Comments on dissertation titled

"Environmental and genetic aspects
of *Saccharomyces cerevisiae* yeast cells' differentiation"

submitted by Mgr. Monika Opałek to the Jagiellonian University in Krakow for a Doctoral degree.

This dissertation contains the findings of an experimental-cum-theoretical investigation into the phenomenon of quiescence as exhibited by yeast cells. It begins with a survey of previous published work; goes on to discuss non-quiescence, the counterpart of quiescence, and considers it, in conjunction with quiescence, as an evolved 'bet-hedging' trait; mentions the identification of candidate genes behind quiescence; and suggests how to address the question of measuring the lag phase that precedes growth and whose prolongation, in a sense, anticipates a quiescent period.

The work is original and comprehensive, and reflects very well on the scientific capabilities of the author. Based on my experience with doctoral dissertations, it has some unique features. The literature review goes much beyond what one expects. It contains a critical survey of the manner in which earlier workers have dealt operationally with the theme of quiescence – and finds that the relevant publications adopt diverse (and not always reconcilable) viewpoints. Then, unusually, it goes on to suggest what to do about the non-uniformity. Next, the work that is presented reflects a mix of mathematical modelling and experimental methods in equal measure, something that is rare. Finally, the last chapter, on estimating the duration of the lag phase, contains a sophisticated appreciation of a tricky subjective criterion as well as a decision tree for workers in the field. Namely, given that real data tend to be noisy, what is the optimal way of deciding when a measurement that has remained almost constant, begins to increase? Most of the thesis's content has already been published in good journals.

The results that are presented are of high quality and deserve to be acclaimed. I have no hesitation in recommending that the dissertation be accepted as it stands for the award of a doctoral degree **with distinction**.

Now let me address a few questions for Ms. Opałek to consider. They could be raised by whoever conducts the viva-voce, assuming there is one. I stress that the questions have nothing to do with my assessment of the work as stated above.

1. Quiescence and non-quiescence have been presented as (binary) alternative phenotypes with different temporal profiles after the onset of starvation. Can one

rule out the possibility that the data are equally well, or better, described by a continuously varying intracellular parameter that is reflected in the proportion of Q or NQ cells? Could it be that each cell in a population goes through a range of Q/(Q+NQ) that, for a 'normally growing' population, varies from almost 0 early in growth to almost 1 in stationary phase? If the answer is yes, the observed phenotypic heterogeneity would reflect cell-to-cell differences in an internal threshold which decides whether a particular cell falls on the Q side or the NQ side of a dividing line that is set by the experimenter.

2. Even if they are at a low density, cells will stop growing when nutrition runs out. They can also stop growing at high density because of the effects of crowding. Are the two forms of quiescence comparable? Is there any way of distinguishing between them?

3. Statements in several places refer to evolutionary advantage, adaptation, and fitness. Are they made with the individual cell or the population of cells in mind? Can Ms. Opałek provide reasons in support of her answer? Can the answer be tested by looking for the distribution of Q and NQ cells in a genetically heterogeneous, but still 'wild-type', population?

4. With regard to the genetic underpinnings of quiescence, is it possible to distinguish experimentally between genes with loss of function phenotypes from those with gain of function phenotypes? If so, how would such a classification help in analysing the data presented? (Incidentally, to which categories do the mutants described here belong?)

5. How might mutations that affect the Q:NQ ratio also affect the cell's energy state? Is it possible to make testable predictions based on the hypothesis that internal energy reserves should be inversely correlated with the probability of entering quiescence?

Minor point. If the dissertation is going to be made available to future readers, a few misprints need to be corrected. The one on page 139 is important, because it occurs in a formula that others may try to use. In the right-hand side of the equation for $d^2N(t_N)/dt^2$, $2N(t_N)$ has been wrongly shown as $N(t_N)$. The correct expression should be $[N(t_{N+1})+N(t_{N-1})-2N(t_N)]/[\{(t_{N+1}-t_{N-1})/2\}^2]$.

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