Anemonia viridis primary cell culture: a new tool for cnidarian studies

Stéphanie BARNAY-VERDIER, Diane DALL'OSSO, Nathalie JOLI, Juliette OLIVRÉ, Fabrice PRIOUZEAU, Thamilla ZAMOUM, Pierre-Laurent MERLE and Paola FURLA

Adresses : UMR SAE 7138, UPMC/CNRS/MNHN/UNS, Equipe Symbiose Marine, Faculté des Sciences de Nice, Parc Valrose, 06108 Nice Cedex 02, France

Corresponding author : Stéphanie Barnay-Verdier, stephanie.barnay-verdier@upmc.fr

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Member of the cnidarian phylum, the temperate symbiotic sea anemone *Anemonia viridis* is a relevant experimental model to investigate, in a post-genomics approach, the molecular and cellular events involved in the preservation or in the rupture of the symbiosis between the animal cells and their symbiotic microalgae, named zooxanthellae (Sabourault *et al*., 2009; Ganot *et al*., 2011; Moya *et al*., 2012).

In this aim, we developed a primary culture from *A. viridis* epidermal and gastrodermal cells. By adapting and optimizing previous published methods, i.e. spontaneous or chemical dissociations (Frank *et al.,* 1994; Domart-Coulon *et al.,* 2004), we extracted cells from whole tentacle or from a separated epithelial cell layers corresponding to the epiderm or the gastroderm. Each plating resulted in a heterogeneous primary culture of different cell types as discharged cnidocytes, free zooxanthella cells (*A. viridis* symbiotes) and many regular, small rounded and adherent cells (of 3-5 μm diameter). The different culture observations showed that this last cell group contains *A. viridis* epithelial undifferentiated cells. Moreover, PCR analyses conducted on primary cultures, maintained for 2 weeks, confirmed a specific signature of *A. viridis*. In parallel, we evaluated the cell viability of these cultures by vital staining. Serial dilutions, led during 4 weeks, of re-suspended small rounded cells isolated using chemical dissociation allowed us to obtain a homogenous primary culture of *A. viridis* epithelial undifferentiated cells.

The maintenance and the propagation of this homogenous primary cell culture for several weeks provide suitable model for *in vitro* cnidarian studies and preliminary step for further investigations on symbiosis mechanisms.