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NON-PHOTOSYNTHETIC *EUGLENA LONGA* REQUIRES AN INTACT PLASTID GENOME FOR THE SURVIVAL IN CONTRAST TO PHOTOSYNTHETIC *EUGLENA GRACILIS*

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Abstract

This report is a short summary of the original article by HADARIOVÁ et al. (2017) entitled "An intact plastid genome is essential for the survival of colorless *Euglena longa* but not *Euglena gracilis*". Wild type strains of the flagellate *E. gracilis* possess three-membrane-bounded photosynthetic chloroplasts of secondary green algal origin. *E. longa* is its close relative possessing non-photosynthetic plastids with a plastid genome. The treatment of *E. gracilis* with antibacterial drugs such as ofloxacin or streptomycin leads to permanent plastid gene loss and bleaching of this flagellate without affecting its growth and viability. In contrast, the treatment of *E. longa* with ofloxacin or streptomycin, which is also accompanied by the loss of plastid genes, kills this non-photosynthetic flagellate. These results suggest that an intact plastid genome is obligatory for the survival of *E. longa* but not *E. gracilis*. A molecular mechanism of an "intermittent bleaching" was proposed for an explanation for the plastid genome reduction in *E. longa*.

Keywords: *Euglena gracilis*, *Euglena longa*, bleaching, antibacterial drugs, plastid genome reduction

The plastids of the flagellate *Euglena gracilis* are bounded by three membranes and they arose through a process of secondary endosymbiosis between a photosynthetic prasinophyte alga (HRDÁ et al. 2012; TURMEL et al. 2009) and an ancestrally heterotrophic euglenozoan host (AHMADINEJAD et al. 2007; VESTEG et al. 2010; O'NEILL et al. 2015a, b; YOSHIDA et al., 2016). The circular plastid genome of *E. gracilis* is of cyanobacterial origin and it is 143 kb in size (HALLICK et al. 1993). There are approximately 200–1000 plastid genomes copies per one *E. gracilis* cell (RAWSON & BOERMA 1976) distributed among approximately ten plastids. This plastid genome encodes one copy of each polypetide-encoding gene, but the rRNA genes are present in

three copies. The growth of *E. gracilis* in the presence of antibacterial drugs such as streptomycin (SM) or ofloxacin (OFL) leads to the process termed bleaching – the irreversible loss of the ability to form green colonies (the loss of photosynthetic ability) (POLÓNYI et al. 1998; for review see KRAJČOVIČ et al. 2002). The influence of various antibacterial agents on plastids (as well as on bacteria) differs. OFL is a specific inhibitor of plastid (and bacterial) DNA gyrase and SM is a specific inhibitor of plastid (and bacterial) protein synthesis (SCHWARTZBACH & SCHIFF 1974).

Euglena (formerly *Astasia*) *longa* is a non-photosynthetic close relative of *E. gracilis*. It was considered to be a naturally bleached *E. gracilis* in the past (for review see BODYL 1996). This opinion challenged after the discovery of a circular 73 kb *E. longa* plastid genome (GOCKEL & HACHTEL 2000). All genes encoding photosynthesis-related protein were lost from the *E. longa* plastid genome, except for the *rbc*L gene encoding a large subunit of RuBisCo. This reduced plastid genome is transcribed (GOCKEL et al. 1994; GOCKEL & HACHTEL 2000).

The goal of the study of HADARIOVÁ et al. (2017) was to compare the influence of SM and OFL on the growth, viability and the plastid DNA content of *E. longa* and *E. gracilis*. The relative number of selected plastid genes (*rrn*16, *rrn*23, *rpl*2, *rpl*16, *rpo*C2, *tuf*A and *rbc*L) in both *Euglena* species treated with SM or OFL was determined by quantitative (real-time) PCR. The treatment of *E. gracilis* with SM or OFL resulted in bleaching and the rapid decrease of the copy number of all studied plastid genes except rpl16 without any influence on viability, growth and copy number of nuclear genes even after six weeks of antibiotic treatments. In contrast, *E. longa* was completely killed during the third week and the fifth week of SM and OFL treatment, respectively. A gradual decrease of the copy number of all studied plastid genes in *E. longa* cells had been observed during SM and OFL treatment, before the cells died. These results suggest that the loss of plastid genes (e.g. induced by antibiotics) is lethal for *E. longa* but not for *E. gracilis*.

The function of the reduced but essential plastid genome of colorless *E. longa* is currently unknown, but it is likely that it encodes at least one protein necessary for *E. longa* survival that has to be expressed. This protein(s) is (are) likely involved in an essential plastid-localized metabolic pathway(s). Such an essential metabolic pathway is unlikely to be localized solely in plastids of *E. gracilis*, but the same or similar metabolic pathway is likely rather localized in another *E. gracilis* compartment (i.e. in cytoplasm), what makes the loss of *E. gracilis* plastid genome if not the entire plastid compartment possible.

The essential non-photosynthetic *E. longa* plastid is currently not assumed to have evolved by the bleaching process. It is more likely that a selective loss of photosynthetic plastid genes (all but one) rather than a random extensive gene loss as during the bleaching process of *E. gracilis* led to the evolution of the reduced *E. longa* plastid genome. HADARIOVÁ et al. (2017) have proposed a molecular mechanism for the *E. longa* plastid genome reduction – an "intermittent bleaching" – the repeated exposures of the *E. longa* ancestor to subsaturating concentrations of reversible bleaching agents followed by the periods of growth without them. After the loss of a single photosynthetic gene, all other photosynthetic could have been lost during repeated rounds of intermittent bleaching, if they were not required for the survival. In contrast, the loss of essential non-photosynthetic plastid genes such as those involved in the expression of at least one essential plastid gene would be lethal and thus only *E. longa* cells retaining them survived.

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References

AHMADINEJAD N., DAGAN T., MARTIN W. 2007: Genome history in the symbiotic hybrid *Euglena gracilis*. — Gene 402 (1-2): 35-39.

BODYL A. 1996: Is the origin of *Astasia longa* an example of the inheritance of acquired characteristics? — Acta Protozoologica 35 (2): 87-94.

GOCKEL G. & HACHTEL W. 2000: Complete gene map of the plastid genome of the nonphotosynthetic euglenoid flagellate *Astasia longa*. — Protist 151 (4): 347-351.

GOCKEL G., HACHTEL W., BAIER S., FLISS C., HENKE M. 1994: Genes for chloroplast apparatus are conserved in the reduced 73-kb plastid DNA of the nonphotosynthetic euglenoid flagellate *Astasia longa*. — Current Genetics 26 (3): 256-262.

HADARIOVÁ L., VESTEG M., BIRČÁK E., SCHWARTZBACH S. D., KRAJČOVIČ J. 2017: An intact plastid genome is essential for the survival of colorless *Euglena longa* but not *Euglena gracilis*. — Current Genetics 63 (2): 331-341.

HALLICK R. B., HONG L., DRAGER R. G., FAVREAU M. R., MONFORT A., ORSAT B., SPIELMANN A., STUTZ E. 1993: Complete sequence of *Euglena gracilis* chloroplast DNA. — Nucleic Acids Research 21 (15): 3537-3544.

HRDÁ Š., FOUSEK J., SZABOVÁ J., HAMPL V., VLČEK Č. 2012: The plastid genome of *Eutreptiella* provides a window into the process of secondary endosymbiosis of plastid in Euglenids. — PLoS One 7 (3): e33746.

KRAJČOVIČ J., EBRINGER L., SCHWARTZBACH S. D. 2002: Reversion of endosymbiosis? — Pp. 185-206. In: Seckbach J. (eds.): Symbiosis: mechanisms and models. Cellular origin in extreme habitats. Springer Netherlands, Dordrecht, 796 pp.

O'NEILL E. C., TRICK M., HENRISSAT B., FIELD R. A. 2015a: *Euglena* in time: evolution, control of central metabolic processes and multidomain proteins in carbohydrate and natural product biochemistry. — Perspectives in Science 6: 84-93.

O'NEILL E. C., TRICK M., HILL L., REJZEK M., DUSI R. G., HAMILTON C. J., ZIMBA P. V., HENRISSAT B., FIELD R. A. 2015b: The transcriptome of *Euglena gracilis* reveals unexpected metabolic capabilities for carbohydrate and natural product biochemistry. — Molecular BioSystems 11 (10): 2808-2820.

POLÓNYI J., EBRINGER L., DOBIAS J., KRAJČOVIČ J. 1998: Giant mitochondria in chloroplast-deprived *Euglena gracilis* late after N-succinimidylofloxacin treatment. Folia Microbiologica 43 (6): 661-666.

RAWSON J. R. Y. & BOERMA C. 1976: Infuence of growth condition upon the number of chloroplast DNA molecules in *Euglena gracilis*. Proceedings of the National Academy of Sciences USA 73 (7): 2401-2404.

SCHWARTZBACH S. D. & SCHIFF J. A. 1974: Chloroplast and cytoplasmic ribosomes of *Euglena*: binding of dihydrostreptomycin to chloroplast ribosomes. — Journal of Bacteriology 120 (1): 334-341.

TURMEL M., GAGNON M. C., O'KELLY C. J., OTIS C., LEMIEUX C. 2009: The chloroplast genomes of the green algae *Pyramimonas*, *Monomastix*, and *Pycnococcus* shed new light on the evolutionary history of prasinophytes and the origin of the secondary chloroplasts of euglenids. — Molecular Biology and Evolution 26 (3): 631-648.

VESTEG M., VACULA R., STEINER J. M., MATEÁŠIKOVÁ B., LÖFFELHARDT W., BREJOVÁ B., KRAJČOVIČ J. 2010: A possible role for short introns in the acquisition of stroma-targeting peptides in the flagellate *Euglena gracilis*. — DNA Research 17 (4): 223-231.

YOSHIDA Y., TOMIYAMA T., MARUTA T., TOMITA M., ISHIKAWA T., ARAKAWA K. 2016: De novo assembly and comparative transcriptome analysis of *Euglena gracilis* in response to anaerobic conditions. — BMC Genomics 17: 182.