Role of the RNA polymerase α subunits in CII-dependent activation of the bacteriophage λ pE promoter: identification of important residues and positioning of the α C-terminal domains

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ABSTRACT

The bacteriophage λ CII protein stimulates the activity of three phage promoters, pE, pI and p40, upon binding to a site overlapping the −35 element at each promoter. Here we used preparations of RNA polymerase carrying a DNA cleavage reagent attached to specific residues in the C-terminal domain of the RNA polymerase α subunit (αCTD) to demonstrate that one αCTD binds near position −41 at pE, whilst the other αCTD binds further upstream. The αCTD bound near position −41 is oriented such that its 261 determinant is in close proximity to σ70. The location of αCTD in CII-dependent complexes at the pE promoter is very similar to that found at many activator-independent promoters, and represents an alternative configuration for αCTD at promoters where activators bind sites overlapping the −35 region. We also used an in vivo alanine scan analysis to show that the DNA-binding determinant of αCTD is involved in stimulation of the pE promoter by CII, and this was confirmed by in vitro transcription assays. We also show that whereas the K271E substitution in αCTD results in a drastic decrease in CII-dependent activation of pE, the pI and p40 promoters are less sensitive to this substitution, suggesting that the role of αCTD at the three lysogenic promoters may be different.

INTRODUCTION

The temperate bacteriophage, λ, is one of the simplest model organisms for the study of developmental regulation. The decision between lytic and lysogenic growth is based on the activity of two phage-encoded transcriptional activators, CI and CII, both of which are required for lysogenization.

Establishment of lysogeny depends on the CII-dependent pE promoter (also known as pRE) that directs the expression of cI necessary for maintenance of the lysogenic state. CII also stimulates pI, which directs expression of the λ int gene, and p40, which directs synthesis of an antisense mRNA that regulates late gene expression [for a review, see Echols (1)].

The mechanism by which CII activates transcription is of special interest. The CII homotetramer regulates all three lysogenic promoters co-ordinately by binding to a tetrad repeat (TTGC) flanking the −35 element, and it was the first regulatory protein suggested to recognize direct repeats [for a review, see Ho et al. (2)]. The location of the CII-binding site at the λ lysogenic promoters is consistent with the idea that it is a class II activator (3). Class II activators bind to target sites that overlap the promoter −35 region and, in most cases, make contact with domain 4 of the RNA polymerase (RNAP) σ subunit, that is bound to the −35 element (4). However, CII binds on the opposite face of the DNA helix to RNAP, and thus, target promoter −35 elements are sandwiched between σ and the activator. Binding of CII to the direct repeats appears to distort the intervening −35 hexamer in some way, although it remains unclear whether this distortion facilitates recognition of the −35 region by σ70 or whether CII makes direct contact with σ (2).

At many bacterial promoters, the C-terminal domain of the α subunit (αCTD) interacts with upstream promoter DNA, the RNAP σ subunit and/or transcription activator proteins (5,6). These interactions are mediated by determinants on the surface of αCTD. For example, residue 265 and neighbouring residues contribute to the αCTD 265 determinant, which is responsible for interactions with DNA (7–10). Similarly, residue 261 and neighbouring residues contribute to the 261 determinant, that can contact σ (11–13). Previous experiments demonstrated that deletion of αCTD greatly reduces CII-dependent activation of pE (14). We have shown that the rpoA341 mutation, specifying the K271E substitution in αCTD, blocks lysogenization of Escherichia coli by phage λ (15). Analysis of reporter gene fusions revealed that this
substitution abolishes activation of pE by CII (16). At many class II or class II-like promoters, both α-CTDs bind immediately upstream of the bound activator and, in some cases, one or both of them make interactions with the activator that contribute to activation. However, CII-dependent promoters present an interesting situation, as CII binds to the opposite face of the DNA to RNAP. Here we have carried out a genetic and biochemical analysis of the positioning and role of α-CTD at the CII-dependent pE promoter. Our results show that α-CTD contacts the DNA immediately upstream of the −35 region at pE, on the opposite face of the DNA helix to CII, and that these contacts are important for CII-dependent activation.

MATERIALS AND METHODS

Bacterial strains, plasmids and gene fusions

The rpoA+ and rpoA341 strains, WAM106 and WAM105, respectively, have been previously described (17). Derivatives of these strains containing single copy pE-lacZ, pr-lacZ and pααAαααα-lacZ fusions were constructed by lysogenization with λ299 derivatives containing the respective fusions. The fusions were constructed according to Giladi et al. (18) and have been reported previously (16,19). Plasmid pJMH1 is a pSC101-based replicon carrying the lacP and kanamycin resistance genes (17). Plasmid pMO23 is a p15A-derived replicon bearing a chloramphenicol resistance gene and the cII and cIII genes, each under the control of the pααAαααα promoter (16). Plasmid pTIJSpE was constructed by cloning the EcoRI–Dral fragment of pHG86 (20), containing the pααAαααα–lacZ promoter fusion, into the RK2 minireplicon, pTJS42, which harbours a tetracycline resistance gene (21). For the expression of mutant rpoA alleles for the alanine scan analysis, derivatives of plasmids pHITIα, encoding alanine substitutions at position 255–271 and 302 in α-CTD (7,22), pREIIα, encoding alanine substitutions at remaining positions in α-CTD (7,22–25), or pLAW2phs, encoding the K271E substitution (17), were used. The control plasmid encoding wild-type rpoA was pLAW2 (26). All the plasmids which encode α and mutant derivatives thereof specify resistance to ampicillin.

Measurement of β-galactosidase activity.

β-Galactosidase assays were performed on mid-logarithmic phase cultures according to the method of Miller (27). Results presented are averages of at least three independent experiments and are shown with standard deviations.

Protein purification and reconstitution of RNA polymerase.

Plasmid pET-CII (28) was used for over-production of N-terminally His-tagged CII protein, which was purified as described previously (28). For the reconstitution of RNAP, inclusion bodies of RNAP β, β′ and σ subunits from strains XL1-Blue (pMKSe2), BL21(DE3)(pT7β′) and BL21(DE3) (pLHN12σ), respectively, were prepared as described previously (29). His-tagged RNAP α subunits were prepared using plasmid pHITI1NHα. Derivatives of pHITI1NHα carrying mutant rpoA alleles were constructed by replacing the HindIII–BamHI fragment, which encodes wild-type α-CTD, with the corresponding fragments from plasmids pHITIα (258A, 261A, 265A, 271A) (7,22,30) or pLAW2phs (271E) (17). Over-expression of the α subunits in strain BL21(DE3) and purification of α by Ni2+-affinity chromatography and reconstitution into RNAP were performed essentially as described previously (7,29). Purification of α subunits with single cysteine residues at positions 273 and 302, conjugation with iron [5]-[p-bromosacamidobenzenyl] ethylenediaminetetraacetate (Fe-BABE), and reconstitution into RNAP were performed as described in Lee et al. (31).

In vitro transcription

Single round in vitro transcription reactions were performed in a total volume of 20 μl in buffer containing 50 mM KCl, 40 mM Tris–HCl (pH 8.0), 10 mM MgCl2, 1 mM dithiothreitol (DTT), 100 μg/ml bovine serum albumin (BSA) and 150 ng of linear template DNA. Template was prepared by PCR amplification of λ DNA using primers 5′-TGG-CGTATGGTGCGATAGTC-3′ and 5′-ACGTCGCTTCTCAAGCTG-3′, for 35 cycles of denaturation at 95°C for 30 s, annealing at 53.2°C for 30 s and extension at 72°C for 1 min. The resultant PCR product (1433 bp) was digested by BsuRI to obtain a fragment of 1172 bp, containing the λ, pE, and ppoop promoters. The binding reaction of CII (40 ng) with the DNA (150 ng) was carried out at 37°C for 10 min, after which RNA polymerase was added and the incubation continued for a further 10 min. After the addition of nucleotides (CTP, GTP and ATP each to a final concentration of 150 μM, UTP to 15 μM, and 0.6 μCi of [α32P]UTP per reaction) and 50 μg heparin/ml, the samples were incubated at 37°C for 15 min and the reactions were stopped by the addition of an equal volume of 95% formamide containing 20 mM EDTA, 0.05% bromophenol blue and 0.05% xylene cyanol. The samples were separated by electrophoresis in 6% polyacrylamide gels containing 46% urea in TBE buffer (32). The gel was dried, and RNA bands were visualized and quantified, following background subtraction, using a PhosphorImager (Bio-Rad). Concentrations of RNAP, calibrated to give the same amount of transcription from the activator-independent ppoop promoter, were: 46 nM wild-type RNAP, 34 nM RNAP αK271A, 54 nM RNAP αK271A, 13 nM RNAP D258A, 28 nM RNAP αE261A and 35 nM RNAP αE265A. Transcriptional activities were calculated from at least three independent experiments and are presented as a percentage (with standard deviation) of transcripts obtained with wild-type RNAP.

Fe-BABE-mediated hydroxyl radical footprinting

A 275 bp DNA fragment containing the λ pE promoter was amplified from bacteriophage λ DNA by PCR using primers 5′-GCCGAACTCCACACCTATGGTGATGC-3′ and 5′-GCCGAATTTCCATGTCGCTCAAGACC-3′, cleaved with EcoRI and HindIII restriction enzymes and cloned into the vector pSR (33). A 355 bp AatII–HindII fragment was purified from the resultant plasmid (pSRpe) and labelled at the HindIII end with either [γ32P]ATP and T4 polynucleotide kinase (for the template strand) or [α32P]ATP and E.coli DNA polymerase Klenow fragment (for the non-template strand). The Fe-BABE-mediated DNA cleavage reactions were carried out in a reaction volume of 25 μl (5 mM MgCl2, 100 mM potassium glutamate, 40 mM HEPES pH 8.0, 50 μg/ml BSA, 10 μg/ml herring sperm DNA). Promoter DNA fragments were incubated with CII protein (3 μM final concentration) at 37°C for 10 min. After 10 min, RNAP
holoenzyme was added (200 nM final concentration) and incubated at 37°C for 15 min. Complexes were then challenged with heparin (50 μg/ml final concentration) for 1 min at 37°C, then DNA cleavage was initiated by the addition of 3 mM sodium ascorbate and 3 mM hydrogen peroxide. The reactions were incubated for at least 2 min before being stopped by the addition of thiourea and EDTA to final concentrations of 7 and 45 mM, respectively. DNA was then extracted with phenol/chloroform, precipitated with ethanol and analysed by electrophoresis on a 6% polyacrylamide gel. The gels were calibrated with Maxam–Gilbert G + A ladders and analysed using a PhosphorImager and Quantity One software (Bio-Rad).

RESULTS
Location of αCTD–DNA interactions at the pE promoter
To determine the location of αCTD at the pE promoter, we exploited the DNA cleavage reagent Fe-BABE that can be attached to specific locations in RNAP α subunits (34,35). Thus, RNAP was reconstituted with purified α subunits that had been covalently modified with Fe-BABE, CII-dependent open complexes were formed at pE, and the DNA cleaving ability of Fe-BABE was triggered by the addition of ascorbate and hydrogen peroxide. In these experiments, the Fe-BABE reagent was tethered either to position 273 or to position 302, located on opposite faces of αCTD (Fig. 1A) (31). Figure 1B shows the patterns of DNA cleavage by Fe-BABE-tagged RNAP, revealed by gel electrophoresis and phosphorimager analysis. These cleavages occur in small clusters, separated by 10–11 bp, suggesting that αCTD binds to successive minor grooves on one face of the promoter DNA [see Murakami et al. (34,35)]. The strongest DNA cleavage on both strands is found near position −41, and we interpret this as due to the binding of one of the two αCTDs. For both strands, cleavage due to Fe-BABE conjugated to residue 302 occurs 4–5 bp downstream of the sites of cleavage due to Fe-BABE conjugated to residue 273. The locations of the different cleavage sites on the two strands are illustrated in Figure 1C and D. The simplest interpretation of these data, based on the model shown in Figure 1A, is that this αCTD binds to the minor groove near position −41, and is oriented such that the 261 determinant points downstream. The 261 determinant would thus be well placed to interact with region 4 (domain σJ) of the RNAP σ subunit, in agreement with results from previous authors (11–13). The results in Figure 1B show that αCTD can also bind to promotor DNA near positions −51 and −61. The signals near these positions are weaker than those near position −41, but it is clear that the cuts, at least around the −51 position, are also staggered. Therefore, we suggest that the position of the second αCTD is not fixed, and that the orientation of this αCTD, bound at −51, is the same as that of αCTD bound at −41.

Determinants in αCTD important for CII-dependent activation of pE in vivo
To determine whether the αCTD–DNA interactions at pE are important for CII-dependent activation, and to identify other amino acid side chains in αCTD that are important for CII-mediated activation, we used an alanine scanning approach. To do this, we exploited a set of plasmids encoding the RNAP α subunit in which residues 255–329 were each changed individually to alanine. These plasmids were introduced into a host strain carrying the pE-lacZ fusion plasmid (pTJSpE) and plasmids specifying inducible CII function (pMO23 and pMH1). In addition, the host strain carried the rpoA341 mutant allele that encodes α subunits with the K271E substitution. Since this substitution greatly reduces expression from pE, CII-induced lacZ expression is low in the absence of plasmid-encoded wild-type α. This provides a simple system to measure the effects of different alanine substitutions in plasmid-encoded αCTD on CII-dependent activation of pE. In this experiment, the cII gene was expressed from an isopropyl-β-d-thiogalactopyranoside (IPTG)-inducible promoter (on pMO23) and, due to the toxicity of overproduced CII (36), induction was allowed to proceed only for 75 min. In our conditions, sufficient incorporation of plasmid-encoded α into RNAP was achieved to assay the effects of the alanine

Figure 1. Location and orientation of αCTD at the pE promoter. (A) Model of αCTD bound to DNA to show the relative location of amino acid residues pertinent to this study. The model of αCTD is adapted from Benoff et al. (10). R265, located within the DNA-binding determinant, is shown in pink; residue 261, which participates in interactions with σ, is shown in yellow; and residues 273 and 302, which were derivatized with Fe-BABE, are shown in blue and red, respectively. The orientation shows the 261 determinant facing downstream, as occurs at pe. (B) Cleavage of pe promoter DNA by Fe-BABE-labelled RNAP. Phosphorimager scan of a polyacrylamide sequencing gel showing DNA cleavage at the pe promoter resulting from attack by RNAP reconstituted with Fe-BABE-derivatized α in the presence of CII. Lanes 1–4 show results for the template strand, and lanes 5–8 show results for the non-template strand. Lanes 1 and 5, RNAP containing Fe-BABE-derivatized E302C α; lanes 2 and 6, RNAP containing Fe-BABE-derivatized E273C α; lanes 3 and 7, no proteins; lanes 4 and 8, Maxam–Gilbert A + G ladder. (C) Model of DNA showing the minor groove locations of DNA cleavage at pe due to RNAP reconstituted with α subunits derivatized with Fe-BABE at position 273 (blue) or 302 (red). The template strand is shown in pale blue and the complementary strand in grey. The arrow indicates the direction of transcription. (D) DNA sequence of the pe promoter upstream region showing sites of Fe-BABE-induced cleavage indicated by coloured stars (colour coded as in A). The −35 region is in bold and the tetrad repeats recognized by CII are boxed.
substitutions, i.e. plasmid-directed synthesis of wild-type α allowed for a 4- to 5-fold stimulation of \( p_E \) activity relative to plasmid-directed synthesis of α containing the K271E substitution (Fig. 2A).

The results show that alanine substitutions at residues 265, 268, 281, 295, 296, 303 and 320 in αCTD most strongly impaired CII-dependent activation of \( p_E \) due to the higher sensitivity of our system in comparison with previous alanine scan experiments with αCTD, which required dominant-negative effects of plasmid-encoded α (11), we regard substitutions that give rise to ≤60% of the activity afforded by plasmid-encoded wild-type α as exerting strong inhibitory effects on \( p_E \) (Fig. 2A). The location of these residues on the αCTD structure is shown in Figure 2B. Residues 265, 268, 295 and 296 fall within the 265 DNA-binding determinant (7,8), suggesting that DNA binding of
αCTD is important for CII-dependent activation of pE. Interestingly, in contrast to the rpoA341-encoded K271E substitution, substitution of K271 by alanine exerted only a small inhibitory effect on activation of pE by CII. Concerning the effects of alanine substitutions at residues 281, 303 and 320, these could be due to either direct or indirect effects. Residues 281 and 303 are buried within αCTD and so the latter possibility appears more likely. Regarding residue 320, the side chain is surface exposed but is located some distance from the other important residues (Fig. 2B).

Determinants in αCTD important for CII-dependent activation of pE in vitro

In the next set of experiments, we reconstituted RNAP containing wild-type or mutant α subunits and used run-off transcription assays to measure its activity at pE. Our primary aim was to quantify the effect of disrupting DNA binding by αCTD, and thus we compared wild-type RNAP with RNAP carrying α subunits harbouring the R265A substitution. Results presented in Figure 3 show that CII-dependent activation of pE is greatly reduced by the R265A substitution (while transcription from the control oop promoter is unaffected). Since our analysis with Fe-BABE suggested that one αCTD was positioned such that it could contact the RNAP σ subunit, we also reconstituted RNAP with α subunits carrying the D258A and E261A substitutions in the 261 determinant, known to be involved in αCTD–σ interactions. Results in Figure 3 show that these preparations of RNAP were also impaired for CII-dependent activation, although to a lesser degree than with the R265A substitution. Finally, we also measured CII-dependent activation at pE with RNAP containing α subunits with the K271E or K271A substitutions. Consistent with the in vivo analysis (Fig. 2A), RNAP reconstituted with K271Eα was severely impaired for CII-mediated activation of pE, whereas RNAP reconstituted with K271Aα supported efficient activation (Fig. 3).

Figure 3. Identification of αCTD residues important for activation of pE by CII in vitro. Single-round in vitro transcription experiments were performed using linear template DNA containing pE and p_opp CII, and RNAP reconstituted with hexahistidine-tagged α derivatives containing alanine substitutions at the positions indicated. The activities of purified RNAPs were normalized at the p_opp promoter. The efficiency of transcription from pE in the presence of each reconstituted mutant RNAP is shown below the corresponding lane from a typical transcription gel. Values (with standard deviation) are expressed as percentages of the transcript yield obtained with wild-type RNAP.

Figure 4. CII-dependent and CII-independent activities of the phage λ, pE (A), pI (B) and paQ (C) promoters in rpoA+ (WAM106) and rpoA341 (WAM105) hosts. Each host strain (rpoA+ and rpoA341), carrying a single copy pE–lacZ, pI–lacZ or paQ–lacZ fusion together with plasmids pMO23 and pMM1, was grown in LB medium containing chloramphenicol (34 μg/ml) and kanamycin (50 μg/ml) at 37°C to OD ~0.2. IPTG was added to the indicated final concentrations and the activities of β-galactosidase (in Miller units) were measured 60 min later.

Effect of the rpoA341 mutation on CII-mediated transcription activation at pI and paQ

To determine whether the K271Eα substitution affects the other CII-dependent promoters, pI and paQ, reporter gene fusions to all three CII-dependent promoters were constructed in single copy using the same genetic system, and promoter activity was measured in the presence and absence of CII. For this experiment, levels of induced CII production were varied by using different concentrations of IPTG. We found that the maximum induced activities of the three lysogenic promoters in the rpoA+ background occurred following induction with 0.05 mM IPTG (Fig. 4). With this level of CII synthesis, the degree of activation afforded by CII at each promoter was similar, i.e. 12.5- to 14-fold. Consistent with previous observations, in the rpoA341 mutant, the activity of the pE promoter was approximately equal to that observed in the wild-type strain in the absence of CII synthesis, i.e. CII
activation of $p_E$ was negligible (Fig. 4A) (16). The $p_I$ promoter was $\sim$30% as active in the rpoA341 mutant in comparison with the wild-type strain when CII synthesis was induced by 0.05 mM IPTG. However, this difference became less pronounced with higher levels of CII induction (Fig. 4B). The efficiency of CII-mediated stimulation of $p_{\alpha Q}$ was least affected by the rpoA341 mutation (Fig. 4C). Only when CII synthesis was induced with 0.05 mM IPTG was there a significant difference between $p_{\alpha Q}$ activity in the wild-type and mutant strains, suggesting that it may be slightly less sensitive to CII in the mutant background. These results suggest differences in the role of $\alpha$CTD at each of these promoters.

**DISCUSSION**

Previous biochemical and genetic studies have suggested a role for $\alpha$CTD in CII-dependent activation of the $\lambda$ $p_E$ promoter (14,15). Using a DNA cleavage reagent that was tagged to two different locations in $\alpha$CTD, we deduced the location and orientation of $\alpha$CTD in RNAP–promoter open complexes at $p_E$. These experiments showed that one $\alpha$CTD binds near position $-41$ and the other $\alpha$CTD binds further upstream. The $\alpha$CTD near position $-41$ must be bound on the opposite face of the DNA helix to CII (Fig. 5A), in accord with previous investigators who showed that CII binds to the opposite face of the DNA helix to $\sigma$ (38). The results of the Fe-BABE analysis are also consistent with previous DMS protection studies (38) showing that protection occurs at positions $-40$ and $-41$ at $p_E$ in the presence of CII and RNAP. Our results show that the $\alpha$CTD near position $-41$ is bound with its 261 determinant pointing downstream such that it could interact with region 4 of the RNAP $\sigma$ subunit bound to the $-35$ element (Fig. 5A). Our observation that substitutions in the 261 determinant slightly impair CII-dependent activation of $p_E$ is consistent with such an interaction, which may be required for full activation of $p_E$ by CII. In this regard, it is noteworthy that, at certain activator-independent promoters such as $rrnB$ P1, where $\alpha$CTD binds at the same location, functional interactions occur between two residues within the 261 determinant, D259 and E261, and the side chain of R603 in region 4.2 of $\sigma$ (12). Also, at the class I CRP-dependent $p_I$ promoter, where $\alpha$CTD binds to the $-42/-43$ region, the 261 determinant also interacts with $\sigma$ and this interaction is required for activation by CRP (10,11,13).

The location of $\alpha$CTD in CII-dependent open complexes at $p_E$ is very similar to that found at factor-independent promoters such as $rrnB$ P1 and lacUV5 (39–41). This contrasts sharply with the situation at most class II activator-dependent promoters, where the activator is positioned on the same side of the DNA helix as $\sigma$, and $\alpha$CTD is displaced to a site upstream of the bound activator (Fig. 5B). An exception is found at the Bordetella pertussis fha promoter, where three dimers of the activator, BvgA, occupy a region extending from positions $-35$ to $-100$ on the same face of the DNA helix as $\sigma$. At this promoter, $\alpha$CTD binds to the same segment of DNA as BvgA, but to a different face of the DNA helix (42). One common pattern which is emerging from studies of class II or class II-like promoters is that, at this class of promoter, $\alpha$CTD appears to bind to the nearest ‘vacant’ segment of upstream DNA to the promoter, with a preference for binding to the same face of the DNA as RNAP (43).

The alanine scanning analysis identified four residues in the $\alpha$CTD DNA-binding determinant where alanine substitution causes at least a 40% decrease in activation in our assays, suggesting that DNA binding by $\alpha$CTD is important for CII-dependent activation of $p_E$. This conclusion was supported by run-off transcription assays in vitro. However, the mechanism of activation of the $p_E$ promoter by CII still remains unclear. In particular, it is not apparent whether there is any direct contact between CII and $\alpha$CTD at this promoter. The alanine scanning analysis showed that substitutions of residues at positions 281, 303 and 320 caused large decreases in activation, but, for reasons discussed above, we think it is unlikely that L281 and I303 contact CII. We are unable to conclude whether N320 is involved in contacts with CII. However, due to its location on $\alpha$CTD, this residue would not be expected to contact an activator bound to the opposite face of the DNA. Interestingly, although the K271E substitution severely inhibits CII-mediated activation of $p_E$, alanine substitution at position 271 had very little effect. This suggests that the effect of the K271E substitution might be to create an interaction (or ‘clash’) which impairs CII function. In the last part of our study, we showed that each of the three CII-dependent $\lambda$ promoters is affected differently by the K271E substitution. This suggests that, although CII binds to identical sequences at identical locations at these promoters, the mechanisms of transcription activation, particularly with respect to the role of $\alpha$CTD, are distinct. This is consistent with previous observations that the initial binding and isomerization steps in the transcription initiation pathway are differentially affected by CII at each of these promoters (2,44). Thus, the $K_E$ step is stimulated 103- to 104-fold at $p_I$ and $p_{\alpha Q}$ whereas at $p_E$, which is distinguished by having an ‘extended’ $-10$ sequence, $K_E$ is increased by only 15-fold (44–46).
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REFERENCES


