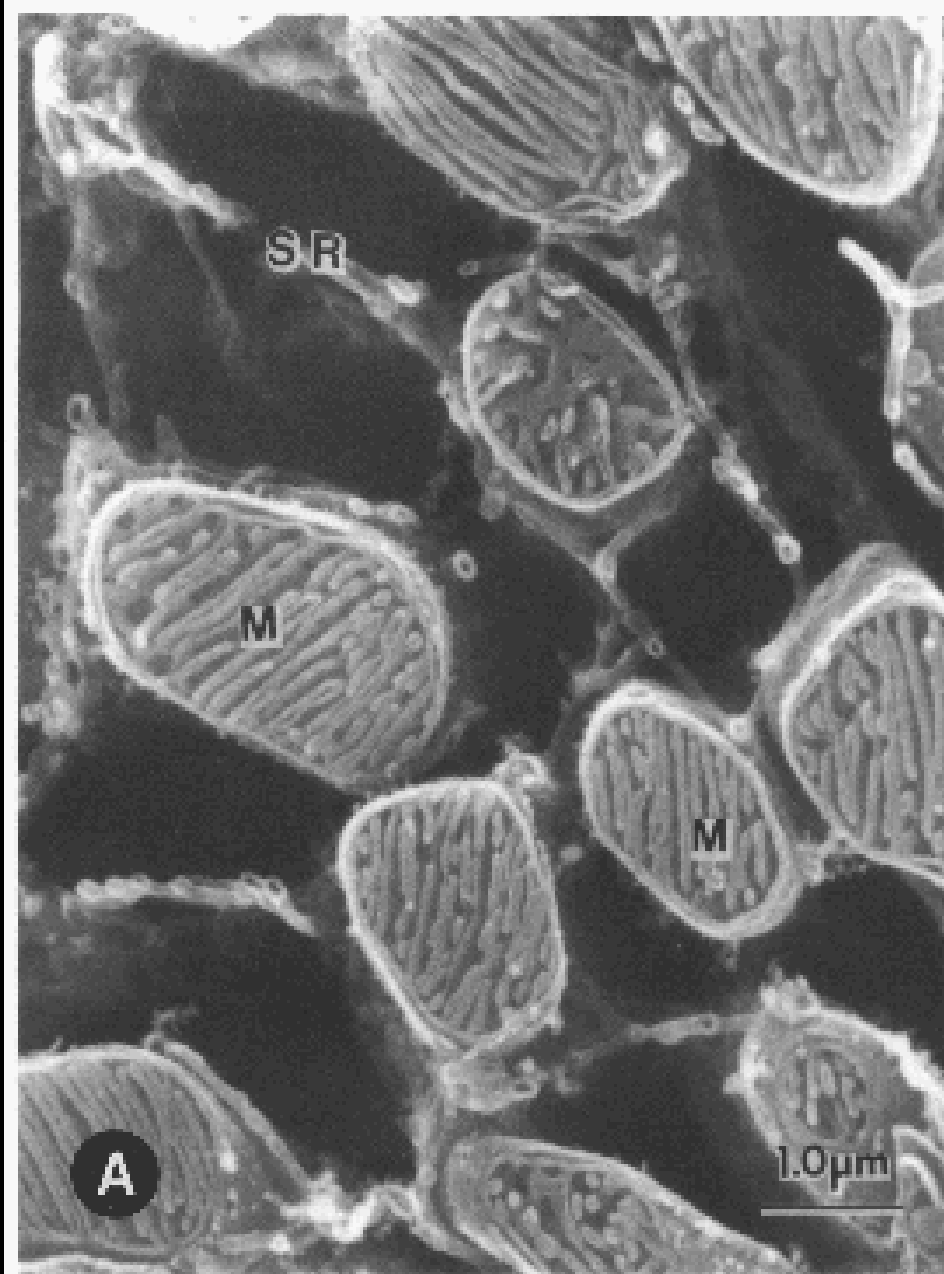
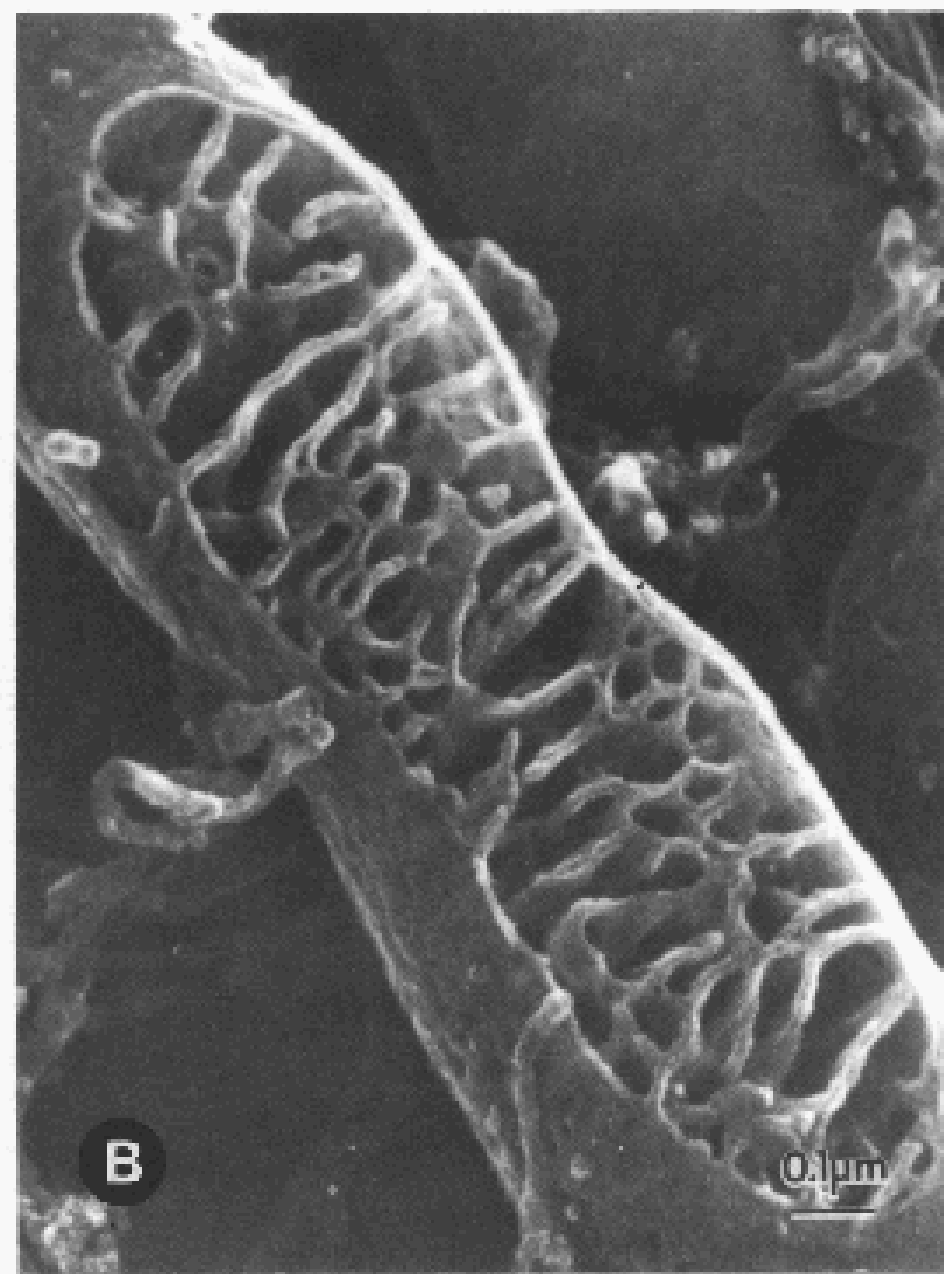


## **Question #1**

**What are mitochondria?**



x12,000



x62,000

Yoshizawa et al. (1986) *J. Submicrosc. Cytol.* 18:623

# Gene products present in animal mitochondria

## General functions (~ 420)

Protein translation and stability (150)

Carriers and transporters (80)

Organelle morphology and inheritance (35)

Protein import and sorting (35)

DNA replication and repair (35)

RNA transcription and stability (25)

Stress response (25)

Signal transduction (20)

Nucleotide metabolism (15)

## Specialized functions (~ 400)

Respiratory chain and oxidative phosphorylation (140)

Carbohydrate metabolism (65)

Apoptosis (60)

Amino acid metabolism (55)

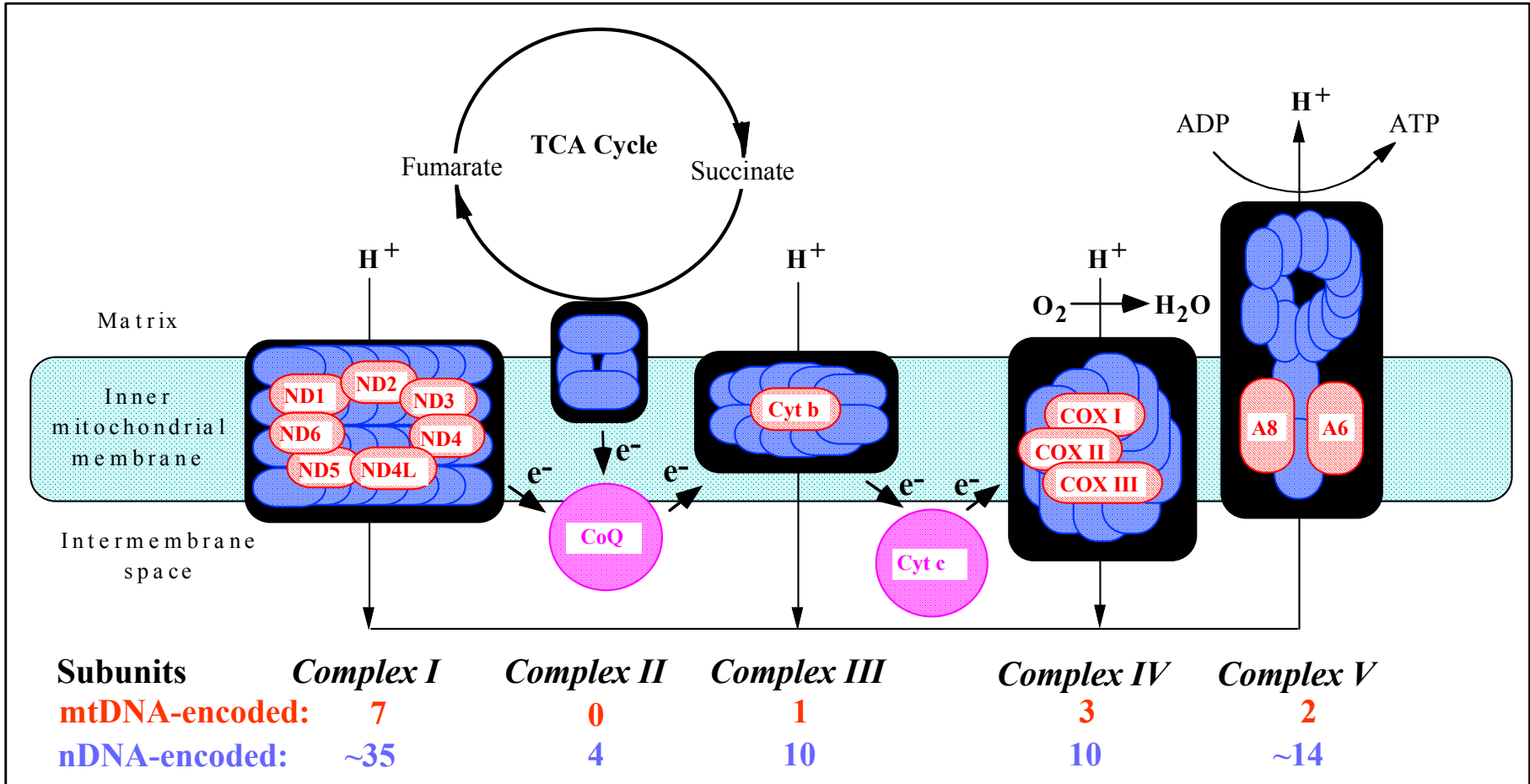
Fatty acid metabolism (30)

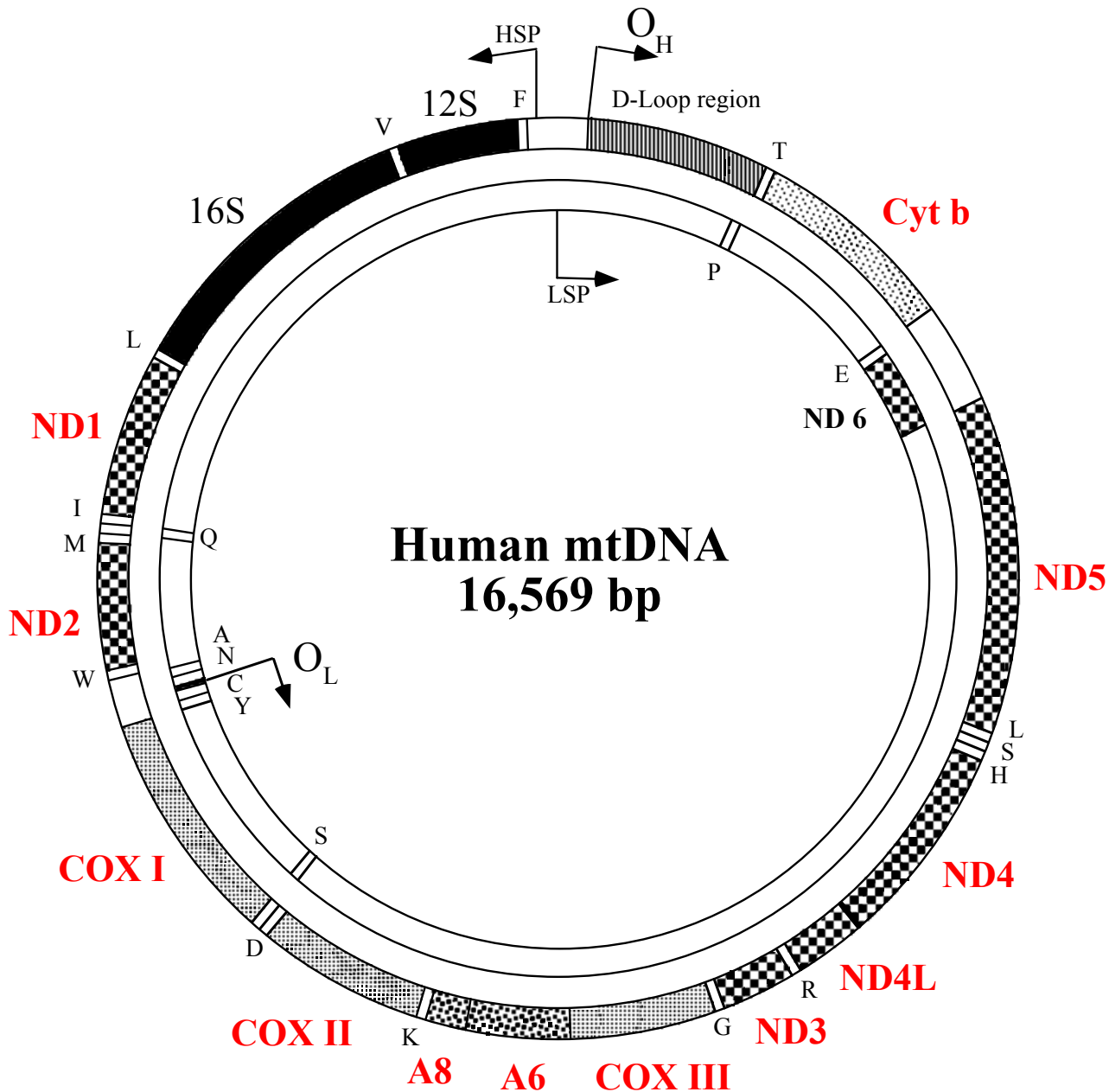
Steroid metabolism (15)

Phospholipid metabolism (15)

Xenobiotic metabolism (15)

# The mitochondrial respiratory chain





## **Question #2**

**How much of a contribution does mtDNA make to the inherited characteristics of an organism?**

## **Nuclear DNA**

99% of total cellular DNA

~3,000,000,000 bp

~30,000 genes

~100,000 polypeptides

## **Mitochondrial DNA**

1% of total cellular DNA

16,569 bp

37 genes

13 polypeptides

# Important concepts in mitochondrial genetics

Population genetics

Hundreds or thousands of organelles/cell

~5 mtDNAs/organelle in somatic cells

~1 mtDNA/organelle in oocytes

Maternal inheritance

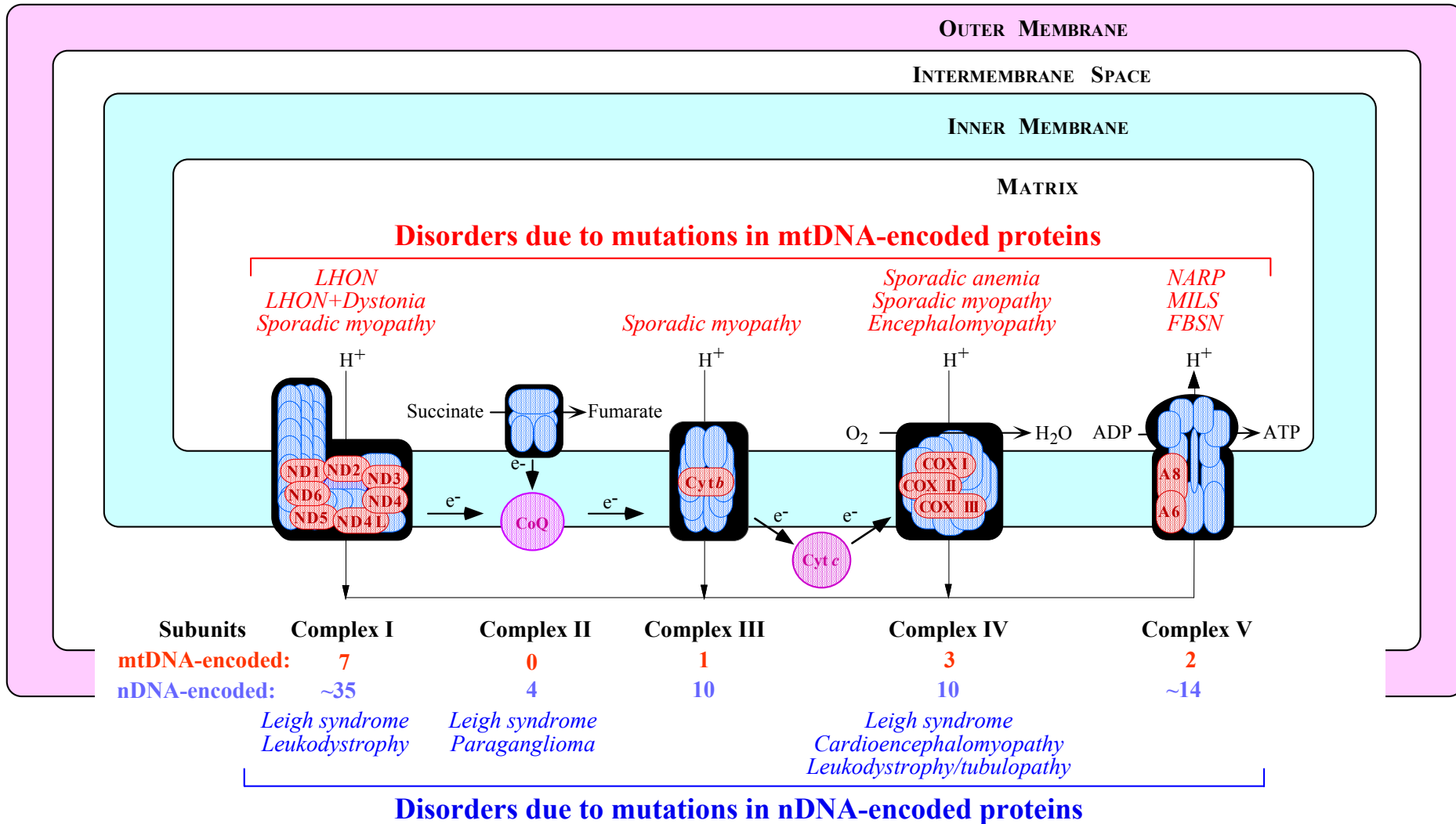
Normal genetic variation among individuals

Homoplasmy vs. heteroplasmy

Mitotic segregation

Threshold effects

# Mitochondrial diseases



## **Question #3**

**Will cloning result in mtDNA heteroplasmy,  
and if so, will it be a problem?**

## **Mitochondria in the maternal germline**

There are ~100,000 mitochondria, and mtDNAs, in mature oocytes.

There is an mtDNA bottleneck in oogenesis.

Oocyte mitochondria are non-randomly distributed (e.g. perinuclear) during meiosis and at fertilization.

Oocytes destroy paternal mitochondria soon after fertilization.

## **Mitochondria in embryonic development**

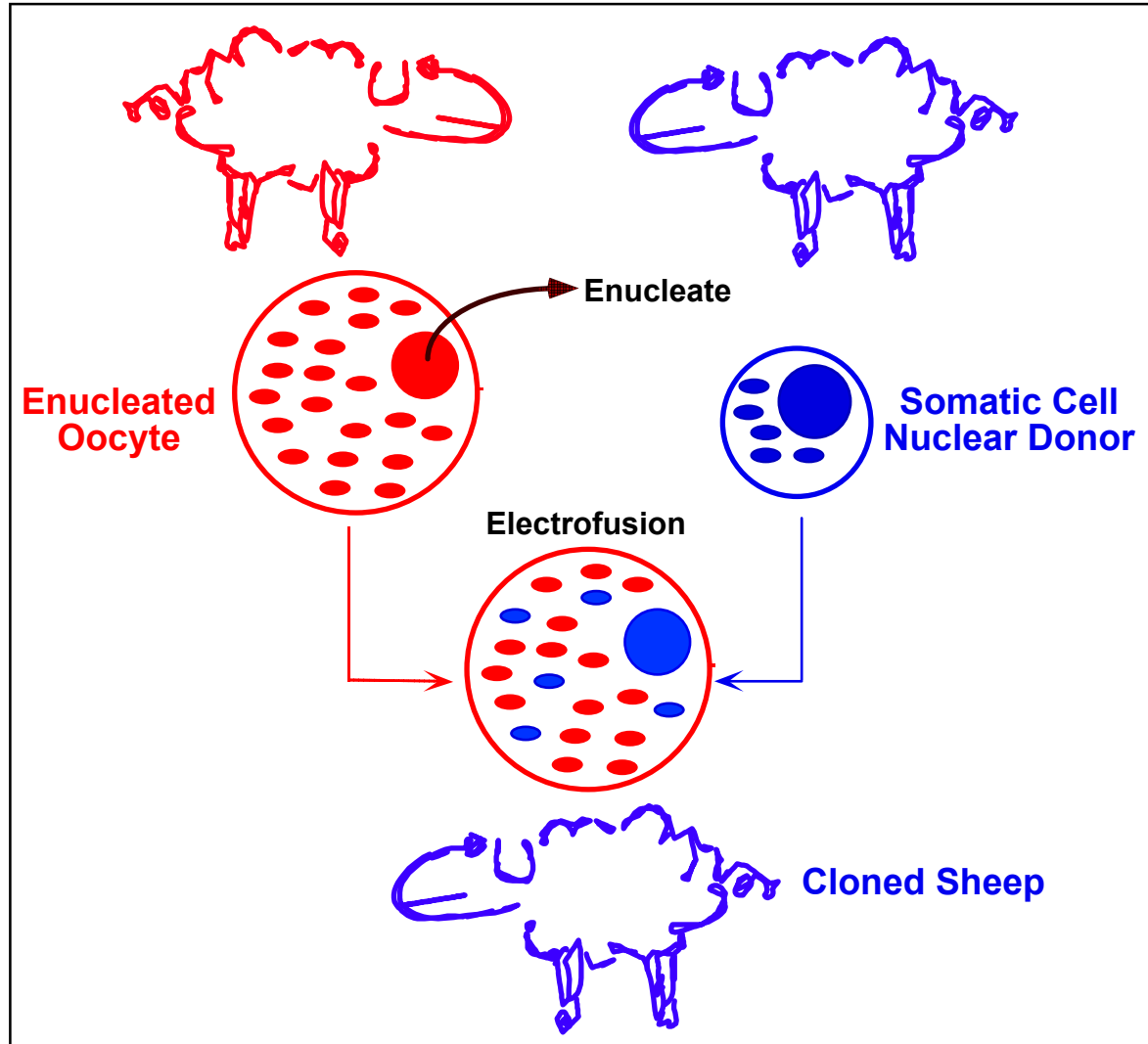
There is no mtDNA replication at or immediately after fertilization, but RNA transcripts are present and are translated.

Organelle division and mtDNA replication resume around the time of implantation.

There is also an mtDNA bottleneck in embryogenesis, as most zygotic mitochondria end up in the extra-embryonic tissues.

Thus, <5% of zygotic mtDNAs repopulate the next generation.

# How to clone a sheep



## Some numbers

A typical somatic cell contains ~1,000 mtDNAs.

An oocyte contains ~100,000 mtDNAs.

Thus, all else being equal, cloning via electrofusion dilutes somatic cell mtDNA copy number by 100-fold. By volume, the dilution is much greater (10,000- to 100,000-fold).

Remember, however, that there is a non-random distribution of mtDNAs in oocytes (e.g. in the perinuclear region).

## Perinuclear localization of mitochondria in oocytes during meiosis



FIGURE 9 Pachytene oocyte. Numerous round or oval mitochondria forming a single or double layers around the nucleus (N) are observed. Several among them show a dense matrix and intracristal spaces (arrows). Chromosomes (chr). X 6,600.

## The data on animal cloning

Sheep cloned by electrofusion were homoplasmic (no somatic cell mtDNA was detected) (Evans, 1999).

Some cows cloned by electrofusion were homoplasmic (Takeda, 1999), but others were heteroplasmic (Takeda, 1999; Hiendleder, 1999; Steinborn, 2000). Cows cloned by cytoplasm-blastomere fusion were also heteroplasmic (Steinborn, 1998).

Data on mice (e.g. Cumulina) are not informative, as the mice were cloned using isogenic mtDNAs (Wakayama, 1998).

## **The data on transmitochondrial mice**

Intraspecific crosses of mice were homoplasmic, as expected, but interspecific crosses of mice were heteroplasmic (Kaneda, 1995).

Mixing ooplasm from two mice resulted in overall heteroplasmy (Jenuth, 1996), but the two "neutral" mtDNAs segregated to homoplasmy in some tissues (Jenuth, 1997). This segregation is under genetic control (E. Shoubridge, pers. comm.)

## **The data on human ooplasmic transfer**

Of 13 human embryos produced via ooplasmic transfer, 6 were heteroplasmic (Brenner, 2000).

Two children produced via ooplasmic transfer were heteroplasmic (Barritt, 2001).

Heteroplasmy, when detected, ranged from 1% - 60%, and varied markedly among tissues.

# Summary

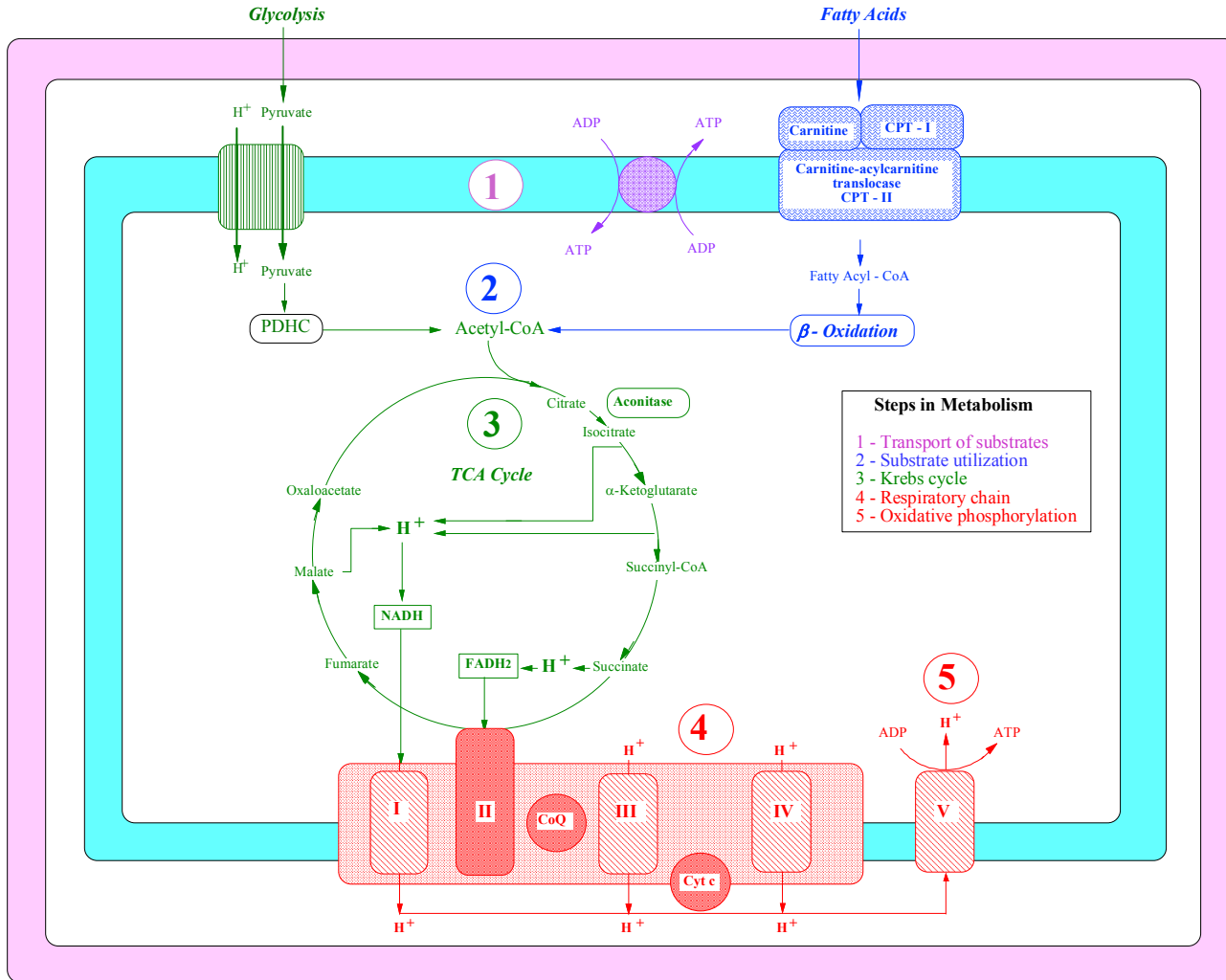
Some somatic cell donor mtDNAs will likely be transferred randomly during cloning, but active processes are also at work to (1) eliminate donor mitochondria and (2) skew segregation.

While “single mutation” heteroplasmy is both common and normal, heteroplasmy of different overall mtDNA genotypes is not.

The presence of heteroplasmy does not seem to affect the phenotype or health of cloned animals. However, donor somatic cell mitochondria, especially from older donor cells, could harbor greater numbers of potentially deleterious mtDNA mutations.

Attempts to clone dissimilar species (e.g. an "extinct" mammoth nucleus transferred to a "surrogate" elephant oocyte) may fail merely on the basis of mtDNA incompatibility.

# Mitochondrial biochemistry



## **Mitochondria in the paternal germline**

There are ~200 mtDNAs in sperm.

All mitochondria are in the sperm neckpiece.

Paternal mitochondria enter the oocyte during fertilization.

Paternal mitochondria are actively destroyed soon after fertilization.