutant of *Arabidopsis*, the abnormal cell division pattern led to irregular cell arrangement and defect of protoderm, the epidermal precursor. Therefore, the

- ◁ **Fig 5.** The Whole mount in situ hybridization of wild type and *acd* mutant embryo of *Arabidopsis*. Details of procedure of hybridization see material and method. (a-e) wild type embryo; the immuno-color response can be followed throughout the whole course of wild type embryogenesis, a)globular stage; b)late globular stage; c)heart stage; d)torpedo stage; e)cotyledon stage. (f-j) *acd* mutant embryo; f) early globular embryo of *acd* embryo shown weak response of color reaction; g-h) the color reaction was declined along with the growth of *acd* embryo; i-j) no color reaction in late stage of arrested globular embryos

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      10          20          30          40          50
GAAAACAATG AATGGTCATT GCTTTCTTCC GAGTTTTGTG GAGGAACCCA

      60          70          80          90         100
CATAACAAAC ACCCGCGAAG CCAAAGCATT TGCTCTTCTA TCGGAGGAGG

     110         120         130         140         150
GAATTGCTAA AGGTATTCGT AGGGTAACTG CTGTGACTAC TGAATGTGCT

     160         170         180         190         200
TTTGATGCAT TGAATGCGGC GTCCTTACTT GAAAGAGAAG TAGAGGATGC

     210         220         230         240         250
CTCCAGAGCG GAGGGAAGTG GATTGGAAAA GAAAGTTTCT GCTTTGAAAA

     260         270         280         290         300
GCCGAGTAGA TGCAGCAATT ATCCCAGCAG CTAAAAAGGC AGATATTAGG

     310         320         330         340         350
ACTAAGATTG CTTTCGCTTCA GAATGAAGTA AGAAAAGCTC AGAAGAAAAT

     360         370         380         390         400
AGCGGAACAA AACCTGAAAA AATCTGTCAA ATTAGCAACA GAGGCAGCTG

     410         420         430         440         450
AGTCCGCAGC ATCAGATGGG AAGACTTTCT GCATAATCCA GCTGGATGTG

     460         470         480         490         500
GGTCTTGATG CAGCAGCTGT GCGAGAGGCC GTTTCAAAAG TCATGAAAAA

     510         520         530         540         550
GAAGGGTATG TCGATAATGG TGTTTCAGCAC AGATGAAAGC ACAAACAAGG

     560         570         580         590         600
CGGTTGTGTG TGCAGGAGTG CCAGAAAAAT CAGACCAGTT TAAGCCGTTA

     610         620         630         640         650
GACGTCAC TG AATGGTTGAC AACTGCATTG GGTCTCTTAA AAGGAAGGTG

     660         670         680         690         700
CGGGAAAGGG AAAGGTGGTC TTGCATCAGG GCAGGGAACG GATGCTTTTC

     710         720         730         740         750
AAGTGCAGGC GGCTTTGGAT ATGGCTTCAT CATTTCATC AATGAAGCTC

     760         770         780         790         800
AACTGATTTG GTCGGGAGGT TACATAATCT GTGAGGTCAG AATAACCTCT

     810         820         830         840         850
ACTTAATTTG CTTTCATTGT ACACCGTTTT ACACATGTTT TAAGAATCTG

     860         870         880         890         900
TTAAATTTCT CTGTTCTTGT GATGGAATTT GTGGTATGAC ACTCTTTTAG

     910         920         930
TCAAAGTTAC CAATGGCTGT TATAAAAAAA AAAAAA

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Fig 6. The cDNA sequence of positive clone NO.1 (EMBL as accession number Y12555).

antichinal and perichinal division were influenced and the tunic and corpus differentiation were seriously disturbed. Furthermore, the electron microscopic investigation indicated that the cells in outer layers of early globular embryo were in degenerating which involved cell vacuolization and the rupture of cell wall. This confirmed the morphological observation that the embryo differentiation of *acd* mutant were interfered in this stage. As to the relationship between cell wall and cell differentiation, the significant roles of cell wall formation in cell differentiation, including cell communication and signal transduction (Lukowitz et al. 1996; Brownlee and Berger 1995)[5; 18] are recently reported.

The developmental fate of the rudimentary embryo

Due to the abnormal cell division pattern in *acd* mutant, rudimentary embryos which contained only groups of misoriented cells were formed. In this case, their early region specification and tissue primordial establishment were disrupted. Since the initiation of organ formation was largely depended on the early region of developing embryo, therefore, it was interesting to know the developmental fate of the cell groups in these rudimentary embryos. In our experiment, most of these rudimentary embryos were finally aborted, but a few of them could germinate in agar plate as rootless seedlings (Fig 4c) which may be raised from the disturbed cell division in central and basal regions of embryo, or as seedlings with distorted shoot meristem and shorter hypocotyl (Fig 4, b, d) which may possibly be originated from embryos with both abnormal apical and basal regions. From these data, it appears that abnormal differentiation of early region could result in abnormal seedlings. The significance of early region on axis formation in plant embryogenesis has been recently reviewed by Jurgens (1995)[12].

*On the possible role of *AlaRS* gene in early development of embryo*

In present paper, the plant DNA sequence from T-DNA/plant DNA junction was characterized as alanyl-tRNA synthetase gene, according to the results of cDNA library screening and sequence similarity searching (Fig 6). To confirm the relationship between the phenotype of abnormal embryo development and the expression of *AlaRS* gene, on the basis of our previous data of single T-DNA insertional site (proved by gel blot) and a segregation ratio of 1:3 for abnormal and normal seeds (Yao et al 1996)[9], we studied further by using whole mount *in situ* hybridization (Fig 5). The results showed that the *AlaRS* transcript were detectable throughout embryogenesis of wild type embryo. However, the expression of this gene was rather weak in early globular embryo of mutant and then declined along with the increasing volume of arrested globular embryo. This altered expression pattern suggests that phenotypic effects of *acd* mutant are closely related to the expression of *AlaRS* gene. As mentioned above, the T-DNA insertional site is in the 3' untranslated region of *AlaRS* gene. Therefore, it is likely the reduced expression of *AlaRS* gene is due to

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T-DNA insertion in its 3' end, since the deletion experiments has confirmed that 3' end region can influence the level of gene expression in higher plant cells (Ingelbrecht et al 1989, An et al 1989)[19, 20]. Recently, the suspensor-derived polyembryony caused by altered expression of vanyl-tRNA synthetase gene was also reported in *twn2* *Arabidopsis* mutant (Zhang JZ and Somerville CR 1997)[21].

In addition, as a possible explanation for the physiological function of alanyl tRNA synthetase, it is thought that this enzyme may possess an essential role in the translational regulation of preformed or maternal mRNA in early embryogenesis. tRNA synthetase is one of the prerequisite factors for the mRNA translation (Marcus 1982, Moras 1992)[22, 23]. In somatic embryogenesis of carrot, five more genes appear to be translationally controlled (Zimmerman 1993)[24] and a clone encoding translation elongation factor EF1- α was isolated by differential hybridization from embryonic and non-embryonic cells (Kawahara et al. 1992)[25]. In the investigation of *Arabidopsis* embryo-defective mutant, many mutants were found to be aborted before globular stage. This indicates that gene expression early in embryogenesis is essential (Castle and Meinke 1993)[1]. Since the presence of stored or maternal mRNA in *Drosophila* (Kimble 1994)[26], *Fucus* (Bouget et al. 1996)[27] and higher plant embryos (Spiegel and Marcus 1975, Dure 1979, Zhu et al. 1981, Ragavan 1986, Paramanic et al. 1992)[28-32], therefore, it appears that alanyl tRNA synthetase could play an essential role in translational regulation during early development of embryo.

ACKNOWLEDGEMENT

The authors want to thank Tang X.H. for enlightening discussion, Bai Y.Y. and Wang, Z.Y. for valuable advice of cDNA library screening and molecular technology, Bai Y.T. for DNA sequence analysis and Wu D.S. for electron microscopy and Rong L. (Shanghai Jiao Tong University) who partly joined this work for his BS degree thesis. This work was financially supported by Chinese National Natural Science Foundation (Project No. 39680013) and Chinese National Opening Lab. of Plant Molecular Genetics.

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Received March-16-1998. Revised May-19-1998. Accepted May-22-1998.