DQ 65–79, A Peptide Derived from HLA Class II, Mimics p21 to Block T Cell Proliferation

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DQ 65–79, a peptide derived from residues 65–79 of the α-chain HLA class II molecule DQA03011, blocks T cell proliferation and induces T cell apoptosis. Using a yeast two-hybrid assay, we previously identified proliferating cell nuclear Ag (PCNA) as an intracellular ligand for DQ 65–79. In this study, we show that three regions of PCNA, residues 81–100, 121–140, and 241–261, interact with DQ 65–79. Residues 241–261 of PCNA also interact with the C terminus (residues 139–160) of the cell cycle regulator, p21, suggesting that DQ 65–79 and p21 might function similarly. We show here that DQ 65–79 competitively inhibits binding of p21 to PCNA and that both DQ 65–79 and p21 139–160 induce T cell apoptosis, suggesting that DQ 65–79 and p21 act similarly to inhibit cell growth. The Journal of Immunology, 2003, 171: 5064–5070.

Major histocompatibility complex molecules present self and foreign protein fragments to T lymphocytes and are the major molecular targets of organ transplant rejection. There have been many reports that MHC molecules or synthetic peptides corresponding to regions of MHC molecules can modulate the alloreponse in vitro or in vivo (1–5). Peptides corresponding to polymorphic or relatively nonpolymorphic regions of MHC class I molecules can block T cell responses in an allele-specific or -nonspecific manner (6–8). Previously, we reported that a synthetic peptide designated DQ 65–79, corresponding to residues 65–79 of the α-chain of the HLA class II molecule DQA03011, blocks T lymphocyte proliferation stimulated by anti-CD3 mAb, mitogens, or alloantigen but not that induced by PMA and ionomycin (9). Substitution of each amino acid with serine indicated that residues 66, 68, 69, 71–73, and 75–79 are critical for DQ 65–79 function. A mutant peptide in which isoleucine at position 75 was changed to serine (DQ 75S) does not inhibit T cell proliferation (9, 10). Murphy et al. (11) reported that a similar synthetic peptide corresponding to residues 51–57 of a rat MHC class II molecule inhibited mixed lymphocyte reactions and differentiation of CTL in a dose-dependent manner and induced apoptosis (12).

We used the yeast two-hybrid system to show that DQ 65–79 specifically binds to the C-terminal portion of proliferating cell nuclear Ag (PCNA) (residues 178–261) (10). PCNA plays important roles in DNA replication, DNA repair, and control of cell cycle regulation (13, 14). PCNA interacts with a number of proteins involved in cell cycle regulation and the DNA damage response pathways, including p21 (15–17), cyclin D (18), and growth arrest and DNA damage (GADD)45 family members (19, 20).

p21 is a key regulator of cell growth and differentiation. p21 interacts with cyclin dependent kinases (CDKs) in the G1 and S phases of the cell cycle (21). In nontransformed cells, p21 associates with PCNA, cyclins, and CDKs to form a quaternary complex (15, 22–24). p21 inhibits PCNA dependent DNA replication and repair in vitro. The crystal structure of PCNA complexed with the C-terminal fragment of p21 revealed that p21 interacts with the interdomain connector loop of PCNA, most likely preventing the association of PCNA with other components of DNA pol assembly complex (25). The same carboxyl region of p21 also inhibits CDK activity (26–28).

This study was undertaken to precisely map the regions of PCNA that interact with DQ 65–79. We demonstrate that major regions of interaction is located in the C terminus of PCNA, a region that interacts with other proteins such as p21. Functional comparison of the p21 and DQ 65–79 peptides indicates that they most likely inhibit cell cycle progression by a similar mechanism.

Materials and Methods

Peptides

DQ 65–79 (NIAVLKHNLNIVIKR), DQ 75S (NIAVLKHNLNSVIKR), and p21 139–160 (GKRRQRTSMTDFYHSKRRLIFS) were synthesized with or without a C-terminal IRS tag (RYIRS) and purified (United Biochemical Research, Seattle, WA). Peptide composition was confirmed by mass spectrometry. Stock solutions (10 mM) were prepared in DMSO.

Cells and cell culture

Escherichia coli strains DH5α and BL21 (DE3) were grown in LB medium at 37°C. 3T3 cells were cultured in DMEM (Invitrogen, Carlsbad, CA) supplemented with 10% bovine calf serum (HyClone Laboratories, Logan, UT), 2 mM l-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES (Invitrogen). One day before transfection, 3T3 cells were trypsinized and plated in six-well plates at 80% confluency. Human HLA-A2-specific CTL were cultured in RPMI 1640 (Irvine Scientific, Santa Ana, CA) supplemented with 10% FBS (HyClone Laboratories), 2 mM l-glutamine, 100 IU/ml penicillin, 100 μg/ml streptomycin, 10 mM HEPES, and 10% T cell-conditioned medium (29). CTL were stimulated weekly with irradiated (10,000 R) human B lymphoblastoid cells JY.

Plasmids

Total RNA from human PBMC was isolated using an RNaseasy mini kit (Qiagen, Valencia, CA). Human PCNA and p21 cDNAs were amplified using RT-PCR. The primers for amplifying PCNA cDNA are 5′-TCTA GACTAAGATCGTCCTTCACCTC-3′ and 5′-TCCTAGACTAAGATCTC TTCTCATCCTC-3′, and for amplifying p21 cDNA are 5′-GGATC

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3 Abbreviations used in this paper: PCNA, proliferating cell nuclear Ag; GADD, growth arrest and DNA damage; IFTG, isopropyl β-D-thiogalactoside; PI, propidium iodide.
CGTGCAGGAGGTCACTGCTTGGT-3' and 5'-TCTAGATATGCGGTT CTCCTTGGAGAAA-3'. The PCR fragments were subcloned into pBIND (Promega, Madison, WI) between the BamHI and XhoI sites.

cDNA encoding DQ 65–79 was produced by two sets of complementary primers of synthetic oligonucleotides, 5'-GATCACAATCCTG CTGGCTCAACATACCTTGAACATCGTGAAGTCAAGTAA-3' and 5'-CTAGTACTAGCTGGTTAATACGAGTGTCAAGTTTTAGC CACACGGATGTT-3', the DNA fragment was subsequently cloned into pACT between the BamHI and XhoI sites. A series of PCR primers were designed to amplify truncated PCNA fragments that sequentially remove 60 base units from the 5' end. These DNA fragments were further cloned into pBIND as described above.

Mammalian two-hybrid analysis

Mammalian two-hybrid analysis was conducted using the Promega Check-Mate Mammalian Two-Hybrid System. A total of 0.5 µg of each of pACT (or its derivatives), pBIND (or its derivatives), and the reporter construct pG5lac were cotransfected into 3T3 cells using LipofectAMINE Plus (Invitrogen) according to the manufacturer's protocol. After 18 h of transfection, the transfected cell extracts were harvested with a PhD cell harvester. The bound proteins were eluted by boiling in 30 µl of loading buffer, and 10 µl of the supernatant were loaded onto a 12% SDS-PAGE gel. The gel was stained with Coomassie Brilliant Blue R250 or silver staining. The proteins were transferred to nitrocellulose membranes and incubated overnight with the indicated antibodies. A 96-well microtiter plate was incubated with 1 µg/ml of anti-CD3 Ab, incubated at 37°C for 24 h, and pulsed with [3H]TdR as above.

Neutralino precipitation

Neutralino precipitation of PCNA (20 µg) were incubated with 3 or 30 µg of DQ 65-79-IRS, or DQ 75S-IRS on ice for 1 h. The mixture was then incubated overnight at 4°C with 2 µl of anti-IRS mAb (1 mg/ml) (Covance, Richmond, CA) and 10 µl of Protein G Plus/Protein A agarose suspension (Novagen). Agarose beads were spun down and washed extensively with NET buffer (50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 1 mM EDTA and 0.1% Nonidet P-40). The bound proteins were eluted by boiling in 30 µl of 1× SDS loading buffer, and 10 µl of the supernatant was loaded onto a 12% SDS-PAGE. PCNA was visualized by Western blotting using polyclonal anti-PCNA Ab (Santa Cruz Biotechnology, Santa Cruz, CA).

ELISA

A 96-well microtiter plate was incubated with 1 µg of purified PCNA overnight at 4°C. The wells were washed extensively with PBS, and incubated overnight with indicated amounts of pG5lac, pDQ4545, or pDQ 139-160-IRS peptide with or without DQ 65–79. The plate was then incubated at room temperature sequentially with 1) 10% BSA in PBST (PBS with 0.1% Tween 20) for 1 h; 2) 1 µg/ml anti-p21 Ab (to detect p21), anti-GADD45 Ab (to detect GADD45), or anti-IRS Ab (to detect p21 139-160 IRS) for 2 h; 3) HRP-conjugated anti-rabbit or anti-mouse IgG for 1 h, and 4) 100 µg/ml MB (3′,3′,5′-tetramethylbenzidine in 0.1 M NaAc with 0.03% H2O2) for 0.5 h. Between each step, the wells were washed three times with 200 µl of PBST. The reaction was stopped by addition of 100 µl of 0.1 M H2SO4, and the OD590 was determined on a SpectraMax 340 Microplate Reader (Molecular Devices, Sunnyvale, CA).

Proliferation assay

CTL were seeded at 5 x 10^4 cells/well in a 96-well plate and stimulated with 50 U/ml rIL-2 (Biological Resources Branch, Frederick Cancer Research and Development Center, National Cancer Institute, Bethesda, MD) plus the indicated concentration of DMSO, DQ 65-79-IRS, DQ 75S-IRS, or pDQ 139-160-IRS for 48 h. Cells were pulsed with [3H]Tdr (1 µCi/well) (DuPont, Boston, MA) for 24 h and harvested with a PhD cell harvester. [3H]Tdr incorporation was determined by liquid scintillation counting. Purified T cells (5 x 10^4 cells/well) were stimulated with 10 ng/ml PMA plus 250 ng/ml ionomycin, and pulsed with [3H]Tdr as above. For activation with anti-CD3 plus anti-CD28, 96-well plates were sequentially coated with 1) 1.5 µg/ml anti-mouse Ab for 2 h at room temperature; 2) 1 µg/ml anti-CD3 Ab at 4°C overnight. Wells were washed once with PBS between each step. Puriﬁed T cells were then added (5 x 10^5 cells/well) with 1 µg/ml anti-CD28 Ab, incubated at 37°C for 24 h, and pulsed with [3H]Tdr as above.

Aptoposis

CTL (2.5 x 10^5 cells/ml) were treated with DMSO, DQ 65-79-IRS, DQ 75S-IRS, or pDQ 139-160-IRS for 20 h and washed twice with ice-cold PBS. Cell pellets were suspended in 100 µl of binding buffer (100 mM HEPES, pH 7.4, 140 mM NaCl, 2.5 mM CaCl2) supplemented with 5 µl of Annexin VFITC (BD PharMingen, San Diego, CA) and 10 µl of 5 µg/ml propidium iodide (PI). Cells were incubated at room temperature for 15 min in the dark and analyzed by FACS within 1 h.

Results

Identification of the DQ 65-79-binding domains of PCNA

A mammalian two-hybrid system was used to map the regions of PCNA that interact with DQ 65–79. A series of truncated PCNA cDNA segments in which 60 bases were sequentially removed from the 5' end were generated by PCR. These fragments, as well as the full-length PCNA cDNA, were subcloned into the pBIND vector, which allows expression in frame with a GAL4 binding domain. Each of these constructs was cotransfected into 3T3 cells with the luciferase reporter plasmid pG5lac and a pACT construct expressing VP16 fused DQ 65–79. Empty pBIND and pACT vectors were used as negative controls. The interaction between DQ 65–79 and PCNA or PCNA deletions was quantified using a dual luciferase assay, in which Remilla luciferase activity allowed the normalization of transfection efficiencies. As shown in Fig. 1, full-length PCNA bound only weakly to DQ 65–79 in this system. However, three PCNA N-terminal deletion segments, 81–261, 121–261, and 241–261 showed stronger interaction with DQ 65–79, implying that residues 81–100, 121–140, and 241–261 of PCNA interact with DQ 65–79 (Fig. 1). These results suggest that at least three regions of PCNA, residues 81–100, 121–140, and 241–261, interact with PCNA and that protein conformation may play a role in epitope availability in the mammalian two-hybrid system.

To verify the interaction of PCNA 81–100, 121–140, and 241–261 with DQ 65–79, individual DNA fragments encoding each PCNA region were cloned into pBIND separately and their interaction with DQ 65–79 examined. A cDNA fragment encoding the first 20 amino acids of PCNA (1–20) was used as a control. PCNA 81–100, 121–140, and 241–261 all showed significant interaction.
with DQ 65–79 (Fig. 2) while PCNA 1–20 did not interact. To confirm that differences in the binding of each PCNA fragment to DQ 65–79 were due to affinity and not to lack of expression, all truncated PCNA fragments were transiently expressed in 3T3 cells. An aliquot of the supernatants was separated by SDS-PAGE followed by Western blotting using an anti-PCNA polyclonal Ab. Equivalent expression of the full-length PCNA and all truncations was detected (not shown).

p21 and DQ 65–79 interact with the same region of PCNA

The cell cycle regulatory protein p21 also binds directly to PCNA (15–17). Residues 139–160 of p21 contain the entire PCNA interaction domain (30–34). The crystal structure of the p21 139–160/human PCNA complex revealed that p21 contacts PCNA through the N-terminal domain, the connector loop, and the C-terminal portion (25). Despite the fact that there is substantial sequence diversity between the p21 C-terminal and DQ 65–79 terminal portion (25), the interaction domain (30–34) of p21 and DQ 65–79–139: GRKRRQTSMTDFYHSKRRLIFS; DQ 65–79–140: LKHNLNIVIKR), both interact with the connector loop and C-terminal portion of PCNA (Fig. 1 and Ref. 25).

To directly compare the interaction of p21 and DQ 65–79 with PCNA, human PCNA and p21 cDNAs were amplified by RT-PCR from PBMC. These cDNAs were cloned into pET29a(+) and pET28a(+) respectively, and the proteins were expressed in E. coli after induction with 0.5 mM IPTG. Most of the expressed PCNA was soluble (Fig. 3A, lane 2), and was easily purified using a Ni²⁺-affinity column (Fig. 3A, lane 6). In contrast, only a small amount of expressed p21 was found in the cell lysate supernatant (Fig. 3B, lane 3). Four liters of supernatant were pooled and run over a Ni²⁺-affinity column, resulting in sufficient amounts of purified p21 (Fig. 3B, lane 6). The purified PCNA and p21 were used for in vitro assays.

Coimmunoprecipitation of DQ 65–79 and PCNA

In the mammalian two-hybrid system, the interaction between full-length PCNA and DQ 65–79 is very weak. We assume that this is an artifact of the two-hybrid system because we previously reported that DQ 65–79 interacts with full-length PCNA in an ELISA format (10). To convincingly demonstrate a specific interaction between DQ 65–79 and full-length PCNA, coimmunoprecipitation experiments were conducted. Purified PCNA was incubated with either DQ 65–79 or DQ 75S, a peptide that is identical to DQ 65–79 except that the isoleucine at position 75 has been changed to serine. DQ 75S does not exhibit any of the immunoregulatory properties of DQ 65–79 (9, 10, 35). In addition, DQ 65–79 and DQ 75S were synthesized with a tag (RYIRS, designated IRS) at their C termini. We previously showed that addition of the IRS tag does not alter the function of either peptide (36). Protein complexes were immunoprecipitated with an anti-IRS mAb, separated by SDS-PAGE, and subjected to Western blotting with polyclonal anti-PCNA Ab. As shown in Fig. 4, PCNA co-precipitated with DQ 65–79-IRS, but not with DQ 75S-IRS, indicating that full-length PCNA interacts specifically with DQ 65–79.

DQ 65–79 competes with p21 for binding to PCNA

Both DQ 65–79 and p21 interact with similar regions of PCNA (Fig. 1 and Ref. 25). To determine whether the regions of PCNA recognized by DQ 65–79 and p21 are identical, a competitive ELISA was performed. p21 was mixed with DQ 65–79 or medium

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**FIGURE 2.** The interactions between DQ 65–79 and residues 81–100, 121–140, and 241–261 of PCNA. Lane 1, Empty pACT and pBIND vectors; lanes 2–6, DQ 65–79-pACT plus the indicated PCNA segment-pBIND. Transfections were conducted as in Fig. 1. Results shown are the average of three independent experiments.

**FIGURE 3.** Expression and purification of human PCNA and p21 from E. coli. A, Expression and purification of PCNA. Lane 1, IPTG induced total cell lysate; lane 2, supernatant of IPTG-induced cells; lane 3, flow through after loading the supernatant onto a Ni²⁺ column; lane 4, wash of Ni²⁺ column; lane 5, protein markers; lane 6, purified PCNA eluted from the Ni²⁺ column. B, Expression and purification of p21. Lane 1, The supernatant of the uninduced cells; lane 2, uninduced total cell lysate; lane 3, the supernatant of the induced cells; lane 4, induced total cell lysate; lane 5, protein markers; lane 6, purified p21 eluted from the Ni²⁺ column.

**FIGURE 4.** Coimmunoprecipitation of PCNA and DQ 65–79. Purified PCNA was incubated with DQ 65–79-IRS (lanes 1 and 4), DQ 75S-IRS (lanes 2 and 5), or medium and the protein complex was immunoprecipitated with anti-IRS Ab. The bound proteins were separated by SDS-PAGE, and PCNA was visualized by Western blot using polyclonal anti-PCNA Ab. Lane 1, PCNA with 3 μg of DQ 65–79-IRS; lane 2, PCNA with 3 μg of DQ 75S-IRS; lane 3, PCNA alone; lane 4, PCNA with 30 μg of DQ 65–79-IRS; lane 5, PCNA with 30 μg of DQ 75S; lane 6, purified PCNA loaded directly onto the SDS-PAGE.
and then added to PCNA-coated wells. p21 binding was determined with a polyclonal anti-p21 Ab. DQ 65–79 competes with p21 for binding to PCNA: addition of 70 μM DQ 65–79 increased the EC₅₀ value of p21 binding to PCNA from 107 to 290 ng/ml (p < 0.005) (Fig. 5A). This competition was specific, as DQ 65–79 did not affect the binding of GADD45, an unrelated protein, to PCNA (Fig. 5B). The ability of DQ 65–79 to compete with p21 139–160-IRS to PCNA was also determined (Fig. 5C). DQ 65–79 competed very efficiently with p21 139–160-IRS for binding to PCNA (p < 0.005). To exclude the possibility that DQ 65–79 might bind directly to p21, thereby decreasing the available pool of p21, the interaction between DQ 65–79 and full-length p21 was explored using the mammalian two-hybrid system. p21 bound strongly to PCNA but did not bind directly to DQ 65–79 (Fig. 5D). Collectively, these findings indicate that DQ 65–79 and p21 interact with the same or proximal sites on PCNA.

DQ 65–79 and p21 139–160 inhibit T cell proliferation and induce apoptosis

We reported previously that DQ 65–79 inhibits T cell proliferation (Refs. 9, 10, and 35). p21 also blocks cell growth through its interaction with PCNA, and promotes cell death by an as yet unidentified mechanism (37). The C terminus of p21 contains the PCNA interaction domain, and a synthetic peptide corresponding to this region mimics full-length p21 in inhibiting the PCNA-mediated DNA replication and repair (30, 38, 39). Because DQ 65–79 and p21 139–160 interact with similar regions of PCNA, we asked whether p21 139–160 could block T cell proliferation and/or induce apoptosis. Addition of up to 100 μM of p21 139–160 did not affect T cell proliferation or induce apoptosis (not shown). However, based on the results of other groups, we reasoned that this might reflect the inability of p21 139–160 to access the cytosol. Ball et al. (28) linked p21 141–160 to a 16-aa sequence from the homeodomain of the Antennapedia protein that allows translocation across the plasma membrane. This chimeric peptide blocked phosphorylation of retinoblastoma protein and induced a potent G₁/S arrest in tumor cells. We speculated that the IRS tag (RYIRS) might be sufficient to allow translocation of p21 139–160 into the cytosol. We used confocal microscopy to investigate this (Fig. 6). Cells treated with either DQ 65-79-IRS or p21 139-160-IRS...
showed staining throughout the cell, while staining for HLA class I was mainly restricted to the plasma membrane.

Having demonstrated that the IRS-tagged p21 139–160 could translocate into cells, we evaluated its effect on T cell proliferation and apoptosis. p21 139–160-IRS blocked IL-2-stimulated DNA synthesis in CTL similarly to DQ 65–79-IRS (Fig. 7A). Both p21 139–160-IRS and DQ 65–79-IRS also inhibited DNA synthesis in T cells stimulated with anti-CD3 plus anti-CD28 (Fig. 7B). However, neither DQ 65–79-IRS nor p21 139–160-IRS inhibited T cell proliferation in cells stimulated with PMA and ionomycin (Fig. 7C). T cells treated with either DQ 65-79-IRS or p21 139-160-IRS, but not DQ 75S-IRS, underwent apoptosis, as judged by annexin V and PI staining (Fig. 8).

**Discussion**

In this study, three regions of PCNA (residues 81–100, 121–140 and 241–261) were shown to interact with DQ 65–79. These regions of PCNA have also been shown to interact with p21 (25). Using competition assays, we demonstrated that DQ 65–79 competes with both intact p21 and p21 139–160 for binding to PCNA. Addition of a pentapeptide tag to the C terminus of p21 139–160 allows it to translocate into cells where it acts similarly to DQ 65–79, blocking T cell proliferation and inducing apoptosis.

PCNA is an essential component of the DNA replication machinery. It functions as the processivity factor for DNA polymerase δ and ε. It also is involved in DNA recombination and repair and interacts with a number of cellular proteins involved in cell cycle regulation and checkpoint control (13, 40). Proteins that have been demonstrated to interact directly with PCNA include p21, GADD45 family proteins (GADD45α, myeloid differentiation factor 118, and cytokine response protein 6), D-type cyclins, p57, CRAMPED, DNA ligase 1 (FEN1), replication factor C, DNA polymerase δ and ε, RNA polymerase, DNA (cytosine-5) methyltransferase, Xeroderma pigmentosum G, MutL homologue 1, MutS homologue 2, and uracil-DNA glycosylase 2 (13, 41). Kelman and Hurwitz (41) noted that these proteins can be divided into two groups: the first is comprised of proteins involved in cell cycle progression, checkpoint control, or cellular differentiation while the second is composed of proteins that have a known enzymatic activity. PCNA is initially synthesized in G1 and peaks in early S phase (42–44). It is rapidly degraded at the end of G2 phase (44). Coincidently, PCNA is completely localized within the nuclei in G1 phase, but is partially exported to the cytosol in S phase (45), and is almost undetectable in nuclei in late G2 and M phase (44).

p21 was the first cyclin-dependent kinase (CDK) inhibitor to be identified (22, 46, 47). The N-terminal domain of p21, which shares homology with the N termini of p27 and p57, is both necessary and sufficient to inhibit CDK activity. The unique C terminus of p21 interacts with a number of proteins including PCNA,
GADD45, calmodulin, SET, and C/EBP-α (37). Scott et al. (48) recently reported that the interaction of p21 with PCNA is regulated through phosphorylation of the C terminus of p21. Although there is no sequence homology between the C terminus of p21 with those of p27 or p57, they may share some function. Watabane et al. (49) reported that the C terminus of p57 also associates with PCNA to inhibit its function.

A number of groups have used either truncation or synthetic peptides to identify regions of p21 that interact with PCNA (16, 25, 30, 32–34, 39, 50). Some of these peptides have also been shown to affect cell growth. Bonfanti et al. (51) reported that fusion of the internalization peptide from Antennapedia to peptides corresponding to residues 17–33 or 63–77 of p21 inhibited growth of two human ovarian cancer cell lines. Similarly, Ball et al. (28) found that linkage of the Antennapedia peptide to residues 141–160 of p21 decreased cells in S phase and blocked phosphorylation of Rb in vivo. Mutoh et al. (52) reported that the Antennapedia-p21 peptide chimeric protein inhibited growth of human lymphoma cells by inducing necrosis. However, the Antennapedia peptide alone also exhibited significant effects on lymphoma growth. Cayrol et al. (53) used p21 peptide mutants that could not interact with PCNA to demonstrate that binding of p21 to PCNA is sufficient to block cell cycle progression and the G1/S and G2/M transitions.

The structural basis for the similarity in function of DQ 65–79 with p21 139–160 is unknown. The best fit using sequence alignment programs to compare residues 146–160 of p21 with DQ 65–79 yields one identical residue (histidine at residue 71 of DQ 65–79 and residue 152 of p21), seven strongly similar residues, four weakly similar residues, and four different residues. Both peptides are largely composed of hydrophobic and charged residues. We previously showed that treatment of cells with DQ 65–79 decreases expression of p21 mRNA and protein (9, 35). This suggests that there is a feedback loop controlling p21 expression and that DQ 65–79 interferes with that loop.

p21 does not directly prevent PCNA from interacting with DNA, nor does it block PCNA moving along DNA strands (54). Rather, the C-terminal region of p21 competes with FEN1 for DNA repair pathways, which further leads to the inhibition of cell proliferation and induction of apoptotic cell death.

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