Extrachromosomal Genetic Elements

Plastid and mitochondrial genomes

Plastid transformation

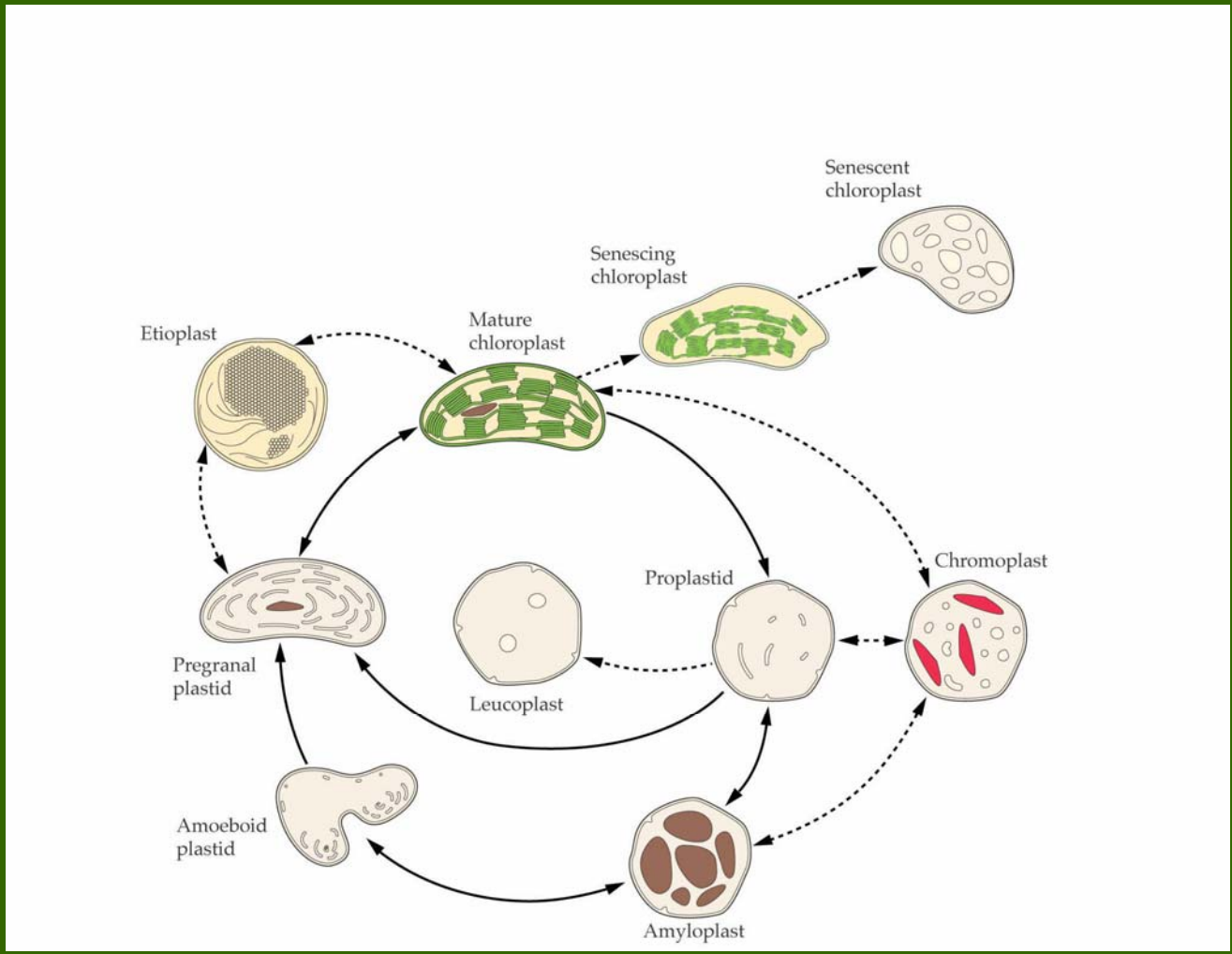
Plastid and mitochondrial genomes 2

- the origin of plastids and mitochondria and their functions
- structure, replication and expression of organellar genomes
- introns in plant genome
- relationship between nucleus and organelles

Types of plastids 3

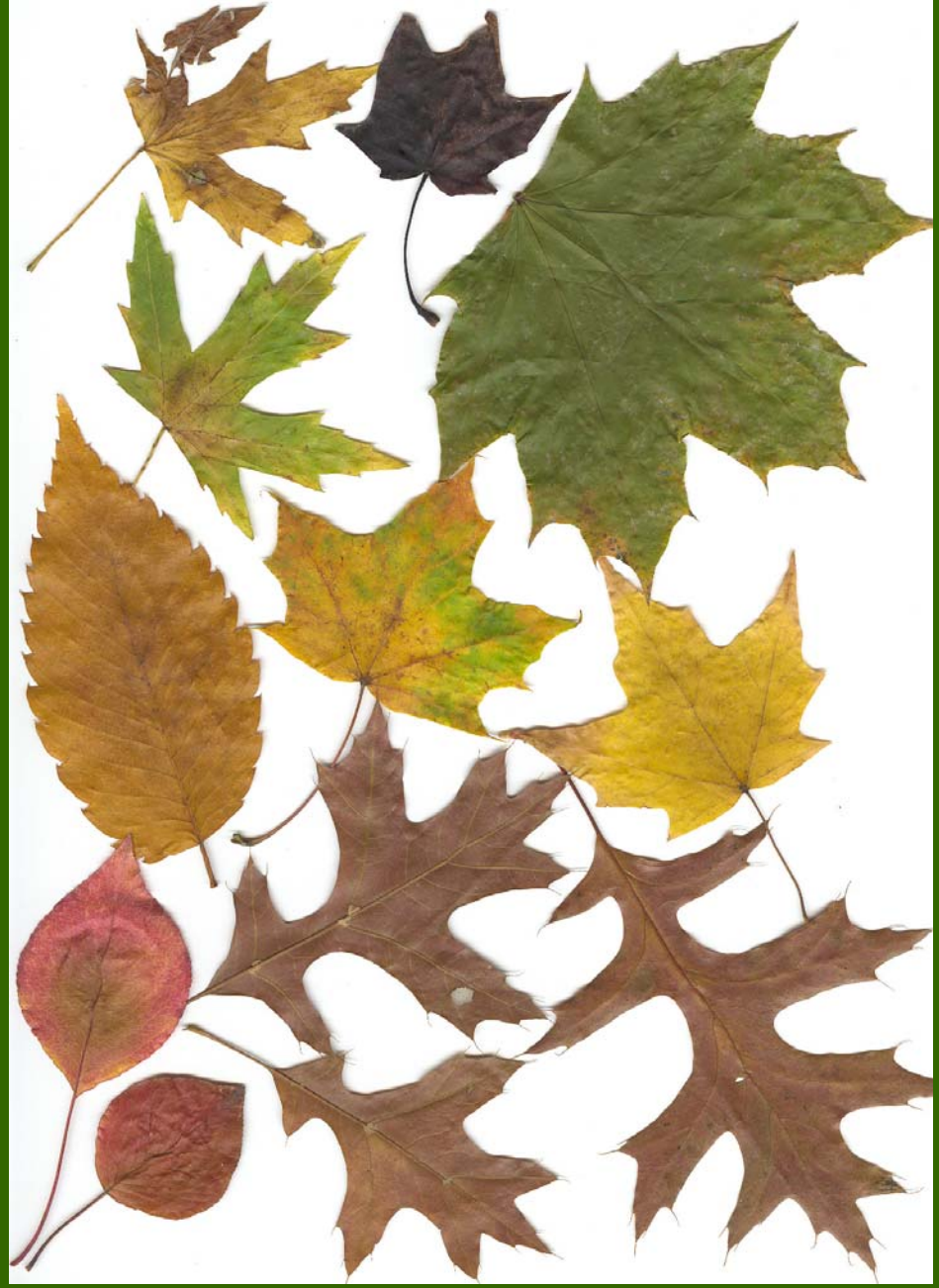
1. Proplastids - precursors of other plastids, in young meristems
2. Amyloplasts – contain starch granules, unpigmented
3. Leukoplasts – colorless, synthesis of monoterpens
3. Etioplasts – develop in absence of light, found in white or pale yellow etiolated leaves, in roots
4. Chloroplasts – in green tissues, contain chlorophyll, photosynthesis
5. Chromoplasts – contain carotens and xantophylls, in flowers, ripe parts of fruit and vegetable

Plastid developmental cycle



New England

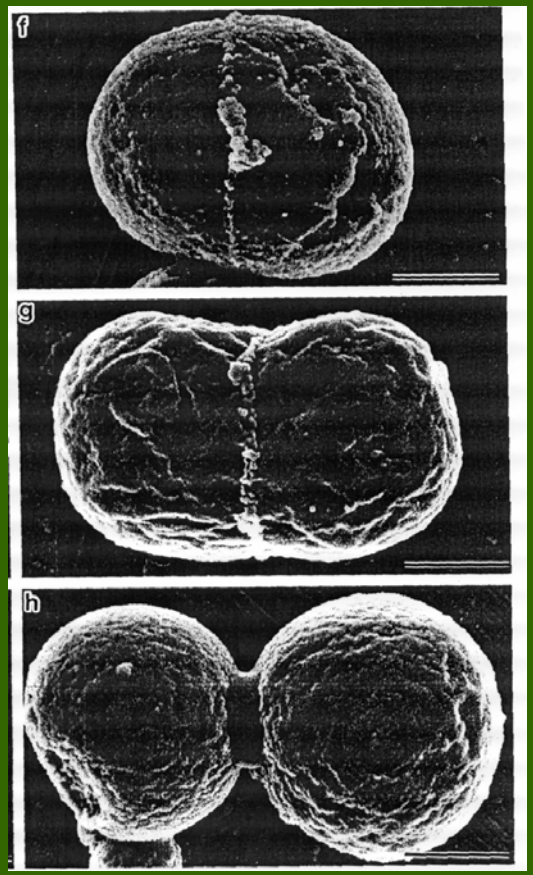
October 2003



Plastid functions 6

photosynthesis
starch synthesis
fatty acids synthesis
amino acid synthesis
pigment synthesis
nucleotide synthesis
nucleic acid and protein synthesis
sulphate and nitrate assimilation

Plastid reproduction by division



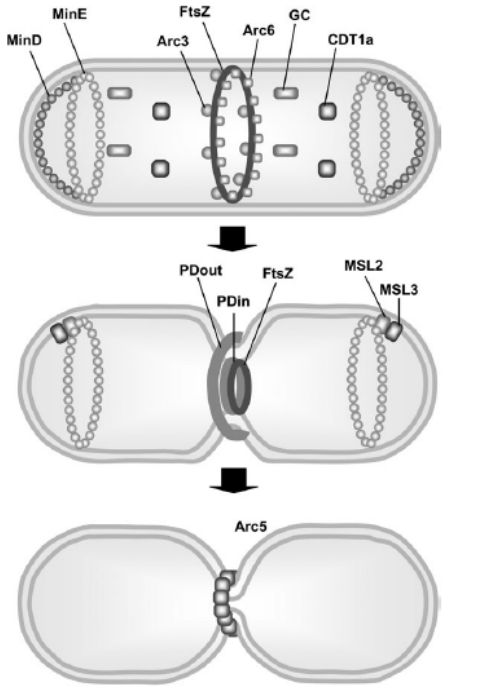


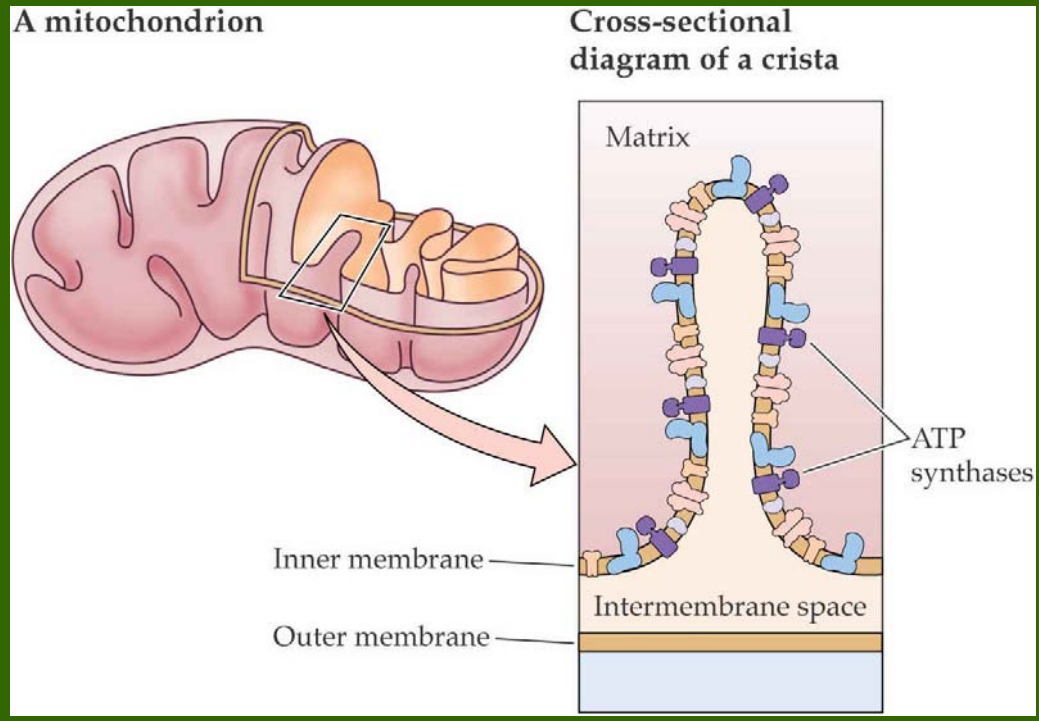
Fig. 2. Plastid division components. Components currently known to play a role in the plastid division process. MinD and MinE play a role in the location of the plastid division apparatus. FtsZ and the plastid division (PD) outer and inner rings physically carry out the constriction. Arc3 and Arc6 help assemble the FtsZ ring. GC plays a poorly-understood role. CDT1a may help co-ordinate plastid and nuclear division. MSL2 and MSL3 probably helps release ionic/hydrostatic pressure generated by the division. Arc5 carries out the final envelope separation. Adapted from hópez-Juez and Pyke (2005) and reprinted by kind permission of UBC press, Vancouver.

Lopez-Juez E., 2007

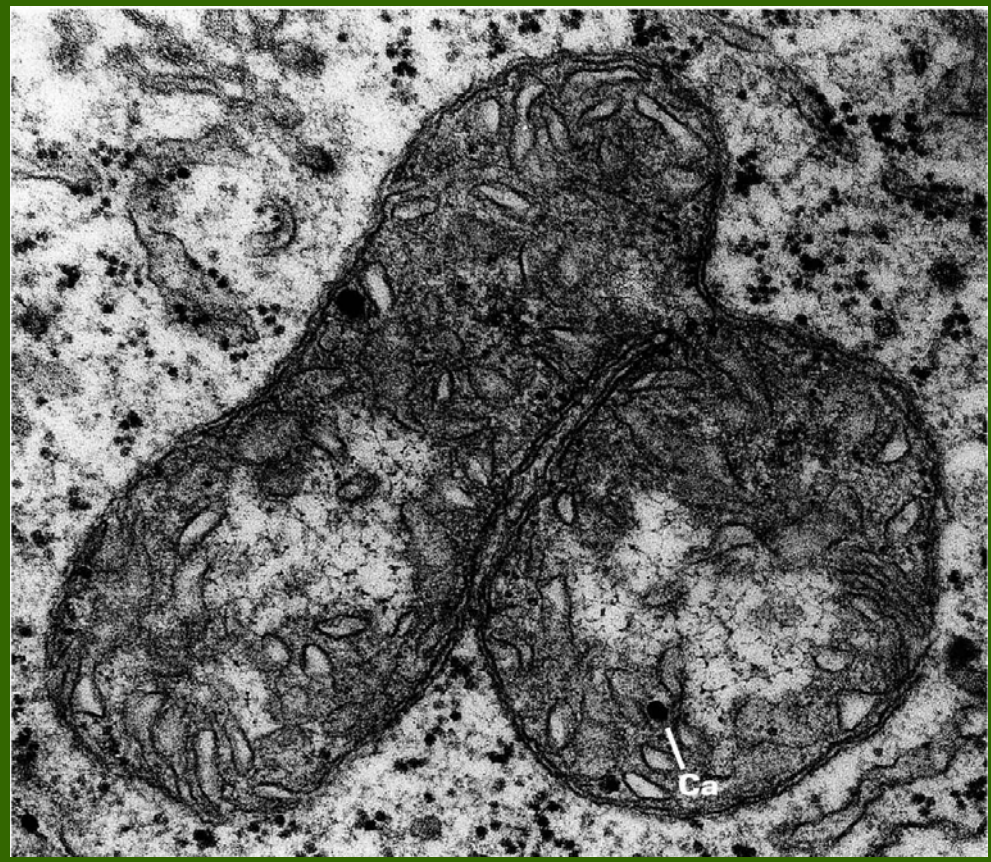
Mitochondrie

inner membrane – cristae (surface enlargement), contain complexes of respiratory chain and enzymes of ATP synthesis

accumulation of energy into energy-rich phosphate bonds



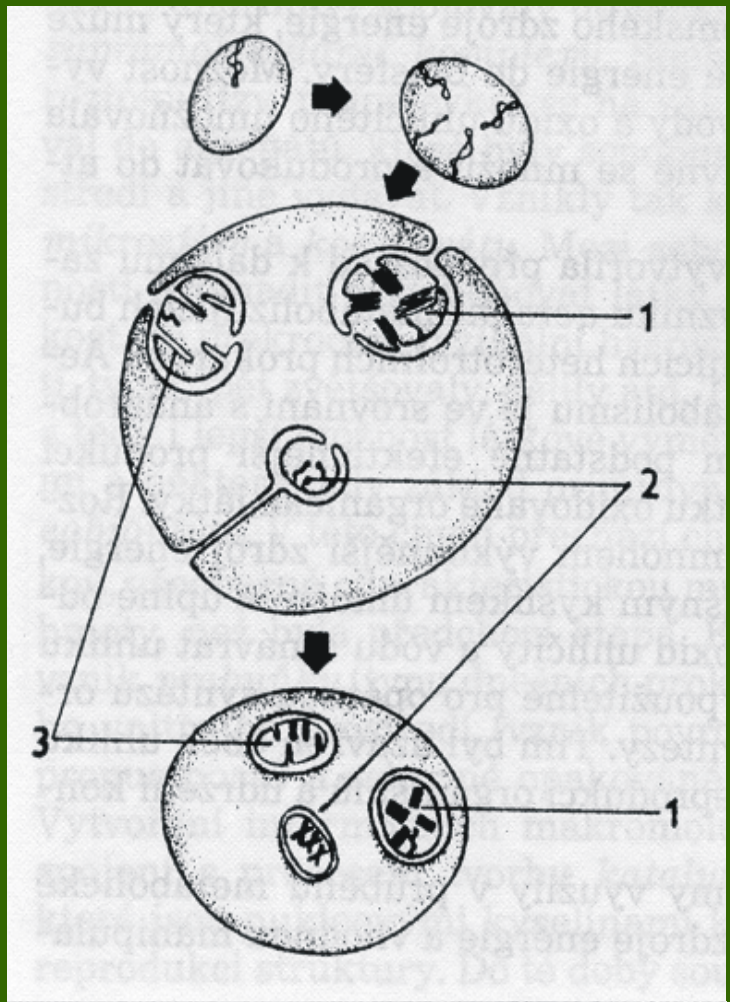
Mitochondria reproduction by division



Organellar features

- defined by double-layer membranes
- highly autonomous – reproduction by division, contain their own DNA and ribosomes
- protein synthesis
- movement of genes to nucleus, gradual extinction in organellar genomes
- plastid stromules (tubular extensions) can mediate the fuse with other plastids (exchange of genetic material)

Endosymbiont hypothesis of organellar origin



3/ Znázornění jedné z představ o evoluci eukaryotické buňky na základě postupného vchlipování biomembrány, členění vnitřního prostoru, zmnožování genetické výbavy a splývání buněk — *1* chloroplast, *2* jádro, *3* mitochondrie

Chloroplast genetics

1. inherited mostly uniparentally (typically maternally)

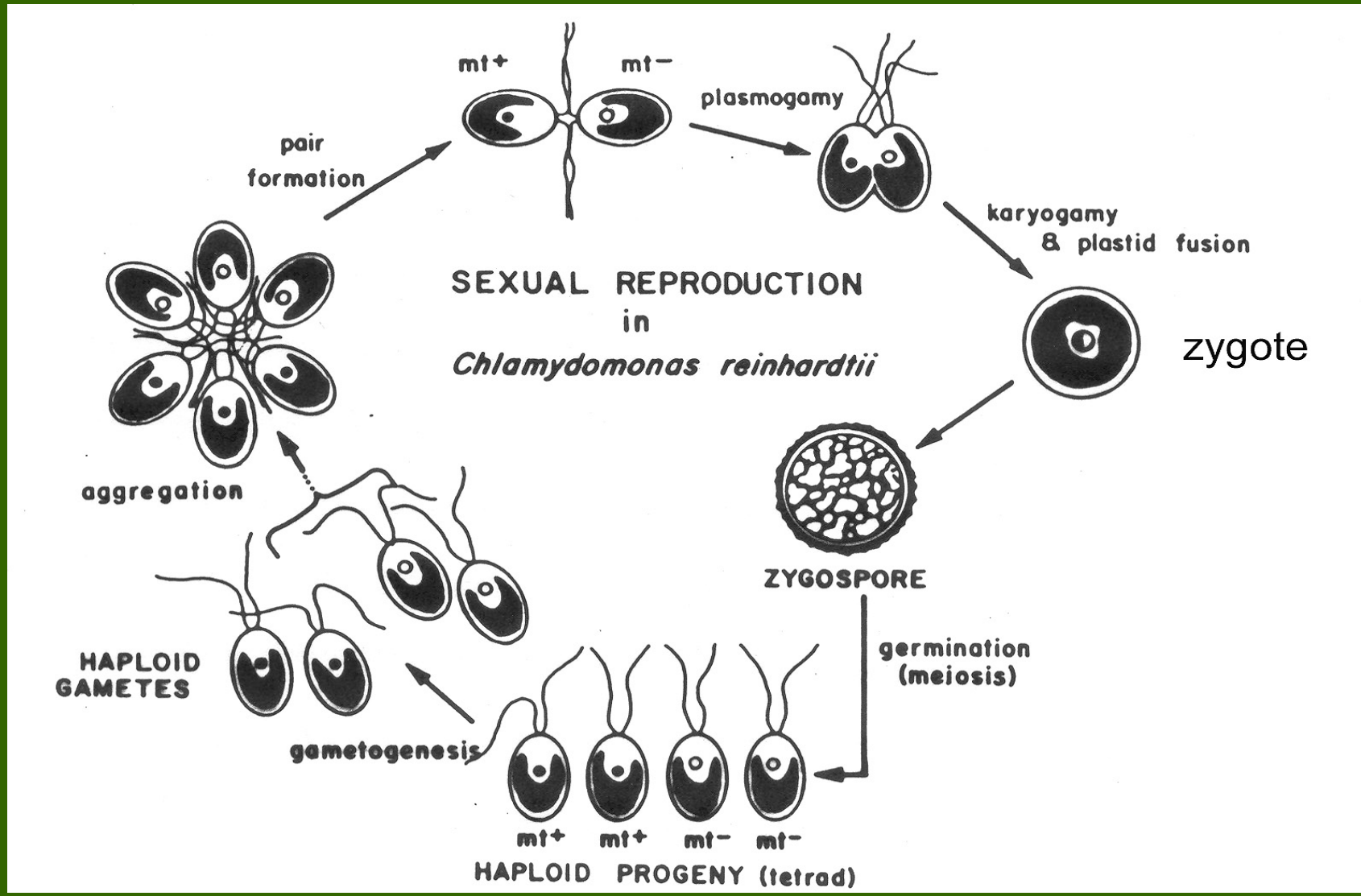
   2 mechanisms:

- *Chlamydomonas*, paternal cpDNA destroyed
- some higher plants – paternal plastids excluded or destroyed during reproduction process

2. Plastid DNA identical in the whole organism
3. genome uniform throughout differentiation

examples of exclusions to 2. a 3.

life cycle of *Chlamydomonas*



Acetabularia

green alga, unicellular, known fossils,
stele 5-10 cm, 1 nucleus, 10^6 chloroplasts,
can regenerate from rhizoid

in vegetative stage up to 30%
chloroplasts do not contain DNA

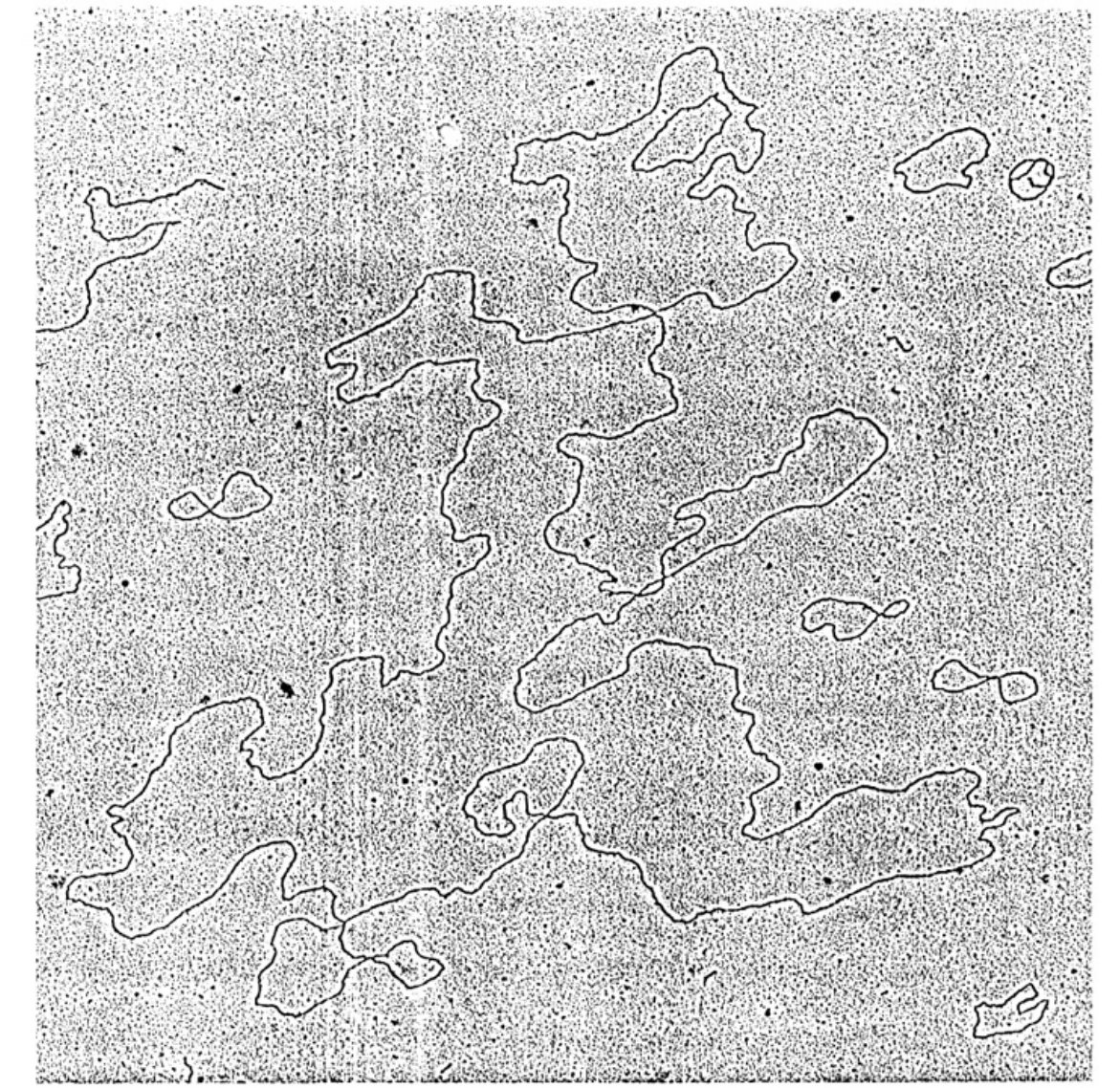
Chloroplast DNA (cpDNA)

General features:

1. dsDNA, circular
2. G-C content lower than in nucleus
3. many copies (~30-100) per plastid
4. 20-40 organelles/genome
5. no histones, bound proteins (*Hu*), organized to nucleoids
6. forms 10-20% of total DNA in leaves

Chloroplast DNA (cpDNA)

Relaxed cpDNA



Chloroplast DNA – not in textbooks

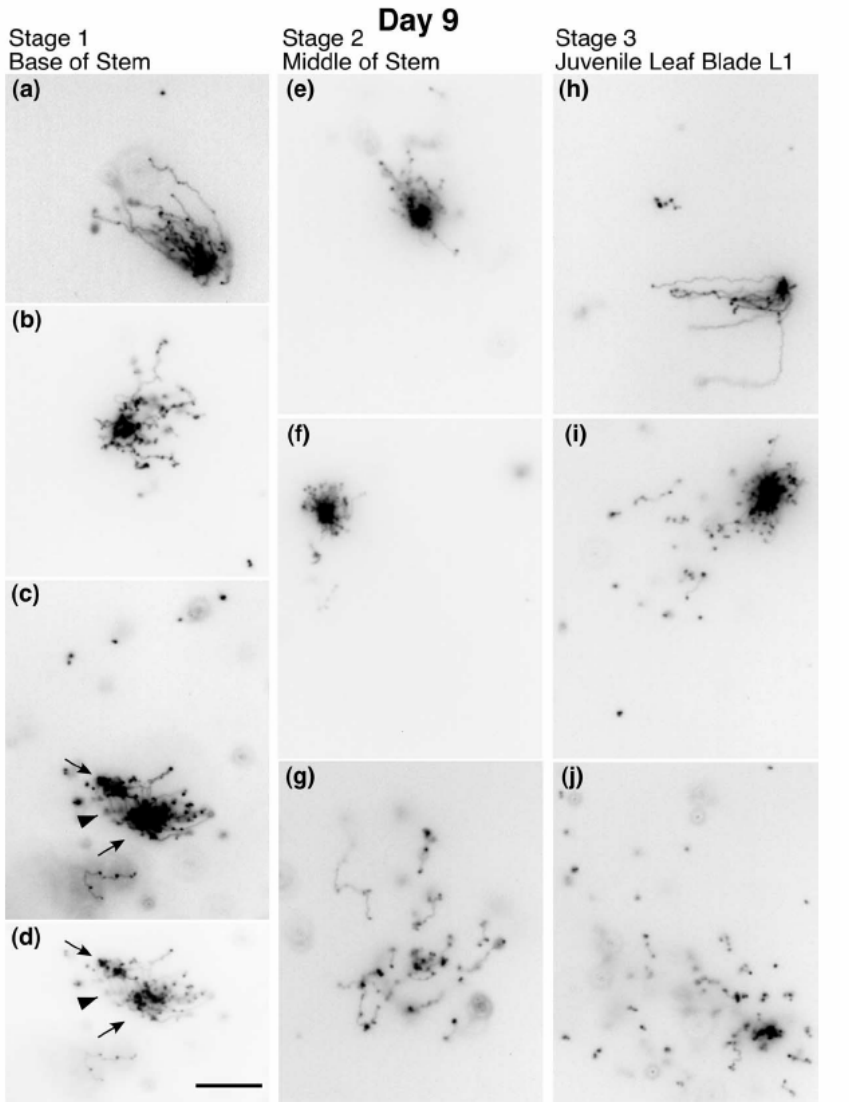
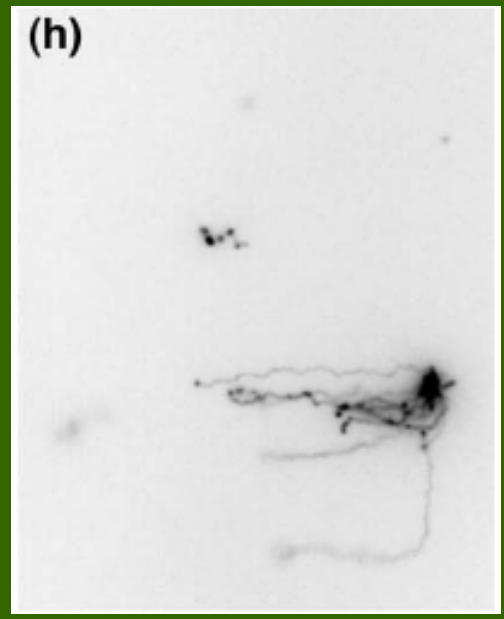


Figure 8. Total EtBr-DNA from individual plastids of nine days old juvenile seedlings. Each image shows the EtBr-DNA from a single plastid, although some unconnected fibers are not included in some images. Stages and structure classes correspond to those described in Figure 7. (a)–(c) Representative class I fields from stage 1. (d) Part of the image shown in (c), except the contrast has been enhanced (decreased). The two arrows in (c) and (d) indicate two DNA cores connected by fibers (arrowhead). This structure may represent the DNA of a dividing nucleoid. (e) and (f) Representative class I fields from stage 2. The connected fibers are shorter than in the class I fields of stage 1. (g) A representative class III field from stage 2. (h)–(j) Representative (h) class I, (i) class II and (j) class III fields from stage 3. The scale bar represents 10 μm and applies to all images.

Oldenburg D.J. and Bendich A.J., 2004

Chloroplast DNA – not in textbooks



Oldenburg D.J. and Bendich A.J., 2004

chloroplast genome size

70 - 200kb

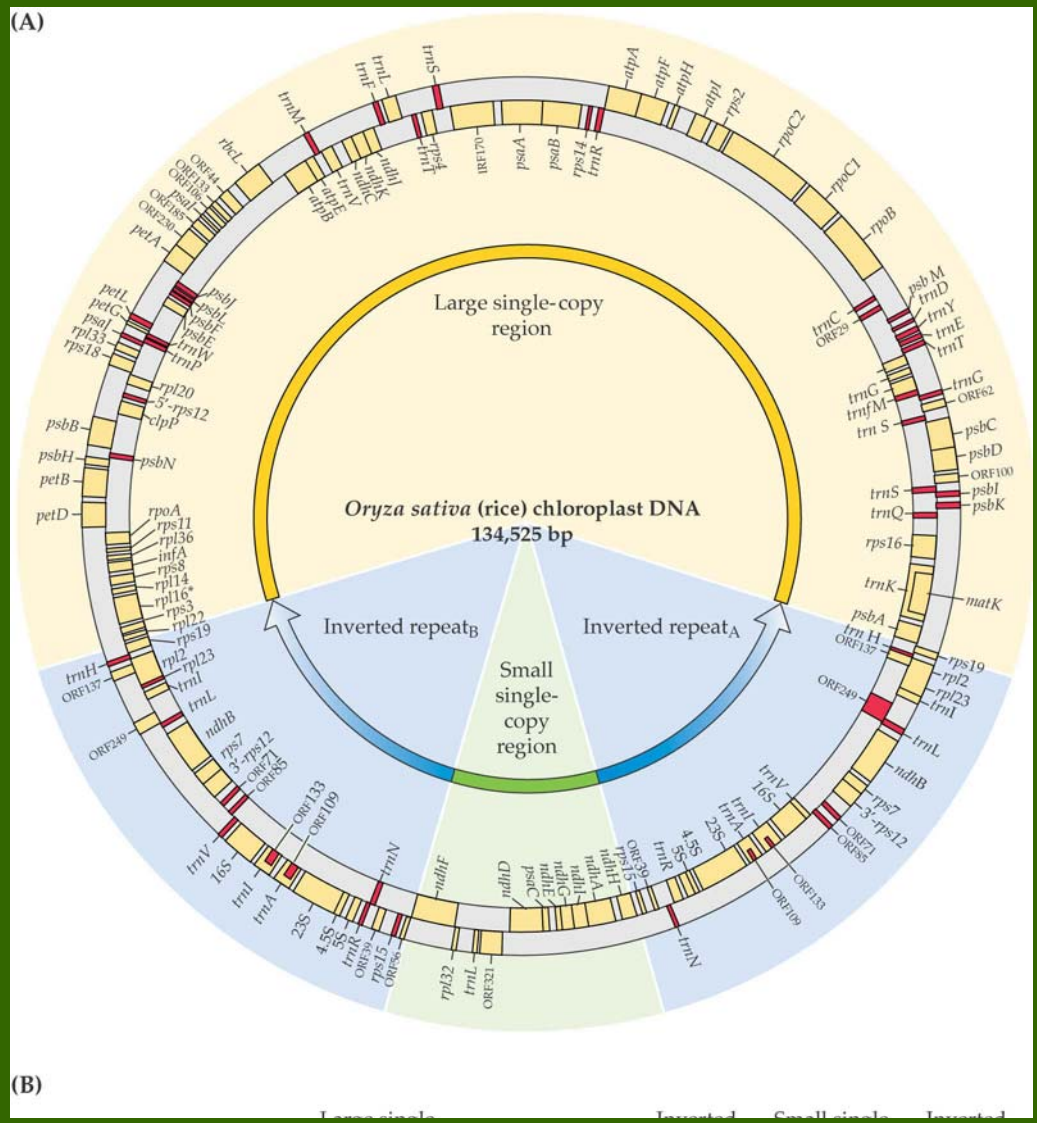
Taxa	Genome size (in kb)	Inverse duplication (in kb)
Angiospermae		
Nicotiana tabacum	156	25
Spinacia oleracea	150	24
Pelargonium hortorum	217	76
Pisum sativum	120	Not present
Epifragus virginiana	70	22
Oryza sativa	134	21
Gymnospermae		
Pinus	120	Not present
Ginkgo biloba	158	17
Pteridophyta		
Osmunda cinnamomea	144	10
Bryophyta		
Marchantia polymorpha	121	10
Chlorophyta		
Codium fragile	85	Not present
Chlamydomonas reinhardtii	195	22
Chlamydomonas moewusii	292	41
Rhodophyta		
Cyanophora paradoxa	127	10
Chromophyta		
Dictyota dichotoma	123	5

Typical cp genome

circular DNA molecule „long" and „short" copy regions (LSC and SSC) - unique copies, divided by IR

rRNA (*rrn*) genes (organized in clusters similarly to v *E. coli*)

recombination between repeats (dividing LSC a SSC) leads to inversion of SSC



cpDNA genes

code for cca 100 proteins

cp genes code for:

1. genetic aparatus (replication, transcription,translation)
2. photosynthesis
3. complexes of thylakoid membranes

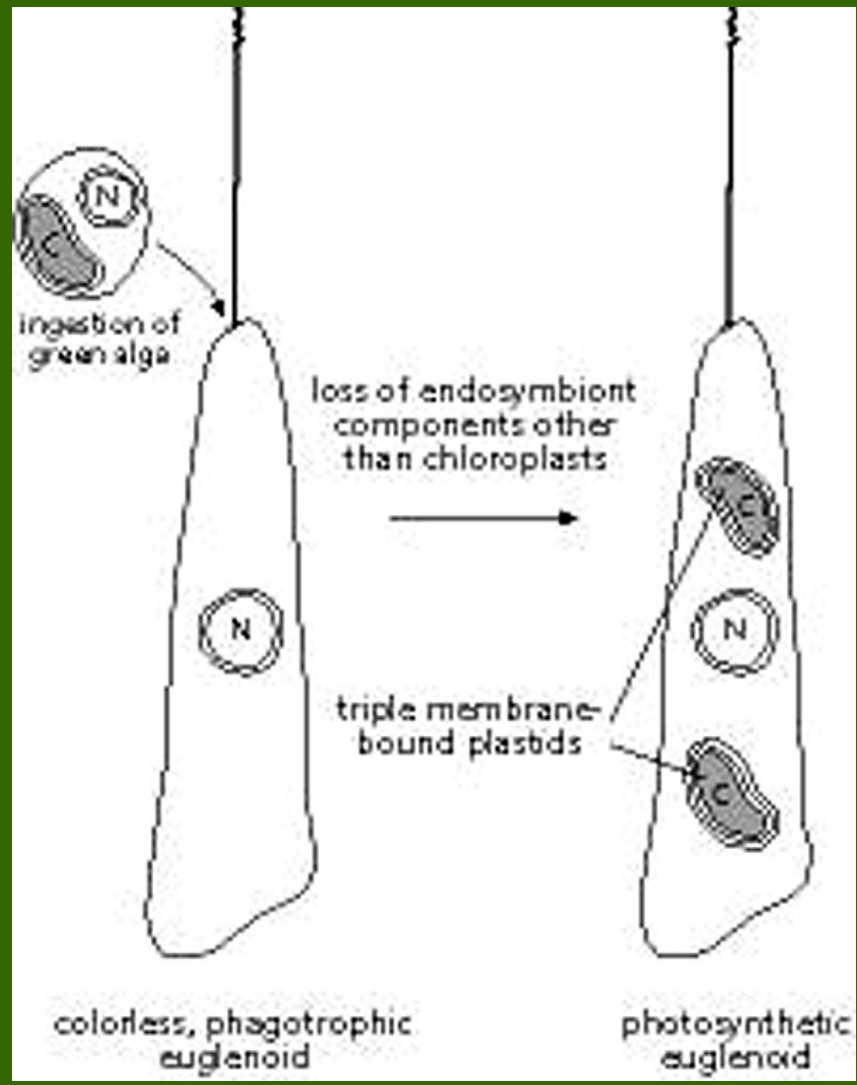
Endosymbiont hypothesis and gene movement

- plastid genome highly conserved
- most genes of endosymbiont extinct or became part of nuclear genome
- Rubisco
- 2 types of introns - chloroplasts evolved before prokaryonts lost introns

More membranes...

some chloroplasts engulfed secondarily: Chromophyta, Dinoflagellata and Euglenoida – 3 or 4 membranes defining chloroplast (outer, inner, cp)

? endosymbiosis of (pre) eukaryont



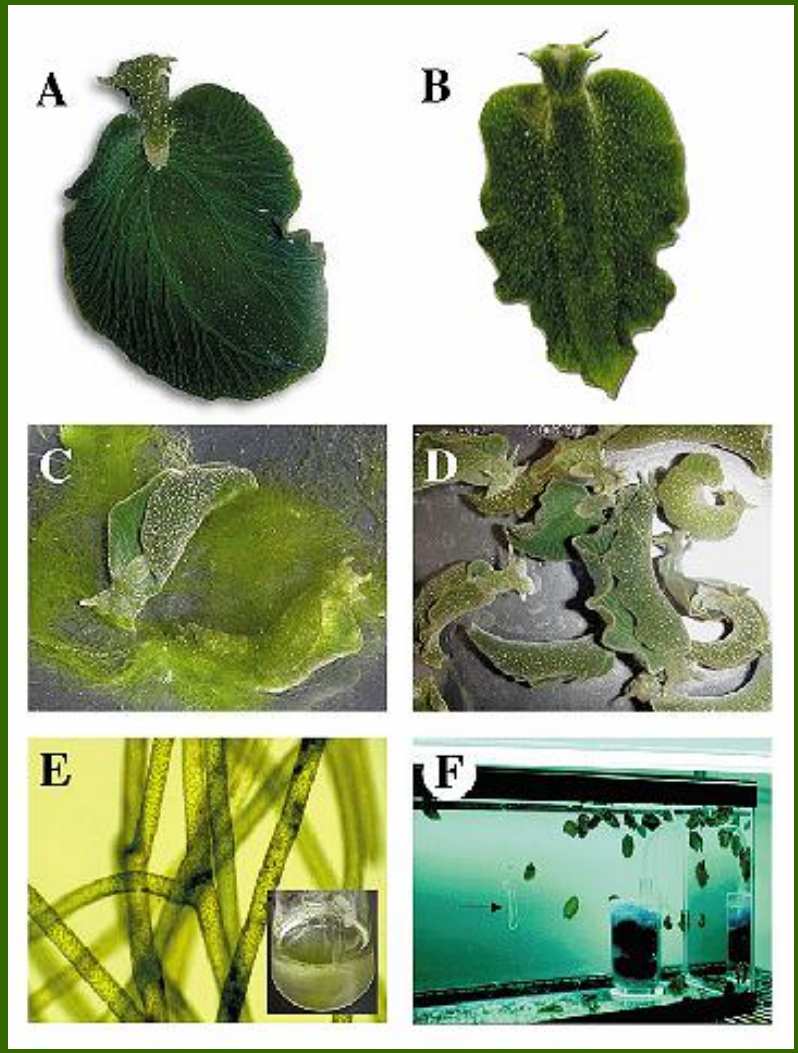
Sea slug *Elysia chlorotica*

Elysia with active chloroplasts of alga *Vaucheria*

Chloroplasts remain active for 8 months

courtesy of Dr. Mary Rumpho

Sea slug *Elysia chlorotica*



***Elysia chlorotica* and *Vaucheria litorea*.** A) Dorsal view of *E. chlorotica*. Animals are typically found in nature as small as 1 or 2 cm to as large as 6 cm, as shown here. B) Ventral view of *E. chlorotica*. C) Two camouflaged *E. chlorotica* specimens feeding on *V. litorea*. D) Several specimens of *E. chlorotica* showing the variation in size and body forms. E) *V. litorea* filaments (about 1 to 2 mm diameter). F) Sea slugs are easily cultured in aquaria containing full-strength artificial sea water and overhead lighting at10°C. Non-pigmented eggs are produced in a mucus mass on the aquaria walls (see arrow). The eggs serve as a source of pure animal DNA since no plastids are found in the eggs.

Rumpho M. et al., 2000

Caulerpa taxifolia

mitochondrial DNA (mt DNA)

- mostly circular
- no histones
- low copy number per organelle
- inherited mostly uniparentally
  - conifers: biparentally
  - Angiosperms : maternally (as cpDNA)
  - *Chlamydomonas*: from minus (-) (cpDNA from parent +)

mt genome size

- *S. cerevisiae* 84 kb
- mammals 16 kb
- similar products
- economization during evolution
- higher plants x algae

mt genome organization

Mitochondrial DNA from different organisms

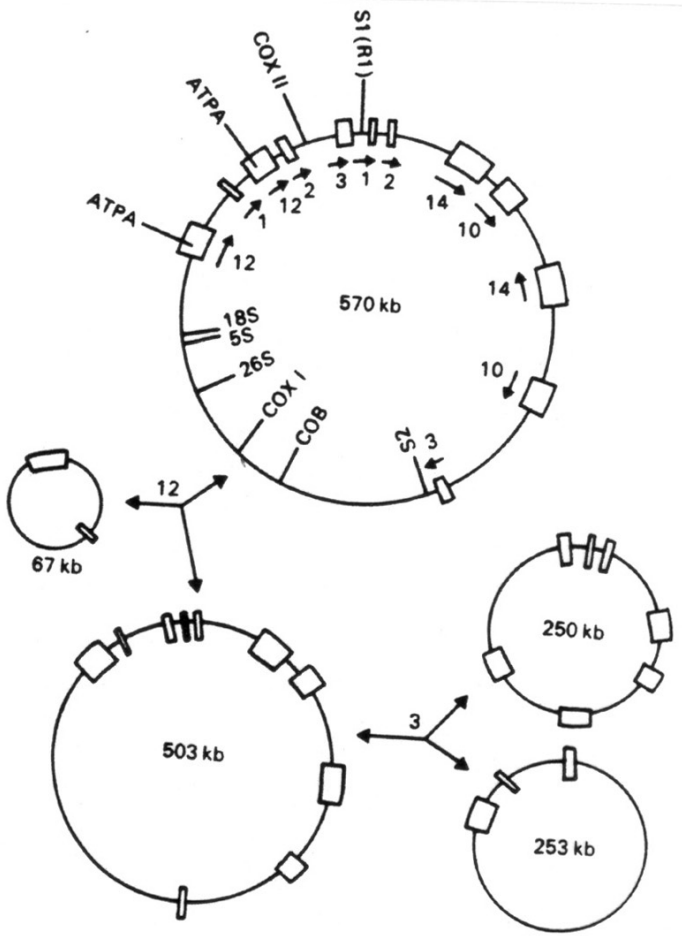
	Number of base-pairs	Number of different molecules per organelle
HIGHER PLANTS		
Brassica ssp.	218 000	3 (circular) 218kb, 135kb, 83kb
Maize	570 000	7 (circular) from 570kb-47kb,
	plus a variable number of plasmid-like DNAs from 1 400–6 000 bp	up to 4 (circular or linear)
Muskmelon	2 400 000	?
ALGAE		
Chlamydomonas	16 000 linear	1
FUNGI		
Podospora anserina	juvenile 95 000	1 (circular)
	senescent 30 000 + 2 400	2 (circular)
Saccharomyces cerevisiae	80 000	1 (circular)
OTHERS		
Cow and man	16 600	1 (circular)

mt genome

maize (*Zea mays*):
7 circular molecules:

„master" molecule
570 kb and derived
subgenomic circular
molecules

subgenomic
molecules produced
by recombination at
direct repeats



The location and orientation of six repeated DNA sequences (the 1, 2, 3, 10, 13 and 14 kilobase repeats) are shown, together with the positions of the integrated S1 (sometimes called R1) and S2 DNA sequences. Subgenomic molecules are generated from the master circle of 570 kilobase-pairs by recombination across the repeated sequences. Examples shown are for recombination across the 12- and 3-kilobase repeats. After Bailey–Serres (1987).

cp genome expression

cotranscription, organization to „operons"

plastids of higher plants cca 30 transcriptional units (defined by promoter and terminator)

subgenomic circular molecules derived from „master" by recombination at inverted repeats

promotors – similar to bacterial (–10 and –35 motifs, mutual distance crucial)

mRNA not capped, not polyadenylated

mt genome expression

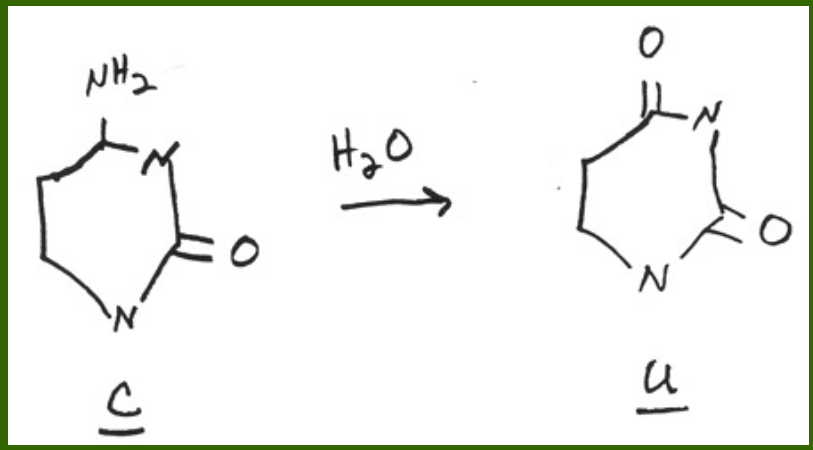
mRNA not capped, not polyadenylated

transcripts „edited"

- RNA Editing
  - -discovered in Trypanozoma mitochondria
  - -frequent in plant mitochondria, also in some chloroplast genes of higher plants
  - -**Definition**: any process (except splicing), leading to a change in RNA sequence which does not correspond to complementary DNA

RNA editing

1. most transcripts edited
2. mainly transition C to U
3. preferential editing of coding regions
4. some transcripts edited incompletely



transition C to U

cytosin deaminase or nucleotide base exchange (elimination)

Introns (splicing)

- in different organisms the same introns can be found at the same positions within the gene

- identical or similar intron found in unrelated genes and organisms

- unusual distribution and phylogenetic analyses confirm that the introns have been gained and lost throughout the evolution

Introns I

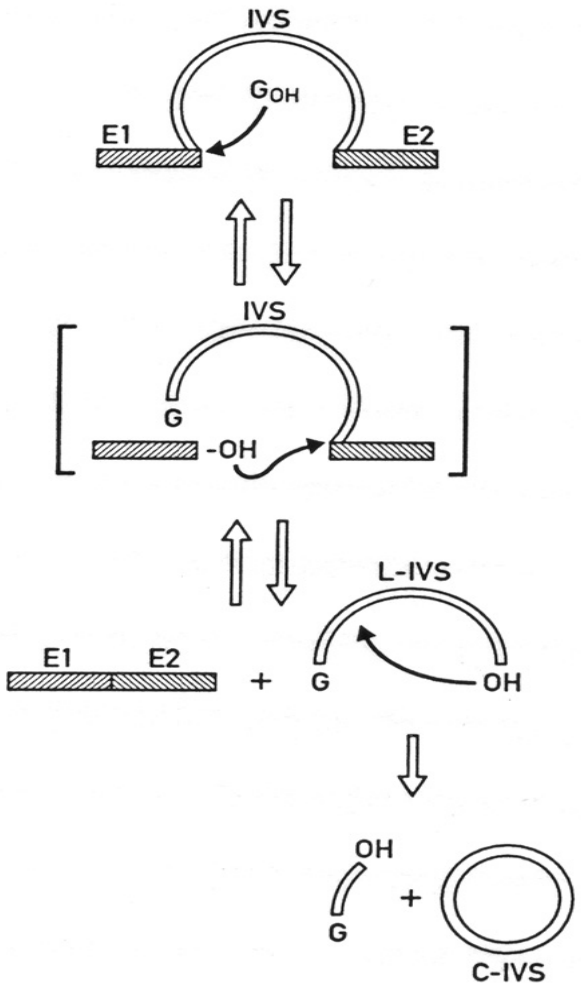


Fig. 1. Mechanism of splicing by Group I introns. The brackets indicate intermediates, which are held together non-covalently. The reader is referred to the text for further details. E1, E2, exons; IVS intervening sequence or intron; G_{OH}, guanosine nucleotide; L-IVS, linear free intron; C-IVS, cyclized intron lacking one or more nucleotides at the 5´ end.

Introns II

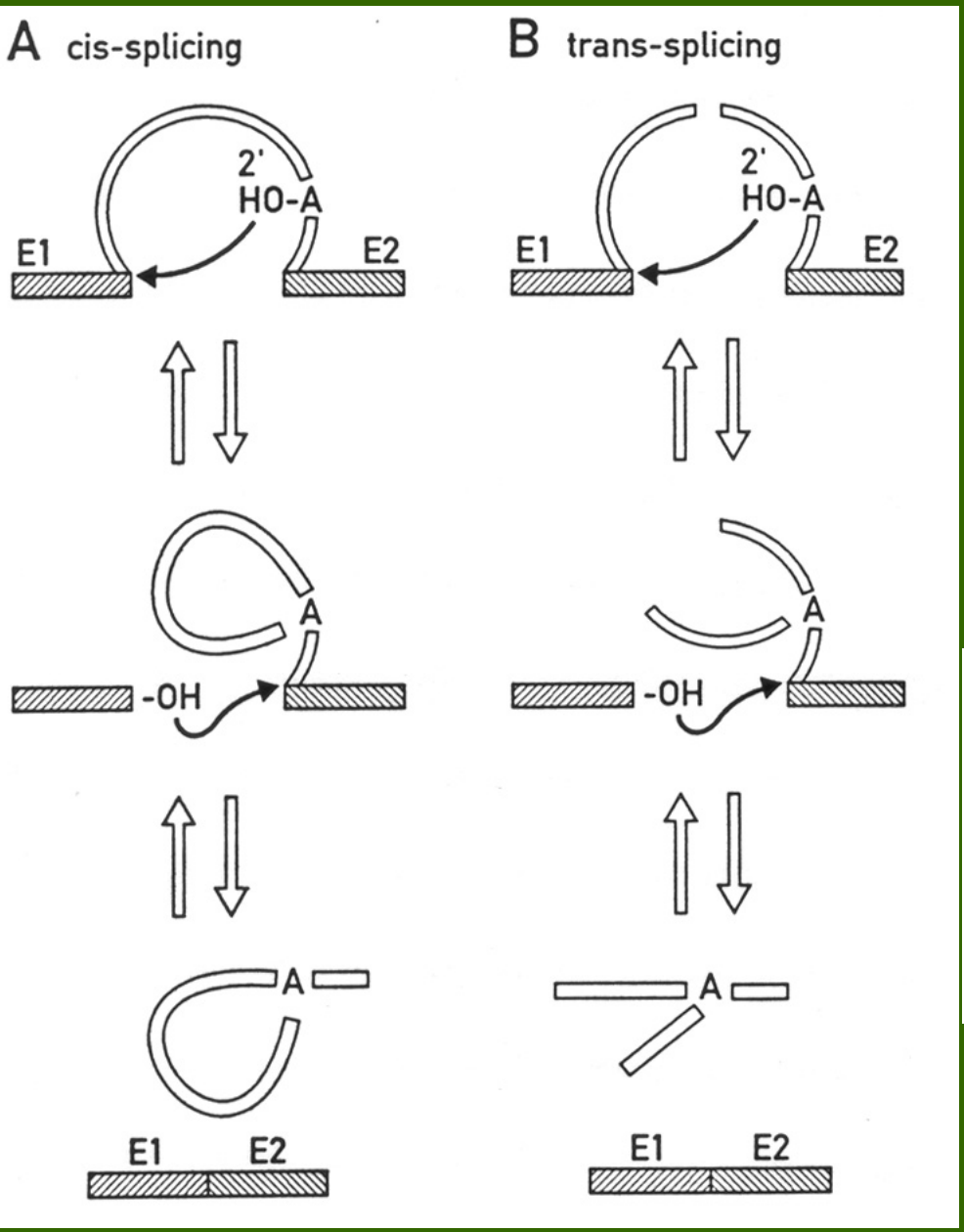


Fig. 4. Mechanisms of splicing by group II introns in vitro. The first step of group II autocatalytic splicing involves a nucleophilic attack on the exon 1 - intron junction by the 2´ OH of the A nucleotide at the branch site (*trans*-esterification). The second reaction proceeds by the nucleophilic attack on the intron - exon 2 junction by the free 3´ OH at the end of exon 1, yielding the ligated exons and the free intron. In *cis*-splicing (panel A) the intron product is a lariat, but in the case of *trans*-splicing (split intron, panel B), the intron is excised as a branched, Y-shaped molecule. Hatched bars (E1, E2): exons; white ribbon: intron.

Trans - splicing

some cp RNAs generated by *trans*-splicing:

- splicing of different RNA molecules
- intron-exon arrangement maintained
- introns II

cp transcriptional regulation

1. total
   - i.e., lowered/enhanced expression of all genes at the same moment (transcription enhances at „greening", lowers when chloroplast turns into chromoplast)
2. gene specific
   - *psbD/psbC* promotor reacts to light

nuclear control of cp gene expression

genetic studies confirmed a potential for nuclear control of cp genes

Mendelian (nuclear) mutants defective in development or function of plastids do not express specific cp - encoded genes

Retrograde signaling pathways

signals from plastid to nuclear „target" promoter elements participating in the response to light

necessary for the plant response to changing and often stressful environmental (nuclear genes for photosynthesis proteins expressed poorly when plastids demaged or underdeveloped)

signal?

- chlorophyll biosynthesis precursors
- functional plastid gene expression (heterotrophy to autotrophy transition)
- components of electron transport in photosynthesis

Plastid transformation

Comparison of the nuclear and plastid genomes of angiosperms

	Nuclear genome	Plastid genome
Chromosomes	Two copies of each of many chromosomes; the number of chromosomes per diploid cell is species-specific	~60 copies of a single circular chromosor per plastid ~50–60 chloroplasts per cell
Genes per chromosome	Could be thousands	~120–150
Arrangement and transcription of genes	Each gene is separate and is transcribed individually	Many genes are in operons and are transcribed together

Plastid transformation

Introducing genes into nuclear and plastid genomes

	Nuclear genome	Plastid genome
Insertion of foreign DNA into genome	Undirected; multiple insertions are common.	Directed to a specific site by homologous recombination.
Transcription of introduced genes	Affected by the promoter, the type of cell and the site of insetion of the gene into the genome. Each introduced gene is expressed individually.	Affected by the promoter, the type of plastid and the type of cell. The location on the chromosome is not known to affect transcription. A set of genes could be introduced as an operon or a individual transcription units.
Current limitations	The level of expression of an introduced gene is unpredictable.	Obtaining homoplasmic transformed strains can be difficult: the devlopment of easier new methods will be important. Alternatively methods are needed to retain transgene(s) in the presence of untransformed plastid chromosomes.
	Each gene in a set required for a new multigenic trait or biosynthetic pathway may have to introduced separately and sequentially. The level of expression of each separately introduced gene is unpredictable.	To date, tobacco is the only crop in which fertile plants with plastic transgenes have been described. Reports on other crops are promising.

Prions and mad cows

Table 1. The prion diseases.

{PRIVATE}

Disease	Mechanism of pathogenesis
Human diseases	
Kuru (Fore people)	Infection through ritualistic cannibalism
Iatrogenic Creutzfeldt-Jakob disease	Infection from prion-contaminated HGH, dura mater grafts, and so forth
Variant Creutzfeldt-Jakob disease	Infection from bovine prions?
Familial Creutzfeldt-Jakob disease	Germline mutations in PrP gene
Gerstmann-Sträussler-Scheinker disease	Germline mutations in PrP gene
Fatal familial insomnia	Germline mutation in PrP gene (D178N and M129)
Sporadic Creutzfeldt-Jakob disease	Somatic mutation or spontaneous conversion of PrP^{C} into PrP^{Sc}?
Animal diseases	
Scrapie (sheep)	Infection in genetically susceptible sheep
Bovine spongiform encephalopathy (cattle)	Infection with prion-contaminated MBM
Transmissible mink encephalopathy (mink)	Infection with prions from sheep or cattle
Chronic wasting disease (mule deer, elk)	Unknown
Feline spongiform encephalopathy (cats)	Infection with prion-contaminated MBM
Exotic ungulate encephalopathy (greater kudu, nyala, oryx)	Infection with prion-contaminated MBM

Scrapie and BSE

Conversion of normal cellular protein PrP^{C} to PrP^{Sc} (isoform causing „scrapie" disease)

- reduced portion of α-helices, increased β-sheets
- infection agents („wrong" prions) do not induce production of antibody against prion protein - the likely reason is the same composition of the „wrong" and the „good" prion proteins
- change in solubility (PrP^{C} soluble in non-denaturing detergents x PrP^{Sc})
- change in degradability by proteazes (PrP^{C} degraded, PrP^{Sc} only partially)

Prions and spongiform encephalopathy

Prion: „proteinaceous infectious particle“

Prusiner S., 1997