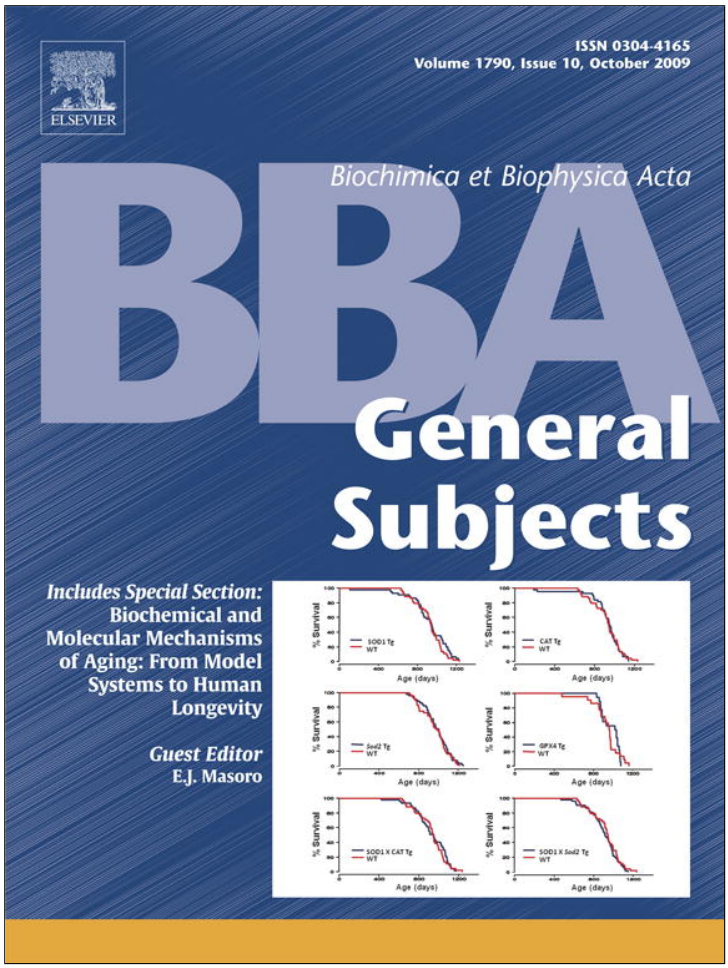

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Oligomerization of *Bacillus subtilis* DesR is required for fine tuning regulation of membrane fluidity

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ABSTRACT

Background: The DesK-DesR two-component system regulates the order of membrane lipids in the bacterium *Bacillus subtilis* by controlling the expression of the *des* gene coding for the delta 5-acyl-lipid desaturase. To activate *des* transcription, the membrane-bound histidine kinase DesK phosphorylates the response regulator DesR. This covalent modification of the regulatory domain of dimeric DesR promotes, in a cooperative fashion, the hierarchical occupation of two adjacent, non-identical, DesR-P binding sites, so that there is a shift in the equilibrium toward the tetrameric active form of the response regulator. However, the mechanism of regulation of DesR activity by phosphorylation and oligomerization is not well understood.
Methods: We employed deletion analysis and reporter fusions to study the role of the N-terminal domain on DesR activity. In addition, electromobility shift assays were used to analyze the binding capacity of the transcription factor to deletion mutants of the *des* promoter.
Results: We show that DesR lacking the N-terminal domain is still able to bind to the *des* promoter. We also demonstrate that if the RA site is moved closer to the −35 region of P*des*, the adjacent site RB is dispensable for activation.
General significance: Our results indicate that the unphosphorylated regulatory domain of DesR obstructs the access of the recognition helix of DesR to its DNA target. In addition, we present evidence showing that RB is physiologically relevant to control the activation of the *des* gene when the levels of DesR-P reach a critical threshold.

1. Introduction

When poikilothermic organisms such as bacteria, plants and fish are exposed to low temperatures, the membrane lipids become rigidified leading to a suboptimal functioning of cellular activities [1,2]. These organisms can acclimate to such new conditions by remodeling its membrane lipid composition. This can be achieved through an increase in desaturation of the acyl chains of membrane phospholipids, because phospholipids containing unsaturated fatty acids have lower transition temperatures than their respective lipids with saturated fatty acids. Hence, membrane fluidity is recovered with restoration of normal cell activity at low temperatures.

Bacillus subtilis has only one desaturase, Δ5-Des, encoded by the *des* gene [3,4]. *B. subtilis* desaturase catalyzes the introduction of a cis-double bond at the Δ5 position of existing saturated fatty acids in membrane phospholipids. *B. subtilis*, *des* gene transcription increases in response to decreased temperature [5]. A canonical two-component regulatory system comprising the histidine kinase DesK and the response regulator DesR regulates *des* expression [6]. The sensor kinase DesK contains five transmembrane (TM) helices and a long cytoplasmic C-terminal tail, which harbors the kinase domain. *In vitro* experiments show that DesK undergoes autophosphorylation on the conserved His188 [7]. The phosphorylated kinase then transfers the phosphoryl group to the Asp54 of the dimeric effector DesR, leading to the stabilization of a DesR-P tetramer [8]. This tetramer binds two adjacent, non-identical DesR-P binding sites within the *des* promoter, named RA and RB, leading to recruitment of RNA polymerase and activation of *des* transcription [8]. We have previously demonstrated that the unphosphorylated form of DesR does not bind to its target DNA, and that the level of phosphorylation of DesR is proportional to its binding activity. These findings suggest that upon DesR phosphorylation, a conformational change would occur to expose previously inaccessible DNA-binding residues in the C-terminus. The promoter region RA is a high affinity binding site containing a palindromic 6-2-6 sequence (IR-L and IR-R), while RB is a low affinity binding site containing a partial direct repeat (DR) (Fig. 1A, [8]). The contacts between DesR-P and the *des* promoter were previously identified by hydroxyl radical footprinting coupled to electromobility shift assays (EMSA) [8]. This study revealed that at low concentration of DesR-P, a

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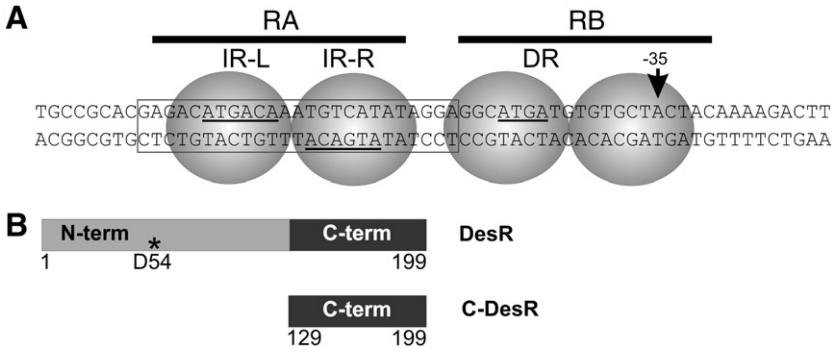

Fig. 1. A. *des* regulatory region. The lines RA and RB at the top show the location of the two DesR-P binding regions. Inverted and directed repeats are the underlined sequences. Boxed is the region that corresponds to probe RA25 used in EMSA (see text and Fig. 2), and the −35 site is indicated with an arrow. The gray circles represent two dimers of DesR-P bound to P*des* (modified from [8]). IR-L, IR-L and DR stand for Inverted repeat-left, Inverted repeat- right and Direct repeat respectively. B. Representation of full length and truncated DesR. The black box represents the C-terminal domain and the gray box represents the N-terminal domain of DesR, which contains the conserved residue Asp54 (asterisk).

dimer binds to the RA forming a fast migrating complex, CI, which is crucial for the cooperative binding of a second dimer to the RB site [8]; while at high concentrations of DesR-P, binding of two dimers, one to RA and the other one to RB, gives rise to a slow migrating complex named CII. Both sites, RA and RB, seem to be critical for *des* expression since point mutations on either the DR site or in the inverted repeats IR-L and IR-R result in abolishment of *des* transcription [8]. Consequently, two DesR-P dimers positioned at RA and RB seem to be required to stimulate transcription. Although these studies established that DesR functions as a phosphorylation-activated switch for the cold inducible *des* gene, the mechanism of control of DesR activity by the regulatory domain is unknown. The physiological relevance that upon phosphorylation DesR oligomerizes into tetramers to stimulate the expression of the *des* gene, also remains unsolved.

In this study, we provide information about the role of the regulatory domain of DesR on the function of its effector domain. We also present data demonstrating that the low affinity binding site, RB, is involved in fine tuning of the output response produced by tetrameric DesR-P and discuss these results in the context of transcriptional regulation of membrane fluidity homeostasis.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Bacterial strains used in this study are listed in Table 1. *B. subtilis* was propagated at 37 °C, with 250 r.p.m. gyration, in Spizizen salts [9] supplemented with 0.2% glucose, 50 μg ml^{-1} each tryptophan and phenylalanine, 0.05% casaminoacids and trace elements [10]. When the strains were complemented with plasmid pDG795, glycerol 0.5% and threonine 50 micrograms/ml were added. Antibiotics were added to media at the following concentrations: chloramphenicol, 5 μg ml^{-1}; kanamycin, 5 μg ml^{-1}; erythromycin, 1 μg ml^{-1}; lincomycin, and 12.5 μg ml^{-1}. β-Galactosidase was assayed in independent triplicates as previously described [11]. It should be noted that the maximum β-galactosidase activities shown in Figs. 2 and 3 are different because DesR is transcribed from different promoters (xylose promoter for Fig. 2 and native *desR* promoter for Fig. 3).

Table 1
B. subtilis strains used in this study.

Strain	Relevant characteristics	Source
JH642	trpC2 pheA1	Laboratory stock.
AKP3	JH642 amyE::[P*des*WT:lacZ]	[6]
AKP9	AKP3 *desR*::Km^R	[6]
CN1	AKP9/pCN1 (P*xyl*:*C-desR*)	This work.
CN2	AKP9/pAG52 (P*xyl*:*desR*)	[6]
AKP8	JH642 *desR*::Km^R)	[6]
MEI1	JH642 *desR*::Km^R amyE::[P*des*ΔDR10:*lacZ*]	This work.
MEI2	MEI1/pCN1 (P*xyl*:*C-desR*)	This work.
MEI3	MEI1/pAG52 (P*xyl*:*desR*)	This work.
SNΔDR15	JH642 amyE::[P*des*ΔDR15:*lacZ*]	This work.
SNΔDR10	JH642 amyE::[P*des*ΔDR10:*lacZ*]	This work.
SNΔDR5	JH642 amyE::[P*des*ΔDR5:*lacZ*]	This work.

2.2. Plasmid and strain constructions

To delete the low affinity site RB, different pairs of oligonucleotides (see Table 2) were used for the amplification of two overlap-extension PCR. A mix of these PCR products was used as DNA template for another PCR using oligonucleotides PECO895 and BAMO [12]. The amplification products were cloned into the integrational vector pJM116 [13]. The resulting plasmids were introduced into the *amyE* locus of strain JH642 giving strains SNDR15, SNDR10 and SNDR5.

Oligonucleotides SalI555 and C-fus789 were used to amplify the C-terminal region of *desR* (*C-desR*). The PCR product was digested with KpnI and SalI, purified and ligated to the vector pGES40 (Gustavo Schujman, personal communication), which has the xylose-inducible promoter (P*xyl*) upstream of the cloning site. The resulting plasmid was digested with BamHI. The BamHI–BamHI fragment including the P*xyl* and the region coding for C-DesR was cloned in the integrational vector pDG795 [14], and recombined into the ThrC locus of strain AKP9, which is *desR*$^-$ and carries the fusion P*des*:*lacZ* in the *amyE* locus. The resulting strain was called CN1, in which the expression of C-DesR is induced upon addition of 0.01% xylose to the growth medium. To construct MEI1, the *desR*$^-$ strain AKP8 (JH642 *desR*::Km^R) was transformed with the integrative plasmid pJM116 carrying the P*des*DRΔ10. To construct MEI2 and MEI3, MEI1 was complemented with plasmid pDG795 carrying DesR-C (pCN1) or DesR (pAG52) respectively. All plasmids were sequenced to confirm the introduction of mutations.

2.3. Gel mobility shift assays

DNA probes including the wild type or different *des* promoter variants were [α-^{32}P]-labeled by PCR using primers PECO895 and BAMO. For the short RA25 probe, RAfor and RArev oligonucleotides were used (see Table 2). RAfor was 5′-end labeled with [γ-^{32}P]-ATP and T4 PNK [8]. Afterwards, both oligonucleotides were heated to 90 °C and let them hybridize by smooth cooling. When phosphorylated DesR was used in the reaction, 6 μM DesR was incubated for 20 min at 0 °C in a mix containing 50 mM Tris–HCl pH 8, 5 mM $MgCl_2$, 0.5 mM EDTA, 1.25 mM dithiotreitol, 10% glycerol and 50 mM acetyl phosphate (AcP). After this incubation, increasing concentrations of DesR-P (0.1–2.4 μM) were incubated with each probe and run in non-denaturing 12% polyacrylamide gels.

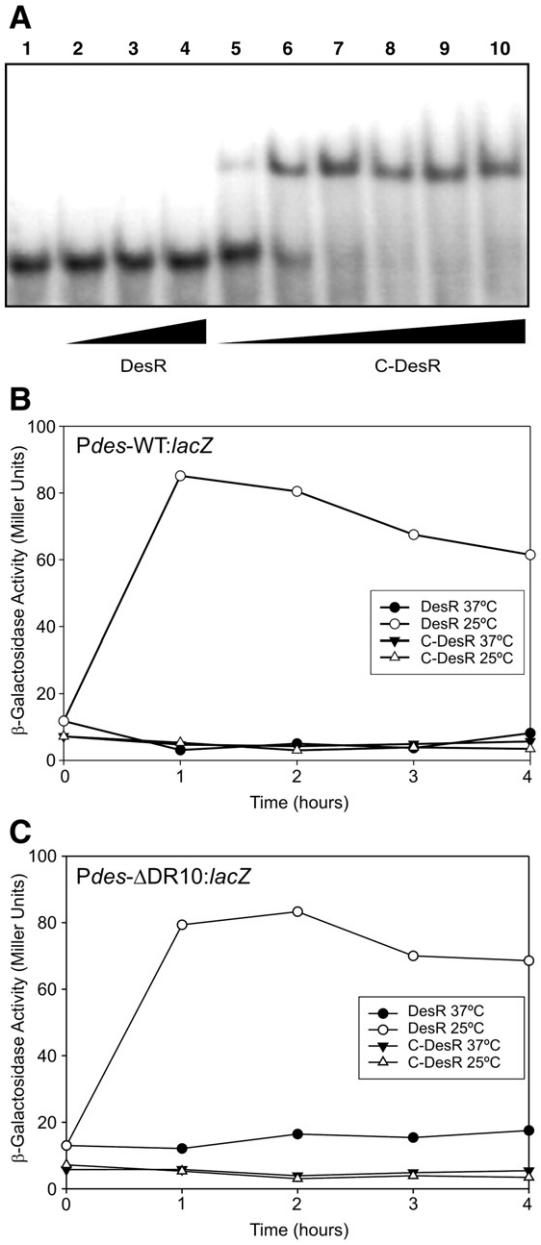

Fig. 2. A. EMSA showing the binding of C-DesR to the RA site of P*des*. A. The radiolabeled RA25 probe (25 bp) was incubated with increasing amounts of either unphosphorylated DesR (lanes 2–4) or C-DesR (lanes 5 to 10) and run in a non-denaturing polyacrylamide gel. CI shows the shift in mobility of the probe due to formation of a C-DesR–DNA complex. B. C-DesR is unable to activate *des* transcription. The *desR*$^-$ *Bacillus subtilis* strain AKP9 harboring the transcriptional fusion P*des*:*lacZ* was complemented with pDG795 carrying P*xyl*:*desR* (●), or P*xyl*:*C-desR* (▲). Cells were grown at 37 °C in the presence of xylose (0.01%) to an optical density of 0.3 and then divided into two samples. One sample was transferred at 25 °C (open symbols) and the other kept at 37 °C (black symbols). β-Galactosidase specific activities were measured as previously described [11]. C. The N-terminal domain of DesR is needed for transcription activation. The *desR*$^-$ *Bacillus subtilis* strain harboring the transcriptional fusion P*des*ΔDR10:*lacZ*, MEI1, was complemented with pDG795 carrying P*xyl*:*C-desR* (▲, MEI2) or P*xyl*:*desR* (●, MEI3) and treated as described in B. The results shown are the average of three independent experiments with a mean error of less than 5%.

2.4. Construction of expression vector and purification of His-tagged C-DesR

The C-terminal portion of DesR, including aminoacids 128 to 199 was amplified using oligonucleotides NdeI571 and Cfus-789 (see Table 2). The PCR product was cloned in pET15b (Invitrogen), and the resulting plasmid used to transform *Escherichia coli* BL21 (DE3). This new construction places a 6×His tag at the N-terminus of the protein. Overexpression and purification of C-DesR were performed as described by the manufacturer (QIAGEN).

2.5. Determination of the molecular weight of C-DesR by Static Light Scattering

The average molecular weight of C-DesR was determined on a Precision Detector PD2010 light scattering instrument connected in tandem to an HPLC system and a LKB 2142 differential refractometer. The samples were loaded on a Superdex S-75 column and eluted with 50 mM Tris–HCl pH 8, 0.3 M NaCl, and 0.5 mM DTT, at a flow rate of 0.4 ml/min. The 90° light scattering and refractive index signals of the eluting material were recorded on a personal computer and analyzed with the Discovery 32 software supplied by Precision Detectors. The 90° light scattering detector was calibrated using 10 mg/ml of *lysozyme* (MW: 14.3 kDa) as a standard. Measurements were performed at least three times, using different protein concentrations ranging from 2–5 mg/ml.

3. Results

3.1. Dual role of the N-terminal regulatory domain of DesR

To analyze whether the N-terminal domain of DesR regulates the interaction of its HTH domain with P*des*, we produced a deletion mutant, C-DesR, lacking the N-terminus (residues 1–128, Fig. 1B). To study the association state of C-DesR, we used Static Light Scattering coupled to size exclusion chromatography, as described in Materials and methods. The molecular mass for purified C-DesR was 18.25 ± 1.8 kDa, suggesting that most of the 10. 491 kDa truncated protein behaves as a dimer in solution. Then, we examined if C-DesR was able to bind to P*des* by EMSA. To this end, oligonucleotide RAfor, containing the high affinity site RA, was labeled at the 5′ end with [γ-^{32}P] ATP and hybridized with its complementary oligonucleotide, RArev. This 25-bp probe, RA25, extending from positions −74 to −51, was incubated with either unphosphorylated DesR or C-DesR and the mixtures were run in a non-denaturing polyacrylamide gel. As shown in Fig. 2A, the probe exhibited changes in its electrophoretic mobility with C-DesR (lanes 5–10), while unphosphorylated DesR (lanes 2–4) was unable to bind to P*des*. This result indicates that removal of the DesR N-terminal domain overcomes the phosphorylation requirement of the transcription factor to bind to its DNA target.

To determine whether C-DesR, in addition to bind to P*des*, is able to activate *des* transcription *in vivo*, genes coding for C-DesR or DesR were cloned under the control of the xylose-inducible promoter, P*xyl*, in the integrative plasmid pDG795 to give plasmids pCN1 and pAG52 respectively. These plasmids were transformed into strain AKP9, which contains a Km^R gene cassette interrupting the *desR* gene and a fusion of the *lacZ* gene to the *des* promoter integrated ectopically at the non essential *amyE* locus of *B. subtilis* (Table 1), to give strains CN1 and CN2, respectively. To analyze induction of P*des* upon a cold shock, these strains were grown in the presence of xylose 0.01% at 37 °C or grown at 37 °C until OD_{525} = 0.3, and then transferred to 25 °C. Fig. 2B shows that, as expected, expression of wild type DesR in strain CN2 allowed stimulation of *des* transcription upon a temperature downshift, whereas the expression of C-DesR was unable to drive transcription from the P*des*:*lacZ* fusion under the same conditions. Together, these experiments indicate that the unphosphorylated regulatory domain of DesR obstructs the access of the recognition helix to DNA. However, when this inhibitory effect is relieved by DesK-mediated phosphorylation, the N-terminal domain becomes essential for transcriptional activation of *des*.

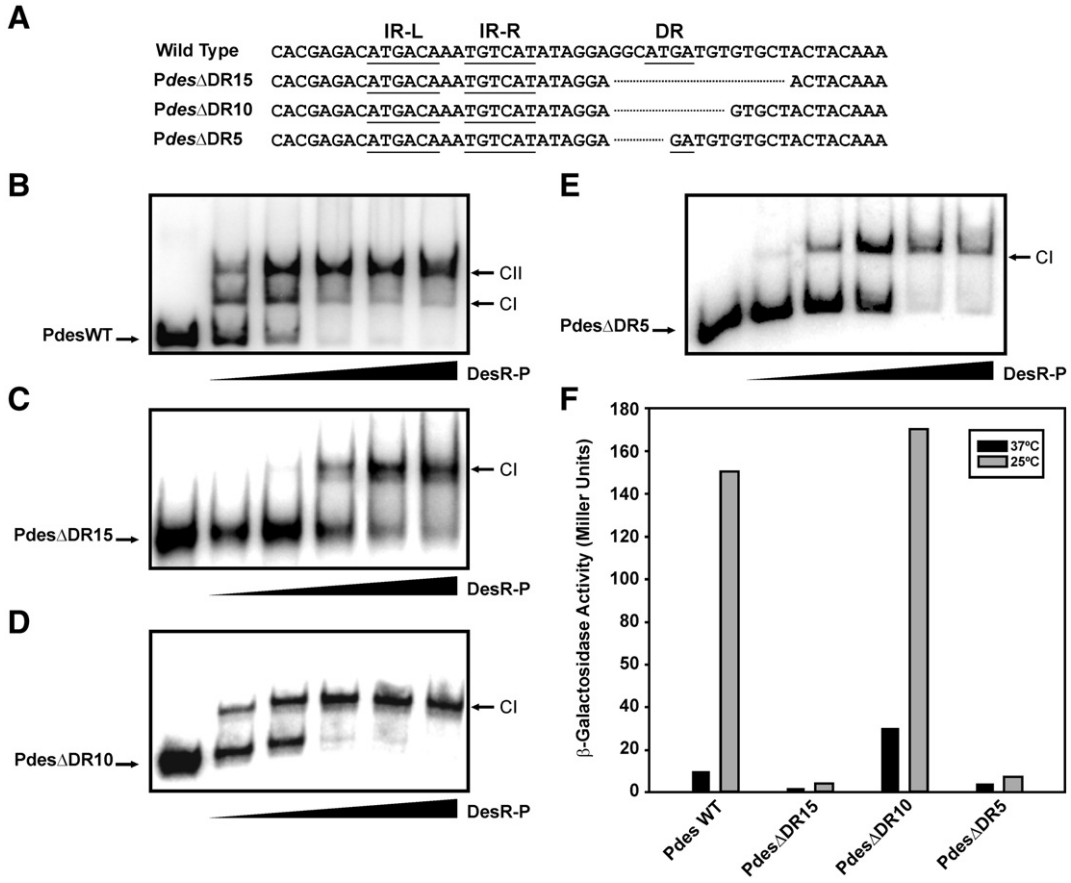

Fig. 3. A, *des* promoter variants. Underlined are the positions that correspond to IR-R, IR-L and DR. Deleted regions in each promoter are indicated by dotted lines. B, C, D and E. EMSAs showing the binding of DesR-P to promoters P*des*WT, P*des*ΔDR15, P*des*ΔDR10 and P*des*ΔDR5 respectively. F. Transcriptional activity of P*des* promoter variants. *B. subtilis* strains AKP3, SNDR15, SNDR10 and SNDR5 harboring a fusion of *lacZ* to either P*des*WT, P*des*ΔDR15, P*des*ΔDR10 and P*des*ΔDR5 respectively, were grown at 37 °C to an optical density of 0.3 and then divided into two samples. One sample was transferred to 25 °C and the other kept at 37 °C. Aliquots were taken every hour and specific β-galactosidase activities were determined. Bars represent the measurements at 4 h of growth at the indicated temperatures. The results represent the average of 3 independent experiments.

3.2. Differential role of the DesR-P DNA-binding sites

We previously showed that at low levels of phosphorylation a dimer of DesR-P binds to RA [8]. However, when the concentration of DesR-P is increased, another DesR-P dimer binds to RB, giving rise to a tetramer which is competent to initiate transcription [8]. To analyze if oligomerization of DesR-P was indeed necessary to stimulate *des* transcription, we constructed a 201-bp DNA fragment (named P*des*ΔDR15) with a 15 bp deletion in the direct repeat DR, located in the low affinity binding site RB, but conserving the high affinity binding site RA, and the −35 region (Fig. 3A). Increasing concentrations of DesR-P were incubated with [α-^{32}P] ATP-labeled DNA fragments containing P*des*ΔDR15 (Fig. 3C) or P*des*WT as a control (Fig. 3B), and analyzed by EMSA. Autoradiographs show that while P*des*WT formed complex I and complex II (CI and CII), as expected for the binding of two DesR-P dimers to the adjacent RA and RB sites of the promoter [8], P*des*ΔDR15 only formed complex CI.

To analyze the capacity of P*des*ΔDR15 to direct *des* transcription, this promoter variant was fused to the *lacZ* reporter gene, and then integrated into the *amyE* locus of strain JH642 yielding strain SNDR15. Under cold-shock conditions the β-galactosidase activity in this strain was very low (Fig. 3F). This result indicates that RB is crucial for P*des* activation, suggesting that two dimers of DesR-P (one bound to RA and another one to RB) are required to activate *des* transcription.

Given that the in P*des*ΔDR15 deletion has eliminated 15 bp (1.5 turns of B-type helix DNA), leaving the RA site on the other face of the DNA turn relative to the −10 and −35 elements of P*des*; it is possible that the orientation of the regulatory elements, rather than the oligomerization state of DesR-P, is crucial for *des* transcription. To test this hypothesis, we changed the spacing between RA and the −35 element of P*des*, by constructing two additional P*des* variants: P*des*ΔDR10 and P*des*ΔDR5 in which 10 and 5 bp were deleted from

Table 2
Synthetic oligonucleotides used in this study.

To amplify the C-terminus of DesR	
NdeI571	CACCTGAACATATGGAGGACTTATACAGCG
Cfus789	CCAGGATCCATTTTTATTTAAACCA
SalI555	GGAAAACGCGTCGACGGAGGTGAACTGATGGAGGACTTATA
EMSA probe	
RAfor	GAGACATGACAAATGTCATATATAGGA
RArev	TCCTATATGACATTTGTCATGTCTC
To amplify Pdes	
PECO895for	ATGCAGAATTCAAGCTATTTCGGGTACATC
BAMOrev	AGTATGGATCCTCTCATTGTGTGTCTCGGTTC
To delete 15 bp of RB	
ΔDR15for	ACTACAAAAGACTTCTCTCATTAGC
ΔDR15rev	CTTTTGTAGTTCCTATATGACATTTGTCATGTCTC
To delete 10 bp of RB	
ΔDR10for	GTGCTACTACAAAAGACTTCTCTCA
ΔDR10rev	GTAGTAGCACTCCTATATGACATTTGTCATGTCTC
To delete 5 bp of RB	
ΔDR5for	AAAAGACTTCTCTCATTAGCGTATA
ΔDR5rev	GAAGTCTTTTTCCTATATGACATTTGTCATGTCTC

the RB site, respectively (Fig. 3A). As shown in Fig. 3D and E, DesR-P binds to either P*des*ΔDR10 or P*des*ΔDR5 giving rise to complex CI. To test *in vivo* the induction of these P*des* variants upon a temperature downshift, we assayed the β-galactosidase activity of strains SNDR10 and SNDR5 bearing either P*des*ΔDR10:*lacZ* or P*des*ΔDR5:*lacZ* ectopically integrated at the *amyE* locus, respectively. When strain SNDR5 was shifted from 37 °C to 25 °C, the levels of β-galactosidase were very low. However, when similar downshift experiments were done with strain SNDR10, the induction levels of the *lacZ* gene were almost identical to those of the wild type strain (Fig. 3F). Taken together, these experiments indicate that if the RA site is moved closer to the −35 site, the adjacent RB promoter region is dispensable for DesR-P-mediated transcriptional activation of P*des*. It follows that in P*des*ΔDR10, a dimer of DesR-P rather than its oligomeric form, is sufficient to establish contacts with the RNA polymerase and activate *des* transcription. It is worth to mention that when we integrated *C-desR* under the control of P*xyl* into strain MEI1, which is *desR*$^-$ and contains the fusion P*des*ΔDR10:*lacZ* (Table 1), the truncated protein was unable to activate transcription from P*des*ΔDR10 (Fig. 2C). This result indicates that the establishment of a complex DesR-P-P*des*ΔDR10-RNAP is strictly dependent on the N-terminal domain of DesR-P.

4. Discussion

DesR belongs to the NarL group of response regulators with a phosphorylatable N-terminal domain, and a C-terminal HTH domain involved in DNA binding and transcription activation [15,16]. Previous studies have shown that phosphorylation of DesR is required for binding to the *des* promoter [8], however the mechanism by which the phosphorylation signal originated in the receiver domain of DesR is propagated to the output domain is unknown. For NarL, it has been shown that the regulatory domain obstructs the access of the recognition helix to DNA, implying that activation involves repositioning of the two domains. Phosphorylation of the N-terminal domain of NarL triggers a global conformational change that weakens interactions at the interdomain interface. This in turn, results in a separation of the two domains exposing critical DNA-binding elements in the C-terminal domain [17,18].

Here, we show that a truncated form of DesR, C-DesR, lacking its N-terminal domain, binds to the *des* promoter with the same apparent affinity that full length phosphorylated DesR. From these results we propose that the mechanism by which the phosphorylation of the Asp54 activates the binding capacity of DesR involves the release of inhibitory effects of the N-terminal domain over the C-terminal DNA-binding domain. Negative regulation imposed by the N-terminus is also observed with the closely related response regulator, OmpR [19]. Another example of this type of negative regulation is observed with CheB in which its unphosphorylated receiver domain inhibits the methylesterase activity of its C-terminal output domain [20]. It should be noted that not all response regulators control their output domains through inhibitory interactions. For example, positive control of an output domain by the N-terminal domain has been demonstrated for the NtrC protein [21].

In spite of the fact that C-DesR binds to P*des in vitro*, this truncated protein was unable to induce *des* transcription *in vivo*. Similar results have been found for NarL, for which DNA binding alone is not sufficient for transcriptional activation [18]. Nevertheless, this seems not to be the rule, since the DNA-binding domain of PhoB not only is able to bind to its target DNA but it is also able to activate transcription of the *phoA* gene [22]. The inability of C-DesR to induce transcription *in vivo* could be due to the fact that it fails to recruit the RNA polymerase or to form tetramers upon phosphorylation. Previous data suggested that two dimers of DesR-P, one positioned at RA and the other at RB site are essential for cold-induced *des* transcription, indicating that deletion of the RB low affinity site would be deleterious for induction. Here we show that a dimer of DesR-P can stimulate transcription if it is correctly positioned regarding the −35 element. Deletions of 5 or 15 bp in RB, representing 0.5 or 1.5 DNA helix turn of B-DNA double helix, respectively, block the activation of P*des* by DesR-P. However, a deletion of 10 bp (a complete helix turn) in RB does not affect the activity of the phosphorylated transcription factor. These results could be explained as follows: deletions of either 0.5 or 1.5 DNA helix turn leave the DesR binding site RA in the opposite face regarding the −35 element abolishing *des* transcription, whereas a deletion of a complete helix turn (10 bp) restores the orientation of the regulatory elements and DesR-P activates normally its target gene. Taken together, these results suggest that the RNAP must interact with DNA and with the transcriptional activator in a specific tridimensional orientation in order to stimulate transcription. These data, combined with previous studies showing that point mutations in the direct repeats of RB allow formation of complex I but hamper *des* transcription [8], suggest that a sole DesR-P dimer bound to RA is far from the −35 region and cannot interact properly with the RNA polymerase.

An important question that arises from this work is why RB is conserved through evolution? We propose that the low affinity RB site at P*des* is designed to ensure that P*des* is activated only when a critical threshold of DesR-P is reached to favor oligomerization of the transcription factor. Additional support for this role of RB is provided by the interesting finding that a 10 bp deletion in this site enhances about three fold of its transcriptional activity at 37 °C, when compared with wild type P*des* (Fig. 3F). The weak activation of the *des* promoter at 37 °C could be the result of some residual DesK-mediated phosphorylation at this repressive temperature or due to unspecific phosphorylation of DesR by intracellular phosphodonors such as acetyl phosphate [23–25]. However, these low levels of phosphorylated DesR are unable to stimulate transcription from the wild type promoter due to the presence of RB, which is only occupied at higher concentrations of DesR-P. Nevertheless, in P*des*ΔDR10 this control mechanism is absent and *des* transcription seems to be stimulated at lower levels of DesR-P. A model that accounts for our present knowledge on the DesR-mediated regulatory pathway is shown in Fig. 4. A kinase dominant state of DesK predominates upon an increase in the proportion of ordered membrane lipids, which autophosphorylates at His188 and transfers the phosphoryl group to Asp54

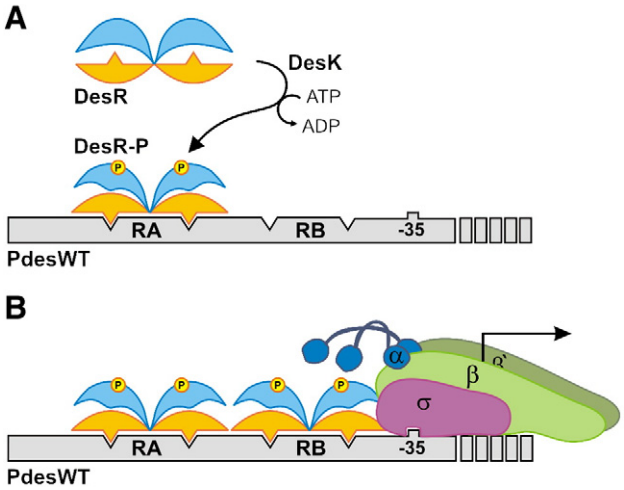

Fig. 4. Phosphorylation-dependent regulation of DesR activity. A. The unphosphorylated regulatory domain of DesR sterically blocks the access of the C-terminal domain to RA. DesK-mediated phosphorylation of DesR relieves the inhibitory interactions between regulatory and effector domains, allowing the binding of dimeric DesR-P to RA. B. When the levels of DesR-P increase, cooperative binding to RB is promoted, followed by oligomerization of the phosphorylated response regulator and recruitment of the RNAP to activate transcription of the desaturase gene. RNA polymerase is shown with the a,β, β′ and σ subunits.

of dimeric DesR. Phosphorylation of the receiver domain results in a repositioning of the regulatory and effector domains. This relieves the inhibition of the effector activity by the regulatory domain, stimulating the interaction of DesR-P with its DNA target. At low levels of phosphorylation DesR-P binds to the 6 bp palindromic sequence localized at RA. Dimeric DesR-P, positioned at RA, is still unable to establish steric collisions with the RNAP to activate transcription. When phosphorylation of DesR increases, cooperative binding of the phosphorylated transcription factor to RB is promoted facilitating the association of two dimers of DesR-P to form tetramers, which in turn are competent to recruit the RNAP and stimulate desaturase expression. Thus, our model argues that DesR-P oligomerization is involved in fine tuning of the switching of *des* expression to maintain the composition of the membrane phospholipids within an optimal fluidity, at which cell growth is more advantageous.

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