Poly(ADP-ribosyl)ation directs recruitment and activation of an ATP-dependent chromatin remodeler

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Posttranslational modifications play a key role in recruiting chromatin remodeling and modifying enzymes to specific regions of chromosomes to modulate chromatin structure. Alc1 (amplified in liver cancer 1), a member of the SNF2 ATPase superfamily with a carboxy-terminal macrodomain, is encoded by an oncogene implicated in the pathogenesis of hepatocellular carcinoma. Here we show that Alc1 interacts transiently with chromatin-associated proteins, including histones and the poly(ADP-ribose) polymerase Parp1. Alc1 ATPase and chromatin remodeling activities are strongly activated by Parp1 and its substrate NAD and require an intact macrodomain capable of binding poly(ADP-ribose). Alc1 is rapidly recruited to nucleosomes in vitro and to chromatin in cells when Parp1 catalyzes PAR synthesis. We propose that poly(ADP-ribosylation) of chromatin-associated Parp1 serves as a mechanism for targeting a SNF2 family remodeler to chromatin.

Results and Discussion

To investigate possible Alc1 interactors, we generated an HEK293/FRT cell line stably expressing Alc1 with an N-terminal FLAG tag (F-Alc1). Initial immunopurification of F-Alc1 from nuclear extracts with M2 agarose suggested that unlike many SNF2 superfamily members, Alc1 does not reside in a stable multisubunit complex (Fig. S1 A); however, MudPIT mass spectrometry indicated that preparations of F-Alc1 contained small amounts of histones and Parp1 and several Parp1-interacting proteins (Table S1).

To gain further insight into the molecular function of Alc1, we expressed and purified recombinant wild-type F-Alc1; a DEAH box mutant F-Alc1(E175Q), which is mutated at a position expected to prevent ATP binding and hydrolysis; a macrodomain mutant F-Alc1(D723A), which is mutated at a position shown previously to decrease substantially the affinity of ADP-ribose binding by AF1521, a macrodomain-containing protein from Archaeoglobus fulgidus (8); and the Alc1 macrodomain (amino acids 666–897) (Fig. 1 A and Fig. S1 B). To determine if Alc1 can bind poly(ADP)-ribose (PAR), purified recombinant proteins were dot-blotted on nitrocellulose after incubation with 32P-labeled PAR (Figs. 1 B and C). Alc1 and the DEAH box mutant Alc1(E175Q) bound PAR. PAR binding was abolished by heat treatment and was substantially reduced by high salt. Indicating that the Alc1 macrodomain is necessary and sufficient for PAR binding, the isolated Alc1 macrodomain bound PAR, while PAR binding by the macrodomain mutant Alc1(D723A) was greatly reduced.

Many SNF2 superfamily members have both DNA- and nucleosome-activated ATPase activities (7). To determine whether Alc1 has similar activities, we assayed anti-Flag agarose eluates from F-Alc1 expressing HEK293/FRT cells and wild-type and mutant versions of recombinant F-Alc1, expressed in and purified from Sf21 cells, for ATPase activity. F-Alc1 from HEK293/FRT cells exhibited robust nucleosome-dependent ATPase activity. However, ATPase activity was lost after size exclusion chromatography (Fig. S1 C), and recombinant F-Alc1 lacking activity (Fig. 2A, compare lanes 3 and 5), suggesting a requirement for an activating factor or cofactor.


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Parp1 catalyzes nicotinamide adenine dinucleotide (NAD)-dependent mono- and PARylation of protein residues in a reaction strongly activated by Parp1 binding to DNA or nucleosomes (12–14). The major PAR acceptor in cells appears to be Parp1 itself; however, many other nuclear proteins, including histones, can be ADP-ribosylated. Biochemical studies have revealed that Parp1 can be incorporated into nucleosomes in place of histone H1 (15). Parp1 has been implicated in both transcriptional regulation and DNA damage repair in vivo (12, 14–17). In addition, Parp1 is localized to a large fraction of active promoters (18), and Parp1 and PAR accumulate at sites of DNA damage (14) in cells.

Our observation that the Alc1 macrodomain binds PAR, together with evidence from MudPIT mass spectrometry that anti-FLAG agarose eluates from F-Alc1 expressing HEK293/FRT cells contained substoichiometric amounts of Parp1, raised the possibility that addition of NAD and Parp1 to reactions might stimulate ATPase. Indeed, we observed that the ATPase activity of recombinant F-Alc1 was strongly stimulated by addition of Parp1 and NAD in the presence of either DNA or nucleosomes (Fig. 2B). ATPase was not activated in the absence of DNA or nucleosomes or when either NAD or Parp1 were omitted from reactions, suggesting Parp1-dependent PAR synthesis is required for the reaction (Fig. 2C). Consistent with this possibility, addition of poly(ADP-ribose) glycohydrolase (Parg), an enzyme known to catalyze the hydrolysis and breakdown of PAR (14), blocks activation of Alc1 ATPase by Parp1 and NAD (Fig. S2).

Suggesting a coupling of ATPase and PAR binding activities, we found that ATPase activity depends on an intact macrodomain. F-Alc1 (D723A), which does not bind PAR, lacks ATPase activity in either the presence or absence of Parp1 and NAD (Fig. 2A, compare lanes 6 and 10). PAR binding is not, however, sufficient to activate ATPase. Neither free PAR nor ADP-ribose activate Alc1 ATPase, even when present at concentrations (expressed in mole equivalents of adenosine) nearly 5 times higher than the maximal amount of poly(ADP-ribose)ated species that could be synthesized in reactions containing Parp1 and NAD (Table S2). Taken together, our data suggest that Alc1 ATPase activity depends on automodification of Parp1 and/or on PARylation of Alc1 itself. As discussed later, our data are most consistent with the former possibility.

Many Snf2 superfamily members, including Chd1, Iswi, and Ino80, can catalyze the ATP-dependent remodeling of nucleosomes in vitro (7, 19–21). To determine if Alc1 also has chromatin remodeling activity, we used a previously described assay (22–24) that takes advantage of the fact that DNA on the octamer surface is largely protected from cleavage by restriction enzymes, while DNA outside the nucleosome boundary is accessible.

We assayed for nucleosome remodeling using mononucleosomes from purified recombinant histones or HeLa oligonucleosomes on a 32P-end-labeled DNA probe containing a nucleosome positioning sequence (Fig. 3A) (25). The accessibility of a HhaI site, initially protected by the positioned nucleosome, is increased after incubation with recombinant F-Alc1, Parp1, and NAD. Arguing that Alc1 moves the nucleosome from its initial lateral position toward a more central position on the DNA, we observe a concomitant decrease in accessibility of an XhoI site outside the initial nucleosomal boundary (Fig. 3B). The DEA box mutant F-Alc1 (E175Q) fails to remodel mononucleosomes (Fig. 3C). Additionally, nucleosome remodeling by F-Alc1 is inhibited by ATPγS (Fig. 3B and C).

Nucleosome remodeling activity depends strongly on Parp1 and NAD (Fig. 3B and C) and is inhibited by benzamidine, a potent inhibitor of Parp1 (Fig. 3C, lane 13). In addition, the macrodomain mutant F-Alc1 (D723A), which exhibits reduced PAR binding, is inactive in our nucleosome remodeling assays (Fig. 3C, lane 12). To confirm further the association of
regions of specifically marked chromatin. However, our observation that Alc1 possesses ATP-dependent nucleosome remodeling activities with Alc1, we subjected anti-FLAG agarose eluates from F-Alc1 expressing HEK293/FRT cells to size exclusion chromatography. F-Alc1 and Parp1- and NAD-dependent ATPase and nucleosome remodeling activities co-eluted from the column as a monodisperse peak (Fig. S1C). Taken together, our findings argue that Alc1 possesses ATP-dependent nucleosome remodeling activity and that nucleosome remodeling, like ATPase, is closely coupled to PAR binding.

Alc1, unlike other chromatin remodeling and modifying enzymes or complexes, lacks targeting domains, such as bromo- or chromodomains, that contribute to targeted recruitment to regions of specifically marked chromatin. However, our observation that Alc1 ATPase and chromatin remodeling activities require Parp1 and NAD raises the possibility that Alc1 could be targeted to chromatin by PARylation via its macrodomain. We tested this hypothesis using biochemical and in vivo assays.

First, we tested Alc1’s ability to bind mononucleosomes formed on biotinylated DNA and immobilized on streptavidin beads (Fig. 3D). In the presence, but not in the absence, of Parp1 and NAD, Alc1 was rapidly recruited to nucleosomes and remained bound after extensive washing. In addition, we observed that F-Alc1 and the ATPase mutant F-Alc1(E175Q), but not the macrodomain mutant Alc1(D723A), could be copurified from cell extracts with Parp1 and histones (Fig. 3E and Table S2). Thus, PARylation and an intact Alc1 macrodomain regulate binding of the Alc1 ATPase to nucleosomes.

Second, we tested whether Alc1 is recruited to locally induced PARylation sites in living cells. We fused the full-length Alc1 cDNA to EYFP and used a pulsed-laser to microirradiate a small section of DNA in a human cell nucleus. The laser rapidly induces a highly localized region of DNA damage that recruits and enzymatically activates cellular PARP1 (26, 27). PARP1 and Alc1 are recruited rapidly to the microirradiated region. Alc1 and PARP1 fluorescence appears within seconds, and most is lost from the irradiated site within 10 min (Fig. 4A and Movie S1). Deletion of the macrodomain results in a complete loss of recruitment to the microirradiated region (Fig. 4B and Movie S2), while the macrodomain point mutant Alc1(D723A), which exhibits greatly reduced PAR binding in vitro, also exhibits reduced recruitment to the microirradiated region (Fig. 4C and Movie S3). Arguing that Alc1 recruitment requires the presence of Parp1 protein and PAR synthesis, we observed a substantial reduction in Alc1 recruitment when endogenous Parp1 was knocked down using short hairpin-mediated RNAi (Fig. 4C and Fig. S3) or in the presence of the Parp inhibitor PJ34 (Fig. 4D).
S4). Thus, Parp1 and Alc1 are co-recruited to irradiation-induced sites of localized PAR synthesis in living cells, and Alc1 association with chromatin in vivo depends on an intact macrodomain.

In summary, our in vivo results indicate that a Parp1-dependent PARylation event directs the recruitment of Alc1 to chromatin in cells. Further, our biochemical assays reveal that Parp1-dependent PARylation promotes the recruitment of Alc1 to nucleosomes and activates its associated ATPase and chromatin remodeling activities. Upon binding to DNA or chromatin, Parp1 can catalyze local PARylation of chromatin associated proteins, including histones; however the primary PAR acceptor in vitro and in cells is Parp1 itself (12, 15, 28, 29). While we cannot rule out the possibility that modification of histones or other proteins contributes to recruitment of Alc1, our biochemical data are most consistent with the model that automodification of Parp1 promotes the PARylation event for Alc1 activation. Indicating that poly(ADPribosyl)ation of histones is not required, Parp1, NAD, and DNA are sufficient to activate Alc1 ATPase activity (Fig. 2B). To address the alternative possibility that modification of Alc1 leads to its activation, we performed order of addition experiments using the Parp1 inhibitor benzamide (30). When added at the beginning of the reaction, benzamide blocked nucleosome remodeling (Fig. 3C, compare lane 3 to lanes 1 and 11); however, when Parp1 was preincubated with nucleosomes and NAD before addition of benzamide and Alc1, robust chromatin remodeling activity was detected (Fig. S2B), suggesting that the essential PARylation events occur before Alc1 addition.

It remains to be determined whether the apparent PARylation-dependent increase in the affinity of Alc1 for nucleosomes is sufficient to explain the activation of its ATPase and nucleosome remodeling activities in the presence of Parp1 and NAD. It will be of interest to determine whether binding of a PARylated species, most likely Parp1 itself, to the Alc1 macrodomain results in allosteric activation of the enzyme.

Our in vivo assays take advantage of the ability to induce DNA breaks by pulsed-laser microirradiation, resulting in Parp1-dependent PAR synthesis and consequent Alc1 recruitment at a discrete nuclear location. In DNA damage repair, Parp1 is thought to bind and be allosterically activated by DNA ends. Parp1 can also be activated by other mechanisms, including interaction with the signaling kinase ERK2 (31) or binding to DNA hairpin and other unbroken DNA structures (32, 33). In transcription regulatory domains (17), Alc1 interacts with nucleosomes (15). In addition, Parp1 has been recently shown to be localized to many promoters and to contribute to transcriptional regulation (18). Thus, our data opens the possibility that Parp1-activated nucleosome remodeling by Alc1 could contribute to the control of chromatin structure during DNA repair, transcription, or other processes requiring Parp1. Future experiments will be necessary to illuminate the precise role of Alc1 in these processes.

**Materials and Methods**

**Purification of Flag-Alc1.** For expression in human cells, Alc1 cDNA (accession no. BC001171) was cloned into pcDNAs with an N-terminal FLAG tag and introduced into HEK293/FRT cells as described (34). Cells were grown to 70–80% confluence. Nuclear extracts were prepared according to the method of Dignam et al. (35), and FLAG-Alc1 and associated proteins were purified on anti-FLAG (M2) agarose beads (Sigma) as described (34). Alternatively, whole cells were lysed as described in *SI Methods*, and Flag-Alc1 and associated proteins were immunopurified as described (34), except beads were washed with 0.2 M KCl. For expression in Sf21 insect cells, Flag-Alc1 was cloned into a pBacPAK8 (Clontech) derivative, and purified from lysates of infected cells as described (36).

**Poly(ADP)ribose Binding Assays.** Recombinant proteins (1 pmol) were incubated for 30 min at 32 °C in 15 μL 40 mM HEPES-NAOH, pH 7.9, 0.1 M NaCl, 0.1 mM EDTA, 10% glycerol, and 32P-labeled PAR purified as described (8). Reaction mixtures were applied to nitrocellulose and washed overnight with TBS-T containing 100 mM NaCl. Bound 32P-labeled PAR was detected using a Typhoon phosphorimagerr.

**ATPase Assays.** ATPase assays were performed as described (34). Where indicated, reaction mixtures contained 100 ng (1 pmol) Flag-Alc1 (wild-type, E175Q, or D723A) from HEK293/FRT cells or SF9 cells, ~115 ng (1 pmol) Parp1 (Trevisen), recombinant Parg (Trevisen), 34 μM nicotine adenine dinucleotide, and 150 mg nono- or oligonucleosomes from HEaLa cells (37).

**Nucleosome Remodeling Assays.** Mononucleosomes were reconstituted by dilution transfer from HeLa oligonucleosomes on a 32P-end-labeled 216-bp DNA fragment (601-lat Gal4) generated by PCR from pGMEMZ-601-Gal4 (37, 38). F-Alc1 (1 pmol) from HEK293/FRT or SF9 cells was incubated at 32 °C for 30 min with mononucleosomes (~0.01 pmol labeled mononucleosome, ~0.25 pmol unlabeled oligonucleosomes) in 20 mM HEPES-NAOH, pH 7.9, 50 mM NaCl, 4.5 mM MgCl2, 2 mM DTT, 0.5 mM PMSF, 45 μg/mL BSA, 10% glycerol, 0.02% Triton X-100, 0.02% Nonidet P-40, and 2 mM ATP. Where indicated, reactions contained 2 mM ATP and 1 pmol Parp1, 34 μM NAD, or 2 mM benzamide. Reaction products were incubated for a further 30 min with 10 U of either HhaI or XhoI and resolved on gels containing 7% polyacrylamide (19:1 acrylamide:bis), 7 M urea, and 45 mM Tris-borate/1 mM EDTA, pH 8.3 (39).

**Nucleosome Binding Assays.** Mononucleosomes (40 pmol) were assembled on a 5'-biotinylated 601-lat Gal4 binding fragment, to 400 μL streptavidin dynabeads, washed, and resuspended in a final volume of 400 μL (100 fmoles mononucleosome/μL beads). Recombinant F-Alc1 (1 pmol) was incubated with 100 fmol immobilized nucleosomes in 45 μL 20 mM HEPES, pH 7.9, 50 mM NaCl, 0.5% Nonidet P-40, 10% glycerol, 5 mM MgCl2, 1 mM DTT, 0.5 mM PMSF, 1 mM ATP, and 100 μg/mL BSA. Where indicated 1 pmol Parp1 and NAD (5 μM) were included in reaction mixtures. Beads were washed three times with 200 μL 40 mM HEPES-NAOH, pH 7.9, 0.2 M NaCl, 0.2% Triton X-100, and 10% glycerol, transferred to a fresh microcentrifuge tube, and bound proteins were eluted with 3 × SDS sample buffer and analyzed by western blot.

**Transient Transfections.** HeLa-Kyoto and AGS cells were grown in HEPES-buffered DMEM-Glutamax-I (Invitrogen) containing 4.5 g/L glucose and 10% FCS US certified (Invitrogen) and supplemented with 90 μM penicillin, 50 μg/mL streptomycin (Invitrogen) and MEM-nonessential amino acids (MEM NEAA; Invitrogen). AGS cells stably expressing scrambled or 2 different short hairpin RNAs targeting PARP1 were generated at the Institute of Veterinary Biochemistry and Molecular Biology (IVMBB) using a shRNA Si-Lentivirus approach. Wild-type and mutant ALC1 cDNAs were amplified by PCR and cloned into the BglII and EcoRI sites of pEYFP-C1 (Clontech) for expression of EYFP-Alc1. PARP1 cDNA was amplified by PCR and introduced into the Xwai and Smal sites of pEYFP-C1 (Clontech) in the expression vector of Parp1-mCherry. For pulsed-laser microirradiation experiments, AGS cells were grown without puromycin. Where indicated, 1 μM PARP inhibitor PJ-34 (Alexis) was added 30 min before laser microirradiation.

**Pulsed Laser Microirradiation, Live Imaging, and Image Analysis.** Pulsed laser microirradiation was performed through a Zeiss C-Apo 63×/1.2 water immersion objective lens on a Zeiss Axiovert 200M epifluorescence microscope equipped with a frequency tripled 355 nm Nd:YAG pulsed laser (JDS Uniphase), scanned with galvo mirrors (40) and an ORCA CCD camera (Hamamatsu Photonics KK). DNA damage was induced by focusing in the nucleus an ~6–8 μm line target including 40–40 points with a pulse energy of 200–300 nJ for 3 times. Cells were imaged every 10 s for 20 min. Cells were kept at 37 °C in a CO2 independent HEPES-based imaging medium (Invitrogen) supplemented with 20% FBS (Invitrogen), 1 mM sodium pyruvate (Sigma), 2 mM L-glutamine (Sigma), 50 μg/mL penicillin, 50 μg/mL streptomycin (Sigma) in MatTek glass bottom dishes. Live images were registered and analyzed using ImageJ. Igor Pro (WaveMetrics) was used for analyzing and plotting the data. Cell motions were corrected using image plug-in MultiStackReg (41). To quantify protein recruitment following laser microirradiation, data were background-subtracted, normalized to premicroirradiation, and corrected for fluorescence loss: R(t) = [I(t) − h(bckgrd)]/M(h(bckgrd)) to [I(t) − h(bckgrd)]/(T − h(bckgrd))/M(h(bckgrd)), where I is the intensity acquired along the laser path region, h(bckgrd) is the background region outside the cell of interest, and T is the total fluorescence within the nucleus.
ACKNOWLEDGMENTS. We thank Kym Delventhal and Paul Hassa for molecular biology assistance, Tingting Yao for helpful discussions, and Julien Colombelli, Ernst Stelzer, and Jan Ellenberg for advice with live cell imaging. This work was supported by National institutes of Health General Medical Sciences Grant R37 GM41628 (to R.C.C.) and the Stowers Institute and by the European Molecular Biology Laboratory (EMBL) and the Human Frontier Science Program (A.L. and G.T.).

Supporting Information

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SI Methods

Size Exclusion Chromatography. Flag-immunopurified F-Alc1 (~35 µg in 100 µL) from HEK293/FRT cells was fractionated on a Superose6 sizing column using a SMART FPLC micro Separation System. The column was equilibrated in 40 mM HEPES-NaOH, pH 7.9, 0.1 M NaCl, 0.1 mM EDTA, 10% glycerol, and was eluted using the same buffer at a flow rate of 50 µL/min. Fifty-microliter fractions were collected and analyzed on silver-stained polyacrylamide gels and western blotting before use in assays.

Purification of F-Alc1 from HEK293T Cell Whole Cell Extracts. To purify F-Alc1 and associated proteins from whole cell extracts, cells were washed with PBS and then lysed by resuspension in 1 mL/dish 40 mM HEPES-NaOH, pH 7.9, 0.2 M NaCl, 1.5 mM MgCl2, 10% glycerol, 1 mM DTT, 0.2% Triton X-100. The resulting suspension was incubated with rotation at 4 °C for 30 min and spun at 40,000 rpm for 60 min at 4 °C in a 70.1 Ti rotor (Beckman-Coulter). Supernatants were subjected to anti-FLAG agarose chromatography as described in the Materials and Methods, except that beads were washed with buffer containing 0.2 M NaCl.
Fig. S1.  (A) SDS/PAGE analysis of F-Alc1 from HEK293/FRT cells. Aliquots of Flag-immunoprecipitates from equivalent amounts of nuclear extract from HEK293/FRT cells or HEK293/FRT cells stably expressing F-Alc1 were analyzed by SDS/PAGE and silver staining. In this panel and in Fig. S2C, the asterisk denotes a protein that nonspecifically binds and is eluted from Flag agarose beads.  (B) SDS/PAGE analysis of recombinant wild-type and mutant versions of Alc1, expressed in insect cells using baculovirus expression system and purified by Flag-immunopurification.  (C) Co-purification of ATPase and nucleosome remodeling activities with Alc1. F-Alc1 purified from nuclear extracts of HEK 293/FRT cells was subjected to Superose 6 chromatography. The indicated fractions were analyzed by SDS/PAGE and silver staining or anti-FLAG western blotting (2 upper panels) or were assayed for ATPase or nucleosome remodeling (lower panels). Assays were performed in the presence (solid lines) or absence (dotted lines) of Parp1 and NAD.
**Fig. S2.** (A) Parp1- and NAD-dependent Alc1 ATPase is inhibited by Parg1. Reactions were performed as described in the *Materials and Methods* with or without 1 ng (1×), 2 ng (2×), or 4 ng (4×) Parg. (B) Alc1 PARylation is not required for nucleosome remodeling. Nucleosome remodeling assays were performed as described with nucleosomes containing HeLa cell histones except that nucleosomes were preincubated for 30 min with Parp1 and NAD before addition of Alc1, with or without 2 mM benzamide.
Fig. S3. Anti-Parp1 and histone H3 western blots of lysates from HeLa cells or from AGS cells expressing 2 different shRNAs targeting PARP1 or a nontargeting shRNA (mock).
Fig. S4. The effect of PARP inhibitor PJ-34 on Alc1 recruitment kinetics. (A) Real-time recruitment kinetics (n ≥ 6) of wild-type Alc1 at the site of laser microirradiation in HeLa cells in the presence (blue dashed line) and absence (black line) of the PARP inhibitor PJ-34.
Table S1. Whole cell lysates from HEK 293/FRT cells expressing wild type or mutant F-Alc1 were immunoprecipitated with anti-FLAG (M2) agarose

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Immunopurified proteins were identified using a modification of the multidimensional protein identification (MudPIT) procedure (1, 2). Shown are the most abundant proteins that were detected by MudPIT mass spectrometry in Flag immunopurified material from cells expressing wild type F-Alc1 but not the macrodomain mutant F-Alc1 (D723A). The normalized spectral abundance factor (NSAF) is proportional to the amount of protein present in the sample (3, 4) and is calculated using the formula:

\[
(\text{NSAF})_k = \frac{(\text{SpC}/L)_k}{\sum_{i=1}^{N} (\text{SpC}/L)_i}
\]

where \( \text{SpC} \) = spectral count, \( L \) = protein length in amino acids, and \( i \) = all proteins detected in the MudPIT runs. ND, not detected.

Table S2. Neither poly(ADP-ribose) nor ADP-ribose activate Alc1 ATPase activity

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Thirty-minute ATPase reactions were performed as described in Methods with or without 1 pmol Alc1, 1 pmol Parp-1, and the indicated concentrations of NAD, free ADP(ribose) (ADPr), or free PAR. All reactions contained 1 pmol HeLa cell long oligonucleosomes, 40 μM ATP. Concentrations of PAR and ADPr are expressed as mole equivalents of adenosine determined using the extinction coefficient of adenosine [A_{260nm} = 15 O.D. (cm^2/μmol) at pH 7]. PAR was prepared as described [Karras GI, et al. (2005) The macro domain is an ADP-ribose binding module. EMBO J 24:1911–1920].
Movie S1. Recruitment kinetics of wild-type Alc1 and PARP1 to DNA damage sites. Representative movie of a HeLa cell transiently co-expressing EYFP-Alc1 (cyan) and PARP1-mCherry (green) following laser microirradiation. Right panel shows the merge.
Movie S2. Recruitment kinetics of a Alc1 fragment lacking its macrodomain and of PARP1 to microirradiated sites. Representative movie of a HeLa cell transiently co-expressing EYFP-Alc1-Δmacrodomain (cyan) and PARP1-mCherry (green) following laser microirradiation. Right panel shows the merge.
Movie S3. Recruitment of wild-type, D723A mutant and macrodomain deletion Alc1 following laser microirradiation. Representative movie of HeLa cells transiently expressing either wild-type, D723A mutant or macrodomain deletion Alc1 tagged with EYFP following laser microirradiation.