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Production of Recombinant Hormones and Growth Factors for Use in Aquaculture

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Extended Abstract

Progress in recombinant DNA technology and development of new expression systems has provided valuable means to produce large quantities of hormones and growth factors from various fish species that otherwise required sacrifice of enormous numbers of fish to obtain only small amounts of the native protein. The motivation to produce recombinant hormones and growth factors for application to fish culture can be attributed to (a) the low amounts of peptides in producing tissues available for purification and characterization by classical methods, i.e., follicle stimulating hormone (FSH = GtH-I) in the pituitary or insulin-like growth factor-I (IGF-I) and IGF-II in the liver, (b) the similarity in chemical structure between two hormones that does not permit efficient separation (FSH and LH = GtH-II), (c) the need for large quantities of hormones for growth enhancement by growth hormone (GH), and (d) interest in studying structure/function relationships, i.e., single or multiple amino acid deletion, amino acid substitution, etc.

The first two recombinant fish GHs were produced twenty years ago from salmon and rainbow trout. Since then, numerous fish GH peptides have been produced from a variety of species, most of them cultured fish species (for a partial list see Funkenstein, 2000, which covers only those reported until 1999). New species are constantly being added to this list as additional GH cDNAs are cloned from fish.

In contrast to the increasing number of recombinant fish GHs produced so far, relatively few studies describe the production of fish recombinant IGFs (Funkenstein, 2000). Yet, the need for fish IGF-I and IGF-II, mainly for developing homologous, or at least of fish origin, quantitative assays has prompted the production of additional IGFs. The shortage of fish IGFs provided the incentive to the Australian company GroPep (www.gropep.com.au) to undertake production of recombinant fish GHs and IGFs from a variety of cultured fish species.

In general, production of recombinant proteins involves cloning of cDNA coding for the desired peptides in appropriate expression vectors. The choice of vectors depends on the expression system employed. An important consideration in the choice of vector and system depends on characteristics of the peptide in question such as post-translational modifications (glycosylation) or formation of

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homo- or heterodimers for biological activity. The most commonly used system is the bacterial system: *E. coli* strains specifically designed for protein expression. The bacterial expression system had been used for hormones and growth factors such as GH, prolactin, somatolactin, IGF-I, and IGF-II, all of which do not present problems in refolding and purification and result in a biologically active peptide. By contrast, production of recombinant FSH and LH lagged behind, mainly due to their complicated production procedure that requires post-translational modification (glycosylation) and the need for α and β subunits to associate non-covalently. The bacterial system is not suitable for this type of peptide and it is only recently through the use of other expression systems (the yeast *Pichia pastoris* and the S2 Drosophila cell line) that functional recombinant gonadotropins were obtained for cultured fish (Kamei et al., 2003; Zmora et al., 2003; Kasuto and Levavi-Sivan, 2005).

Another growth factor that presents a challenge for expression using the bacterial system is myostatin, known also as growth and differentiation factor-8 (GDF-8), and a member of the transforming growth factor-β superfamily. This peptide is produced *in vivo* as a precursor having a signal peptide, an N-terminal prodomain, and a C-terminal mature domain. For myostatin to be active, it needs to be cleaved from the signal peptide and the prodomain, be refolded, and form a disulfide-bonded homo-dimer. As we recently discovered (Funkenstein et al., 2006), the mature peptide (C-terminal) cannot be refolded properly into the disulfide bonded dimer following expression in bacteria, and it is only the precursor form that can be refolded properly. The refolded protein needs then to be cleaved by furin-like enzymes to yield the mature active dimer.

In addition to yeast and insect expression systems, a widely used system in cases where post-translational modifications are needed (glycosylation, disulfide bond formation, and proteolytic processing) are mammalian cell lines like CHO-K1 and HEK293. In the case of myostatin, cells transfected with a DNA containing the precursor form secrete into the medium the mature dimer (C-terminal) and its binding protein prodomain (N-terminal). Work is in progress in our lab to produce both recombinant myostatin and follistatin (a binding protein to myostatin) using the mammalian expression system.

Another aspect of significance in the process of producing recombinant hormones and growth factors is testing their biological activity. This can be achieved by using *in vitro* (cell culture, receptor binding assays) or *in vivo* (induction or stimulation of various physiological responses) assays. However, when employing biological assays with fish peptides, an important decision is whether to use a homologous (or at least fish) or heterologous (mammalian) test system. The second is related to reagents used for comparison, usually of mammalian origin.

The hormones and growth factors mentioned above were produced from a large variety of fish species currently used in the aquaculture or mariculture industry. Among these are the common carp, Indian major carp, tilapia, gilthead sea bream, red sea bream, tuna, barramundi, coho salmon, rainbow trout, turbot, flounder, striped bass, sea bass, and eel.

Potential uses of recombinant hormones and growth factors in aquaculture can vary from enhancing growth rate (GH) to developing quantitative assays such as radioimmunoassay (RIA) and enzyme linked-immunosorbent assay (ELISA) for determination of plasma and tissue levels of the specific peptides under various physiological conditions such as stress, reproduction, adaptation to salinity, nutritional state, etc.

In conclusion, the main factors that should be considered when producing and employing recombinant hormones are cost effective production methods, efficient methods for renaturation (folding) of the recombinant peptides and purification, appropriate assays to determine their biological activity, extension of half-life, and methods of delivery (injection, diet, immersion and osmotic shock, immersion and ultrasound).

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