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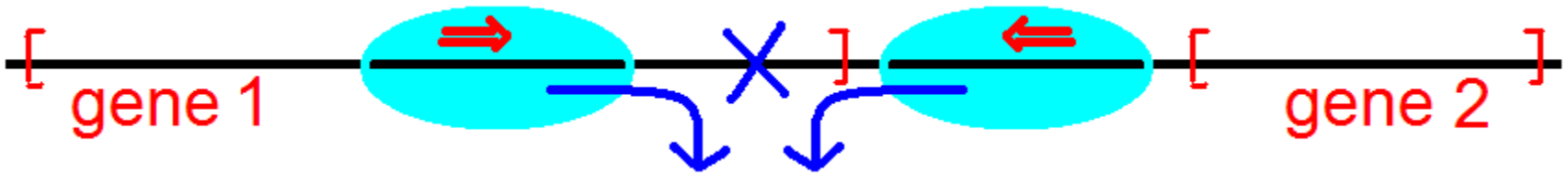
Human with the MELAS mutation,
Rat with hyposecretion of the thyroid hormone

http://lab6.iitp.ru/

Our model of RNA polymerases interaction:

1) Transcription initiations from each promoter form a Poisson process;

2) RNA polymerase elongation ceases when an oncoming RNA polymerase or a terminator is encountered:



Plant plastids in experiments and the model			
external impact	**internal result**	**measurement**	**model**
sig3 or *sig4* knockout	decrease of trans-cription initiation rate	complex changes of gene transcription levels: some genes increase their transcription levels while others decrease	the same changes of transcription levels within experimental errors
heat shock	increase of RNAP speed	transcription level of *rpl23* and *rpl2* genes was measured cumulatively under heat shock	the same changes of transcription levels within experimental errors

Mammal mitochondria in experiments and the model			
external impact	**internal result**	**measurement**	**model**
MELAS mutation: A→G at position 3243	reduction of mTERF affinity to the termination site: 7–10-fold decrease of dissociation time of mTERF•DNA complex	small changes of gene transcription levels; considerable changes of some enzyme activity. **phenotype:** myopathy, encephalopathy, lactic acidosis, stroke-like episodes	the same: small decrease of tRNA and rRNA, considerable decrease of protein concentrations
phenotype: hypo-thyroidism (induced by a drug)	changes of mtDNA methylation: possibly caused by transcription initiation rate changes	changes of mRNA/rRNA in mitochondria	the same changes of transcription levels within experimental errors

For mammals, the **model adopts the parameters estimated for the frog**,

for which peculiar experimental evidence is available.

This significantly lowers the search complexity when we decide for mammals in the model.

Main results

1) Our model accurately **reproduces all relevant experimental data** available for **plant plastids** and the **mitochondria of chordates**.

2) Using these experimental data, the model **estimates**:

a) **binding intensities** of RNA polymerases to their promoters, and predicts the **terminator characteristics**, including polarization;

b) **absolute values** of **all gene transcription levels** and

c) makes **functional predictions** (MELAS and hypothyroidism)

3) We suggest **mechanisms** of disorder.

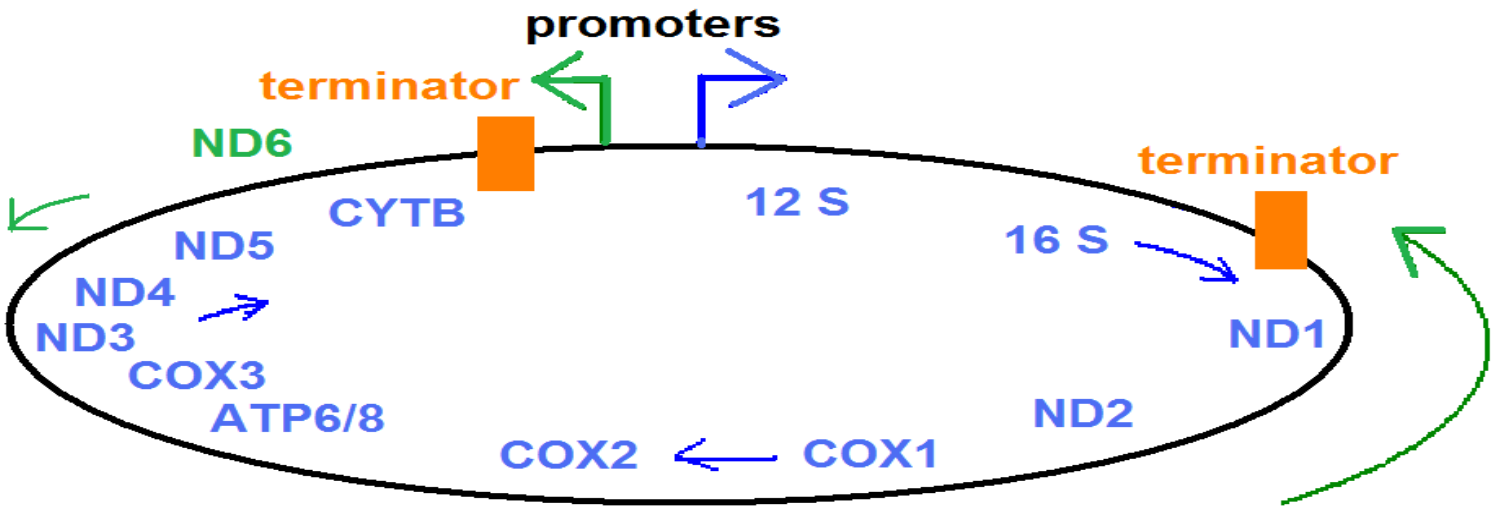
Plastid gene transcription levels obtained in the model and experiment in *Arabidopsis* and *Hordeum*:

Standard deviations are provided where applicable

ген	**эксперимент**	**модель**
Arabidopsis thaliana, sig4 knockout, MT/WT		
ycf1	0.73 ± 0.04	0.76 ± 0.01
ndhF	0.43 ± 0.10	0.47 ± 0.19
*rpl*32	1.52 ± 0.06	1.55 ± 0.02
Hordeum vulgare, heat shock, HT/WT		
*rpl*23– *rpl*2	2.42 ± 0.27	2.64 ± 0.02
psbA	0.53 ± 0.01	0.54 ± 0.04

Mechanism: **in plant** plastids **RNA polymerase competition provides an** explanation of selective increase in transcription level of some genes.

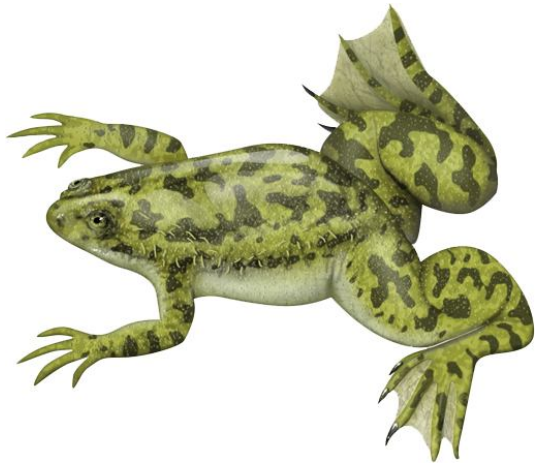
Mitochondrial RNA polymerase **interaction in mtDNA**



The entire mitochondrial DNA is considered, which enables RNA polymerases to complete more than one circle on the DNA strand.

(Mitochondrial and plastid mRNA are uncapped and possess the bacterial-type translation)

Again: transcription levels obtained in the model and experiment (mtDNA) **are very close to each other**. It is true **for all relevant experimental data available** on mitochondria of chordates: human (*Homo sapiens*), rat (*Rattus norvegicus*), frog (*Xenopus laevis*).

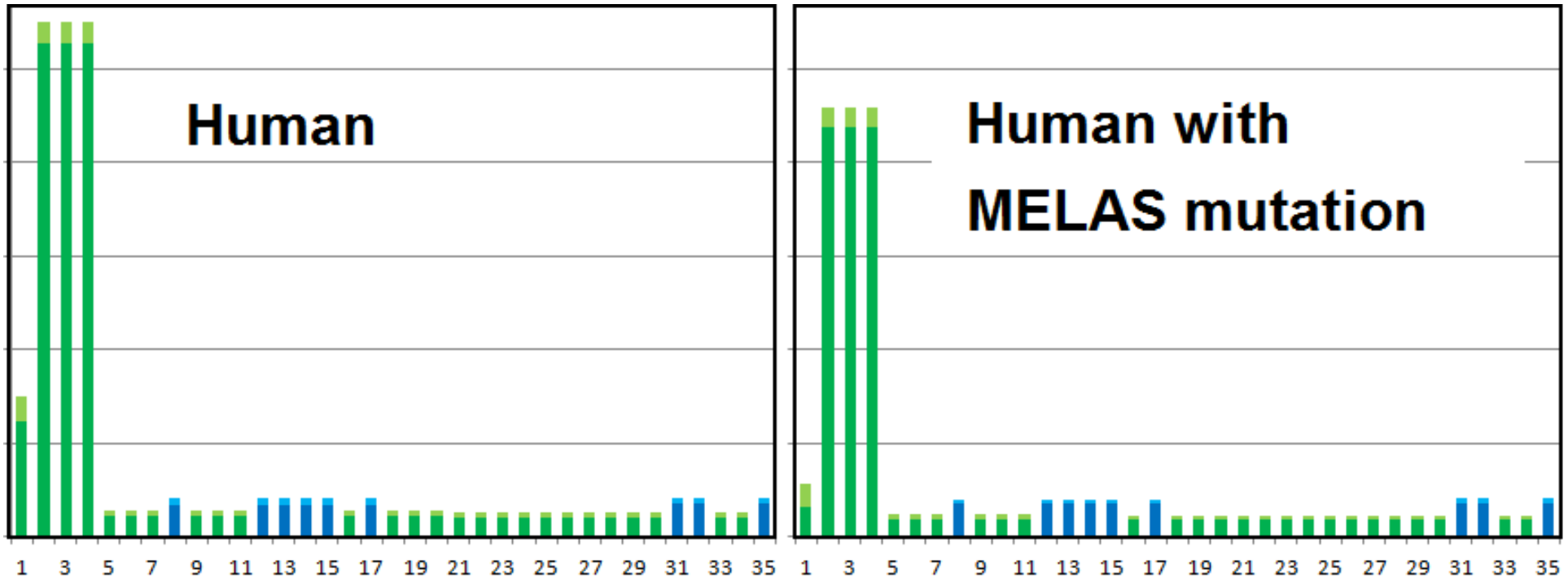


Mechanism: in the model the **competition is negligible** in human and is much lower in rat and frog compared to that in plastids

Functional predictions:

Human (*Homo sapiens*)

Healthy and MELAS mutated humans



Number of transcriptions predicted for 35 genes. Genes on the heavy strand are in green, on the light strand are in blue. Shaded are mean values, standard errors are not shaded.

In MELAS human, the model predicts a 1.21-fold decrease of the mTERF·DNA binding intensity and a 7.75-fold decrease of the HSP1 promoter efficiency. HSP1 is also activated by mTERF. Transcription levels of tRNA-Phe and rRNA drop 3.84- and 1.2-fold, respectively

We propose a putative mechanism mediating the development of **MELAS syndrome** in human:

The A→G transition at position 3243, i.e. in the middle of the mTERF binding site, substantially decreases the mTERF affinity to mtDNA

↓

Decrease of Phe-tRNA, Val-tRNA and rRNA concentration in mitochondria

↓ (*)

Decrease of the protein concentration

↓

Mitochondrial disorder

Explanation of (*):

1) Lowering the concentration of tRNA and rRNA lowers the protein translation rate (not critically?).

2) Due to **interaction between the polysomal mRNA and ribonuclease**, **a small decrease** in rRNA concentration (i.e. the ribosome content) causes a **critical decrease** of the mRNA **half-life**:

$$\tau = \frac{1}{\mu}(1 + d\lambda)\exp(w\lambda)\ln 2$$

This can lead to a severe disease.

$\lambda = \frac{\nu N}{1 + \alpha N}$ is the intensity of ribosome binding to its site,

N is the number of ribosomes.

N depends on the expression of especially **ribosome genes**. Unfortunately, values of ν and α are unknown and vary depending on the ribosome binding site and **mRNA** itself.

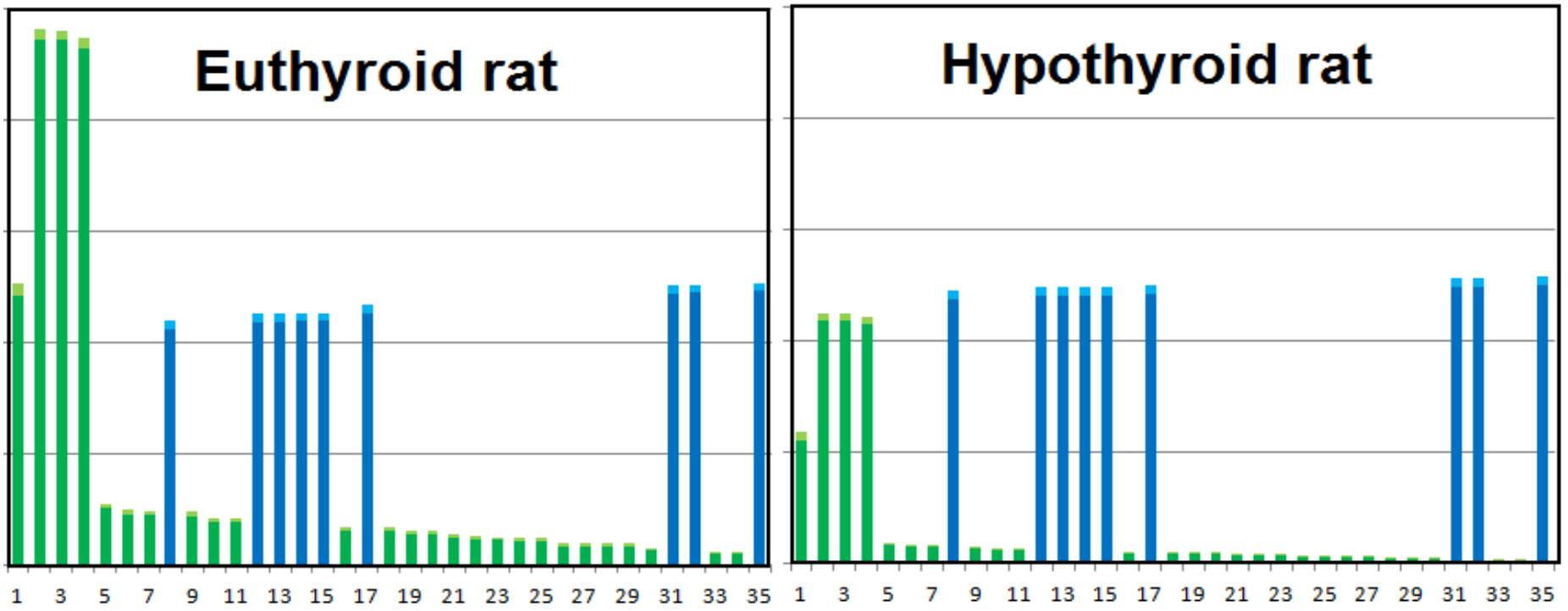
w is the linear size (in sec) of the ribonuclease on mRNA, *d* is the linear size (in sec) of the ribosome on mRNA. The **window** length between ribosomes **depends on** λ, i.e. ***N***, i.e. expression of **ribosome genes**.

μ is the intensity of ribonuclease-mRNA interaction that causes RNA cleavage.

Functional predictions:

Rat (*Rattus norvegicus*)

Healthy and hypothyroid rats



Transcription levels predicted for 35 genes. Genes on the heavy strand are in green, genes on the light strand are in blue.

1st disorder manifestation:

In the model, **intensities** of the mTERF binding and LSP transcription initiation are **equal** in **eu-** and **hypo**thyroid rats.

In the experiment, **methylation** remains unaffected in the mTERF binding site, and exhibits **minor changes** in the LSP promoter [Enríquez *et al*, 1999], which well agrees with the stability of their **predicted binding intensities** to mTERF and LSP.

2^{d} disorder manifestation:

To the contrary,

in the model, the total **intensity** of transcription initiation from promoters HSP1 and HSP2 is 2.15-fold **lower** in the **hypo**thyroid rat.

In the experiment, **methylation changes considerably in** the HSP1 region, but negligibly in HSP2, which is again in good agreement with the decrease of **predicted binding intensities** to HSP1 and HSP2.

About interaction of RNAP:

in a head-on collision, two RNA polymerases approaching one another on the same DNA may pass by one another. In this case antisense mRNA form a duplex, what is inaccessible for translation
[Ma, McAllister: J Mol Biol 2009, 391:808-812].

Thank you

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