

**European Society of Photobiology  
Education and Training Program - Short-Term Fellowships 2004**

**Report of Maria Ntefidou**

This is a report on my visit to the lab of Margaret Ahmad in Paris carried out with the short term fellowship from the ESP. The duration of the visit was from November 15<sup>th</sup> to December 10<sup>th</sup>, 2004.

The aim of the visit was to express photoactivated adenylyl cyclase (PAC) in insect cells. In the past I had expressed different domains of PAC in *E. coli* but I still had not succeeded with expression of the full-length protein.

Two months before arrival Margaret sent me a transfer vector in which I have cloned separately the cDNA of PAC<sub>1</sub> and PAC<sub>2</sub>. In Paris I started to cultivate insect cells until the cells reached the appropriate density for transfection. At this point each of the plasmids containing PAC<sub>1</sub> and PAC<sub>2</sub> were co-transfected with a Baculovirus vector into the insect cells. By recombination the PAC genes were transferred from the transfer vector to the Baculovirus vector. After cultivation for a few days the cells were pelleted and the supernatant containing the recombinant Baculovirus (Baculovirus with either PAC<sub>1</sub> or PAC<sub>2</sub>) formed the primary transfectant used for all further steps. After amplification of the primary recombinant Baculovirus, new cells were co-infected with recombinant PAC<sub>1</sub> and PAC<sub>2</sub> and cultivated at different temperatures (27°C, 22°C, 20°C). At day 3, 4, 5 after infection samples were taken and the presence of PAC was determined with Western blot using PAC<sub>1</sub> and PAC<sub>2</sub> antibodies. It was shown that approximately half of the protein was insoluble and the other half of the recombinant protein was soluble. Transfected insect cells were taken back to Erlangen to further purify them and to determine activity. Additionally, we plan to infect a larger volume of cells for further experiments.

This month in the lab of M. Ahmad I had the opportunity to learn new techniques and we might be able to continue to work with this expression system.

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