

Chapter 4: Organelle Division

- mitochondria
 - division and fusion
 - some of the players
 - mouse
 - yeast
 - images from plants

organelles can divide and then fuse

- chloroplasts
 - overview
 - chloroplast division rings
 - comparing ring structures al little bit between mitochondria and chloroplasts
 - molecular details for chloroplast division

tDNA screens; mutant screens, will cover later--> screen things with odd looking chloroplasts; what comes out are arc mutants (accumulation and replication of chloroplasts: all are nuclear) they looked for anything changing size/number of chloroplast; started to look for sequences similar to bacterial sequences

some similarities and differences

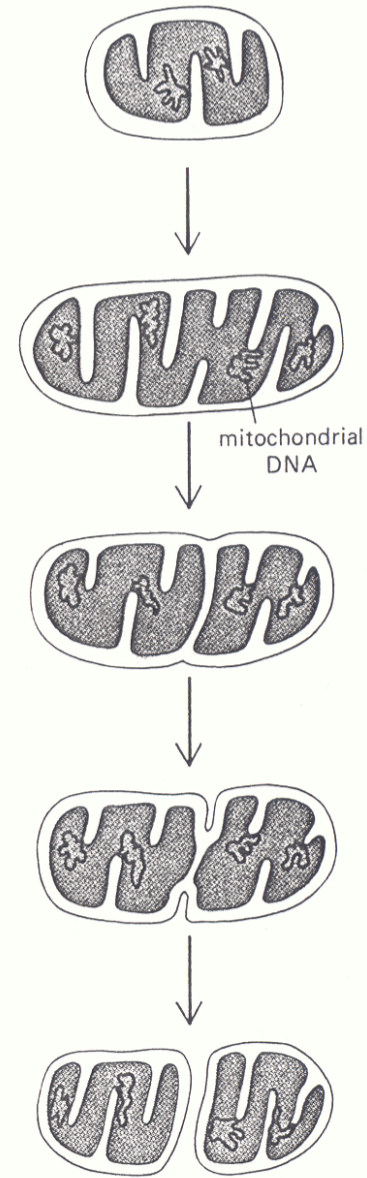
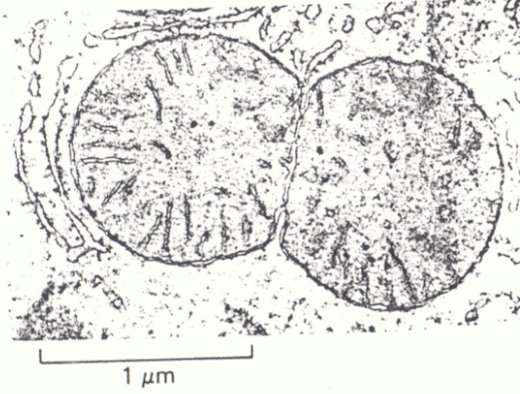
mitochondriokinesis

plastid kinesis

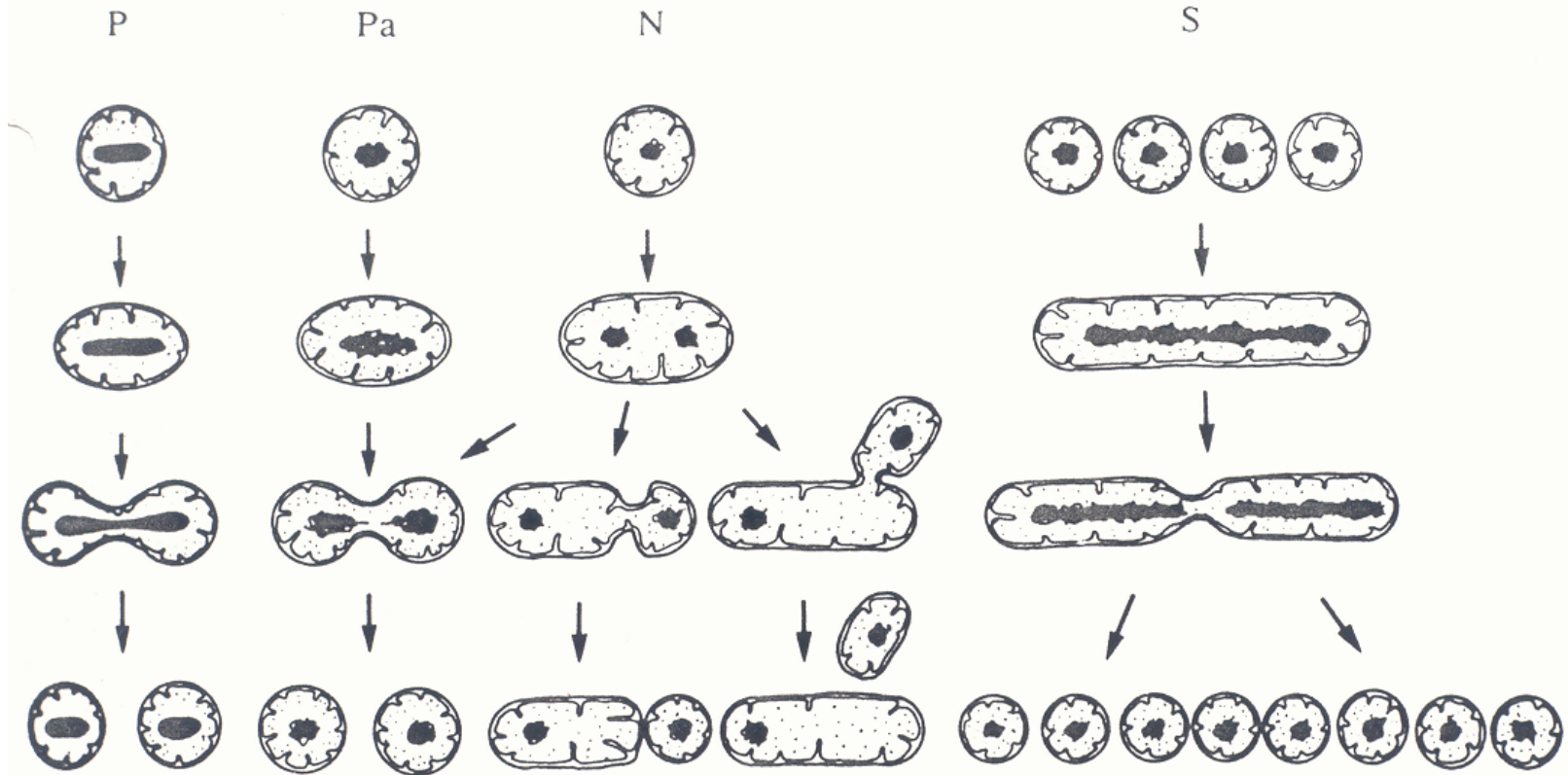
- can separate out the two processes, replication of the genome from organelle division
- can replicate without actually dividing the organelle
- often the two processes are linked, depends on the species; for example- trypanosomes- have one kinetoplast and a mess of mini and maxi circles, replication generally means separating since it is so complex and the two are tightly linked- there are some with looser links- another example is that replication will allow upregulation of the copy number per organelle and then not separating organelles between daughter cells allows upregulation
- plastid kinesis and mitochondriokinesis refers to the division process of organelles rather than replication

Before, only had SEMs to visualize mitochondria

organelles can be fused back together, its not just fission occurring -just images, don't know what way they are going since they look pretty much the same either way



see how genomes are replicated in relation to organelle division across different species- don't have to know the species; more complicated than we think



replication and separating happens simultaneously

replication is almost completed before organelles divide

replication occurs and then can have budding off of new organelles

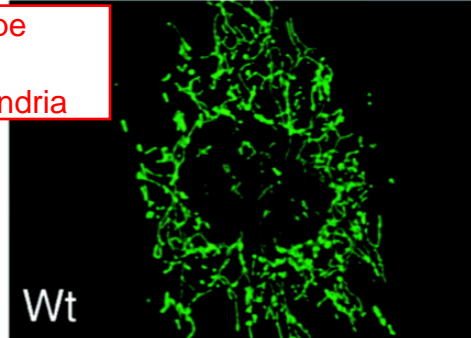
saccharomyces can have genomes which fused and then can divide again
-think of the implications of these methods

In a mouse
Mfn- mitofusions
Drp- dynamic related protein

Wild Type
-tagged
mitochondria

we have mutants that affect one process but not the other with regards to fusion and fission
-fluorescently tagged mitochondria in cell

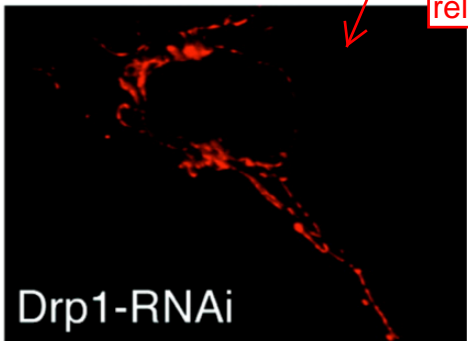
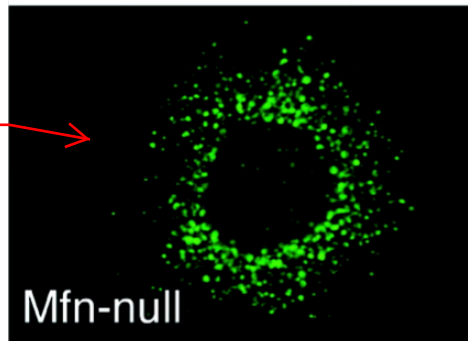
We know that there are fusion and fission processes that occur and that they occur **independently** from one another



No fusion

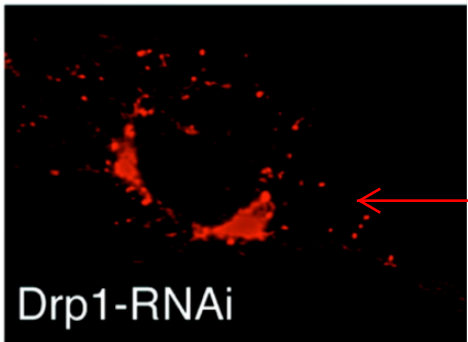
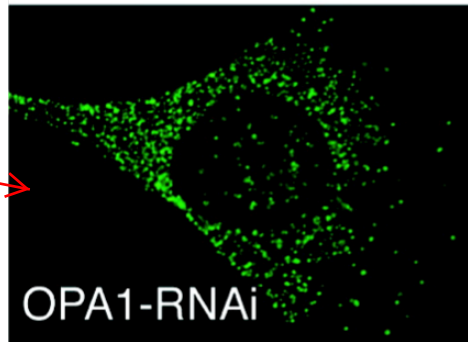
No fission

with mutated mitofusion protein;
No fusion



mutation in gene encoding dynamic related protein

fission still occurs;
mitochondria seem to get smaller and smaller compared to wild type without any fusion occurring

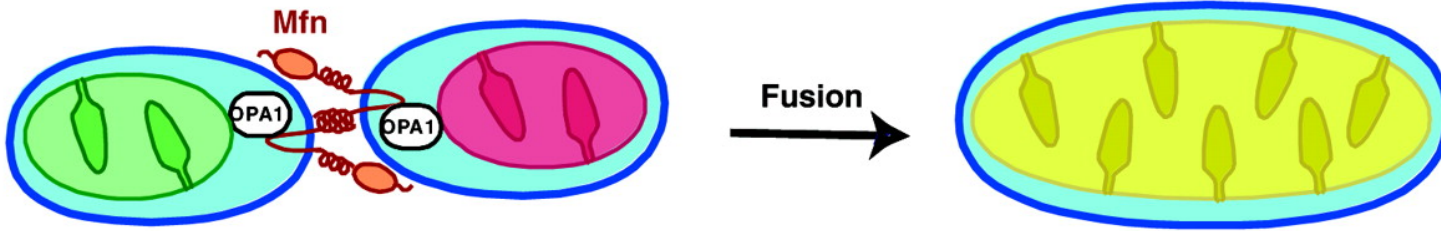


No fission but fusion still occurs; mitochondria seem to cluster more and get larger, seem to cluster near the nucleus

Fis1- fission 1

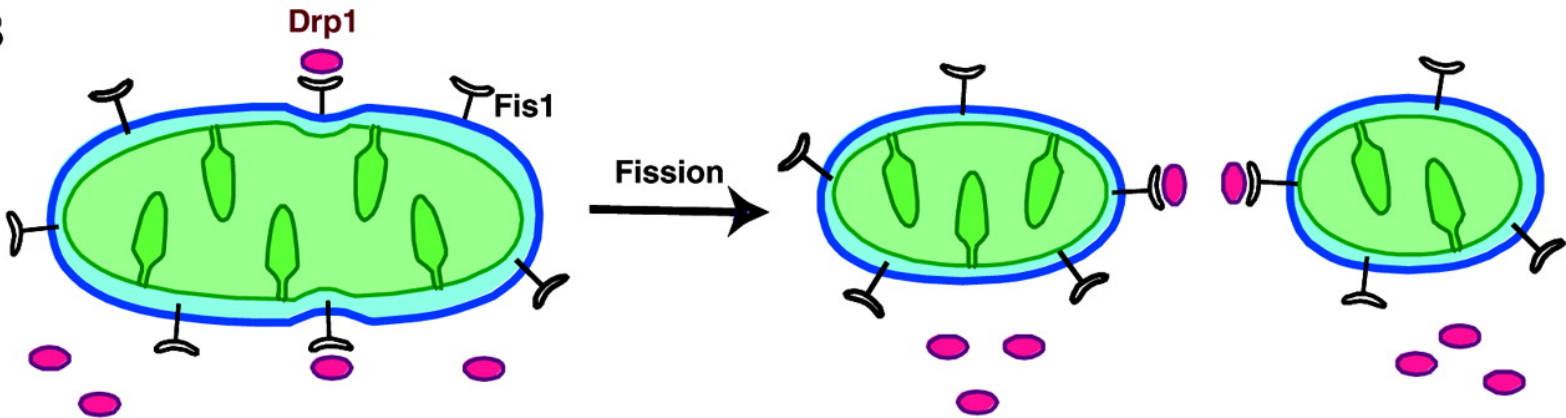
FUSION- mitofusion1 and mitofusion2 are involved, seem to form homo and heterodimers and the dimerization is important, required for fusion process; often they have GTPases have helices that can be formed which are interaction domains; proteins are often anchored (**mitofusions** are in the outer membrane of the mitochondria sticking into the cytosol) (**opa1** is in the inter-membrane space and is also linked to the inner membrane and outer membrane) people propose that there is an interaction occurring between the proteins which allow mitochondria to come together, which mediate fusion between outer and inner membranes to make unit

A



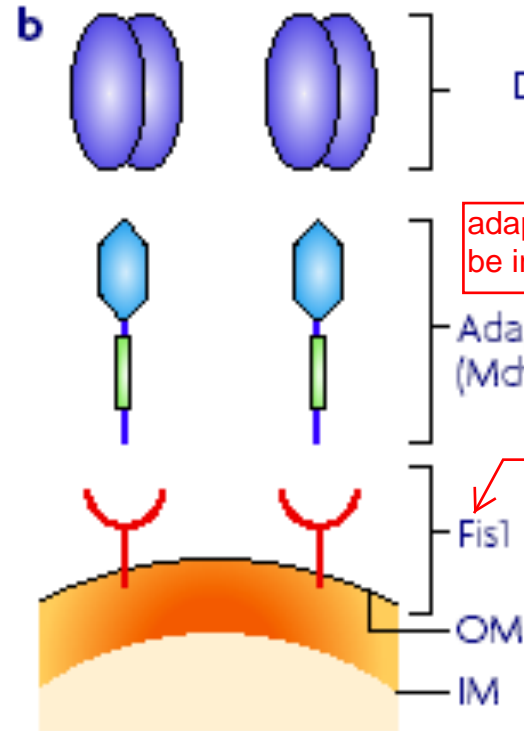
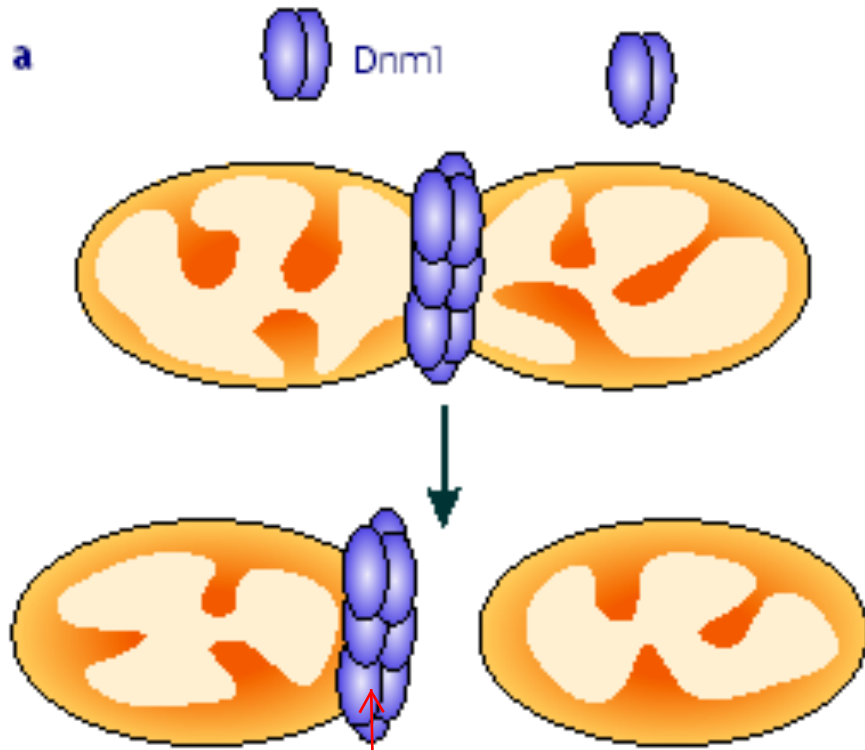
FISSION- different proteins involved, Fis1 is an outer membrane protein, seems to be everywhere in the outer membrane of the mitochondria and interacts with Drp1 (dynamic related protein) which is a cytosolic protein; seems to only interact with Fis1 at the division plane so there must be other players involved in this process to mediate and ensure this interaction only occurs at one particular site; now that there is a division plane (where fission occurs) you will generate two daughter mitochondria, in this case

B



Yeast system
-a bit different compared to mice
-can see some similar players

dynammin is a contractile protein

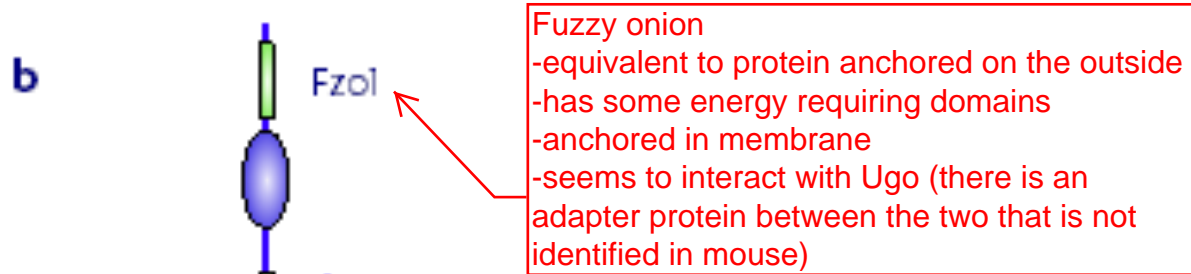


adaptor proteins seem to be involved

outer membrane fission protein; similar to previous model

Dynammin can make a ring structure along the fission plane, which constricts, leading to fission

Fusion process for yeast



Japanese for fusion → Ugo1

mitochondrial genome maintenance protein

OM outer membrane

IMS intermembrane space

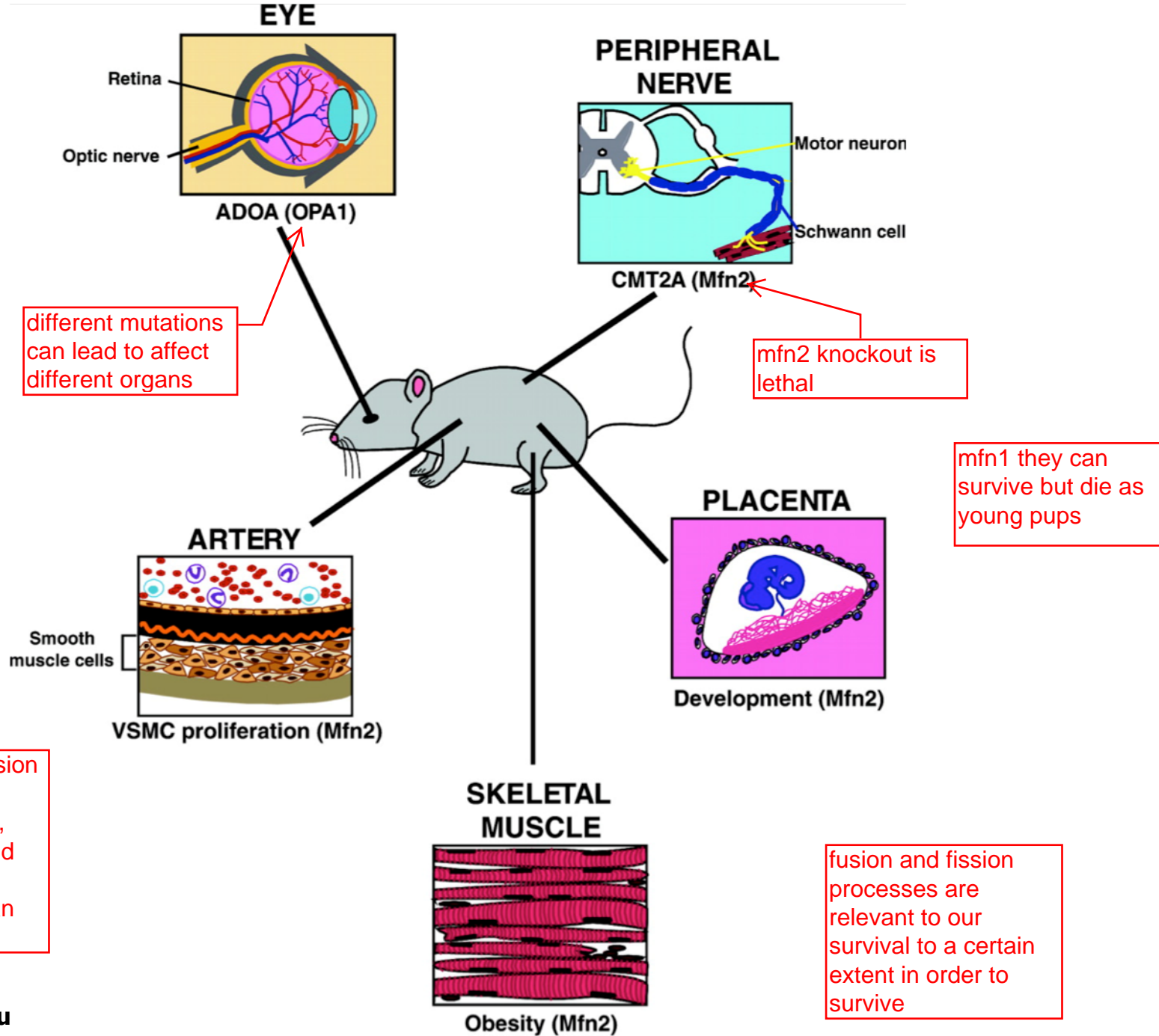
IM inner membrane

there are 2 versions

anchored in inner membrane sticking into the intermembrane space

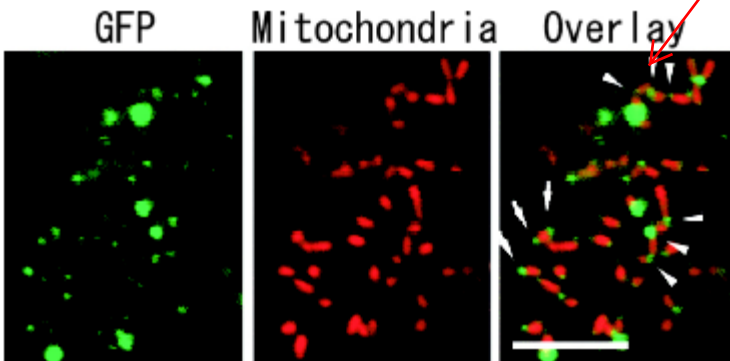
Mgm is also required for shaping the morphology of cristae in mitochondria as well as fusion; more than one function

Cristae



visualization of fusion processes

mitochondria onion bulb cells



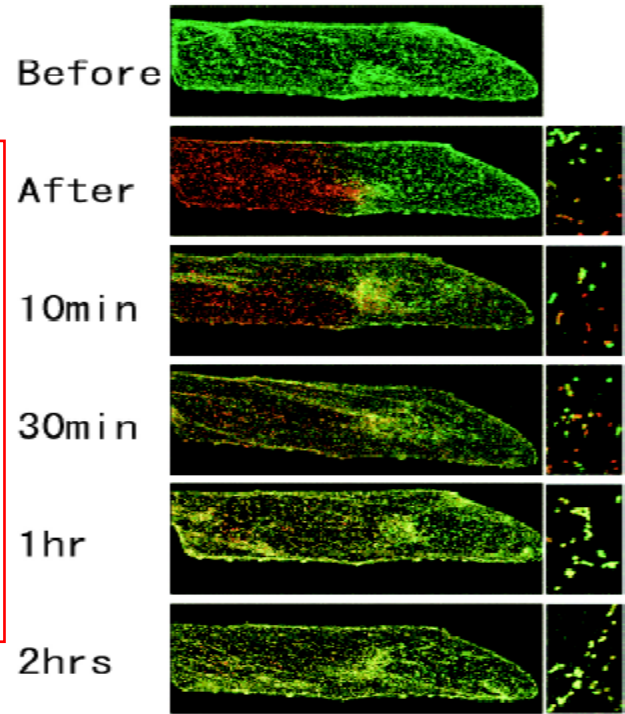
GFP labeled dynamic related proteins, DRP3

shows co-localization, not visualization

DRP is colocalizing with the mito

GFP with Eos can take one wave length, take another area and use different light excitation and make it glow another colour, can watch what is happening and suddenly, can see yellow mitochondria take over more and more indicating fusion processes; have green and red proteins in the same mitochondria

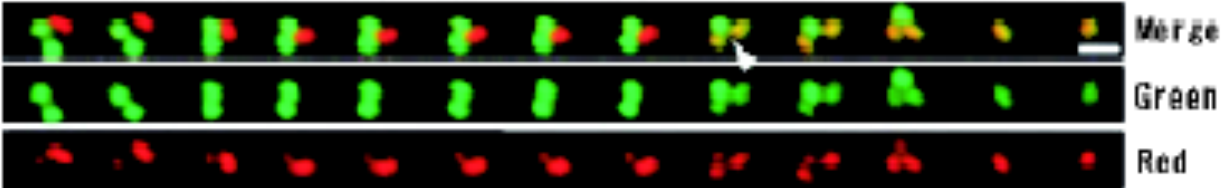
Mitochondria



0 6 9 12 15 18 21 24 27 30 33 51 sec



0 6 12 18 24 30 36 39 42 60 63 66 69 sec

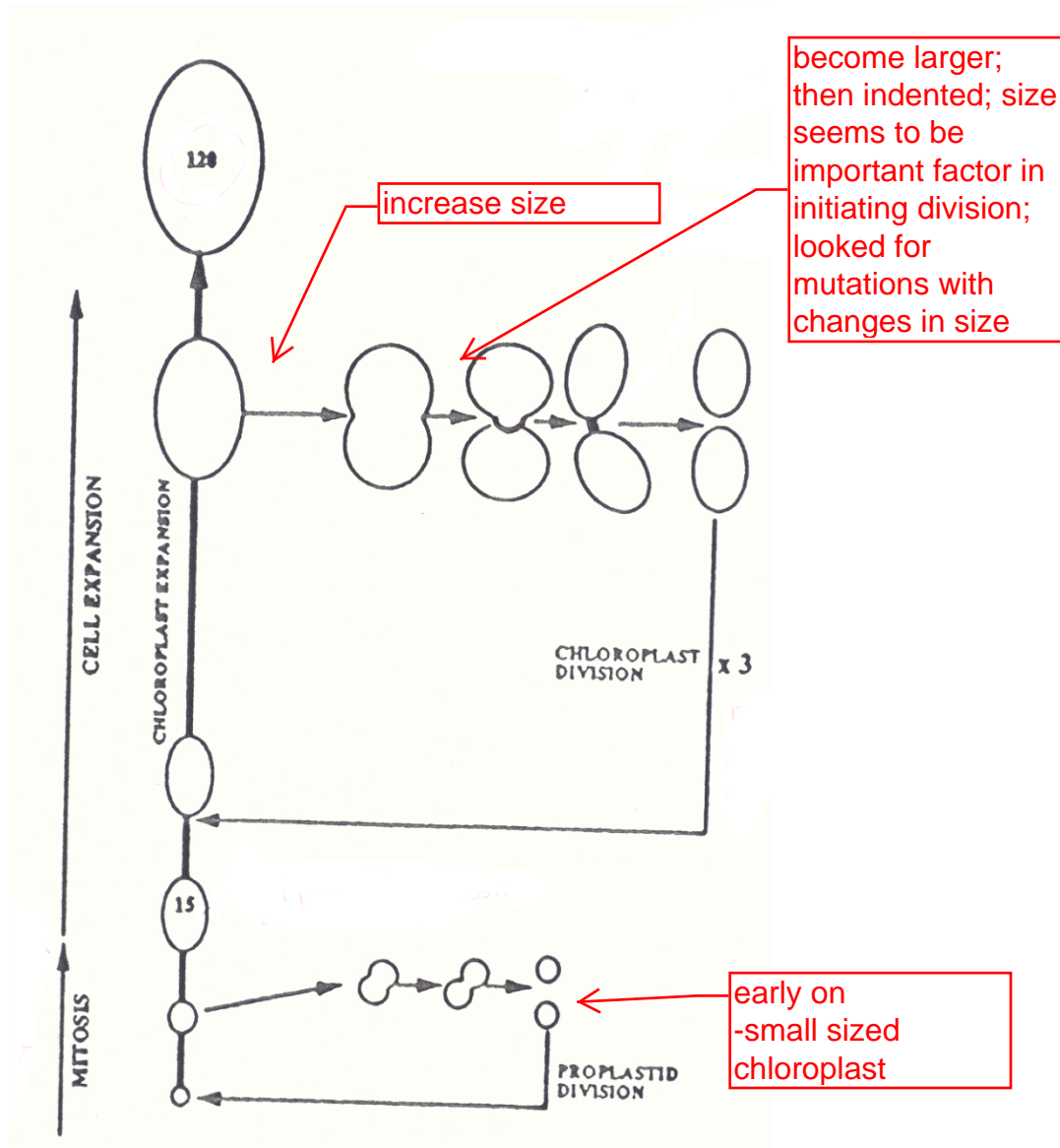


very close up image of mitochondria; Can see the merging pattern and exchange of protein when the two are merged, showing yellow -this shows fusion, not just separation processes -this imaging is taken at different time points

the top part is looking at the same images and can view under different wavelength

the 3 rows are the same images but seen at different wave lengths



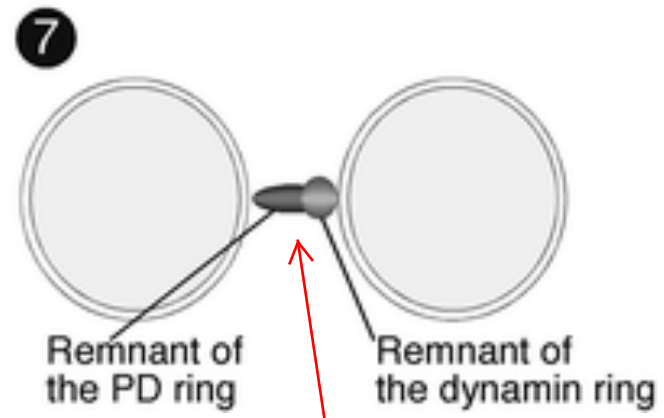
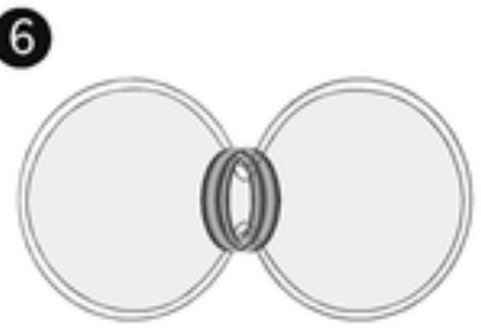
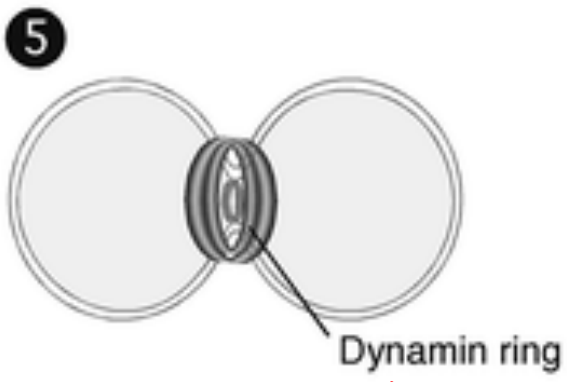
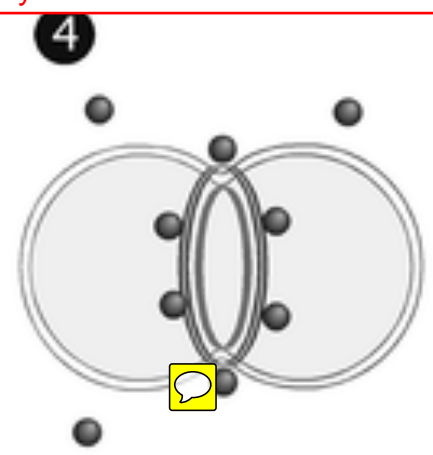
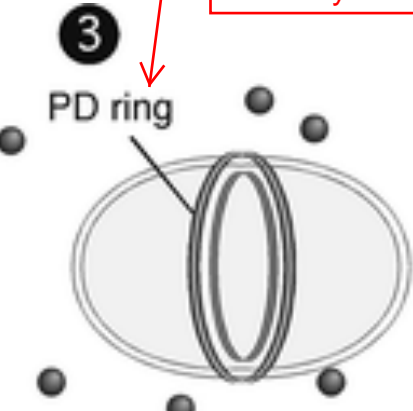
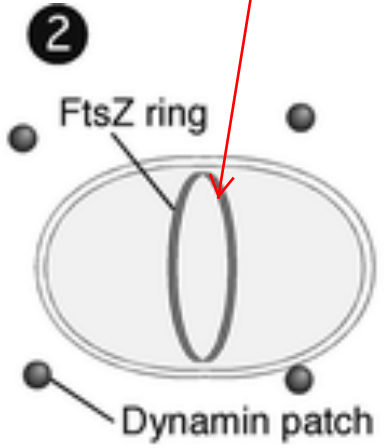
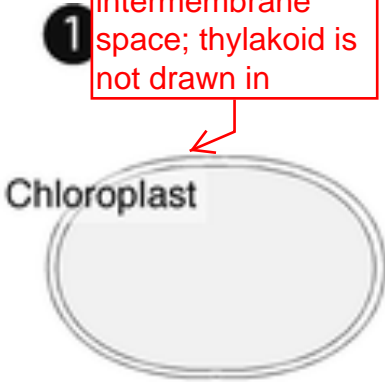


division rings

ring on the inside of the inner membrane and it is attached to the membrane
FtsZ-filamentous temperature sensitive

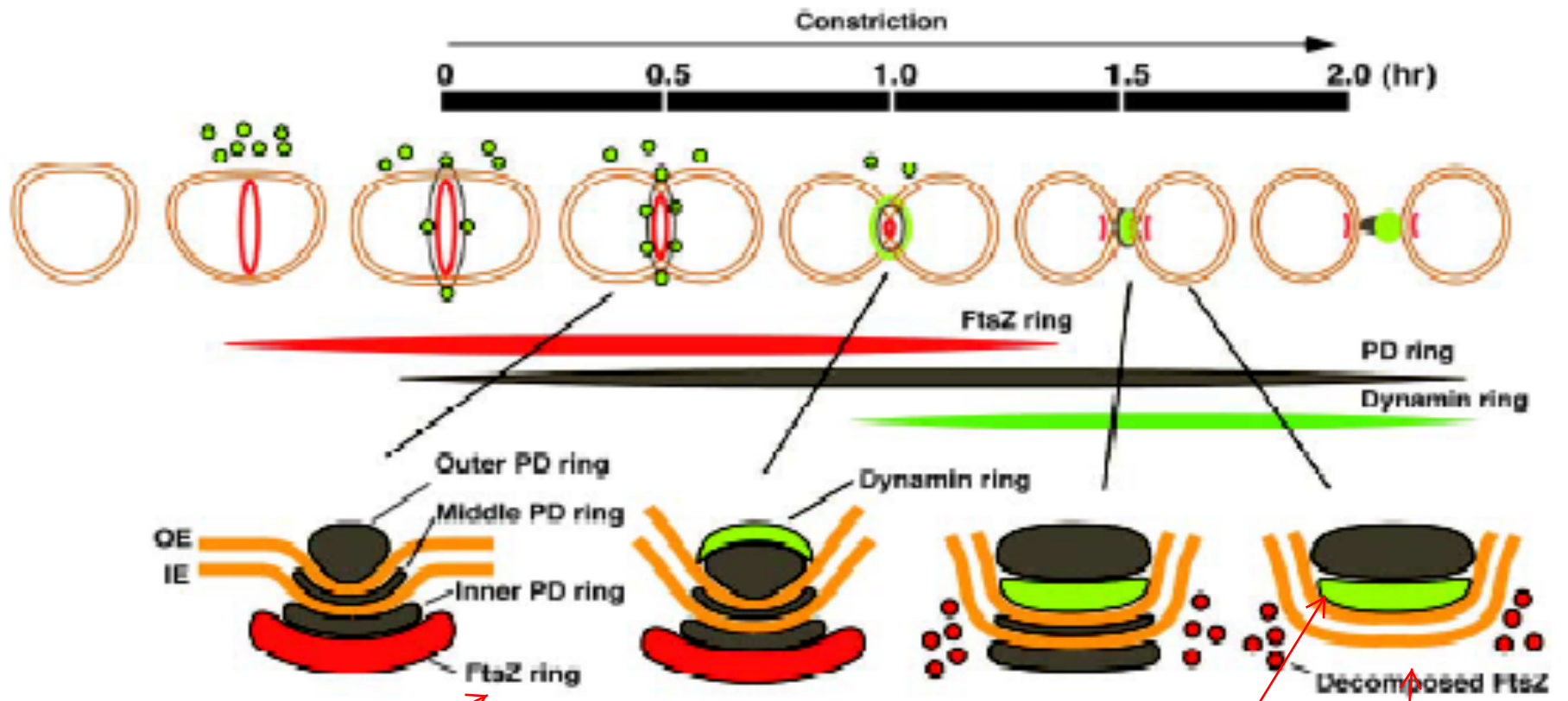
PD (plastid division) ring; in arabidopsis there are 2 PD rings; there is one on the inside of inner and outside of outer; sometimes there are 3 pd rings; here they have only drawn one

outer and inner membrane and intermembrane space; thylakoid is not drawn in



dynammin=ARC5
-double nomenclature

rings then disassemble; chloroplast typically divide in relatively equal daughter chloroplast cells



FtsZ ring is in the inside

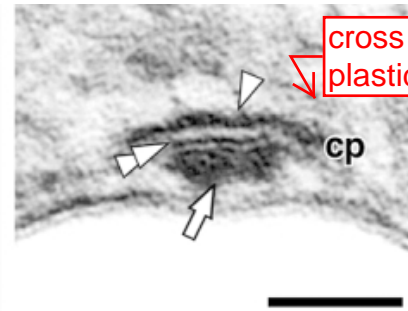
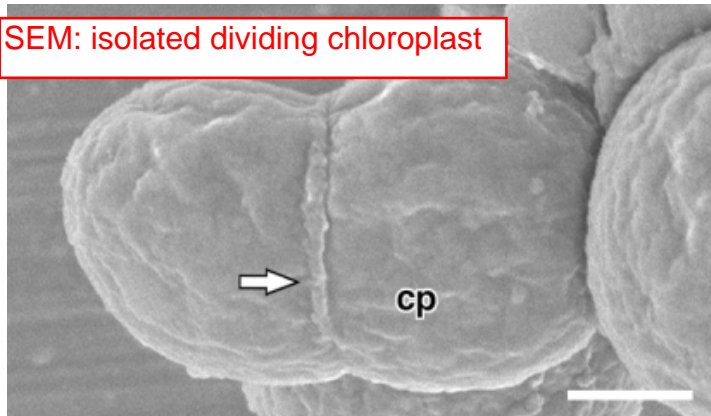
dynamnin is on the outside of the outer membrane but switches to the other side of the outer PD ring

things get dissembled from the inside out

Miyagishima et al., Plant Cell 2003

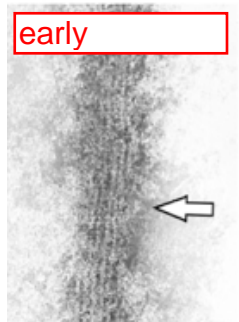
dynamic process; know inside/ outside ring on what membrane

SEM: isolated dividing chloroplast

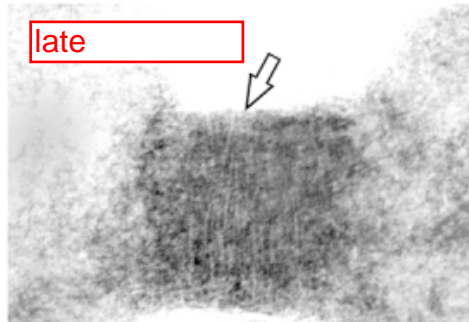


cross section of the plastid dividing ring

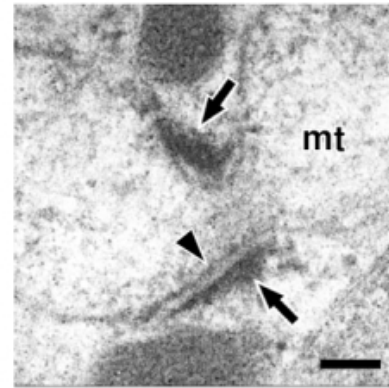
early



late

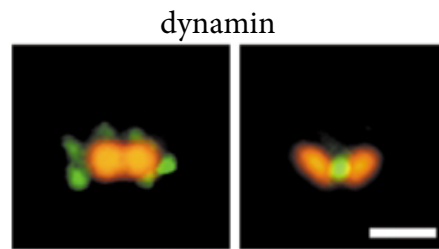
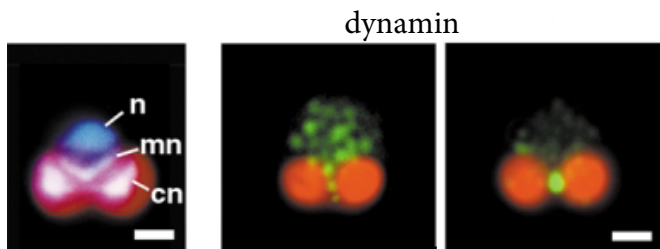
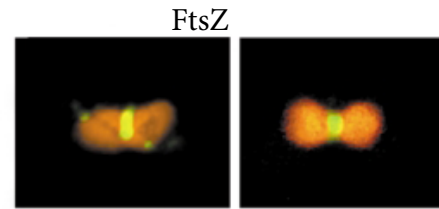
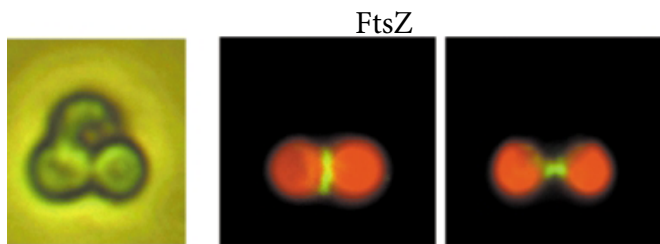


EMs of outer rings



Miyagishima et al Trends Plant Sci 2003

a lot of proteins seem to be difereing but the ring structure is relatively similar



phase contrast -immunofluorescence images

chloroplast

mitochondrion

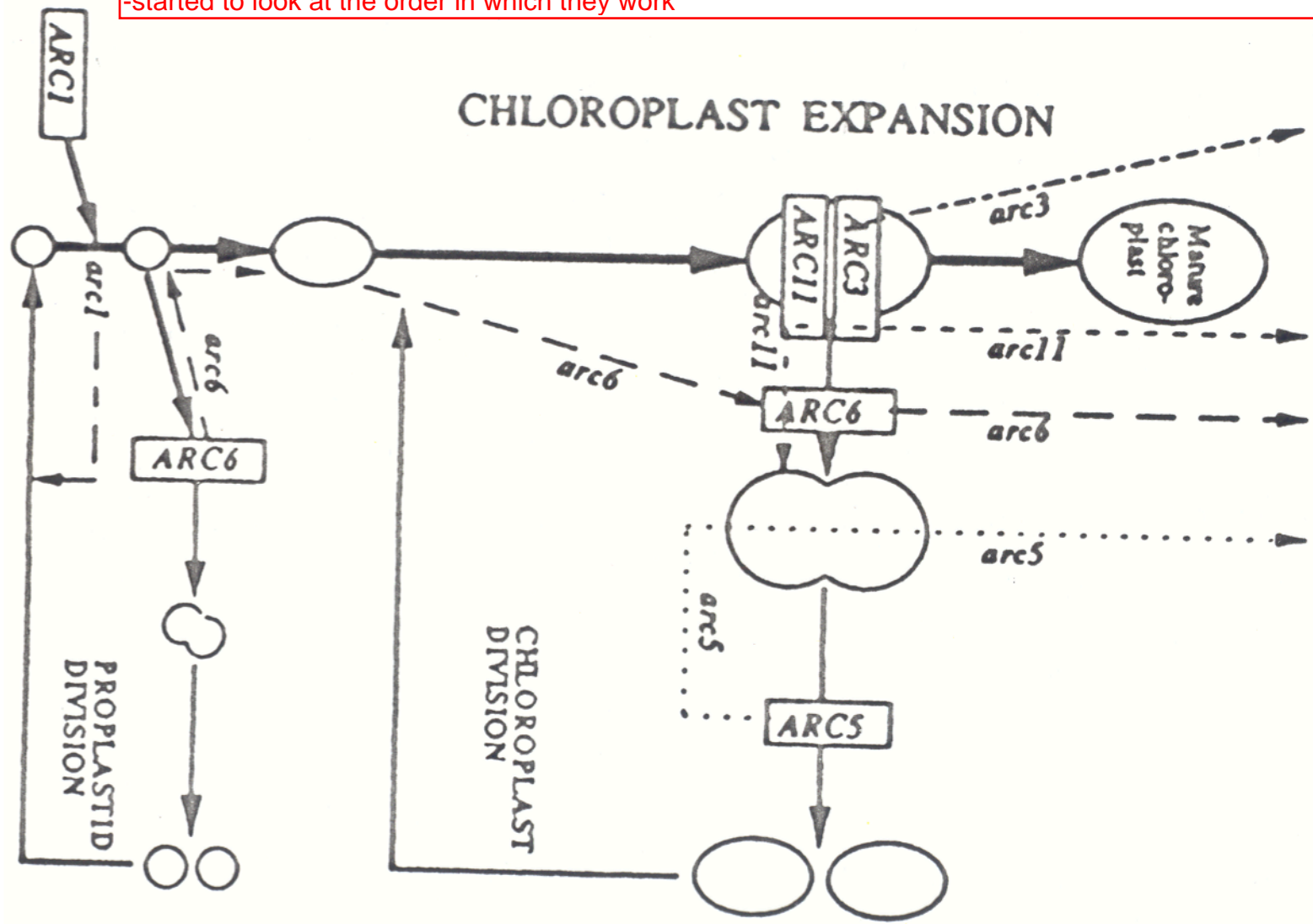
Table 1. A summary of 11 *Arabidopsis arc* mutants indicating their phenotype, chloroplast size and chloroplast number

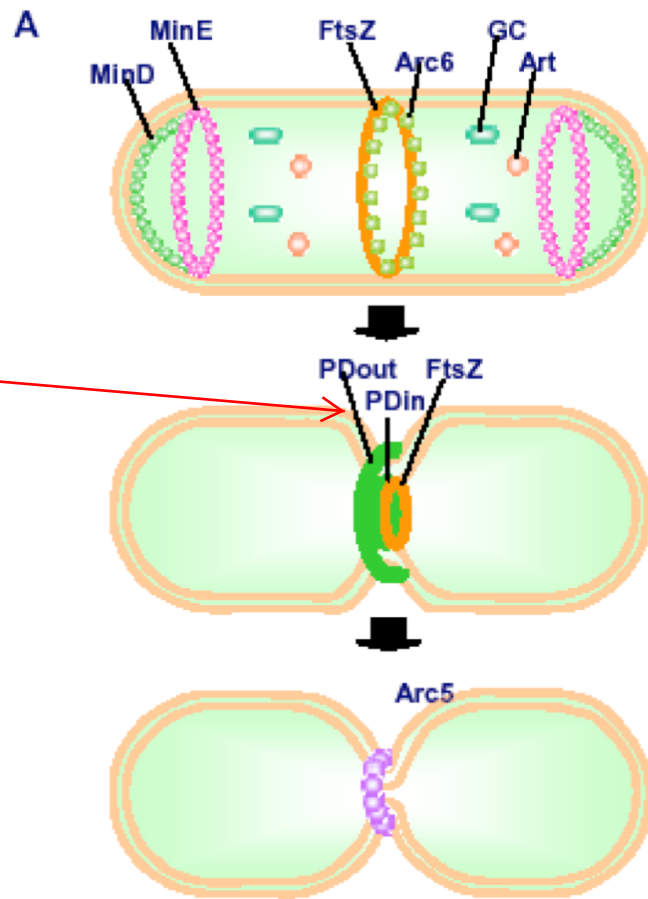
Genotype	Ecotype	Chloroplast size (μm^2)	Chloroplast number/cell	Chloroplasts/ nm^2 mesophyll cell plan area	Notes	Reference
WT ^a	Ler	50	120	25	Spherical	Pyke and Leech, 1992
WT	Ws	50	80–90	20–23	Spherical	Pyke <i>et al.</i> , 1994; Rutherford, 1996
WT	Col	50	100	23	Spherical	Osteryoung <i>et al.</i> , 1998
<i>arc1</i>	Ler	25	108	32	Increased number of smaller chloroplasts	Pyke and Leech, 1992; Marrison <i>et al.</i> , 1999
<i>arc2</i>	Ler	110	40	9	Fewer chloroplasts/cell than WT	Pyke and Leech, 1992
<i>arc3</i>	Ler	200–300	18	4–5	Heterogeneous chloroplast size	Pyke and Leech, 1992; Pyke and Leech, 1994; Marrison <i>et al.</i> , 1999
<i>arc5</i>	Ler	300–900	3–15	1–4	Dumbbell-shaped chloroplasts	Pyke and Leech, 1994; Robertson <i>et al.</i> , 1996; Marrison <i>et al.</i> , 1999; Gao <i>et al.</i> , 2003
<i>arc6</i>	Ws	1000	2	0.5	One or two large chloroplasts	Pyke <i>et al.</i> , 1994; Robertson <i>et al.</i> , 1995; Vitha <i>et al.</i> , 2003
<i>arc7</i>	Ws	40	80	26	Pale first leaves	Rutherford, 1996; Pyke, 1999
<i>arc8</i>	Ws	110	45	10	Moderately enlarged chloroplasts	Rutherford, 1996
<i>arc9</i>	Ws	140	34	12	Moderately enlarged chloroplasts	Rutherford, 1996
<i>arc10</i>	Ws	170	38	6	Highly variable in size	Rutherford, 1996; Pyke, 1999
<i>arc11</i>	Ler	110	30	7	Heterogeneous chloroplast size	Marrison <i>et al.</i> , 1999; Colletti <i>et al.</i> , 2000; Fujiwara <i>et al.</i> , 2004
<i>arc12</i>	Col	ND	1–2	ND	Similar to <i>arc6</i>	Pyke, 1999; Yamamoto <i>et al.</i> , 2002

^a WT=wild type.

many mutations have been identified
 -ARC5 (dynamain)- makes chloroplast dumb bell shaped chloroplast
 -ARC6 is very large; odd looking
 -chloroplast number is changing
 -often when you have large ones, there are very few in number and vice versa, smaller chloroplast have a greater number

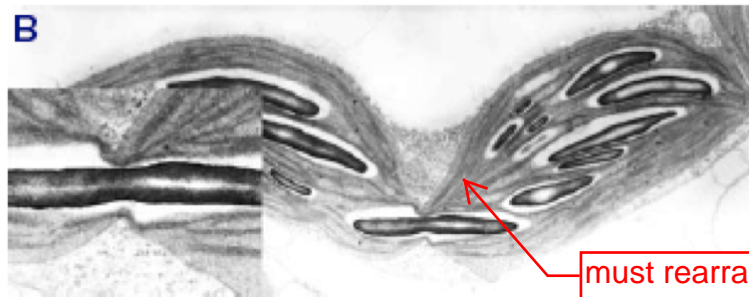
chloroplast division; some genes seem to be required for chloroplast division and plastid division (like ARC6)
 -ARC1 is affected in plastid division but not chloroplast division
 -by looking at mutant phenotype and also doing complementation assays to see when they did crosses
 -started to look at the order in which they work





Plastid Division rings are known -but the composition of the rings are unknown

ARC6 anchors it into inner membrane
 MinE and MinD are polar proteins and are shown in a gradient where the gradient is higher at the pole
 -rings are assembled when the gradient for MinE and MinD are less; MinE and MinD prevent rings from forming at the poles, it interacts with ARC3
 -rings form where there is no ARC3
 -polarity and gradients seem very important
 -ARC3 mutants show multiple rings forming; rings are forming more frequently along the chloroplast
 -GC-giant chloroplast
 -Art- artemis

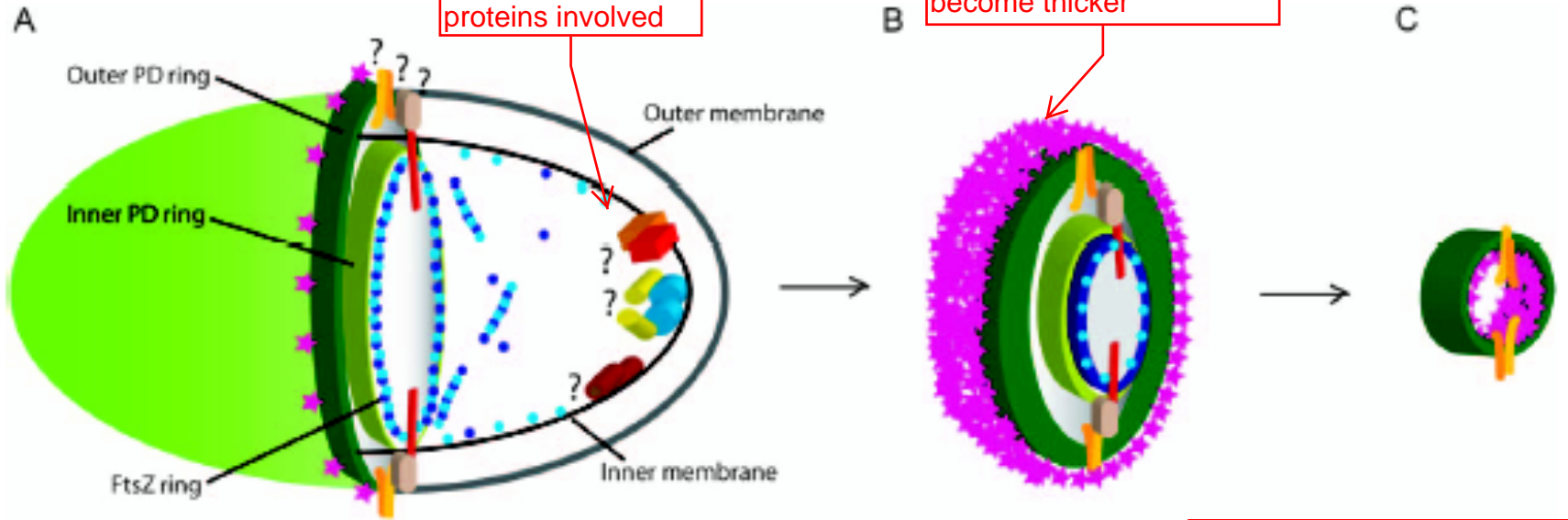


must rearrange thylakoid membrane

dont know what PD is;
dnt know where it is
encoded; no gene; no
protein sequence

anticipate that
there are a lot of
proteins involved

what happens to the rings
when they contract;
become thicker



FtsZ- 3 versions in arabidopsis
-they first assemble into filaments
and then ring structure;
dimerization; interaction between
proteins to form ring

- ★ ARC5
- PDV1
- PDV2
- UNKNOWN
- ARC6
- FtsZ2
- FtsZ1
- MinE
- MSL2
- MSL3
- MinD
- GC1

anticipate many more
proteins are involved;
regulatory proteins would be
involved; some kind of signal
to the nucleus and back