Characterization of a gonad-specific transforming growth factor-β superfamily member differentially expressed during the reproductive cycle of the oyster *Crassostrea gigas*

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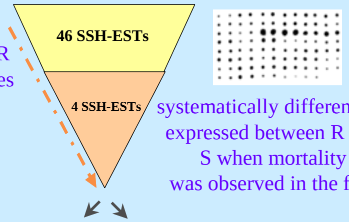
Introduction

To study the complex and non identified host causes of summer mortality in *C. gigas*, divergent selection criteria based upon summer survival have been applied to produce resistant (R) and susceptible (S) oyster families (Samain et al., 2007). A previous analysis by suppression-subtractive hybridization has characterized 46 differentially-expressed cDNAs between R and S families (Huvet et al., 2004). The present study reports the screening, using nylon array, of the 46 oyster SSH cDNAs from R and S selected families. Among the few differentially expressed genes characterized between R and S samples, one encodes a Transforming-Growth-Factor-β-related (TGFβ) protein. Members of the TGFβ superfamily are critical growth factors regulating a variety of important processes. We report the characterization and the spatio-temporal expression of *oyster-gonadal-TGF*β *-like* (*og-TGF*β*-like*), a TGFβ superfamily member specifically expressed in the gonad of the cupped oyster, *C. gigas*.

Results

1) cDNA macroarray analysis

cDNA macroarray hybridized by R and S samples collected in 4 experiments to focus on the genetic effect (4 and 16 month-old oysters of 2^{d} and 3^{d} generations, collected in two sites, and in a nursery)



Screening of R and S progenies sampled in several conditions

46 SSH-ESTs

4 SSH-ESTs

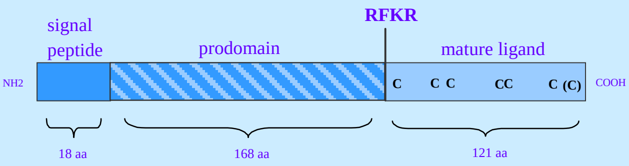
systematically differentially expressed between R and S when mortality was observed in the field

3 ESTs encode unknown protein

1 EST encode putative member of the **TGFβ superfamily** →

- full length cDNA
- spatio temporal expression
- localization

2) Isolation of *og-TGFβ-like* cDNA



Nucleotide sequence encoding a preproprotein of 307 amino acids

- consensus cleavage site type « RXXR »
- 6 conserved cysteine (lacks the seventh characteristic cysteine)
- percentage of identity with other related ligands very low

➡ **derived member of the TGFβ superfamily**

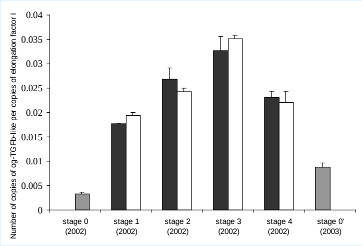
3) Spatio-temporal expression of *og-TGFβ-like*

➢ No significant relative level of transcript detected in any early oyster developmental stage

➢ High relative level of transcript detected **exclusively in the gonad,** and not in other tissues, of both male and female

➢ Relative quantity of transcript increased continuously during the development of gonadic tubules in both sexes (figure)

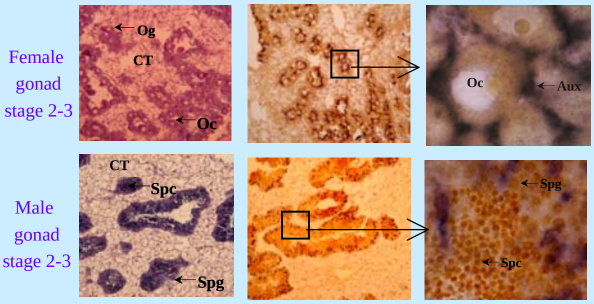
➡ **maximum level** during **active gametogenesis** (stage 3)



Level of relative *og-TGFβ-like* transcript in male (white bar) and female (black bar) during a complete annual reproductive cycle of *C. gigas*

4) Localization of *og-TGFβ-like* by *in situ* hybridization

og-TGFβ-like mRNA detected exclusively in cells **between oocytes**



CT: conjunctive tissu
Og: oogonia
Oc: oocyte
Aux: auxiliary cell

CT: conjunctive tissu
Spg: spermatogonia
Spc: spermatocytes

og-TGFβ-like mRNA detected exclusively in cells **between spermatogonia**

Conclusion

- ✓ *og-TGFβ-like* has only 6 conserved cysteine as observed for teleost gonadal soma-derived growth factor (Sawatari et al., 2006)
- ✓ tissue-specific expression, exclusively in the gonad of both sexes and not during developmental stages nor in other tissues
- ✓ strongly expressed when germ cells are fully mature
- ✓ localized in somatic cells surrounding the germ cells, supposed to play a regulatory role in the differentiation and maturation of germ cells

➡ **derived member of the TGFβ superfamily involved in gonadal development**

- ✓ summer mortality is suggested to interplay with reproduction
- ✓ significant differences in reproduction observed between R and S
- ✓ some TGFβ members (BMP15, GDF9) play crucial regulatory roles in ovarian follicle or testis development

Is that different expression of *og-TGFβ-like* significant to the higher rate of summer mortality of S progeny ?

Huvet et al., 2004, The identification of genes from the oyster *Crassostrea gigas* that are differentially expressed in progeny exhibiting opposed susceptibility to summer mortality, Gene
Samain et al., 2007, Genetically based resistance to summer mortality in the Pacific oyster (*Crassostrea gigas*) and its relationship with physiological, immunological characteristics and infection process. Aquaculture
Sawatari et al., 2006, A novel transforming growth factor-beta superfamily member expressed in gonadal somatic cells enhances primordial germ cell and spermatogonial proliferation in rainbow trout. Dev Biol