# EXPRESSION OF $Na^+,K^+$-ATPase mRNA IN GILLS OF THE EURYHALINE CRAB *PACHYGRAPSUS MARMORATUS* ADAPTED TO LOW AND HIGH SALINITIES

Céline Spanings-Pierrot[1,2] and David W. Towle[2]
[1]Université Montpellier II, UMR 5000, 34095 Montpellier cédex 5, France
[2]Mount Desert Island Biological Laboratory, Salsbury Cove, ME 04672

$Na^+,K^+$-ATPase, a heterodimeric integral membrane protein composed of a catalytic α-subunit and a smaller glycosylated β-subunit, enables transepithelial ion transport either directly through movements of ions across epithelial cell membranes or indirectly through the generation of electrochemical gradients (Towle, J. Exp. Zool. 265: 387-396, 1993; Pequeux, J. Crust. Biol. 15: 1-60, 1995). In crustaceans, the $Na^+,K^+$-ATPase has been located in the basolateral membrane of gill epithelial ionocytes (Towle and Kays, J. Exp. Zool. 239: 311-318, 1986; Spanings-Pierrot et al., Amer. Zool. 40: 1217, 2000) where the enzyme pumps $Na^+$ from the cytosol into the hemolymph in exchange for $K^+$ or $NH_4^+$. An important role of $Na^+,K^+$-ATPase in osmoregulation has been demonstrated in different hyperosmoregulating crabs where the enzyme activity is much higher in ion-transporting posterior gills than in respiratory anterior gills. Moreover, the $Na^+,K^+$-ATPase activity increases following transfer from seawater to low salinity (Towle, In "Comparative Aspects of Sodium Cotransport Systems" 7: 241-263, 1990; Pequeux, 1995). Whether the difference in enzyme activity is due to changes in abundance of the $Na^+,K^+$-ATPase protein or to up-regulation of pre-existing enzyme remained unclear. The aim of the present study was to determine the salinity-related differences in $Na^+,K^+$-ATPase α-subunit mRNA abundance that could result from differential gene activity, during short-term acclimation of the euryhaline crab *Pachygrapsus marmoratus* to different salinities.

*P. marmoratus* is a grapsid crab with strong abilities to hyper-/hypoosmoregulate (Pierrot et al., Arch. Physiol. Biochem. 103: 401-409, 1995). The last two pairs of anterior gills (n°5 & 6) and the three pairs of posterior gills (7, 8 and 9) from *P. marmoratus* acclimated for 2, 4, 6, 24 and 48 hours to three different salinities of 10 ppt (DSW), 36 ppt (SW) and 45 ppt (CSW) were dissected in Montpellier and immediately kept in RNA*later*™ (Ambion) to preserve RNA for molecular analyses at MDIBL. Total RNA was extracted with the RNAgents Total RNA Isolation System from Promega. Poly-A mRNA in 2 µg of total RNA was reverse transcribed using oligo-dT and SuperScript II reverse transcriptase (Invitrogen). The resulting cDNAs were used as templates for real-time quantitative PCR. Specific primers were designed according to the partial sequence of the $Na^+,K^+$-ATPase α-subunit in *P. marmoratus* sequenced previously at MDIBL (Spanings-Pierrot et al., 2000, GenBank Accession No. AF375957). In real-time quantitative PCR, $Na^+,K^+$-ATPase cDNA in 1-µl aliquots was amplified in the presence of SYBR Green dye (Qiagen) using the Stratagene MX4000 Multiplex Quantitative PCR System. A dilution series demonstrated a linear relationship between threshold cycle and $\log_{10}$ of template availability (Fig. 1). Despite a search for an invariant 'housekeeping' gene against which α-subunit gene expression could be compared, we have not yet identified such a standard in gills of *P. marmoratus*. Thus α-subunit mRNA abundance is expressed as a relative value, using one of the test conditions as the basis for comparison.

At low salinity (DSW, 10 ppt), where hemolymph NaCl is strongly hyperionic to the medium, a high expression of $Na^+,K^+$-ATPase mRNA was measured in all tested gills, i. e. in the posterior but also in the anterior gills, two days after transfer to DSW (Fig. 2A). Moreover, the

increase in $Na^+,K^+$-ATPase mRNA expression during the short-term acclimation was different according to the gills. In gill 7, which is the largest of the posterior gills, the mRNA expression significantly increased 2h after transfer to low salinity while it stayed almost unchanged in posterior gill 9 until 24h after transfer (Fig. 2A).

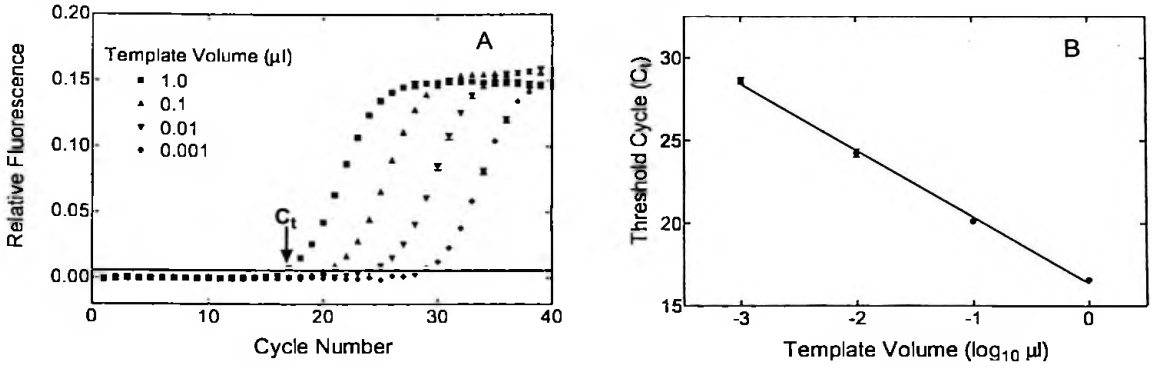


Fig. 1: Real-time quantitative PCR performed with a dilution series (in quadruplicate measurements) of cDNA from DSW-acclimated crabs as template (A) and the corresponding relationship between threshold cycle and $\log_{10}$ of template abundance (B). Error bars may be obscured by symbols.

In concentrated seawater (45 ppt), where hemolymph NaCl is hypoionic to the medium, the mRNA expression of $Na^+,K^+$-ATPase α-subunit increased exclusively in posterior gill 7, 4h after transfer, to reach a 10-fold level at 6 to 48h, while it remained almost unchanged in all other tested gills (Fig. 2B).

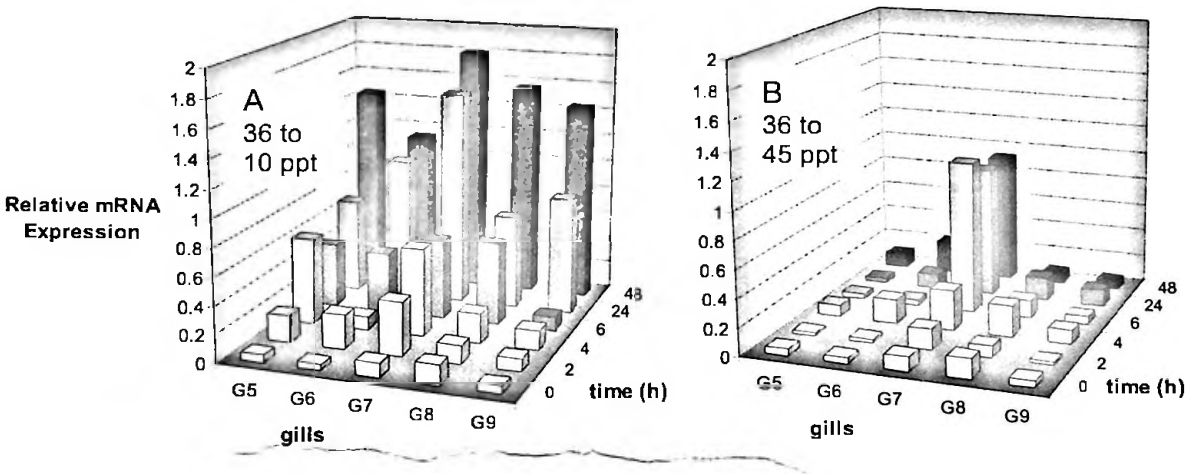


Fig. 2. Relative abundance of $Na^+,K^+$-ATPase α-subunit mRNA in individual gill samples at various intervals following transfer of the shore crab *Pachygrapsus marmoratus* from 36 ppt salinity to 10 ppt (A) or to 45 ppt (B). In 10 ppt salinity, hemolymph osmolality is much higher than the medium; in 45 ppt salinity, hemolymph osmolality is significantly lower than the medium. Three to six gill samples (100 mg) were pooled for each time point and mRNA abundances were measured by real-time quantitative PCR in quadruplicate. Standard deviations (not shown for clarity) averaged 11% of the mean values. cDNA transcribed from RNA of gill 6 at 48 h exposure to 10 ppt salinity served as the reference standard.

Results at low salinity indicate that $Na^+,K^+$-ATPase in posterior gills of *P. marmoratus* is involved in $Na^+$ uptake as it has already been demonstrated in other crab species (Towle, 1990; Pequeux, 1995). But it seems that anterior gills are also implicated in hyperosmoregulatory processes to counter balance the influx of water and loss of ions at low salinity, although it was believed until now that crab anterior gills are mainly involved in respiratory gas exchanges (Pequeux, 1995). This result corroborates observations on immunolocalization of $Na^+,K^+$-ATPase α-subunit in anterior gills at low salinity (Spanings-Pierrot et al., 2000).

In high salinity, it appears that only posterior gill 7 may be involved in $Na^+$ secretion, maintaining hemolymph osmolality lower than the osmolality of the external medium. This finding is in agreement with an earlier flux study on isolated gills of the mangrove crab *Ucides cordatus*, in which gill 6 (out of 7) was implicated in salt secretion while gill 5 accomplished active salt uptake (Martinez et al., Comp. Biochem. Physiol. A 120: 227-236, 1998).

Although little is known about developmental patterns giving rise to specific gill function in crustaceans, it appears that all of the tested gills possess the regulatory machinery to induce expression of $Na^+,K^+$-ATPase α-subunit mRNA, albeit at different times, after transfer to low salinity. However, the ability to respond in this way to concentrated seawater is possessed only by gill 7, suggesting that gill 7 can differentiate from salt-absorbing to salt-secreting function.

This research was supported by an MDIBL New Investigator Award to C.S.-P. and by National Science Foundation grants (IBN-9807539 and DBI-0100394) to D.W.T.