Ve Vestci 13. ledna 2016

Posudek k diplomové práci Jana Vazače

Study on the chromosome number in the alveolate alga *Chromera velia* by TSA-FISH

Jan Vazač se ve své diplomové práci zabývá chromerou, evolučně velmi zajímavým organizmem příbuzným parazitickým výtrusovcům, který však obsahuje funkční plastid, fotosyntetizuje a žije pravděpodobně jako mutualista v buňkách korálů. Během výzkumu chromery se ukázalo, že tento organizmus je zajímavý v mnoha směrech. Nejinak tomu bude zřejmě také z hlediska karyotypu a organizace jádra jako takového, což se Jan Vazač ve své práci rozhodl studovat.

Cílem předložené práce bylo zjistit počet chromosomů a ploidii chromery metodami FISH a Southern blot, a to se podařilo. Chromera, přesněji řečeno její kokální stádium pěstované v kultuře, je podle všeho haploidní a jádro zřejmě obsahuje jen jeden obrovský chromosom. To je situace u eukaryot velmi neobvyklá.

Práce má klasické členění a je psána slušnou a čtivou angličtinou, ve které jsem našel jen malé množství chyb. Práce je dále doplněna kvalitní obrazovou dokumentací. V úvodu o přiměřeném rozsahu autor seznamuje čtenáře s chromerou, s životními i buněčnými cykly myzozoí, kam Chromera spadá, s uspořádáním genomu u těchto organizmů a konečně také se samotnou metodou FISH. Až na jednu drobnost jsem si nevšiml žádné nepravdy či nepřesnosti. Metodické postupy i výsledky jsou podrobně popsány. V kapitole diskuse autor výsledky i použité protokoly hodnotí a vyvozuje z nich závěry. Jasně tím ukazuje schopnost kriticky myslet a zasazovat získaná data do kontextu. Jeden ze závěrů však považuji za ukvapený a ještě se k němu vrátím v otázkách.

Mám-li diplomovou práci celkově zhodnotit, tak nemohu než konstatovat, že zadané cíle jasně splnila, obsahově i formou je na vysoké úrovni a jednoznačně splňuje nároky kladené a magisterskou práci. Doporučuji ji proto k obhajobě.

Vladimír Hampl

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Otázky a komentáře:

1. Na straně 4, kde autor popisuje životní cyklus apikomplex, tvrdí, že „First, a diploid zygote is formed inside a cyst after the fusion of male and female gametes. The encysted zygote enters a new host and meiotically divides…" To není pravda. Pokud je mi známo, encystovaná zygota „oocysta" pokaždé prochází meiózou (sporuluje) dříve, než se dostane do dalšího hostitele. Infekčním stádiem bývá vysporulovaná oocysta s haploidními sporozoity.
2. Proč byla na design SC3 próby použita sekvence topoizomerázy II z genomu *Toxoplasma gondii*? Sekvence *Chromera velia* není dostupná? Pokud opravdu není, proč byl tedy tento gen zvolen jako vhodný single copy gen pro analýzu ploidie?
3. Při pokusech s FISH sondami proti SC genům by mě zajímalo, kolik jader chromery bylo vyšetřeno? Očekával bych, že určité procento jader bude vykazovat dva signály, protože se buňky budou nacházet v G2 fázi životního cyklu. Jak je možné, že na žádné takové jádro autor nenarazil? Ze stejného důvodu bych očekával, že při FISH proti telomerám bude určité procento jader vykazovat čtyři signály.
4. Genom *Chromera velia* byl již publikován. Kolik lokusů s telomerami se v něm nachází?
5. Skutečnost, že sondy proti SC genům vytvořily na Southern blotu vždy jeden signál, považuje autor na podpůrný důkaz haploidie chromery. Podle mého názoru jde však jen o důkaz, že tyto geny mají v haploidním genomu jednu kopii. O ploidii to vůbec nevypovídá. I u diploidního organizmu by taková sonda vytvořila jeden pruh, protože značené fragmenty z obou genomových sad by byly stejně dlouhé.
6. Autor v diskusi přichází s myšlenkou, že *Chromera* „quite possibly lacks an interim diploid stadium in its life cycle, or, the diploid stadium could emerge only for a very short time or just sporadically". Dále dokonce píše, že „C. velia lost the interim diploid stadium". Z čeho tak soudí? Žádné jeho výsledky na toto přece neukazují. Jak sám píše v úvodu práce, životní cyklus chromery je poměrně komplexní a zahrnuje stádia cysty a zoospor, které nevyšetřoval. Zrovna stádium cysty by mohlo představovat diploidní částí cyklu, jak je tomu u obrněnek a apikomplex.

Seznam drobných chyb, překlepů nebo neobratných formulací:

Strana 2	Dinoflagellates are **of high ecological importance algae**….
Figure 3, legenda	The zoospores **exscyst** (h).
Strana 7	… apicomplexan invasion-related motility genes were co-regulated with **encoding** genes of flagellar apparatus…
Strana 20	… yield of approximately **75 µg/1 L** of culture.
Strana 29	… there was only one fluorescent signal in all observed **nukleus**.
Strana 36	Pernthalern and Pernthalern (2007) faced the same difficulties in **varied** environmental microbes
Strana 38	Apicomplexa and parasitic dinoflagellates such as **Oxyrrhis marina**… (Oxyrrhis není parazitický dinoflagelát).

RNDr. Iva Fuková, Ph.D.
Laboratory of molecular cytogenetics
Institute of Plant Molecular Biology, Biology Centre, CAS
Branišovská 31, 370 05 České Budějovice
Phone: (+420) 387 775 511
Fax: (+420) 385 310 356
E-mail: ifukova@umbr.cas.cz

Referee report on the master thesis: "Study on the chromosome number in the alveolate alga *Chromera velia* by TSA-FISH" by Bc. Jan Vazač

The topic of the thesis is a basic cytogenetic characteristics of a very interesting organism closely related to an important parasitic group of protists, Apicomplexa, green-brown alga *Chromera velia*. The alga *C. velia* has a high potential in addressing questions on the evolution of parasitic form of life in Apicomplexa and evolution of their specific organelle, apicoplast.

The introduction covers the background information for all topics the thesis deals with. I would only have a remark to the description of two chromosome landmarks in the chapter 1.4.2. (p. 10): A telomere and centromere. First, the author is mentioning telomere shortening: I would think the principal cause of telomere shortening is the incomplete replication of the lagging strand during each cell cycle although other environmental and genetic factors have their role as well. And second, I cannot agree with the statement that centromeres are missing in some organisms which the author supports with citations of works by Lysák (2014) and Tran et al. (2015). To me it seems as a misunderstanding of the two papers. They both describe the evolutionary dynamics and rearrangements of the centromere and hence the genome and karyotype. Centromere function is very conserved but in comparison with the telomere the underlying DNA sequence is indeed very far from that. It can be highly variable even between different chromosomes of one species. The centromere is established and maintained epigeneticaly. In any case it is not a good marker even in very close species.

Methods are mostly well described. Maybe only the separation of the subsections of chapter 3.5. "Southern blot" is a bit unfortunate. Dividing the chapter according to the system of detection may have been clearer for the reader. In the Methods' section there are only a few inaccuracies, e.g. the usage of 10 mM primers instead of 10 μM solutions (p. 15) or expresing the acceleration during centrifugation in revolutions per minute (rpm) on p. 17 onwards. In this case the use of the relative centrifugal force (rcf) in multiples of *g* is more accurate as rpm without an information on a rotor diameter is not very helpful. Beside that I wish to clarify a few things:

- What are the genbank accession numbers of the single-copy marker genes?

- Did the author make any gene models to be able to avoid intron sequences in hybridization probes?

- Geneious is a very nice platform but it would be more useful if the author wrote which tool or algorithm did he use. For example the note that he performed a blast search is more important than that it was done in Geneious (p. 13).

- Which camera was used for the image capturing? All the components of the imaging system are listed properly, only this information is missing.

- How many copies of a single-copy gene are in theory in 1 μg of *C. velia* genomic DNA? Which plasmid dilution corresponds to this?

The results are well documented. Figures placed in the text make it easier to follow the results description. Again, there are some inaccuracies and mistakes but I do not want to waste time listing everything and have only a few questions:

- How many nuclei did the author score? What was the percentage of nuclei showing the presented hybridization pattern?

- Why did the author use confocal microscopy? What new did it bring compared to the fluorescent microscope?

- Has the author tried to examine the ploidy in other life stages of *C. velia* by FISH? Has he tried to synchronize the nuclei to obtain metaphase chromosomes? How long is the cell cycle?

- From the absence of bands after the gDNA restriction digest and gel separation the author infers the cutting was random. What could he draw from a gel with some bands? Does it say anything about the analyzed DNA?

- Has author had a chance to test more probes with the DIG labeling and detection system? How did the gels look like before the Southern blot?

In the Discussion the author evaluates the results and provides some suggestions to tackle the problems encountered during the experimental work. I agree that the nuclei isolation is a crucial step. The failure to overcome the cell wall was probably also a reason why the flow cytometry was not successful to provide the accurate genome size information during the original research performed in the auhor's laboratory. I have the following comments to the improvements and possible explanations suggested by the author:

- What would be a better standard for the flow cytometry?

- I do not think there should be any concerns about increasing the probe length for FISH: The nick-translation used for the probe labeling leads to the template fragmentation. In such case I guess the introns may be more of a trouble. Their presence may be avoided by using cDNA as a template for the probe generation.

- Among the possible causes of the telomeric sequence detection failure by Southern blot the presence of other inserted sequences seems most likely. The interruption of the monomers or short oligomers of the Arabidopsis type of telomeric repeat by other minisatellites may cause excessive fragmentation of the target gDNA and its loss during gel separation. Presence of alternative telomeric repeats is also in line with the results presented by Fulnečková et al. (2013). In this work, a dot blot with *C. velia* gDNA shows multiple positive hybridization signals with a range of alternative telomeric sequences. On the other hand, the interspersed location of the major repeat would not necessarily hamper the detection on a dot blot or during FISH procedure. What optimization would the author suggest for the Southern blot?

Overall the presented work is written fluently and in detail with clearly documented and discussed results. Bc. Jan Vazač proved his laboratory skills in adopting various molecular techniques and also a great deal of patience and persistence while dealing with a non-model species. His results are necessary prerequisite for further work on the species and I believe his effort will be rewarded by success in case he continues the study. I recommend to grant Bc. Jan Vazač the Msc title and grade his thesis as excellent.

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Iva Fuková