DQ 65–79, a Peptide Derived from HLA Class II, Induces IκB Expression

Yun Jiang, Daniel Chen, Shu-Chen Lyu, Xuefeng Ling, Alan M. Krensky and Carol Clayberger

J Immunol 2002; 168:3323-3328; doi: 10.4049/jimmunol.168.7.3323
http://www.jimmunol.org/content/168/7/3323
DQ 65–79, a Peptide Derived from HLA Class II, Induces IκB Expression

Yun Jiang,∗ Daniel Chen, † Shu-Chen Lyu,∗ Xuefeng Ling, † Alan M. Krensky, † and Carol Clayberger∗‡†

A synthetic peptide corresponding to residues 65–79 of the α helix of the α-chain of the class II HLA molecule DQA03011 (DQ 65–79) inhibits the proliferation of human T lymphocytes in an allele nonrestricted manner. By using microarray technology, we found that expression of 29 genes was increased or decreased in a human CTL cell line after treatment with DQ 65–79. This study focuses on one of these genes, IκB-α, whose expression is increased by DQ 65–79. IκB proteins, including IκB-α and IκB-β, are increased in T cells treated with DQ 65–79. Nuclear translocation of the NF-κB subunits p65 and p50 is decreased in T cells after treatment with DQ 65–79, while elevated levels of p65 and p50 are present in cytosol. DQ 65–79 inhibits the degradation of IκB-α mRNA and inhibits the activity of IκB kinase. These findings indicate that the DQ 65–79 peptide increases the level of IκB proteins, thereby preventing nuclear translocation of the transcription factor, NF-κB, and inhibiting T cell proliferation. The Journal of Immunology, 2002, 168: 3323–3328.

Maj or histocompatibility complex molecules, designated HLA in man, play a central role in Ag recognition by T lymphocytes. MHC molecules bind to processed peptides from both internal and external sources and present them to TCRs (1). Other regions of MHC molecules interact with T cell coreceptors CD4 and CD8 (2–6). In addition to their natural role in Ag presentation, MHC molecules are the principal targets of the immune response to allografts. Graft rejection results from both the direct recognition of foreign MHC as intact molecules and the indirect recognition of foreign MHC as processed peptides in the context of recipient MHC molecules (7, 8). MHC molecules have also been implicated in the prevention of graft rejection. There are many reports that administration of purified MHC molecules (9, 10) or synthetic peptides corresponding to regions of class I or class II MHC molecules (11–17) induce allograft tolerance in animal models.

During the past decade we designed and characterized a number of synthetic peptides corresponding to the linear sequence of HLA molecules as potential therapeutic immunosuppressive reagents. We described several synthetic peptides corresponding to class I HLA sequences that inhibit CTL responses in allele-specific or nonspecific ways (6, 18–20). One such peptide, corresponding to residues 75–84 of the α helix of HLA-B2702, prevents allograft rejection in rodent models (14, 15, 21) and has been evaluated in a clinical trial (22). More recently, we described a synthetic peptide corresponding to residues 65–79 of the α helix of the α-chain of the class II HLA molecule DQA03011, designated DQ 65–79, that inhibits T cell proliferation in an allele nonspecific manner (23). We demonstrated that this peptide is internalized by T cells, blocks cell cycle progress at the late G1 to S transition, and acts as an antagonist of phosphatidylinositol 3-kinase (PI-3K),3 repressing downstream signaling through the IL-2R (24).

To further understand the mechanisms of action of DQ 65–79, we used microarray technology to identify mRNAs that are increased or decreased in T cells treated with DQ 65–79. This study focuses on one of these genes, IκB (inhibitor of NF-κB), whose expression is up-regulated by DQ 65–79. We show in this work that the DQ 65–79 peptide increases the expression of IκB proteins, thereby preventing nuclear translocation of the transcription factor, NF-κB, and thus inhibiting cell activation.

Materials and Methods

Peptides

DQ 65–79 (NIAVLKHNLSNIKR) and DQ 75S (NIAVLKHNLSNIKR) peptides were synthesized and purified by United Biochemical Research (Seattle, WA), and their compositions were confirmed by mass spectrometry. Stock solutions were made by dissolving the peptides in DMSO (10 mM). A final concentration of 40 μM was used unless otherwise indicated.

Cell culture

Cells were grown in a 5% CO2 incubator at 37°C in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 2 mM L-glutamine (Irvine Scientific), 100 IU/ml penicillin, 100 μg/ml streptomycin (Irvine Scientific), 10 mM HEPES (Irvine Scientific), and 10% FCS (HyClone Laboratories, Logan, UT). Human CTL specific for HLA-A2 were generated as described previously (19). Long-term CTL lines were carried in RPMI 1640 supplemented with 10% T cell-conditioned medium (25) and stimulated weekly with the irradiated (10,000 rad) human B lymphoblastoid cells JY at a 1:10–20 ratio. rIL-2 (50 U/ml; National Cancer Institute, Frederick, MD) was used to activate CTL (1 × 106/ml) harvested 4 days after stimulation with JY cells.

RNA

Total RNA was prepared using TRIZol (Life Technologies, Gaithersburg, MD), followed by a phase separation using chloroform (Sigma-Aldrich, St. Louis, MO). RNA was precipitated using isopropyl alcohol (Sigma-Aldrich) and washed with 75% ethanol (Gold Shield Chemical, Hayward, California). RNase-free water was added, and RNA was dissolved in 10 μl of water.

Abbreviations used in this paper: PI-3K, phosphatidylinositol 3-kinase; GEArray, gene expression array; IKK, IκB kinase; NPCP, nuclear pore complex protein.

© 2002 by The American Association of Immunologists

Copyright © 2002 by The American Association of Immunologists

0022-1767/02/$02.00

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 This work was supported by grants from the National Institutes of Health.

2 Address correspondence and reprint requests to Dr. Carol Clayberger, CCSR 2105, Stanford University, Stanford, CA 94305-5164. E-mail address: cclay@stanford.edu

3 Abbreviations used in this paper: PI-3K, phosphatidylinositol 3-kinase; GEArray, gene expression array; IKK, IκB kinase; NPCP, nuclear pore complex protein.
CA), mRNA was isolated with an Oligo-tex mRNA purification kit (Qiagen, Valencia, CA) following the manufacturer’s instructions.

**Differential gene expression using cDNA microarrays**

Details of the methods used for preparation, hybridization, and analysis of microarray can be found at the Stanford microarray web page (www.brownlab.stanford.edu/protocols.html). Briefly, mRNA was extracted from CTL activated with 50 U/ml rIL-2 in the presence or the absence of DQ 65–79 (40 μM) for 24 h. cDNA was synthesized and fluorescently labeled with Cy3- or Cy5-DUTP (Amersham Pharmacia Biotech, Piscataway, NJ) by a single round of reverse transcription using oligo(dT) primer and SuperScript II reverse transcriptase (Life Technologies). Equal masses of the two cDNA probes were combined and allowed to hybridize on a chip displaying cDNA fragments and expressed sequence tags expressed in human lymphocytes. The microarray chip was then scanned, and mono-chrome images were imported into software and analyzed. Data from a single hybridization experiment were viewed as normalized ratios in which significant deviations from 1 (no change) were indicative of increased or decreased levels of gene expression relative to the reference sample. Results are representative of three similar experiments.

**Differential gene expression using the pathway-specific cDNA array system**

Total RNA was extracted from CTL activated with rIL-2 in the presence of DMSO, DQ 75S, or DQ 65–79. Reverse transcription and PCR amplifications of IκB-α and IκB-β were performed using the Platinum Quantitative RT-PCR ThermoScript One-Step System (Life Technologies) and the Qiagen one-step RT-PCR kit, respectively. Reverse transcription and PCR amplification of cDNA was performed and the products were separated on a 1.8% agarose gel containing 0.5% of ethidium bromide (Sigma–Aldrich) and quantitated by densitometry. For DNA decay analysis, actinomycin D (Sigma–Aldrich) was dissolved in ethanol and added at a concentration of 1 μg/ml at the time of peptide treatment.

**RT-PCR analysis**

Total RNA was prepared from CTL activated with rIL-2 in the presence of DMSO, DQ 75S, or DQ 65–79. Reverse transcription and PCR amplifications of IκB-α and IκB-β were performed using the Platinum Quantitative RT-PCR ThermoScript One-Step System (Life Technologies) and the Qiagen one-step RT-PCR kit, respectively. β-Actin was amplified parallel to IκB-α or IκB-β to serve as an internal control. PCR products were separated in a 1.8% agarose gel containing 0.5 μg/ml ethidium bromide (Sigma–Aldrich) and quantitated by densitometry. For DNA decay analysis, actinomycin D (Sigma–Aldrich) was dissolved in ethanol and added at a concentration of 1 μg/ml at the time of peptide treatment.

**Intracellular immunofluorescent staining and flow cytometry**

A Cytofix/Cytoperm kit (BD Pharmingen, San Diego, CA) was used for intracellular staining. CTL (0.5 × 10⁶) as described were fixed and permeabilized in Cytofix/Cytoperm solution. After washing, cells were resuspended in 50 μl Perm/Wash buffer (BD PharMingen) with anti-IκB-α or anti-IκB-β Ab (Santa Cruz Biotechnology, Santa Cruz, CA) for 30 min on ice, followed by a secondary incubation with PE-conjugated goat anti-mouse/rabbit IgG Ab (Caltag Laboratories, Burlingame, CA). After washing, cells were analyzed on a FACSscan cytometer (BD Biosciences, Mountain View, CA).

**Cell lysate preparation**

CTL were activated with rIL-2 in the presence of DMSO, DQ 75S, or DQ 65–79. Whole-cell lysate was prepared using a lysis buffer consisting of 250 mM NaCl, 1% Nonidet P-40, 0.25% sodium deoxycholate, 50 mM Tris buffer, and 2 mM EDTA and supplemented with protease inhibitor mixture (BD Pharmingen). Cytoplasmic and nuclear extracts were prepared as follows. Cells were suspended in buffer A (10 mM HEPES-KOH, 1.5 mM MgCl₂, 10 mM KCl, 1 mM DTT, and 1 mM PMSF) and incubated for 10 min on ice. After vortexing for 10 s the supernatant was harvested as the cytoplasmic extract. The pellet was resuspended in buffer C (250 mM glycerol, 20 mM HEPES-KOH, 1.5 mM MgCl₂, 420 mM NaCl, 0.2 mM EDTA, 1 mM DTT, and 1 mM PMSF) and incubated for 20 min on ice. Cellular debris was removed, and the supernatant was collected as the nuclear extract.

**Western blot**

Proteins were separated by SDS-PAGE and transferred electrophoretically to polyvinylidene difluoride membranes (Amersham Pharmacia Biotech). After blocking with a solution of 5% nonfat dry milk/0.1% Tween 20, the membrane was probed with anti-IκB-α (BD Pharmingen), anti-IκB-β (On-cogene Research Products, Boston, MA), anti-NF-κB p65 (Santa Cruz Biotechnology), or anti-NF-κB p50 (Upstate Biotechnology, Lake Placid, NY). Ab. After washing, the membrane was incubated with anti-mouse/rabbit Ab conjugated with HRP (Amersham Pharmacia Biotech). Proteins were visualized by chemiluminescence. For loading control, a DQ labeled rabbit IgG agarsase conjugate (Santa Cruz Biotechnology) for 1 h at 4°C. Three hundred to 500 μg total protein were then incubated with 5 μl packed anti-IκB kinase (IKK)-α or control rabbit IgG agarsase conjugate (Santa Cruz Biotechnology) for 2 h at 4°C. Agarose beads and supernatants were collected after centrifugation at 3000 rpm for 5 min at 4°C. Actin in the supernatant was determined by Western blot using an actin kit purchased from Oncogene Research Products. The agarose beads were washed with a kinase buffer (20 mM HEPES, 2 mM MgCl₂, 2 mM MnCl₂, 20 mM β-glycerophosphosphate, 10 mM NaF, 10 μM ATP, 500 μM Na₂VO₃, 500 μM PMSF, 1 mM DTT, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1 μg/ml pepstatin) before they were subjected to kinase assay. In vitro kinase assay was performed in the kinase buffer supplemented with 2 μg IκB-α (Santa Cruz Biotechnology) and 3–10 μCi [γ-³²P]ATP (Amersham Pharmacia Biotech) at 30°C for 30–60 min. The reaction was stopped by addition of 6× SDS-PAGE sample buffer, subjected to SDS-PAGE analysis, and visualized by autoradiography.

**Results**

**Differential gene expression mediated by DQ 65–79**

The DQ 65–79 peptide inhibits the proliferation of CTL lines, PBL, and purified T cells stimulated with anti-CD3 Ab, PHA, or alloantigen in an allele nonspecific manner (23). To identify genes that are differentially expressed in T cells following treatment with DQ 65–79, we used cDNA microarray technology. mRNA extracted from CTL treated with rIL-2 in the presence or the absence of DQ 65–79 for 24 h was processed and hybridized onto a human lymphocyte microarray chip as described in Materials and Methods. Over 8000 transcripts were screened, and the expression levels of <1% of these were altered by treatment with DQ 65–79. The change in expression of 29 transcripts was significant (regression correlation, >0.6; altered fluorescence ratio, >1.6); the expression of 11 of these genes was increased by DQ 65–79, while the expression of 18 was decreased (Table I).

Many of the mRNAs listed in Table I encode proteins involved in cell survival/apoptosis/proliferation, including IκB-α and Jun-B (transcription factors); TNF-related apoptosis-inducing ligand, inhibitor of apoptosis c, pim-1 kinase, c-Myc, and IκB-α (survival/apoptosis); initiation factor 4A1 (initiation of translation); IL-2R β-chain (proliferation); and p21 (cell cycle progression). Because IκB-α plays a key role in regulating the activity of NF-κB (26, 27), an important transcription factor for cell activation and survival (28), we chose to further investigate the effect of DQ 65–79 on the IκB/NF-κB pathway. We used the GEArray system to compare the expressions of genes specifically associated with the NF-κB pathway. CTL were treated for 1, 5, or 20 h with DQ 65–79 or DQ75S, a peptide in which the isoleucine at position 75 has been substituted with serine and which does not block cell cycle progression. This system independently confirmed that the expression of IκB-α mRNA was increased in CTL 5 h after treatment with DQ 65–79 (data not shown), while the expression of NF-κB genes (c-rel and p105) was not altered by DQ 65–79 (data not shown). No changes were observed in cells treated with DQ 75S (data not shown).
IκB-α and IκB-β mRNAs are increased in CTL treated with DQ 65–79

IκB-β also plays an important role in regulating the activity of NF-κB. Because IκB-β was not included in either the microarray chip or the GEArray membrane, we used semiquantitative RT-PCR to examine the effect of DQ 65–79 on IκB-β expression in CTL. Expression of IκB-β was increased in CTL after treatment with DQ 65–79 (Fig. 1). This technique also showed that IκB-α mRNA was similarly increased in CTL treated with DQ 65–79 (data not shown).

IκB proteins are increased in cells treated with DQ 65–79

The expression of IκB-α and IκB-β proteins in CTL treated with DQ 65–79 was examined using intracellular immunofluorescent staining and flow cytometry. IκB-α increased in CTL treated with DQ 65–79 for 24 or 48 h (Fig. 2). The expression of IκB-β was increased in CTL after treatment with DQ 65–79 for 48 h, and the augmentation was more significant at 72 h (Fig. 2). These findings were confirmed using Western blot analysis of lysates from peptide-treated CTL (Fig. 3).

Translocation of NF-κB to the nucleus is blocked in CTL treated with DQ 65–79

We next asked whether DQ 65–79-mediated up-regulation of IκB proteins affects nuclear translocation of the transcription factor NF-κB. Cytoplasmic and nuclear extracts from DQ 65–79-treated

<table>
<thead>
<tr>
<th>Increased mRNAs</th>
<th>Cy3:Cy5</th>
<th>Decreased mRNAs</th>
<th>Cy3:Cy5</th>
</tr>
</thead>
<tbody>
<tr>
<td>MIP1β</td>
<td>1024:551</td>
<td>pim-1 kinase</td>
<td>217:743</td>
</tr>
<tr>
<td>Follicular lymphoma variant translocase</td>
<td>1913:1035</td>
<td>Initiation factor 4A1</td>
<td>386:1020</td>
</tr>
<tr>
<td>G-26 (MIP1)</td>
<td>1290:700</td>
<td>HLA-DRα</td>
<td>559:1464</td>
</tr>
<tr>
<td>TNF-related apoptosis-inducing ligand</td>
<td>1853:1071</td>
<td>Ribosomal protein L3</td>
<td>594:1447</td>
</tr>
<tr>
<td>Inhibitor of apoptosis c</td>
<td>1913:1112</td>
<td>c-myb</td>
<td>290:708</td>
</tr>
<tr>
<td>IκB-α</td>
<td>3279:1959</td>
<td>B cell serine/threonine kinase</td>
<td>336:765</td>
</tr>
<tr>
<td>Jun-B</td>
<td>831:499</td>
<td>HLA-DR</td>
<td>389:766</td>
</tr>
<tr>
<td>IL-2R β-chain</td>
<td>3401:2086</td>
<td>T cell receptor αδ region</td>
<td>2858:5528</td>
</tr>
<tr>
<td>Syndecan 2</td>
<td>2682:1652</td>
<td>B cell receptor-associated protein</td>
<td>513:968</td>
</tr>
<tr>
<td>Unknown expressed sequence tags</td>
<td>8074:3646</td>
<td>Glycogen synthase</td>
<td>1081:1781</td>
</tr>
<tr>
<td>Unknown expressed sequence tags</td>
<td>1796:852</td>
<td>Brouton’s tyrosine kinase</td>
<td>614:1114</td>
</tr>
<tr>
<td></td>
<td></td>
<td>polyA binding protein</td>
<td>777:1378</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prothymosin α</td>
<td>3418:5831</td>
</tr>
<tr>
<td></td>
<td></td>
<td>p21</td>
<td>413:693</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Laminin-binding protein</td>
<td>636:1019</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown expressed sequence tags</td>
<td>423:872</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown expressed sequence tags</td>
<td>401:759</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Unknown expressed sequence tags</td>
<td>642:1087</td>
</tr>
</tbody>
</table>

**Table 1. mRNAs differentially expressed in DQ 65-79-treated CTL**

**FIGURE 1.** DQ 65–79 increases the expression of IκB-β mRNA. RNA was extracted from CTL activated with rIL-2 in the presence of DMSO, DQ 75S, or DQ 65–79 for the indicated periods of time. IκB-β and β-actin were reverse transcribed and PCR-amplified, and the products were separated in an agarose gel. Results are expressed in densitometric units normalized to the expression of β-actin. Representative results from one of three similar experiments are shown.

**FIGURE 2.** DQ 65–79 increases the expression of IκB proteins analyzed by FACS. CTL treated with DMSO (dotted line), DQ 75S (dashed line), and DQ 65–79 (solid line) were harvested at 24 (A and B), 48 (C and D), or 72 (E and F) h and stained intracellularly for IκB-α and IκB-β. Representative results from one of three similar experiments are shown.
CTL were prepared, and the expression of p65 and p50, the subunits of a classical NF-κB dimer (29, 30), was determined by Western blot. Elevated levels of p65 and p50 were found in the cytoplasmic extract of CTL treated with DQ 65–79, while lesser amounts of p65 and p50 were found in the nuclear extract (Fig. 4). These findings indicate that DQ 65–79 inhibits the translocation of NF-κB from the cytosol to the nucleus.

**DQ 65–79 retards the degradation of IκB-α mRNA**

Accumulation of IκB mRNA may result from increased transcription and/or inhibition of mRNA degradation. The turnover of IκB mRNA was examined by RT-PCR in CTL treated with actinomycin D, which blocks synthesis of new RNA. IκB-α mRNA was more stable in CTL treated with DQ 65–79 (Fig. 5), indicating that the peptide retards IκB-α mRNA degradation.

**DQ 65–79 inhibits the activity of IKK**

We next asked whether DQ 65–79 affects the activity of IKK, which phosphorylates IκB and targets the protein for ubiquitination and degradation (31). Whole-cell lysate was prepared from peptide-treated CTL and was immunoprecipitated with anti-IKK-α Ab. An in vitro kinase assay was then performed using IκB-α as the substrate. Only low level phosphorylation of IκB-α was observed using lysates from DQ 65–79-treated cells (Fig. 6), indicating that DQ 65–79 inhibits IKK activity.

**Discussion**

NF-κB is a transcriptional regulator that mediates key immune and inflammatory responses (29). The activity of NF-κB is strictly controlled by IκB (26, 27). In resting cells, NF-κB is sequestered in the cytosol and bound to IκB. When cells are activated, IκB is phosphorylated, causing the release of NF-κB, which then translocates to the nucleus to activate the transcription of target genes. Several immunosuppressants, including cyclosporine A (32, 33), rapamycin (34), FK506 (35, 36), and PNU156804 (37), block the activation of NF-κB by targeting the regulatory IκB protein. Glucocorticoids also can inhibit NF-κB expression either by directly interacting with and affecting activated NF-κB subunits (38–41) or by increasing transcriptional activation of the IκB gene (42, 43). We show in this study that the expression of IκB-α and IκB-β is increased in T cells treated with the synthetic immunosuppressive peptide DQ 65–79. Nuclear translocation of the NF-κB subunits, p65 and p50, is decreased after treatment.
with DQ 65–79, while elevated levels of p65 and p50 are found in the cytosol. The effects of DQ 65–79 on IκB and NF-κB may be central to its inhibition of T cell proliferation.

IκB is regulated at both the mRNA and protein levels. Nuclear NF-κB can cause the transcriptional activation of the IκB gene, leading to reaccumulation of IκB that, in turn, represses NF-κB activity (29, 44). The processing or decay of IκB mRNA is also regulated (45). IκB proteins are phosphorylated by IKK, which consists of two catalytic subunits, IKKα and IKKβ, and a third regulatory subunit, IKKγ. Once phosphorylated, IκB undergoes a conformational change, dissociates from NF-κB, and is degraded (31). DQ 65–79 retards the degradation of IκB-α mRNA and inhibits the activity of IKK. Thus, DQ 65–79 up-regulates IκB expression by at least two mechanisms.

The kinase Akt lies at the intersection of the two pathways, IκB and PI-3K, that we have shown to be affected by DQ 65–79. T cells activated through the IL-2R exhibit decreased Akt kinase activity following treatment with DQ 65–79 (24). Akt has been reported to phosphorylate many different targets, a subset of which is involved in cell survival/proliferation. For example, activated Akt phosphorylates Bad, creating a binding site for the 14-3-3 proteins, which, in turn, inhibits the formation of heterodimers between Bad and the antiapoptotic proteins Bcl-2 and Bcl-xL (46). Akt also affects the NF-κB pathway by associating with and activating IKKs (47). Akt activates NF-κB at least in part at the level of IκB degradation (48). Ramoshkova et al. (49) reported that Akt transiently associates with IKK and induces IKK activation, while Ozes et al. (50) found that Akt directly binds to and phosphorylates IKKα. However, IKKs, IκBs, and NF-κB inhibitory kinase do not contain a consensus sequence for Akt (51), suggesting that none of these is the direct target of Akt. As suggested by Weiss and coworkers (48), it is possible that Akt activates IKK through a mitogen-activated protein kinase kinase kinase family.

Weiss and coworkers (48), it is possible that Akt activates IKK, with no apparent involvement of PI-3K (54). This finding suggests that the inhibitory effects of DQ 65–79 on IκK and NF-κB are limited to pathways involving PI-3K.

cDNA microarray technology is an important new technique for identifying genes that are differentially expressed under a variety of conditions (55, 56). We used this technology to examine genes differentially regulated in response to an immunosuppressive peptide, many of which encode proteins involved in cell survival/apoptosis/proliferation. We selected one of these, IκB, confirmed the microarray data and showed that IκB proteins were also increased. Although we do not know whether the effect of DQ 65–79 on IκB is a primary effect of the peptide, the observed up-regulation in IκB protein is due to both an increase in the IκB mRNA and a decrease in the activity of IKK, the kinase central to IκB degradation. These studies are another example of the utility of microarray technology and provide additional insights into signaling pathways affected by the immunoregulatory DQ 65–79 peptide.

Acknowledgments

We thank Drs. Pat Brown and Chris Barry for providing microarrays and for helping with hybridization, fluorescent scanning, and data analysis.

References


