# Green genes – DNA inside chloroplasts
# Teachers' guide

## Summary

Chloroplasts evolved from photosynthetic bacteria and contain DNA encoding a remnant of their original bacterial genes. This booklet discusses how the study of chloroplast genes has helped biologists understand plant evolution, and in particular the transfer of genes from the chloroplast to the nucleus during evolution.
The section *Plant Biotechnology - Future Hopes* considers the potential for manipulating chloroplast biochemistry to improve the nutritional qualities of crops, and also discusses the introduction of genes encoding pharmaceutical proteins directly into chloroplast DNA. The development of transgenic crops is controversial and can provide a rich subject for debate. A series of questions designed to stimulate discussion about the production of pharmaceuticals in GM crops is available from SAPS.
The booklet also contains a box called *Stromules* which briefly describes the dynamic, narrow tubules that project from many plastids and considers their function. There are good web-based images and movies of stromules, see *Web Resources,* below.

## Assumed knowledge

This booklet is directed at post-16 students studying A-level biology or Scottish Advanced Highers. It may also be useful for those studying a range of other F.E. or H.E. courses. The text assumes a basic knowledge of the structure of plant and animal cells, of the role of chloroplasts in photosynthesis, and of the way in which genes encode proteins. The booklet introduces several terms that may be unfamiliar to students: these are given first time in bold text and defined in the glossary.
Some questions are deliberately quite challenging and demand additional knowledge. For example, question 4 tests an understanding of the evolutionary implications of sexual reproduction. Question 7 tests whether students understood the section on signal sequences, and question 10 needs some mathematical skill.

## Answers to questions

1. Movement, respiration, growth, reproduction, excretion, excitability and nutrition.
***Movement and excitability:*** *chloroplasts rotate and/or move towards dim light and away from bright light.*
***Respiration****: In darkness, chloroplasts can release energy by metabolising carbohydrates through glycolysis (they also import ATP from the cytosol).*
***Growth and reproduction****: For each type of cell and/or species, chloroplasts grow to a typical size and then divide by binary fission.*
***Excretion****: eg: oxygen produced by photosynthesis.*
***Nutrition****: Chloroplasts take water, carbon dioxide and mineral nutrients from the plant cell. In the dark, chloroplasts may also import sugars and ATP.*

2. Variegated plants are at a disadvantage because the white sections of the shoot do not photosynthesise. Also, for most species, the variegated phenotype can only be reproduced vegetatively. Because chloroplasts usually display maternal inheritance, seeds from variegated plants produce either all green or all white (and therefore non-viable) seedlings. *(In some cases, the white clone of cells in a variegated plant lacks a nuclear gene for chlorophyll synthesis. In these cases, each seedling is still either all-green or all-white, but with normal Mendelian inheritance.)*

3. Mitochondria evolved first since they are present in both types of cell. They are also present in fungi. A primitive ancestor of plants, animals and fungi engulfed a purple bacterium, leading to the evolution of mitochondria before the three kingdoms diverged. (Like chloroplasts, mitochondria contain DNA and a few of their original bacterial genes.)

4. Sexual reproduction shuffles genes before they are transferred to the next generation. This produces variation and means that deleterious mutations in parental genes are not transferred to all of the offspring—'lucky' offspring that inherit the best combination of genes will be the most likely to survive and reproduce. Asexual organisms produce uniform offspring and have no means of removing deleterious mutations during reproduction—all of an individual's offspring are equally unfit.

5. The main processes required for gene expression are the transcription of the gene to produce messenger RNA (mRNA) and the translation of the mRNA to produce a protein.

6. It implies that the genetic code is the same in prokaryotes and eukaryotes, otherwise when prokaryotic genes moved from the chloroplast to the nucleus they would not encode functional proteins.
The fact that the code is virtually identical in all living organisms suggests that once the code evolved, it was unchangeable. Any change in the code would change the amino acid sequence of many different proteins in the organism and almost certainly be fatal.

7. To ensure that the enzyme that the bacterial gene encoded was imported into plastids, the gene was attached to the DNA encoding a suitable signal sequence(from the pea Rubisco small subunit gene).

8. Wild plants that produced B.t. toxin might outgrow other wild plants because they would suffer less from pest damage. This could cause ecological problems. Wild plants that were resistant to glyphosate would have to be controlled by farmers using other herbicides. This could make weed control more difficult, causing economic problems.

9. The factory has to pay for energy to keep the culture warm, nutrients to feed the culture, and skilled technicians to monitor and maintain the culture– controlling the number of bacteria, removing waste products and of course processing the products of the factory. All this is made far more expensive by the fact that the culture vats must be sterilised before use and continually protected from other microorganisms.

10 a. 46.3 kg
b. 54 hectares
c. About £0.07 per gram

*Notes: The data for this question were taken from Hood, E.E., Woodard, S.L. & Horn, M.E. (2002) Current Opinion in Biotechnology, vol. 13, pp 630-635.*
Maize kernels contain amyloplasts rather than chloroplasts. Production of foreign proteins in transgenic amyloplasts has been achieved in potato tubers.

**Background notes**

***Chloroplast inheritance.***
In most flowering plants, plastids in the pollen tube degenerate at fertilisation so plastid inheritance is maternal. However, some species have more complex inheritance patterns. For example, geranium *(Pelargonium zonale)* plastids may be inherited from the egg only, the pollen only, or from both parents.
The demonstration that chloroplasts carry genes depended not only on Correns' work with the four-o'clock plant, but also on work by Baur on geranium (also published in 1909). When Baur pollinated an all-green geranium with pollen from a white section of a variegated plant, sometimes the offspring inherited both 'green' and 'white' chloroplasts. Crucially, Baur showed that although both types of plastid must have been present in the zygote, they rapidly segregated (by chance) so that most cells in seedlings contained either all green or all white chloroplasts. This segregation suggested that the chloroplast colour depended on chloroplast rather than nuclear genes.

***The chloroplast double membrane.***
The chloroplast double membrane is not mentioned in Section 3 because its origin is uncertain. Cyanobacteria are enclosed by two membranes, the plasma membrane and the 'outer' membrane, which are separated by the bacterial cell wall (the 'gram-negative' arrangement). An engulfed cyanobacterium, therefore, would initially be surrounded by three membranes, the two bacterial membranes and the food vacuole membrane of the host cell.
It used to be thought that the double membrane surrounding chloroplasts represented the bacterial plasma membrane enclosed by the host vacuole membrane. This arrangement is likely to be true for mitochondria (which also arose from gram-negative ancestors), but it now appears more likely that the chloroplast outer membrane is equivalent to the bacterial outer membrane (based on similarities in membrane proteins) and that the food vacuole membrane has been lost.

***Carotene-enriched rice.***
Mammals make vitamin A from ß-carotene, a carotenoid that is synthesized inside chloroplasts and other plastids. In rice grains, ß-carotene is absent from the endosperm but present in the oily aleurone layer that surrounds it. Unfortunately, the aleurone layer goes rancid when rice is stored in tropical regions, so rice is 'polished' down to the endosperm before storage.
Children whose diet consists largely of polished rice often suffer from vitamin A deficiency, leading to blindness and exacerbating diseases such as diarrhoea and measles. It is estimated that worldwide 124 million children are deficient in vitamin A, and UNICEF predicts that improving vitamin A nutrition could prevent between 1 and 2 million child deaths each year.
To create carotene enriched rice the genes for three enzymes in the carotenoid pathway were introduced into the rice nucleus. All three genes were attached to endosperm-specific promoters to ensure that only the rice grain was affected and that the metabolism of the rest of the plant was not diverted to carotene synthesis.

***Somatotropin.***
This is a growth hormone produced by the pituitary gland (a pea-sized organ at the base of the brain) in bursts after exercise, eating and in the early phase of sleep. Somatotropin production is highest at puberty and declines throughout adulthood. Excess production causes giantism, whereas somatotropin deficiency causes dwarfism.

**Further information** *Reviews*

For a general overview of plant cell and chloroplast biology, see Alberts, B. (2002) Molecular Biology of the Cell, 4th Edition. Garland Science.

For an excellent review of the history of chloroplast molecular biology, see Bogorad, L. (2001) Emergence of plant molecular biology viewed through the portal of chloroplast research. *Current Science,* vol. 80, pp. 153-160.
For a discussion of chloroplast biotechnology, see Daniell, H., Khan, M.S. & Allison, L. (2002) Milestones in chloroplast genetic engineering: an environmentally friendly era in biotechnology. *Trends in Plant Science,* vol 7, pp. 84-91.

**Web Resources**

An excellent, well-illustrated review of photosynthesis, chloroplast structure, and chloroplast DNA is available in Prof. John Kimball's online biology textbook at 'http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/C/Chloroplasts.html'.

More general information about biotechnology, as well as practical resources and training, is offered by the National Centre for Biotechnology Education at the University of Reading. Their website is: 'www.ncbe.reading.ac.uk'.

A web essay on stromules by Maureen Hanson and Rainer Köhler of Cornell University, including photographs and movies, is available at http://www.plantphys.net/article.php?ch=e&id=122

*Illuminating DNA* by Dean Madden of the National Centre for Biotechnology Education at the University of Reading (published in 2000). The document can be downloaded free of charge at: http://www.ncbe.reading.ac.uk/dna50/protocols.html.

*Photosynthesis* by M J Farabee an on-line introduction to photosynthesis is available at: http://www.emc.maricopa.edu/faculty/farabee/BIOBK/BioBookPS.htm

*Photosynthesis and the Web:* 2002 by L Orr and Govindjee is a comprehensive listing of information on photosynthesis-related web sites and is available at http://www.life.uiuc.edu/govindjee/photoweb/.

*Book*
*DNA Science: A First Course in Recombinant DNA Technology* by David Micklos and Greg Freyer. Published by Cold Spring Harbor Laboratory Press (published in 2002).

**Suggested practical work**

A practical 'Can leaf discs make starch in the dark?' is available on the SAPS website (see below). This demonstrates that starch synthesis can occur independently of photosynthesis if leaf tissue is provided with sucrose. The practical could be a useful starting point for a discussion of the range of chloroplast metabolism and of the variety of plastids, for example starch-synthesis by amyloplasts in underground storage organs.

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**About Science and Plants for Schools (SAPS)**

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