



Supervisor' review of dissertation thesis by Ambar Kachale

Ambar arrived in our lab directly from India. He was very young, restless, naive, enthusiastic, and interested in all aspects of life (sometimes too many). I gave him a topic that probably looked boring and, on top of that, not easy at all. I suggested to him that he will turn a weird trypanosome that we have at that time discovered in our liquid nitrogen (where it was for half a dozen of years) into a genetically tractable organism and that we will afterwards experimentally explore vagaries of its genetic code.

Ambar had pretty good background in cell and molecular biology and a decent notion about how research is done. He did not waste time and started playing with the culture, going early for a research stay at the University of Ostrava, trying to learn how to deal with these flagellates. The problem right from the start was that they grew terribly slowly. Fortunately, in collaboration with Jan Votypka from Charles University, he was able to accelerate them surprisingly quickly, and thus make them amenable for any further work.

Next critical step was much more difficult – to electroporate DNA into *Blastocrithidia*, have it transcribed, translated and stable. Numerous attempts led us to realize that at this stage we don't have time for that and that we shall transfer as much of the experiments as possible to the established model *Trypanosoma brucei*. The bioinformatic analysis of the genome, transcriptome and proteome of *Blastocrithidia*, done in parallel by another PhD student Anna Nenarokova (now at the University of Bristol), was very helpful and provided many questions to be experimentally addressed. And they seemed to orbit around tRNAs. This is where Ambar and all of us were lucky, since a tRNA expert worked right next doors. Zdenek Paris helped Ambar, on a truly daily basis, to extract tRNAs, run them on various types of gels, enrich for specific tRNA class, isolate mitochondrial tRNA etc. This is difficult molecular biology, where even a bad look can degrade the miniscule amounts of tRNAs, but in the hands of Ambar, it worked very well. In fact, he became soon an expert on tRNA gels, perfectionizing them in overnight gels with hourly changes of the buffer and other intricate adjustments. All this started producing intriguing results.

And that's when another of Ambar's strengths, of which neither he nor I was initially aware, came into play. He is a very critical and fast thinker, how frequently came to me or Zdenek with proposals of smart experiments and interpretation. After we decided to do this or that experiment, Ambar did not



waste time – he started doing it 10 minutes after the labmeeting. I can't stop myself from adding that he remained me of my youth in those moments.

Ambar is a rather rare species of a born scientist, on top of it with an artistic touch, to say the least. This is a creative combination with a promising potential, but it is also burdened with what usually goes with that – by which I mean certain level of disorganization and ignorance for some of life's necessities, also instantly grabbing whatever is needed for experiments wherever, which I and Zdenek are trying to teach him to overcome or at least to reflect on. Sometimes I simply had to give up – f.e. on my effort to convince Ambar to sign up in the lab book for insurance purposes, as everyone else. Importantly, this was not because he is arrogant or self-centered, quite the opposite; in his research-centered mind these are simply trivia not worth attention. Again, since I used to be similar, I have a lot of understanding for that. Another small weakness of Ambar is the writing, which due to his predilection for genuine bench work came later than usual. However, since Ambar is a very fast learner, I enjoyed correcting his thesis and seeing instant improvements.

Ambar is a nice person to be with, liked by his colleagues, and with a colorful social life. He is humble, listens to and respects others around him. I believe that unless he screws something, he has a big potential in science, and wish that it materializes.

Finally, I would like to state that the presented dissertation thesis fulfils, in my opinion, all postulations and I recommend it to be accepted as a partial fulfillment of the requirements for the degree of Doctor of Philosophy at the Faculty of Science of the University of South Bohemia.

Thanks Ambar for a great work, it was a joy to have you around, and all the best in your next life – although I hope you will still stay for a bit to reap the fruit of your ongoing work.

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