Regulation of FANCD2 and FANCI monoubiquitination by their interaction and by DNA

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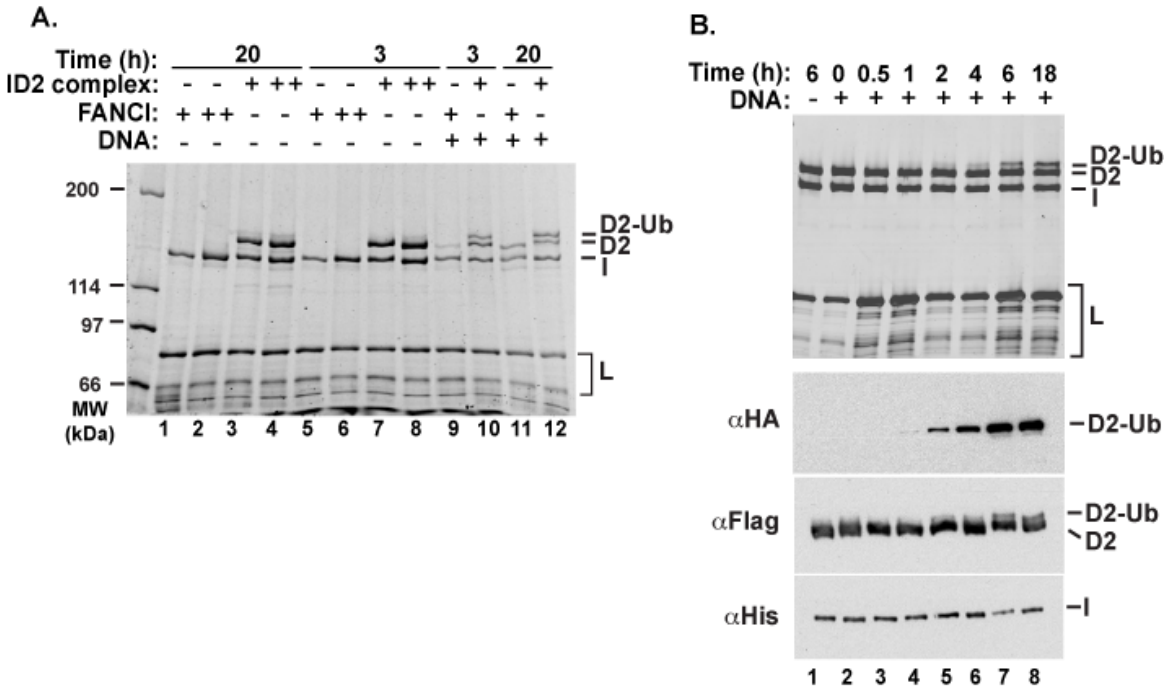
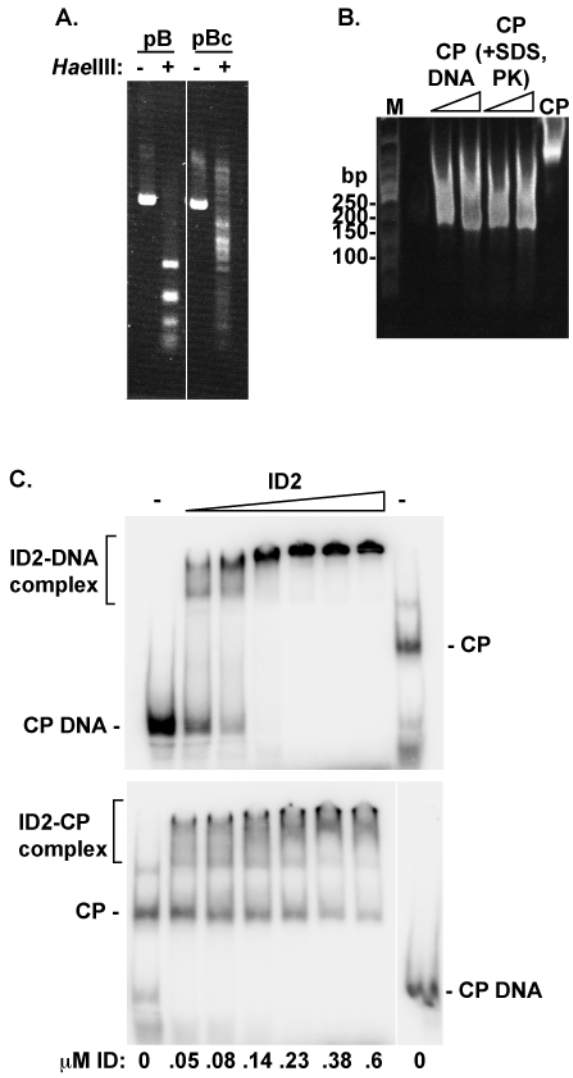
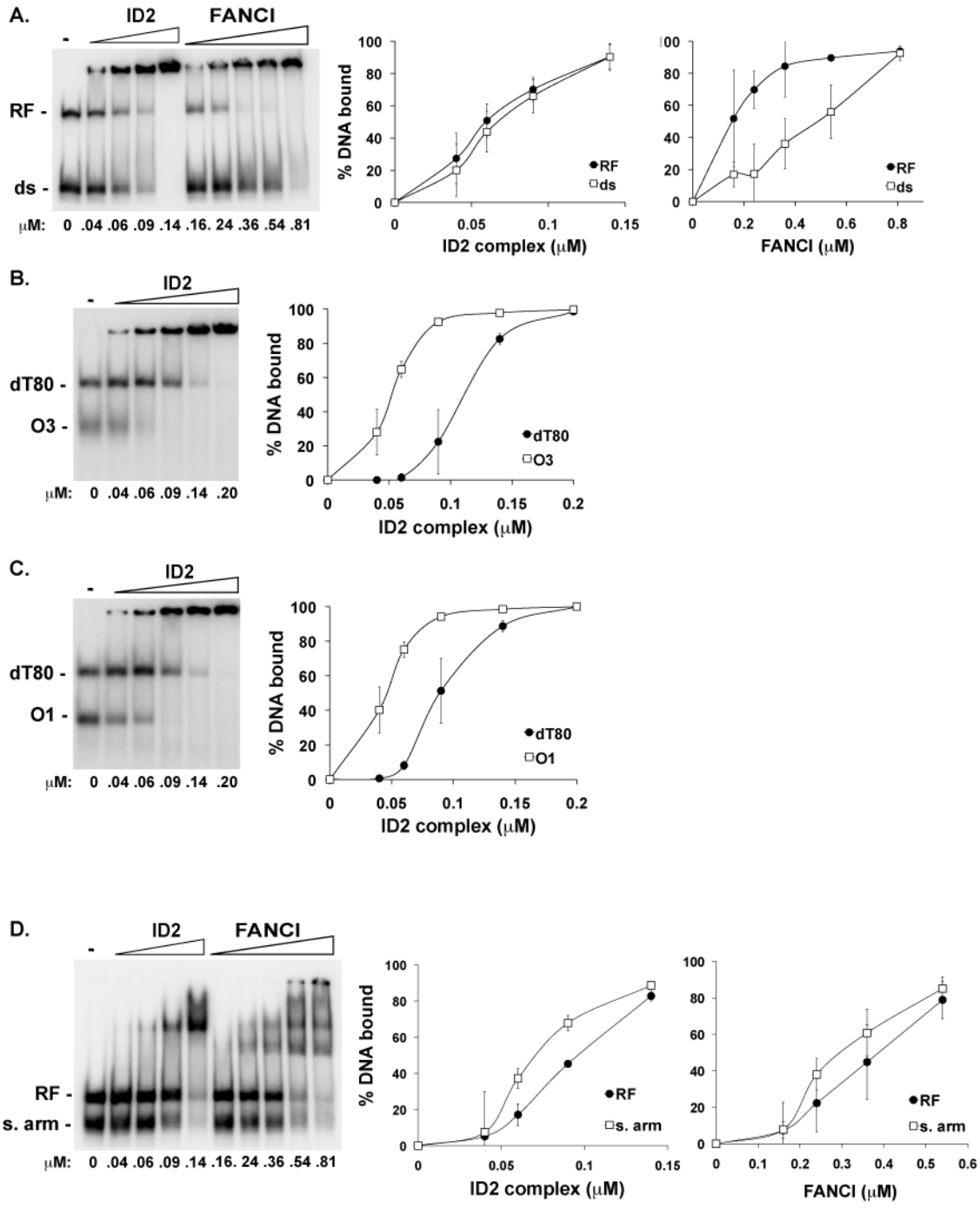
Supplementary Data:



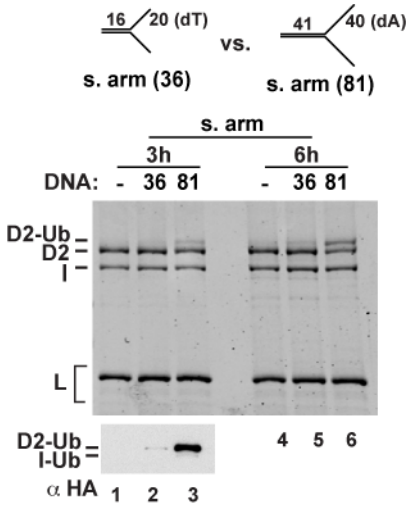
Supplementary Figure S1 A. The whole gel of the experiment shown in Figure 1A is shown, including protein markers, and side-by-side ubiquitination reactions using FANCI or ID2 complex as substrates, in the presence or absence of DNA. **B.** A time-course experiment like the one shown in Figure 6 (except using 0.3 uM UBE2T) was performed and analyzed by staining proteins with a fluorescent dye following 5.5% SDS-PAGE (upper panel), and (lower panels) by Western blotting using anti-HA (to detect ubiquitinated species), anti-Flag (to detect FANCD2) and anti-His (to detect FANCI).



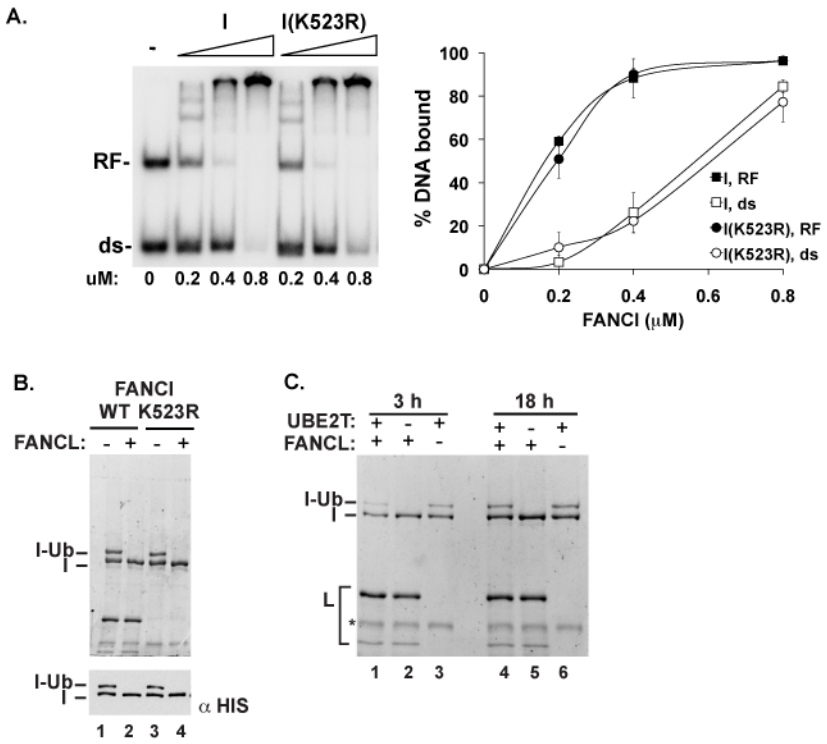
Supplementary Figure S2 A. Preparation of nucleosomal DNA substrates. Plasmid DNA, either mock-treated (pB) or assembled with histones (pBc), was analyzed by gel electrophoresis with or without prior restriction with *Hae*III. **B.** Core particles (CP) with or without SDS and proteinase K treatment, and purified core particle DNA (CP DNA) were analyzed by native gel electrophoresis. **C.** Binding of the ID2 complex to DNA released from core particles (CP DNA), or core particles (CP) containing an equivalent amount of DNA, was analyzed.



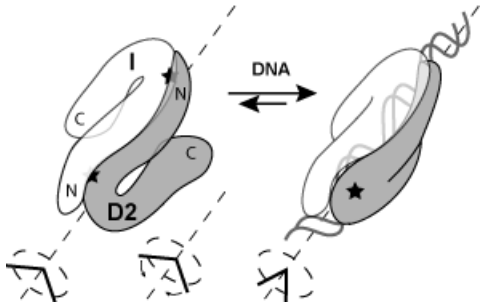
Supplementary Figure S3. Comparison of DNA substrate preference of the ID2 complex and FANCI for double-stranded linear (ds) versus a replication fork structure (RF) (**A)**, the ID2 complex for various single-stranded oligonucleotides (**B** and **C**), or the ID2 complex and FANCI for a replication fork structure versus a splayed arm structure (with single stranded arms of random sequence) (**D**). Assays were performed and quantified (right panels) as in Figure 4B. dt80 is an 80-mer oligonucleotide of dT residues.



Supplementary Figure S4 Comparison of two different splayed-arm substrates in supporting ID2 ubiquitination**.** ID2 ubiquitination was examined with two splayed arm substrates diagrammed at the top which differ in sequence and length (as indicated, 36- or 81- nucleotides in total length). Proteins were analyzed either by fluorescent staining (3 or 6 h incubation) or by Western blotting (3 h incubation).



Supplementary Figure S5 A. FANCI and FANCI(K523R) bind DNA with the same affinity and preference as examined with double-stranded DNA (ds) and a replication fork structure (RF). DNA binding assays were performed and quantified (right panel) as in Figure 4B. Ubiquitination reactions were performed with FANCI or FANCI(K523R) (**B)** or with and without UBE2T or FANCL (**C**). Analysis was performed as in Figure 8.



Supplementary Figure S6 Model for the licensing of ID2 ubiquitination by DNA. Based on available crystallographic information [10], the model on the left shows FANCD2 (D) K561 and FANCI (I) K523 sequestered in the dimer interface (stars). Results presented herein suggest that, upon DNA binding, a conformational change in the ID2 complex results in the exposure of the ubiquitin-targeted lysines in the complex. Moreover, our results implicate the FANCI C-terminus in the mediation of this conformational change.

Supplementary Table S1 Oligonucleotides used as, and to generate, substrates for DNA binding and stimulation of ubiquitination

Name	# Nts	Sequence	Lowest ΔG (kcal/ mole)*
O1	**64**	TTTCCCAGCACCAGATTCAGCATACGTTACCGATCGTACGTT CGATGCTGGCTACTGCTAGCTT	**-6.4**
O2	**81**	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAGTA GAGTCGACGGTGCTGGGATCCAACATGT TTTCAATCTG	**0.07**
O3	**81**	CAGATTGAAAACATGTTGGATCCCAGCACCGTCGACTCTACT CCGTTTCCGATCGTCCGT TCGATGCTGGCTCCTGCTTGC	**-5.11**
O4	**41**	TTAAGCTCTAAGCCATGAATTCAAATGACCTCTTATCAATT	**-1**
O5	**41**	TTGCTAGCAGTAGCCAGCATCGAACGTACGATCGGTAACGT	**-6.2**
O6	**64**	TTTTGATAAGAGGTCATTTGAATTCATGGCTTAGAGCTTAATT GCTGAATCTGGTGCTGGGATT	**-1.58**
O7	**40**	ATGAAGCTCGAAGCCATGAATTCAAATGACCTCTGATCAA	**-1**
O8	**40**	GCAAGCAGGAGCCAGCATCGAACGGACGATCGGAAACGGA	**-3.79**
O9	**81**	CAGATTGAAAACATGTTGGATCCCAGCACCGTCGACTCTACA AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA	**-0.83**
O10	**81**	TTGATCAGAGGTCATTTGAATTCATGGCTTCGAGCTTCATGT AGAGTCGACGGTGCTGGGATCCAACATGTTTTCAATCTG	**-2.78**
O11	**81**	CAGATTGAAAACATGTTGGATCCCAGCACCGTCGACTCTACA TGAAGCTCGAAGCCATGAATTCAAATGACCTCTGATCAA	**-5.11**

* Calculated using UNAFold[1]

Supplementary Table S2 Primers used for cloning

Name	Sequence (5'-3')
FL1	AAAGAATTCATGGCGGTGACGGAAGCGAGC
FL2	TTGTCGACTCAGTGTTTCCTTCCAGACATTTTTAAG
FL3	GAAATCGATGAGAAGACCGCGGTAGCTGAGCCAGAAAAACCTCCACGG
FANCI R1285Q F	CAGCACCTCACAAGACTTCAAGATCAAAGG
FANCI R1285Q R	CTTGAAGTCTTGTGAGGTGCTGAGCTTCATGTGC
FANCI R1285X F	CAGCACCTCATGAGACTTCAAGATCAAAGG
FANCI R1285X R	CTTGAAGTCTCATGAGGTGCTGAGCTTCATGTGC
FANCI K294E F	GAAACACTTAGAGGTAGGACAGCAAGGAGATTC
FANCI K294E R	CTGTCCTACCTCTAAGTGTTTCACGAGTTC
FANCI K339E F	AAGAGCTTTGAGGATCTTCAACTCCTCCAAG
FANCI K339E F	TTGAAGATCCTCAAAGCTCTTTACAACCG
FD1	TGATAAGAAGGCAGCTCTCTAGCACCG
FD2	CTAGAGAGCTGCCTTCTTATCACCAAGTGC

Supplementary Table S3 Oligonucleotides annealed to generate fork structures

Structure name	Oligonucleotides annealed
Splayed arm	O2+O9
5' Flap	O2+O3+O8
3' Flap	O7+O9+O10
Replication fork (RF)	O3+O7+O8+O10
Double-strand (ds)	O10+O11

Reference:

1. Markham, N.R. and Zuker, M. (2008) UNAFold: software for nucleic acid folding and hybridization. *Methods in molecular biology*, **453**, 3-31.