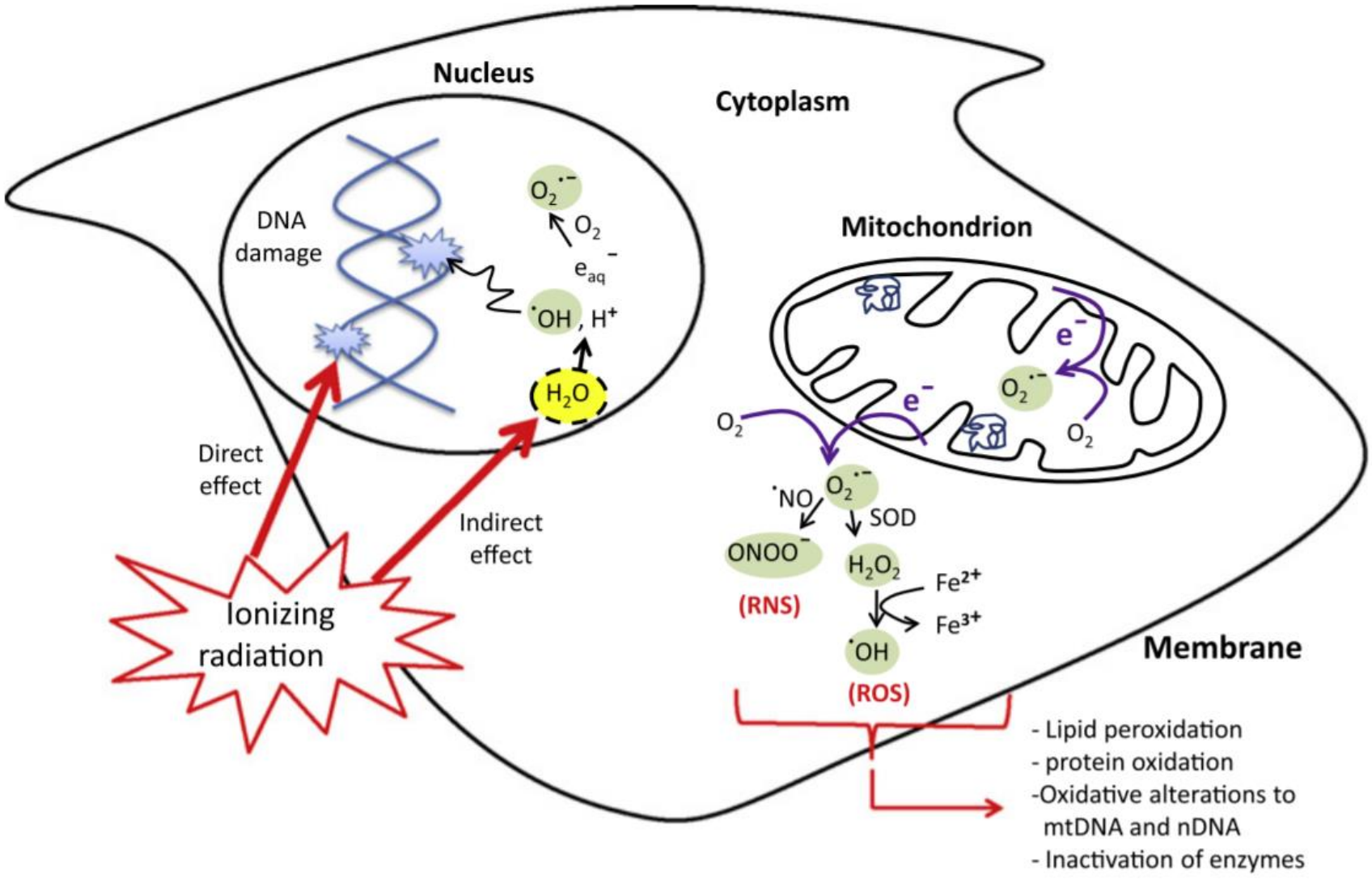
The XXIV International Scientific Conference of Young Scientists and Specialists (AYSS-2020)

Omic-technologies in radiation biology

Dr. Polina Volkova,
Russian Institute of Radiology and Agroecology
volkova.obninsk@gmail.com

How does ionizing radiation influence a cell?



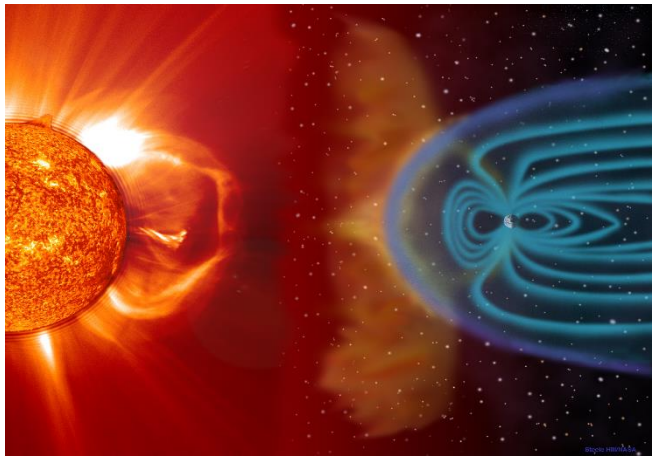
Azzam et al., 2012

Medicine

Agronomy

Space research

Ecology



Reductionism *vs.* holism

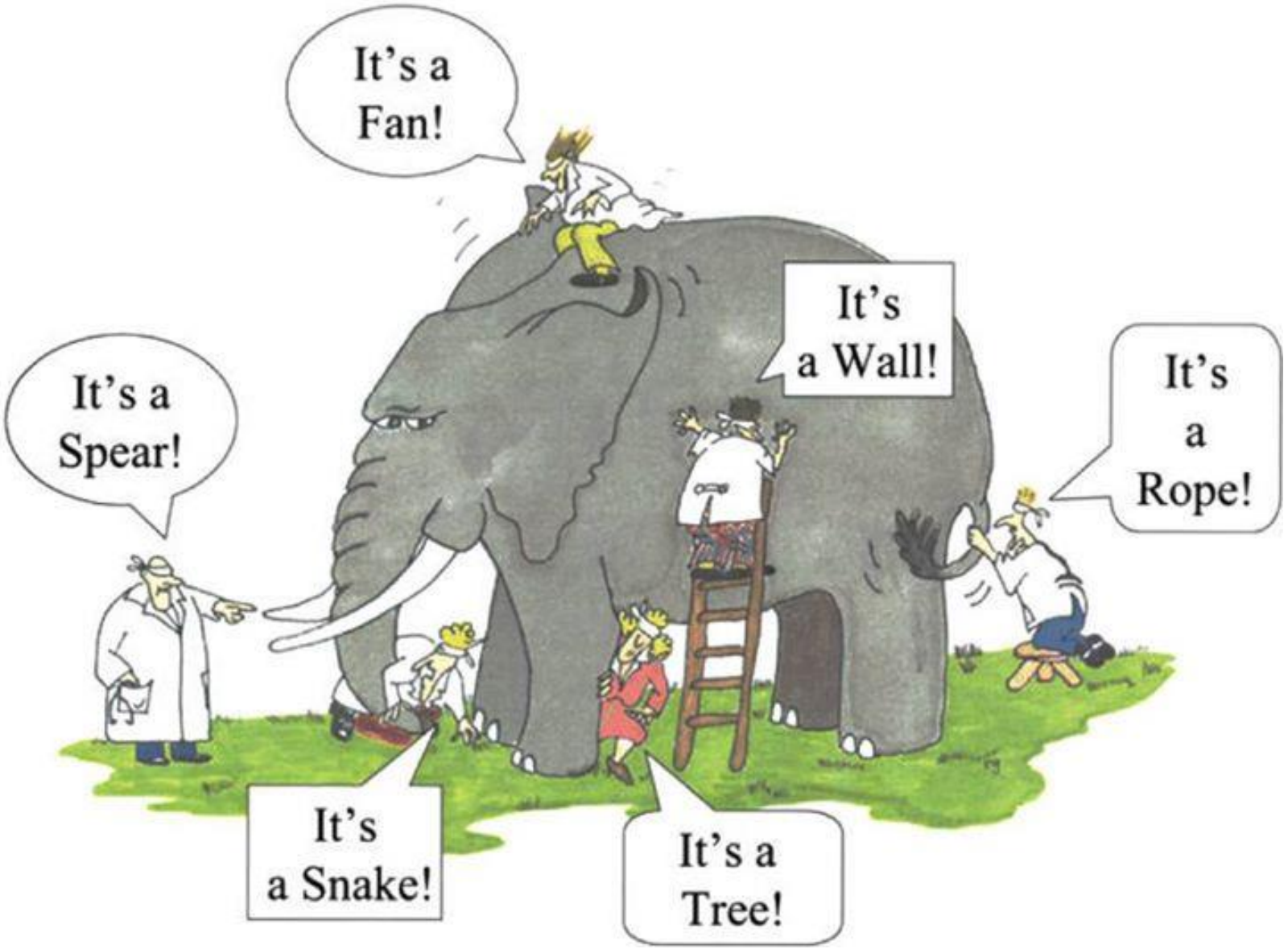
Reductionism:

- A complex system can be understood by studying its constituent components.
- **Example:** Watson and Crick deciphered the structure of DNA and made conclusions about its role in the transmission of hereditary information.

Holism:

- It postulates that the properties of a higher-level system cannot be fully explained by examining only its individual components.
- The whole organism is more than just the sum of the parts that make it up.
- **Example:** a cell disassembled into chemical constituents is no longer a cell.
- However, the analysis of the whole thing without decomposing it into simpler components can be challenging.

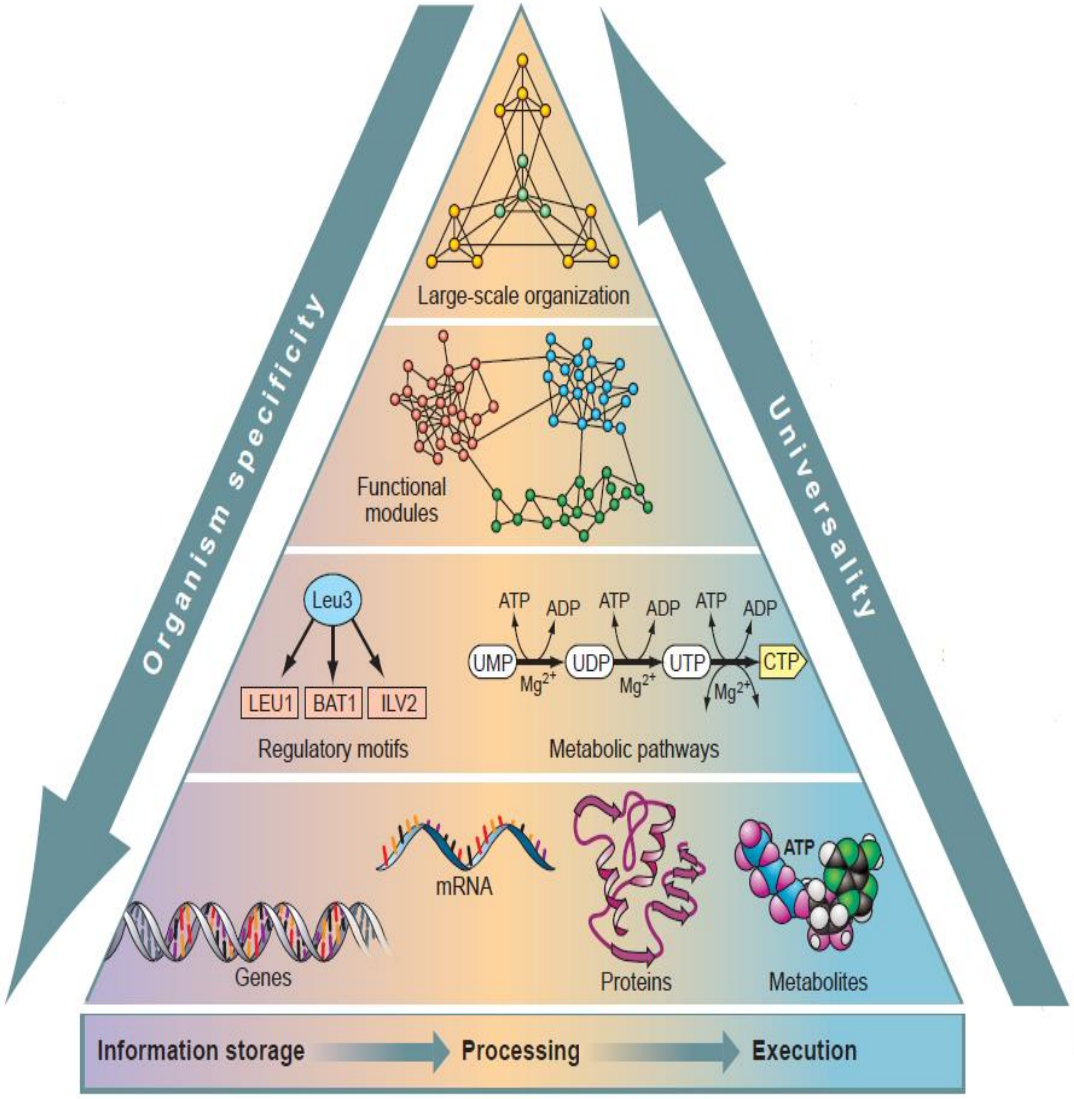
How to identify an elephant?



What is systems biology?

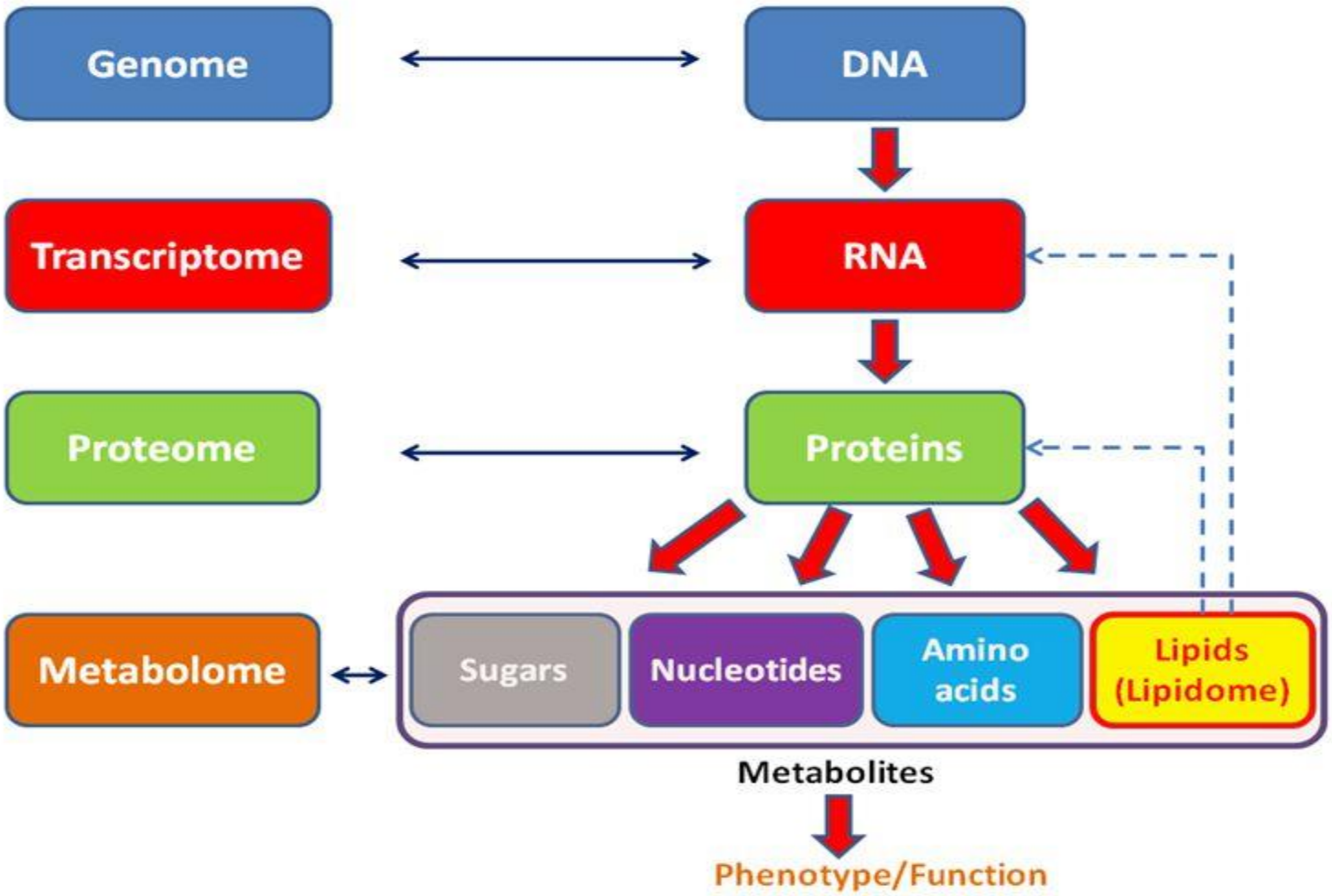
Systems biology studies a living organism as an interacting network of genes, proteins, and biochemical processes.

Using computer technology, systems biology organizes and integrates information from different functional levels in order to create plausible models of living systems.



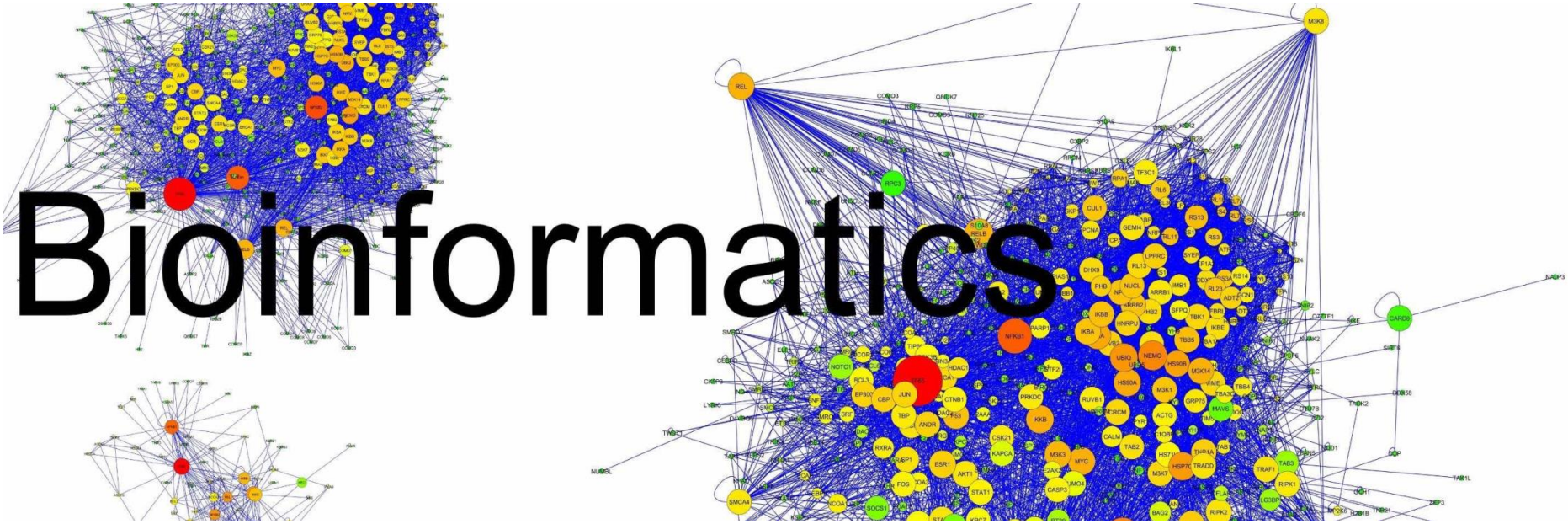
Oltvai and Barabási, Science, 2002

Omics Technologies

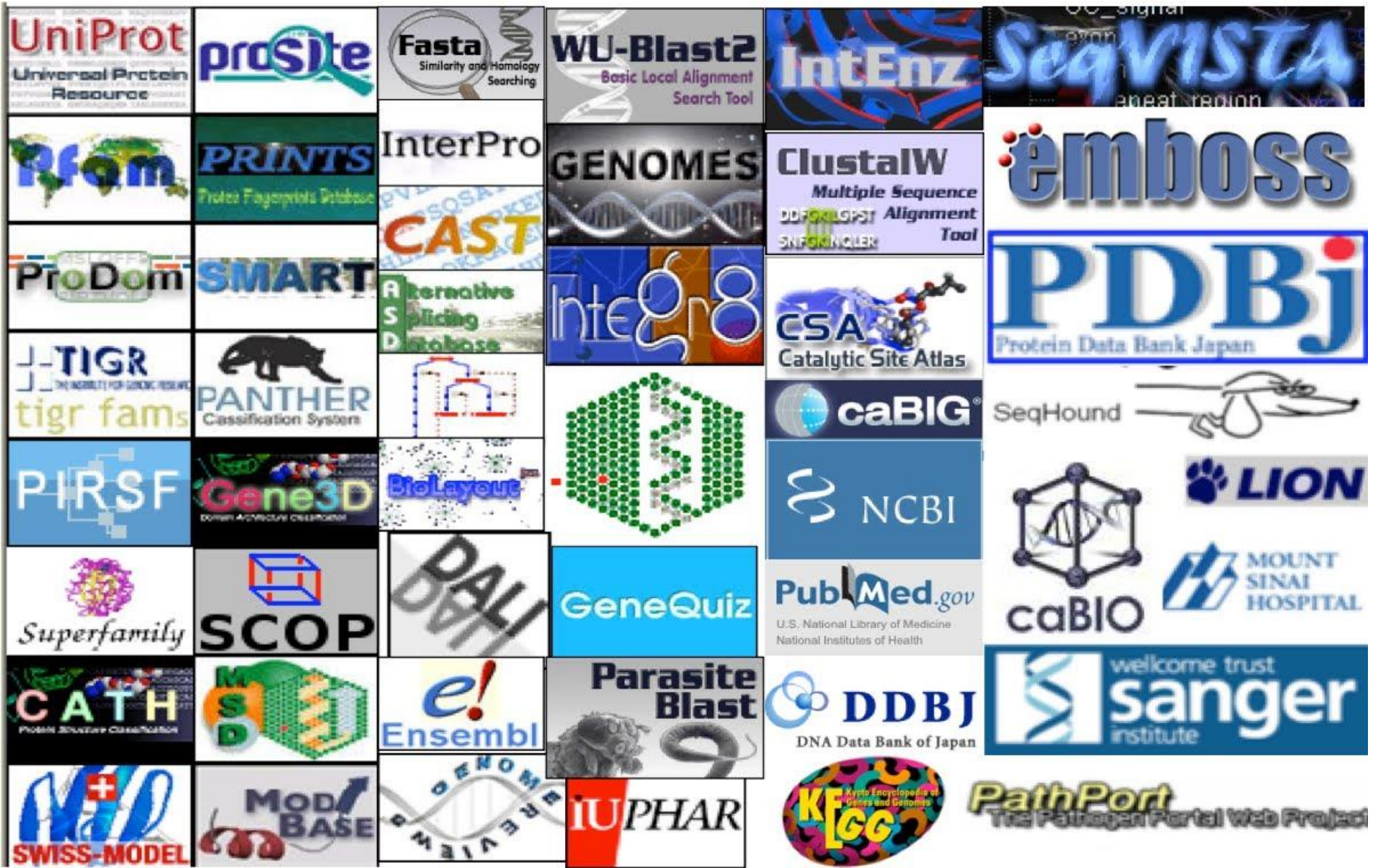


Bioinformatics

Bioinformatics is an interdisciplinary field of research, including the development of methods and software for the analysis of biological “big” data.

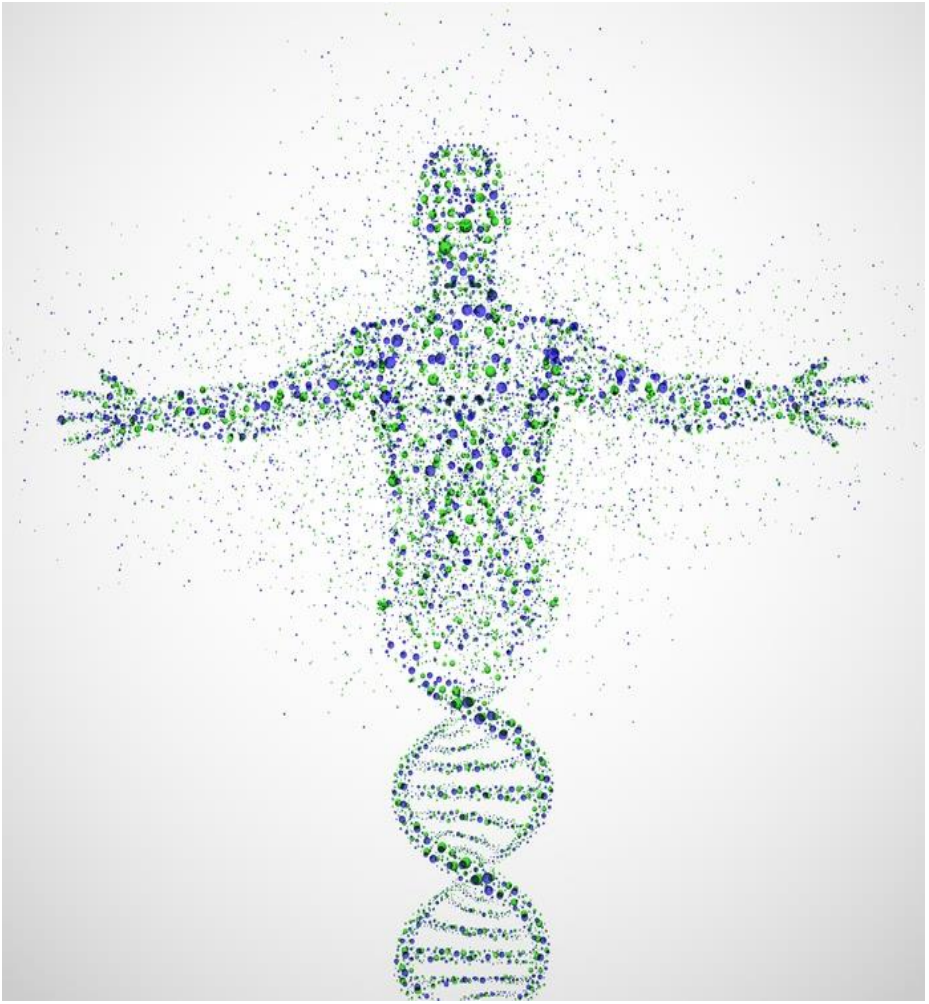


Bioinformatics. Databases.



Genomics

Genomics is an interdisciplinary field of biology focusing on the structure, function, evolution, mapping, and editing of genomes.



Genetics *vs.* genomics

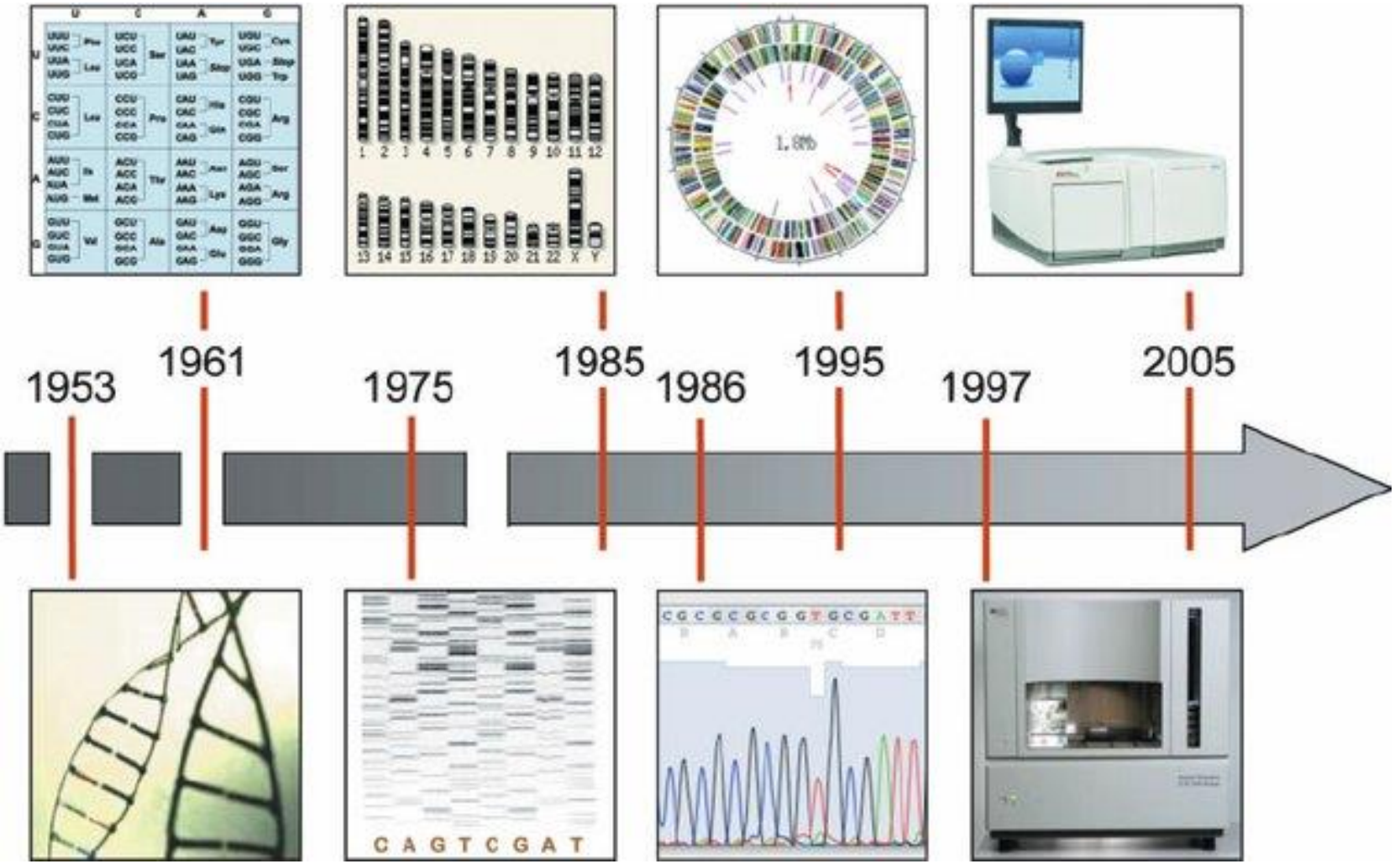
Genetics:

- Genetics studies heredity.
- A gene is a specific DNA sequence on a chromosome.
- Genetics examines the function and structure of a single gene.

Genomics:

- Genomics studies the entire set of genes of an organism.
- Therefore, a genome includes the entire set of genes of a particular organism.
- Genomics explores all genes of the organism and their interactions.

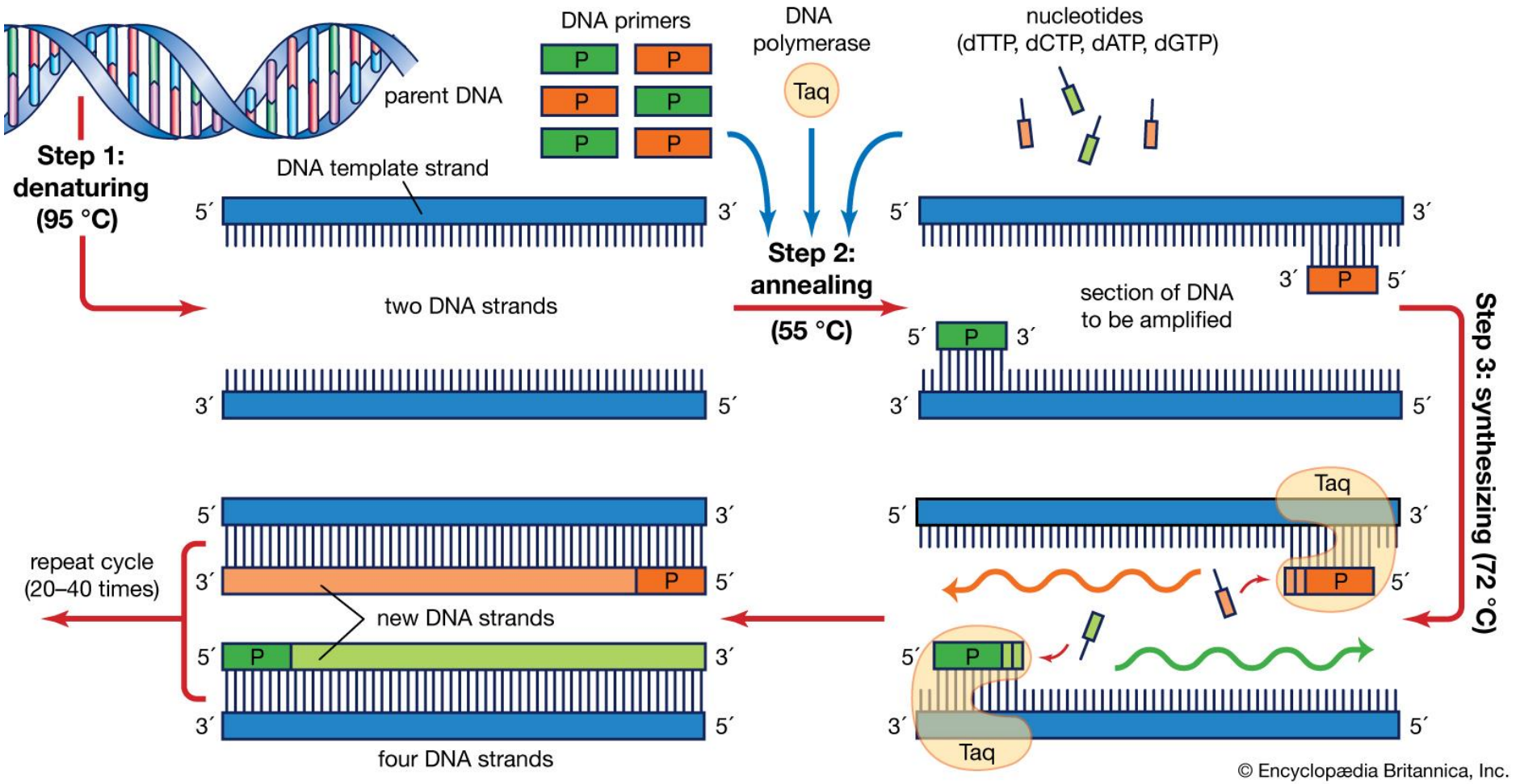
Genomics



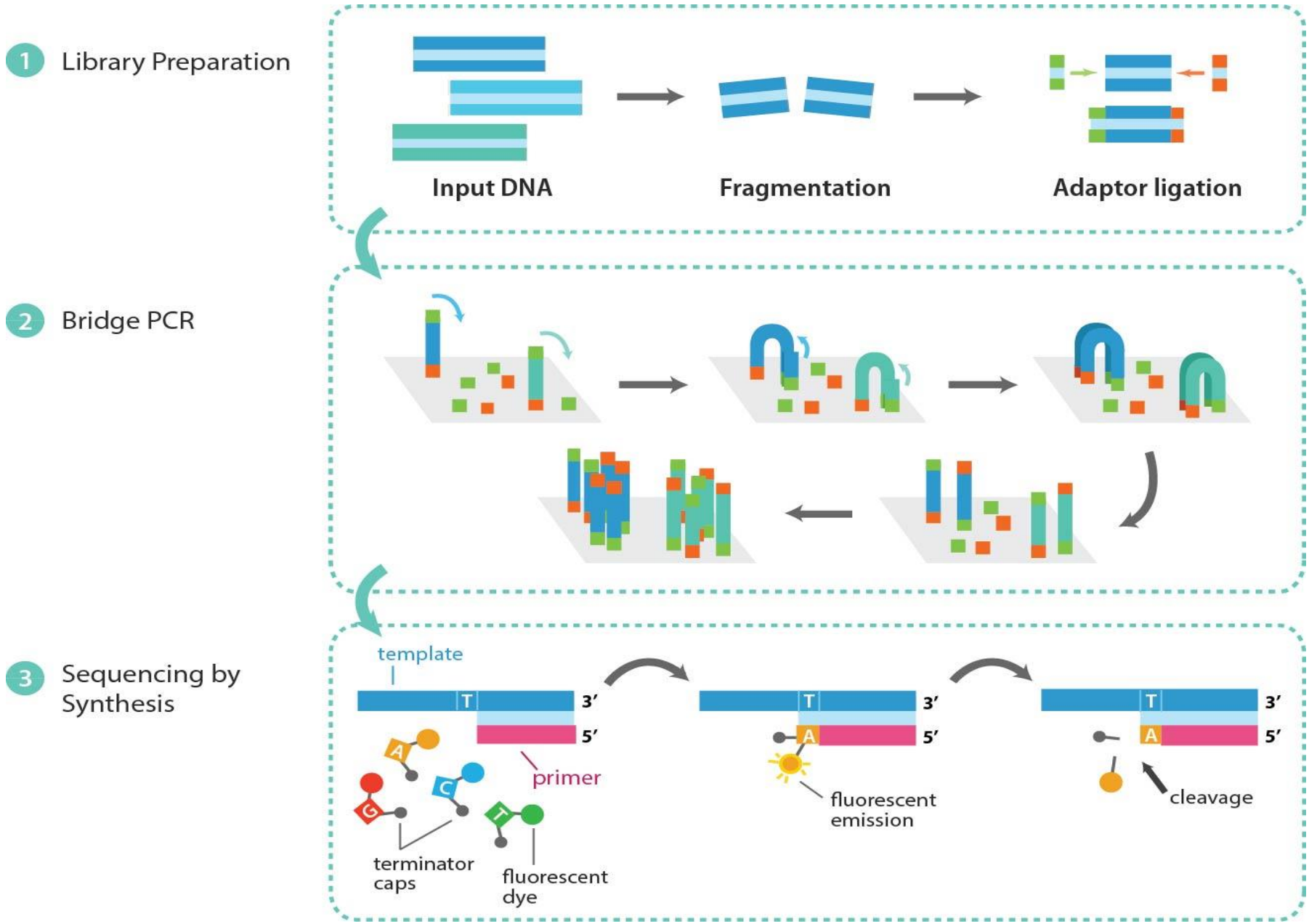
1953, DNA double helix structure discovery; 1961, first step in the cracking of the genetic code; 1975, chain-termination nucleic acid sequencing method developed by Sanger; 1985, international consortium set up to sequence the human genome (HGP); 1986, increased sequencing capacities by the use of fluorescent dyes; 1995, first microbial genomes published by J. C. Venter and colleagues; 1997, development and commercialization of capillary sequencers; 2005, development and commercialization of GS20 pyrosequencer.

Bertin et al., 2008

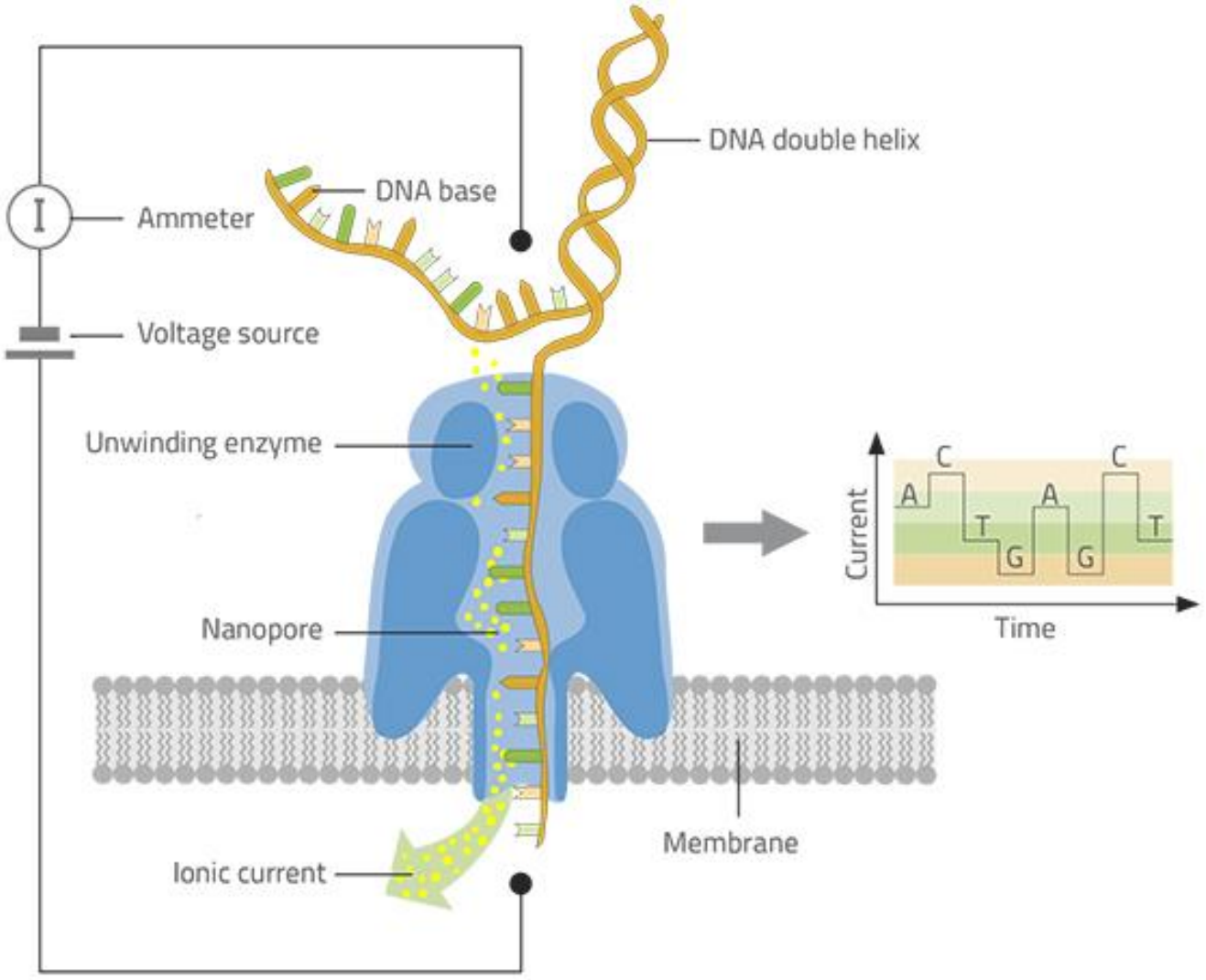
DNA sequencing – Polymerase chain reaction



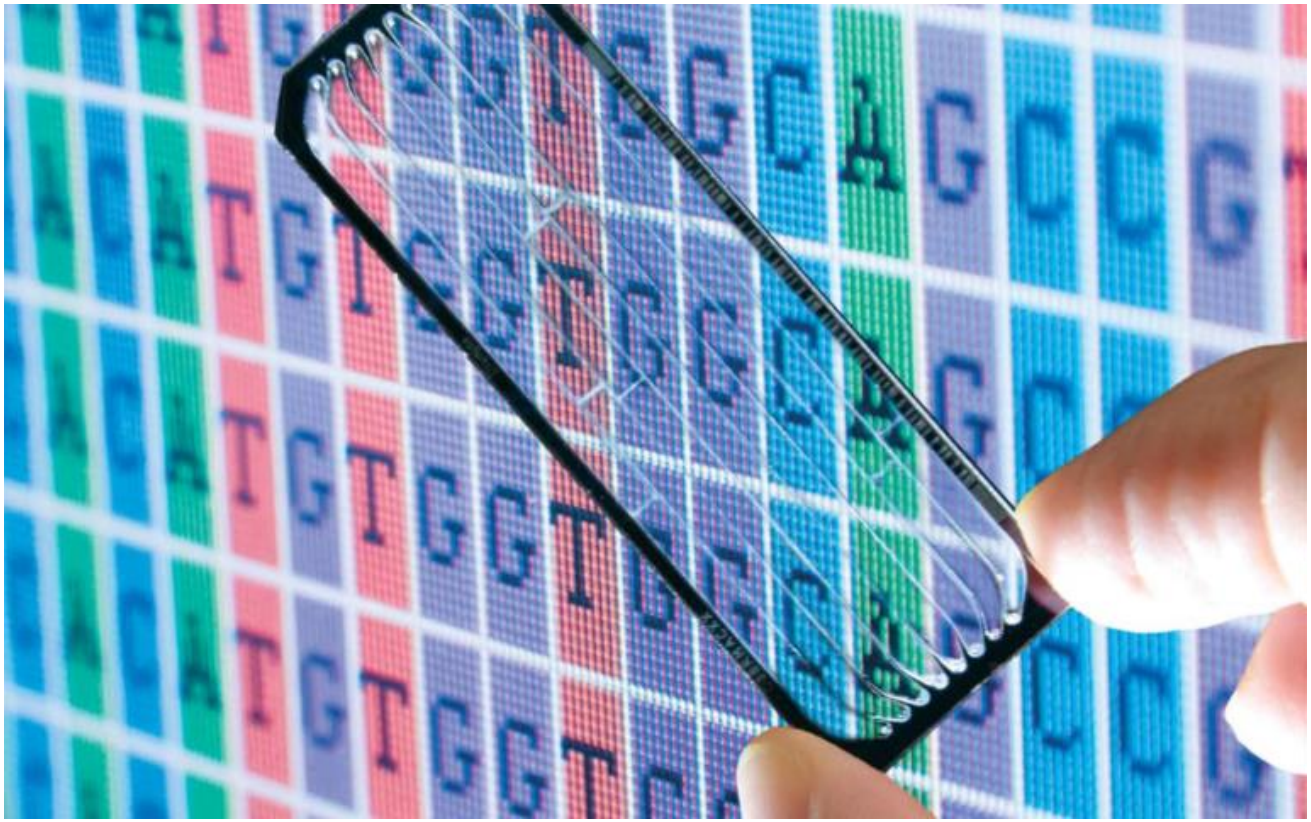
DNA sequencing – Next Generation Sequencing



DNA sequencing – Third-Generation Sequencing

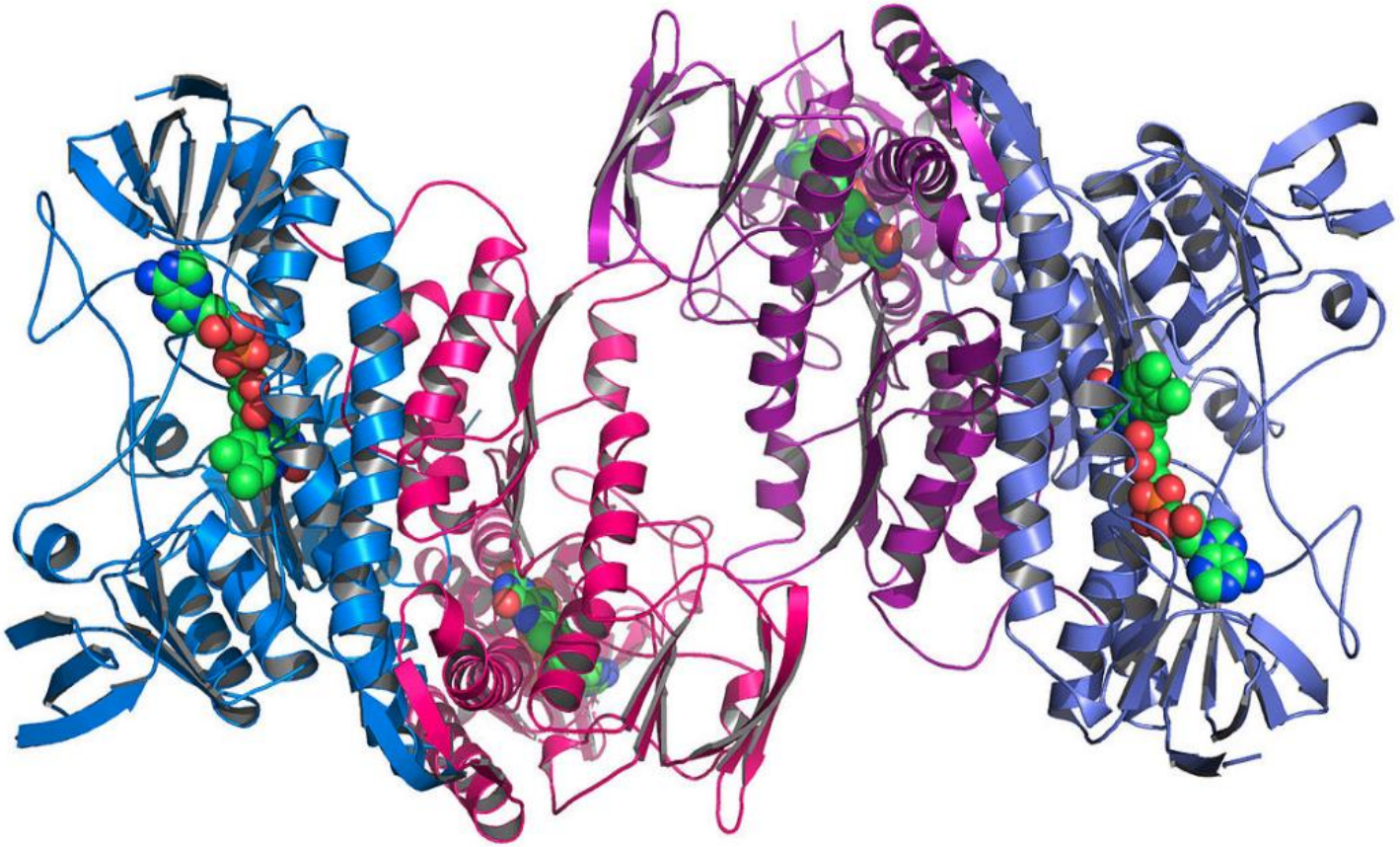


Genomics is divided into two basic areas: structural genomics, characterizing the physical nature of whole genomes; and functional genomics, characterizing the transcriptome (the entire range of transcripts produced by a given organism) and the proteome (the entire array of encoded proteins).



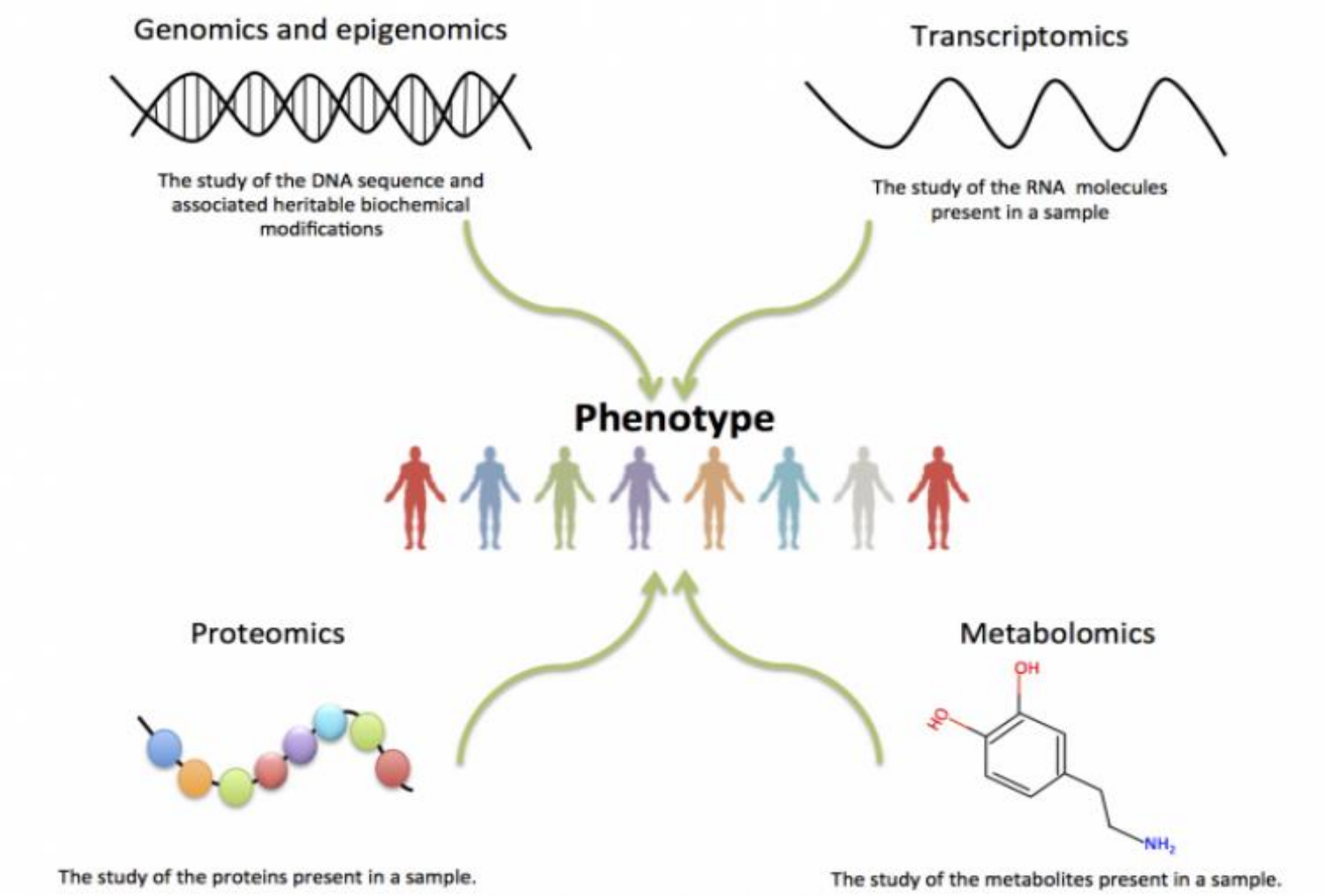
Structural genomics

Structural genomics studies the content and organization of genomic information: examines genes with a known structure to understand their function, and also studies the spatial (3D) structure of protein molecules encoded in the genome.



Functional genomics

Functional genomics studies how the information recorded in the genome is realized: from the gene to the trait.

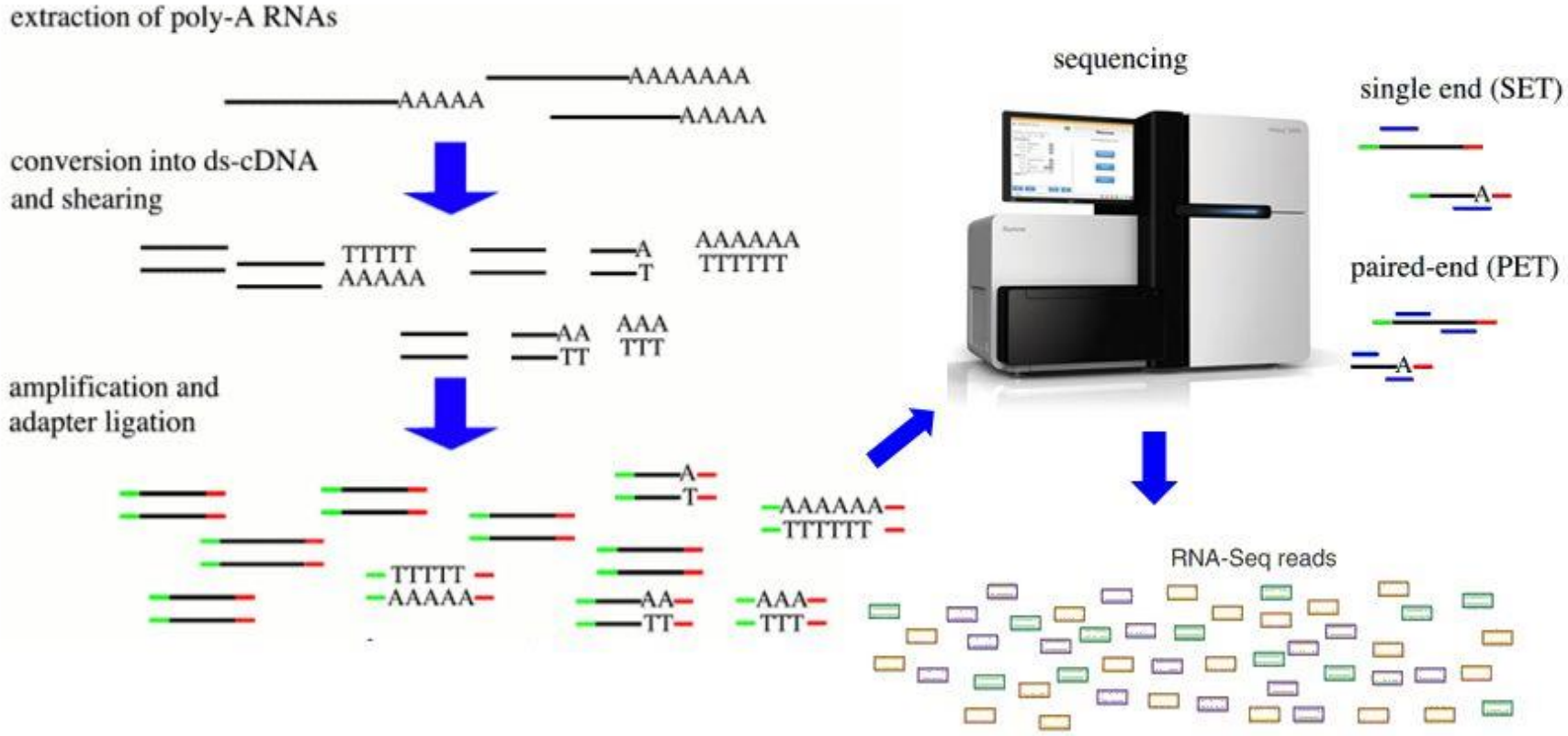


Transcriptomics

Transcriptomics studies the transcriptome — the complete set of RNA transcripts that are produced by the genome, under specific circumstances or in a specific cell — using high-throughput methods, such as microarray analysis and RNA sequencing.

Overview of RNA-Seq

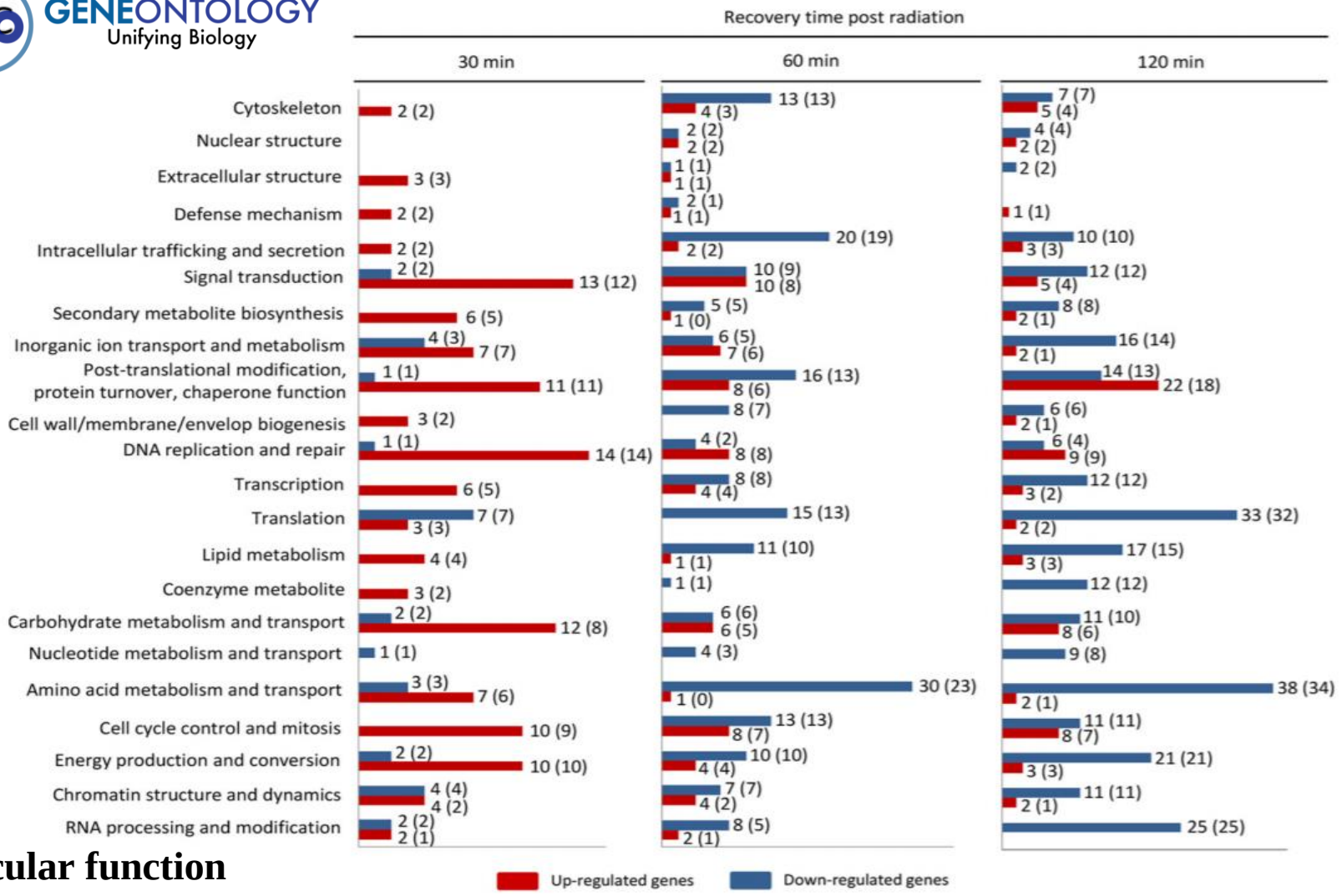
Transcriptome profiling using NGS



What can the transcriptome tell?

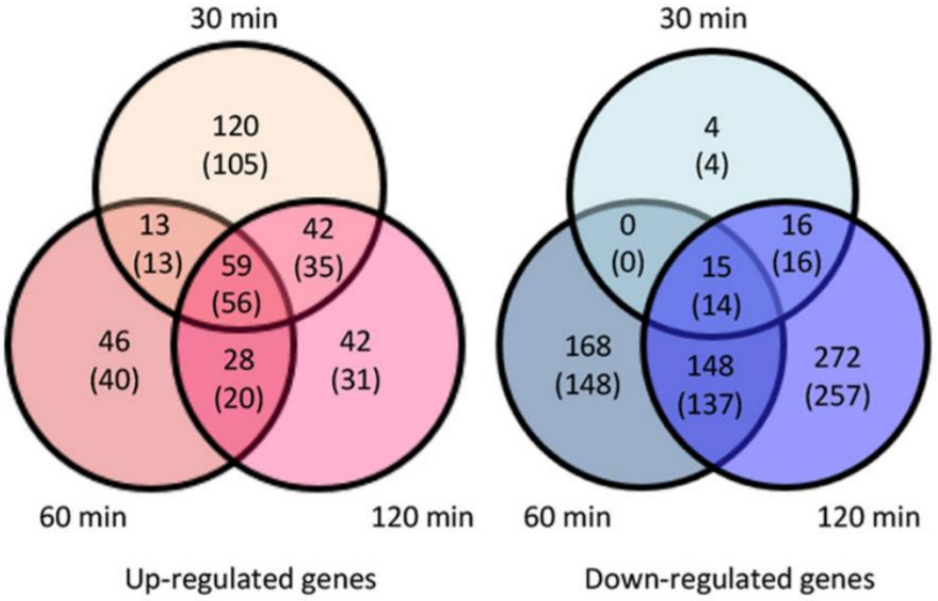
Jung et al., 2016. Unraveling Fungal Radiation Resistance Regulatory Networks through the Genome-Wide Transcriptome and Genetic Analyses of *Cryptococcus neoformans*

GENEONTOLOGY
Unifying Biology



Molecular function
Biological process
Cellular compartment

"Most importantly, we discovered a number of novel *C. neoformans* genes, the expression of which was modulated by gamma radiation exposure, and their deletion rendered cells susceptible to gamma radiation exposure, as well as DNA damage insults. Among these genes, we found that a unique transcription factor containing the basic leucine zipper domain, named Bdr1, served as a regulator of the gamma radiation resistance of *C. neoformans* by controlling expression of DNA repair genes, and its expression was regulated by the evolutionarily conserved DNA damage response protein kinase Rad53. Taken together, the current transcriptome and functional analyses contribute to the understanding of the unique molecular mechanism of the radiation-resistant fungus *C. neoformans*." **Jung et al., 2016.**



AMERICAN SOCIETY FOR MICROBIOLOGY | mBio®

RESEARCH ARTICLE

CrossMark

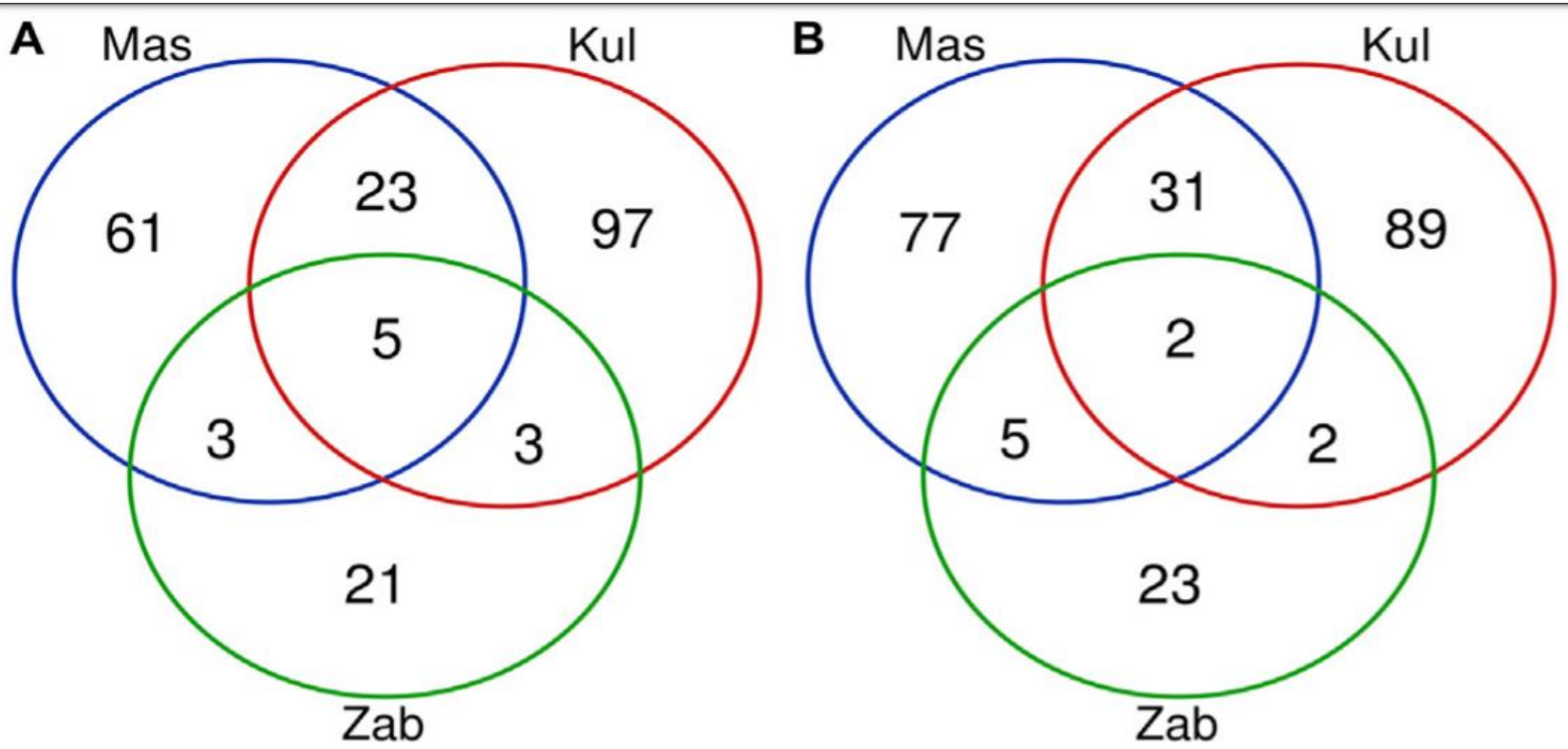
Unraveling Fungal Radiation Resistance Regulatory Networks through the Genome-Wide Transcriptome and Genetic Analyses of *Cryptococcus neoformans*

Kwang-Woo Jung,[a] Dong-Hoon Yang,[b] Min-Kyu Kim,[a] Ho Seong Seo,[a] Sangyong Lim,[a] Yong-Sun Bahn[b]

Research Division for Biotechnology, Korea Atomic Energy Research Institute, Jeongeup, Republic of Korea[a]; Department of Biotechnology, College of Life Science and Biotechnology, Yonsei University, Seoul, Republic of Korea[b]

ABSTRACT The basidiomycetous fungus *Cryptococcus neoformans* has been known to be highly radiation resistant and has been found in fatal radioactive environments such as the damaged nuclear reactor at Chernobyl. To elucidate the mechanisms underlying the radiation resistance phenotype of *C. neoformans*, we identified genes affected by gamma radiation through genome-wide transcriptome analysis and characterized their functions. We found that genes involved in DNA damage repair systems were upregulated in response to gamma radiation. Particularly, deletion of recombinase *RAD51* and two DNA-dependent ATPase genes, *RAD54* and *RDH54*, increased cellular susceptibility to both gamma radiation and DNA-damaging agents. A variety of oxidative stress response genes were also upregulated. Among them, sulfiredoxin contributed to gamma radiation resistance in a peroxiredoxin/thioredoxin-independent manner. Furthermore, we found that genes involved in molecular chaperone expression, ubiquitination systems, and autophagy were induced, whereas genes involved in the biosynthesis of proteins and fatty acids/sterols were downregulated. Most importantly, we discovered a number of novel *C. neoformans* genes, the expression of which was modulated by gamma radiation exposure, and their deletion rendered cells susceptible to gamma radiation exposure, as well as DNA damage insults. Among these genes, we found that a unique transcription factor containing the basic leucine zipper domain, named Bdr1, served as a regulator of the gamma radiation resistance of *C. neoformans* by controlling expression of DNA repair genes, and its expression was regulated by the evolutionarily conserved DNA damage response protein kinase Rad53. Taken together, the current transcriptome and functional analyses contribute to the understanding of the unique molecular mechanism of the radiation-resistant fungus *C. neoformans*.

IMPORTANCE Although there are no natural environments under intense radiation, some living organisms have been found to show high radiation resistance. Organisms harboring the ability of radiation resistance have unique regulatory networks to

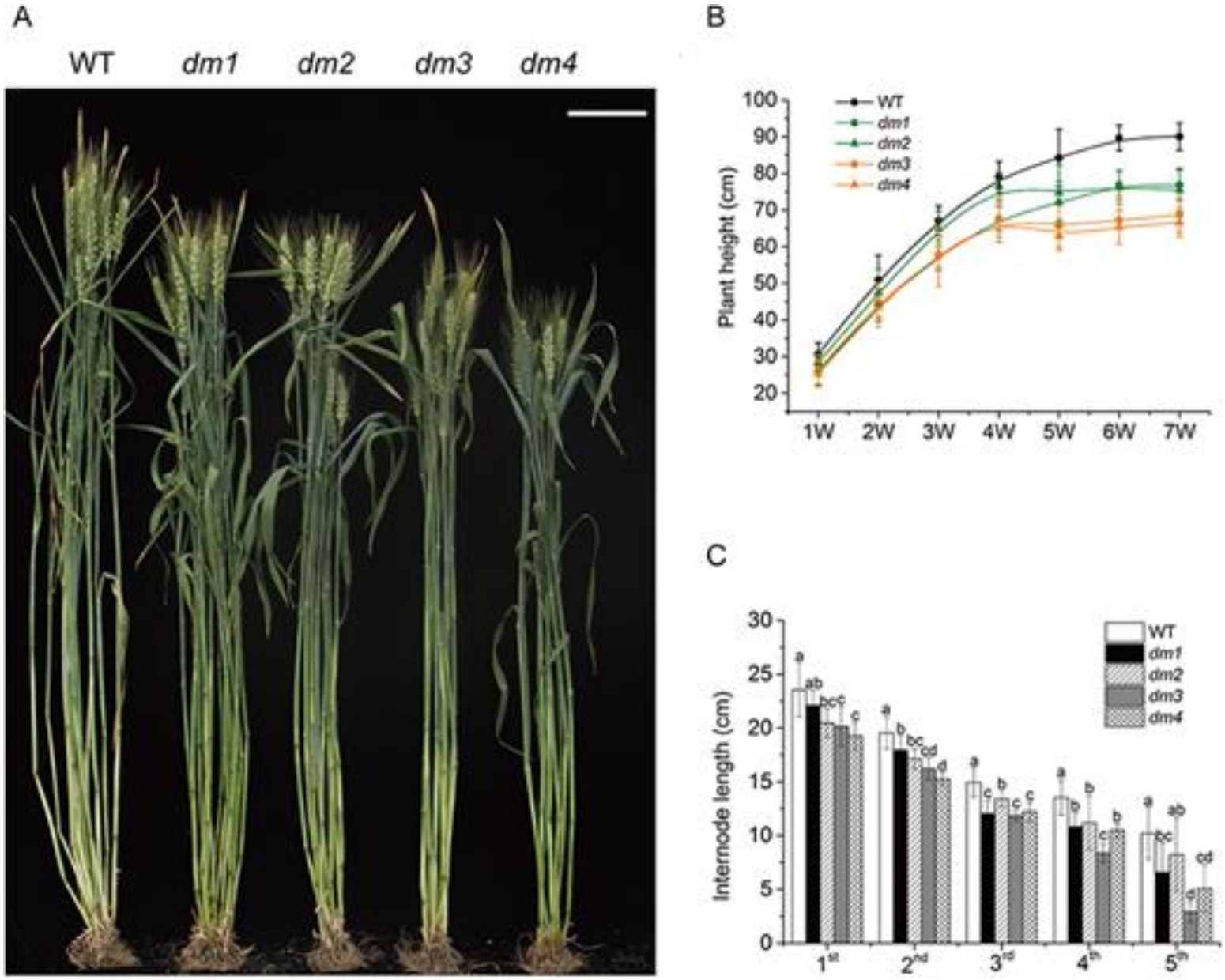


Overlapping transcripts among the three radioactively contaminated plots.

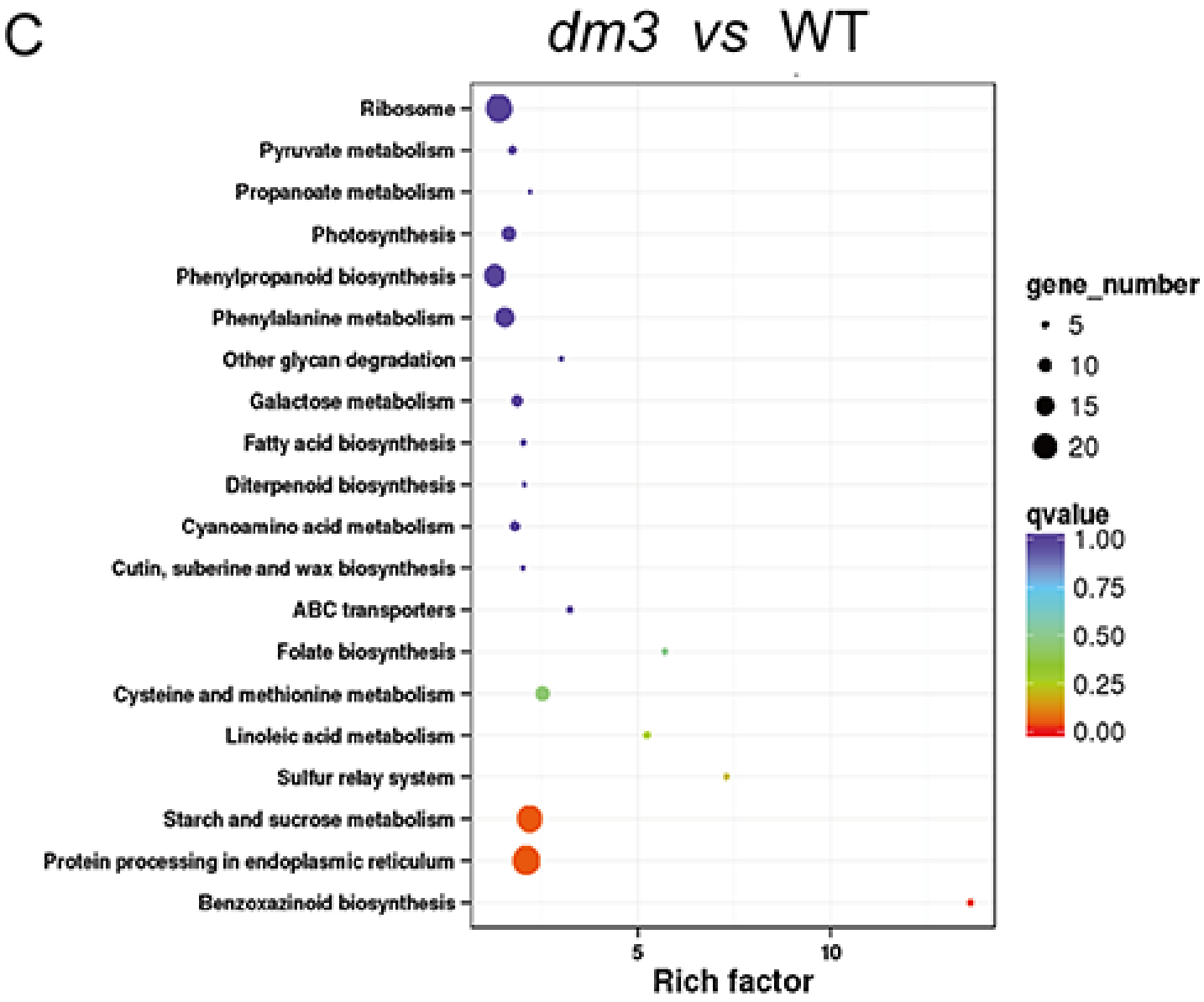
Transcript ID	UniProt ID	Function	Description	$\log_2$ FC		
				Mas	*Kul*	*Zab*
evgTrinTRINITY_DN60304_c1_g1_i1	WUN1_SOLTU	Cell death	Wound-induced protein 1	2.8	2.4	1.8
evgSOAPd26480654005	HY5_SOLLC	Photomorphogenesis	Transcription factor HY5	2.2	1.7	1.1
evgSOAPd244693109749	CIPK5_ORYSJ	Abscisic acid signalling	CBL-interacting protein kinase 20	−1.3	−2.3	−1.3
evgSOAPd25748839369	CIPKI_ORYSJ		CBL-interacting protein kinase 10	−3.2	−4.7	−1.9
evgSOAPd241551103465	SLAC1_ARATH	Stomatal closure	Guard cell S-type anion channel SLAC1	−1.6	−2.0	−1.7
evgTrinTRINITY_DN60799_c3_g2_i4	P2B11_ARATH	Protein ubiquitination	F-box protein PP2-B11	−4.0	−4.2	−3.1
evgSOAPd203292154205	–	Uncharacterized protein	–	−2.0	−2.3	−1.5

Note. The expression values are given as $\log_2$ Fold Change in comparison to the *Ref* population.

Duarte, Volkova. Geras'kin, 2019. The response profile to chronic radiation exposure based on the transcriptome analysis of Scots pine from Chernobyl affected zone



Xiong et al., 2019. Transcriptome sequencing reveals hotspot mutation regions and dwarfing mechanisms in wheat mutants induced by γ-ray irradiation and EMS.



Xiong et al., 2019. Transcriptome sequencing reveals hotspot mutation regions and dwarfing mechanisms in wheat mutants induced by γ-ray irradiation and EMS.

Table 4

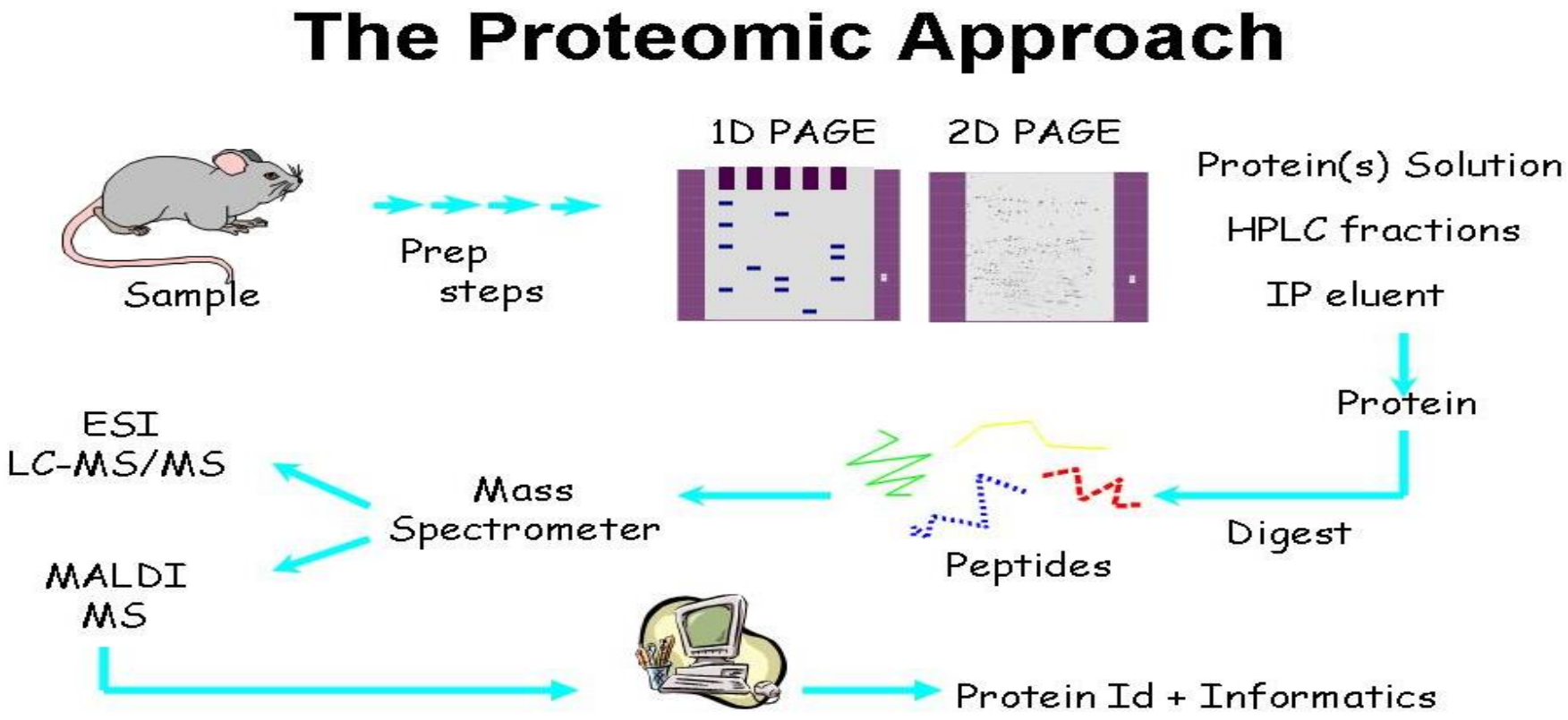
Representative list of over-represented pathways in higher dose group (Group IV) as compared to NLNRA (Group I).

Over-represented Pathways	No. of genes / database count	P-value
Up-regulated		
MAPK signaling pathway	(24 / 272)	9.87E-013
T cell receptor signaling pathway	(14 / 108)	5.81E-010
B cell receptor signaling pathway	(9 / 65)	4.21E-007
Cytokine-cytokine receptor interaction	(14 / 263)	1.46E-005
Jak-STAT signaling pathway	(10 / 155)	5.34E-005
Ubiquitin mediated proteolysis	(12 / 139)	5.83E-007
p53 signaling pathway	(6 / 69)	0.0003
Focal adhesion	(10 / 203)	0.0004
Gap junction	(7 / 96)	0.0003
Down-regulated		
Ubiquitin mediated proteolysis	(17 / 139)	2.08E-007

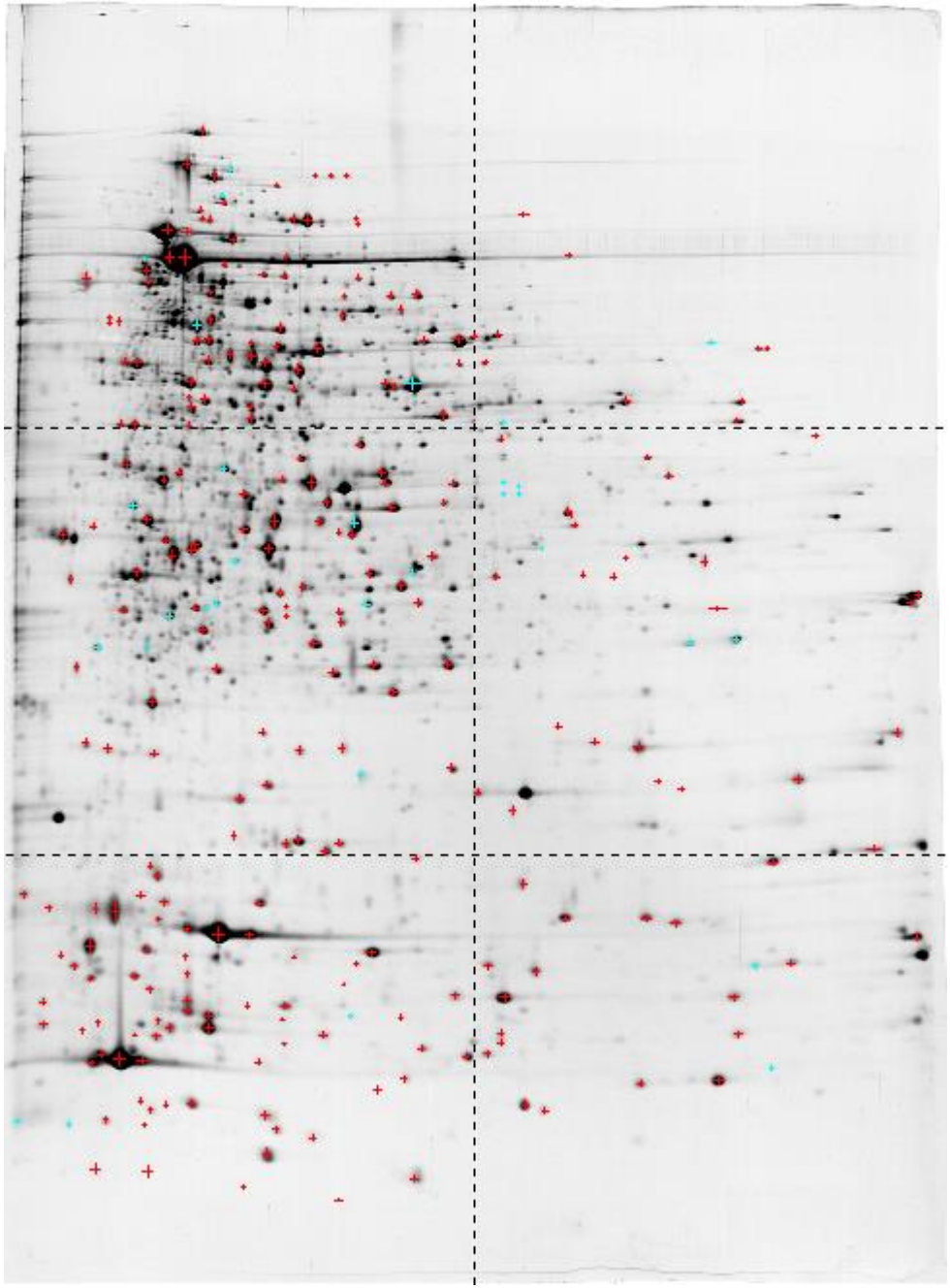
Jain, Das, 2017. Global transcriptome profile reveals abundance of DNA damage response and repair genes in individuals from high level natural radiation areas of Kerala coast

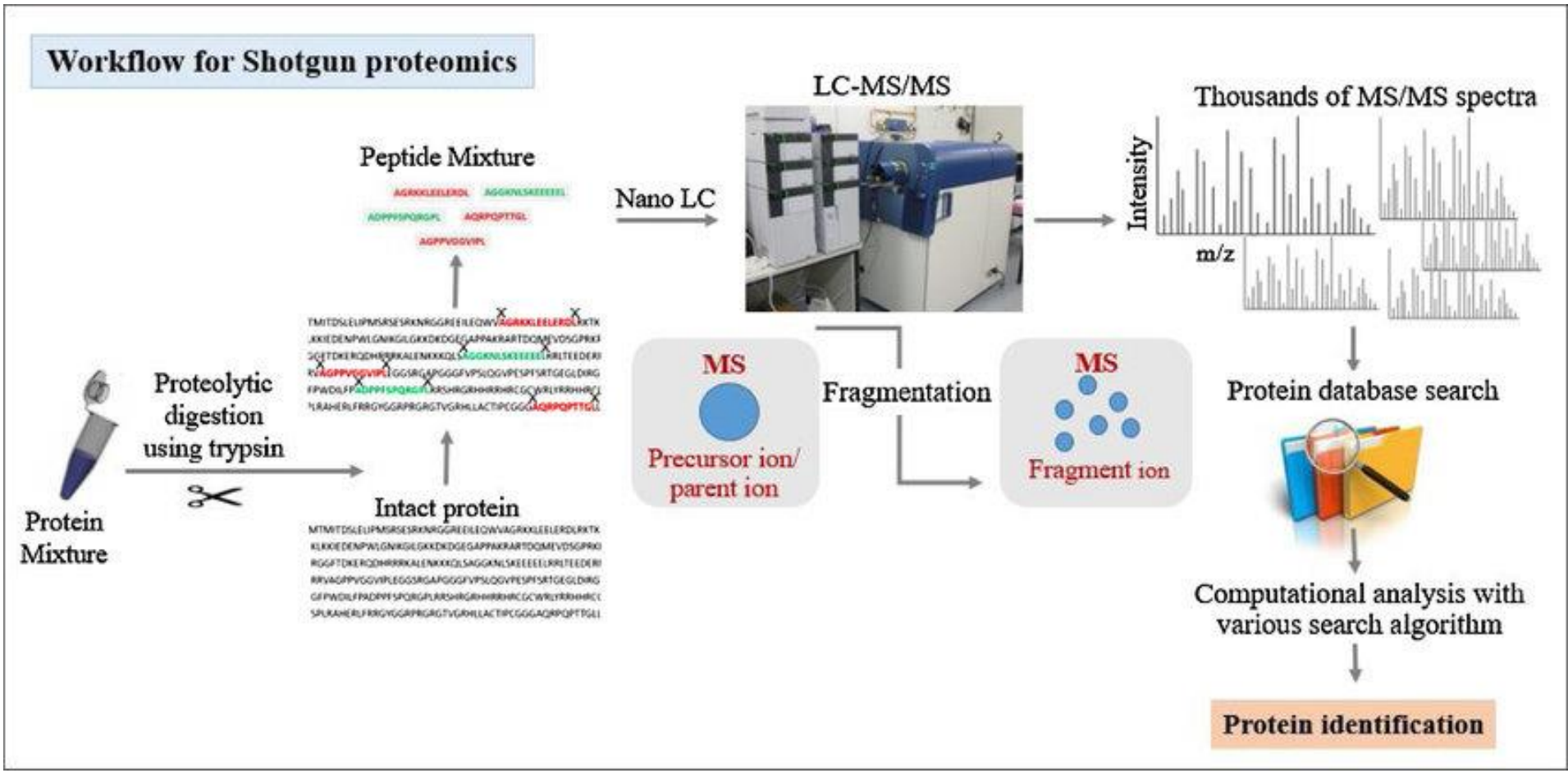
Proteomics

Proteomics is the large-scale study of proteomes. A proteome is a set of proteins produced in an organism, system, or biological context.



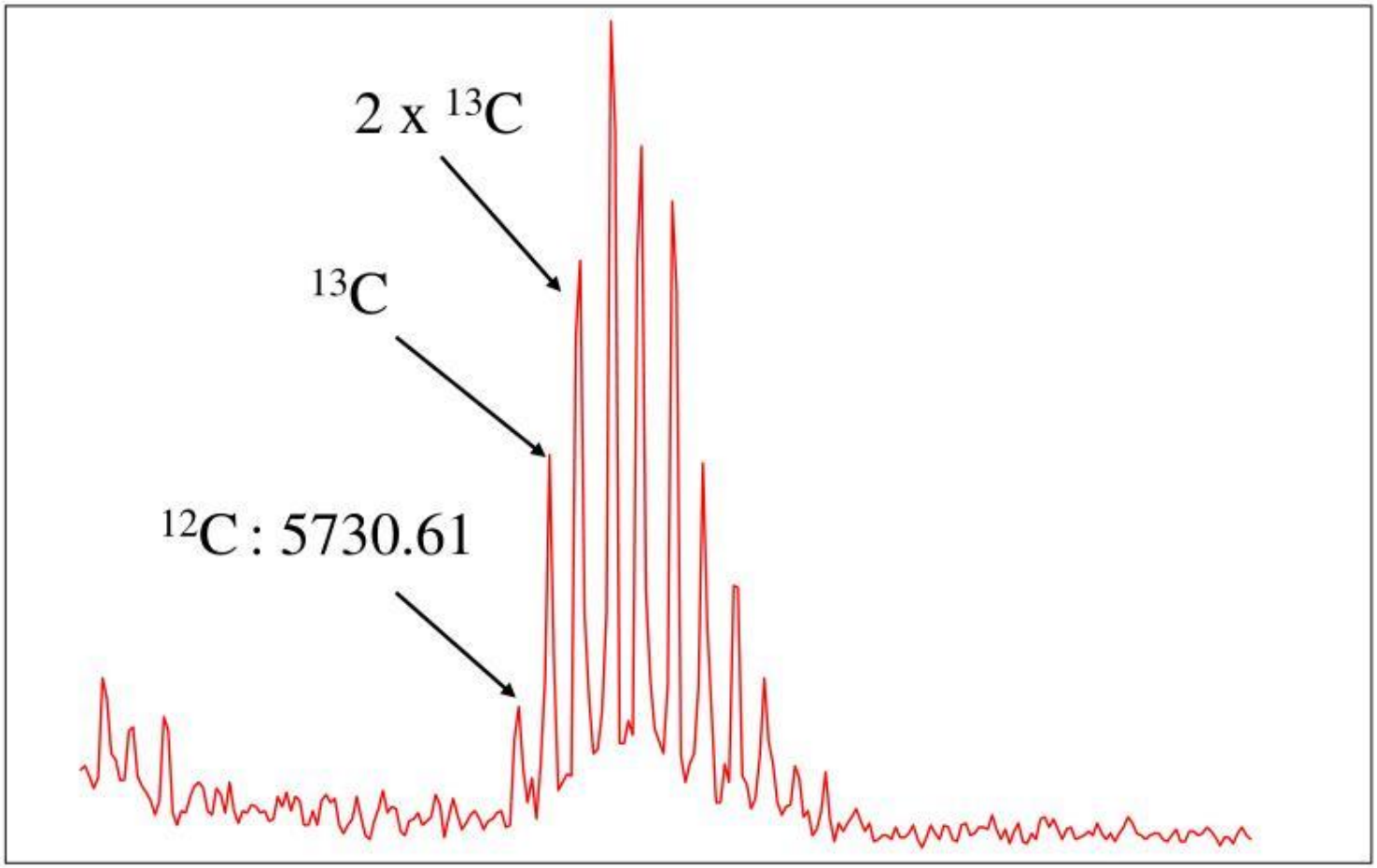
A two-dimensional electrophoregram with visualized thousands of proteins can be considered the first proteome – it was the first type of analysis in which the entire set of proteins from a biological sample was determined (or their significant proportion).





Ghodasara et al., 2017

Mass spectrum of insulin



Proteomics

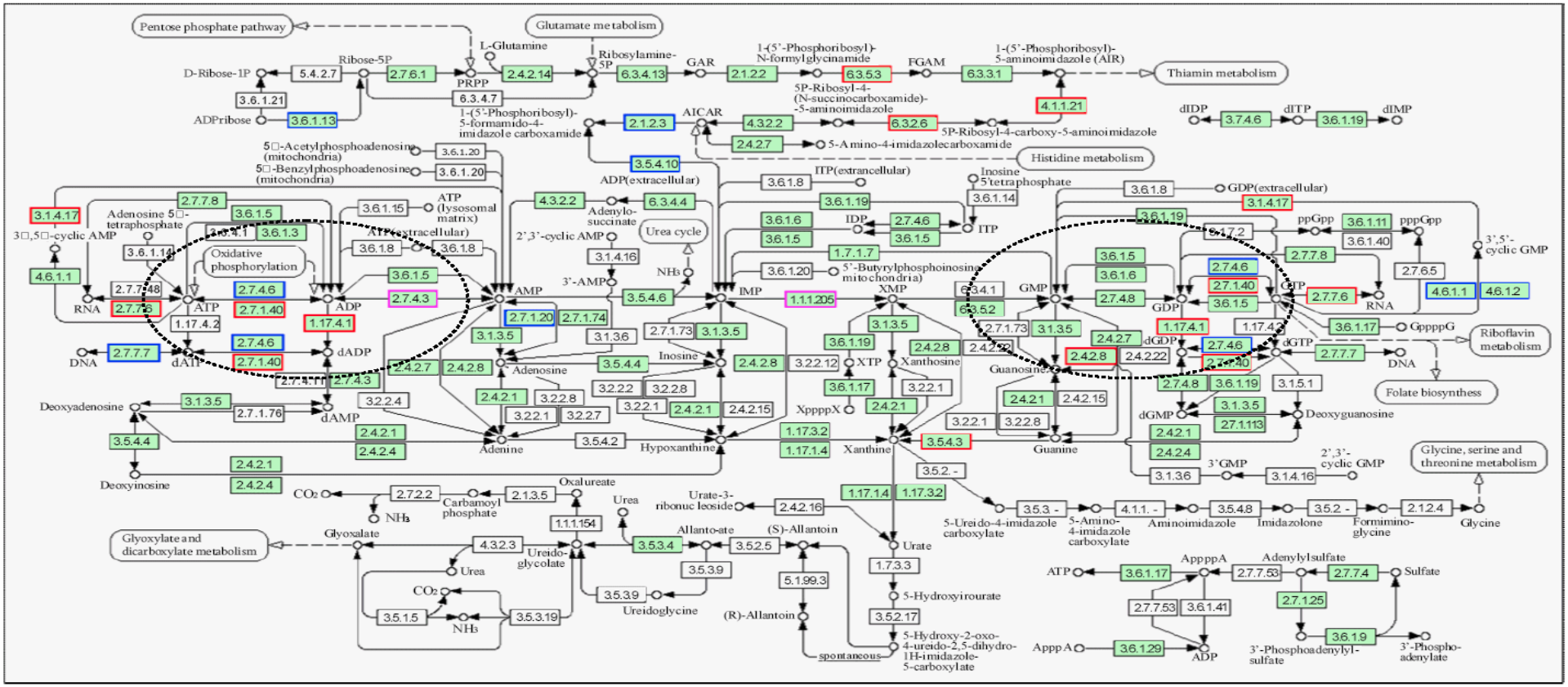


Figure 4. Differentially expressed enzymes in purine metabolism identified from irradiated AT5BIVA and ATCL8 cells. Enzyme Commission numbers (EC#, e.g. 1.17.4.1) are used to represent enzymes in metabolism. Highlighted in green background are known human enzymes annotated in the KEGG database. Differentially expressed enzymes in purine metabolism (Table 3) are superimposed onto this pathway diagram: blue-boxed are enzymes changed in AT5BIVA cells, red-boxed those in ATCL8 cells, and pink-boxed those from both cells. Areas circled with broken lines highlight closely related biochemical steps surrounding ADP/ATP (left) or GDP/GTP (right) metabolisms, which include most of these differentially expressed enzymes from either cell type.

Hu et al., 2008. Integrated Bioinformatics for Radiation-Induced Pathway Analysis from Proteomics and Microarray Data

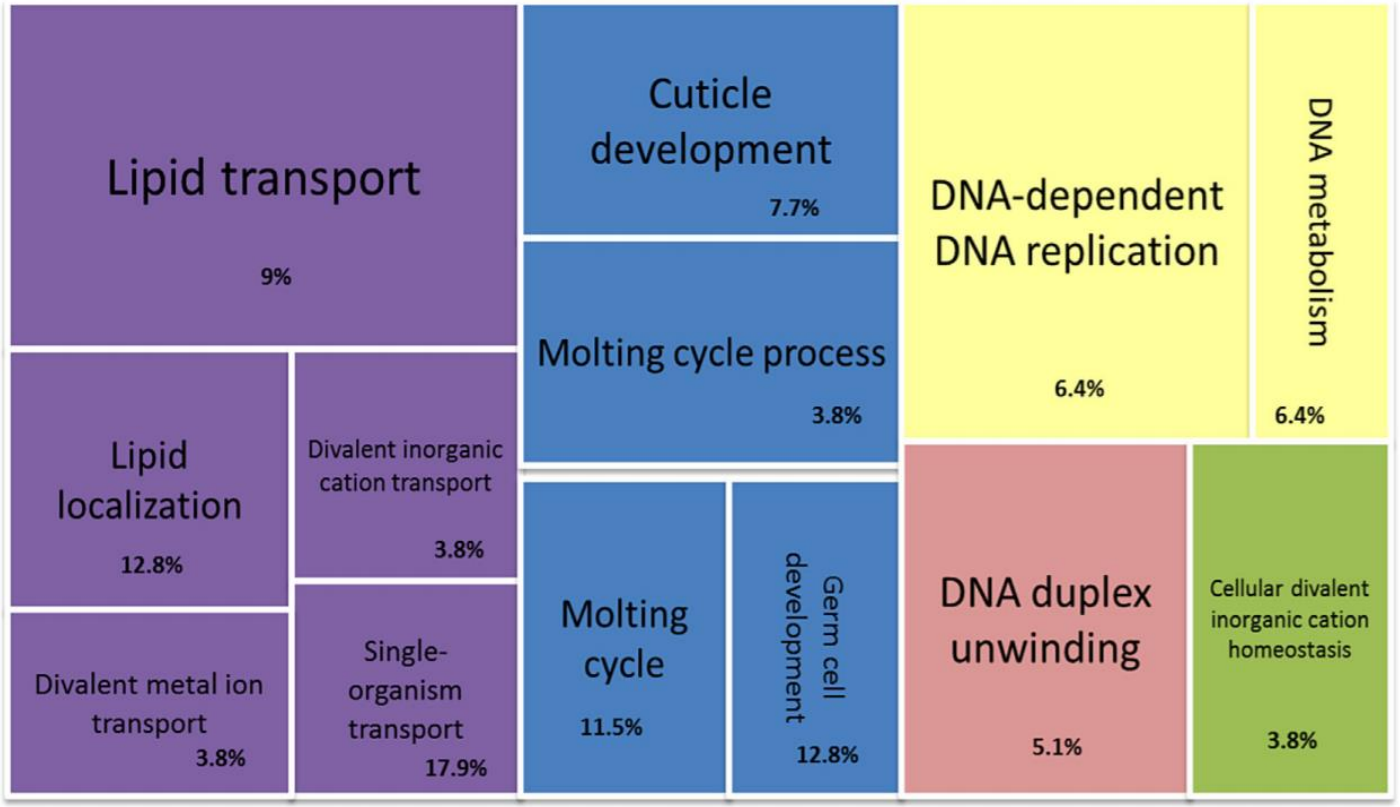
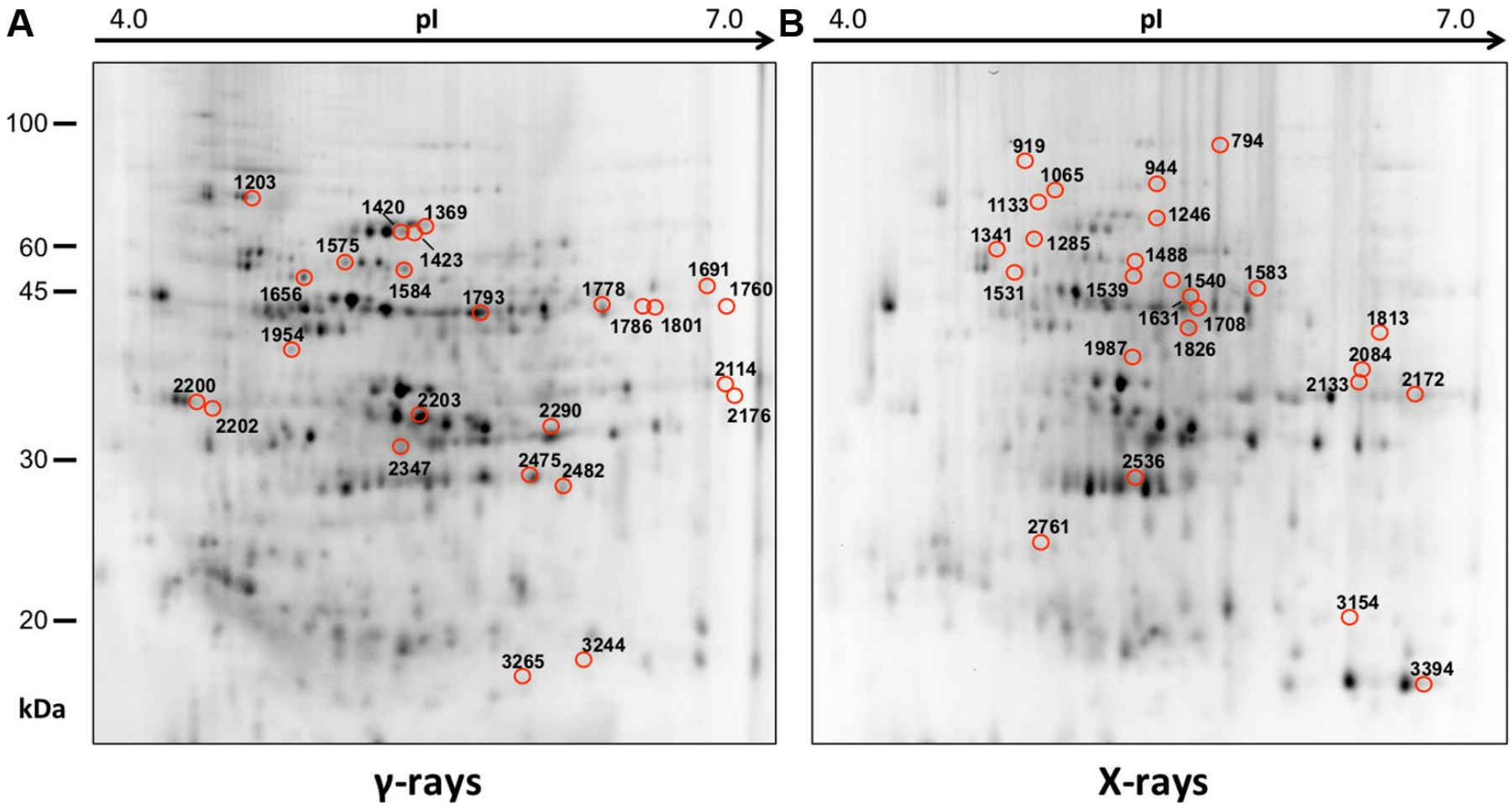
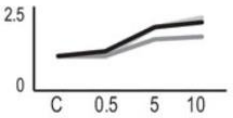
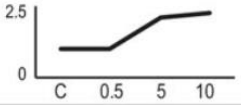
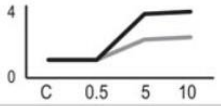
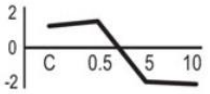
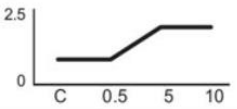
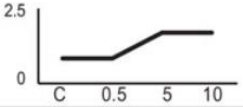


Fig. 2. Independent DAVID gene functional enrichment analysis based on results of all proteins identified as modulated after chronic irradiation (*t*-test, pairwise comparison). Significant GO-term biological processes (p. value <0.05) were then summarized using REVIGO. The % of proteins involved in the process is written in each case. Tree maps show a two-level hierarchy of GO terms (main clusters and cluster members); the size of the rectangles is relative to the log10 (p. value) absolute. With a same color, biological processes belonging to one main head process: lipid transport (purple), cuticle development (blue), DNA-dependent DNA replication (yellow).

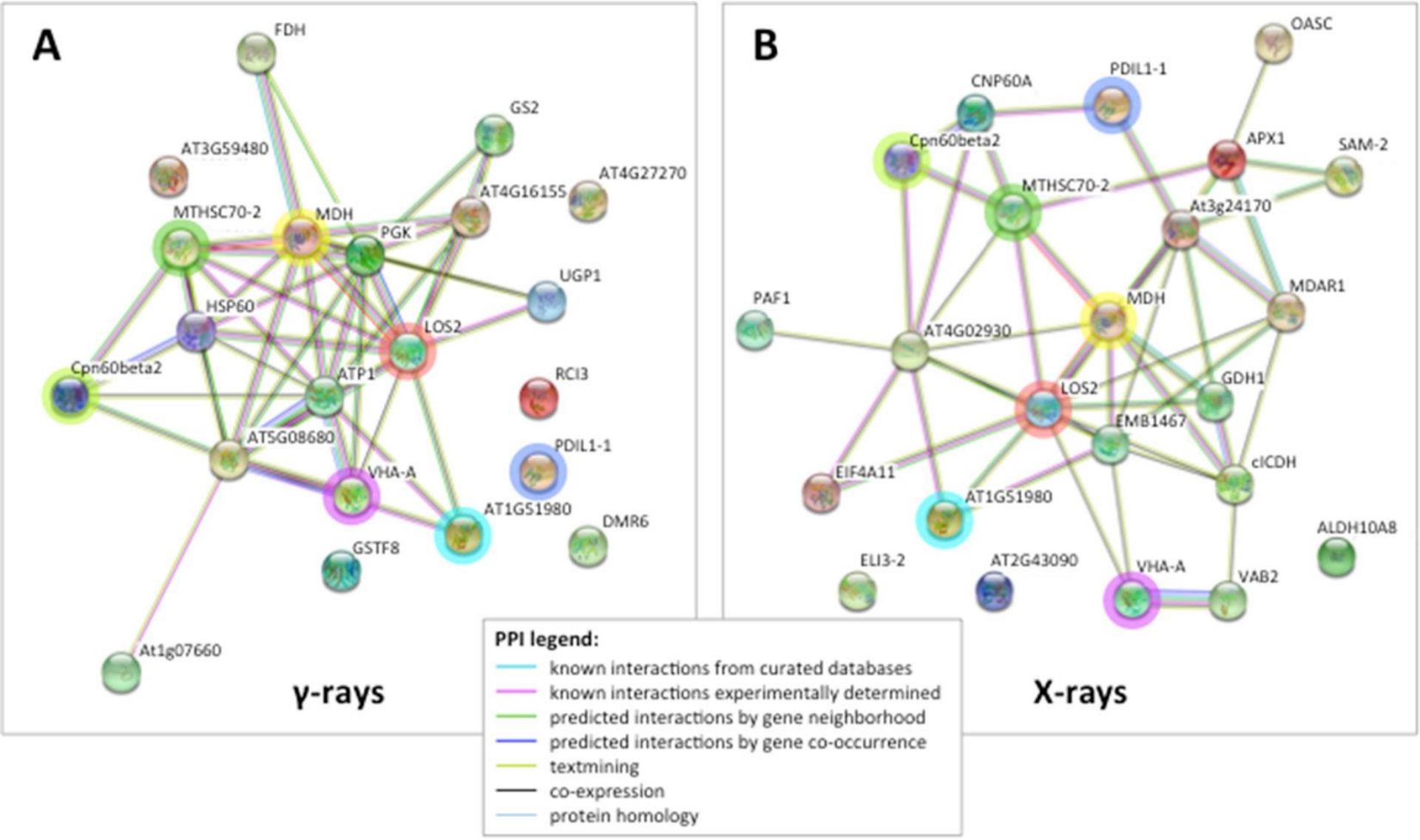
Dubois et al., 2019. Differential modification of the *C. elegans* proteome in response to acute and chronic gamma radiation: Link with reproduction decline



Desiderio et al., 2019. Effects of Simulated Space Radiations on the Tomato Root Proteome

Spot number	Protein identification	Accession number	Abbreviation (STRING reference)	Functional category	Relative protein abundance[1]	Average ratio[1] (0.5 Gy; 5 Gy; 10 Gy/not exposed)	pI[2] (Theor/Exp)	MW[3] (kDa–Theor/ Exp)	Coverage/N. peptides[4]
1203 1778	Enolase	AT2G36530.1	LOS2	Carbon metabolism		1203: 1.0; 1.9; 2.1 1778: 1.0; 1.9; 2.2	1203: 5.68/5.15 1778: 5.68/5.91	1203: 48.054/72.119 1778: 48 054/44.802	1203: 23.6/7 1778: 51.4/23
1203 1778 1786 1801	UDP-glucose pyrophosphorylase	AT3G03250.1	UGP1	Carbon metabolism		1786: 1.0; 1.5; 1.6 1801: 1.2; 1.9; 2.0	1203: 5.84/5.15 1778: 5.84/5.91 1786: 5.84/6.02 1801: 5.84/6.13	1203: 52.014/72.119 1778: 52.014/44.802 1786: 52.014/44.599 1801: 52.014/44.206	1203: 15.1/5 1778: 43.8/22 1786: 22.6/9 1801: 41.1/18
1369	Heat shock protein 70, mitochondrial	AT5G09590.1	MTHSC70-2	Protein folding/ refolding		1369: 1.0; 2.1; 2.3	1369: 5.75/5.52	1369: 73.153/65.870	1369: 27.0/17
1420 1423	ATP synthase subunit A, vacuol	AT1G78900.2	VHA-A	Amino acid metabolism		1420: 1.0; 2.1; 2.2 1423: 1.0; 3.5; 3.6	1420: 5.20/5.42 1423: 5.20/5.51	1420: 68.798/64.219 1423: 68.798/64.023	1420: 46.2/29 1423: 32.6/18
1575	Heat shock protein 60	AT3G23990.1	HSP60	Protein folding/ refolding		1575: 1.3; -1.7; -1.8	1575: 5.80/5.35	1575: 61.438/58.531	1575: 45.5/25
1584	TCP-1/cpn60 chaperonin family protein	AT3G13470.1	Cpn60beta2	Protein folding/ refolding		1584: 1.0; 2.3; 2.3	1584: 5.72/5.48	1584: 63.238/51.622	1584: 40.6/24
1656	Protein disulfide isomerase	AT1G21750.2	PDIL1-1	Protein folding/ refolding		1656: 1.0; 2.0; 2.1	1656: 4.96/5.22	1656: 49.612/48.553	1656: 42.2/15
1691	ATP synthase subunit 1	ATMG01190.1	ATP1	Amino acid metabolism		1691: 1.1; 1.6; 1.8	1691: 5.30/6.52	1691: 18.381/46.021	1691: 48.3/8

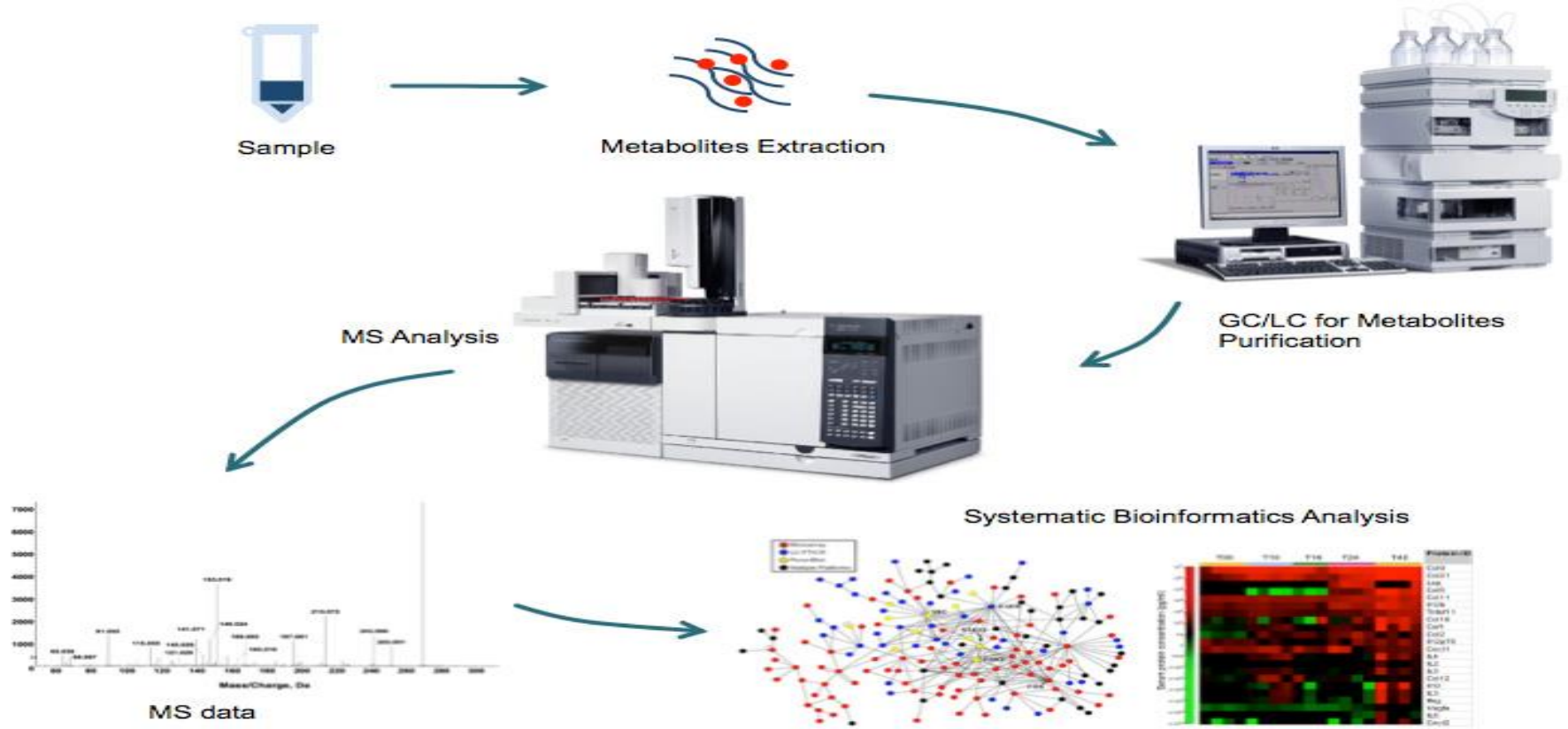
Desiderio et al., 2019. Effects of Simulated Space Radiations on the Tomato Root Proteome



Desiderio et al., 2019. Effects of Simulated Space Radiations on the Tomato Root Proteome

Metabolomics

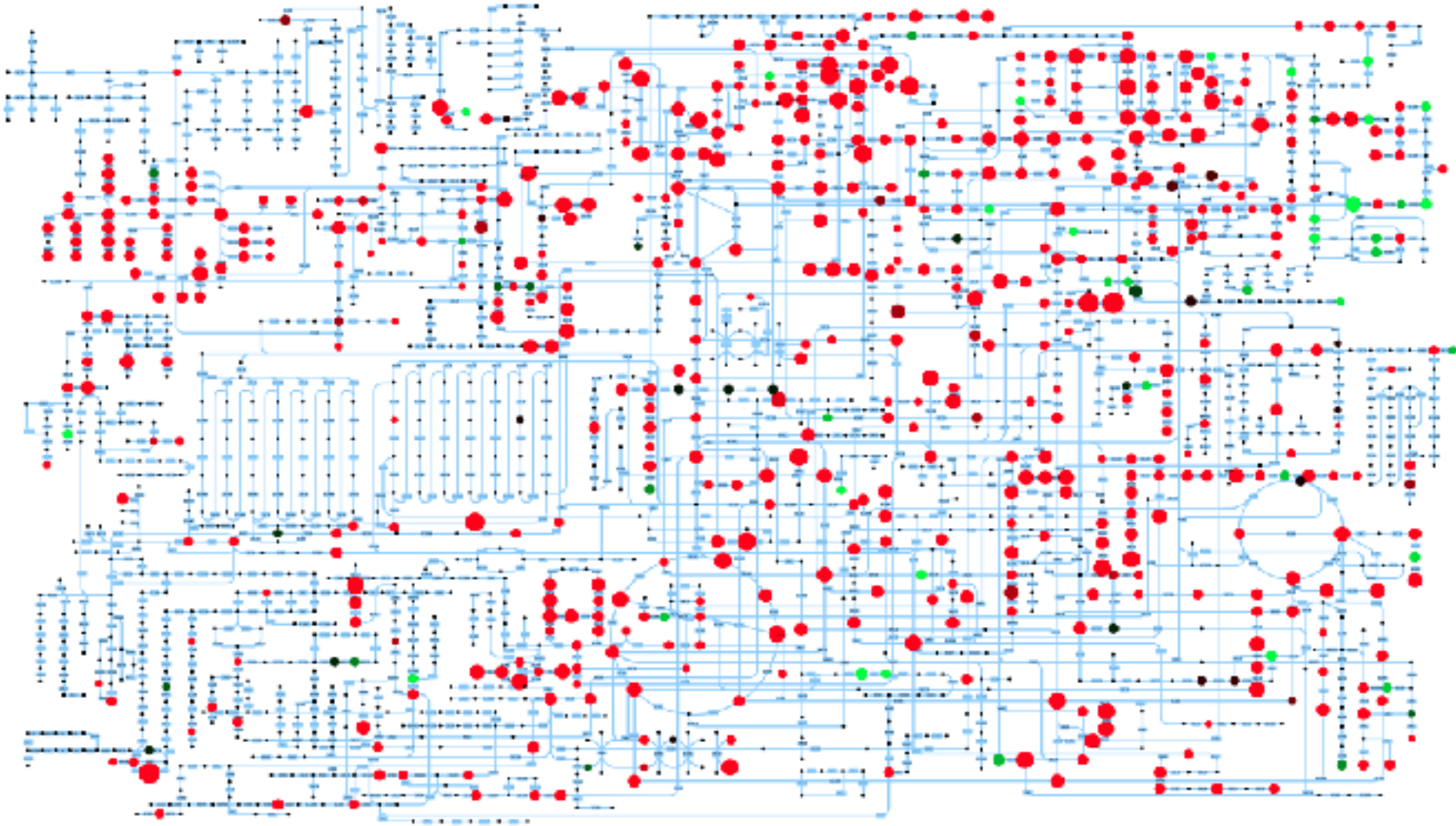
Metabolomics studies the totality of all metabolites in a cell, tissue, organ or the whole organism at a given time and their interaction with other functional levels.



The word "metabolome" describes a set of small metabolite molecules that can be found in a cell, tissue or the whole body. Metabolites include molecules with a molecular weight less than 1 kDa (as small peptides, some hormones, antibiotics, lipids, and other secondary metabolites).

The metabolic approach is a powerful tool in the diagnosis and prognosis of diseases, since metabolites and their concentrations, unlike other “omics”, directly reflect the biochemical processes that occur in cells and tissues. Thus, the metabolome is a molecular phenotype.

Metabolomics



http://www.imsb.ethz.ch

Metabolomics

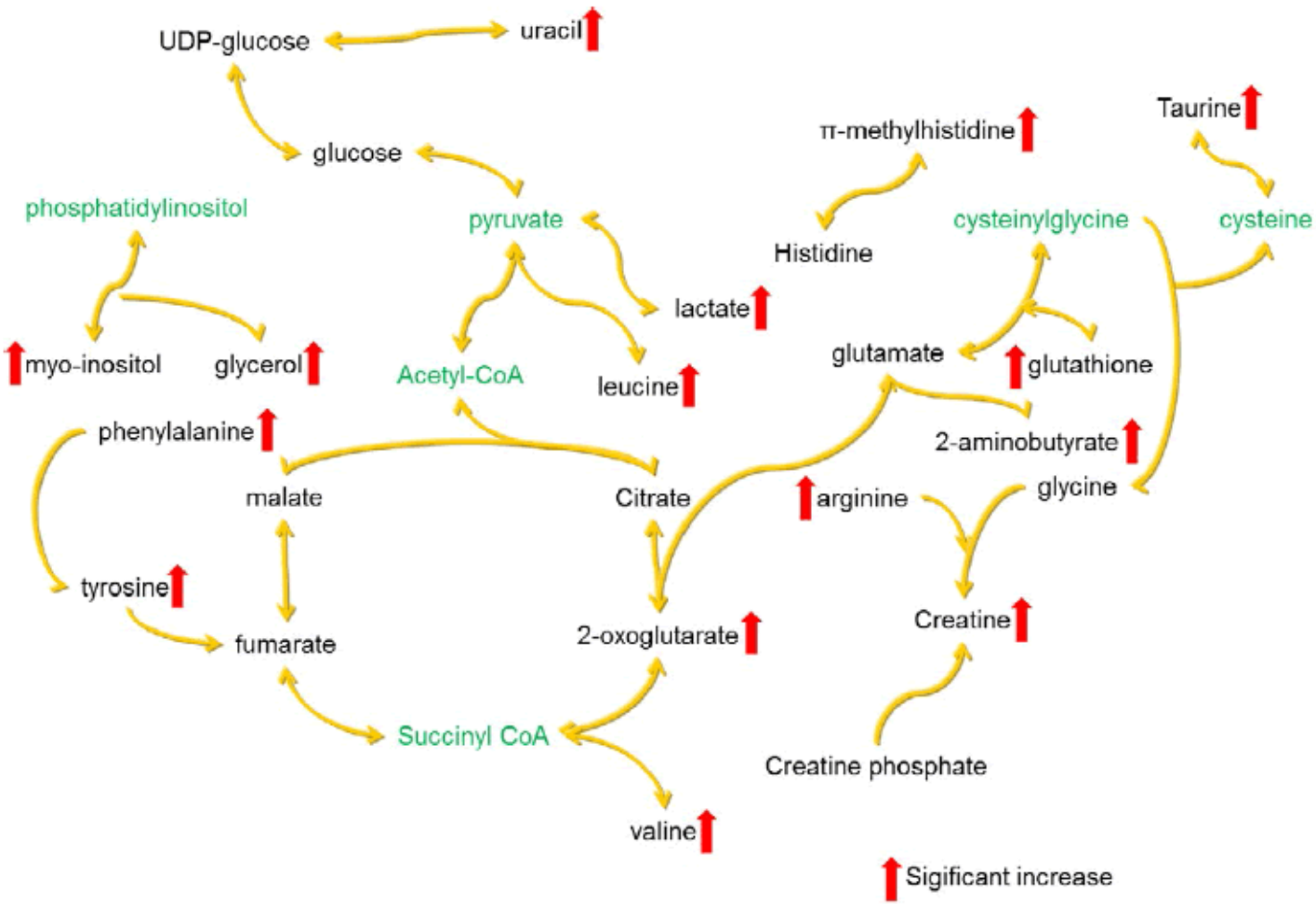
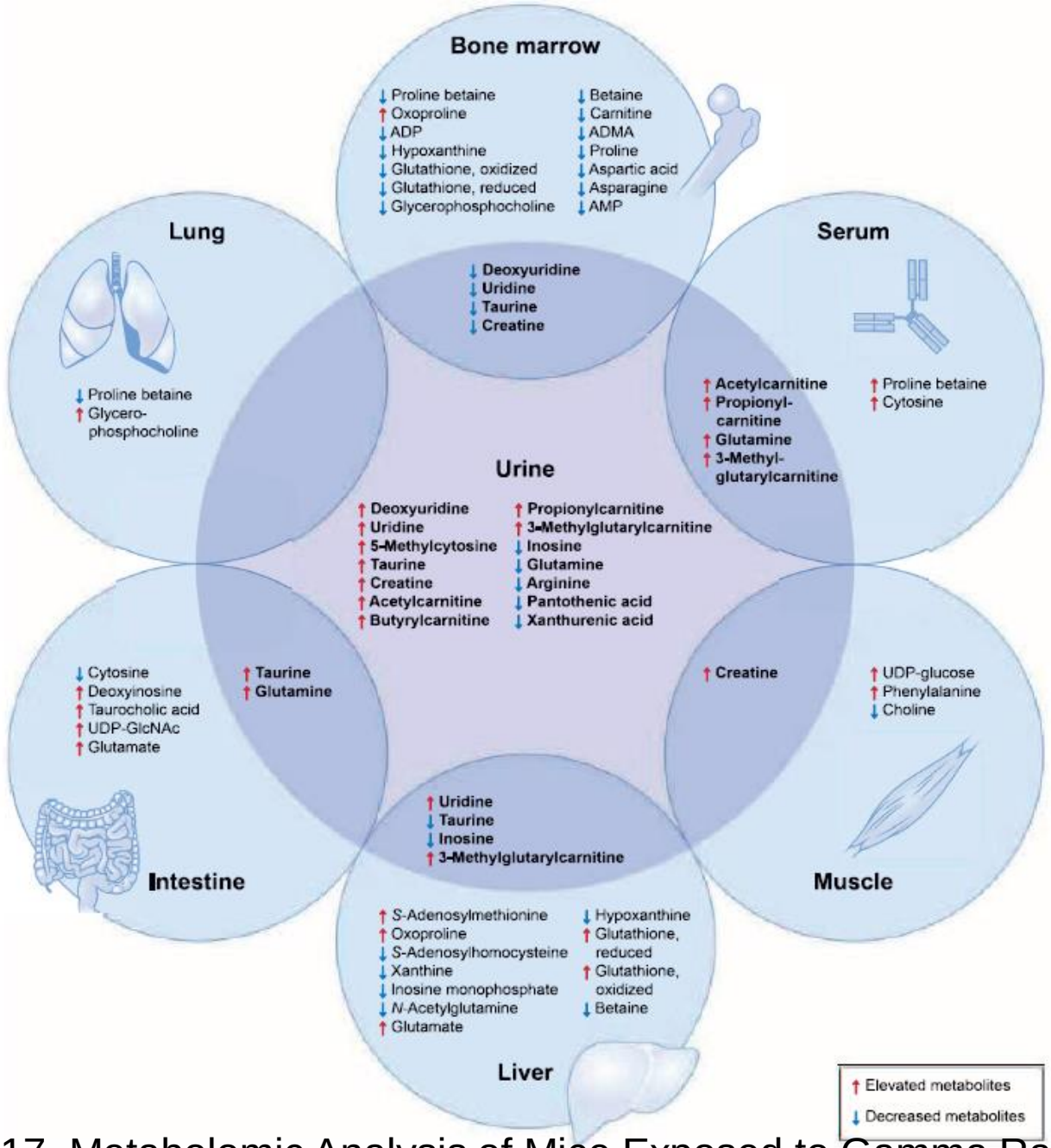
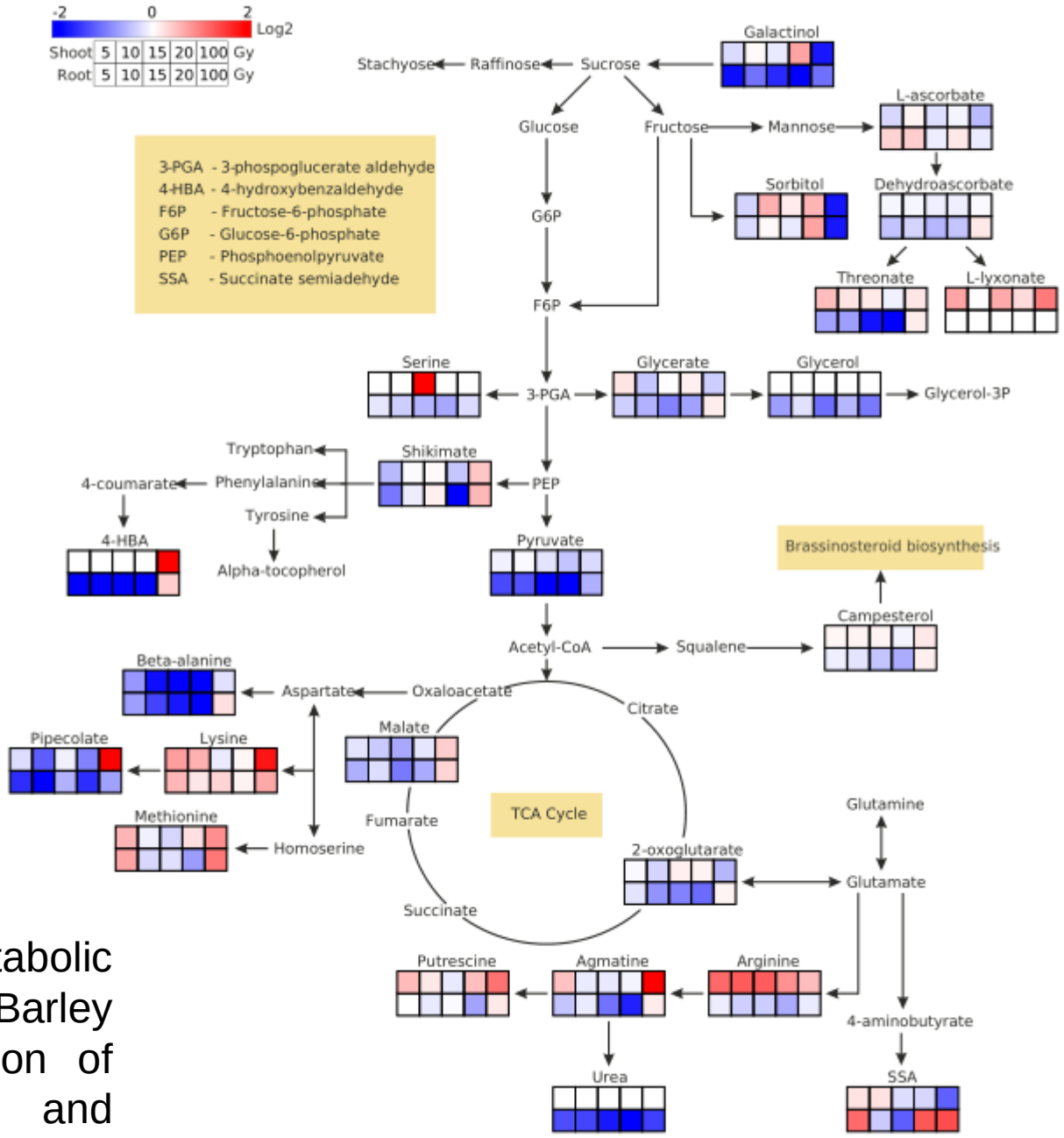


Figure 4: Proposed metabolic pathway networks associated with the significantly altered metabolites after exposed to gamma radiation based on the findings from this work and the diverse metabolic fates depicted in the small molecule pathway database (SMPDB) (http://www.smpdb.ca/). Metabolites colored green are not detected.

Xiao et al., 2016. ^{1}H NMR Metabolomics Study of Spleen from C57BL/6 Mice Exposed to Gamma Radiation



Golla et al., 2017. Metabolomic Analysis of Mice Exposed to Gamma Radiation Reveals a Systemic Understanding of Total-Body Exposure



Volkova et al., 2020. Metabolic Profiling of γ-Irradiated Barley Plants Identifies Reallocation of Nitrogen Metabolism and Metabolic Stress Response

The advantage of omic technologies over traditional approaches is their ability to simultaneously analyze a large number of molecules and get a holistic picture of the biological process or developmental stage, integrating data obtained from different omic levels.

Resources for self-education:

A Brief Guide to Genomics
https://www.genome.gov/about-genomics/fact-sheets/A-Brief-Guide-to-Genomics

Online courses for independent study in bioinformatics and omic-technologies
https://edu.t-bio.info/

Proteomics Academy
https://www.proteomics-academy.org/online-education

Metabolomics Workbench
https://www.metabolomicsworkbench.org/training/online.php