

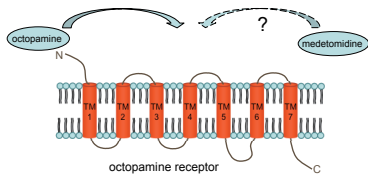
Cloning and expression of octopamine receptors from the barnacle *Balanus improvisus*



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Introduction

The octopaminergic system in invertebrates has been suggested to be the correlate of the adrenergic system in vertebrates and octopamine has been shown to be a major neuromodulator with neurotransmitter and neurohormone functions. Settling of the barnacle *Balanus improvisus* has been shown to be inhibited by the synthetic α_2 -adrenergic receptor agonist medetomidine. The action of medetomidine is believed to be mediated by its binding to octopamine receptors, which belong to the G-protein coupled receptor family. In *Drosophila melanogaster* two classes of octopamine receptors have been identified, one class is more similar to the α -adrenergic receptors in vertebrates and the other is more similar to the β -adrenergic receptors.

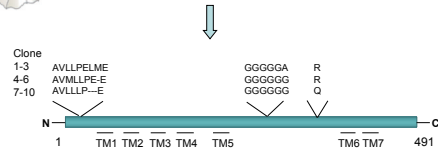


Aim

The aim is to clone octopamine receptors from *Balanus improvisus* and characterize them with regard to sequence and function. The receptors will be expressed in *Saccharomyces cerevisiae* and in mammalian cells. Studies to investigate if medetomidine binds to and activates these receptors will be performed.

Cloning of the *B. improvisus* octopamine receptor from a single adult

1x → Genomic DNA → Sequence 10 octopamine receptor clones

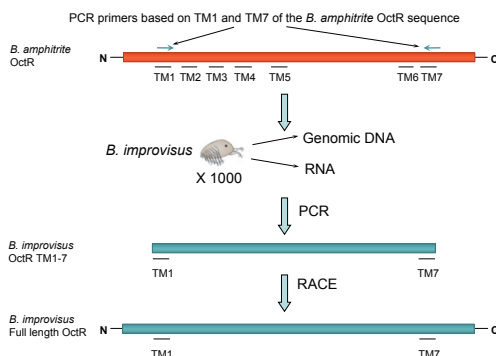


Three different sequences were found!



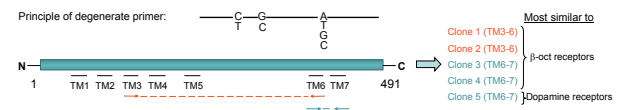
Three receptor sequences were found within one single adult. One possible reason that there were more than two sequences, which is expected for a diploid organism, could be the existence of isogenes.

Cloning of an α -adrenergic like *B. improvisus* octopamine receptor from cyprid larvae, based on sequence similarity with *B. amphitrite*



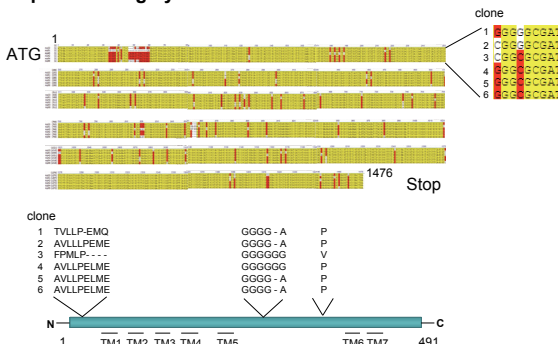
PCR primers based on the sequence of transmembrane region 1 and 7 (TM1 and TM7) of the previously cloned *B. amphitrite* octopamine receptor (Isoai et al 1996) were designed and used to clone the TM1-TM7 region of the octopamine receptor from *B. improvisus*. "Rapid Amplification of cDNA ends" (RACE) was then used to obtain the full length receptor.

Cloning of putative β -adrenergic like octopamine receptors in *B. improvisus* using degenerate primers annealing to conserved regions of biogenic amine receptors



PCR with degenerate primers annealing to conserved regions of biogenic amine receptors was performed on genomic DNA from *B. improvisus* cyprid larvae. Four of five receptor clones obtained showed highest sequence similarity to the β -adrenergic-like octopamine receptors.

Comparison of the nucleotide sequence of six receptor clones shows high sequence variability, whereas the amino acid sequence is highly conserved

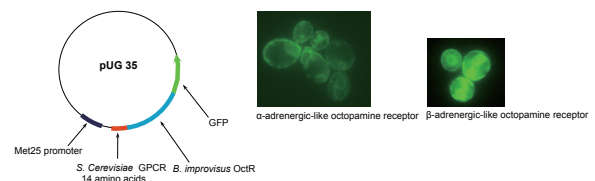


Comparison of the nucleotide sequences of six receptor clones revealed a high sequence variability. Positions with red markings show where one or more nucleotides differs between the clones. In the 1476 bp long sequence around 80 such differences were found between the six clones. Pairwise comparison gave 30-40 nucleotide differences. Despite the high variation at the nucleotide level, the amino acid sequence is very conserved and varies only within three regions.

Expression of cloned octopamine receptors in *Saccharomyces cerevisiae*

S. cerevisiae expression vector

Expression in *S. cerevisiae*



The pug35 vector was used to express the cloned octopamine receptors in *S. cerevisiae*. In this vector the receptor is expressed from the Met25 promoter as a fusion protein with green fluorescent protein (GFP). To enable expression, the receptor had to be N-terminally fused to a small part of an endogenous *S. cerevisiae* GPCR. The *B. improvisus* receptor is expressed, but most receptors seem to be located to the endoplasmic reticulum (ER) instead of the plasma membrane.

CONCLUSIONS

- The octopamine receptor in *B. improvisus* shows a high sequence variation at the nucleotide level, whereas the amino acid sequence is highly conserved
- Isogenes of the octopamine receptor in *B. improvisus* might exist
- Both α -adrenergic like and β -adrenergic-like octopamine receptors were cloned
- The cloned octopamine receptors can be expressed in *Saccharomyces cerevisiae*, but might be mislocated to the ER