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A possible fission of chloroplasts spawn microinjected Zebrafish

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Abstract
An odd phenomenon was observed in an experiment of microinjecting plant extracted chloroplasts to AB wildtype zebrafish (*Danio rerio*) spawn. Chloroplasts was extracted from spinach (*Spinacia oleracea Linn.*) (provided by Southwest University, China) (density information not provided by Southwest University, China). Extracted chloroplasts were diluted to a level that won't cause spawn lethal and then microinjected into the yolk of spawn. The chloroplasts were RFP labeled for later confirmation of existence in zebrafish spawn and larvae. The microinjected spawn was then hatched and observed in PTU (propylenethiourea) dealed fresh water so the pigment won't sedate (the zebrafish will be kept transparent). The spawn and hatched larvae later were kept fasting condition. The spawn and hatched larvae were kept in a condition of 14-hour light and 10-hour darkness and 28 ℃ fresh water every day. The hatched larvae of microinjected chloroplasts were observed in number increase (no mortal found after hatching even under fasting condition). Assumption of possible fission of chloroplasts microinjected zebrafish larvae was therefore made. The experiment was repeated three times by Ting Lu and each time the phenomenon was confirmed.

Keywords: chloroplasts, zebrafish

Introduction
Chloroplasts in plants conduct photosynthesis to transfer light energy into biochemical energy plants need. Therefore, a bold idea of microinjecting plant extracted chloroplasts into wildtype zebrafish spawn to screen the possible outcomes was made.

Methods
Chloroplasts was extracted from spinach (*Spinacia oleracea Linn*) (provided by Southwest University, China) (density information not provided by Southwest University, China). Extracted chloroplasts were diluted to a level that won't cause spawn lethal and then microinjected into the yolk of spawn (nearby the position of animal pole). The chloroplasts were RFP labeled for later confirmation of existence in zebrafish spawn and larvae. The microinjected spawn was then hatched and observed in PTU (propylenethiourea) dealed fresh water so the pigment won't sedate (the zebrafish will be kept transparent). The spawn and hatched larvae later were kept fasting condition. The spawn and hatched larvae were kept in a condition of 14-hour light and 10-hour darkness and 28 ℃ fresh water every day.

Control groups included microinjected shreded chloroplasts (provided by Southwest University, China) (density coherent with microinjected chloroplats sample) group and phenol red microinjected group. Chloroplasts microinjected spawn, shreded chloroplasts microinjected spawn and phenol red microinjected spawn were splited into two teams, one team was dealed with 14 hour light and 10 hour darkness condition, and the other team was dealed with absolute darkness.

Results
The existence of chloroplasts was shown by followed RFP fluorescence figures (Fig.1 - Fig.9):

0h Post Microinjection

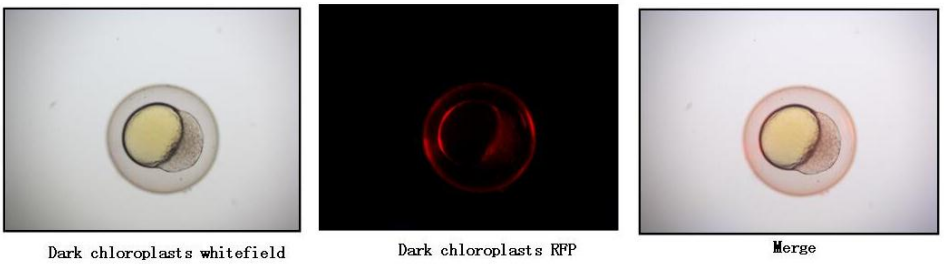


Fig 1: 0h post microinjection of chloroplasts (light dealed group and absolute darkness dealed group respectively).

0h Post Microinjection

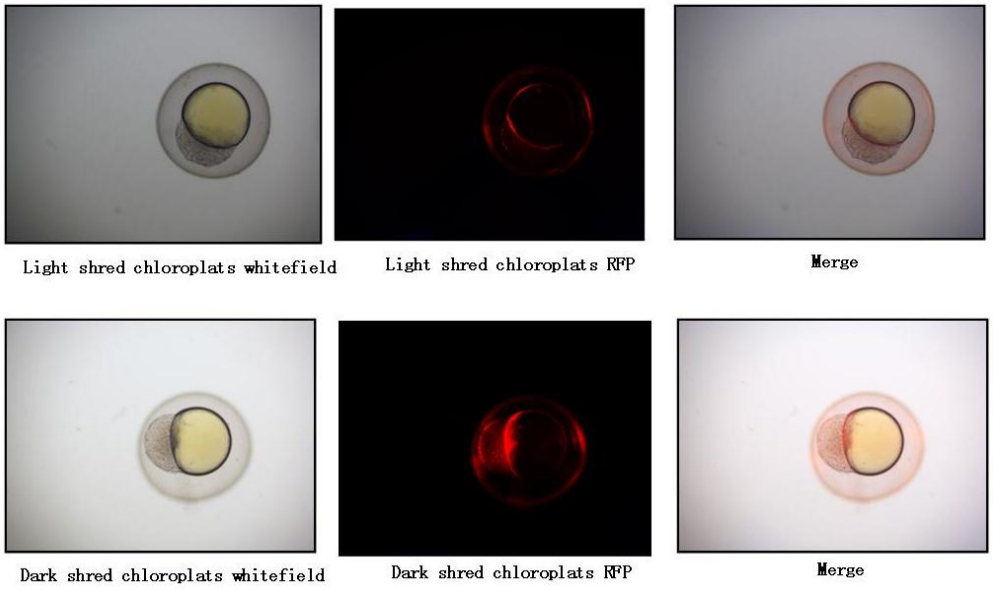


Fig 2: 0h post microinjection of shred chloroplasts (light dealed group and absolute darkness dealed group respectively).

0h Post Microinjection

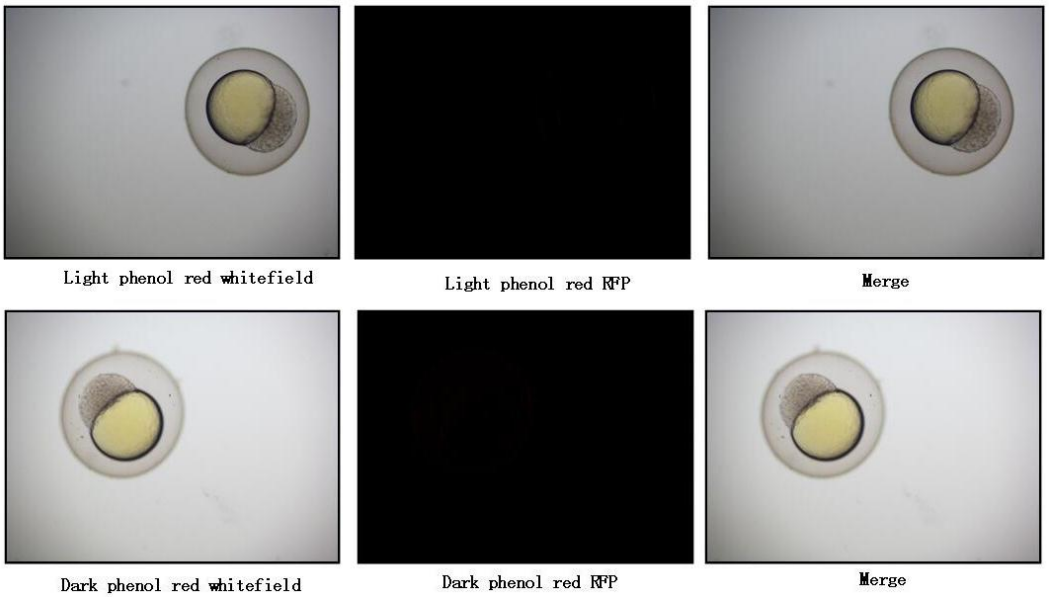


Fig 3: 0h post microinjection of phenol red (light dealed group and absolute darkness dealed group respectively).

24h Post Microinjection

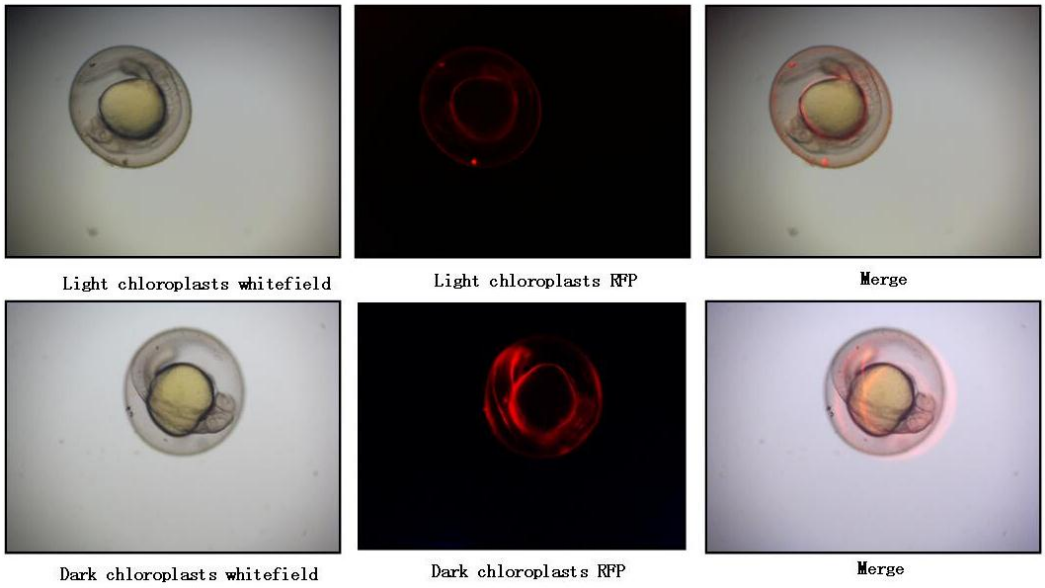


Fig 4: 24h post microinjection of chloroplasts (light dealed group and absolute darkness dealed group respectively).

24h Post Microinjection

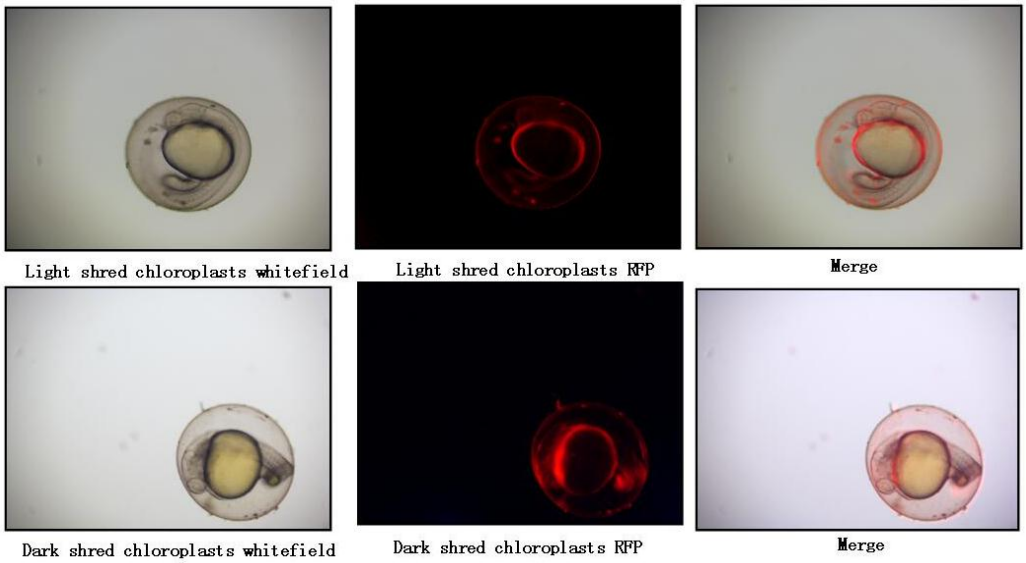
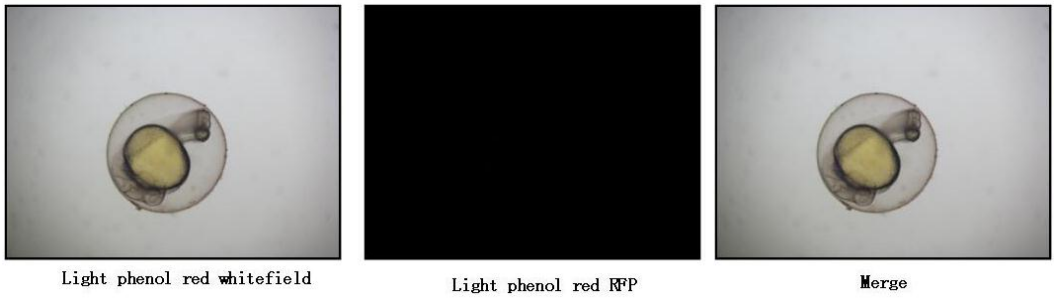


Fig 5: 24h post microinjection of shred chloroplasts (light dealed group and absolute darkness dealed group respectively).

24h Post Microinjection



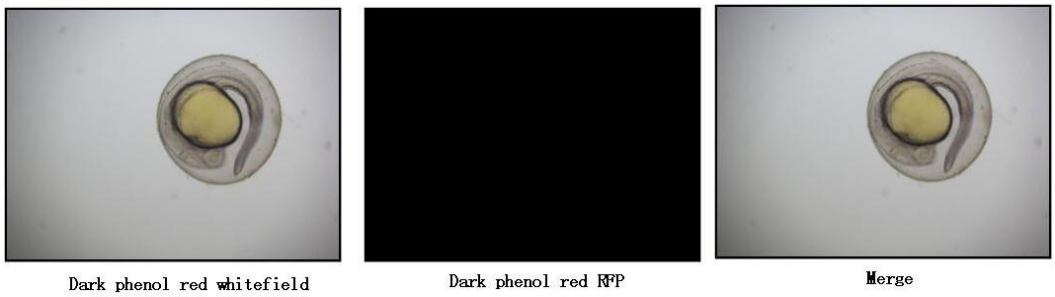


Fig 6: 24h post microinjection of phenol red (light dealed group and absolute darkness dealed group respectively).

48h Post Microinjection

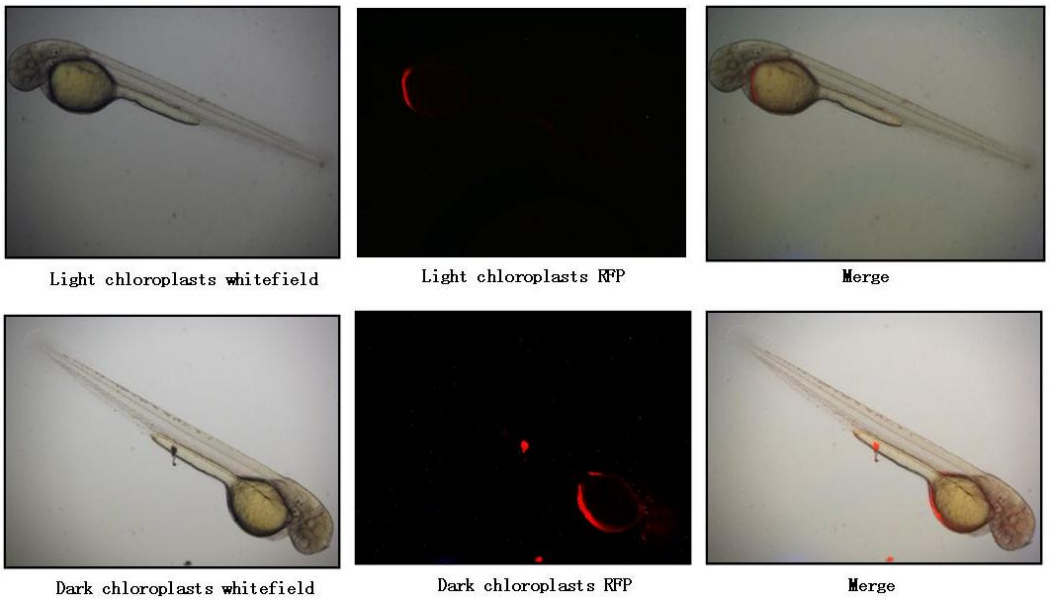


Fig 7: 48h post microinjection of chloroplasts (light dealed group and absolute darkness dealed group respectively).

48h Post Microinjection

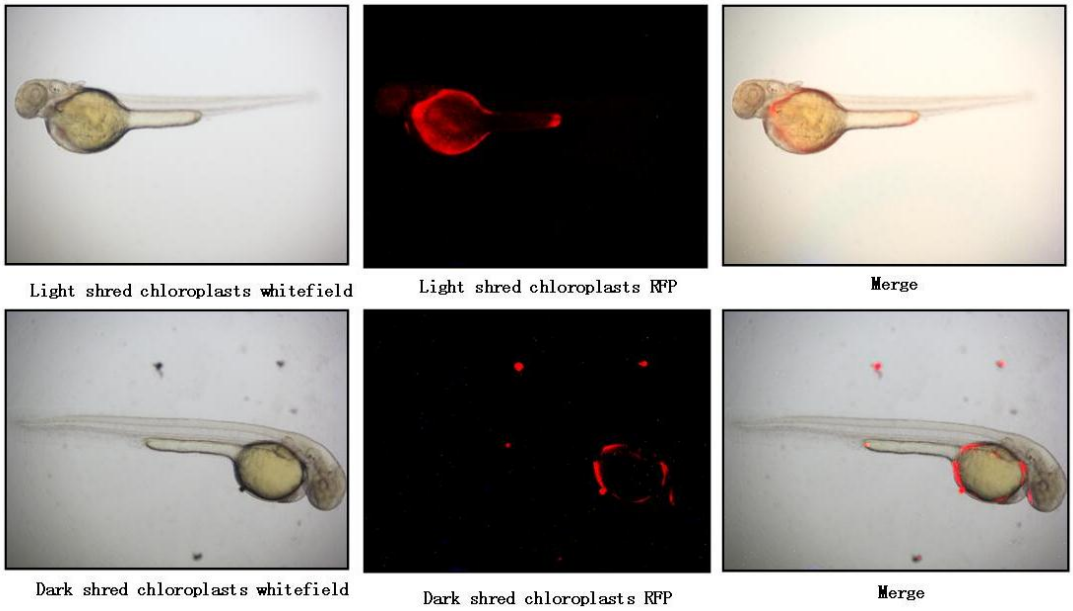


Fig 8: 48h post microinjection of shred chloroplasts (light dealed group and absolute darkness dealed group respectively).

48h Post Microinjection

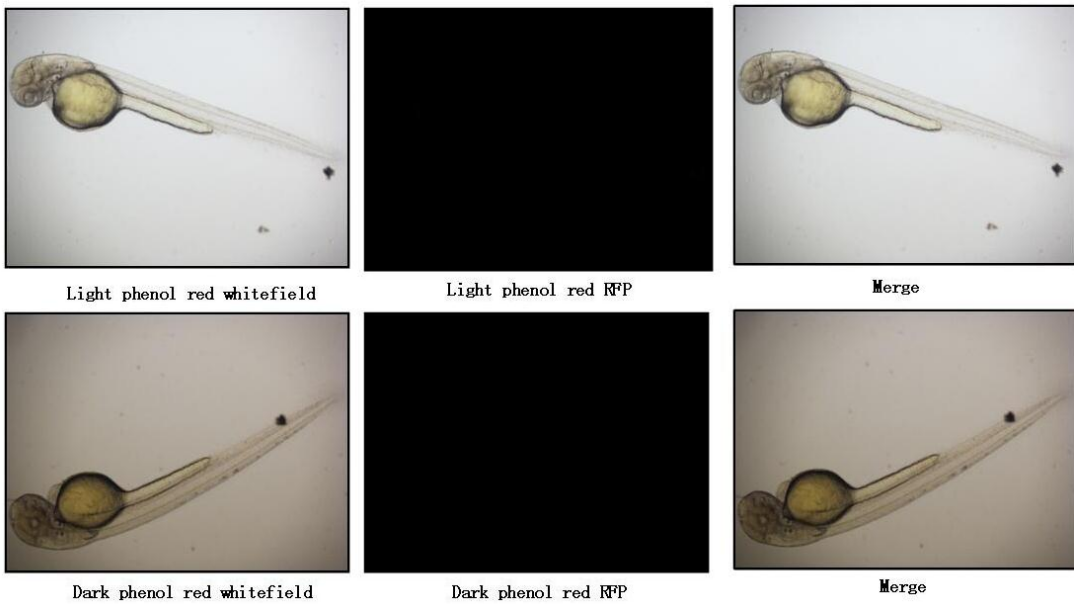


Fig 9: 48h post microinjection of phenol red (light dealed group and absolute darkness dealed group respectively).

Only the chloroplasts microinjected zebrafish group under 14 hour light/10 hour darkness condition group showed number increase hence lead to assumption of larvae fission. In the first trail, the number increase happened on the 9th day post oviposition (larvae number increased from 19 to 21), in the second repeat, the number increase happened on the 5th day post oviposition (2 larvae coming out of 1 well from 96-well plate while only just one microinjected spawn was placed in each well), in the third repeat, the number increase happened later on the 3rd day post oviposition (larvae number increased from 13 to 17). Constant observation has acquired a picture of suspicious inkling of potential fission. (Fig.10) Therefore, assumption of possible fission of chloroplasts spawn-microinjected wildtype zebrafish (dealed with PTU to make skin transparent) under the condition of 14 hour light/10 hour darkness was therefore made.

Fig 10: Constant observation of suspicious inkling of potential fission of zebrafish larvae.

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