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Short Communication

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The Sea Star IGKappa Cloning by the Use of E.Coli and CHO Protocol: A New Production of Young Protein

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ABSTRACT

This previous work has been performed with UMR-INRA ISP1282 (Nicolas Aubrey). It allows, by the use of CHO (Chinese Hamster Ovarian) protocol cloning to produce in a relative great quantity of the Young Protein or anti-HRP (Horse-Radish Peroxydase) from the sea star IGKappa gene which corresponds to the IPA: Invertebrate Primitive Antibody.

Keywords: Immunology, Protein, Antibody, Cells.

INTRODUCTION

10 years ago, we tried to clone, for the first time, the sea star IGKappa gene by the use and the help of E.coli as amplificator [1]. It allowed, in a second time, to verify that the Young Protein, or anti-HRP Protein recognizes the HRP antigen [1].

But, this verification of the affinity between the IPA (Invertebrate Primitive Antibody) and the antigen, seemed unclear at that time, for many of us. Thus, we decided to operate a new cloning [2] of the IGKappa gene with new parameters and new affinity tests. This second one did not allow to obtain the protein of interest or Young Protein. We attempt, in these conditions, a third assay: It used a CHO protocol, as described in various experiments [3].

RESULTS

First, the percentage of Young Protein production, was greatly ameliorated with the CHO protocol. A best rate occurs when compared to the E.coli one sensu stricto.

A western-blot determined exactly the M.W of the Young Protein, as shown in Figure 1: It is 12,49271 Kda.

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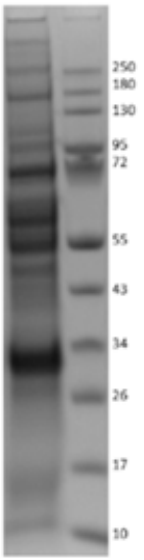

Figure 1. Purification of Young Protein (Production en ExpiCHO. Western Blot)

DISCUSSION

We think now to perform an Elisa test to verify the affinity between this young protein and the HRP antigen. In first analysis this Elisa seems positive: It would be the second time a primitive invertebrate antibody recognizes the antigen HRP [1]. It's almost incredible for many immunologists!

We envisage also (My colleagues and me) in a next future, to immunize other sea stars with anti-tumoral antigens to product specific nanobodies [4] from sea stars, against cancer activity (in a general way): sequencing and cloning, after ''ll be applicated to obtain a specific recombinant specific protein we 'll test against cancerous cells.

We envisage the future with serenity.

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