Syndrom fragilního chromozómu X - expanzivní repetice v lidském genomu

Lukáš Tichý

- Syndrom fragilního X chromozómu je nejčastější příčinou dědičných mentálních retardací s frekvencí výskytu 1:4000 u mužů a 1:8000 u žen.
- Je způsoben expanzí CGG repetic v 5′UTR fragile X mental retardation genu (*FMR1*, Xq27).
- První dokázaný příklad choroby způsobené expanzí trinukleotidových repetic.


Repetitivní oblasti v lidské DNA

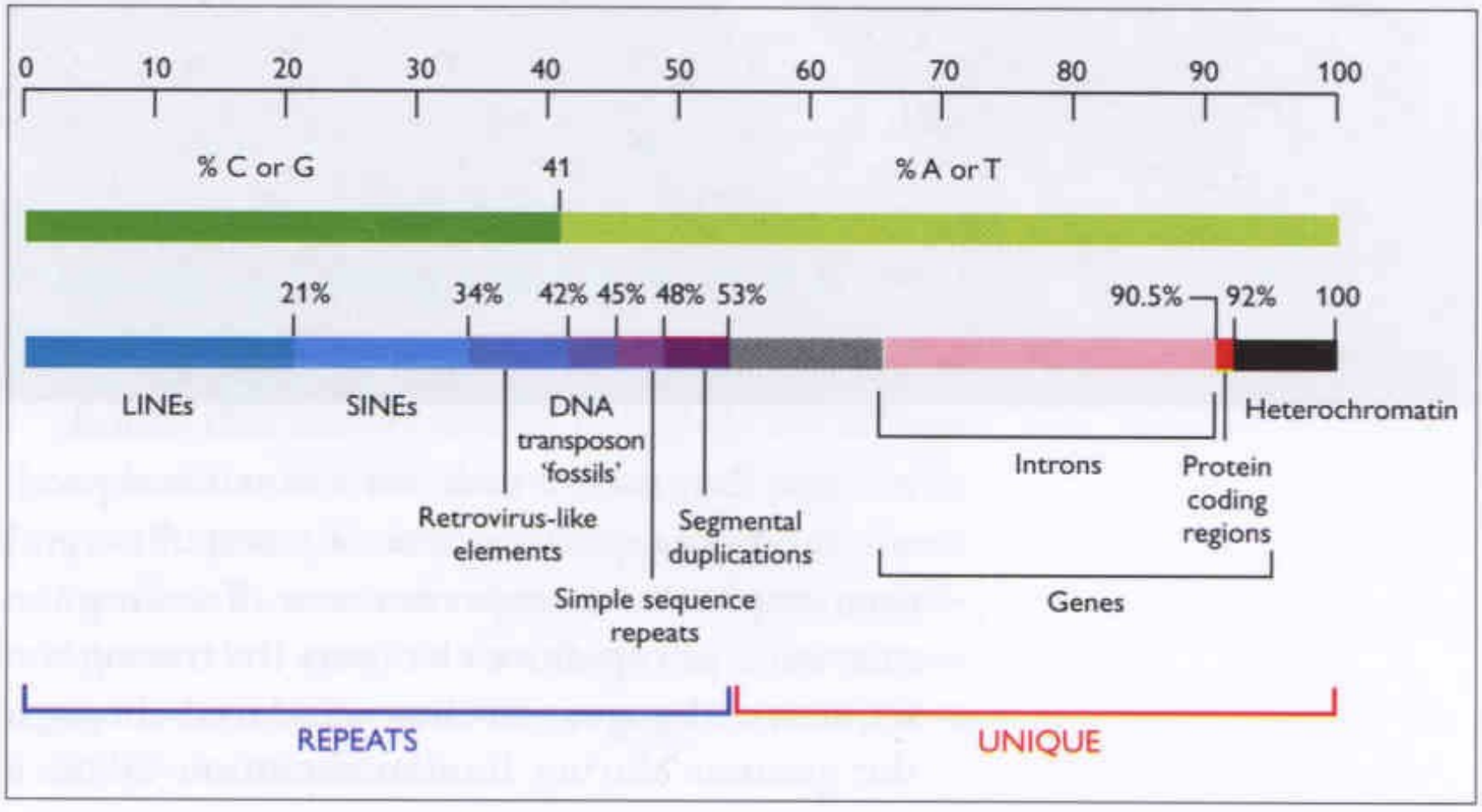
Table 20.1 Genome Sizes and Estimated Numbers of Genes*

Organism	Haploid Genome Size (Mb)	Number of Genes	Genes per Mb
Hemophilus influenzae (bacterium)	1.8	1,700	940
Escherichia coli (bacterium)	4.6	4,400	950
Saccharomyces cerevisiae (yeast)	12	5,800	480
Caenorhabditis elegans (nematode)	97	19,000	200
Arabidopsis thaliana (plant)	118	25,500	215
Drosophila melanogaster (fruit fly)	180	13,700	76
Oryza sativa (rice)	430	60,000	140
Danio rerio (zebrafish)	1,700	22,000	13
Mus musculus (house mouse)	2,600	25,000	11
Homo sapiens (human)	2,900	25,000	10
Fritillaria assyriaca (plant)	120,000	ND	ND

*Strictly defined, "genome" refers to the *haploid* genome of an organism. Some values given here are likely to be revised as genome analysis continues. Mb = million base pairs. ND = not determined.

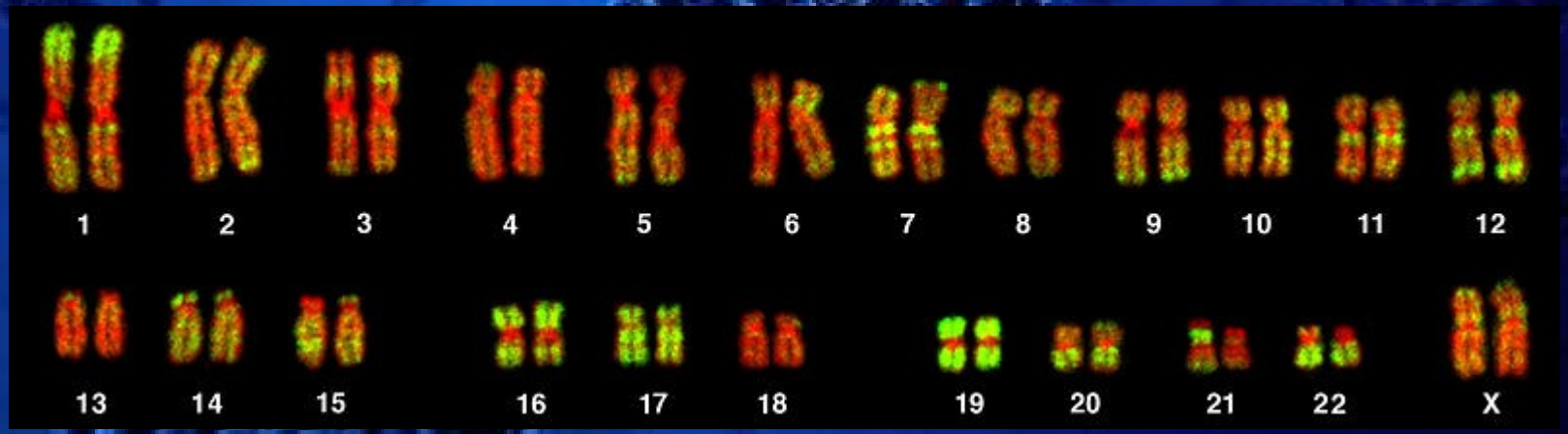






0
10
20
30
40
50
60
70
80
90
100
% C or G
41
% A or T
21%
34%
42%
45%
48%
53%
90.5%
92%
100
LINEs
SINEs
DNA transposon 'fossils'
Retrovirus-like elements
Segmental duplications
Simple sequence repeats
Introns
Protein coding regions
Heterochromatin
Genes
REPEATS
UNIQUE

Arthrobacter luteus – Alu repetice

- zbytek restrikční endonukleázy (7SL RNA)
- 299 bp dlouhá, v lidském genomu je cca 1.000.000 kopií (10,7% genomu)

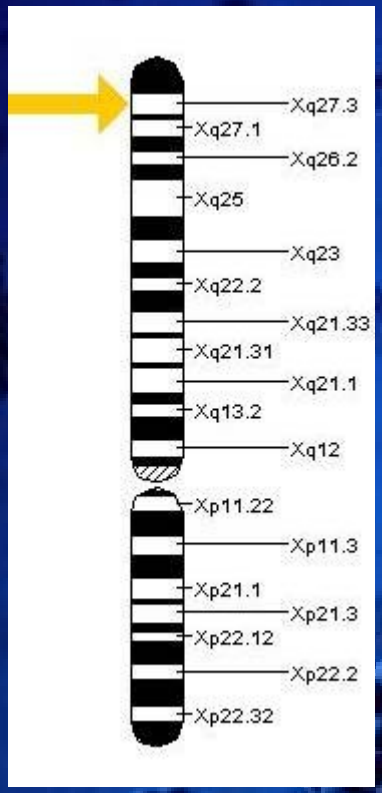


http://en.wikipedia.org/wiki/File:PLoSBiol3.5.Fig7ChromosomesAluFish.jpg


Syndrom fragilního chromozómu X

Klinické projevy

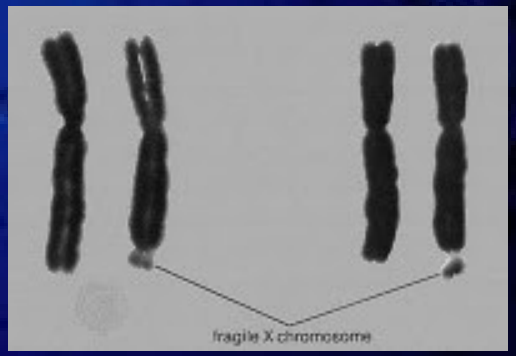
FMR1 gene



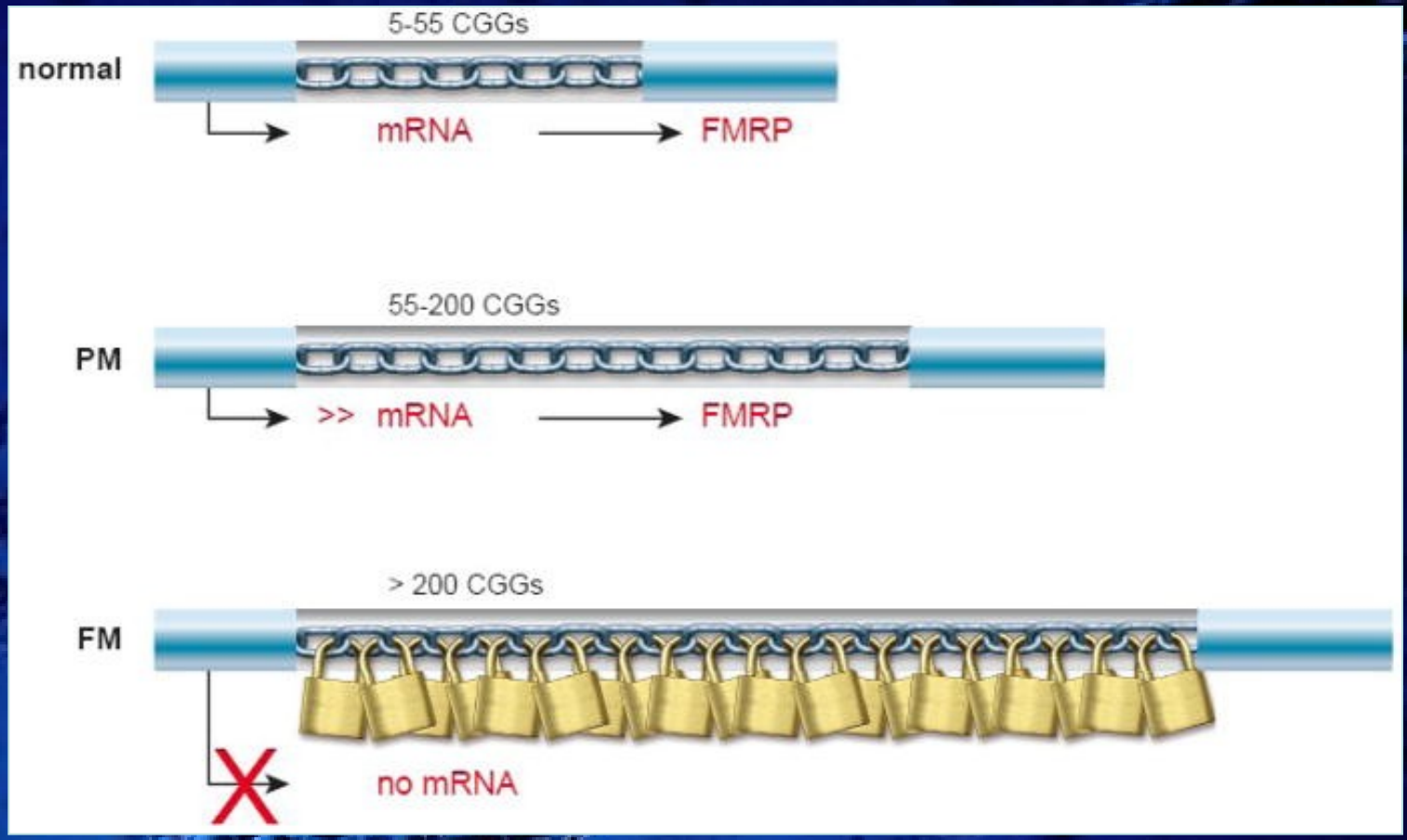
http://en.wikipedia.org/wiki/File:Fmr1.jpeg

http://www.erasmusmc.nl/47421/51019/2004677/hoogeveen-figuur.JPG

- Xq 27.3
- 39,179 bp
- Několik sestřihových variant
- CGG repetitivní oblast v 5'-UTR



http://4.bp.blogspot.com/_FoiEZNQLqOI/S-6APdSyIII/AAAAAAAABfM/pZLSLHtrpC0/s1600/fragile43.jpg



The CGG repeat in the *FMR1* gene Schematic representation of normal, PM (premutation) and FM (full mutation) alleles of the *FMR1* gene and the effect of the expansion on transcription and translation. Methylation due to extensive elongation of the CGG repeat in the 5′-ÚTR of the *FMR1* gene is depicted as a lock.

Biochim Biophys Acta. 2009 June; 1790(6): 467–477.

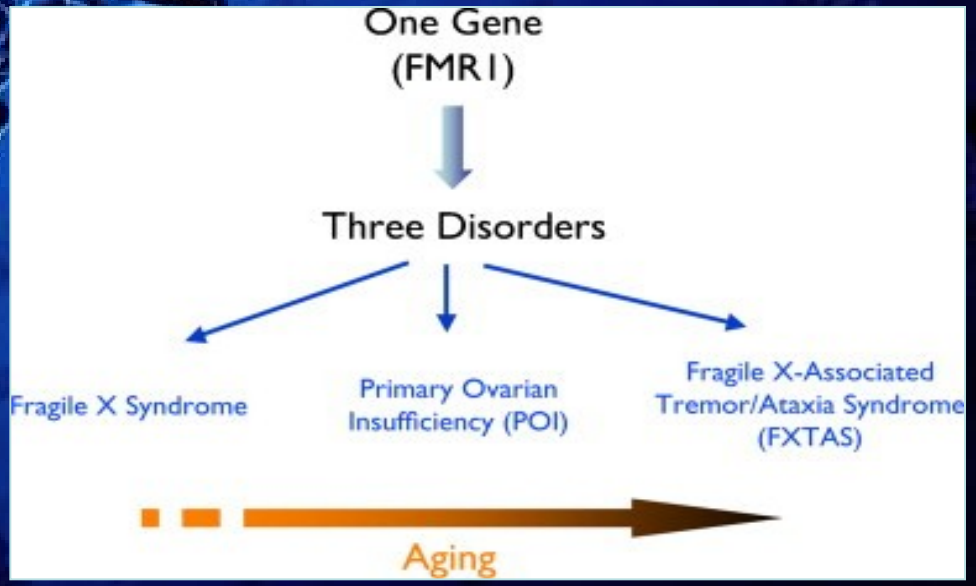
Mutace ve *FMR1* genu vedou ke třem rozdílným projevům.

• Normální lidský *FMR1* gen má počet CGG repetic v rozmezí 5 - 54.

• Rozsáhlé expanze na 200x CGG vedou k metylaci CpG ostrovů a transkripčnímu umlčení *FMR1* genu → **syndrom fragilního X chromozómu** (FXS).

• Expanze CGG repetic mezi 55 až 200 opakováními (premutace) je spojena s progresivním neurodegenerativním onemocněním zvaným **fragile X-associated tremor/ataxia syndrome (FXTAS)** (onemocnění manifestující se v nebo po páté dekádě života). U žen nosiček se přítomnost premutace může projevit **předčasnou ovariální insuficiencí (FXPOI)**.

•

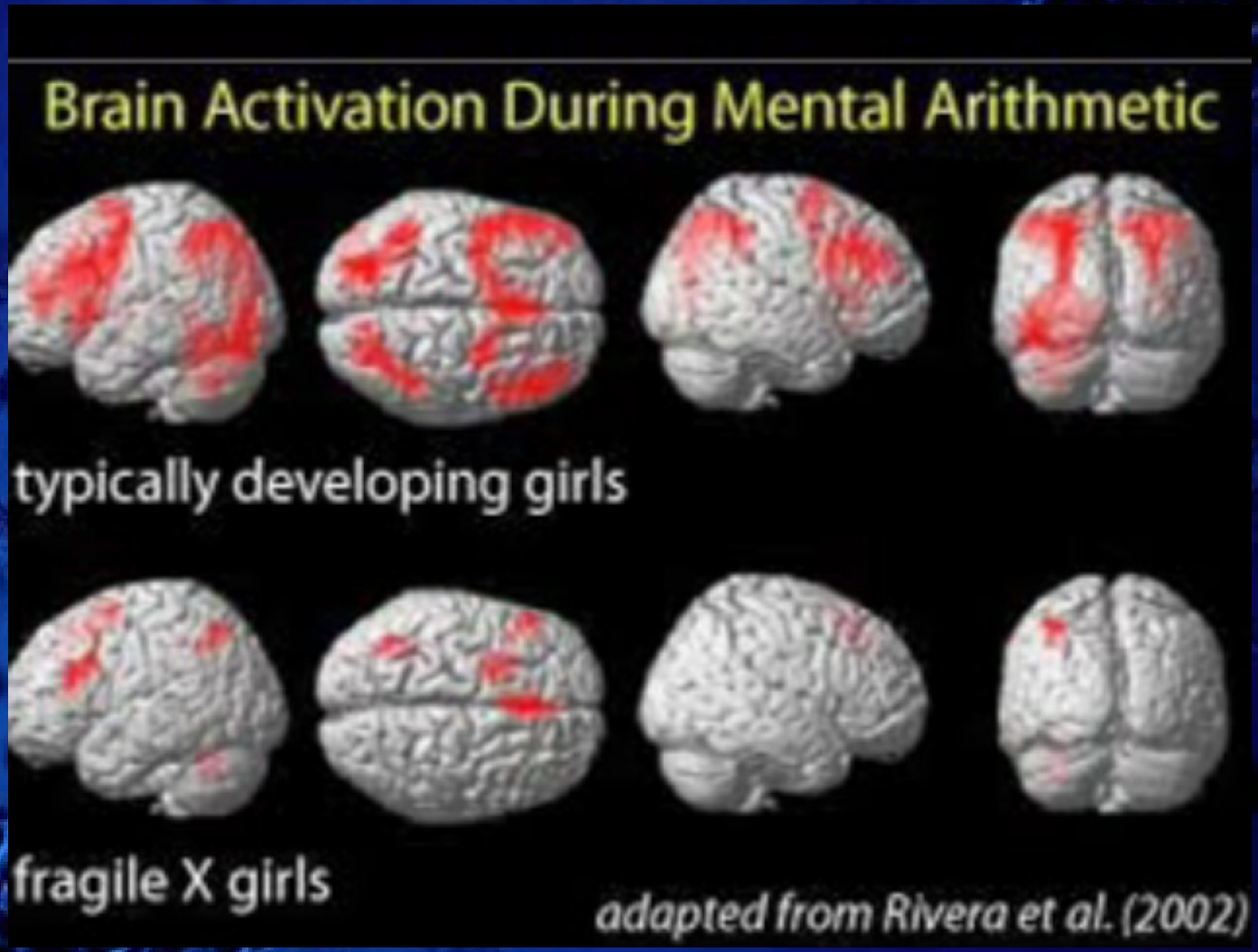


Morfologické projevy FXS

- normal structure
- broad forehead
- elongated face
- large prominent ears
- strabismus (crossed eyes)
- highly arched palate
- hyperextensible joints
- hand calluses (from self-abuse)
- pectus excavatum (indentation of chest)
- mitral valve prolapse (benign heart condition)
- enlarged testicles
- hypotonia (low muscle tone)
- soft, fleshy skin
- flat feet
- seizures (in about 10 percent)

Psychosociální projevy FXS

- Snížené IQ.
- Hyperaktivita, hypersenzitivita spojená se senzorickými podněty, úzkost a zpoždění vývoje.
- 30% pacientů jsou autisté (2–5% autistických dětí má FXS).
- 25% pacientů trpí epilepsií.


Brain Activation During Mental Arithmetic
typically developing girls
fragile X girls
adapted from Rivera et al. (2002)

Fragile X-assosiated tremor/ataxia syndrom (FXTAS)

- Nejčastěji se projevuje v pozdějším věku cca nad 50 let. Důležitým stanovujícím diagnostickým vodítkem mohou být oboustranné změny v oblasti středního mozečku viditelné na MRI (magnetická rezonance).
- Nejčastějšími příznaky onemocnění jsou tremor (třes) a ataktická chůze (nejistá, vrávoravá, přirovnává se k chůzi opilecké). Dalších příznaků může být řada, nejčastěji porucha funkce mozečku, demence, duševní úpadek, ztráta krátkodobé paměti, ztráta matematické i pravopisné dovednosti, parkinsonský syndrom (třes částí těla, svalová ztuhlost, mimovolní pohyby, zpomalená řeč), neuropatie, necitlivost nebo naopak přecitlivělost až pálení rukou a nohou, nízký krevní tlak, změny nálad, zvýšená podrážděnost až výbuchy vzteku, nevhodné, impulsivní chování, obtíže s organizováním, plánováním a prováděním běžných úkolů každodenního života, autonomní dysfunkce (ztráta ovládání některých orgánů např. svěračů střev, močového měchýře).

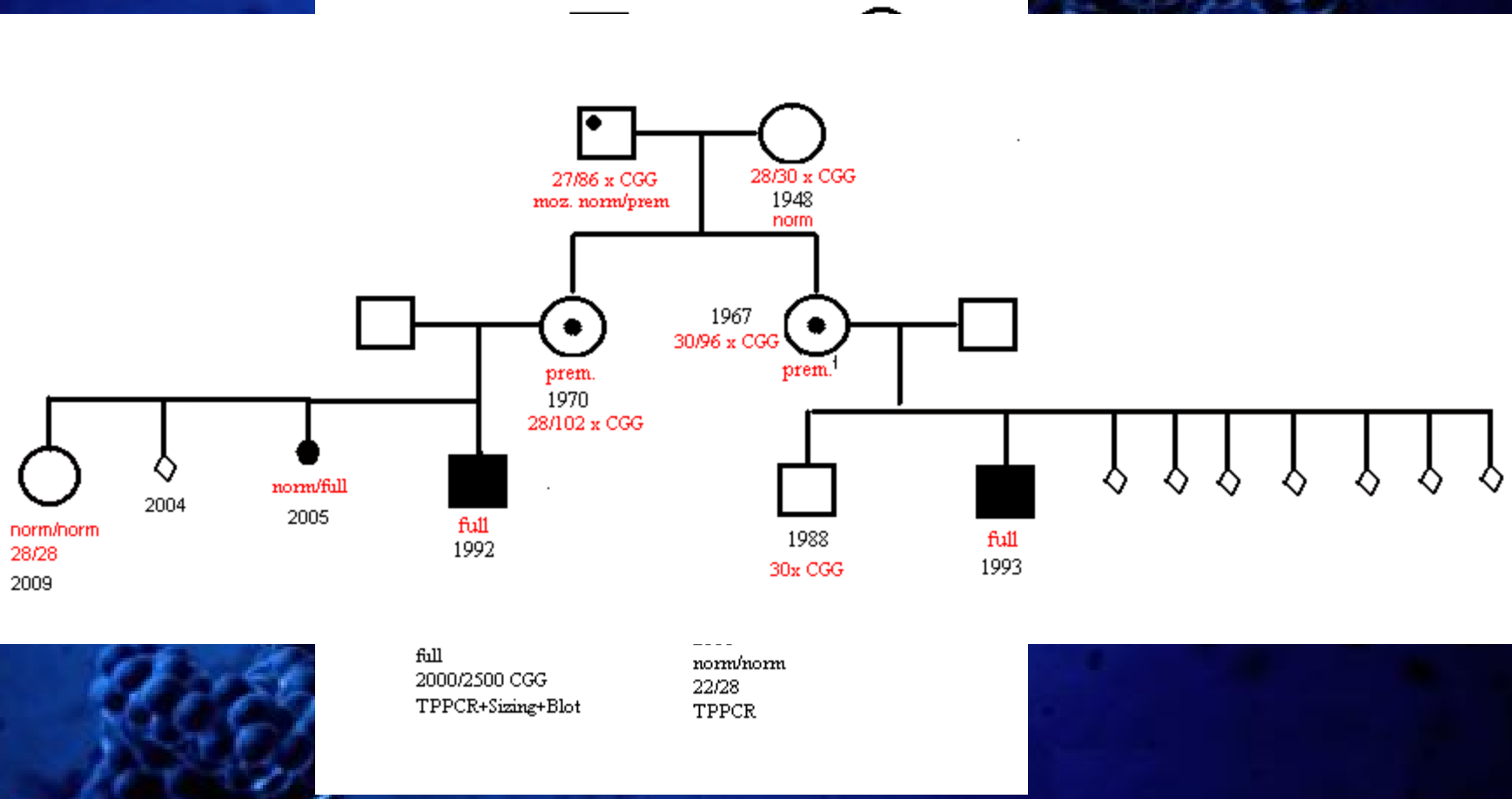
Fragile X associted primary ovarian insufficiency (FXPOI)

- Approximately 20-28% of women with an FMR1 premutation (55-200 CGG repeats) experience fragile x primary ovarian insufficiency and another 23% experience early menopause (i.e., menopause before the age of forty five).

(http://www.fragilex.org/html/menopause.htm)

Premutace – časovaná bomba v genomu

- Nemetylované expanze 55-200 jednotek CGG jsou nestabilní během meiózy a mohou expandovat do plné mutace.
- K velkým expanzím dochází pouze při maternálním přenosu.

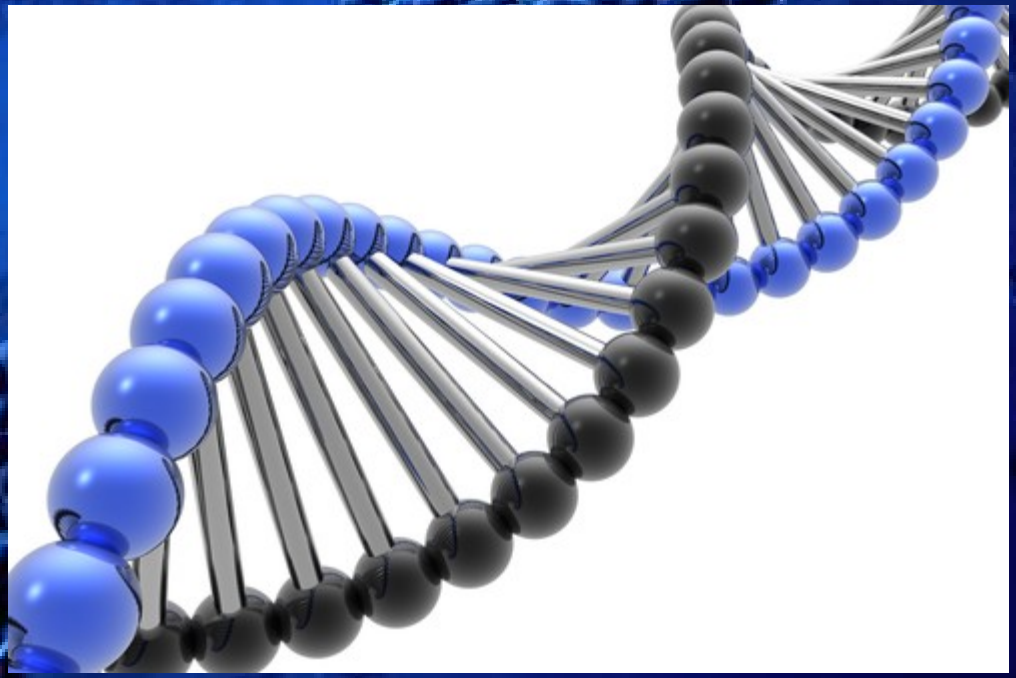

27/86 x CGG
moz. norm/prem
28/30 x CGG
1948
norm
1967
30/96 x CGG
prem.1
prem.
1970
28/102 x CGG
norm/norm
28/28
2009
2004
norm/full
2005
full
1992
1988
30x CGG
full
1993
full
2000/2500 CGG
TPPCR+Sizing+Blot
norm/norm
22/28
TPPCR

IMPORTANCE OF REPEAT SIZE IN PREMUTATED INDIVIDUALS: RISK FOR THE OFFSPRING

Number of Maternal Premutation CGG Repeats	Total Maternal Transmissions	Expansions to Full Mutations (%) [1]
55-59	27	1 (3.7%)
60-69	113	6 (5.3%)
70-79	90	28 (31.1%)
80-89	140	81 (57.8%)
90-99	111	89 (80.1%)
100-109	70	70 (100%)
110-119	54	53 (98.1%)
120-129	36	35 (97.2%)
130-139	18	17 (94.4%)
140-200	19	19 (100%)

Saul, RA and Tarleton JC. FMR1-related disorders GeneReviews 2008

Molekulární podstata

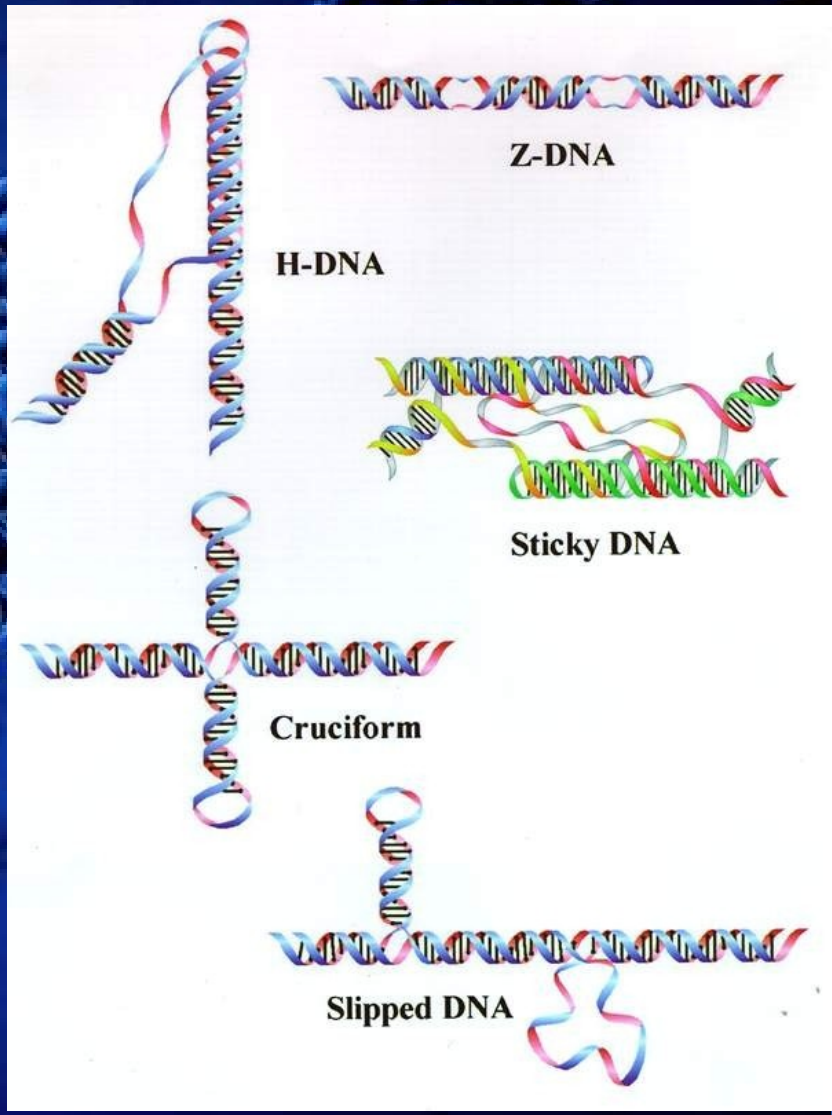


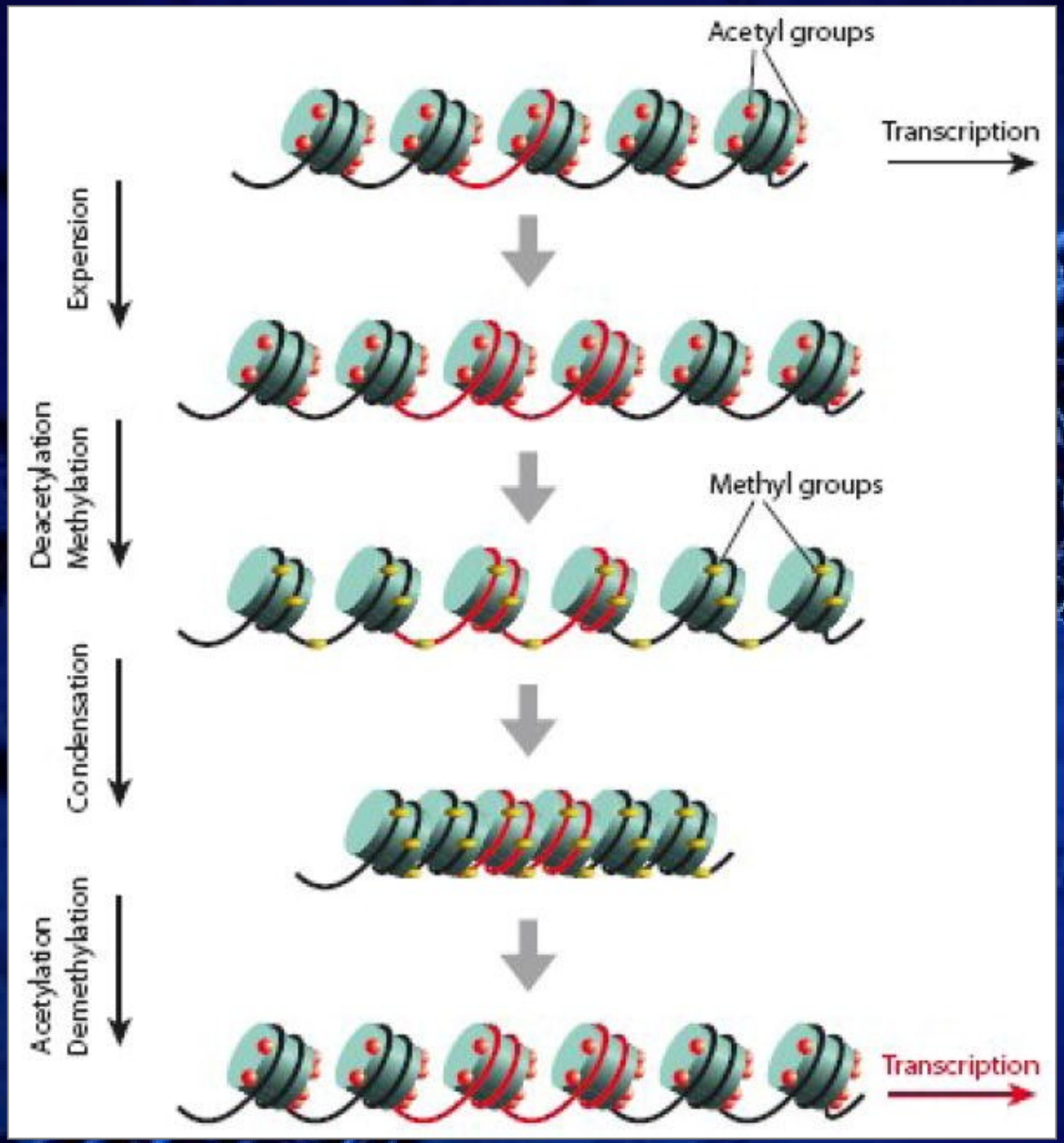
The simplest explanation of an repeat expansion – an slippage of DNA polymerase during DNA replication.

• Trinucleotide repeat sequences (TRS) - dynamic mutations - the number of repeats tends to increase in size over generations.

• Several factors contribute to mutational dynamics - number of repeats, composition and length of the repeating motif, presence of interruptions within the sequence and the rate of intracellular processes such as replication, transcription, repair, or recombination.

• **A significant feature of tandem repeats is ability to form unusual DNA structures (left-handed Z-DNA, cruciforms, slipped-stranded DNA, triplexes, and tetraplexes). Such non-B-DNA structures potentially may be hazardous for genome stability if not removed by repair mechanisms.**

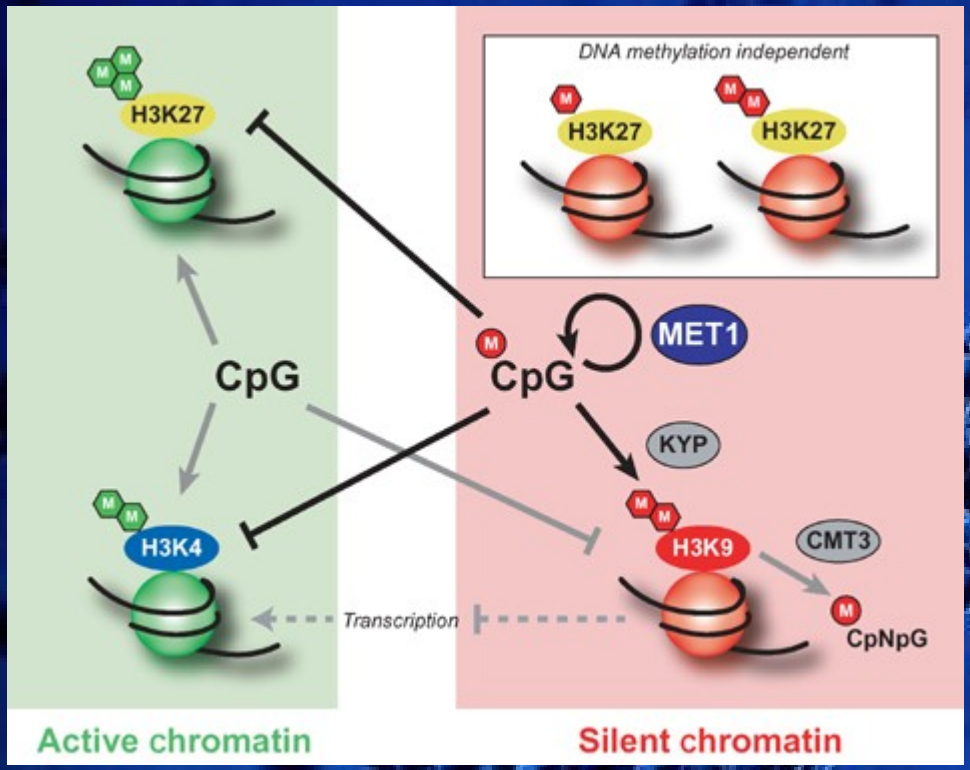




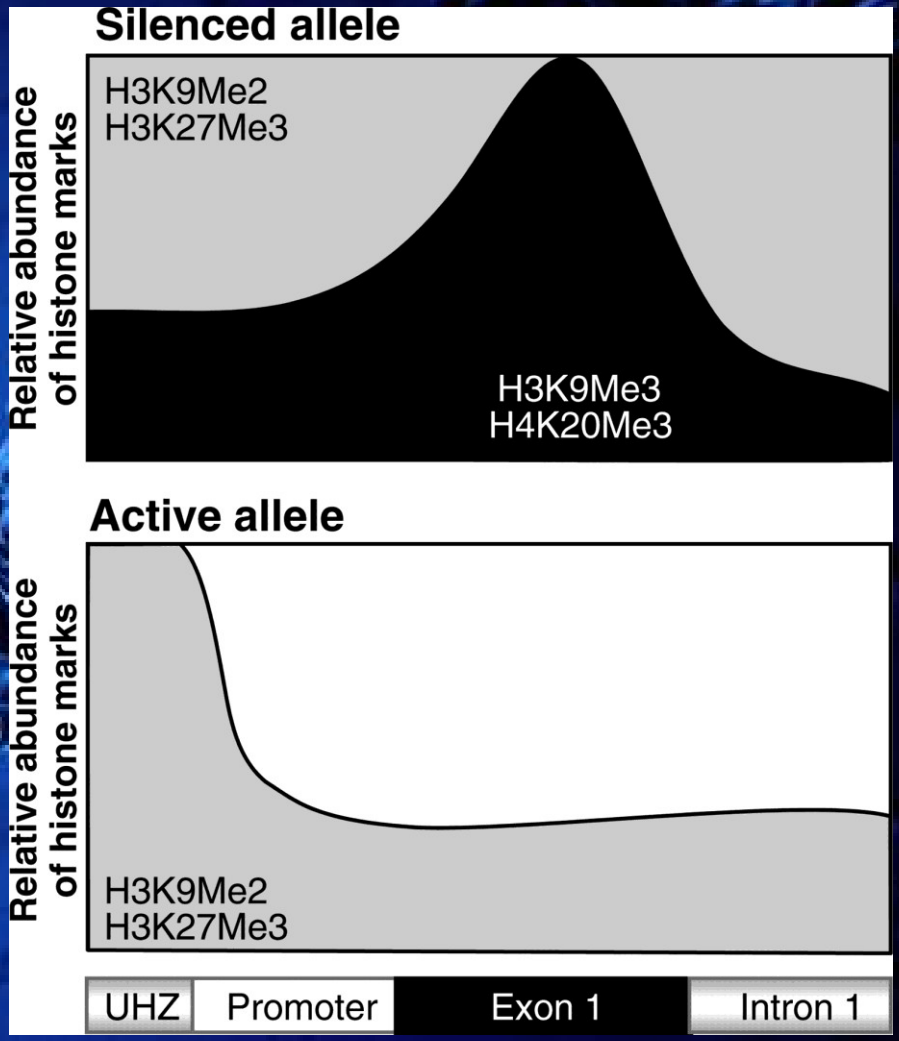
Schematic representation of the chromatin structure of the *FMR1* gene

In the normal situation the active gene has an open chromatin structure. **When the CGG repeat (red line) is expanded, deacetylation and methylation of the promoter and CGG region takes place leading to a packaged and less accessible chromatin structure causing inactivation of the *FMR1* gene.** Treatment with 5-azadC results in demethylation and acetylation leading to an open chromatin structure and transcription will be (partly) restored.

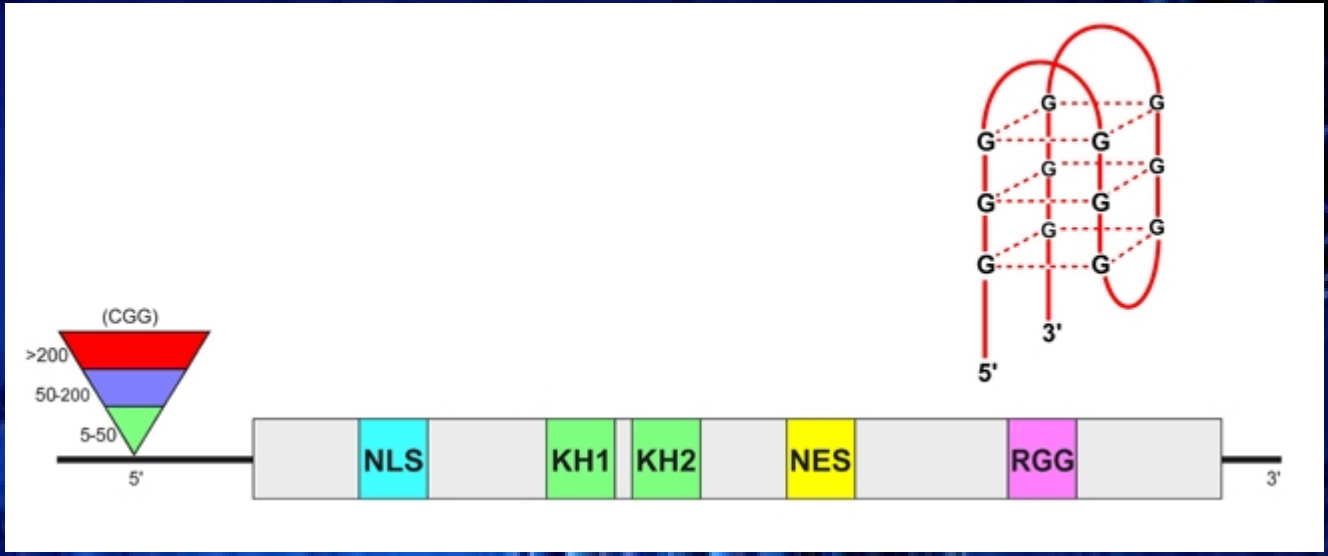
Biochim Biophys Acta. 2009; 1790(6): 467–477.



http://www.nature.com/emboj/journal/v24/n15/images/7600743f6.jpg



Kumari D , Usdin K Hum. Mol. Genet. 2010;hmg.ddq394



http://www.erasmusmc.nl/47421/51019/2004677/hoogeveen-figuur.JPG

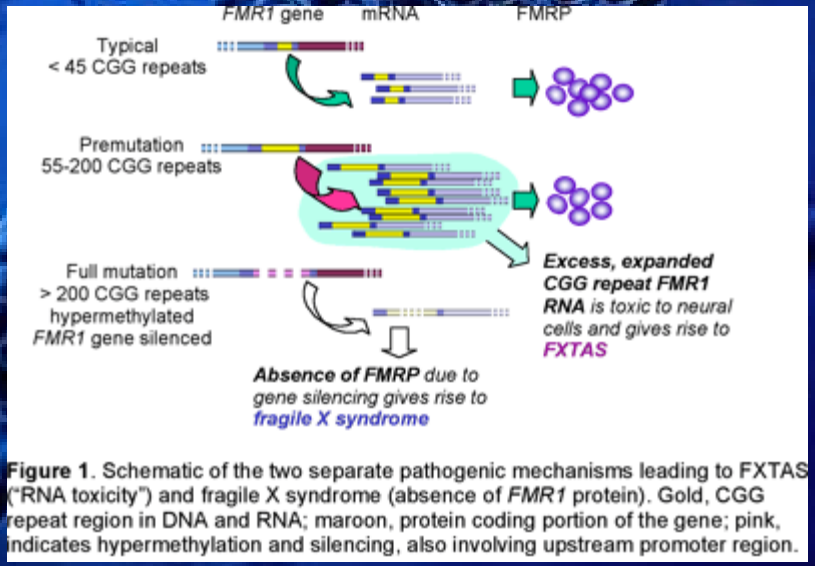
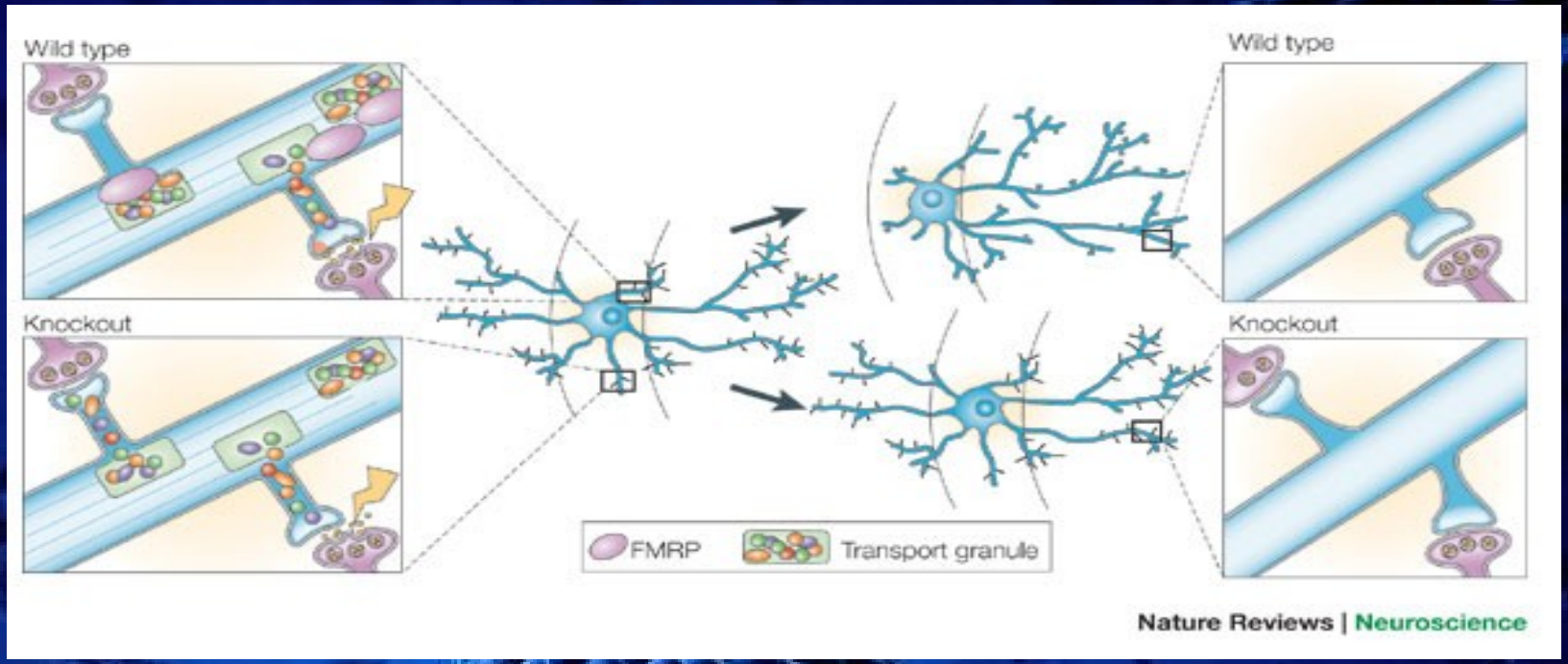


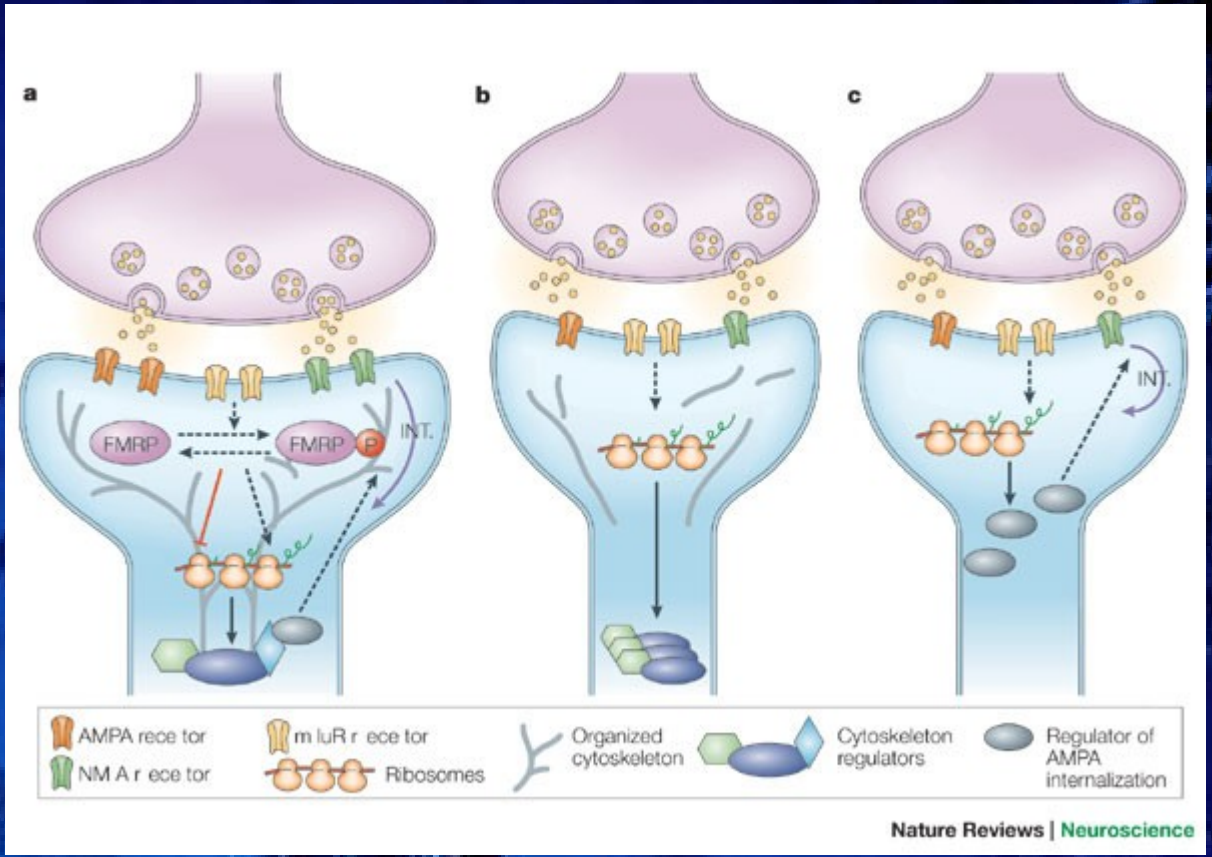
Figure 1. Schematic of the two separate pathogenic mechanisms leading to FXTAS ("RNA toxicity") and fragile X syndrome (absence of *FMR1* protein). Gold, CGG repeat region in DNA and RNA; maroon, protein coding portion of the gene; pink, indicates hypermethylation and silencing, also involving upstream promoter region.

http://www.ucdmc.ucdavis.edu/ntri/images/body/figure1.gif



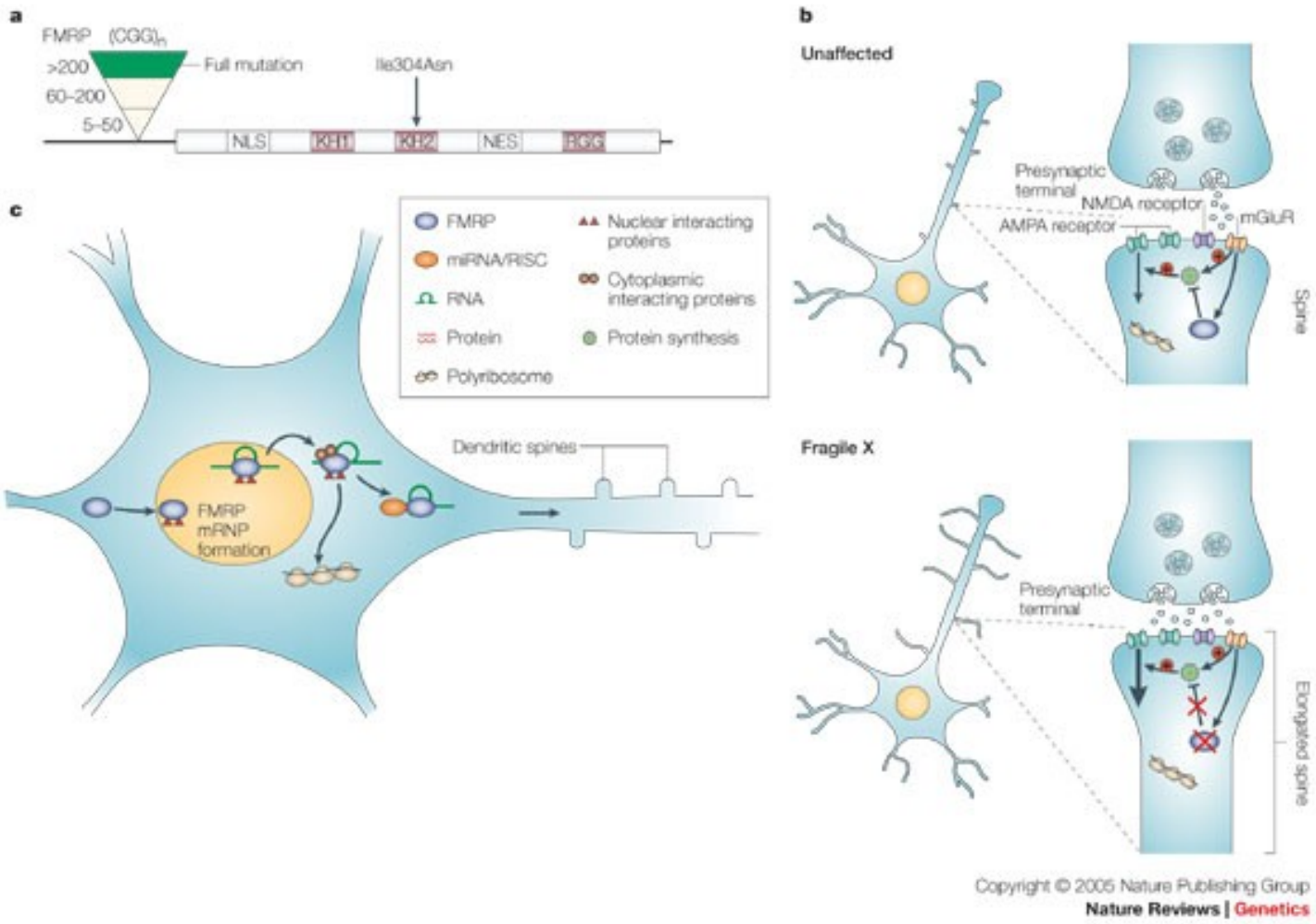
The figure shows a hypothetical mechanism through which the absence of fragile X mental retardation protein (FMRP) could lead to failure of synapse pruning and, as a consequence, dendrite pruning, in a typical spiny stellate neuron in a whisker barrel (centre). The model assumes that FMRP regulates the synthesis of structural proteins (for example, postsynaptic density protein 95 (PSD-95)) or signalling proteins that form part of a complex that is important for stabilizing and maturing developing synapses (see next fig. for one possible conceptualization of this process). When FMRP is present, this stabilization complex (carried by the transport granule) is selectively targeted to active synapses (upper left), which results in selective maturation and stabilization of spines (upper right) and pruning of non-stabilized synapses. In the absence of FMRP (lower left), the stabilization complex is equally targeted to active and inactive synapses, which results in a weaker form of maturation and stabilization, and gives rise to greater numbers of synapses and an immature morphology (lower right).

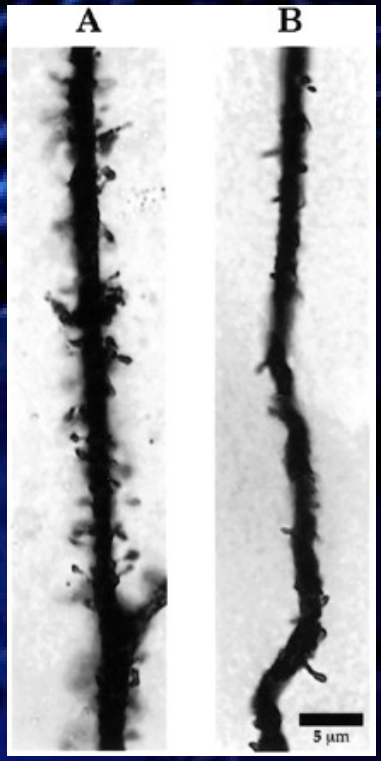
From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Claudia Bagni & William T. Greenough
***Nature Reviews Neuroscience* 6, 376-387 (May 2005)**



At synapses, protein synthesis is initiated by different cellular stimuli, and this leads to an independent response of a single synapse that can influence synaptic plasticity. **a** | In a wild-type spine, stimulation of metabotropic glutamate receptors enhances the synthesis of fragile X mental retardation protein (FMRP), which could act to negatively regulate the translation of proteins that are involved in ionotropic receptor internalization during long-term depression and of proteins that regulate the cytoskeleton (such as microtubule-associated protein 1B (MAP1B), activity-regulated cytoskeletal-associated protein (ARC), arginine-binding protein 2 (ARGBP2), postsynaptic density protein 95 (PSD-95) and Rac1). This receptor-coupled signalling pathway might also be responsible for FMRP phosphorylation and the consequent release of mRNAs from translational inhibition and/or the activation of translation of other specific dendritic mRNAs. The correct balance between synthesis and degradation of these proteins would promote and maintain the mature shape of the synapse. **b** | In a spine of a patient with fragile X syndrome, or in the mouse model of the syndrome, the absence of FMRP would lead to an increase and/or decrease in the translation of protein regulators of the cytoskeleton, both of which might have an effect on the lengthening of dendritic spines. **c** | The absence of FMRP could also lead to an increase in the translation of proteins that are involved in ionotropic (AMPA (-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) and NMDA (***N***-methyl-**D**-aspartate)) receptor internalization (INT.) during hippocampal long-term depression, which could lead to fewer receptors being present on the postsynaptic membrane and to thinner spines. mGluR, metabotropic glutamate receptor.

From mRNP trafficking to spine dysmorphogenesis: the roots of fragile X syndrome. Claudia Bagni & William T. Greenough
***Nature Reviews Neuroscience* 6, 376-387 (May 2005)**



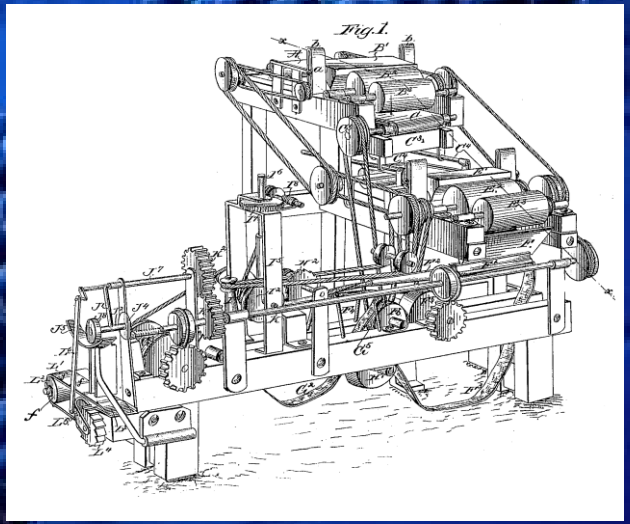


Typical spine morphologies from (A) a human afflicted by fragile-X syndrome and (B) an unaffected control.

Abnormal dendritic spine morphology in patients with FRAXA (an increased density of long, immature dendritic spines). During transport FMRP-mRNA, FMRP suppresses translation. Stimulation of postsynaptic glutamate receptors (mGluRs) results in increased protein synthesis. FMRP, which is also upregulated by mGluRs, serves to dampen this process. The absence of FMRP in FRAXA results in over-amplification of this response.

Dendritic Spine Structural Anomalies in Fragile-X Mental Retardation Syndrome: Scott A. Irwin, Roberto Galvez and William T. Greenough. Cereb. Cortex (2000) 10 (10): 1038-1044.

Metodické přístupy - diagnostika



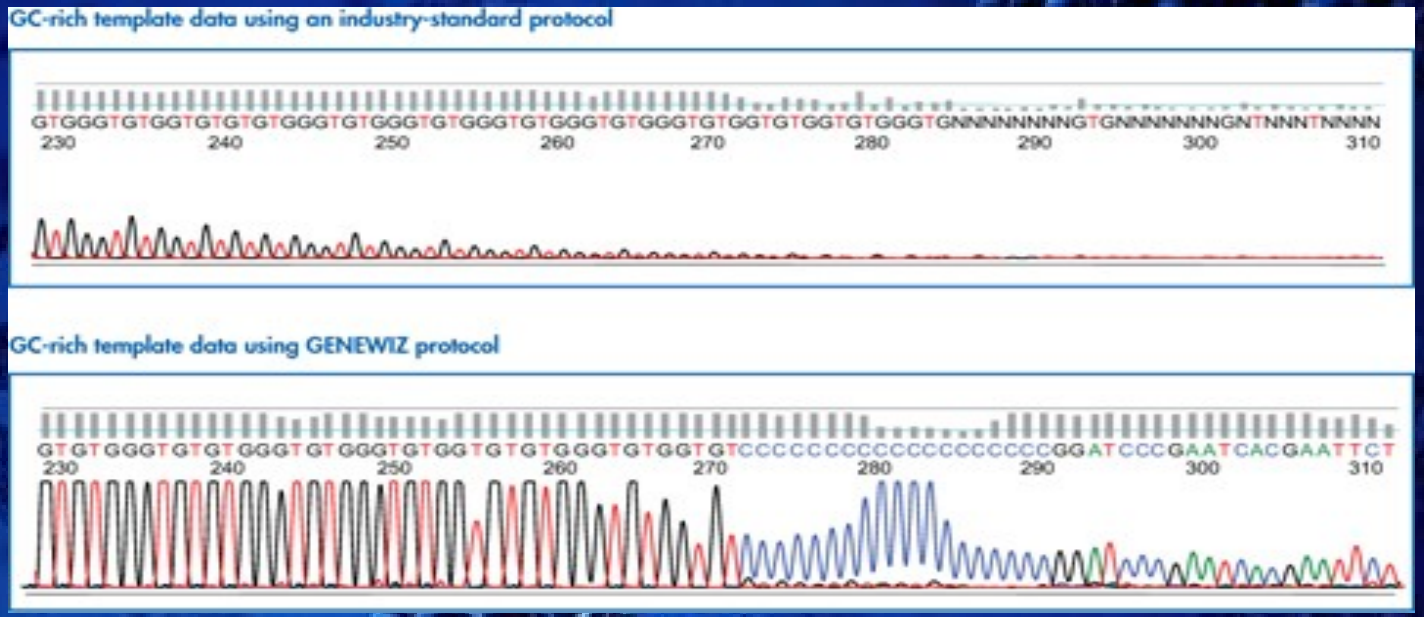
Spektrum metod

- Home-made metody
- GC-rich PCR systémy
- Metylačně specifická PCR
- Repeat Primed PCR (Asuragen)
- Southern blotting

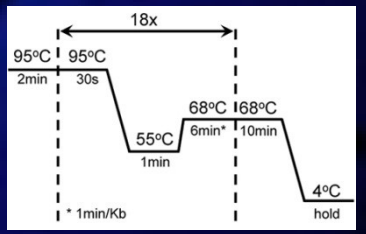
Home-made metody

- Většinou založeny na prosté amplifikaci daného úseku
- Většinou odhalí pouze alely o normální velikosti
- Nutno použít polymerázy s *proof reading* aktivitou

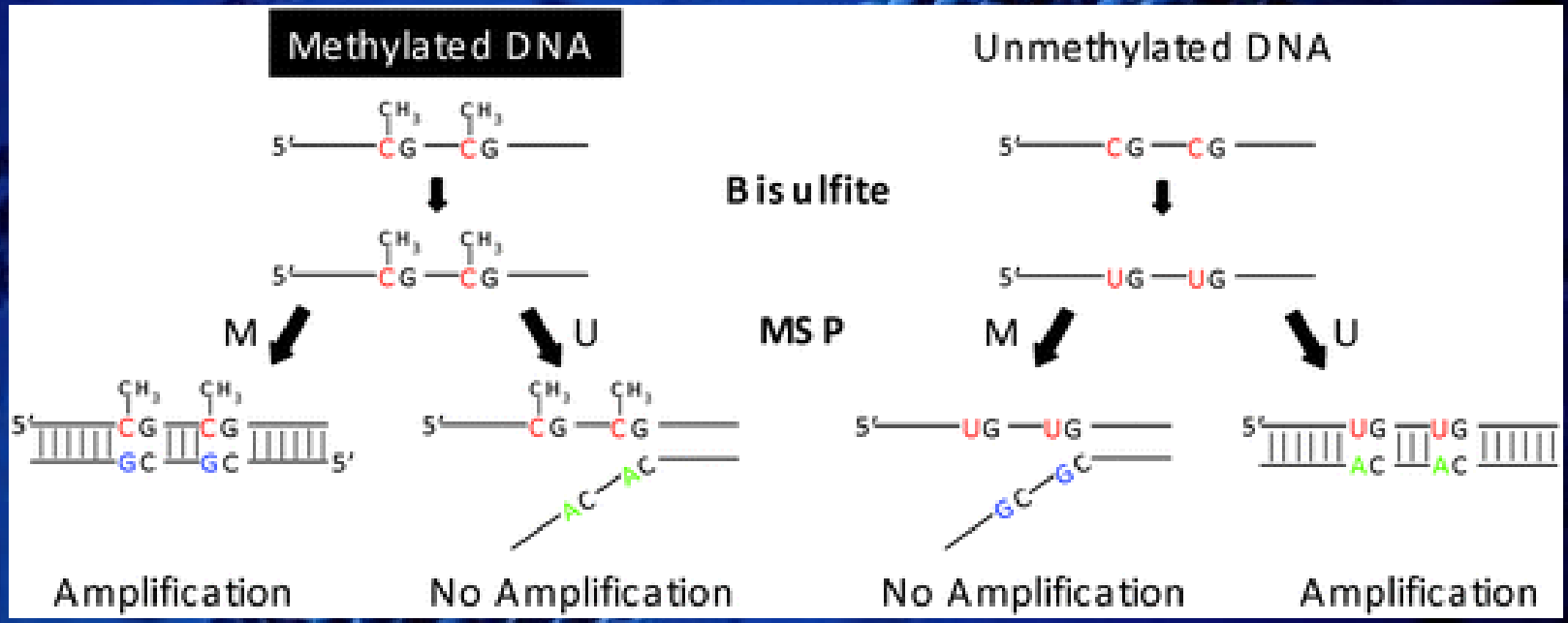
GC-rich PCR systémy



- Amplifikace GC-rich templátů složitá – speciální pufry, speciální polymerázy, speciální PCR programy
- Většinou pouze alely o normální velikosti, dolní hranice premutací



Metylačně specifická PCR



Repeat primed PCR (Asuragen)

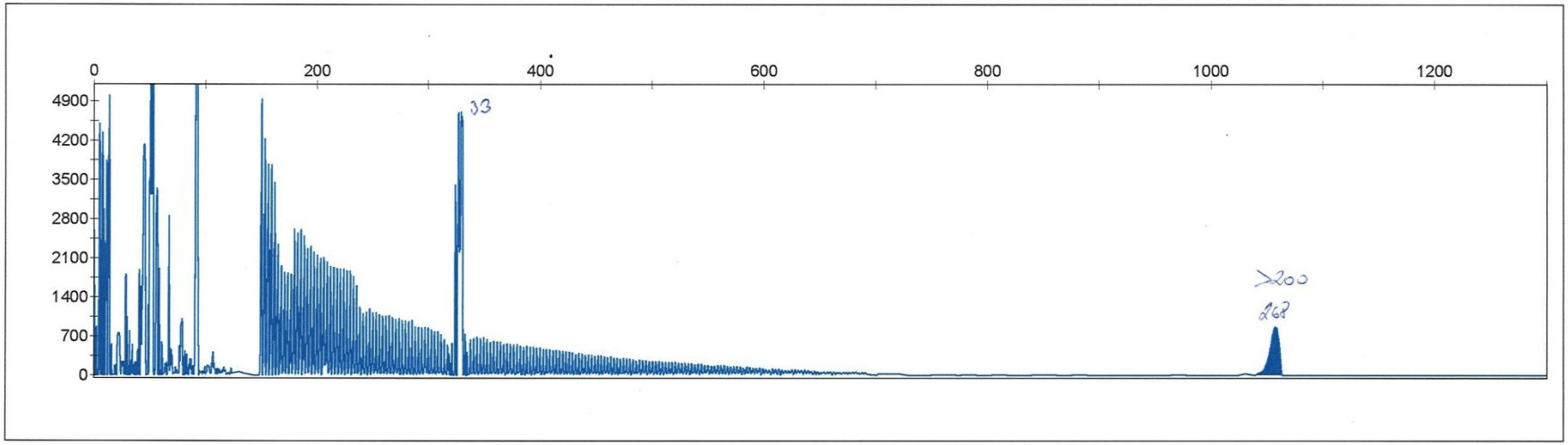
- Malé vstupní množství DNA
- PCR + fragmentační analýza (5 + 1 h)
- Robustní, rychlá, jednoduchá
- Drahá

2517

Applied Biosystems

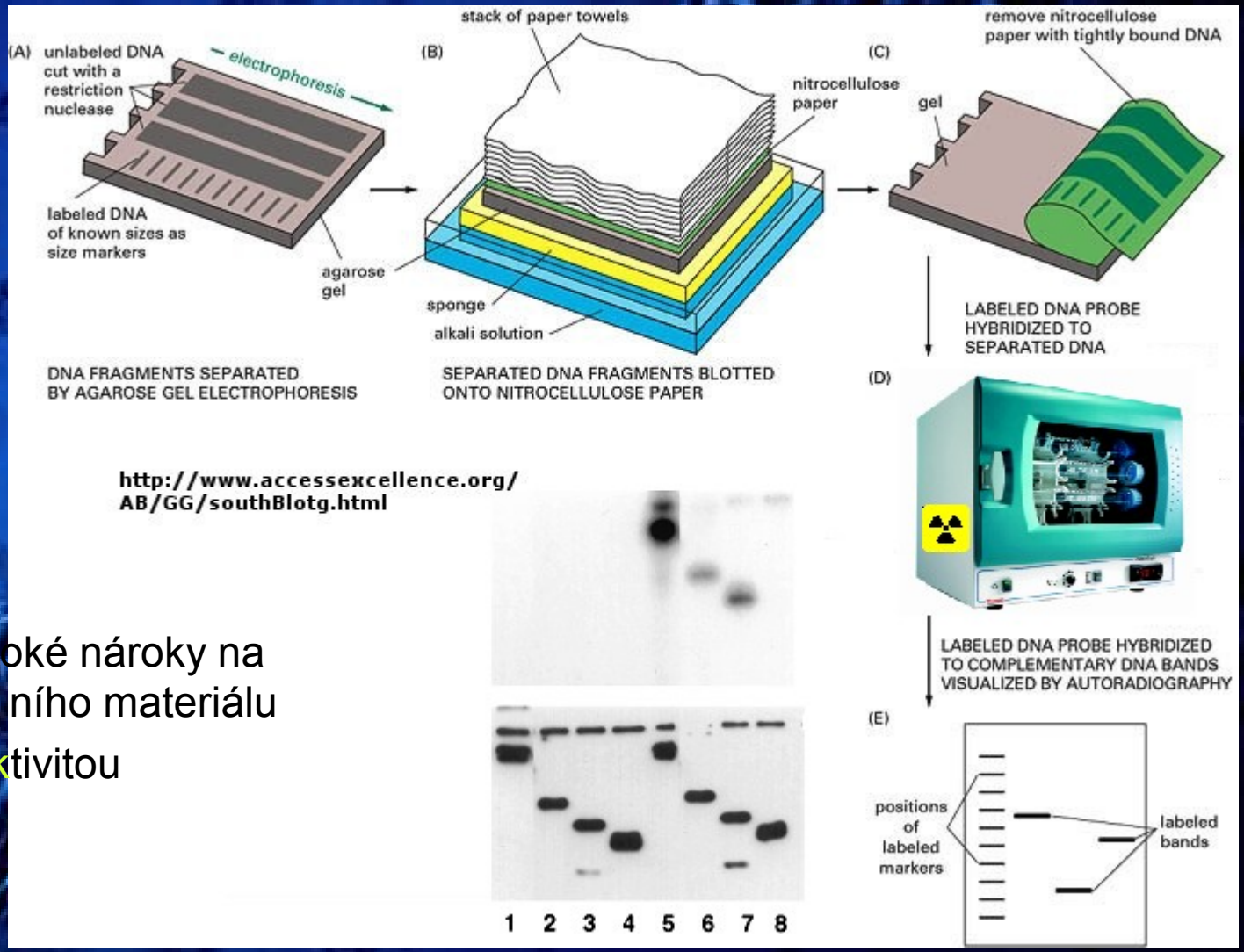
GeneMapper 4.1

27-6-2014 lukas



		Sample File Name	Size	Height	Area
1		1_2014-06-30_08-30-22.fsa	329.06	4720	70380
2		1_2014-06-30_08-30-22.fsa	1057.26	877	18272

Southern blotting



- Zdlouhavé, vysoké nároky na množství vstupního materiálu
- Práce s radioaktivitou
- Poměrně levné

Děkuji za pozornost