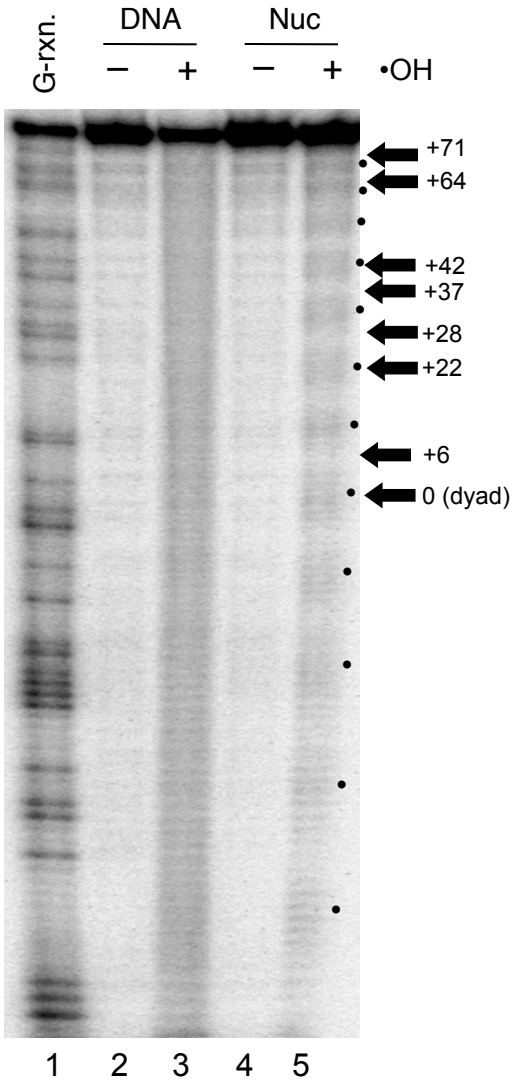

Supplemental Fig. S1. Hydroxyl radical cleavage analysis of 154 (+22U) nucleosomes. Samples of the reconstitution with the 154 (+22U) template were subjected to hydroxyl radical cleavage as described in the Experimental Procedures and reaction products separated on 0.7% nucleoprotein gels. DNA within the nucleosome and naked DNA bands was recovered, purified and the cleavage patterns analyzed on a 6% sequencing gel. Lane 1, G-reaction marker, lanes 2 and 3, naked DNA prepared without or with hydroxyl radical cleavage, respectively; lanes 4 and 5, nucleosome DNA prepared without or with hydroxyl radical cleavage, respectively.

Supplemental Table S1. Oligos used to make the top strand for the 154-mer single U incorporation templates. *Sequence* of *oligomers* shown correspond to those identified by length in Fig. 1A for construction of the top strand of the 154 bp templates. The 66-mer was used for all 154 bp constructs. The (U-less) 42-mer was ligated to individual U-containing 46-mers using the complementary 42+46 splint for the *Templates* indicated, as shown in Fig. 1. Conversely, the U-less 46-mer was ligated to the U-containing 42-mers. Each resulting 88-mer was also simultaneously ligated to the 66-mer using the 66+42 splint as diagramed in Fig. 1A. U's are shown in bold.

Oligomer	Sequence	Templates
66-mer	AAT TCG AGC TCG CCC CGG GAT CCG GCT GGG CCC CCC CCA GAA GGC AGC ACA AGG GGA GGA AAA GTC	All 154 nt top strands
42-mer	AGC CTT GTG CTC GCC TAC GGC CAT ACC ACC CTG AAA GTG CCC	154U(+37), (+42), (+64), (+71)
46-mer	GAT ATC GTC TGA TCT CGG AAG CCA AGC AGG GTC GGG CCT GGT TAG T	154U(+22), (+28), (0), (+6)
0U 42-mer (dyad)	AGC CTT GTG C**U**C GCC TAC GGC CAT ACC ACC CTG AAA GTG CCC	154 0U
+6U 42-mer	AGC CTT GTG CTC GCC **U**AC GGC CAT ACC ACC CTG AAA GTG CCC	154 +6U
+22U 42-mer	AGC CTT GTG CTC GCC TAC GGC CAT ACC ACC C**U**G AAA GTG CCC	154 +22U
+28U 42-mer	AGC CTT GTG CTC GCC TAC GGC CAT ACC ACC CTG AAA G**U**G CCC	154 +28U
+37U 46-mer	GAT A**U**C GTC TGA TCT CGG AAG CCA AGC AGG GTC GGG CCT GGT TAG T	154 +37U
+42U 46-mer	GAT ATC GTC **U**GA TCT CGG AAG CCA AGC AGG GTC GGG CCT GGT TAG T	154 +42U
+64U 46-mer	GAT ATC GTC TGA TCT CGG AAG CCA AGC AGG G**U**C GGG CCT GGT TAG	154 +64U
+71U 46-mer	GAT ATC GTC TGA TCT CGG AAG CCA AGC AGG GTC GGG CC**U** GGT TAG T	154 +71U

42+46 Splint	TTC CGA GAT CAG ACG ATA TCG GGC ACT TTC AGG GTG GTA T	All 154 nt top-strands
66+42 Splint	CCG TAG GCG AGC ACA AGG CTG ACT TTT CCT CCC CTT GTG C	All 154 nt top-strands

Table S2. Oligos used to make the bottom strand for the 154-mer single U incorporation templates. *Sequence* of *oligomers* shown correspond to those identified by length in Fig. 1A for construction of the bottom strand of the 154 bp templates. The 74-mer was ligated to the 80-mer using the complementary 74+80 splint to generate a 154 nt bottom strand. The 154-mer bottom strand was annealed to all top strands to generate the 153 bp templates as shown in Fig. 1 and described in the *Experimental Procedures*.

Oligomer	Sequence	Template
74-mer	ACA AGG CTG ACT TTT CCT CCC CTT GTG CTG CCT TCT GGG GGG GGC CCA GCC GGA TCC CGG GGC GAG CTC GAA TT	154 nt bottom-strand
80-mer	ACT AAC CAG GCC CGA CCC TGC TTG GCT TCC GAG ATC AGA CGA TAT CGG GCA CTT TCA GGG TGG TAT GGC CGT AGG CGA GC	154 nt bottom-strand
74+80 Splint	GGA GGA AAA GTC AGC CTT GTG CTC GCC TAC GGC CAT ACC A	154 nt bottom-strand

Table S3. Oligos annealed to generate the 42 bp U-containing construct. The +22 42-mer (Table S1, shown again here) was radiolabeled as described in the *Experimental Procedures* and annealed with a complementary 42 nt oligo to generate a 42 bp double-stranded oligonucleotide, which served as the internal naked DNA control in the UDG reactions with nucleosomes.

+22U 42-mer	AGC CTT GTG CTC GCC TAC GGC CAT ACC ACC C**U**G AAA GTG CCC	+22U 42 bp ds oligo
42-mer Bottom Strand	GGG CAC TTT CAG GGT GGT ATG GCC GTA GGC GAG CAC AAG GCT	+22U 42 bp ds oligo