Epigenetics from HIV to Immunization:

New Prospects on Health and Disease

Christl Meyer, AIDS Research, Vienna/
Austria, 2018

„Nothing in Biology makes sense, except in the light of evolution“

This is from a 1973 essay by the evolutionary biologist and Russian Orthodox Christian Theodosius Dobzhansky, Criticising Young Earth creationism and espousing Evolutionary Creationism.

(American Biology Teacher, volume 35, 125 –129)

Overview

1. Genetics, HIV and the Immune System
2. Epigenetics
3. Microbes in Evolution
4. Immunization
5. What does it mean to be Human?

1. Genetics, HIV and the Immune System
2. Epigenetics
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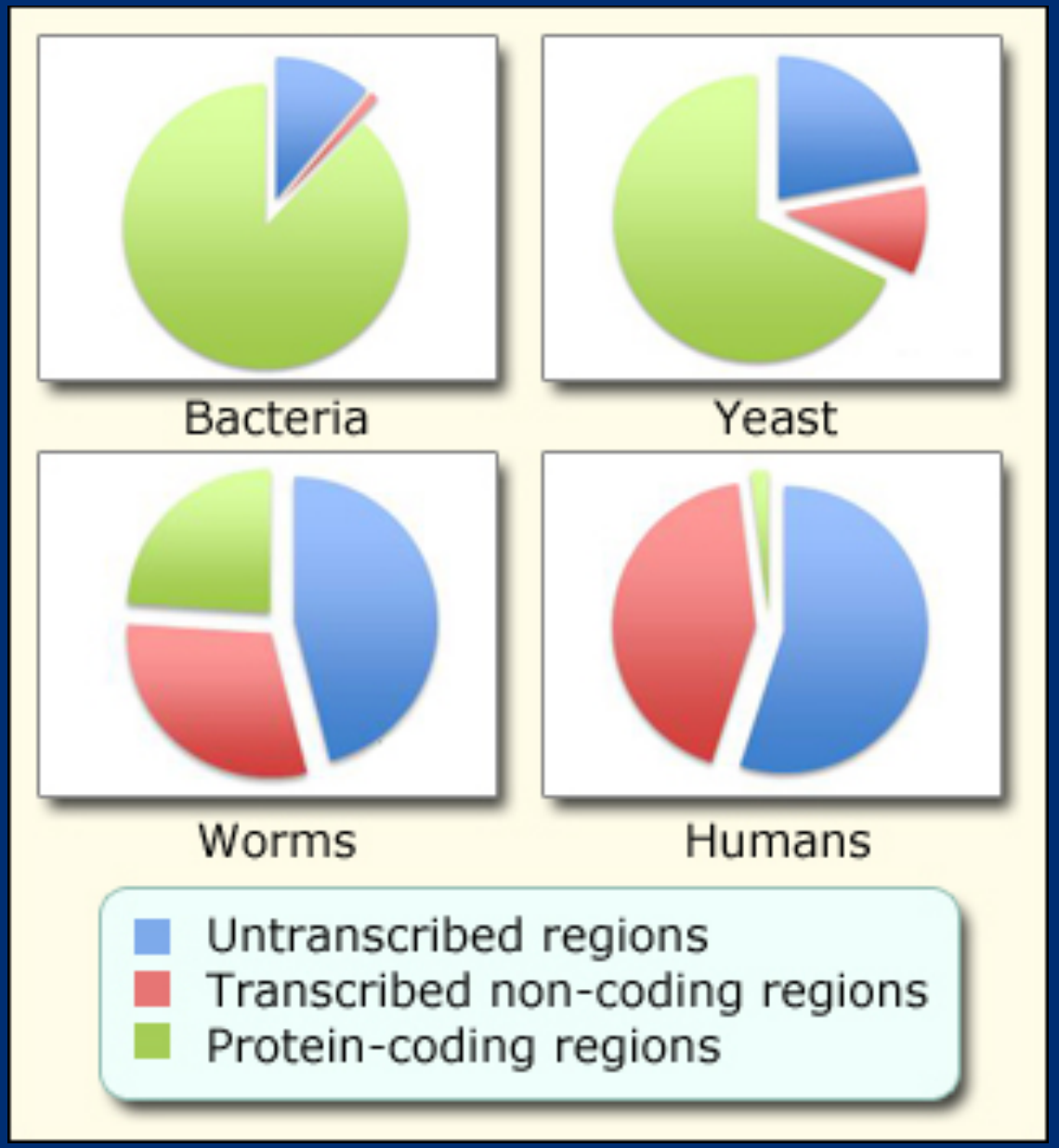
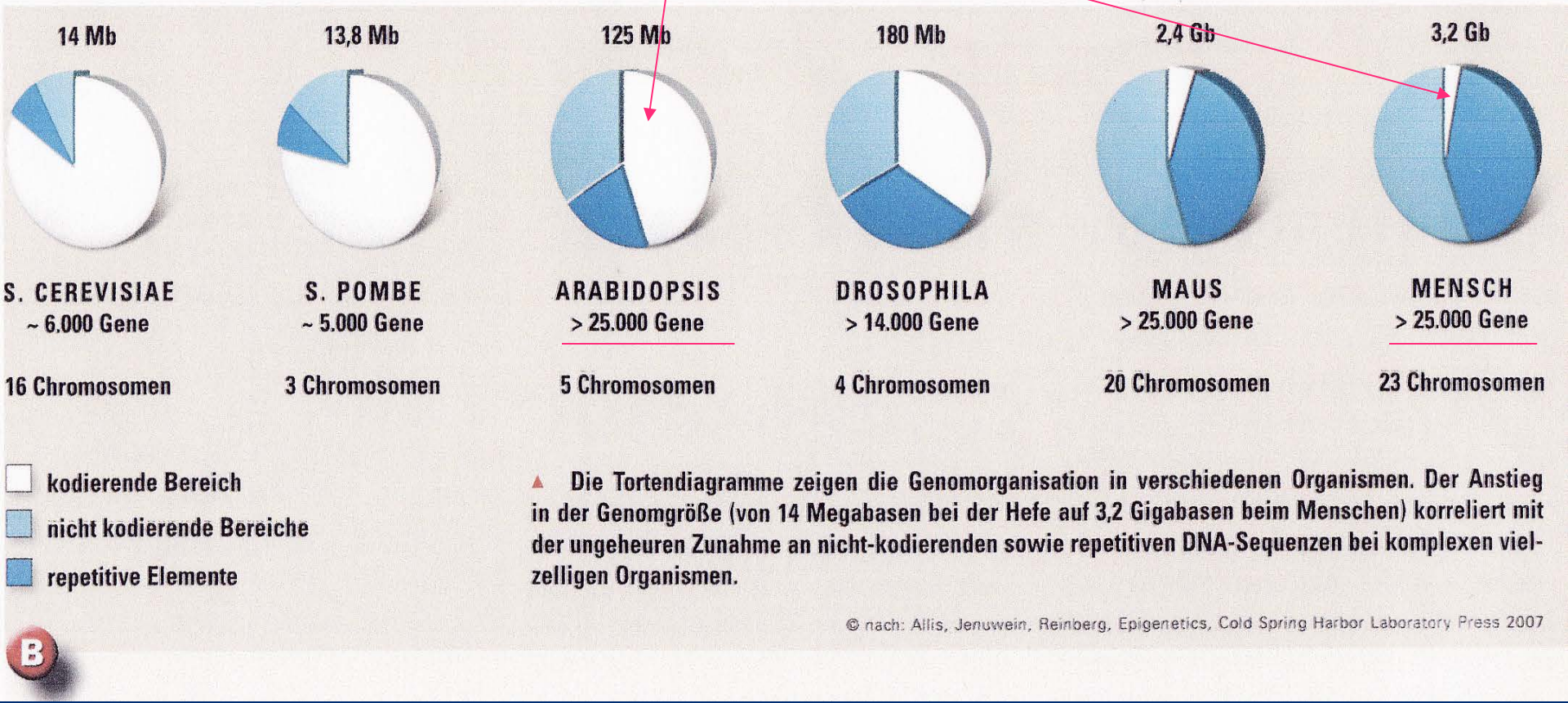
Genes for proteins
RNA molecules
???



Genomes: Coding, non coding, repetitive elements

PLANT and HUMAN: Both harbour about 25.000 genes



▲ Die Tortendiagramme zeigen die Genomorganisation in verschiedenen Organismen. Der Anstieg in der Genomgröße (von 14 Megabasen bei der Hefe auf 3,2 Gigabasen beim Menschen) korreliert mit der ungeheuren Zunahme an nicht-kodierenden sowie repetitiven DNA-Sequenzen bei komplexen vielzelligen Organismen.

Effect of the „*kit*“-gene

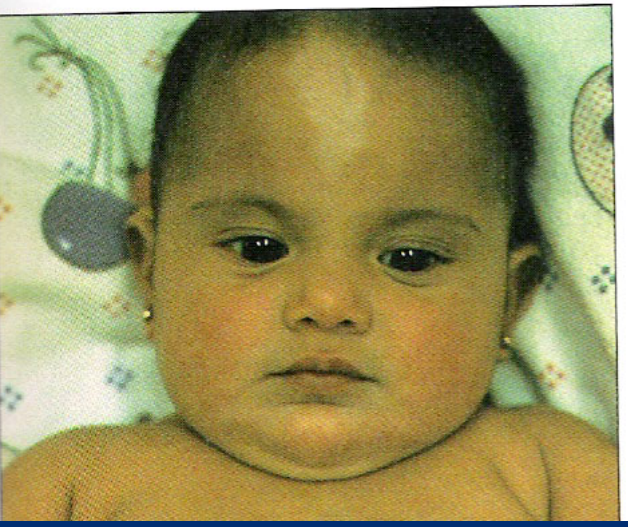
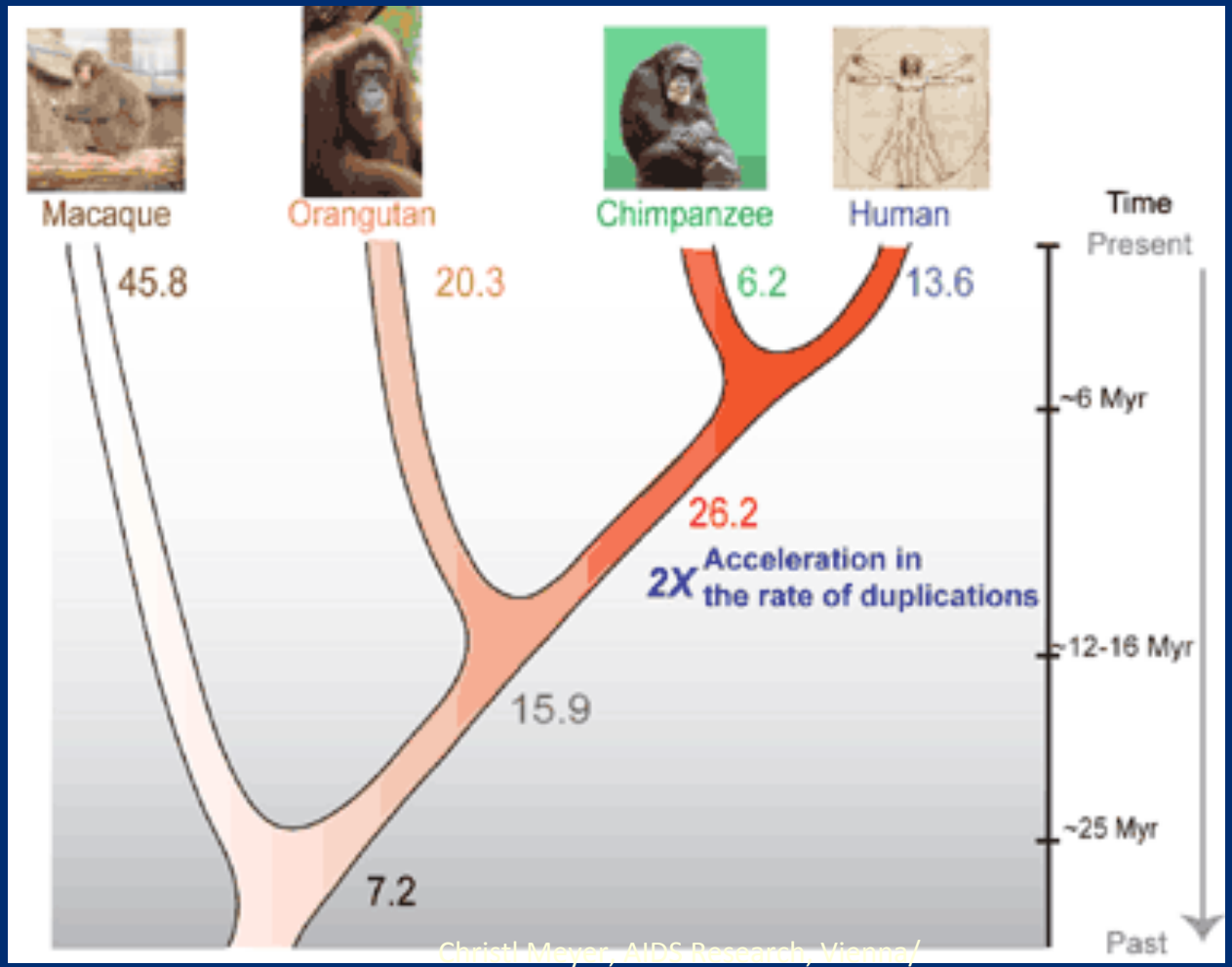
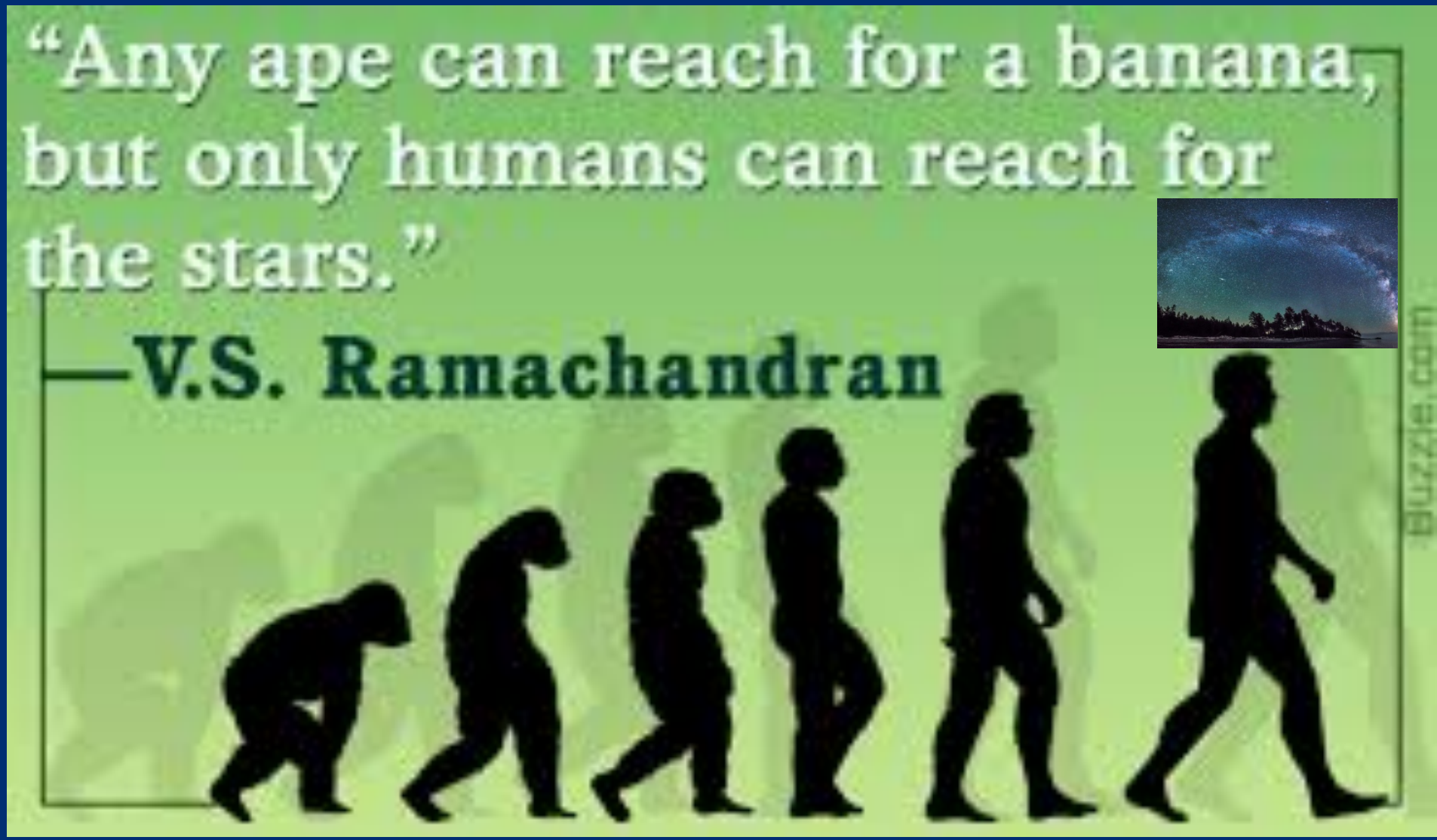


Figure 1–53 Human and mouse: similar genes and similar development. The human baby and the mouse shown here have similar white patches on their foreheads because both have mutations in the same gene (called *kit*), required for the development and maintenance of pigment cells. (From R.A. Fleischman, *Proc. Natl. Acad. Sci. USA* 88:10885–10889, 1991. © National Academy of Sciences.)

Example: Trisomie 21 (Down Synrom)

Genduplication as the driving force behind Evolution



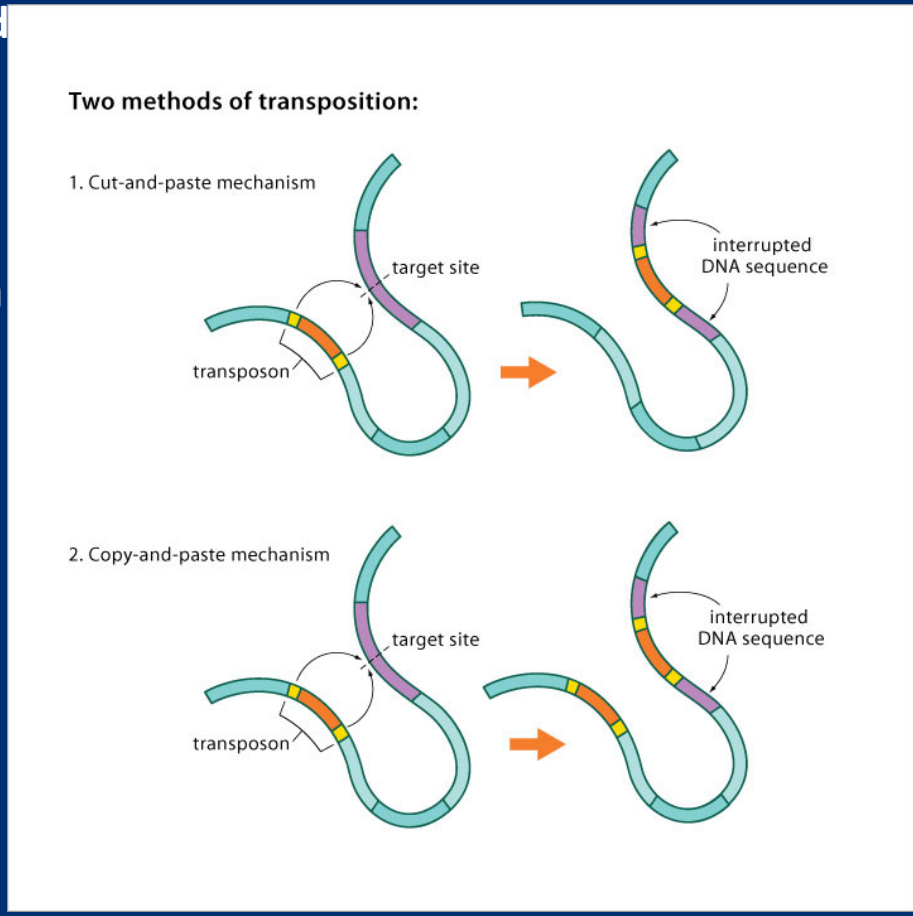

"Any ape can reach for a banana, but only humans can reach for the stars."
—V.S. Ramachandran
Buzzle.com

Over half of our DNA comes from retro-transposons,

genetic elements that independently multiply and spread through the genome
(de Koning et al., 2011).

On the move

The mechanisms by which a retrotransposon called LINE-1 duplicates
itself and spreads through the human genome are becoming clearer.
Martin. eLife 2018;7:e34901. DOI: https://doi.org/10.7554/eLife.34901



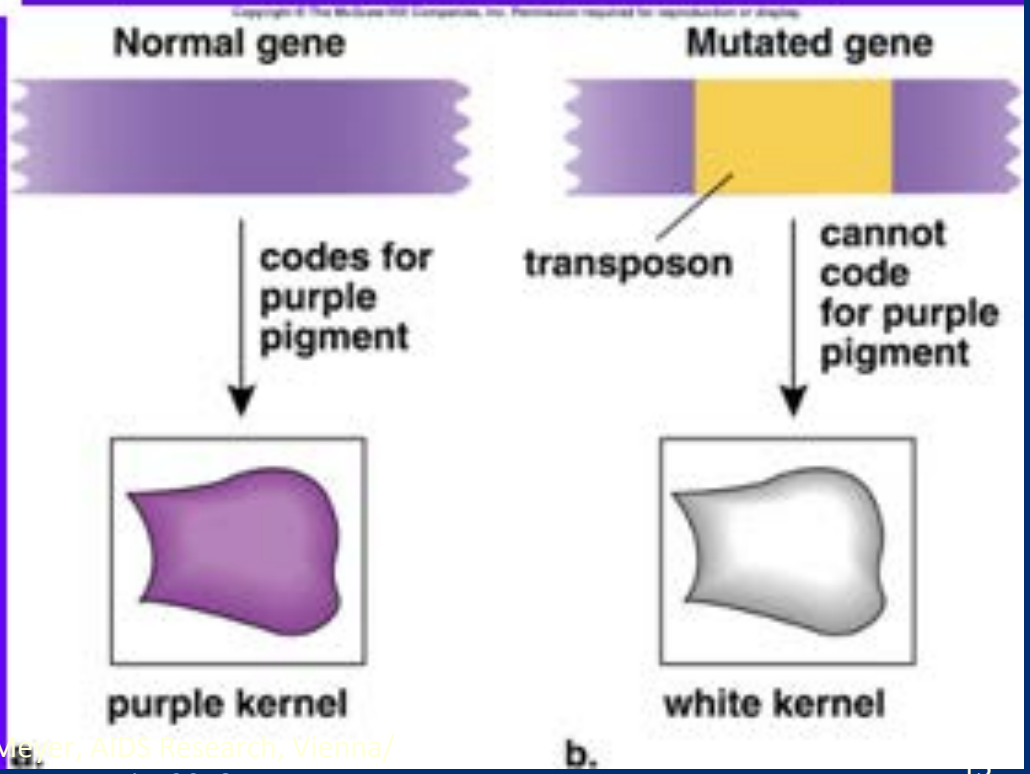
In mammals, a retrotransposon called LINE-1 is responsible for most of these multiplication events, either duplicating itself or lending its 'photocopying' machinery to other genetic elements.

Barbara Mc. Clintock (Nobelprice for Physiology and Medicine 1983) Transposons „jumping genes“

Christl Meyer, AIDS Research, Vienna/ Austria, 2018

Transposons are jumping genes!

Transposons are small pieces of DNA that get cut from one spot and spliced into an inappropriate spot– often right in the middle of a gene. The gene's sequence is altered and it can no longer be used to produce the protein it codes for.



Humans and chimpanzees, which diverged from a common ancestor some 5 million years ago, differ in their genome sequences only in about 1 – 2 %.

Proviruses fixed in the germ line can provide us with a fossil record of viruses long extinct in the population.

In human DNA only 2- 3 % of the genome are coding.
97 % are noncoding (formerly called „junk DNA“).
This DNA might be involved in gene regulation processes.
In humans, there are about 80.000 proviruses or their remnants, comprising about 6-8% of the genome, or about twice as many as genes.

John M. Coffin, Prof of Molecular Biology and Microbiology, Tufts University :

„ There is more provirus in us than there is us in us “.

The Human Genome Project discovered our genetic background by reducing the amount of protein coding genes to approximately 20.000 and augmented the RNA transcriptions.

1. Alvarez-Dominguez JR, Hu W, Yuan B, et al. Global discovery of erythroid **long noncoding RNAs** reveals novel regulators of red cell maturation. Blood. 2014;123(4): 570-581.
2. Sabin LR, Del´as MJ, Hannon GJ. Dogma derailed: the many influences of RNA on the genome. Mol Cell. 2013; 49(5):783-794.
3. Ulitsky I, Bartel DP. lincRNAs: genomics, evolution, and mechanisms. Cell. 2013;154(1):26-46.
4. Batista PJ, Chang HY. Long noncoding RNAs: cellular address codes in development and disease. Cell. 2013; 152(6):1298-1307.
5. Bergmann JH, Spector DL. Long non-coding RNAs …..

Aleksandra E. Kornienko, Christoph P. Dotter, Philipp M. Guenzl, Heinz Gisslinger, Bettina Gisslinger, Ciara Cleary, Robert Kralovics, Florian M. Pauler and Denise P. Barlow

"Long non-coding RNAs display higher natural expression variation than protein-coding genes in healthy humans"

Genome Biology 2016, 17:14, DOI: 10.1186/s13059-016-0873-8.

Human Genome

Nature **409**, 860-921 (15 February 2001) / doi: 10.1038/35057062;
Received 7 December 2000; Accepted 9 January 2001

Initial sequencing and analysis of the human genome

Internationbal Human Genome Sequencing Consortium Eric S. Lander...

...Here we report the results of a collaboration involving 20 groups from the United States, the United Kingdom, Japan, France, Germany and China to produce a draft sequence of the human genome.

...Hundreds of human genes appear likely to have resulted from horizontal transfer from bacteria at some point in the vertebrate lineage.

...Dozens of genes appear to have been derived from transposable elements.

...large recent segmental duplications...much more frequent in humans...

A major part of the immune system:

MHC - Major Histocompatibility –Complex

and

HLA - Human Leucocyte Antigen – System

give new insights on the co-evolution of the species, particularly of mammals (and primates) with microbes.

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DNA Methylation Profiling of the Human Major Histocompatibility Complex: A Pilot Study for the Human Epigenome Project

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The Human Epigenome Project aims to identify, catalogue, and interpret genome-wide DNA methylation phenomena. Occurring naturally on cytosine bases at cytosine–guanine dinucleotides, DNA methylation is intimately involved in diverse biological processes and the aetiology of many diseases. Differentially methylated cytosines give rise to distinct profiles, thought to be specific for gene activity, tissue type, and disease state. The identification of such methylation variable positions will significantly improve our understanding of genome biology and our ability to diagnose disease. Here, we report the results of the pilot study for the Human Epigenome Project entailing the methylation analysis of the human major histocompatibility complex. This study involved the development of an integrated pipeline for high-throughput methylation analysis using bisulphite DNA sequencing, discovery of methylation variable positions, epigenotyping by matrix-assisted laser desorption/ionisation mass spectrometry, and development of an integrated public database available at http://www.epigenome.org. Our analysis of DNA methylation levels within the major histocompatibility complex, including regulatory exonic and intronic regions associated with 90 genes in multiple tissues and individuals, reveals a bimodal distribution of methylation profiles (i.e., the vast majority of the analysed regions were either hypo- or hypermethylated), tissue specificity, inter-individual variation, and correlation with independent gene expression data.

Citation: Rakyan VK, Hildmann T, Novik KL, Lewin J, Tost J, et al. (2004) DNA methylation profiling of the human major histocompatibility complex: A pilot study for the human epigenome project. PLoS Biol 2(12): e405.

Introduction

DNA methylation is indispensable for vertebrate genome function. It is involved in diverse genomic processes such as gene regulation, chromosomal stability, and parental imprinting (Bird 2002), and interest in the function of DNA methylation is further heightened by the various human diseases associated with epigenetic dysfunction, a notable example being cancer (Laird 2003). However, the DNA methylation profile of the human genome is still largely a mystery.

The sequencing of the human genome (IHGSC 2001) and creation of a whole-genome map of single nucleotide polymorphisms (SNPs) (Sachidanandam et al. 2001) laid the foundation for the Human Epigenome Project (HEP). For the HEP, we aim to analyse DNA methylation in the regulatory regions of all known genes in most major cell types and their diseased variants, along with producing high-density snapshots of non-genic regions spread evenly across the human genome. Although genome-wide DNA methylation analyses have been performed previously (Costello et al. 2000; Strichman-Almashanu et al. 2002), the HEP is the first systematic whole-genome study of DNA methylation at the sequence level.

As a prelude to the HEP, here we report the results of the HEP pilot study: DNA methylation profiling of the human major histocompatibility complex (MHC). The MHC, located on Chromosome 6 (6p21.3), is one of the most gene-dense regions in the human genome, containing genes with a high diversity of function, many of which are involved in the innate and adaptive immune systems. We chose to analyse the MHC for the pilot HEP study for three main reasons. (i) The MHC is associated with more diseases than any other region of the human genome, and therefore the generated data will be of interest to researchers with diverse biomedical interests. (ii) It is also the most polymorphic region in the genome, and therefore the data will allow study of the potential effects of the loss or gain of cytosine–guanine dinucleotide (CpG) methylation sites (due to SNPs) on gene expression and possibly other phenotypes. (iii) At the time when the HEP pilot study was initiated in 1999 (Beck et al. 1999), the MHC was one of the few regions within the human genome for which finished sequence and annotation were readily available (MHC Sequencing Consortium 1999).

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Abbreviations: CpG, cytosine–guanine dinucleotide; DAS, distributed annotation system; HEP, Human Epigenome Project; *IGF2, insulin-like growth factor 2;* MHC, major histocompatibility complex; MALDI-MS, matrix-assisted laser desorption/ionisation mass spectrometry; METHANE, METHylation ANalysis Engine; MVP, methylation variable position; ROI, region of interest; SNP, single nucleotide polymorphism; *TNXB, tenascin-XB*

Academic Editor: Peter B. Becker, University of Munich

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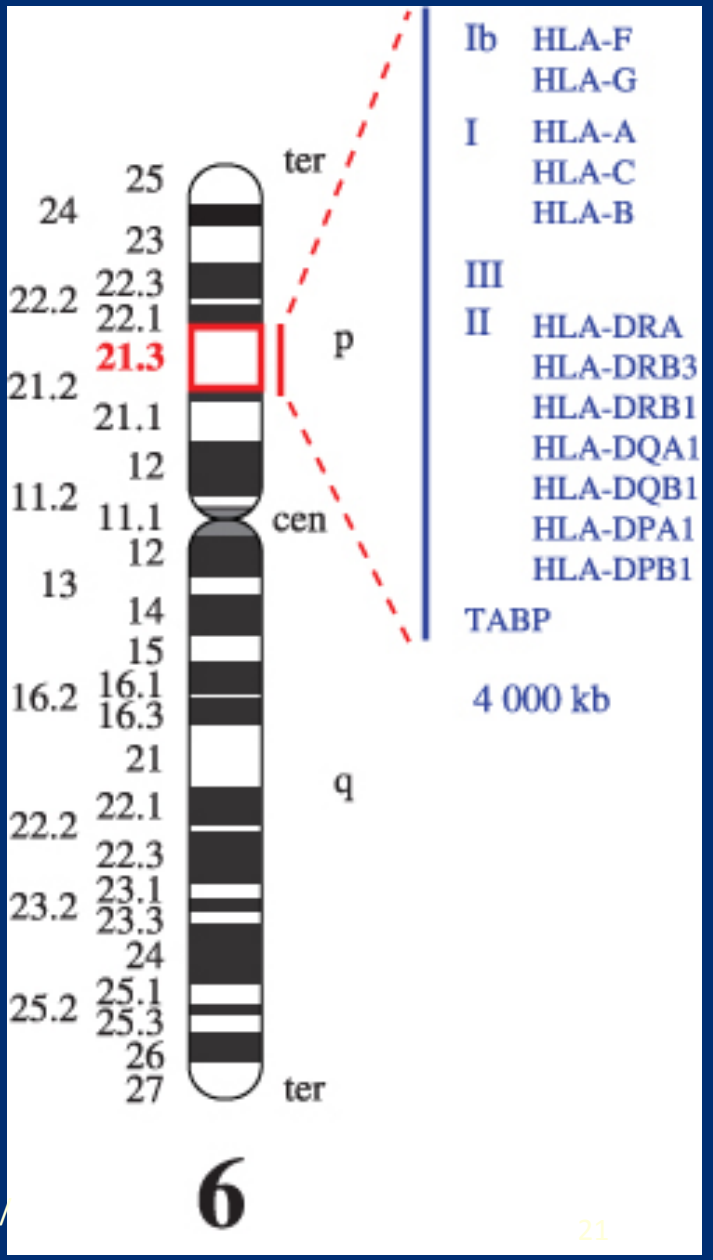
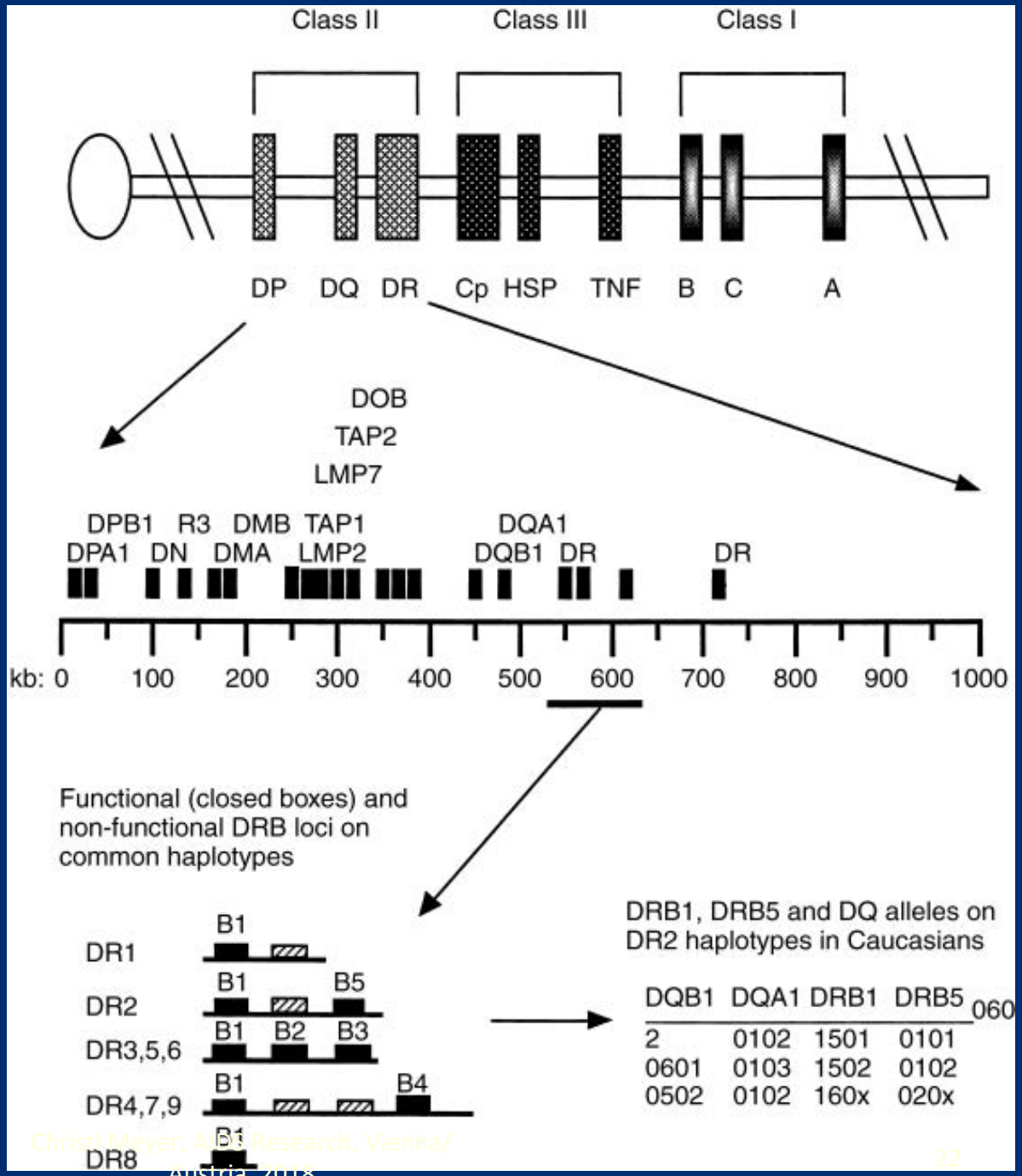
HLA is the most gene dense and variable region and is related to health and disease!

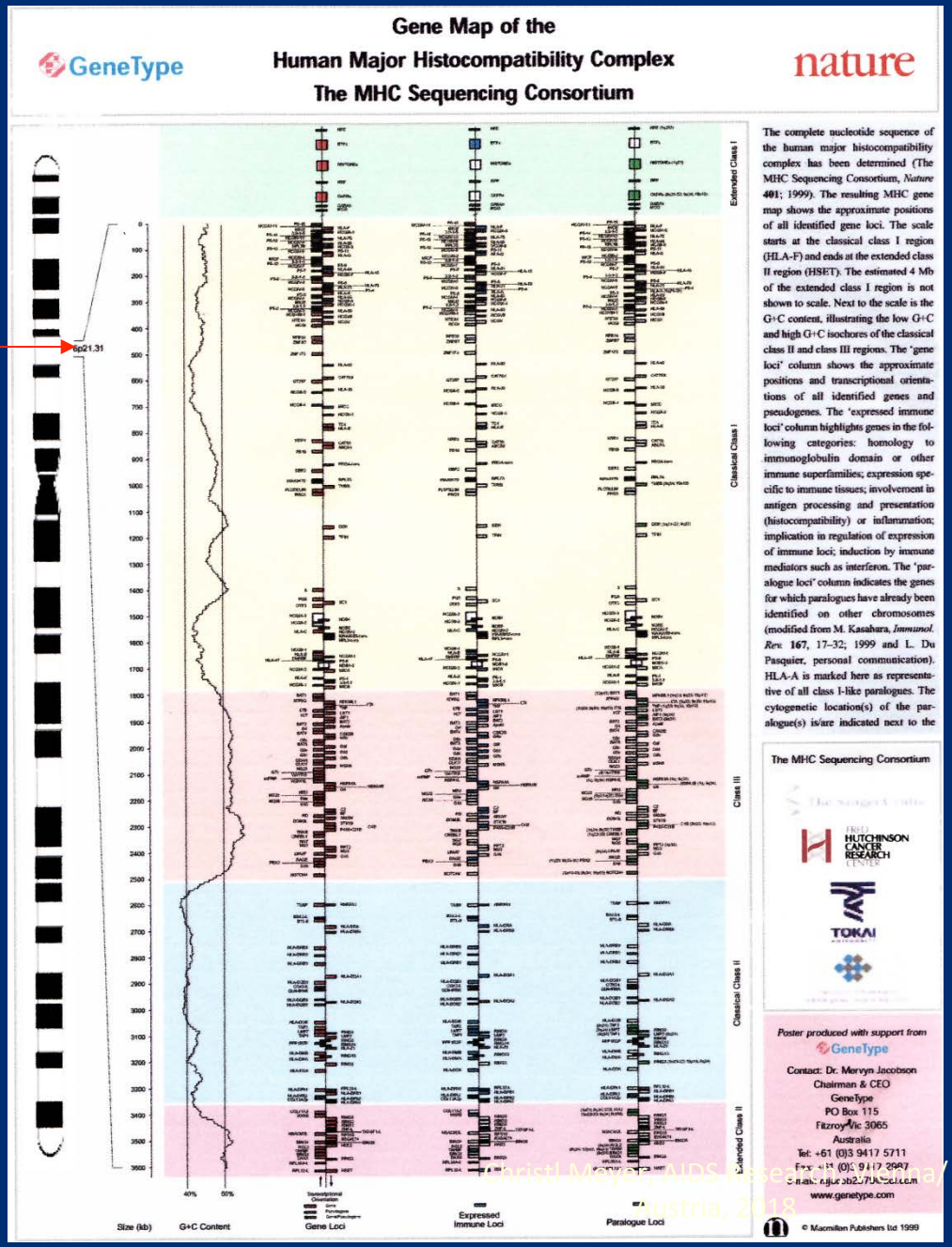
HLA-sequences on Chromosome 6



HLA classes


Class II
Class III
Class I
DP DQ DR Cp HSP TNF B C A
DOB
TAP2
LMP7
DPB1 R3 DMB TAP1 DQA1
DPA1 DN DMA LMP2 DQB1 DR DR
kb: 0 100 200 300 400 500 600 700 800 900 1000
Functional (closed boxes) and non-functional DRB loci on common haplotypes
DR1
DR2
DR3,5,6
DR4,7,9
DR8
B1
B5
B2
B3
B4
DRB1, DRB5 and DQ alleles on DR2 haplotypes in Caucasians
DQB1 DQA1 DRB1 DRB5
2 0102 1501 0101
0601 0103 1502 0102
0502 0102 160x 020x

6p21.31


Gene Map of the
Human Major Histocompatibility Complex
The MHC Sequencing Consortium
GeneType
nature
The complete nucleotide sequence of the human major histocompatibility complex has been determined (The MHC Sequencing Consortium, Nature 401; 1999). The resulting MHC gene map shows the approximate positions of all identified gene loci. The scale starts at the classical class I region (HLA-F) and ends at the extended class II region (HSET). The estimated 4 Mb of the extended class I region is not shown to scale. Next to the scale is the G+C content, illustrating the low G+C and high G+C isochores of the classical class II and class III regions. The 'gene loci' column shows the approximate positions and transcriptional orientations of all identified genes and pseudogenes. The 'expressed immune loci' column highlights genes in the following categories: homology to immunoglobulin domain or other immune superfamilies; expression specific to immune tissues; involvement in antigen processing and presentation (histocompatibility) or inflammation; implication in regulation of expression of immune loci; induction by immune mediators such as interferon. The 'paralogue loci' column indicates the genes for which paralogues have already been identified on other chromosomes (modified from M. Kasahara, Immunol. Rev. 167, 17–32; 1999 and L. Du Pasquier, personal communication). HLA-A is marked here as representative of all class I-like paralogues. The cytogenetic location(s) of the paralogue(s) is/are indicated next to the
The MHC Sequencing Consortium
Poster produced with support from GeneType
Contact: Dr. Mervyn Jacobson
Chairman & CEO
GeneType
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Fitzroy Vic 3065
Australia
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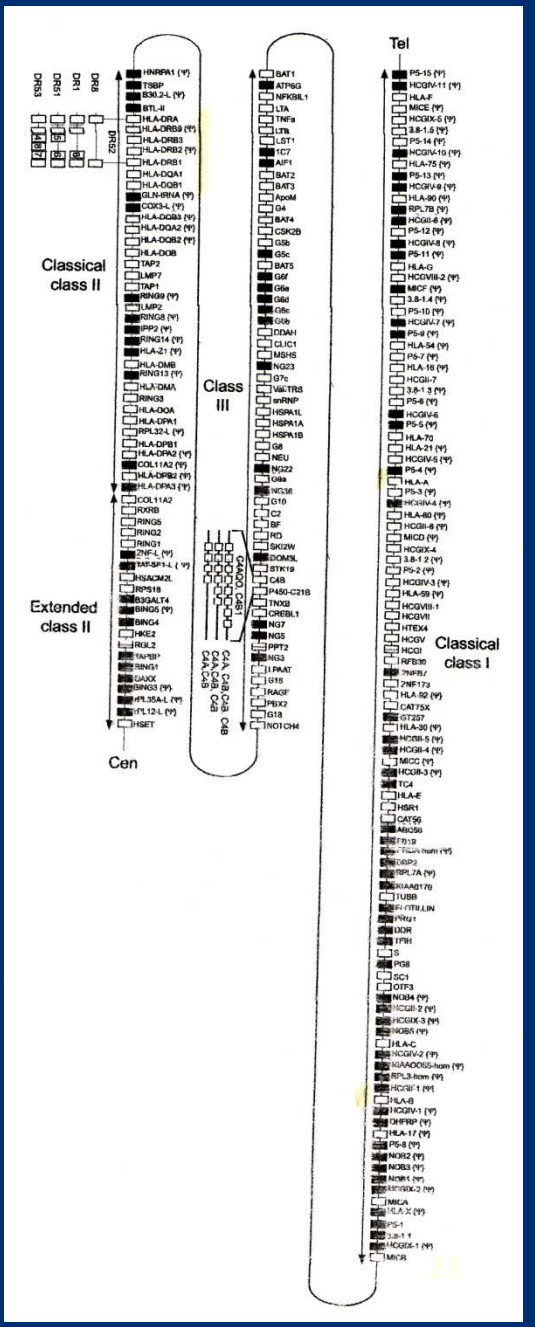
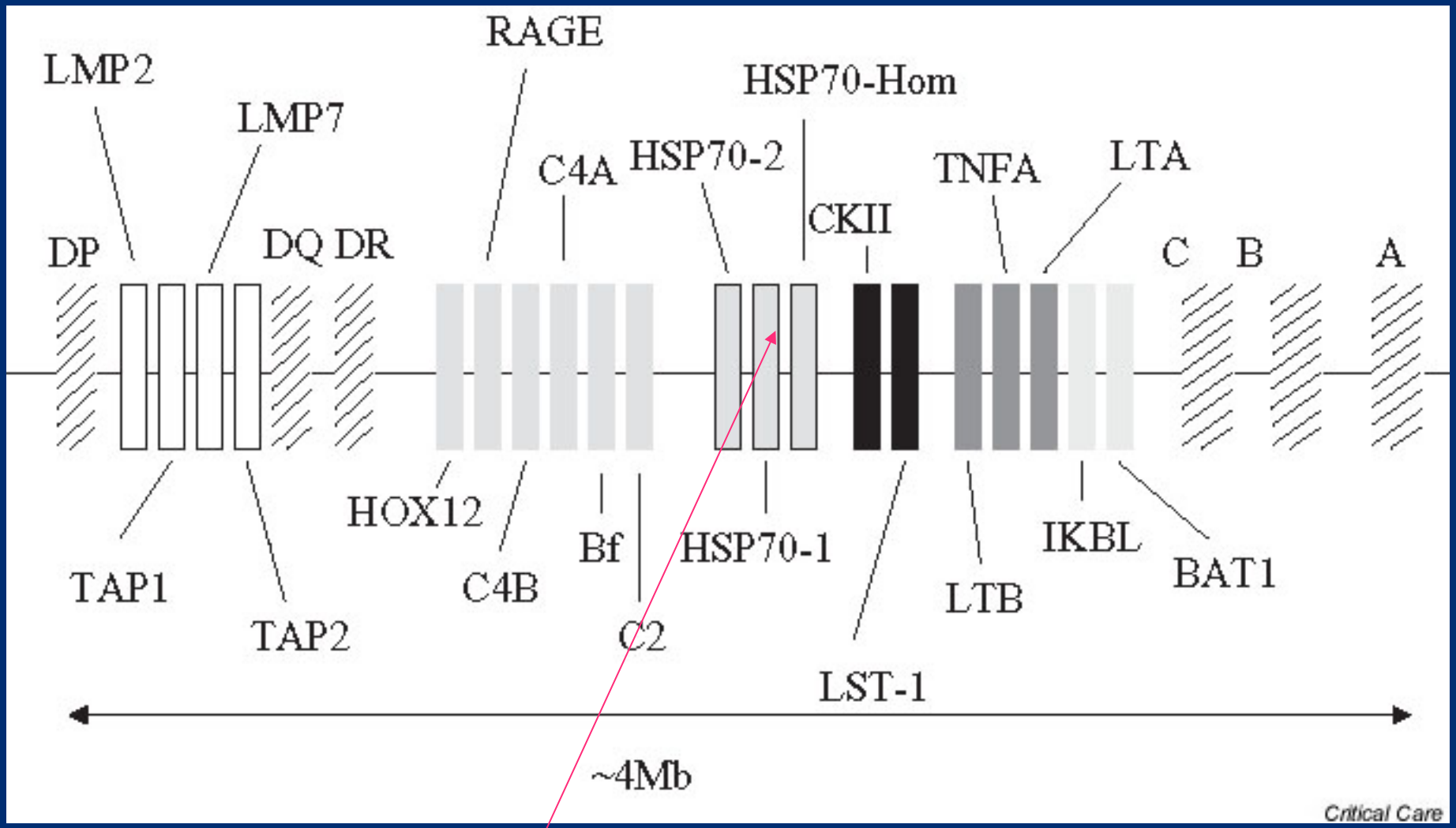
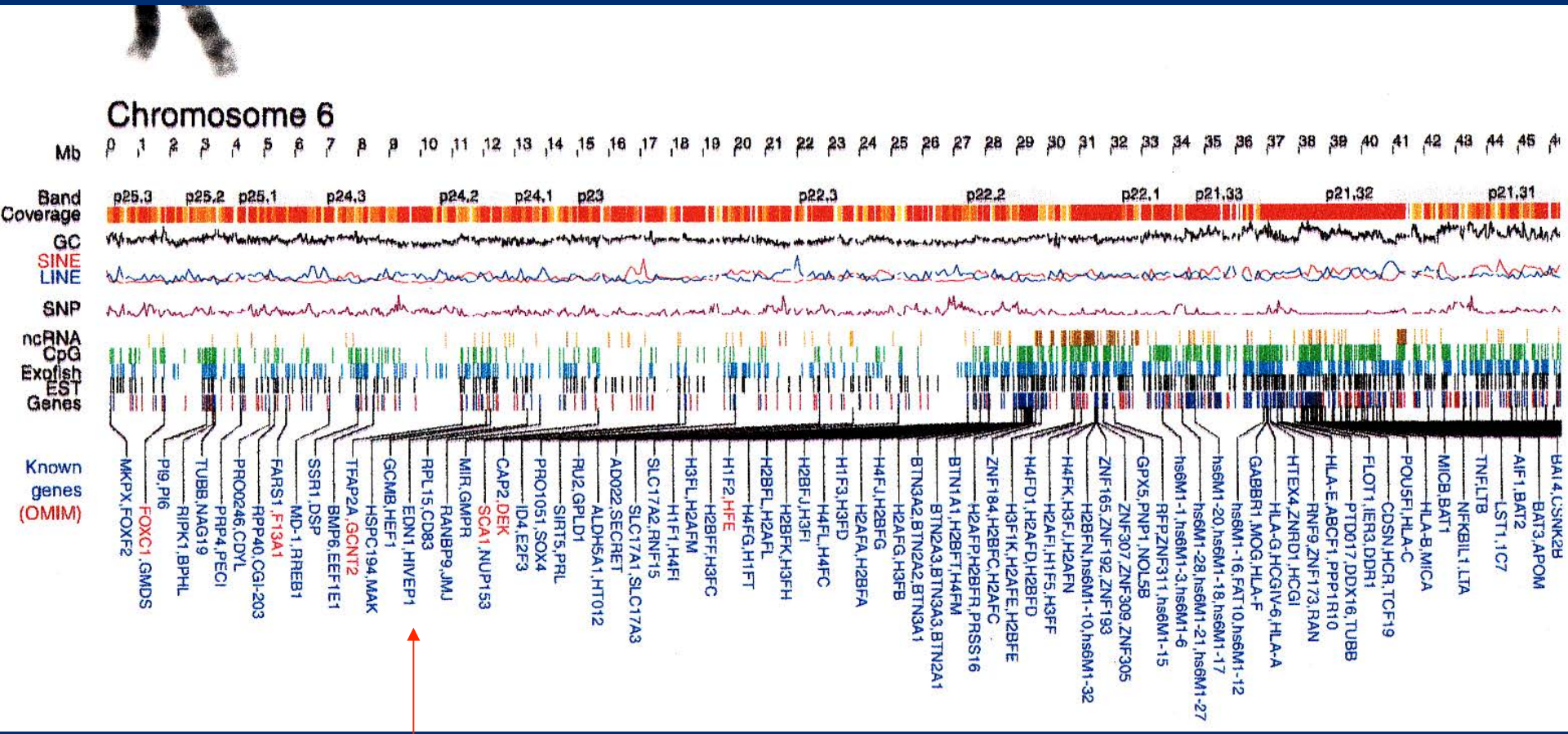
© Macmillan Publishers ltd 1999
Size (kb)
G+C Content
Gene Loci
Expressed Immune Loci
Paralogue Loci
Class I
Class III
Class II


Tel
Cen
Classical class II
Extended class II
Class III
Classical class I

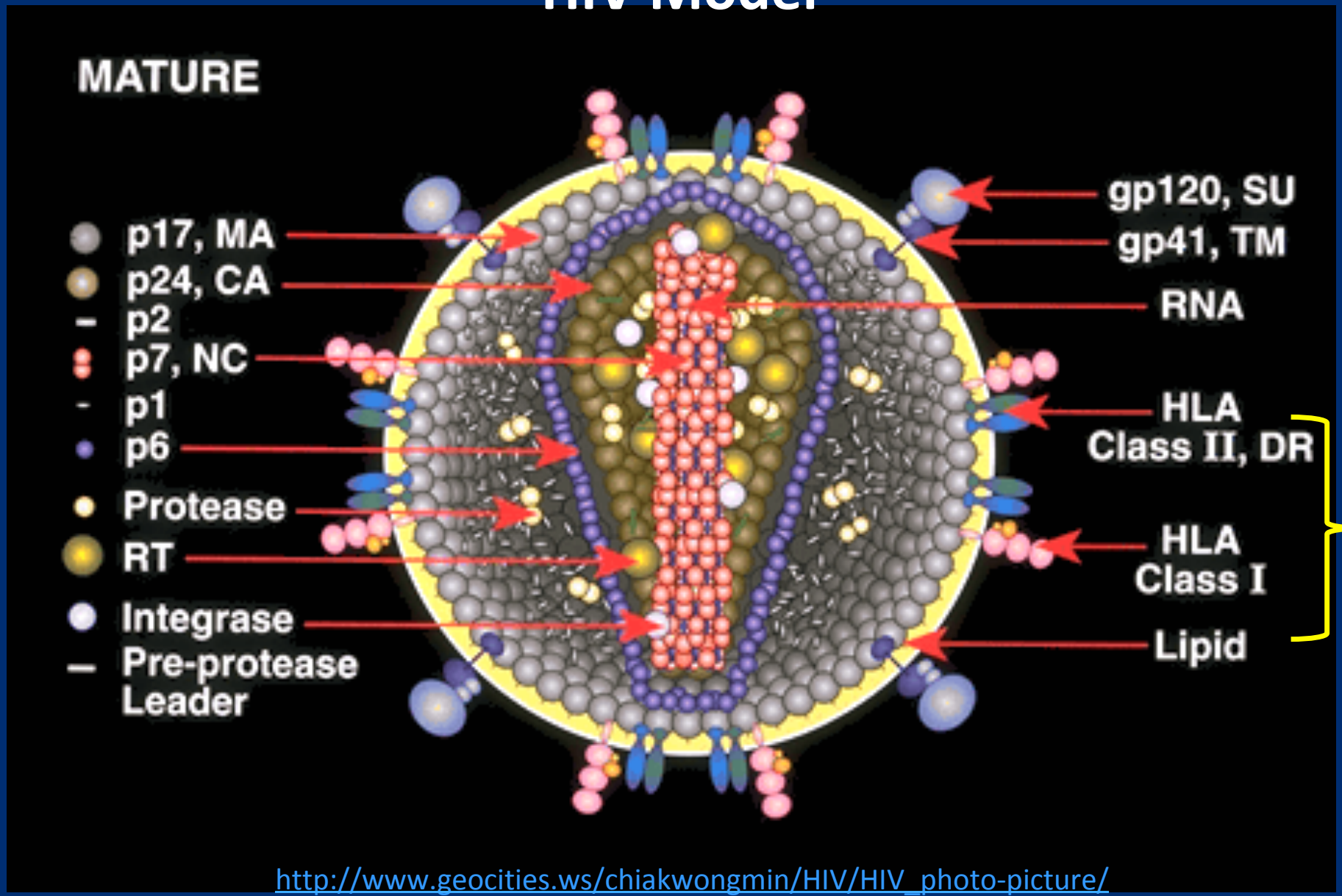

RAGE
LMP2
HSP70-Hom
LMP7
C4A
HSP70-2
TNFA
LTA
CKII
DP
DQ DR
C
B
A
HOX12
Bf
HSP70-1
IKBL
TAP1
C4B
LTB
BAT1
TAP2
C2
LST-1
~4Mb
Critical Care



HLA 6p21.3

„Virus“ binding site

HIV Model



http://www.geocities.ws/chiakwongmin/HIV/HIV_photo-picture/

Supplementary Table 1a: Full list of HLA allele-associated HIV polymorphisms in Nef

Brumme et al

Gene	Codon	HLA	Escape	Reversion
Nef	5	A11	C	
Nef	7	B57		K
Nef	7	B83	-	
Nef	8	A02		G
Nef	8	A24	M	
Nef	8	C04		L
Nef	11	A68		A
Nef	11	B57	A	V
Nef	11	C03		K
Nef	11	C06		V
Nef	12	B40		G
Nef	14	B08	Y	
Nef	15	A31	D, T	A
Nef	15	B51		A
Nef	15	B57		A
Nef	21	A11		R
Nef	21	A33	T	
Nef	21	B46		R
Nef	21	B58	T	
Nef	23	A34	Q	
Nef	23	B13	-	
Nef	24	B54	-	
Nef	24	C02		E
Nef	24	C06		E
Nef	28	A11		D
Nef	28	B14		D
Nef	28	C05		D
Nef	28	C08		D
Nef	30	A31		V
Nef	33	A11		A
Nef	33	A68	A	V
Nef	38	B37	G	E
Nef	39	A24	K	
Nef	39	B37		R
Nef	39	B44		T
Nef	39	C06		K
Nef	40	B37	R	
Nef	43	B55	V	I
Nef	43	C03		I
Nef	45	B38		S
Nef	49	B57	P	
Nef	50	B14	T	
Nef	50	B35		T
Nef	50	B53	G	A
Nef	50	B57	E	
Nef	50	B58	D, E	A
Nef	51	B58	N	T
Nef	53	B14	P	A
Nef	53	B81	S	
Nef	53	C08	P	A
Nef	54	B14	A	D
Nef	54	C08	A	D
Nef	56	B54	C	
Nef	58	A33	V	L
Nef	61	C05		Y
Nef	62	A74	S	
Nef	65	B35		D
Nef	65	B40		E
Nef	65	B45	D	E
Nef	65	C06	D	E
Nef	71	A03	K	R
Nef	71	B07	K	R
Nef	71	B14	R	K
Nef	71	B35	R	K
Nef	71	C04	R	K
Nef	71	C07	K	R
Nef	71	C08	R	K
Nef	71	C16	R	
Nef	73	A69	R	Q
Nef	74	B45		V
Nef	76	B81		L
Nef	81	A29		F
Nef	81	A30		F
Nef	81	A32		F
Nef	81	A33		F
Nef	81	A66		F
Nef	81	B07		F
Nef	81	B13		F
Nef	81	B14	S	
Nef	81	B18		F
Nef	81	B35	F	Y
Nef	81	B40	Y	F
Nef	81	B41		F
Nef	81	B42		F
Nef	81	B44		F
Nef	81	B45		F
Nef	81	B46		F
Nef	81	B49		F
Nef	81	B52		F
Nef	81	B55		F
Nef	81	C04	F	Y
Nef	81	C16		F
Nef	82	A03	R	
Nef	82	B14		K
Nef	82	B15		R
Nef	83	A03	G	A
Nef	83	A11	G	A
Nef	83	B15	G	A
Nef	83	B40	G	A
Nef	83	B44		G
Nef	83	B55	G	A
Nef	83	C03	G	A
Nef	83	C07	A	G
Nef	85	A02		L
Nef	85	A11	L	V
Nef	85	A68	L	V
Nef	85	B07	V	L
Nef	85	B14	F, M, R	L
Nef	85	B15	L	V
Nef	85	B55	L	V
Nef	85	C03	L	V
Nef	85	C07	V	L
Nef	85	C08	F, M, R	L, V
Nef	87	B08		L
Nef	89	B14	F	H
Nef	89	C08	F	H
Nef	91	B14	I	L
Nef	91	C08	I	L
Nef	92	A11	R	K
Nef	92	B55		K
Nef	94	A01	E, N, Q	K
Nef	94	B08	E, M, N, Q	K
Nef	94	B15	K	
Nef	94	C03		N
Nef	94	C07	E, Q	K
Nef	98	B40	D	E
Nef	100	A03		I
Nef	100	B40	M	L
Nef	100	C03		L
Nef	101	B14	I	V
Nef	101	B40	I	V
Nef	101	C01	V	
Nef	101	C08	I	
Nef	102	A29	H	Y
Nef	102	B14	H	
Nef	102	B44	H	Y
Nef	102	C08	H	
Nef	102	C16	H	Y
Nef	105	A01	R	K
Nef	105	A02	K	
Nef	105	A03	Q	
Nef	105	B07	Q	
Nef	105	B08	R	K
Nef	105	B15	K	
Nef	105	B44		Q
Nef	105	B49		K
Nef	105	C07	Q, R	K
Nef	105	C16		Q
Nef	107	A30		Q
Nef	107	B13	R	Q
Nef	107	C06	R	
Nef	114	A30		I
Nef	114	B08		V
Nef	114	B13	V	I
Nef	114	B57		I
Nef	114	C06	V	I
Nef	114	C07	I	V
Nef	115	B18	H	Y
Nef	115	C07	H	Y
Nef	116	B57	N	H
Nef	116	B58		H
Nef	116	C06	N	H
Nef	120	B51	F	Y
Nef	120	C14	F	Y
Nef	125	A30	H	Q
Nef	125	B51		Q
Nef	125	C07		H
Nef	125	C14		Q
Nef	126	A26	S	
Nef	126	B51	C	N
Nef	126	C14	C	N
Nef	133	A24	T	I
Nef	133	A26		T
Nef	133	B35	T	I
Nef	133	B38	I	T
Nef	133	B57	I	T
Nef	133	C02	P	
Nef	135	A02	Y	F
Nef	135	A03	Y	F
Nef	135	A11		F
Nef	135	A24	F	Y
Nef	135	C01	F	Y
Nef	139	A24	L	
Nef	143	A23	Y	F
Nef	143	B58		F
Nef	150	B51		Q
Nef	151	B39		E
Nef	151	B41	S	
Nef	153	A02	I	V
Nef	153	B37		I
Nef	156	A30	D	
Nef	158	B40		E
Nef	161	B15		D
Nef	163	C16		C
Nef	168	A31		I
Nef	168	C06	L	
Nef	169	B18		C
Nef	173	A03	T	
Nef	173	B18	I	M
Nef	174	B40	D	E
Nef	175	B44		S
Nef	176	B44	T	
Nef	177	C12		E
Nef	178	B40	R	K
Nef	182	A68		I
Nef	182	A69	Q	
Nef	182	B18		V
Nef	182	B27		E
Nef	182	B37		V
Nef	182	C03	K	
Nef	182	C06		V
Nef	184	A02		K
Nef	184	A36	E	K
Nef	184	B14	K	R
Nef	184	B27		R
Nef	184	B57	R	K
Nef	187	B39		S
Nef	188	A31		R
Nef	188	B27	H	R
Nef	188	C16	R	
Nef	191	B07		Y
Nef	191	B14		F
Nef	191	C08		F
Nef	192	B39	R	H
Nef	194	A01		R
Nef	194	A31	M	V
Nef	194	B08	M	V
Nef	194	B35	V	M
Nef	194	B48		M
Nef	194	C04	V	
Nef	194	C06	V	
Nef	196	A02	R	
Nef	196	A31	K	R
Nef	198	B15	L	
Nef	198	B35		Q
Nef	198	B57	M	L
Nef	198	C04		Q
Nef	198	C14	K, Q	L
Nef	201	A33		E
Nef	201	B58		E
Nef	202	B50		Y
Nef	206	C02		C
Nef	206	C03	*	C
Nef	206	C07	C	

Supplementary Table 1b: Full list of HLA allele-associated HIV polymorphisms in Protease, Reverse Transcriptase and VPR

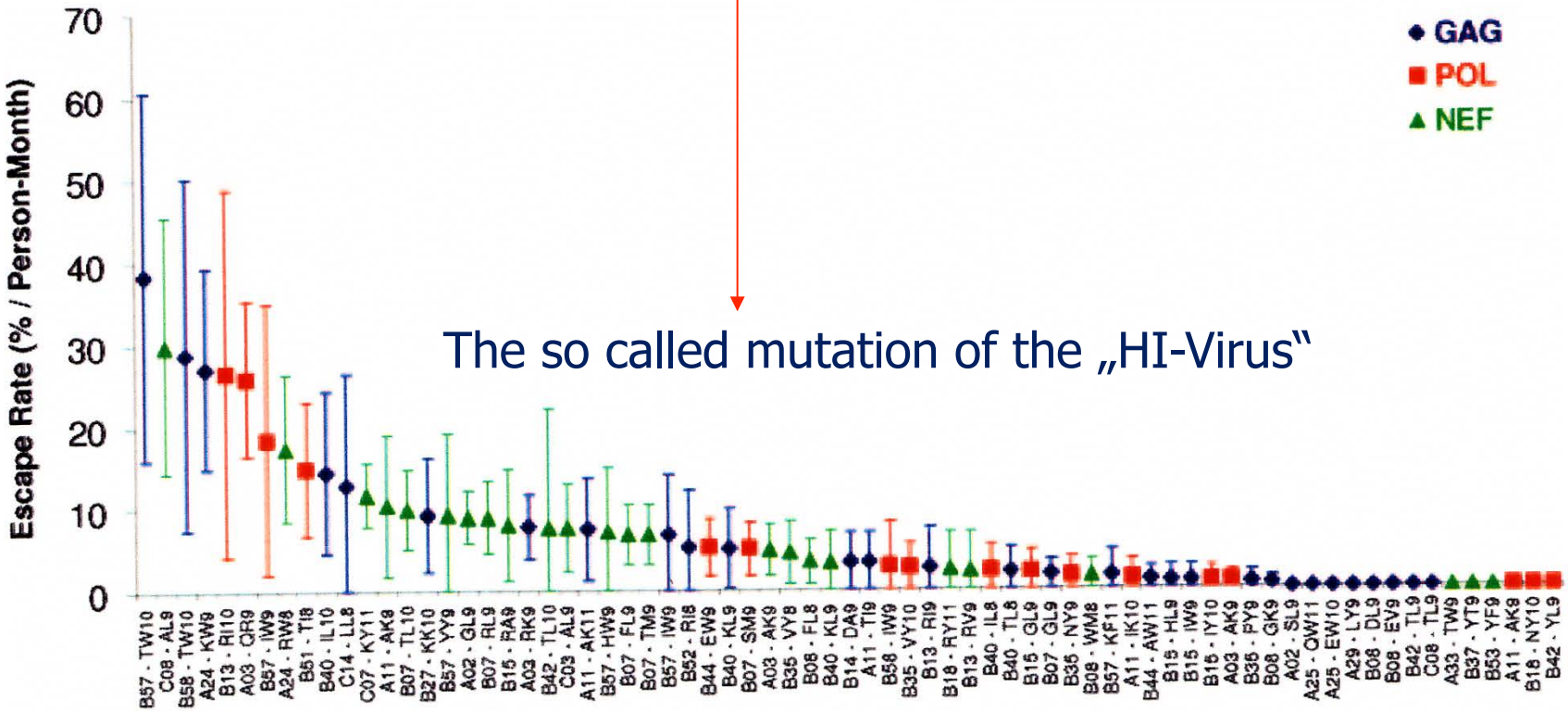
Gene	Codon	HLA	Escape	Reversion
PR	10	B15	I	L
PR	12	B51		T
PR	12	B52	A	T
PR	14	A68	R	K
PR	14	B51	R	K
PR	14	C14	R	K
PR	14	C15	R	K
PR	15	B51	V	I
PR	35	B44	D	E
PR	35	C05	D	E
PR	35	C16		E
PR	37	C16	S	
PR	63	B13	S	P, C
PR	64	B13	M	
PR	93	B15	L	I

Gene	Codon	HLA	Escape	Reversion
RT	11	B40	R	K
RT	11	C03	R	K
RT	35	B57		I
RT	102	B48	R	K
RT	102	C08	R	
RT	123	B35	E	D
RT	123	B44	D	E
RT	123	C04		D
RT	135	A02		V
RT	135	A25		T
RT	135	A29		T
RT	135	B13		T
RT	135	B51	T	I
RT	135	B58		T
RT	135	C08		T
RT	135	C14	T	I
RT	135	C15	T	I
RT	162	B07	C	S
RT	165	B07	I	T
RT	173	C07		T
RT	174	B15	R	Q
RT	177	B35	E	D
RT	200	B08	T	
RT	200	B40	I	
RT	203	A29	D	E
RT	207	B15	E, R	Q
RT	211	B44		R
RT	245	B57	E	V
RT	245	B58	E	V
RT	245	C06		V
RT	250	B53		D
RT	275	C17		K
RT	277	A02	K	R
RT	277	A03	R	K
RT	309	A31		I
RT	321	A66		P
RT	321	B81		P
RT	329	C05		I
RT	335	C02	D	
RT	345	A11		P
RT	369	A30		T
RT	369	B13		T
RT	376	C12	S	
RT	379	B38	G	
RT	379	B58	G	S
RT	386	B53	I	T
RT	399	A32	D	E

Gene	Codon	HLA	Escape	Reversion
VPR	28	B40	H	
VPR	32	B27	K	R
VPR	32	C01	K	R
VPR	32	C02	K	R
VPR	37	B51	A	V
VPR	37	C14	T	
VPR	48	A25		E
VPR	55	A33	T	A
VPR	63	A02	T	
VPR	63	A26		I
VPR	63	B38	V	
VPR	84	A29		T
VPR	84	B50		I
VPR	84	C16		T
VPR	85	A31		Q
VPR	86	C02		Q
VPR	86	C06	P	
VPR	87	C01		R
VPR	88	C01	G	
VPR	93	A25	S	

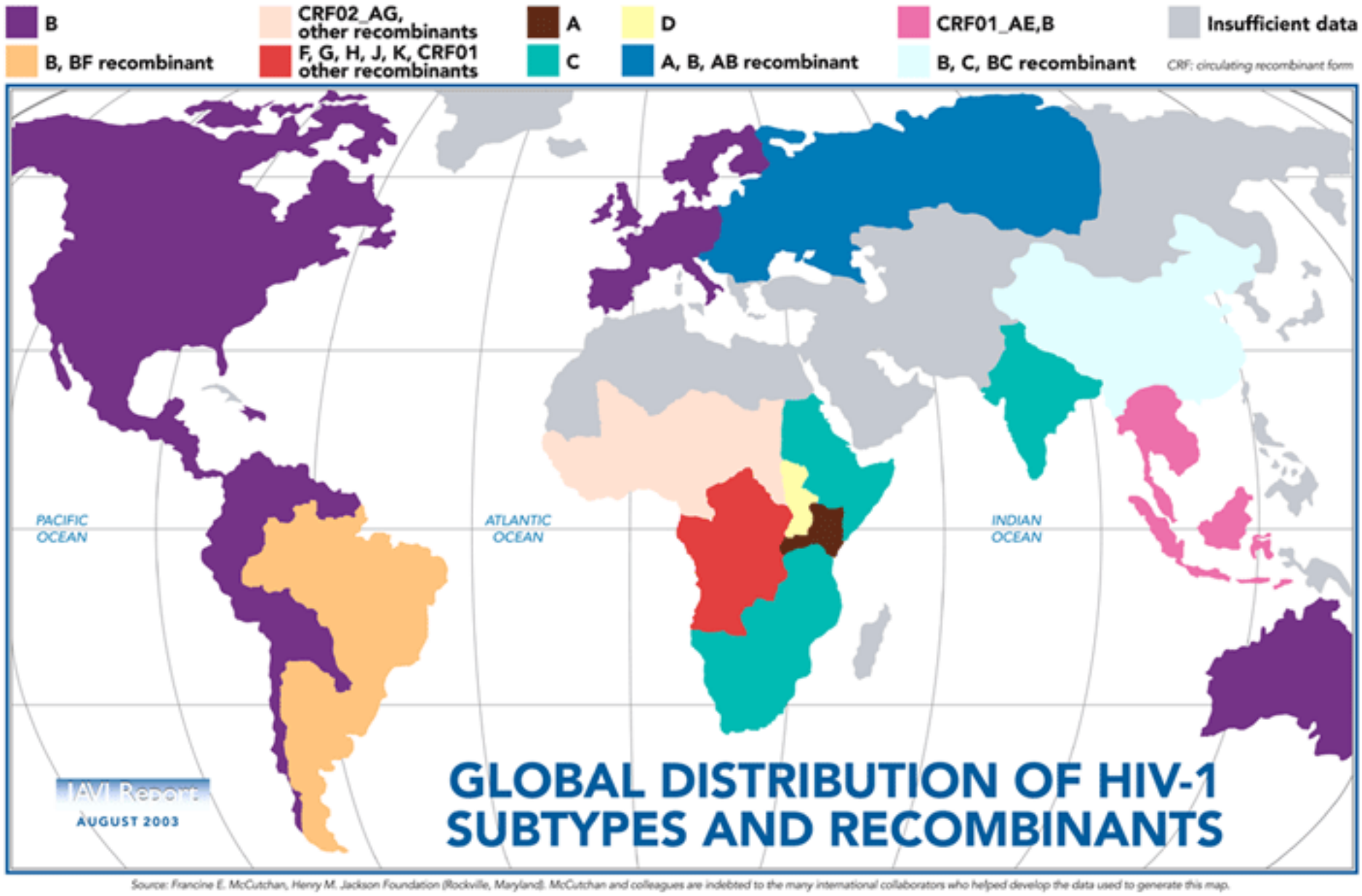
Marked Epitope- and Allele-Specific Differences in Rates of Mutation in Human Immunodeficiency Type 1 (HIV-1) Gag, Pol, and Nef Cytotoxic T-Lymphocyte Epitopes in Acute/Early HIV-1 Infection

Zabrina L. Brumme et al. *Partners AIDS Research Center, Massachusetts General Hospital, Harvard Medical School, Boston, Massachusetts*



JOURNAL OF VIROLOGY, Sept. 2008, p. 9216–9227 Vol. 82, No. 18 0022-538X/08/$08.000 doi:10.1128/JVI.01041-08

„Life has a tendency to plurality, being colourfully, to independence, simply to freedom. The totaliarian system demands monolithical unity, uniqueness and discipline.“ (Vaclav Havel, dissident and president – Charta 77 /Chech Republik)



Source: Francine E. McCutchan, Henry M. Jackson Foundation (Rockville, Maryland). McCutchan and colleagues are indebted to the many international collaborators who helped develop the data used to generate this map.


What we realize
Das, was wir sehen...
...and what
is hidden.
... und das, was
verborgen ist.

HIV can not be the cause of AIDS – it is a genetic factor in evolution

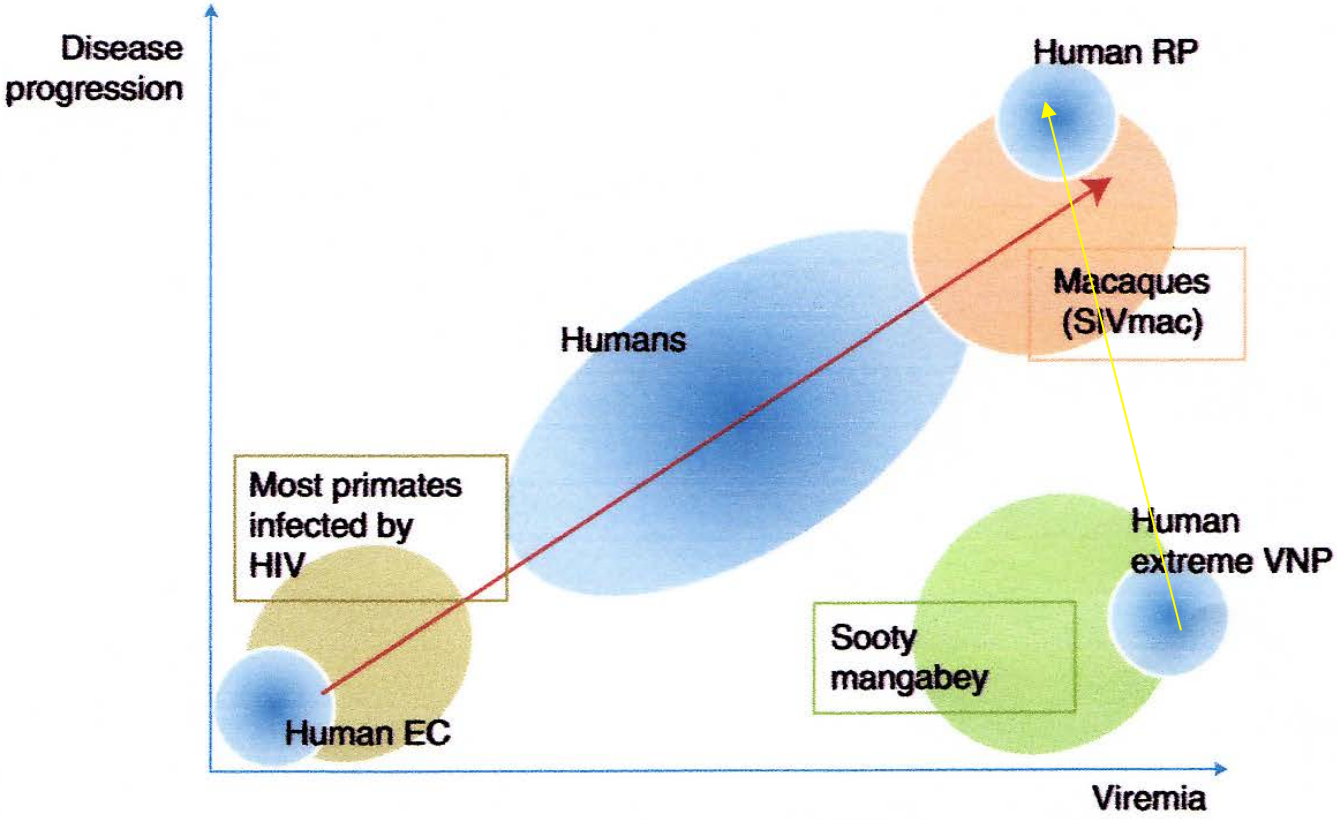


Figure 2. Schematic representation of the parallelism between human and nonhuman primate (NHP) models of HIV/SIV pathogenesis. EC, elite controllers; RP, rapid progressors; VNP, extreme viremic nonprogressors. (Adapted, with permission, from Guido Silvestri 2010.)

Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a007203

The GAG „viral protease“ is under positive selective pressure

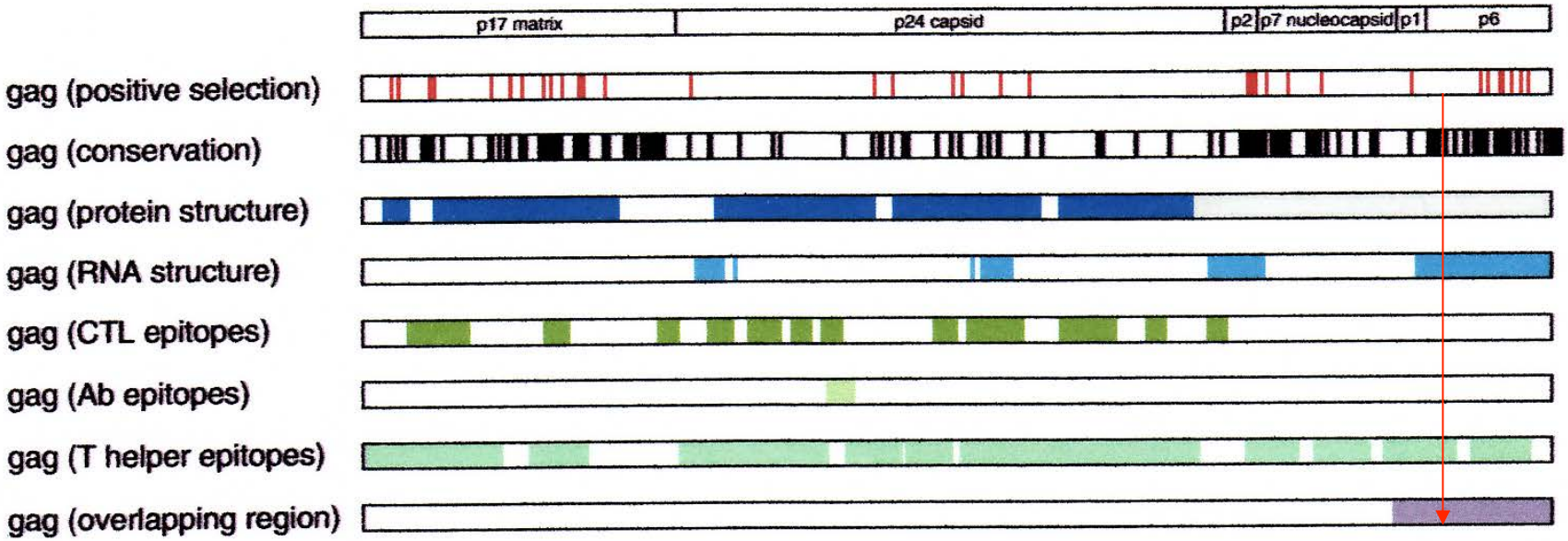


Figure 3. Multilayer representation of HIV-1 clade B Gag. The various information layers align the sites under positive selective pressure (red), conservation scores (<90% conserved, black), the structured domains at the protein (dark blue), and viral RNA level (light blue) (Watts et al. 2009), the position of CTL (dark green), antibody (light green), and T helper epitopes (turquoise) compiled in the Los Alamos HIV database, and the Gag region overlapping with the viral protease (purple).

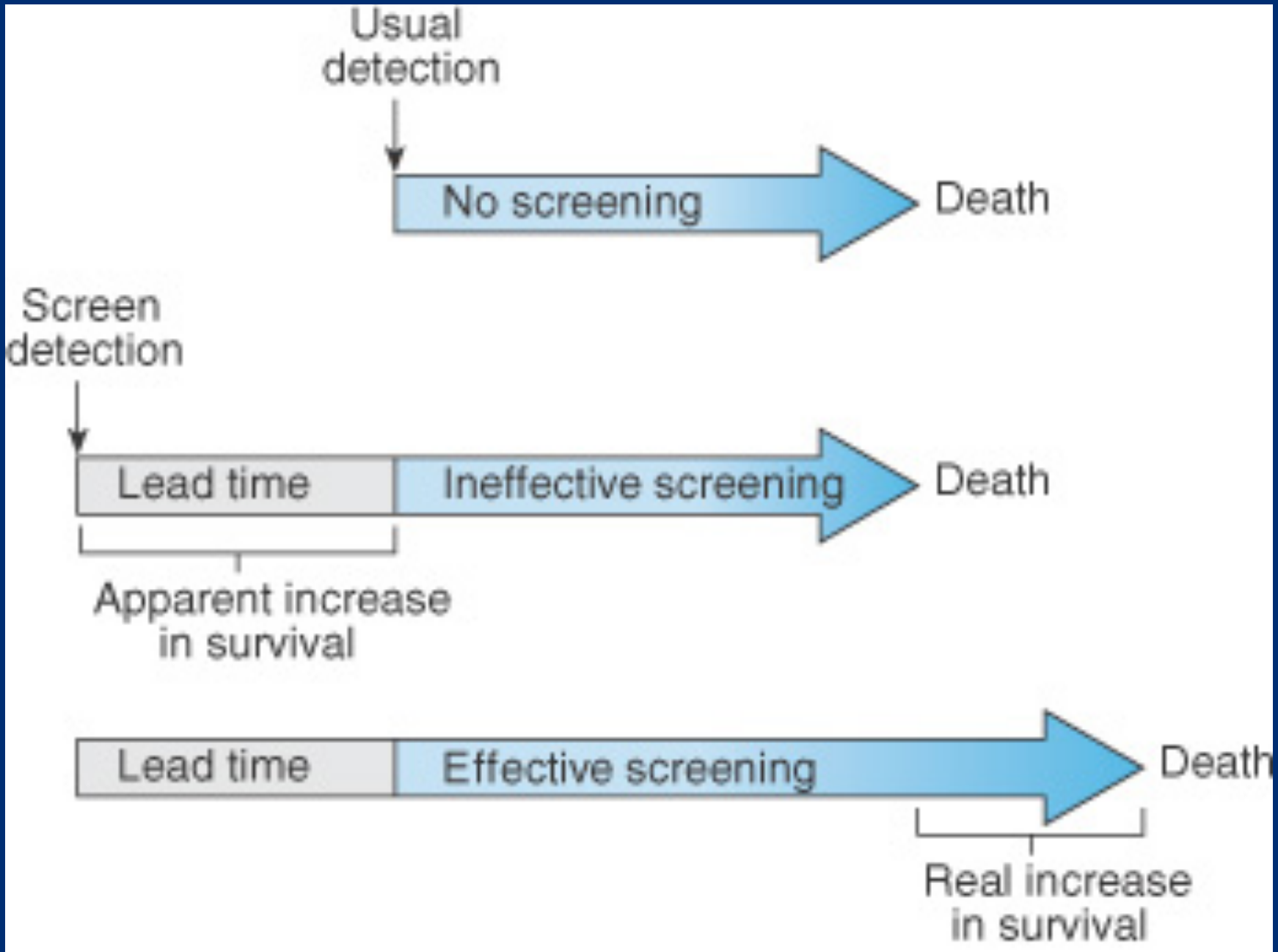
Cite this article as *Cold Spring Harb Perspect Med* 2012;2:a007203

„Dark matter“ of the genome may play a major biological role in cell development and metabolism including diseases like cancer.

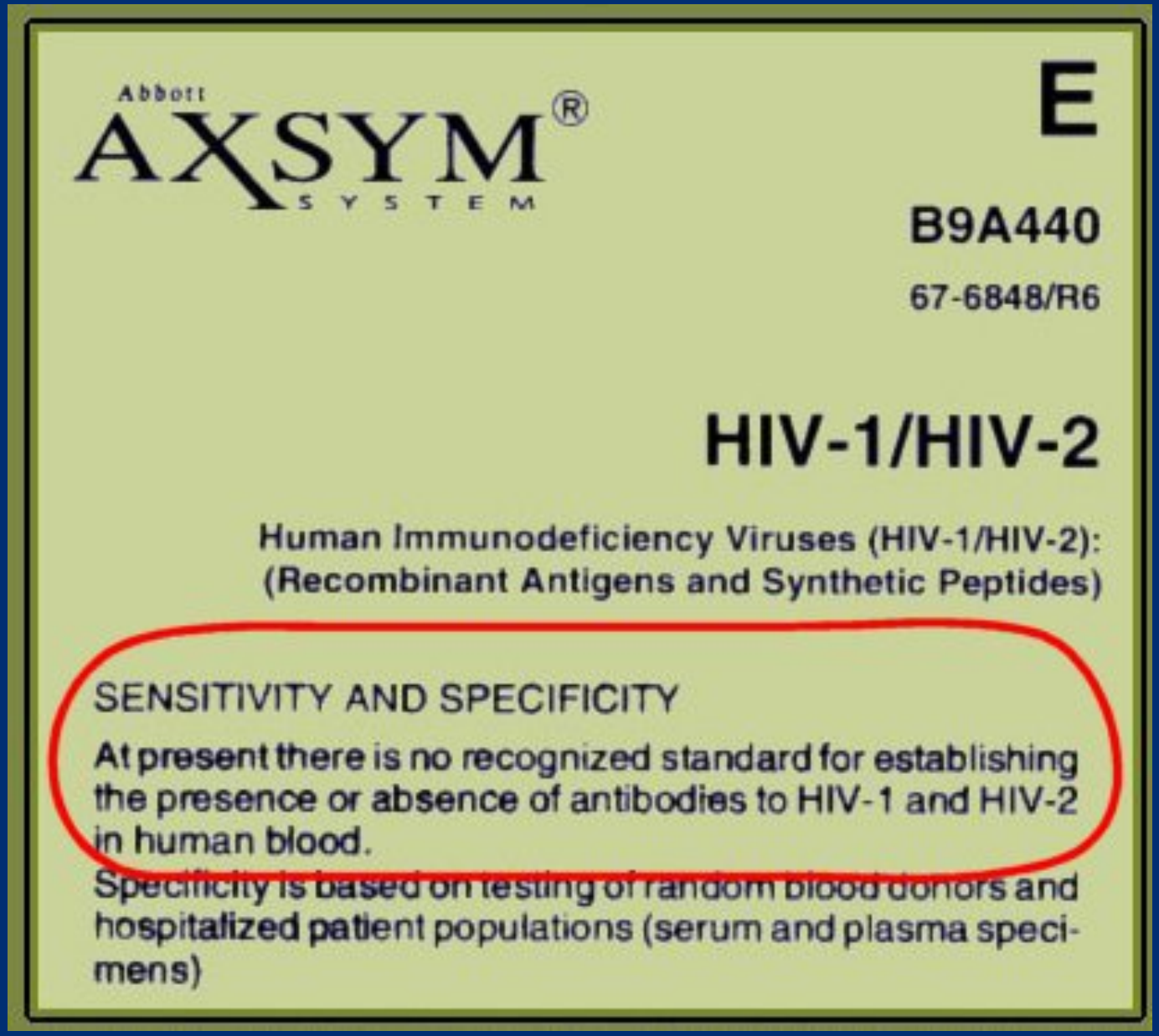
Involved are
many different
Non coding RNA genes
that have recently been detected.

Questions (concerning tests):

1. Is the test save and valid?
2. Does early detection prolonge life-span?
3. Does early detection improve life-quality?



HIV-Test Insert:



Abbott
AXSYM® SYSTEM

E

B9A440

67-6848/R6

HIV-1/HIV-2

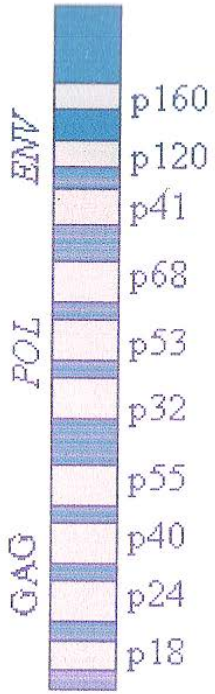
Human Immunodeficiency Viruses (HIV-1/HIV-2):
(Recombinant Antigens and Synthetic Peptides)

SENSITIVITY AND SPECIFICITY

At present there is no recognized standard for establishing the presence or absence of antibodies to HIV-1 and HIV-2 in human blood.

Specificity is based on testing of random blood donors and hospitalized patient populations (serum and plasma specimens)

Western Blot Test



AFR	AUS	FDA	RCX	CDC 1	CDC 2	CON	GER	UK	FRA	MAC
ANY 2	ANY 1	ANY 1	ANY 1	p160/ p120 AND p41	p160/ p120 OR p41	p160/ p120 OR p41	ANY 1	ANY 1	ALL 3	3 WEAK BANDS OR ANY STRONG BAND
	ANY 3 GAG OR POL	p32 AND p24	ANY 1 AND ANY 1		AND p24	p32 OR p24	ANY 1 GAG OR POL	p32 AND p24	ANY 1 OR ANY 1	

AFR = Africa; AUS = Australia; FDA = US Food and Drug Administration; RCX = US Red Cross; CDC = US Center for Disease Control; CON = US Consortium for Retrovirus Serology Standardization; GER = Germany; UK = United Kingdom; FRA = France; MACS = US Multicenter AIDS Cohort Study 1983-1992.

Source: Val Turner

What does this mean?

If you are tested HIV-positive in Germany or Africa, you can fly to Australia or France with your Western-blot test result and be claimed HIV-negative only because of the different standards that are applied.

Sayre, K. R., Dodd, R. Y., Tegtmeier, G., Layug, L., Alexander, S. S. and Busch, M. P. (1996),

False-positive human immunodeficiency virus type 1 western blot tests in noninfected blood donors.

Transfusion, 36: 45–52. doi: 10.1046/j.1537-2995.1996.36196190514.x

Low-risk blood donors *(PCR negative)* can have false-positive results on WB tests. Increased detection of env-only and p24/env-only WBs appears related to the

enhanced sensitivity of newer enzyme immunoassays to gp41 and p24 antibodies.

Article first published online: 28 FEB 2003

Keith R. Sayre, MBA, Product Manager, AIDS/Hepatitis Business Unit, **Ortho Diagnostic Systems**, Inc., Raritan, NJ.
Roger Y. Dodd, PhD, Head, Transmissible Diseases Laboratory, **American Red Cross, Rockville**, MD.
Gary Tegtmeier, PhD, Director of Research, **Community Blood Center of Greater Kansas City**, Kansas City, MO.
Lynne Layug, MPH, MT(ASCP), Technical Officer, National Reference Laboratory for Infectious Disease, **American Red Cross,** Rockville, MD.
Steve S. Alexander, PhD, Principal Scientist, AIDS/Hepatitis Research and Development, Ortho Diagnostic Systems.
*6Michael P. Busch, Associate Professor in Residence, Department of Laboratory Medicine, **University of California**, San Francisco; and **Vice President, Research and Scientific Services, Irwin Memorial Blood Centers, 270 Masonic Avenue, San Francisco, CA 94118-4496.**

The diagnosis (prognosis) of a serious disease can lead to disease and death – even if the person was not sick before.

This is called the NOCEBO-effect!

The contrary – the PLACEBO-effect is scientifically better examined and proven.

"Combivir is classified by the FDA as a pregnancy category C drug. Pregnancy category C means that animal studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans"

http://www.aidsmeds.com/archive/Combivir_1083.shtml

"RPD [rapid disease progression] was three times more likely to occur in infants born to [AZT] treated mothers- compared with findings in untreated mothers."

de Souza RS et al. Effect of prenatal zidovudine on disease progression in perinatally HIV-1-infected infants. J Acquir Immune Defic Syndr. 2000 Jun 1;24(2):154-161

Comparisons of Causes of Death and Mortality Rates Among HIV-Infected Persons: Analysis of the Pre-, Early, and Late HAART (Highly Active Antiretroviral Therapy) Eras

Crum, Nancy F MD, MPH[*†]; Riffenburgh, Robert H PhD[‡]; Wegner, Scott MD[†§]; Agan, Brian K MD[† //]; Tasker, Sybil A MD[†¶]; Spooner, Katherine M MD[†#]; Armstrong, Adam W DO[†**]; Fraser, Susan MD[††]; Wallace, Mark R MD[*†]on Behalf of the Triservice AIDS Clinical Consortium

JAIDS Journal of Acquired Immune Deficiency Syndromes:
February 1st, 2006 - Volume 41 - Issue 2 - p 194-200
doi: 10.1097/01.qai.0000179459.31562.16
Epidemiology and Social Science

Methods: Comparisons of death-related variables during the 3 eras were performed.
Results: The number of deaths declined over the study period, with 987 deaths in the pre-HAART era, 159 deaths in the early HAART era (1997-1999), and 78 deaths in the late HAART era (2000-2003) ($P < 0.01$). The annual death rate peaked in 1995 (10.3 per 100 patients) and then declined to <2 deaths per 100 persons in the late HAART era ($P < 0.01$). The proportion of deaths attributable to infection decreased, but infection remained the leading cause of death in our cohort, followed by cancer. Of those who died, there was an increasing proportion of non-HIV-related deaths (32% vs. 9%; $P < 0.01$), including cardiac disease (22% vs. 8%; $P < 0.01$) and trauma (8% vs. 2%; $P = 0.01$) in the post-HAART versus pre-HAART era. Despite the absence of intravenous drug use and the low prevalence of hepatitis C coinfection in our cohort, an increasing proportion of deaths in the HAART era were attributable to liver disease, although the numbers are small.

„Medical error—the third leading cause of death in the US“, British Medical Journal (3.5.2016).

Martin Makary from Johns Hopkins University School of Medicine in Baltimore.

PERSPECTIVE

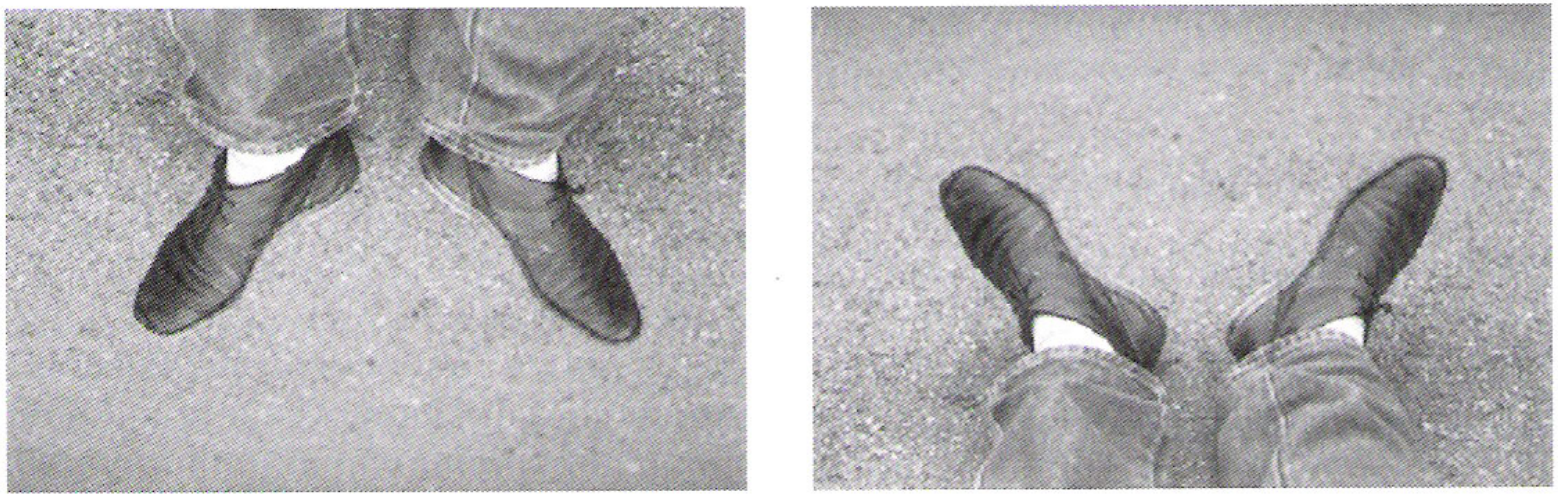


Figure 1.1
The problem with perspective is that you need to know where you stand. ('Ottawa shoes' Patrick Brennan 1989. Reproduced with kind permission of the artist.)

In medical history decision making was paternalistic. Replaced by patient autonomy. This shift is exemplified in the legal requirement of

informed consent **to treatment.**

Shared decision making is now the ideal.

But:

The patient is the ultimative and authoritative decision maker**, because he or she alone determines what will be done to his or her body and how this action will affect his or her life.**

Chemical Substances (Medications) can lead to Mutations !

Mutation frequency in ribavirin-treated poliovirus populations

Population	G to A mutations	C to T mutations	Total mutation frequency[a]
Normal population	0.5	1.2	2.1
100 μM Ribavirin	—	1.3	2.5
400 μM Ribavirin	4.4	5.0	9.3
1000 μM Ribavirin	6.8	12.0	20.8

[a] Mutations per 10,000 nt sequenced (reprinted with permission from Crotty et al., 2001)

HAART drives Evolution

of

HLA / HIV = Human genes!

AIDS. 2012 Jul 17;26(11):F21-9. doi: 10.1097/QAD.0b013e328355fe8f.

Abacavir induces loading of novel self-peptides into HLA-B*57: 01: an autoimmune model for HLA-associated drug hypersensitivity.

Norcross MA, Luo S, Lu L, Boyne MT, Gomarteli M, Rennels AD, Woodcock J, Margulies DH, McMurtrey C, Vernon S, Hildebrand WH, Buchli R.

Source

Laboratory of Immunology, Division of Therapeutic Proteins, Office of Biotechnology Products, Center for Drug Evaluation and Research, Food and Drug Administration, Bethesda, MD 20892, USA. Michael.norcross@fda.hhs.gov

CONCLUSION:

Our results support a model of drug-induced autoimmunity in which abacavir alters the quantity and quality of self-peptide loading into HLA-B57:01. Drug-induced loading of novel self-peptides into HLA, possibly by abacavir either altering the binding cleft or modifying the peptide-loading complex, generates an array of neo-antigen peptides that drive polyclonal T-cell autoimmune responses and multiorgan systemic toxicity.

Chem Immunol Allergy. 2012;97:18-31. doi: 10.1159/000335613. Epub 2012 May 3.

Genetics and the potential for predictive tests in adverse drug reactions.

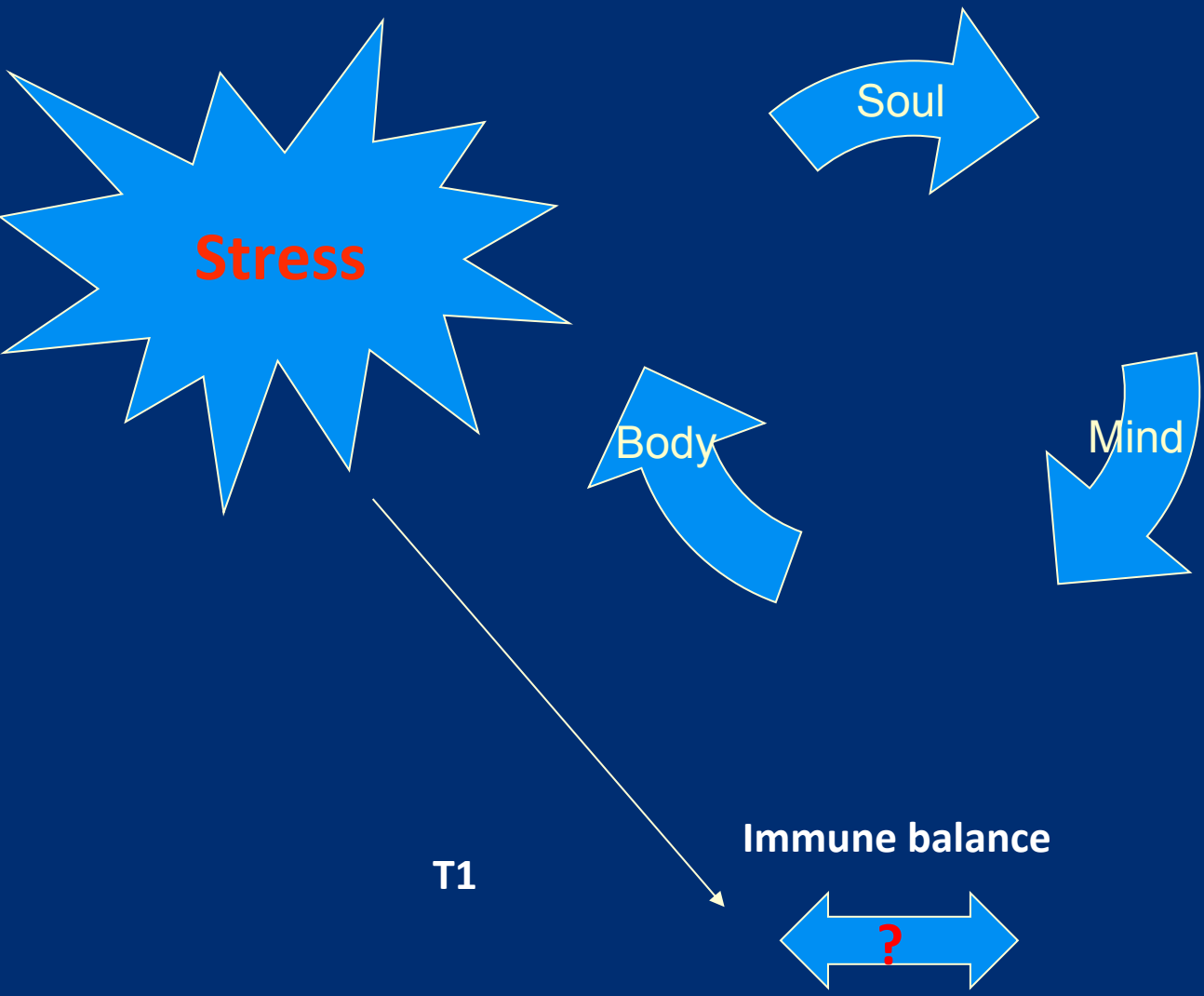
Pirmohamed M. The Wolfson Centre for Personalised Medicine, Department of Molecular and Clinical Pharmacology, University of Liverpool, Liverpool, UK. munirp@liv.ac.ukAbstract

Drug hypersensitivity reactions are an immune-mediated reaction to otherwise innocuous antigens derived from drugs. These reactions can affect many different organs, with the skin being the commonest. Skin involvement can range in severity with hypersensitivity syndrome (or DRESS) and the blistering reactions (Stevens-Johnson syndrome, toxic epidermal necrolysis), also termed serious cutaneous adverse drug reactions, being the most severe and most feared. There is increasing evidence for the role of the immune system in the pathogenesis of these reactions, with drug-specific T cells having been identified in many patients.....

HLA genes as genomic biomarkers of predisposition. The 'revolution' started with abacavir where the predisposition to hypersensitivity was linked to HLA-B*57:01, which was confirmed in a clinical trial, and where its implementation has shown to reduce the incidence of hypersensitivity in a cost-effective manner. Since then, associations have also been shown for **allopurinol (HLA-B*58:01)**- and **carbamazepine (HLA-B*1502 and HLA-A*3101)**-induced serious cutaneous adverse drug reactions. The latter is interesting since the association with **HLA-B*1502 is present in certain South-Eastern Asian populations**, and the predisposition is phenotype specific (only for SJS/TEN). The utility of this biomarker has been shown in a prospective cohort study performed in Taiwan. By contrast, the association with **HLA-A*3101 is seen in more diverse ethnic groups,** and predisposes to mild as well more severe cutaneous reactions associated with carbamazepine. It is important to note that strong HLA associations have also been shown with a number of drugs that cause **liver injury** including flucloxacillin, lumiracoxib, lapatinib and ximelagatran, indicating that the immune system is also important in the pathogenesis of other forms of **drug-induced organ toxicity.**

Paracelsus:

Everything is dependent on the concentration


Stress
Soul
Body
Mind
Immune balance
T1
T2
?
cancer
cell proliferation,
lack of apoptosis
antibody production,
T-cell decline
„AIDS“

A symbiotic interaction that originates from the genes of the immune system leads to communication particles (exo- and endosomes) that act differently within the immune system.

The whole cell-communication in and between cells of an organism depends on the activity of molecules like RNAs, Proteins, Exo- and Endosomes, Informosomes and Clathrines, to mention just some of them.

The Journal of Immunology, 2005, 174: 4779–4788.

HIV Type 1 Can Act as an APC upon Acquisition from the Host Cell of Peptide-Loaded HLA-DR and CD86 Molecules1

Jocelyn Roy, Genevie`ve Martin, Jean-Franc¸ois Gigue`re, Dave Be´langer, Myriam Pe´trin,and Michel J. Tremblay2

The Human Microbiome Project (HMiP) tries to specify the genes of the microbes living on
and in our bodies.
We have 10-fold more microbes than we have cells in a human being.

Evolution is accelerated by (ancient) lateral (horizontal) gene transfer from microbes to their hosts.

Humans underwent an evolutionary adaption process which was influenced by geography. This resulted in differences including the immune system. People of African descent show reduced neutrophil count due to a regulatory variant.

There is also an extensive genetic diversity in the HLA class II region of Africans from Gambia and Malawi. **This diversity is twice as extensive as found in northern Europeans.**

In consequence we find differences in humoral responses between Ethiopian and Swedish persons who are claimed to be “infected” by HIV.

Doxiadis et al. state a phylogenetic evidence that supports the notion of the generation of new HLA- DRB genes as a dynamic and steadily ongoing process. This is due to the presence of indels (insertions/deletions), mainly mapping to intron.

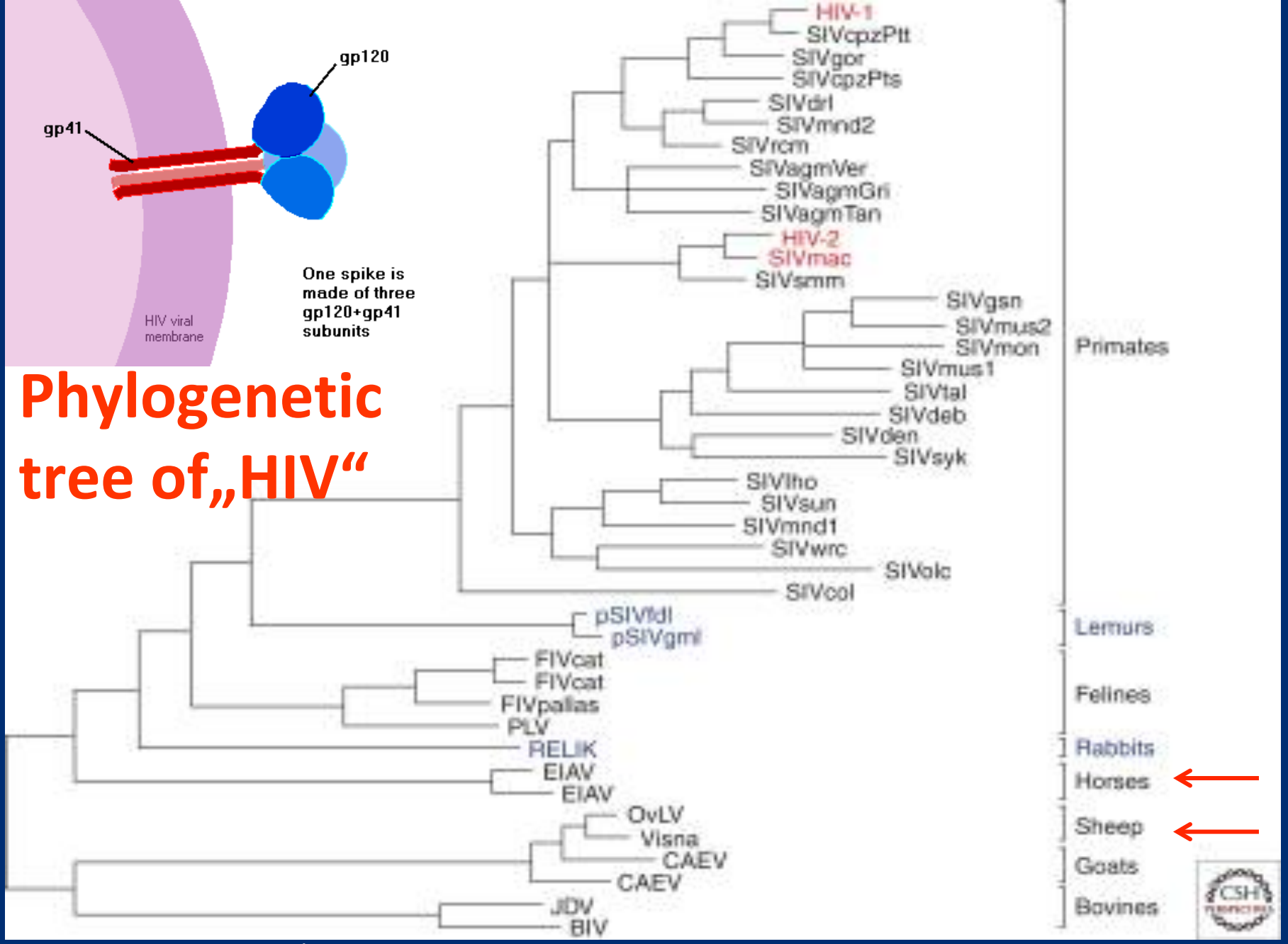
THE HIV GENOME

„ ...analysis of the proteins of the virus demands mass production and purification...

I repeat, we did not purify.“

LUC Montagnier, Pasteur Institute, July 18th 1997

From: Eleni PapadopulosEleopulos Biophysicist, Department of Medical Physics, Royal Perth Hospital, Perth, Western Australia



Phylogenetic tree of „HIV“

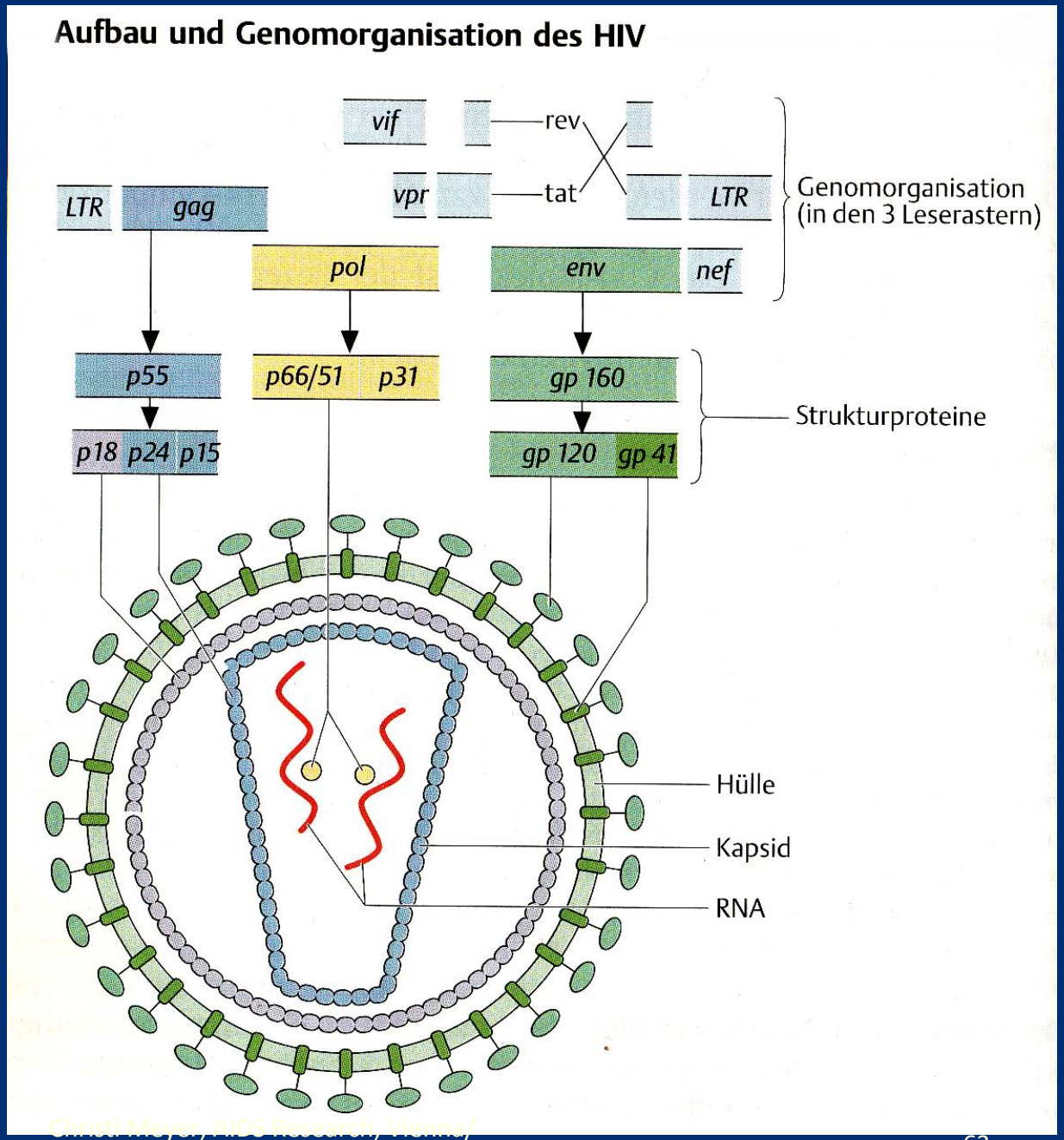
P.M. Sharp and B.H. Hahn / Cold Spring Harb Perspect Med 2011;1:a006841
www.perspectivesinmedicine.org

Conclusion:

HIV means evolutionary adapted genetic variation instead of virus mutation!

„Open Reading Frames“ Leading to variations

HIV-model



Human Genome

Nature **409**, 860-921 (15 February 2001) / doi: 10.1038/35057062;
Received 7 December 2000; Accepted 9 January 2001

Initial sequencing and analysis of the human genome

Internationbal Human Genome Sequencing Consortium Eric S. Lander...

...Here we report the results of a collaboration involving 20 groups from the United States, the United Kingdom, Japan, France, Germany and China to produce a draft sequence of the human genome.

...Hundreds of human genes appear likely to have resulted from horizontal transfer from bacteria at some point in the vertebrate lineage.

...Dozens of genes appear to have been derived from transposable elements.

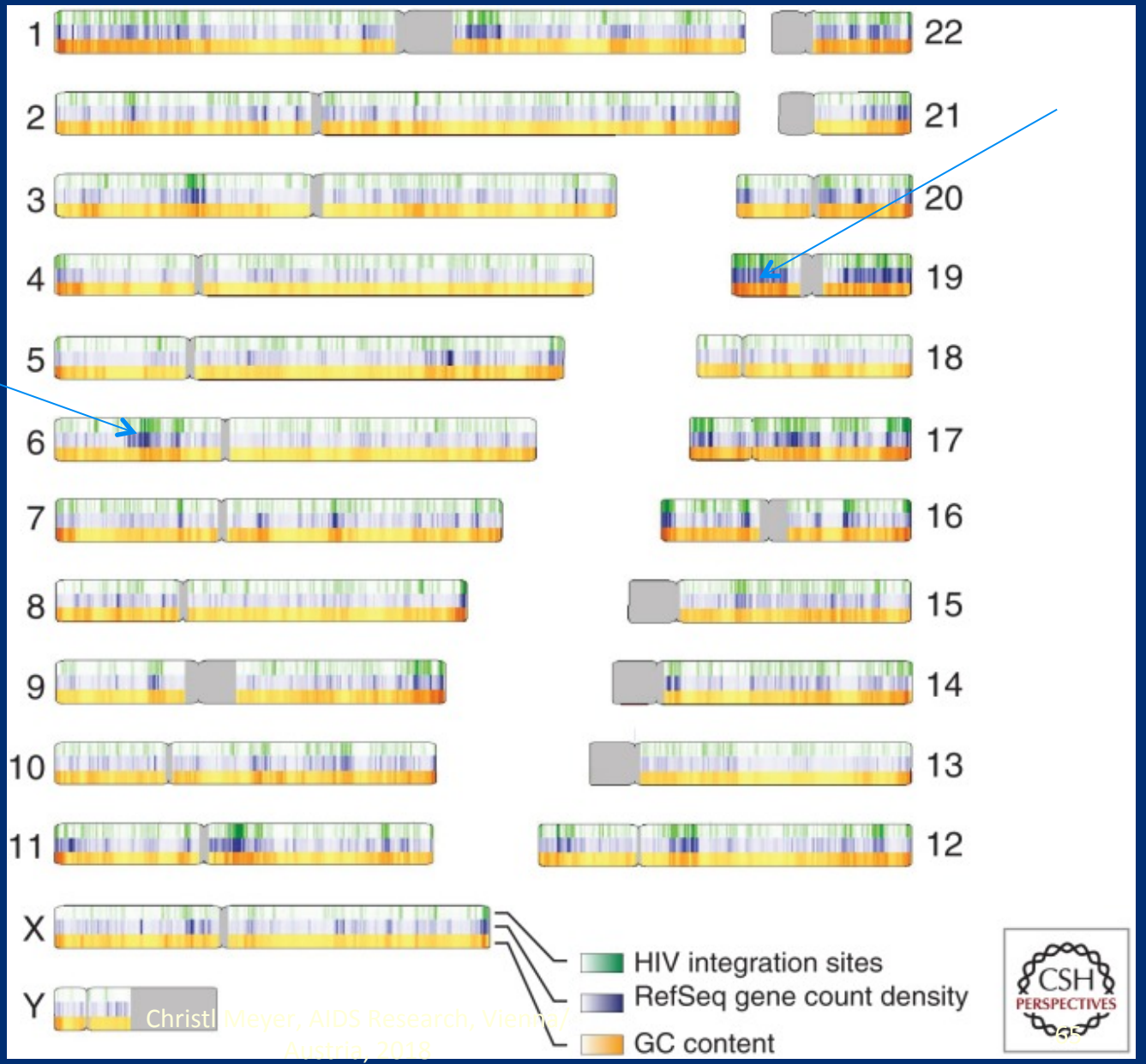
...large recent segmental duplications...much more frequent in humans...

„HIV“ is found most near genes and CG sequences / bacterial origin;

Population genomics of intrapatient HIV-1 evolution

Zanini et al. eLife 2015;4:e11282. DOI: 10.7554/eLife.11282

https://www.sciencedirect.com/science/article/pii/S0092867402008644



MHC of the vertebrate immune system:

- Non-random distribution
- Increased gene density in the MHC/HLA

Crisp, A et al.
Expression of multiple horizontally acquired genes is a hallmark of both vertebrate and invertebrate genomes. Genome Biology; 12 March 2015

Transcriptional analysis for host factors required by HIV-1 was performed by RNA interference. More than 250 HIV-dependency factors were identified. These proteins participate in cellular functions. Transcriptional analysis revealed that these genes were enriched for high expression in immune cells.

Jeremias et al. claim that human semen is both an inducer of an anti-inflammatory TH2 immune response and an inhibitor of TH1 cell mediated immunity.

Why?

This protects the male genes in the fetus.

HIV is a natural product in sperms which has its origin in the HLA and protects the fetus from maternal rejection of paternal antigens by shifting T1 to T2.
Heterosexual transmission of HIV is only suggested with additional pathogens in STDs.
Homosexual transmission is due to rejection of alloantigens.
Allogeneic immunity protects from infection but can be related to allergies also in the offspring.

Thus HIV positivity means an allergic reaction!

Multiple interactions in cell communication are proved concerning HIV, specifically in GALT (gut associated lymphoid tissue) which makes sense for protecting the body from strange invaders from nutrition!

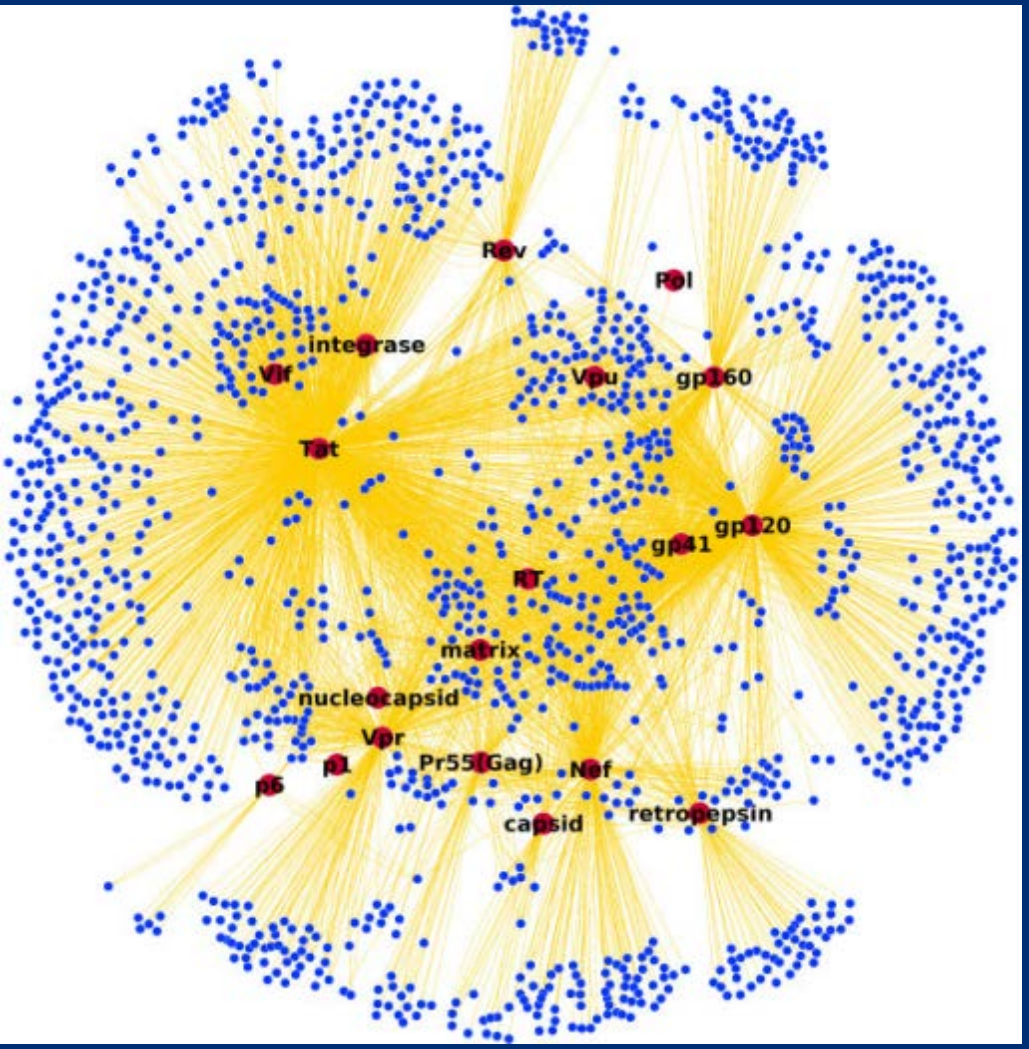
retrotransposon-mediated diversity.

https://openi.nlm.nih.gov/detailedresult.php?img=PMC2913931_1752-0509-4-96-1&req=4

Figure 1: HIV-Human protein interaction network.

19 HIV proteins that interact with 1452 human proteins through 3959 interactions.

Blue nodes are human proteins and red nodes are HIV proteins.



Gp 120 is active as a superantigen that increases Th2 related antibody production in infections as a "booster" and might be due to allergy and autoimmunity.

Stress is involved in gene expression.

„HIV" can be protective to cancer.

Medications and HAART might have different (negative) impacts on the balance of the Th1 / Th2- system.

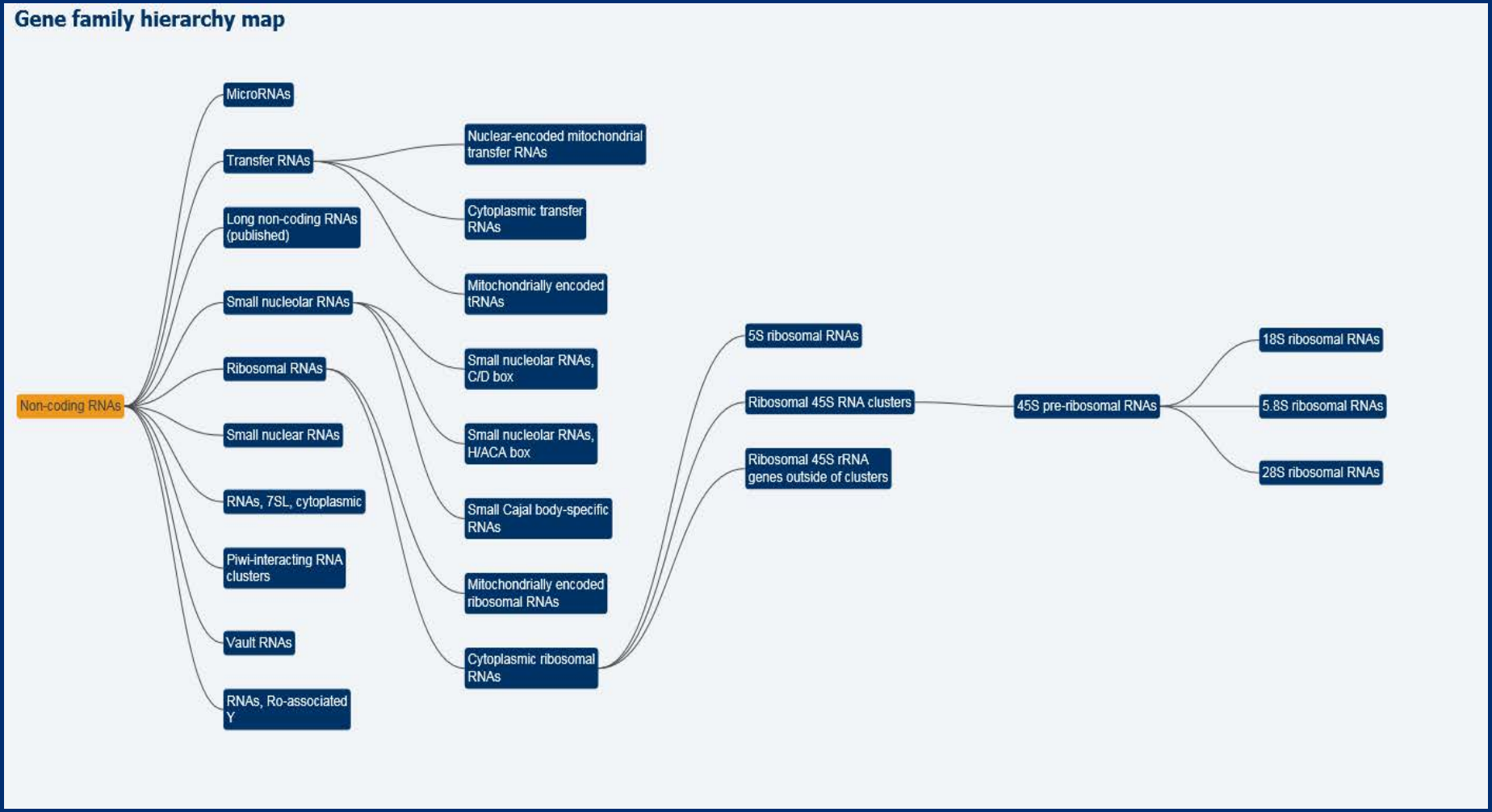
The whole cell-communication in and between cells of an organism depends on the activity of molecules like RNAs, Proteins, Exo- and Endosomes, Informosomes and Clathrines, to mention just some of them.

The Journal of Immunology, 2005, 174: 4779–4788.

HIV Type 1 Can Act as an APC upon Acquisition from the Host Cell of Peptide-Loaded HLA-DR and CD86 Molecules1

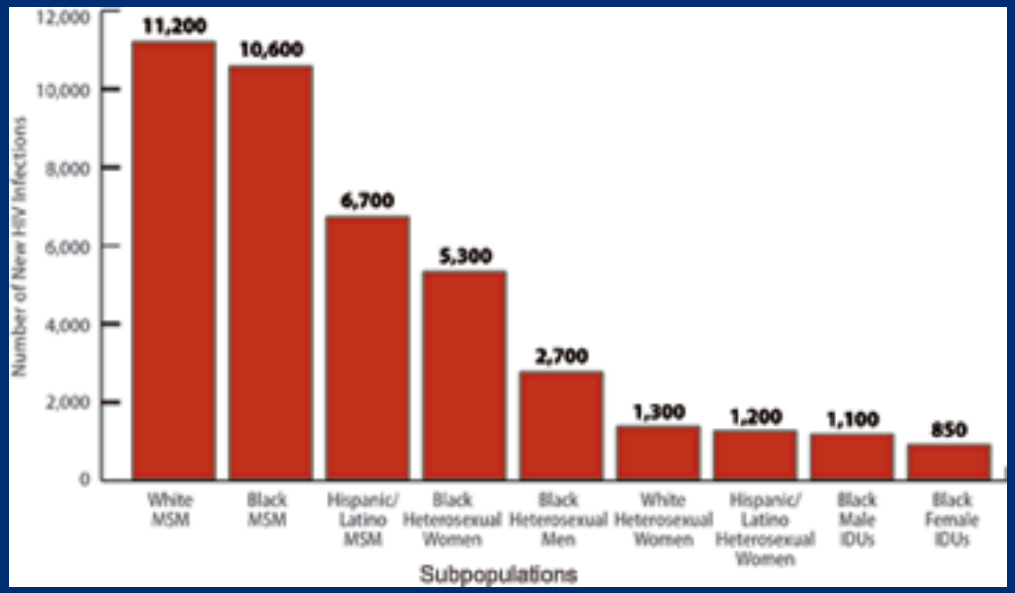
Jocelyn Roy, Genevie`ve Martin, Jean-Franc¸ois Gigue`re, Dave Be´langer, Myriam Pe´trin,and Michel J. Tremblay2

The RNA-family



African Americans are the racial/ethnic group most affected by HIV.

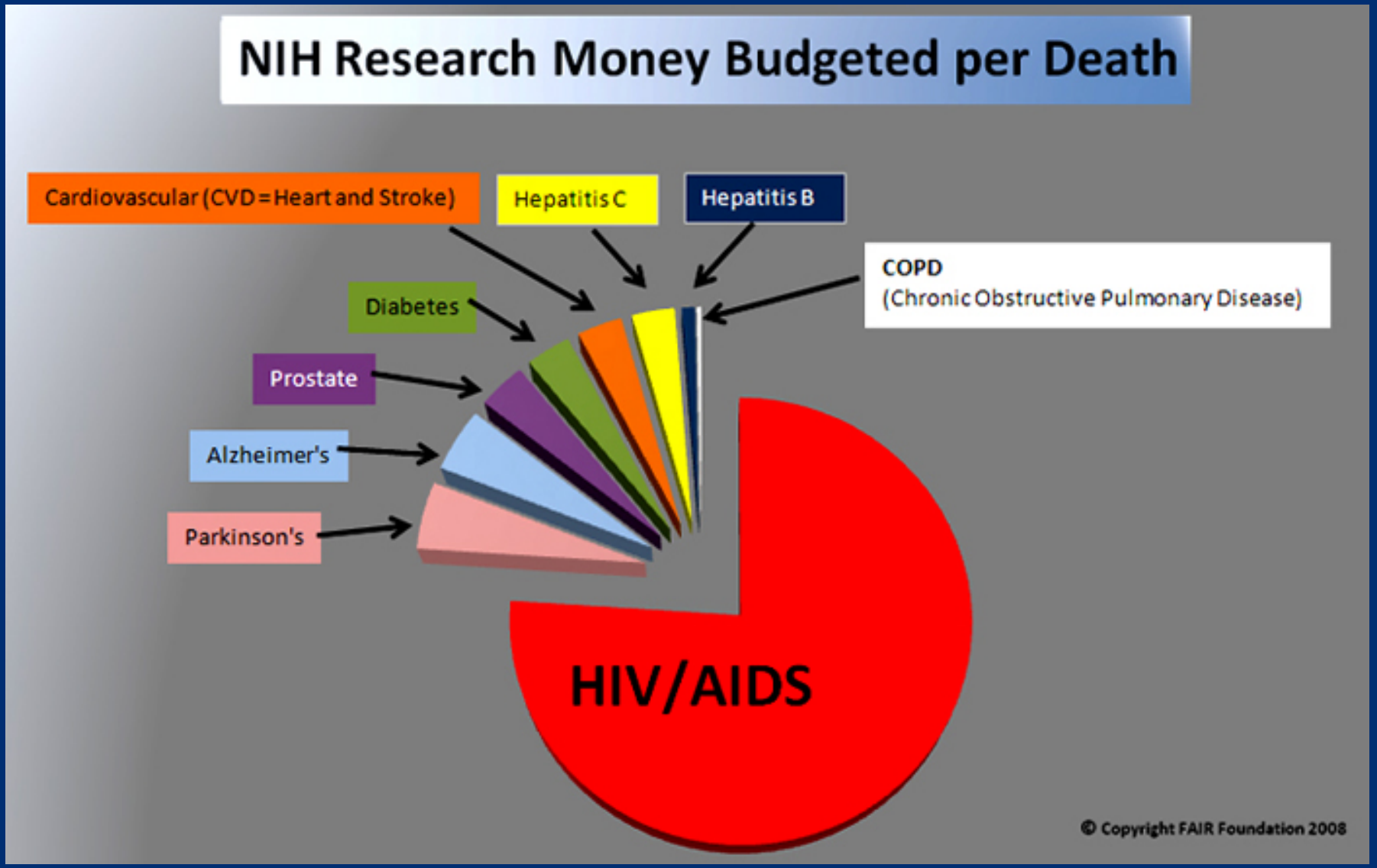
African Americans accounted for an estimated 44% of all new HIV infections among adults and adolescents (aged 13 years or older) in 2010, despite representing only 12% to14% of the US population.



Source: CDC. Estimated HIV incidence among adults and adolescents in the United States, 2007–2010. HIV Surveillance Supplemental Report 2012;17(4)

http://www.cdc.gov/hiv/topics/surveillance/resources/reports/index.htm#supplemental.

Subpopulations representing 2% or less of the overall US epidemic are not reflected in this chart. Abbreviations: MSM, men who have sex with men; IDU, injection drug user.


NIH Research Money Budgeted per Death
Cardiovascular (CVD = Heart and Stroke)
Hepatitis C
Hepatitis B
COPD
(Chronic Obstructive Pulmonary Disease)
Diabetes
Prostate
Alzheimer's
Parkinson's
HIV/AIDS
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"Combivir is classified by the FDA as a pregnancy category C drug. Pregnancy category C means that animal studies have shown an adverse effect on the fetus and there are no adequate and well-controlled studies in humans"

http://www.aidsmeds.com/archive/Combivir_1083.shtml

"RPD [rapid disease progression] was three times more likely to occur in infants born to [AZT] treated mothers- compared with findings in untreated mothers."

de Souza RS et al. Effect of prenatal zidovudine on disease progression in perinatally HIV-1-infected infants. J Acquir Immune Defic Syndr. 2000 Jun 1;24(2):154-161

The nervous and the immune system share a lot of biochemical molecules, i. e.

Hormons (Cortisol)
Transmitter (Gamma-Amino-Butyrat)
Interleukine Il 2, Il 10...)

Both systems are involved in learning processes!

The Immune-System comprises high variability and fluctuation through Transposable Elements.

It has to distinguish between SELF and NON-SELF!

Learning means to add NEW informations to knowledge!

Anergy

Anergy is the missing reaction to an antigen by shuting down the immune reaction. Anergy is a mechanism by which the immune system prevents T-cells from attacking the bodies own tissues. Anergy is a permanent characteristic. Under normal conditions it is not reactivated. Some systems are activated despite anergy by Interleukin-2.

Problems: puberty and infectious tissues (lung cells with virus-infection).

ALLOANTIGENs

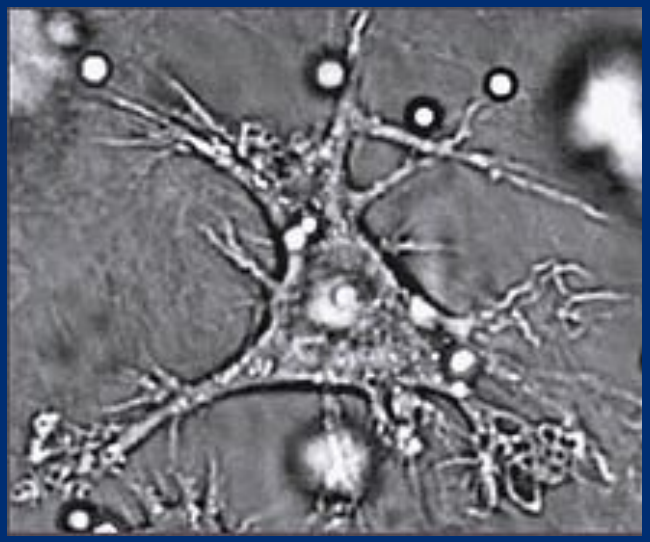
An allergic reaction against variants of molecules (glyco-proteins, nucleic acids) of the same species might occur if the variants of contact (blood-components, leucocytes, sperms) activate the immune-system.
Those people might also test „HIV-positive“

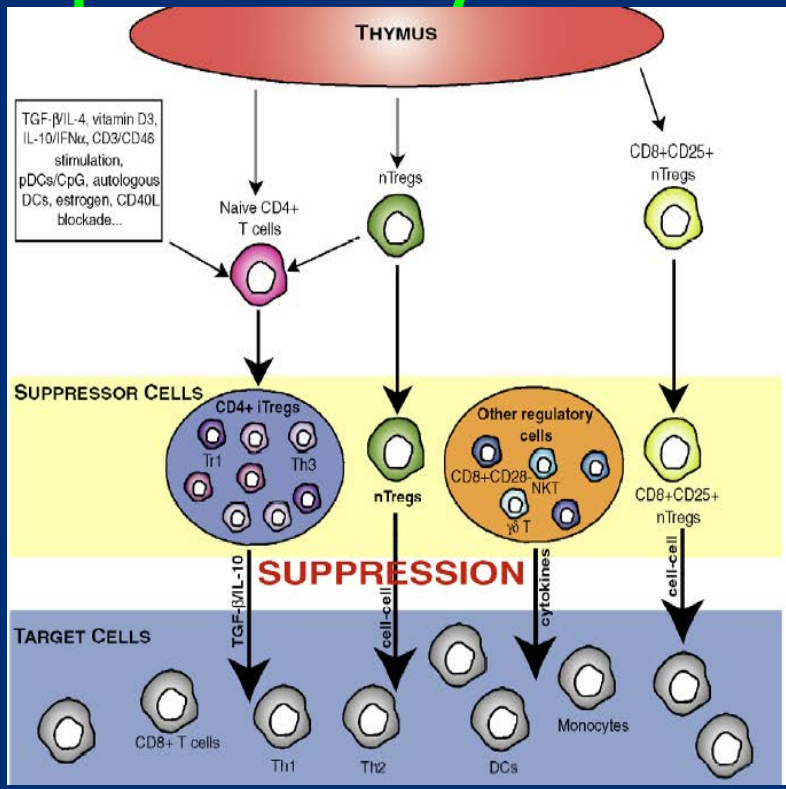
The Nobel Prize in Physiology or Medicine 2011

Bruce A. Beutler and Jules A. Hoffmann:
Discoveries concerning the Activation of Innate Immunity

Steinman : DendriticCells

Discovery of the Dendritic Cell and its Role in Adaptive Immunity: Mediating the Communication between innate and adaptive immunity.





Toll-like receptor ligands will directly alter the functions of regulatory T cells

HIV- antibodies are

- Autoantibodies as we all produce them.
- Allergy-antibodies, which are induced by molecular similarity.
- A hint for an active or
- Imbalanced immune-system (stress).
- Increased after some vaccinations.

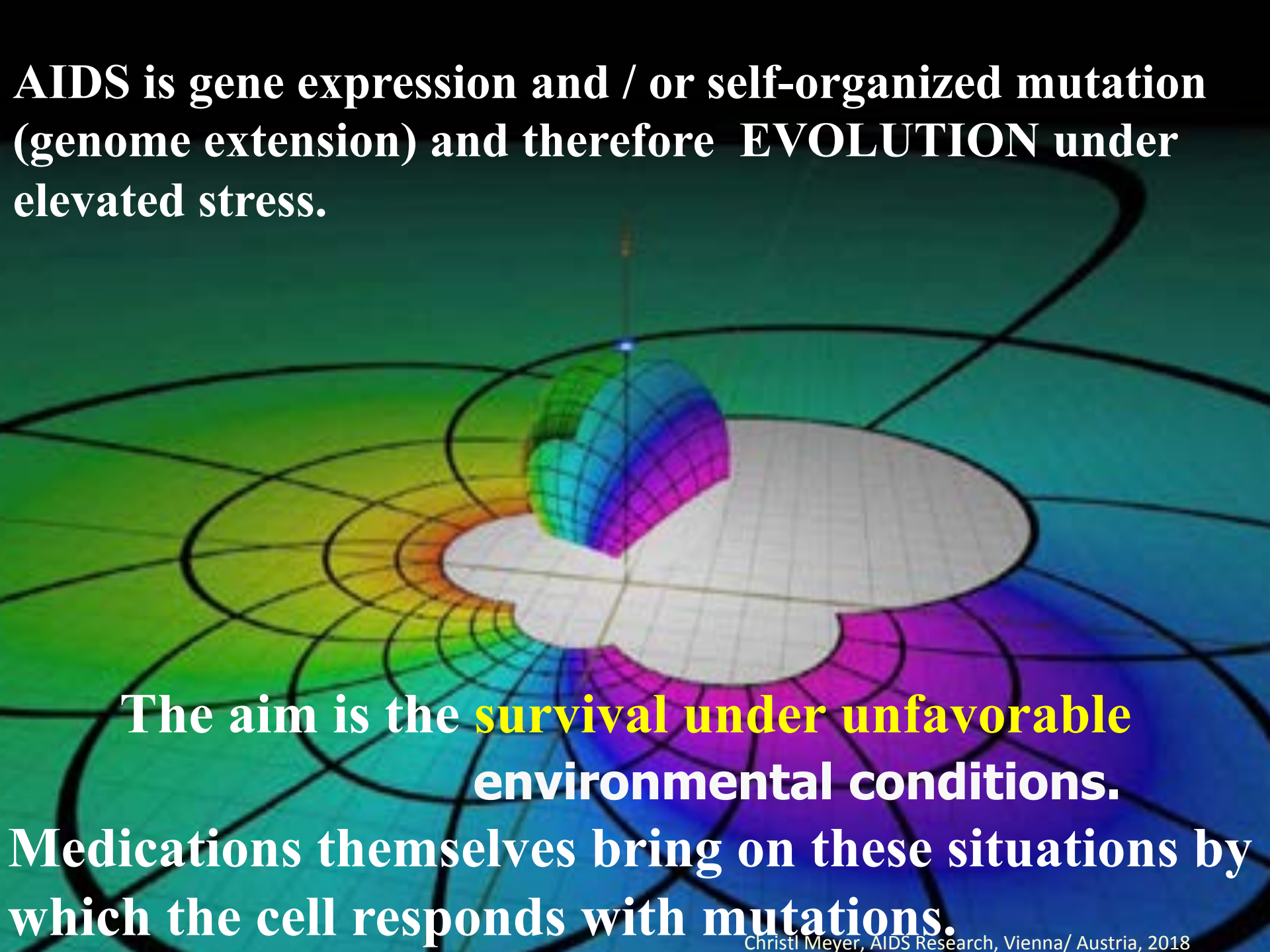
HIV – „Viruses" are

- Particels of cell-communication (Endo- and Exosomes).

The Rest belongs to other diseases or wrong diagosis!

Like hunger, malaria, tuberculosis, lung- and gut-infections, drugs.

AIDS is gene expression and / or self-organized mutation (genome extension) and therefore EVOLUTION under elevated stress.

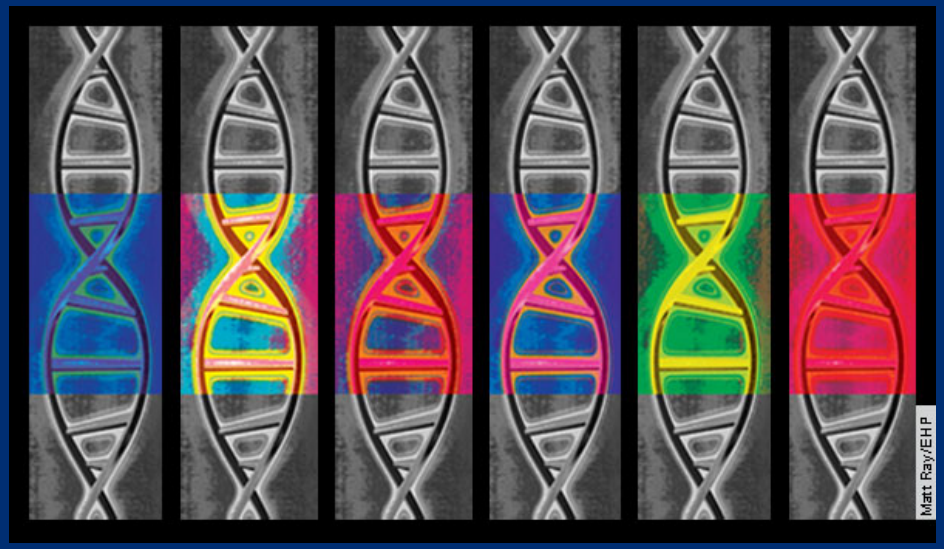


The aim is the survival under unfavorable environmental conditions.

Medications themselves bring on these situations by which the cell responds with mutations.

1. Genetics, HIV and the Immune System
2. **Epigenetics**
3. Microbes in Evolution
4. Immunization
5. What does it mean to be Human?

Epigenetics

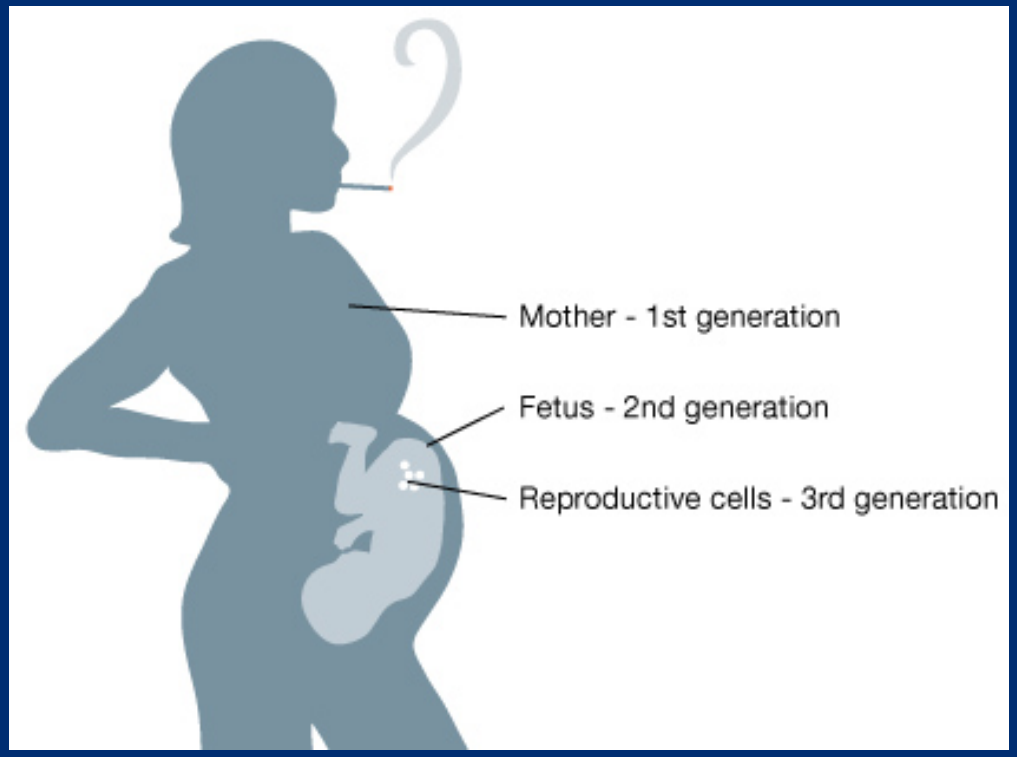


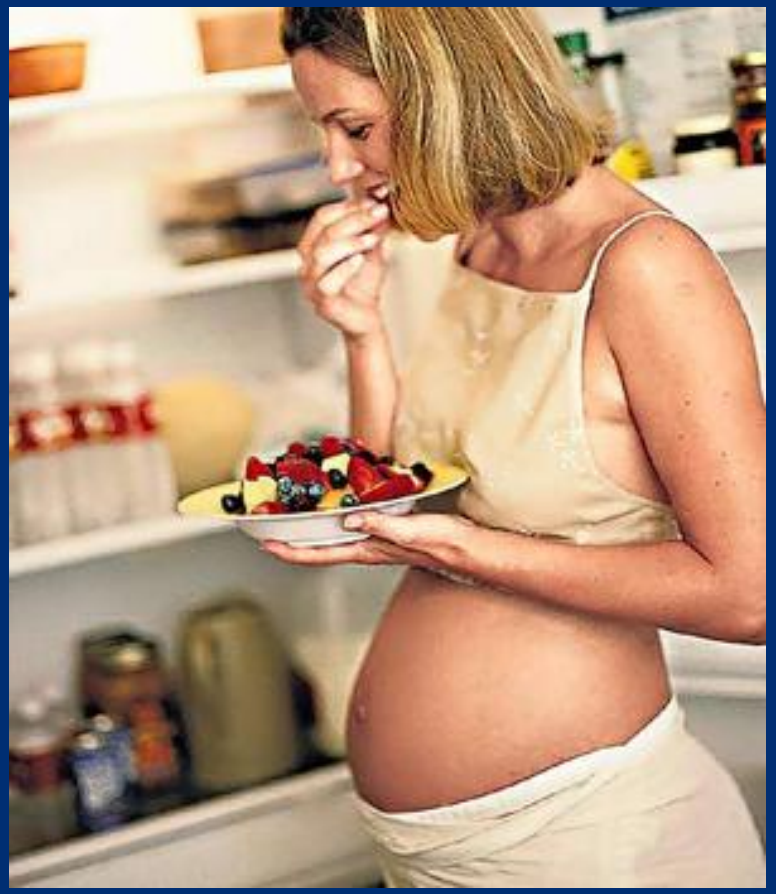
Stimuli from the environnement change gene expression.
This effect can be transmitted through generations.

Psychoneuroimmunology

Psychoneuroimmunology (PNI) is an interdisciplinary field of research, which deals with the interaction of the **nerve system, the hormone system and the immune system**. The scientific basis is that messenger molecules (like hormones and other substances) of the nervous system influence the immune system and vice verse.

Pregnancy and the future of the next generations:





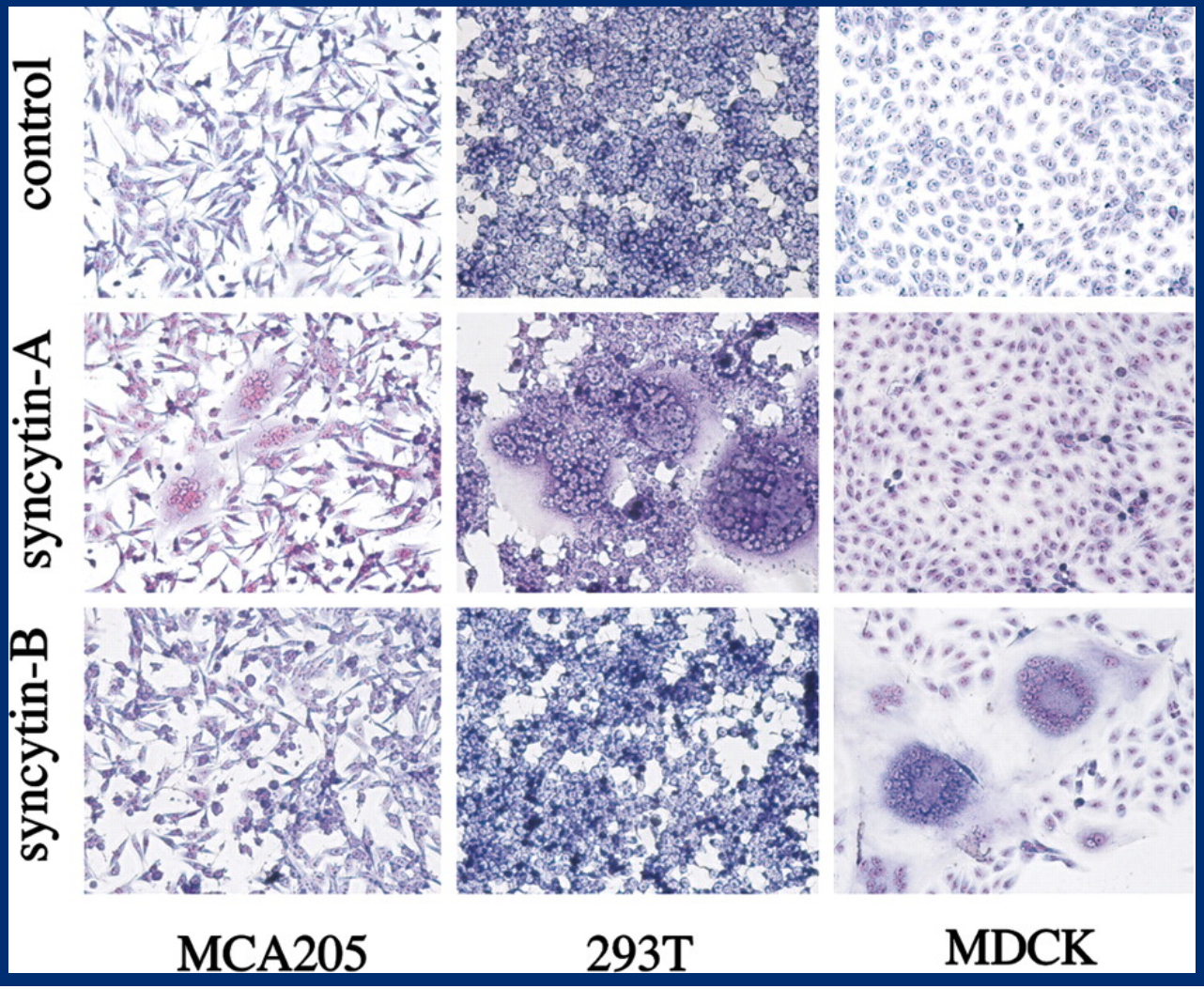
Institute of Science in Society Report 30/09/15

Organic Food during Mother's Pregnancy Reduces Risk of Male Birth Defects

Expecting mothers eating mostly organic produce reduce risk of urogenital anomalies in male offspring ***Dr Eva Sirinathsinghji***

Pesticides are the other obvious candidate for the observed disparity in disease rates between those eating organic versus conventional foods. Several classes of pesticide have been shown to have endocrine disrupting effects including glyphosate, 2,4-D, atrazine, endosulfan, linurin, vinclozolin and dichlorodiphenyldichloroethylene (DDE).

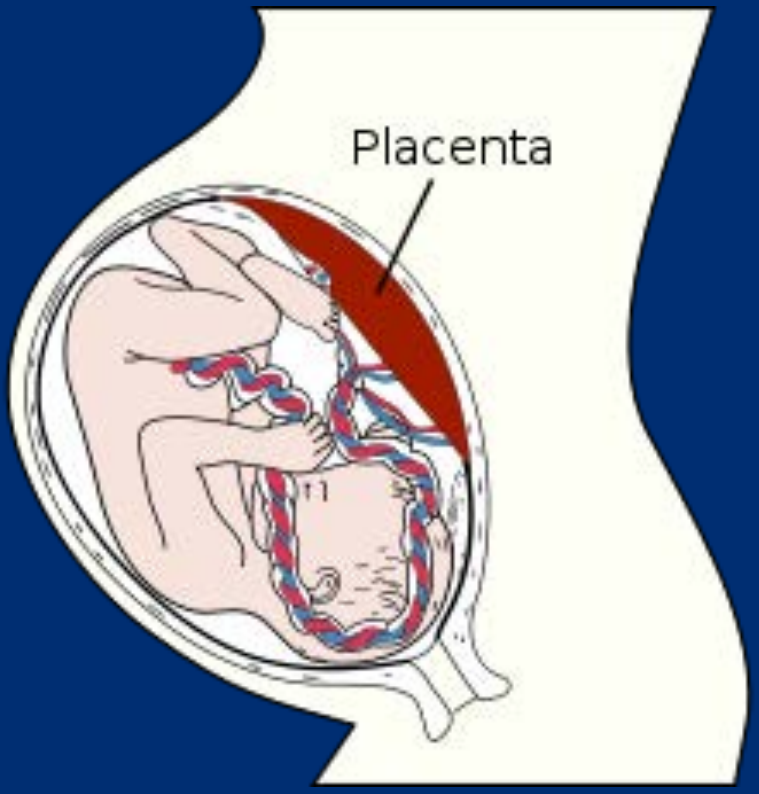
Syncytin-mediated cell–cell fusion

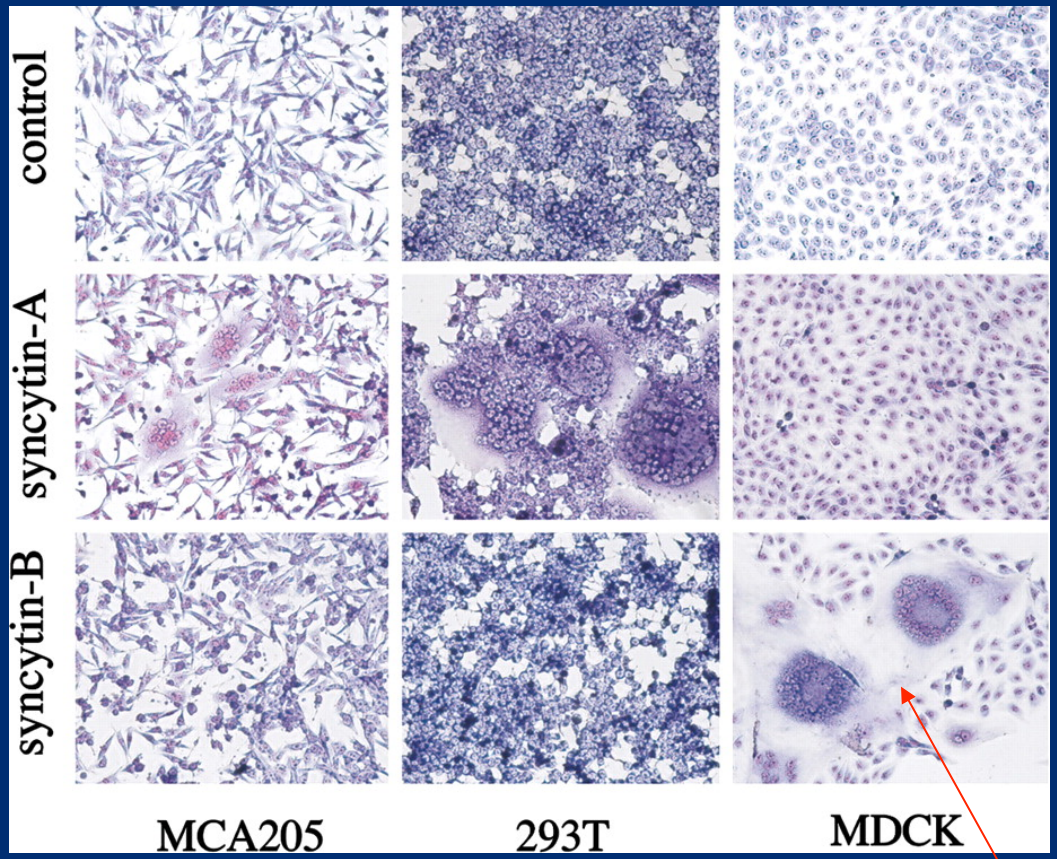


Syncytin and placenta development

Dupressoir A et al. PNAS 2005;102:725-730

Syncytin, which is expressed by endogenous retroviruses of the pregnant women, leads to cel
fusion by building up the placenta in normal development.





Humans have 400% more of retroviral genes than of human genes

Dupressoir A et al. PNAS 2005;102:725-730

PNAS

Why it is so important that mothers breastfeed their babies:

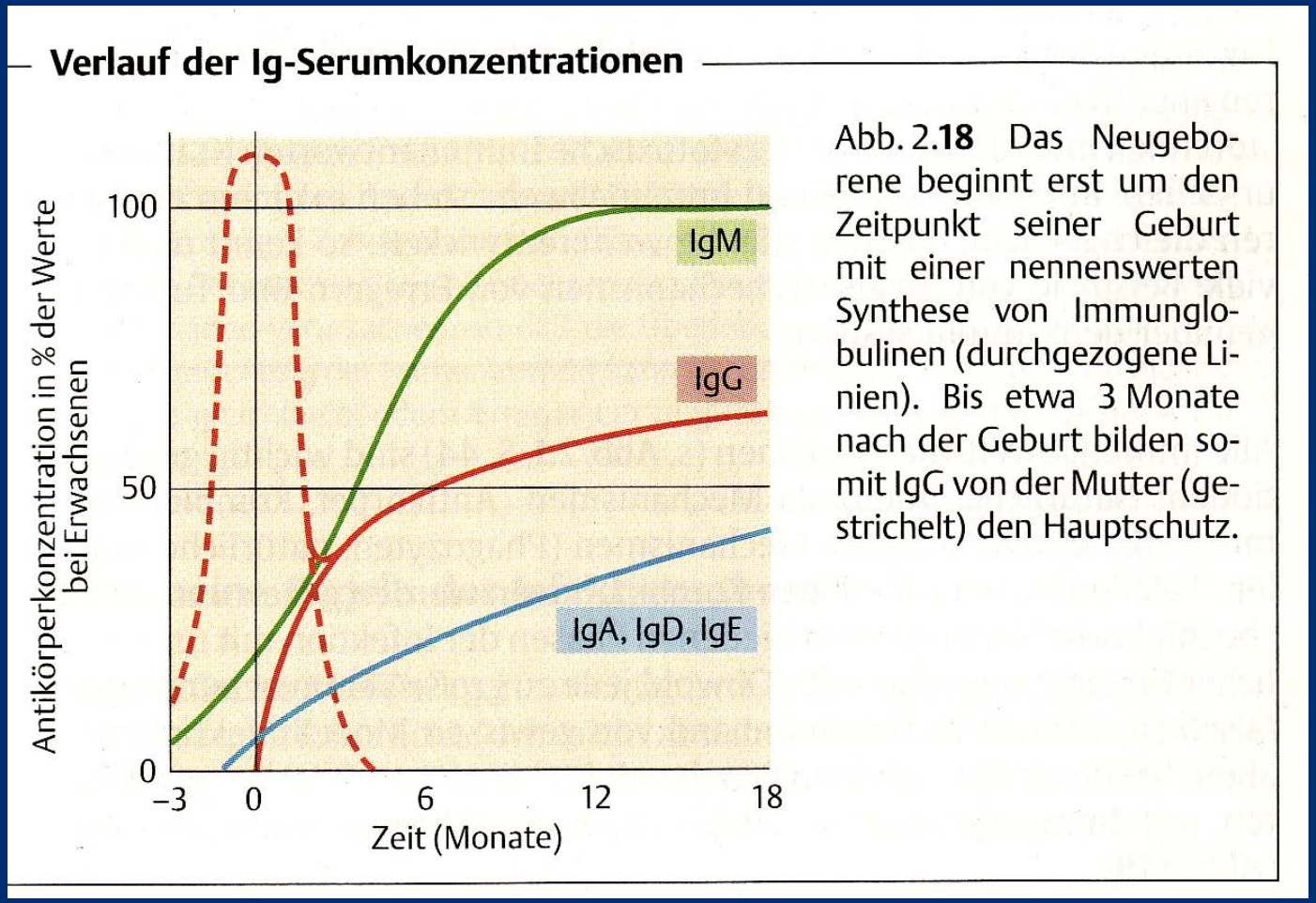
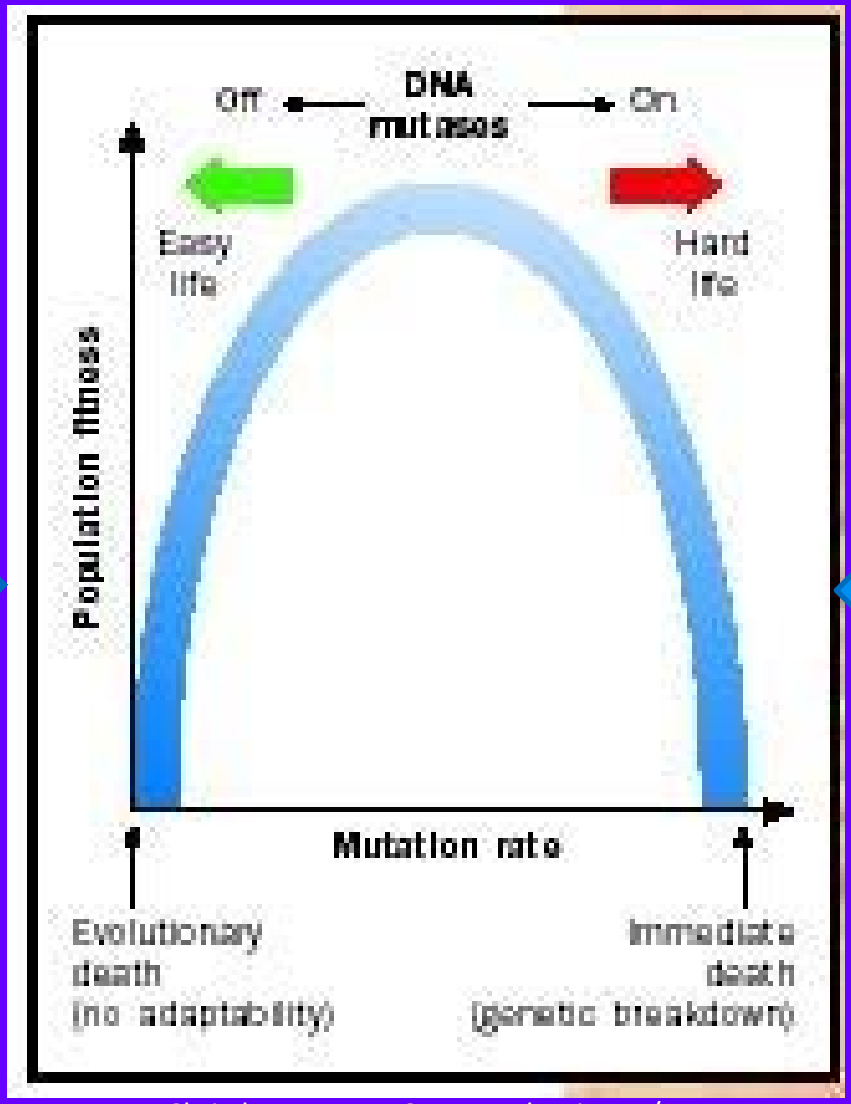


Abb. 2.**18** Das Neugeborene beginnt erst um den Zeitpunkt seiner Geburt mit einer nennenswerten Synthese von Immunglobulinen (durchgezogene Linien). Bis etwa 3 Monate nach der Geburt bilden somit IgG von der Mutter (gestrichelt) den Hauptschutz.

- **The antibodies of the mother protect the child from infections.**
- **The emotional relationship and the primal sense of trust are strengthened.**

EVOLUTION and MUTATION

Lack of genetic diversity



„gene chaos“

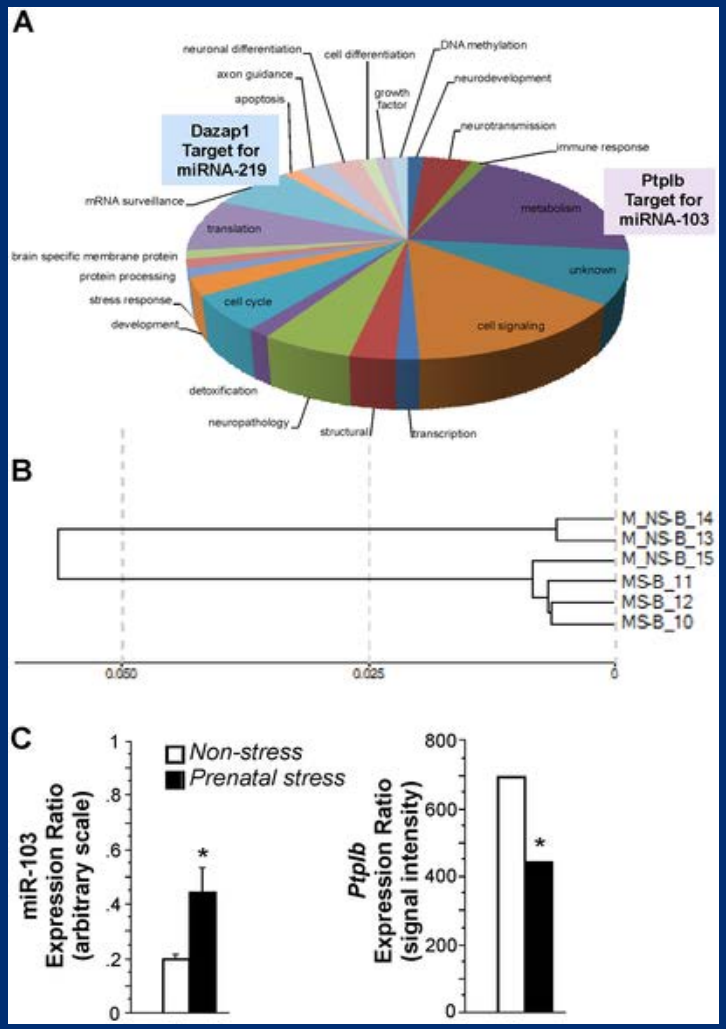
Extinction of the Population

Extiction of the Individual

Result:

The more of Stress
The more of Mutations.

Prenatal stress alters the brain transcriptome in male newborn offspring.

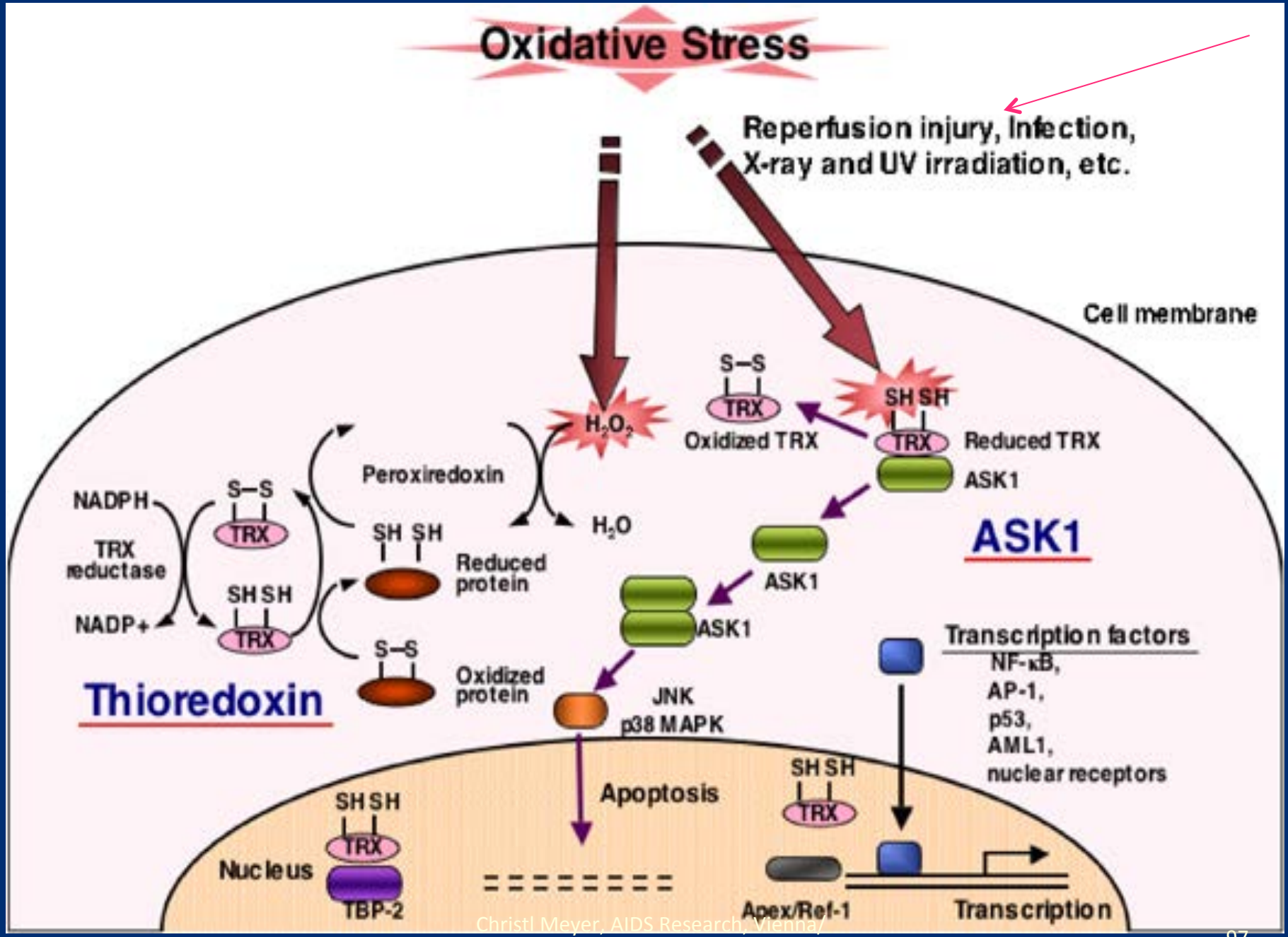


Zucchi FCR, Yao Y, Ward ID, Ilnytskyy Y, et al. (2013) Maternal Stress Induces Epigenetic Signatures of Psychiatric and Neurological Diseases in the Offspring. PLoS ONE 8(2): e56967. doi:10.1371/journal.pone.0056967

http://www.plosone.org/article/info:doi/10.1371/journal.pone.0056967

Normal cellular *homeostasis* is a *delicate balance* between the rate and magnitude of *oxidant formation* and the *rate of oxidant elimination*.

Oxidative stress can, therefore, be defined as the pathogenic outcome of the overproduction of oxidants that overwhelms the cellular antioxidant capacity.


Oxidative Stress
Reperfusion injury, Infection,
X-ray and UV irradiation, etc.
Cell membrane
H_2O_2
S–S
TRX
Oxidized TRX
SH SH
TRX
Reduced TRX
ASK1
Peroxiredoxin
H_2O
NADPH
TRX reductase
NADP+
S–S
TRX
SH SH
TRX
SH SH
Reduced protein
S–S
Oxidized protein
Thioredoxin
ASK1
ASK1
ASK1
JNK
p38 MAPK
Transcription factors
NF-κB,
AP-1,
p53,
AML1,
nuclear receptors
Apoptosis
SH SH
TRX
Nucleus
TBP-2
SH SH
TRX
Apex/Ref-1
Transcription