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The *CRISPR* game: the latest hit in genetics.

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Author Block: Alexandra Iliadi[1], Paraskevi Ioannidou[1], George TSOLAKIS[2]
[1]3rd General Lyceum of Nea Ionia, Nea Ionia [Athens], Greece[2]3rd General Lyceum of Nea Ionia, Athens, Greece

Essay:
The galloping advances in genetics during this decade have led scientists to the development of a method characterized by such a subtle precision in genome interference of almost any living species, human included, that we get effortlessly puzzled to wonder: what extent are life scientists of our post-genomic era open to get to? The technique in question, known as *CRISPR/Cas9* [C/C9], was put on the map so soon after the detailed description of the native bacterial process on which is based, and since then has been so sweepingly implemented by life scientists as enthusiastically happened with PCR in the past.[1,2,3,4]
In certain bacteria and archaea, the C/C9 system consists of two molecular components: the **CRISPR** region (***C****lustered* ***R****egularly* ***I****nterspaced* ***S****hort* ***P****alindromic* ***R****epeats*) of the prokaryotic genome and the **Cas9** DNA endonuclease. Their role: both components cooperate as a molecular defense mechanism against invading DNA molecules, mainly those from bacteriophages. The bacterial host cells under attack develop resistance against the intruding forces when they integrate fragments of the viral genome within their CRISPR locus [this is the 1st stage]. In the 2nd stage, the CRISPR region containing the foreign DNA information is transcribed into CRISPR-RNA molecules (crRNAs) that become attached to the Cas9 enzyme guiding the latter to the invading sequences. In the 3rd stage, the guided

endonuclease recognizes its target via base homology (of □20 nucleotides length) and pairing between the crRNA and the invading genome, which is then snipped and destroyed by Cas, thus averting the host cells from further infective phage hijacking. It is this very mechanism that scientist have been exploiting and refining upon as a method for editing genes/other genomic regions and other Cas9-based applications in any model eukaryotic cell type: by synthesizing a chimeric single guide RNA (sgRNA) which combines the crRNA properties with a customized 20 nucleotides region able to base pair with the DNA sequence of interest, they can retarget Cas9 to any genomic locus where the endonuclease is free to act by creating double-stranded breaks. The rest of the task, i.e. DNA repairing of the snipped sequence, is undertaken by the intrinsic cellular mechanisms leading to site-specific sequence mutagenesis and corrections. [5,6]

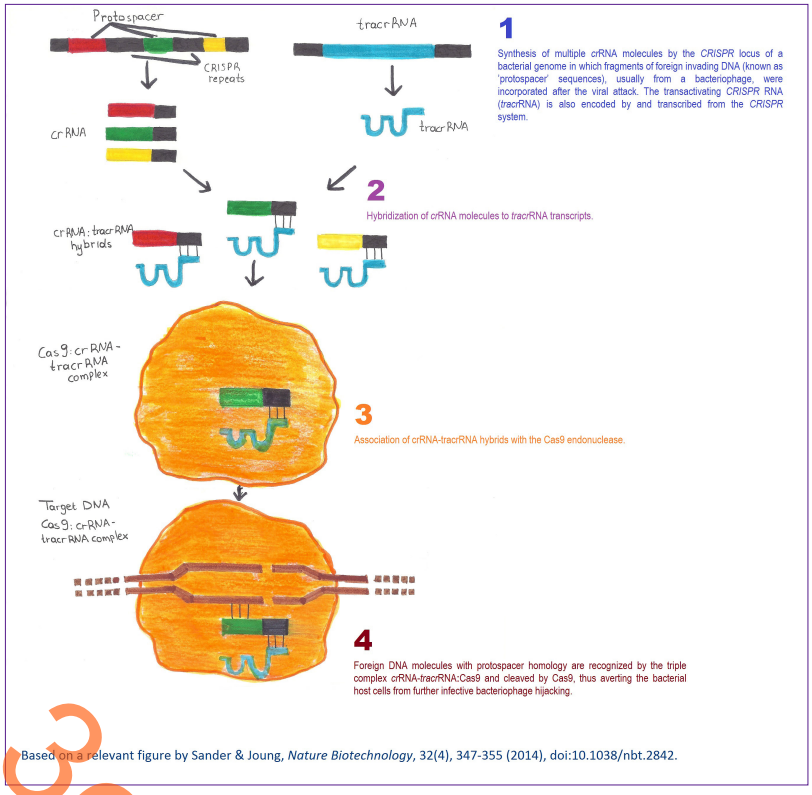
Genome editing, a new term coined for the controlled revising interference in the genomes of organisms, is greatly boosted by the C/C9 technique. We deem that the impact of this method in the near future is going to be even stronger than today that the meaning of genome editing will coincide with any C/C9 application. The so far comfortable repair of dysfunctional genes by C/C9 in human cell lines, in laboratory mice, or even in human embryos opens new frontiers for the treatment not only of major genetic diseases but also of ailments like cancer or viral diseases that still tantalize humanity.[7] In this respect, C/C9 sounds as a blessing; however, ethical issues raised by bioethicists focus on how this versatile method should be applied

i) with the highest degree of safety for the individuals to benefit from it therapeutically, and ii) without misuse for predetermination of genetic traits, something which ultimately leads to *positive eugenics*.[8,9]

The application of the C/C9 method in the world of animals promises well in the days to come and is full of impressive achievements, ranging from genetically modified mosquitoes unable to transmit malaria or other deadly parasites, engineered hornless cows with 'elite' meat quality or high dairy yield, lab animals for modeling human diseases, up to improved pets and ‘de-extinction’ animals (like mammoth-mimicking elephants)![10] Yet more ethical concerns have been set off about such animal interferences: the unknown ecological impact on wild populations (*gene drives*) of releasing such insects in nature, or the likely harmed animal well-being are aspects of this theme that should be taken seriously into consideration.

Plant genome editing by C/C9 technology has not been left behind, perhaps with a less revolutionary impact than in humans/animals. For sure, higher crop yields, better plant resistance to pests, or increased nutritional benefit are desired agricultural traits that always went hand-in-hand with a sustainable increase in global food production. Will CRIPSR/Cas9 make the difference compared with the classical genetic engineering methods? Time will tell until the dispute on whether crops produced by CRIPSR/Cas9 should be classified as genetically modified organisms is settled by regulatory authorities.[11]

The important ethical questions raised by the possible effects of the progress made by C/C9 system, we believe, should always be guided by the common good, the respect to nature/organisms, and the ancient Greek “μέτρον ἄριστον”[12].



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ESHG c/o Vienna Medical Academy
Alser Strasse 4, A-1090 Vienna, Austria
Tel: (+43/1) 405 13 83-22
Fax (+43/1) 407 82 74

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