# Heterologous coexpression of the blue light receptor psRII and its transducer pHtrII from *Natronobacterium pharaonis* in the *Halobacterium salinarium* strain Pho81/w restores negative phototaxis

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**Abstract The photophobic receptor (psRII) and its transducer pHtrII from *Natronobacterium pharaonis* were heterologously coexpressed in the phototaxis-deficient *Halobacterium salinarium* strain Pho81/w which lacks all four bacterial rhodopsins, i.e. the two ion pumps bacteriorhodopsin and halorhodopsin as well as the two sensory pigments SRI and SRII. This genetically transformed Pho81/w strain showed a photophobic response upon illumination with blue light. The action spectrum of the psRII/pHtrII mediated phototactic behavior was determined in the range of 420–600 nm. The shape of the action spectrum was similar to the absorption spectrum of psRII, clearly indicating that the psRII-specific photophobic response in Pho81/w was restored. These results suggest that the *pharaonis* photoreceptor-transducer complex (psRII/pHtrII) is functionally competent to substitute the corresponding *salinarium* receptor system. Although the two archaea are phylogenetically quite distant from each other the two signal transduction chains are homologous systems which can replace each other.**



*Key words:* Photorhodopsin; Sensory rhodopsin; Phototaxis; Signal transduction

## 1. Introduction

Phototaxis of halophilic archaea is mediated by two sensory rhodopsins (SRI and SRII) which are responsible for positive and negative responses of the bacteria. SRI and SRII are membrane proteins with seven transmembrane spanning helices and an all-*trans* retinal bound to the opsin. On light excitation the bacterial sensory rhodopsins undergo a photocycle during which the physiological reaction is triggered. Sensory rhodopsins have been isolated from *Halobacterium salinarium* and in the case of the photophobic receptor (psRII) also from the phylogenetically distinct *Natronobacterium pharaonis* (reviewed in [1]). In earlier work it has been shown that psRII is functionally quite similar to SRII from *H. salinarium* [2,3]. Excitation of psRII and SRII with blue-green light leads to a photophobic response of both species. The underlying signal transduction chain is homologous to the eubacterial chemotaxis cascade with the cytoplasmic Che proteins as members. After the halobacterial receptor is activated the signal is conducted to the so called signalling domain of transducer proteins. In the archaeal system the photoreceptors interact with their corresponding 'halobacterial transducers of rhodopsins' (Htr) which subsequently transmit the signal to the cytoplasmic components of the signal transduction chain. Finally, the stimulus results in a switching of the rotational sense of the flagellar motor. In the case of SRII and psRII an increase in the intensity of blue-green light increases the probability of cell reversals whereas decreases in the intensity suppress the reversal probability.

It has been shown that the halobacterial sensory rhodopsins are coexpressed with their transducer molecules [4–6]. This observation indicates that the interaction of the photoreceptor with its own transducer is quite specific. Indeed, a chimeric rhodopsin-transducer dimer has so far not been published. In this work we describe the heterologous coexpression of the psRII and pHtrII from *N. pharaonis* in the phototactic minus *H. salinarium* strain Pho81/w. Coexpression of the two proteins in Pho81/w restores the photophobic response.

## 2. Materials and methods

*H. salinarium* strain Pho81/w, which lacks all of the four rhodopsins as well as the two transducers HtrI and HtrII [7,8], was used for transformation using the methods described in [9]. Briefly, to obtain overexpression, *phtrII* and *psopII* sequences from *N. pharaonis* were cloned under the control of the strong *bop* promoter from *H. salinarium*. The *phtrII*/*psopII* operon arrangement from *N. pharaonis* was not changed, but the 5′ end of *phtrII* was mutated by PCR to get a *Sph*I restriction site (which changes the second amino acid from serine to leucine). *phtrII*/*psopII* was cloned downstream of the 0.36 kbp *bop* promoter region which was obtained by PCR with specific primers. The construction of the *Escherichia coli*/*H. salinarium* shuttle vector started with pBluescript SK$^-$ and a 2.3 kbp *Xba*I/*Bam*HI novobiocin resistance gene fragment (plasmid pUSNovo was obtained from D. Oesterhelt) from *Haloferax volcanii* [10]. The coexpression cartridge with *bop* promoter/*phtrII*/*psopII* was ligated into the *Bam*HI/*Hin*dIII site of the plasmid (Fig. 1). Transformed Pho81/w was grown at 37°C on peptone medium containing 0.4 μg/ml novobiocin (Sigma). Transformed colonies were cultured to a volume of 6 ml with $OD_{578} > 1.5$.

Motility of the cells was controlled by light microscopy and only the most motile cells were chosen for measurements. Cells were diluted 1:2 in 3.5 M NaCl, 80 mM $MgSO_4 \cdot 7H_2O$, 26 mM KCl, 0.3% sodium citrate·$2H_2O$, 2 mM HEPES supplemented with 0.1% arginine and incubated for 1–12 h at 37°C in the dark without agitation. Data acquisition and evaluation were done as described [11]. The light intensity was measured at each wavelength using a photometer (Model 1010; K. Lambrecht corporation). The data were corrected accordingly.

## 3. Results and discussion

The determination of the primary structure of psRII and pHtrII [5] allowed us to express the two proteins heterologously in *H. salinarium* strain Pho81/w. These cells are especially suited for this kind of experiment because they are deficient in phototaxis. Furthermore, this strain does not possess

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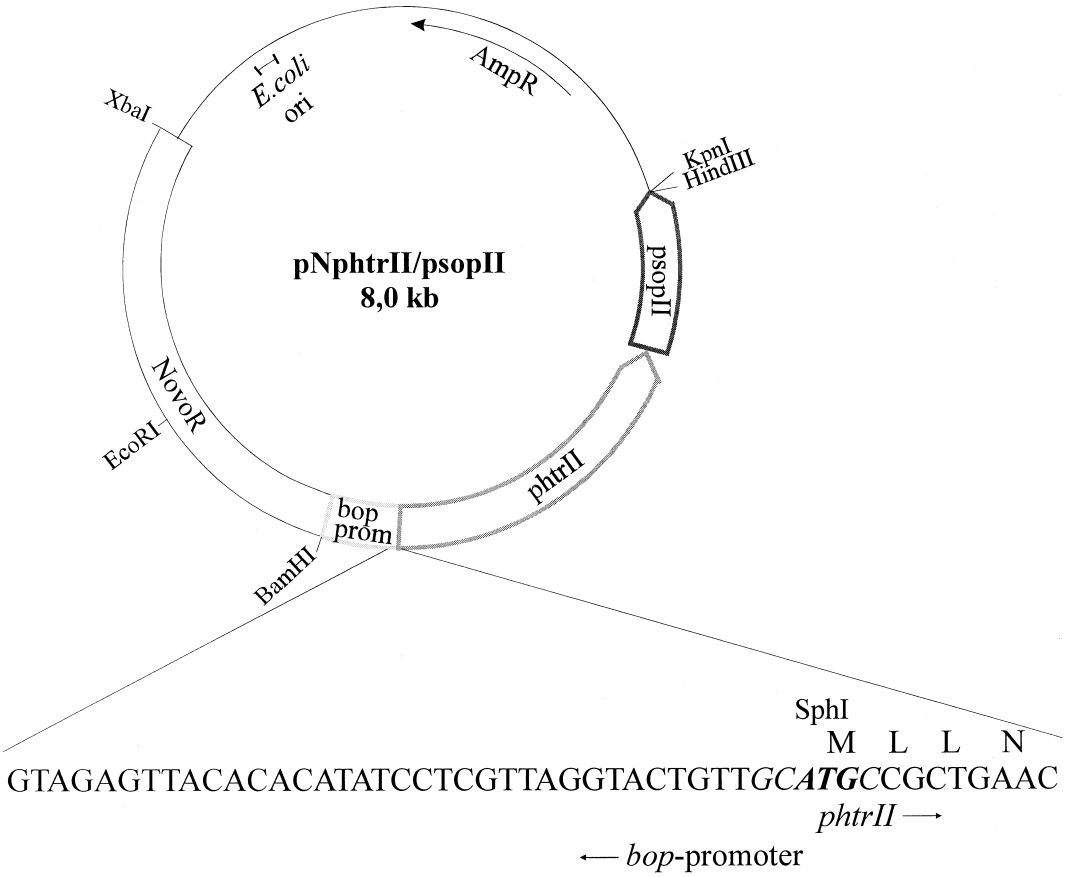


Fig. 1. Construction of the *H. salinarium*/*E. coli* shuttle vector pNphtrII/psopII for the coexpression of pSRII and pHtrII in *H. salinarium* strain Pho81/w.

functional genes for the expression of the two ion pumps, bacteriorhodopsin and halorhodopsin.

Pho81/w was transformed with the *E. coli*/*H. salinarium* shuttle vector (pNphtrII/psopII) containing the coexpression cartridge *bop* promoter/*phtrII*/*psopII* (Fig. 1). In *H. salinarium* Pho81/w the *bop* coding region is disrupted by the insertion element ISH2 (data not shown) [12]. After transformation one copy of the expression vector had been integrated into the *bop* locus by homologous site-specific recombination (data not shown). The presence of the *phtrII*/*psopII* genomic sequence was verified by PCR analysis. At the protein level, the expression of psRII was determined spectroscopically. The absorption spectra from membrane fractions displayed the psRII-specific absorption at 500 nm together with a shoulder at 465 nm. Taking the molar extinction at 498 nm ($\varepsilon_{498}$=48 000 $M^{-1}$ $cm^{-1}$) into account, the copy number of psRII molecules can be calculated to be about 5000 molecules/cell.

For the phototaxis experiments motile cells had to be chosen. Generally, motile cells can be selected by using swarmplates (0.2% agar-agar) similar to the procedure described in [13]. However, using this method cells containing the transformed DNA disappeared gradually during the selection procedure. For this reason, a large number of single colonies were screened for motility using a light microscope. As controls original Pho81/w as well as a Pho81/w strain overexpressing psRII (pho81/wpsopII) were screened similarly. The responses of the two transformed strains (Pho81/wpsopII; Pho81/wphtrII/psopII) as well as the response of 'wild-type' Pho81/w towards 500 nm light are shown in Fig. 2. As expected, Pho81/w and Pho81/wpsopII do not show phototactic responses. However, in Pho81/wphtrII/psopII, which coexpresses psRII and pHtrII, the bacteria flee the illuminated spot. This is indicated by the decreasing light scattering (measured as negative photocurrent $I_{Pm}$) displaying the typical photophobic response as seen in *N. pharaonis* [2,7]. This negative phototaxis could also be restored in Pho81/w by using an extrachromosomal stable plasmid for transformation which contained the naturally occurring halobacterial plasmid pGRB1 (data not shown) [14].

For the determination of the action spectrum, data were collected every 5 nm in the range of 420–600 nm in two independent experimental data sets. At each point the initial slope was determined, normalized to the reference wavelength at 500 nm and corrected for the light intensity as described in [11]. Fig. 3 shows the action spectrum in comparison to the psRII absorption spectrum. Generally, the action spectrum of Pho81/wphtrII/psopII shows the same overall shape as the absorption spectrum. The maximum is found at around 500 nm. The fine structure of the absorption spectrum is also indicated in the action spectrum, although the quality of the data is not sufficient for an unequivocal resolution. Below 500 nm the values of the action spectrum decrease more rapidly and reach the *x*-axis – where no photophobic response is observed – at 430 nm. At this point the absorption of psRII still has a value around 0.4. One reason for the discrepancies between the two curves at the short wavelength side of the

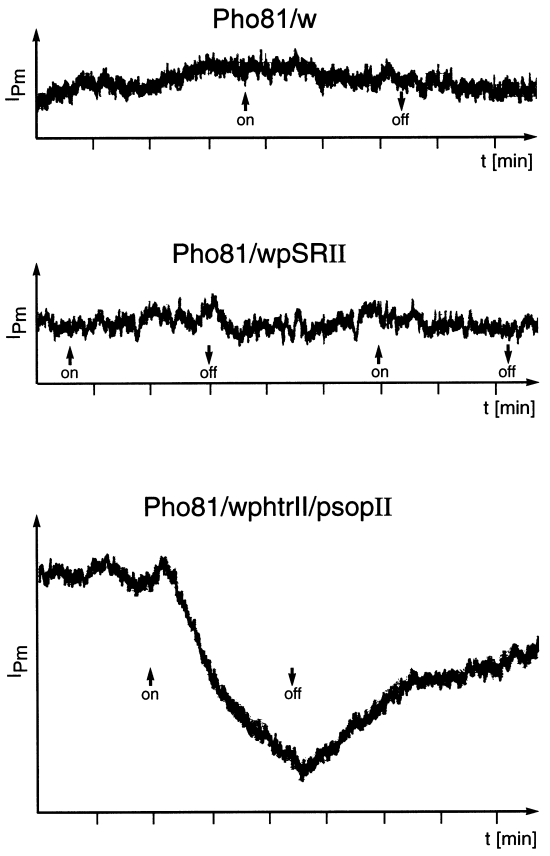


Fig. 2. Light-induced response of *H. salinarium* strain Pho81/w as well as the response of the transformants Pho81/wpsopII and Pho81/wphtrII/psopII which express pSRII and HtrII, respectively. The beginning and the end of the illumination (500 nm) period are indicated by the on/off arrows.

maxima might be found in the light scattering of the sample. The light scattering which increases with decreasing wavelengths contributes only to the absorption spectrum and it is not reflected in the action spectrum. Another reason might be a two photon process, in which the photoexcitation of the long-lived intermediate $psRII_M$, whose maximum is found at about 400 nm (I. Chizhov, personal communication), inhibits the physiological response. A similar effect has been described for the proton pump activity of bacteriorhodopsin [15].

The phototaxis-deficient *H. salinarium* strain Pho81/w is still chemotactically active which implies a functional cytoplasmic machinery to regulate the flagellar motor. In *H. salinarium* the cytoplasmic pathway with the Che gene products influences the reversal frequency of the flagellar motor [16]. The change in the rotational sense of the monopolar inserted flagellar bundle enables the bacteria to move forward or backward. This motility can also be observed with the monopolar flagellated *N. pharaonis* [17]. To restore a phototactic response in *H. salinarium* Pho81/w it is necessary to enable the interaction between the cytoplasmic signal transduction pathway and the heterologously expressed pHtrII. The results presented here indicate a functional interaction between pHtrII and the *salinarium* cytoplasmic signal transduction proteins. Furthermore, this interaction results not only in phototactic activity but leads to the same photophobic response of the transformed *H. salinarium* cells on illumination with blue light as in the native pHtrII/psRII host *N. pharaonis*.

In summary, although the two archaea *N. pharaonis* and *H. salinarium* are phylogenetically quite distant from each other, even belonging to separate genera [18], and although their natural habitats differ considerably the photophobic receptor/transducer complex from *N. pharaonis* can restore the negative phototaxis in *H. salinarium*.

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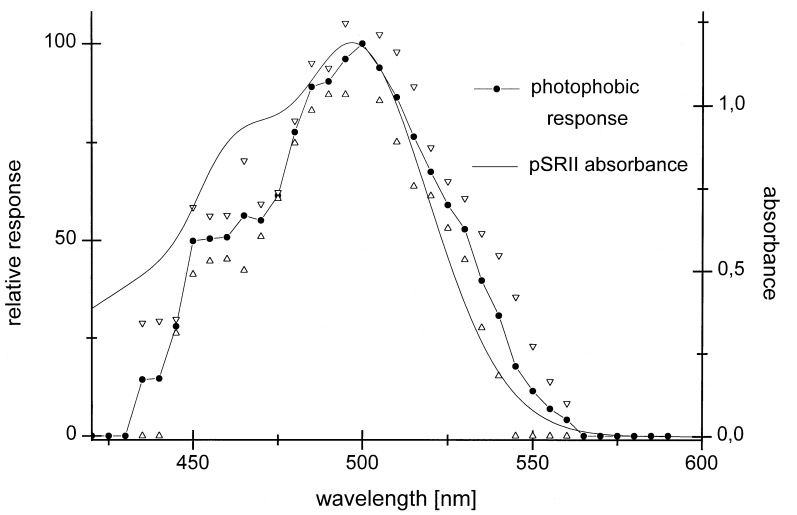


Fig. 3. Action and absorption spectra of pSRII. Closed circles depict the mean value of two measurements which are represented by the open triangles. For experimental details, see text.

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