Phosphorylation Targets of DNA-PK and Their Role in HIV-1 Replication

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Abstract: The DNA dependent protein kinase (DNA-PK) is a trimeric nuclear complex consisting of a large protein kinase and the Ku heterodimer. The kinase activity of DNA-PK is required for efficient repair of DNA double-strand breaks (DSB) by non-homologous end joining (NHEJ). We also showed that the kinase activity of DNA-PK is essential for post-integrational DNA repair in the case of HIV-1 infection. Besides, DNA-PK is known to participate in such cellular processes as protection of mammalian telomeres, transcription, and some others where the need for its phosphorylating activity is not clearly elucidated. We carried out a systematic search and analysis of DNA-PK targets described in the literature and identified 67 unique DNA-PK targets phosphorylated in response to various in vitro and/or in vivo stimuli. A functional enrichment analysis of DNA-PK targets and determination of protein–protein associations among them were performed. For 27 proteins from these 67 DNA-PK targets, their participation in the HIV-1 life cycle was demonstrated. This information may be useful for studying the functioning of DNA-PK in various cellular processes, as well as in various stages of HIV-1 replication.

Keywords: DNA-PK; HIV-1; DNA-damage; HIV-1 transcription regulation; post-integrational repair

1. Introduction

DNA-dependent protein kinase (DNA-PK) is a heterotrimeric complex that consists of Ku70 (XRCC6), Ku80 (XRCC5), and DNA-PKcs (PRKDC). The last one belongs to the phosphatidyl inositol 3-kinase-like kinases (PIKKs) family [1]. DNA-PK is a major component of DNA double-strand break (DSB) repair system, which initiates the non-homologous end joining pathway (NHEJ). DNA-PK also participates in V(D)J and class-switch recombination [1]. More and more non-canonical functions of DNA-PK have recently been reported [2]; for instance, this complex has been shown to participate in ageing processes and metabolism regulation [3], transcription [4], and telomere maintenance [5,6].

NHEJ pathway begins with the recognition of a DNA DSB (double-strand break) by Ku70/Ku80 heterodimer. After that, DNA-PKcs is recruited to the DNA damage site and binds to the Ku70/Ku80-DNA complex, which triggers its kinase activity. The phosphorylating activity of this enzyme is crucial for efficient recruitment and activity regulation of DNA repair factors [1]. Activation of DNA-PK due to binding to DNA is a canonical activation mode, which is typical for such cellular functions of DNA-PK as DSB repair, V(D)J and class-switch recombination, and foreign DNA sensing [1,7,8].

However, it has recently been shown that DNA-PK activation is also possible under its interaction with RNA. This non-canonical activation has been described for RNA helicase A and heterogeneous nuclear ribonucleoprotein A1 phosphorylation [5,6,9].
One more example of the non-canonical activation of DNA-PKcs has been discovered by studying how the DNA-PK complex is involved in HIV-1 post-integrational repair (PIR) [10]. The integration of HIV-1 genome is vital for efficient production of new viral particles. However, this process is the key source of danger for further viral reproduction, because HIV-1 DNA integration into the cellular DNA causes its damage [11]. The integration of viral DNA results in the formation of proviral DNA with five-nucleotide single-strand gaps in genomic DNA on both sides of the viral DNA, and the unmatched dinucleotides located on the 5′ ends of the viral DNA (the so-called integration intermediate) (Figure 1). Cellular systems of DNA repair restore the integrity of this integration intermediate, which eventually results in the formation of a provirus—a template for the production of new virions [11].

![Model of HIV-1 post-integrational DNA repair](image)

**Figure 1.** Model of HIV-1 post-integrational DNA repair based on our previously published data [10]. HIV-1 integrase (IN) that marks integration sites recruits the Ku70/Ku80 heterodimer by direct interaction with the Ku70 subunit; then, the catalytic subunit of DNA-PK (DNA-PKcs) binds to this complex and phosphorylates some unknown protein targets that results in DNA repair. This process strongly depends on the interaction between IN and Ku70.

Using a modified variant of gag-alu specific PCR [12], we found that DNA-PK components, Ku70, Ku80, and DNA-PKs, are involved in PIR of the integration intermediate [10]. Moreover, the recruitment of DNA-PK to viral DNA integration sites and the activation of its catalytic subunit is mediated by the direct interaction of HIV-1 integrase (IN) (the enzyme accomplishing the integration of viral DNA into the cell’s genome) and Ku70, a part of DNA-PK [10] (Figure 1). The impaired interaction between these proteins decreases the infectivity of VSV-G-pseudotyped HIV-like particles. We also showed that the phosphorylating activity of DNA-PKcs is crucial for the efficient accomplishment of PIR, since the low-molecular specific inhibitor of DNA-PKcs kinase activity Nu7441 decreases both the PIR efficiency and the pseudovirus infectivity [10]. Thus, it is obvious, that DNA-PK as well
as its targets, phosphorylated during PIR, are necessary for the efficient completion of this process. Importantly, there are no double-stranded DNA breaks in the product of viral DNA integration. In this case, DNA-PK activation is triggered by IN binding to Ku70, since IN mutations disturbing this binding significantly reduce the PIR efficiency and disrupt the pseudovirus sensitivity to Nu7441 [10]. Therefore, this is an example of non-canonical activation of DNA-PK due to protein–protein binding.

The necessity of DNA-PK phosphorylating activity has also been shown for some other cellular processes. For example, phosphorylation of TRIM28 by DNA-PK converts it to an elongation factor during transcription regulation [13]. Just recently, the necessity of RNA polymerase II phosphorylation by DNA-PK for the efficient transcription from the HIV-1 promoter and reactivation of the latent provirus has been clearly demonstrated [14]. However, the mechanism of DNA-PK activation during transcription remains obscure, since the DNA-binding component of DNA-PK, the Ku protein, has high affinity to DNA ends, whereas its ability to interact with internal DNA sequences is not proved clearly [15].

To date, there is a lack of systematic research of phosphoproteome alterations upon DNA-PK activation. In one of the most grand-scale studies aimed at finding DNA-PKcs targets, 26 proteins have been found to be extensively phosphorylated upon the activation of this kinase [16]. However, among these proteins, there are no some classical DNA-PK targets (e.g., p53), which indicates that this list is not complete. The lack of such systematic studies, unfortunately, complicates the understanding of details of regulation of DNA-PK-dependent processes including the further study of HIV-1 PIR and transcription regulation.

In the present study, we first performed a search and analysis of DNA-PK targets described in literature. We managed to collect information on 67 unique DNA-PK targets, phosphorylated in response to various stimuli in vitro and/or in vivo. The represented targets could be conventionally divided into the following functional groups: DNA-repair, cell response to heat, post-translational RNA processing, transcription regulation, and a less functionally homogeneous cluster with proteins involved in cell cycle regulation (e.g., TP53 and MDM2), RNA biogenesis regulation (e.g., Jun, POLR2A, and POU2F1), and response to exo- and endogenous stimuli (e.g., AKT1 and AKT2). We also separately analyzed the literature data on the involvement of the indicated DNA-PK targets in HIV-1 replication.

2. Cellular Functions of DNA-PK

DNA-PK is a huge heterotrimeric complex comprised of the Ku70 and Ku80 that form the Ku-heterodimer and a 470-kDa catalytic subunit DNA-PKcs. The involvement of the DNA-PK protein complex, as well as its DNA-binding component Ku, in various cellular processes is being studied quite extensively [1–6]. Nevertheless, the functional role of DNA-PK has been thoroughly described only for DNA double-strand break repair by NHEJ pathway [1].

DNA-PKcs as a member of PIKKs family, together with two other kinases from the same family called ATM and ATR, maintains genome stability through regulation of the cellular DNA damage response (DDR). These kinases are activated in response to DNA damage, which leads to cell cycle arrest and DNA repair due to phosphorylation of different protein targets [1]. When activated, DNA-PKcs preferentially phosphorylates protein targets at canonical for PIKK S/T-Q sites, but the phosphorylation within non-S/T-Q contexts has also been shown [1].

DNA-PK mediates DSB repair by NHEJ-pathway, which is a major cell cycle independent repair pathway for this type of DNA lesions. The less error-prone way to repair DSB is a homologous recombination (HR), which is orchestrated by ATM. It takes place only in late S-G2 phases of cell cycle, and here NHEJ competes with HR pathway. Despite the significant difference between HR and NHEJ mechanisms in the DNA integrity restoration, DNA-PKcs may also participate in negative regulation of HR [17]. This regulation depends on kinase activity of DNA-PKcs [18], and may be explained by phosphorylation of ATM [19].

In the NHEJ pathway several principal stages can be distinguished: (1) DSB sensing; (2) recruitment of repair factors to the damage site and synopsis of DNA ends; (3) processing of DNA ends; and
(4) ligation of these ends together. Ring-shaped Ku-heterodimer rapidly binds DNA ends after DSB formation [20,21] and increases the affinity of DNA-PKcs to DNA ends [22], which results in DNA-PK complex assembly and the activation of the catalytic subunit [23]. DNA-PK complex acts both as a scaffold platform for NHEJ participants (XLF, XRCC4, APLF, Ligase IV, etc.) that are essential for synapsis formation, end-processing, and ligation and as a kinase that modifies chromatin around the DSB, regulates the activity of repair factors as well as promotes DNA-PK disassembly from DSB sites to allow DNA ends’ ligation [1,23–25]. Noteworthy, DNA-PKcs autophosphorylation is important for DNA-repair regulation. DNA-PKcs undergoes autophosphorylation in a DNA-damage dependent manner at multiple S/T-Q (S2056, T2609, S2612, S2620, T2638, T2647, and T3950) as well as non-S/T-Q site (S2624) in vivo [26]. Neither S2056 nor T2609 is required for DNA-PKcs kinase activity, but both are important for DNA repair. The current model suggests that their phosphorylation causes conformational changes that promote DNA-PK disassembly from DSB sites to allow DNA-end ligation [1]. Another phosphorylation site with a known effect on DNA repair is located in the kinase domain (T3950) and may act to switch off DNA-PKcs kinase activity when phosphorylated [1].

V(D)J and class switch recombination during B- and T-cell differentiation is another example of a cellular process involving DNA-PK. The DSBs are generated in a programmed enzymatic manner in both processes, and as a result, the NHEJ pathway is an integral part of both processes. The absence or mutation of NHEJ factors results in defective recombination leading to immune deficiencies and/or predisposition to cancers such as leukemias and lymphomas [8,27].

Recently, DNA-PK has been identified as a foreign DNA sensor in the cytoplasm that activates innate immunity. The DNA binding properties of Ku are obligatory for this activity [7,28]. For some viruses such as Vaccinia virus, Human adenovirus 5, and Herpes simplex virus 1, the mechanisms that counteract the DNA sensing by DNA-PK have been described [29,30]. Another cytoplasmic function of DNA-PK is its participation in translation regulation, in particular Ku has been found to bind p53 mRNA and this results in repression of p53 protein synthesis [31]. Of note, the need for phosphorylating activity of DNA-PK for this process has not been established.

In addition, there is a number of other cellular processes involving the components of the DNA-PK complex, however their role cannot be explained by the mechanical ability of DNA-PK to bind the ends of DNA. DNA-PK is shown to be implicated in the regulation of mitosis, telomere maintenance, hypoxic response, metabolism, and transcription regulation. Some of these issues are discussed in detail in other reviews [2,3,6,32]. The involvement of DNA-PK in the regulation of transcription of cellular genes and HIV-1 is discussed in detail below in Sections 3 and 4, respectively.

3. Phosphorylation Targets of DNA-PK

As mentioned above, DNA-PK kinase activity is important for various cellular processes. Although the components of NHEJ pathway exposed to phosphorylation have been identified [16,33–43] and are discussed below, the detailed role of DNA-PK catalytic activity in DSB repair and other processes is not yet completely understood, and our knowledge of phosphoproteome alterations upon DNA-PK activation is not complete.

We found and analyzed DNA-PK targets described in 63 articles, in which the relation between DNA-PK activation and phosphorylation of a human protein is revealed. These studies describe 67 unique human proteins phosphorylated by DNA-PK (Table 1). For 22 of them, phosphorylation has been described in vitro, for 25 of them only in vivo, and for 20 targets both in vitro and in vivo.
Table 1. DNA-PK targets and effects of their phosphorylation.

| Gene Name   | Protein Name                        | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo)                                      | Ref.     |
|-------------|-------------------------------------|--------------------|-----------------------------|----------------------------------|---------------------------------|--------------------------------------------------------------------------|---------|
| Akt1; Akt2; Akt3 | RAC-alpha serine/threonine-protein kinase | in vitro/in vivo   | CpG ODN, UVB                | Phosphorylated Akts activity; WB; $^{32}$P-incorporation | AKT1: T308, S473; AKT2: T309    | Cell survival after UVB treatment, Akt translocation to nucleus after CpG ODN treatment | [44,45] |
| ATM          | Serine-protein kinase ATM            | in vitro/in vivo   | bleomycin                   | WB; $^{32}$P-incorporation; mutagenesis | S85/T86, T372/T373 and S1985/T1987/T1988 | Negative regulation of ATM                                              | [19]    |
| C1D          | Nuclear nucleic acid-binding protein C1D | in vitro           | C1D, dsDNA                  | $^{32}$P-incorporation            |                                 |                                                                          | [46]    |
| DCLRE1C      | Protein Artemis                      | in vitro/in vivo   | dsDNA, bleomycin            | MS; changes in gel mobility of phosphorylated forms; $^{32}$P-incorporation; WB | S385, T410, S417, S503, S509, S516, S518, S572, S589, T601, S645, T676, S679, S688, T692 | Increase Artemis association with chromatin                              | [37,47,48] |
| DHX9         | ATP-dependent RNA helicase A         | in vitro           | poly(rG)                    | $^{32}$P-incorporation            |                                 |                                                                          | [9]     |
| DSP          | Desmoplakin                          | in vivo            | Dbait32H                    | ProQ-Diamond staining + MS       |                                 |                                                                          | [16]    |
| EIF2B2       | Translation initiation factor eIF-2B subunit beta | in vitro           | dsDNA                       | $^{32}$P-incorporation            |                                 |                                                                          | [49]    |
| EIF4A1       | Eukaryotic initiation factor 4A-I    | in vivo            | Dbait32H                    | ProQ-Diamond staining + MS       |                                 |                                                                          | [16]    |
| FKBP4        | Peptidyl-prolyl cis-trans isomerase FKBP4 | in vivo            | Dbait32H                    | ProQ-Diamond staining + MS       |                                 |                                                                          | [16]    |
| Gene Name | Protein Name | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo) | Ref. |
|-----------|--------------|--------------------|---------------------------|---------------------------------|---------------------------------|-----------------------------------|------|
| FUS       | RNA-binding protein FUS | in vitro/in vivo | Calicheamicin γ1, Dbait32H | WB; changes in gel mobility of phosphorylated forms; mutagenesis | S/T-Q located in N-terminal region of FUS (1-165 aa) | Translocation to cytoplasm | [50] |
| GOLPH3    | Golgi phosphoprotein 3 | in vitro/in vivo | Camptothecin, doxorubicin, IR | MS; 32P-incorporation | T143 | Cell survival following DNA damage | [51] |
| GTF2B     | Transcription initiation factor IIB | in vitro | dsDNA | 32P-incorporation | | | [52] |
| H2AFX     | Histone H2AX | in vitro/in vivo | dsDNA, IR, Dbait32H | WB; IF; changes in gel mobility of phosphorylated form | S139 | Assembly of DNA repair proteins at the DNA-damage sites | [16,53–58] |
| HMGCS1    | Hydroxymethylglutaryl-CoA synthase, cytoplasmic | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| HNRNPA1   | Heterogeneous nuclear ribonucleoprotein A1 | in vitro/in vivo | dsDNA, hTR, hnRNP | 32P-incorporation; WB; mutagenesis | S95, S192 | Essential for capping of the newly replicated telomeres and prevention of telomeric aberrations | [5,9,59] |
| HNRNPC    | Heterogeneous nuclear ribonucleoproteins C1/C2 | in vitro | hnRNP | 32P-incorporation | | | [9] |
| HNRNPF    | Heterogeneous nuclear ribonucleoprotein F | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| HNRNPU    | Heterogeneous nuclear ribonucleoprotein U | in vitro/in vivo | dsDNA, etoposide, Calicheamicin γ1 | MS; WB; changes in gel mobility of phosphorylated form | S59 | | [60,61] |
| Gene Name  | Protein Name                          | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo)                                                                 | Ref.       |
|-----------|---------------------------------------|--------------------|---------------------------|---------------------------------|---------------------------------|----------------------------------------------------------------------------------------------------------------|-----------|
| HSP90AA1  | Heat shock protein HSP 90-alpha       | in vitro/in vivo    | dsDNA; Dbait32H, IR       | ProQ-Diamond + MS; 32P-incorporation; WB | T5, T7                          | pThr7-HSP90α accumulates at repair foci, that is necessary for maintenance of γ-H2AX Foci and efficient DNA repair | [16,62–64]|
| HSPA1A    | Heat shock 70 kDa protein 1A          | in vivo             | Dbait32H                  | ProQ-Diamond + MS               |                                 |                                                                                                                 | [16]      |
| HSPA4     | Heat shock 70 kDa protein 4           | in vivo             | Dbait32H                  | ProQ-Diamond + MS               |                                 |                                                                                                                 | [16]      |
| HSPA8     | Heat shock cognate 71 kDa protein     | in vivo             | Dbait32H                  | ProQ-Diamond + MS               |                                 |                                                                                                                 | [16]      |
| HSPH1     | Heat shock protein 105 kDa            | in vivo             | Dbait32H                  | ProQ-Diamond + MS               |                                 |                                                                                                                 | [16]      |
| ILF2      | Interleukin enhancer-binding factor 2 | in vitro            | dsDNA                     | 32P-incorporation               |                                 |                                                                                                                 | [49]      |
| ILF3      | Interleukin enhancer-binding factor 3 | in vitro            | dsDNA                     | 32P-incorporation               |                                 |                                                                                                                 | [49]      |
| JUN       | Transcription factor AP-1             | in vitro            | dsDNA                     | 32P-incorporation; mutagenesis  | S249                            |                                                                                                                 | [65]      |
| LMNB1     | Lamin-B1                              | in vivo             | Dbait32H                  | ProQ-Diamond + MS               |                                 |                                                                                                                 | [16]      |
| MDM2      | E3 ubiquitin-protein ligase Mdm2       | in vitro            | dsDNA                     | 32P-incorporation; mutagenesis  | S17                             | Mdm-2 Phosphorylation by DNA-PK Prevents Interaction with p53                                                | [66]      |
| MRE11     | Double-strand break repair protein MRE11 | in vitro            | dsDNA                     | 32P-incorporation               |                                 |                                                                                                                 | [19]      |
| Gene Name   | Protein Name                  | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo) | Ref.  |
|-------------|-------------------------------|--------------------|-----------------------------|----------------------------------|----------------------------------|-------------------------------------|-------|
| MYC         | Myc proto-oncogene protein    | in vitro           | dsDNA                       | $^{32}$P-incorporation           |                                  |                                     | [67]  |
| MYH9        | Myosin-9                      | in vivo            | Dbait32H                    | ProQ-Diamond + MS                |                                  |                                     | [16]  |
| NBN         | Nibrin                        | in vitro           | dsDNA                       | $^{32}$P-incorporation           |                                  |                                     | [19]  |
| NFKBIA      | NF-kappa-B inhibitor alpha    | in vitro           |                             | $^{32}$P-incorporation; MS       | S36, T273                        |                                     | [68]  |
| NFKBIB      | NF-kappa-B inhibitor beta     | in vitro           |                             | $^{32}$P-incorporation           |                                  |                                     | [68]  |
| NHEJ1       | Non-homologous end-joining factor 1 (XLF) | in vitro/in vivo | dsDNA, IR | $^{32}$P-incorporation; MS | S245 | Dispensable for DSB repair | [69]  |
| NPM3        | Nucleoplasmin-3               | in vivo            | Dbait32H                    | ProQ-Diamond + MS                |                                  |                                     | [16]  |
| NR4A2       | Nuclear receptor subfamily 4 group A member 3 | in vitro/in vivo | dsDNA, IR | WB; MS; IF; mutagenesis | S337 | Promotes DSB repair | [70]  |
| NSFL1C      | NSFL1 cofactor p47            | in vivo            | Dbait32H                    | ProQ-Diamond + MS                |                                  |                                     | [16]  |
| PARP1       | Poly-(ADP-ribose) polymerase 1| in vitro           | dsDNA                       | $^{32}$P-incorporation           |                                  |                                     | [71]  |
| PLIN3       | Perilipin-3                   | in vivo            | Dbait32H                    | ProQ-Diamond + MS                |                                  |                                     | [16]  |
| PNKP        | Bifunctional polynucleotide phosphatase/kinase | in vitro/in vivo | IR | $^{32}$P-incorporation; mutagenesis | S114, S126 | Regulates DSB repair | [72]  |
| POLL        | DNA polymerase lambda, involved in BER, NHEJ and HR | in vitro | dsDNA | WB; $^{32}$P-incorporation; mutagenesis | T204 |                                     | [73]  |
| Gene Name | Protein Name | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo) | Ref. |
|-----------|--------------|--------------------|---------------------------|---------------------------------|----------------------------------|-------------------------------------|------|
| POLR2A    | DNA-directed RNA polymerase II subunit RPB1 | in vitro/in vivo | dsDNA; unknown transcriptional signal | WB; $^{32}$P-incorporation | Heptapeptide repeats of CTD; S2; S5 | Increase transcription efficiency | [14,74] |
| POU2F1    | POU domain, class 2, transcription factor 1 (octamer transcription factor 1, Oct-1) | in vitro/in vivo | dsDNA, IR, zeocin | $^{32}$P-incorporation | | Stabilizes Oct-1, decreases Oct-1 dependent transcription | [75] |
| PRKDC     | DNA-dependent protein kinase catalytic subunit | in vivo | Dbait32H | WB; IF; MS; $^{32}$P-incorporation; mutagenesis | S2056, T2609, S2612, T2620, S2624, T2638, T2647; S3205; S3821; S4046; T4102 | | [16,33,36,37] |
| RAD50     | DNA repair protein RAD50 | in vitro | dsDNA | $^{32}$P-incorporation | | | [19] |
| RBBP7     | Histone-binding protein RBBP7 | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| RPA2      | Replication protein A 32 kDa subunit | in vitro/in vivo | Camptothecin, UV, 4NQO, Etoposide | WB; mutagenesis; $^{32}$P-incorporation; changes in gel mobility of phosphorylated forms | S4, S8, T21 | Regulates fork restart, new origin firing, HR, mitotic catastrophe, and cell survival in response to replication stress. RPA2 hyperphosphorylation by DNA-PK in response to DSBs blocks unscheduled homologous recombination and delays mitotic entry. | [76–79] |
| RPSA      | 40S ribosomal protein SA | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| Gene Name | Protein Name | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo) | Ref. |
|-----------|--------------|--------------------|----------------------------|--------------------------------|---------------------------------|-----------------------------------|------|
| SP1       | Transcription factor Sp1 | in vitro | dsDNA | $^{32}$P-incorporation | | | [80] |
| SRF       | Serum response factor | in vitro/in vivo | IR | $^{32}$P-incorporation; two-dimensional separation of phosphopeptides on thin-layer cellulose plates | S435, S446 | | [81] |
| TBP       | TATA-box-binding protein | in vitro | dsDNA | $^{32}$P-incorporation | | | [52] |
| TP53      | Cellular tumor antigen p53 | in vitro | dsDNA | WB; $^{32}$P-incorporation; SPR | S6, S15, S37, S46, S166 | | [82–85] |
| TRIM28    | Transcription intermediary factor 1-beta | in vivo | IR; Heat-shock induced gene transcription | WB | S824 | TRIM28 phosphorylation induces chromatin changes in response to DNA breaks. | [13,86,87] |
| TUBB      | Tubulin beta chain | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| TUBB2C    | Tubulin beta-4B chain | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| TUBB6     | Tubulin beta-6 chain | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| UBQLN1    | Ubiquilin-1 | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| VCP       | Transitional endoplasmic reticulum ATPase | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
Table 1. Cont.

| Gene Name  | Protein Name      | Type of Experiment | DNA-PKcs Activation Method | Phosphorylation Event Validation | Identified Phosphorylation Sites | Effect of Phosphorylation (In Vivo) | Ref. |
|------------|-------------------|--------------------|---------------------------|----------------------------------|----------------------------------|-------------------------------------|------|
| VIM        | Vimentin          | in vitro/in vivo   | Dbait32H                  | ProQ-Diamond + MS; WB; 32P-incorporation | S459                             | Regulates cell migration and adhesion | [16] |
| WRN        | Werner syndrome ATP-dependent helicase | in vitro/in vivo | dsDNA; bleomycin; 4NQO | WB; MS; 32P-incorporation; mutagenesis | S440, S467 | Inhibits both the helicase and exonuclease activities of WRN. Phosphorylation of S440 and S467 are important for relocalization of WRN to nucleoli, and that it is required for efficient DSB repair. | [38,39,88] |
| XRCC4      | DNA repair protein XRCC4 | in vitro/in vivo | dsDNA, IR | MS; 32P-incorporation; mutagenesis | S260, S318, S320 | Not essential for DSB repair | [34,40–43] |
| XRCC5      | XRCC5 X-ray repair cross-complementing protein 5 (Ku80) | in vitro | dsDNA | WB; MS; 32P-incorporation; amino acid sequencing | S577, S580, T715 | | [35,89] |
| XRCC6      | XRCC6 X-ray repair cross-complementing protein 6 (Ku70) | in vitro | dsDNA | WB; MS; 32P-incorporation; amino acid sequencing | S6 | | [35,89] |
| YWHAQ      | 14-3-3 protein theta | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
| YWHAZ      | 14-3-3 protein zeta/delta | in vivo | Dbait32H | ProQ-Diamond + MS | | | [16] |
The kinase assay using [γ-32P]-ATP was the most frequent method to detect phosphorylation in vitro. In this case, a putative target protein was incubated with DNA-PK complex components under appropriate conditions including the activator of DNA-PKcs. Phosphorylation events were detected by 32P-incorporation into a band in SDS-PAGE that corresponds to the target protein (Table 1, 32P-incorporation). In the case of in vivo targets identification, phosphorylation of proteins was analyzed using Pro-Q Diamond, which is a specific dye for phosphoproteins, followed by mass-spectrometry (Table 1, ProQ-Diamond staining + MS). In this case, the protein that is differentially phosphorylated in the experiment vs. control can be identified, but the identification of phosphorylated peptides needs additional research, which is why phosphorylated amino acid residues have not been identified for some of the targets in Table 1. The site-directed mutagenesis was also used to confirm identified amino acid residues as phosphorylation sites (Table 1, mutagenesis). When it was possible to obtain antibodies against phosphorylated forms of targets, they were used to investigate the details of their phosphorylation (Table 1, WB (Western blot) and IF (immunofluorescence)). In rarer cases, protein phosphorylation was detected by changes in the mobility of the target protein in SDS-PAGE, which is applicable both for in vitro and in vivo studies (Table 1, changes in gel mobility of phosphorylated forms).

DNA end mimicking molecules such as Dbait32H (in vitro and in vivo studies), sheared genomic DNA (in vitro activation), etc. were commonly used as DNA-PK activators: upon their introduction, phosphorylation of the targets was observed (Table 1). Besides, DNA-PK targets were phosphorylated upon exposure to classical DNA-damaging agents: ionizing radiation (IR), UV, bleomycin, etoposide, camptothecin, doxorubicin, zeocin, calicheamicin γ1, and 4-nitroquinoline-1-oxide (4NQO) (Table 1). In some studies, DNA-PKcs activation and the subsequent phosphorylation of the targets were observed in response to non-canonical stimuli: C1D protein, heterogeneous ribonucleoprotein particle (hnRNP), and telomerase RNA component (hTR) (Table 1).

Phosphorylation sites were identified for 29 of the 67 described proteins (Table 1), most of them belonging to SQ or TQ motifs—sequences found in proteins phosphorylated by the members of PIKKs family: ATM, ATR, and DNA-PKcs [1]. Three (T2624, S3205, and S4026) out of nine DNA-PKcs autophosphorylation sites are not located in SQ or TQ sites [37]. The DNA-PKcs phosphorylation sites on Ku70, Ku86, and XRCC4 are also located in sites other than this consensus [35,40].

DNA-PK-dependent phosphorylation of the proteins with the determined phosphorylation sites had various effects on their functionality. Thus, phosphorylation of well-known DNA-repair proteins NHEJ1 [40] and XRCC4 [40,43] was dispensable for DNA repair in vivo. In contrast, phosphorylation of such proteins as ATM [19], DCLRE1C [37], GOLPH3 [51], H2AFX [90], HSP90AA1 [63], NR4A2 [70], POU2F1 [75], PNKP [72], RPA2 [77], and WRN [39] was important for the DNA-repair regulation. Phosphorylation of ATM leads to its inhibition, which may explain how the cell makes its choice between homologous recombination, stimulated by ATM, and NHEJ, given that DNA-PKcs and ATM are both simultaneously recruited and activated at the same DSB ends [19]. C-terminal phosphorylation of Artemis increases its endonucleolytic activity that may affect DNA-end processing in NHEJ-pathway [37]. T7-phosphorylated Hsp90α is accumulated at the site of DNA damage, where it appears to be important for maintaining phosphorylated histone H2AX [63]. Mutations in WRN that impair DNA-PK-dependent phosphorylation change the kinetics of WRN dissociation from DSBs and decrease the efficiency of DSB repair [39].

A well-known transcription factor POU2F1 (Oct-1) was phosphorylated by DNA-PK under IR or zeocin treatment, and this event regulated Oct-1 dependent transcription, leading to an increased cell survival after DNA damage [75]. The same effects on the cell survival were observed in the case of DNA-PK-dependent phosphorylation of GOLPH3, that regulates Golgi dispersal [51]. Interestingly, the phosphorylation of another transcription factor NR4A2 promotes DSB repair, but NR4A2 transcriptional activity is entirely dispensable in this function. Instead, NR4A2 functions directly at DNA repair sites [70]. Human polynucleotide kinase/phosphatase (PNKP) has the dual function as 5′-DNA kinase and 3′-DNA phosphatase and plays a role in NHEJ and other DNA repair
processes. The phosphorylation of PNKP on S114 and S126 in DNA-PK and ATM-dependent manner was demonstrated in vitro, and was confirmed on S126 in vivo. This phosphorylation promotes the retention of PNKP at sites of DNA damage, and may regulate its catalytic activity near DSB sites that helps to successfully complete the DNA repair process [72]. The phosphorylation events described above are important for the successful NHEJ. Moreover, the phosphorylation of RPA32 at S4/S8 by DNA-PKcs is essential for cell survival under replicative stress [77]. Even this shows that the role of the kinase activity of DNA-PK goes beyond the NHEJ-pathway.

In several reports, protein translocation after phosphorylation was detected, and this may be important for the cellular response to DNA-damaging agents [16,44,50]. For example, a cell treatment with antibiotic calicheamicin γ1, which causes DNA double-strand breaks, led to cytoplasmic accumulation of FUS, which depended on phosphorylation of its N-terminus by DNA-PK [50]. Besides, regulation of cell mobility and adhesion was observed in the case of DNA-PK-dependent phosphorylation of VIM under Dbait32H treatment [16].

We performed a functional enrichment analysis of DNA-PK targets to elucidate the cellular processes, in which DNA-PK targets are involved. Annotation of 67 genes was performed using the DAVID online analysis tool v. 6.8 [91]. Genes were divided into 17 functional clusters with enrichment score higher than 1.7 (Table S1). Most of them were enriched with following terms: DNA-repair (36 targets), nucleotide binding (26 targets), DNA-binding (26 targets), transcription regulation (23 targets), regulation of cellular response to heat (8 targets), RNA processing and splicing (8 targets), PI3K-Akt signaling pathway (8 targets), telomere maintenance (7 targets), cell cycle (7 targets), DNA-unwinding (7 targets), and cytoskeleton (6 targets).

To determine protein–protein associations of DNA-PK targets listed in Table 1, we used STRING database v. 11.0 [92] and revealed a network of directly interacting proteins. We excluded the “text mining” option from the active interaction sources because of the low level of reliability of this type of data, and also because our protein targets were retrieved by the articles’ analysis. It turned out that, among 67 proteins, 56 were directly interconnected (Figure 2A). The resulting network had meaningfully more interactions than expected (218 vs. 64 expected edges) with protein–protein interactions (PPI) enrichment $p$-value $< 10^{-16}$. Markov Cluster Algorithm (MCL) analysis revealed five main clusters of the most interconnected nodes.

The proteins in these clusters could be divided according to their functions into DNA repair related proteins, transcription regulation, cell response to heat or unfolded proteins, RNA processing, and cell survival/signaling factors (Figure 2A). Of note, the mathematical approach to protein clusterization in the interaction network (MCL analysis) led to practically the same results as the functional classification.

Taking into account that our target list (Table 1) contained some proteins for which DNA-PK-dependent phosphorylation has been confirmed only in vitro, we analyzed “in vitro” and “in vivo” target groups separately, 22 and 45 targets, respectively. The second group contained both in vivo and in vitro/in vivo confirmed targets. Analysis of the “in vitro” group showed significant PPI enrichment: $p$-value $< 10^{-16}$, 43 vs. 7 expected edges. This group can be divided into three clusters containing at least four proteins. The clusters contain proteins with the following functions: DNA-repair, transcription regulation, and RNA-processing (Figure 2B). For the “in vivo” group (PPI enrichment $p$-value $< 2.22 \times 10^{-16}$, 80 vs. 27 expected edges), five clusters were identified. According to their functions, these proteins could be classified as DNA-repair, RNA processing, cell response to heat or unfolded proteins, and cell survival/signaling factors (Figure 2C). The full list of the enriched biological processes and molecular functions (GO) is presented in Table S2.
and cell survival/signaling factors (Figure 2A). Of note, the mathematical approach to protein clusterization in the interaction network (MCL analysis) led to practically the same results as the functional classification.

Taking into account that our target list (Table 1) contained some proteins for which DNA-PK-dependent phosphorylation has been confirmed only in vitro, we analyzed “in vitro” and “in vivo” target groups separately, 22 and 45 targets, respectively. The second group contained both in vivo and in vitro/in vivo confirmed targets. Analysis of the “in vitro” group showed significant PPI enrichment: \( p \)-value < \( 10^{-16} \), 43 vs 7.7 expected edges. This group can be divided into three clusters containing at least four proteins. The clusters contain proteins with the following functions: DNA-repair, transcription regulation, and RNA-processing (Figure 2B). For the “in vivo” group (PPI enrichment \( p \)-value < \( 2.22 \times 10^{-16} \), 80 vs 27 expected edges), five clusters were identified. According to their functions, these proteins could be classified as DNA-repair, RNA processing, cell response to heat or unfolded proteins, and cell survival/signaling factors (Figure 2C). The full list of the enriched biological processes and molecular functions (GO) is presented in Table S2.

Figure 2. Protein–protein interactions network of DNA-PKcs targets described in Table 1 (A). PPI networks of DNA-PKcs targets identified only in in vitro experiments (B) and in in vivo or both in vitro and in vivo experiments (C). The most connected nodes are the same color and are grouped in a cluster using Markov Cluster Algorithm (MCL) with inflation parameter 3.1.

The analysis of Table 1 and Figure 2 demonstrates that most of the research of DNA-PK targets was focused on proteins anyway involved in DNA repair processes. This is not surprising, given that the role of DNA-PK in DNA repair is studied best. Furthermore, it was a priori assumed that DNA-PK is activated only upon binding to DNA ends forming as a result of a double-strand break. However, DNA-PK may also be activated in the absence of DNA ends \([5,9,46]\). For instance, hTR dependent phosphorylation of HNRNPA1 by DNA-PK is essential for telomere maintenance \([5]\). Although these studies are not numerous, the variety of systems with confirmed DNA-independent activation of DNA-PK lends credibility to these data. All these data additionally demonstrate that DNA-PK may regulate a broad spectrum of cellular processes. In particular, the ability of DNA-PK to become activated upon binding to RNA may explain the presence of a substantial number of target proteins involved in RNA processing in the “in vitro”, as well as in the “in vivo” group.
As shown in Figure 2B, among DNA-PK phosphorylation targets identified in vitro, there is a significant group consisting of proteins involved in transcription regulation, such as TBP, JUN, MYC, etc. Transcription regulation factors can also be found in the “in vivo” group (POU2F1, TRIM28, POLR2A, NR4A2, and RBBP7). Such targets seem quite plausible, given that the association between activation of DDR kinases (ATM and DNA-PKcs) and transcriptional regulation has already been demonstrated. On the one hand, transcription from certain promoters is initiated through the introduction of DSB by topoisomerase IIβ, and DNA-PK is involved in the repair of such breaks [93].

It was also shown that expression regulation of some stimulus-inducible and developmental genes can occur by RNA polymerase II (RNAP II) pausing and pause release [94]. Typically, RNAP II pauses at around +30–100 relative to the transcriptional start site until activating cellular signals induce elongation [95]. Interestingly, the DNA-repair factor TRIM28 is a main player that maintains RNAP II paused state. To release RNAP II, topoisomerase II should introduce the DSB at the regulated genes. This event activates DDR kinases ATM and DNA-PKcs, which phosphorylate protein targets including H2AX and TRIM28. The phosphorylated TRIM28 is no longer able to retain RNAP II near the transcriptional start, which results in processive elongation of RNAP II and effective synthesis of RNA [13,87,94]. Interestingly, these transcription-induced, topoisomerase II-mediated DSBs can also be exploited therapeutically and propose that, in hormone-dependent tumors such as breast and prostate cancers, a hormone-cycling therapy, in combination with topoisomerase II poisons or inhibitors of the DNA repair component DNA-PKcs, could overwhelm cancer cells with transcription-associated DSBs [96]. On the other hand, the occurrence of DSBs in a human gene transcribed by RNAP II is known to lead to inhibition of transcription elongation and reinitiation. Upon inhibition of DNA-PK (or ATM) activity, RNAP II bypasses the break and continues transcription elongation, suggesting that it is not the break per se that inhibits the processivity of RNAP II, but the activity of the kinases [97,98]. However, it is known that DNA-PK-dependent phosphorylation of such NF-κB inhibitors as IκBα (NFKBIA) and IκBβ (NFKBIB) favors the association of NF-κB with DNA [68]. It has been shown in other studies that NF-κB is activated as part of the DNA damage response and is thought to orchestrate a cell survival pathway, which, together with the activation of cell cycle checkpoints and DNA repair, allows the cell in cases of limited damage to restore a normal life cycle [99]. DNA-PK likely utilizes several mechanisms to regulate transcription and is capable of exerting both positive and negative effects.

4. DNA-PK Targets in HIV Replication

HIV-1 replication cycle is conventionally divided into early and late replication stages. The early stages are aimed at the formation of proviral DNA, i.e., that of the copy of the viral genome integrated into the genome of the infected cell. They include viral attachment and entry, reverse transcription, nuclear import, integration, and post-integrational DNA repair (PIR) [11,100]. The late stages include virion component biosynthesis and assembly of new viral particles [100]. The dependence of the HIV-1 replication on the DNA-PK-complex components has been previously shown by different groups [10,101–107]. We previously showed that the phosphorylating activity of DNA-PKcs was necessary for HIV-1 PIR [10]. To search for possible downstream proteins involved in this process, we analyzed the data on the role of DNA-PK targets in Table 1 in HIV-1 replication.

For 27 proteins out of 67 represented in Table 1, information on their involvement in HIV-1 replication cycle is available. Thus, 13 proteins favor HIV-1 replication, 6 proteins impair it, data for 3 proteins are controversial, and 5 proteins have opposite effects on HIV-1 replication, depending on the life cycle stage (Table 2). Table 2 also provides information on HIV-1 life cycle stages influenced by these factors.

It has been suggested that certain factors are involved in post-integrational DNA repair or integration (Table 2). Unfortunately, the discrimination of their effects on each of these two stages was not possible up until recently, since there were no methods allowing the quantitative assessment of post-integrational DNA repair. That is why the effects of cellular proteins on the repair were studied
only by indirect methods, for instance, based on the decrease of integrated HIV-1 DNA levels 48 hpi vs. 24 hpi [108]. Only three proteins, DHX9, EIF4A1, and p53 (TP53), have been shown to participate in reverse transcription, the latter being also involved in other stages of viral life cycle (Table 2). However, the significance of phosphorylation of these proteins for their functionality in HIV-1 replication has not yet been demonstrated.

Here, we dwell on DNA-PK phosphorylation targets involved in post-integrational DNA repair, starting with DNA-PKcs (PRKDC) itself, as well as Ku70 (XRCC6) and Ku80 (XRCC5), which form DNA-PK complex together. Their beneficial effect on HIV-1 replication was shown in numerous studies [101–106]. It was speculated that DNA-PK complex is involved in PIR and our method of the quantitative assessment of PIR efficiency [12] allowed directly proving the involvement of this complex in this process [12].

Based on indirect data, kinase ATM was supposed to be involved in PIR [108]. Thus, the presence of small-molecule ATM inhibitor Ku55933 was shown to have no effect on the level of integrated viral DNA at 24 hpi; however, at 48 hpi, the levels of integrated DNA in inhibitor-treated cells drastically decreased, but remained practically unchanged in the control cell line [108]. The authors attributed this effect to the impossibility of post-integrational DNA repair with ATM being inhibited. The role of MRN complex components MRE11 and Nibrin (NBN), the main factors recruiting ATM to DNA damage sites [109], in the replicative cycle is controversial [106,110]. Thus, Smith et al. showed that the decreased levels of MRN complex components decreases the efficiency of transduction with an HIV-1-based vector [110], whereas, in the study by Sakurai et al., the transduction of cells with defective components of this complex was similar to that in control cell lines [106]. However, integration site sequencing revealed the higher mutation rate in integration sites in the absence of NBN and MRE11, which supports the dysfunctional repair in these cells [106]. It may indirectly support these proteins being involved in the PIR of proviral DNA.

Table 2. Effects of DNA-PKcs targets on HIV-1 replication.

| Gene Name (Common Protein Name) | Role in HIV Life Cycle | Comments/Life Cycle Step | Publications |
|--------------------------------|------------------------|--------------------------|--------------|
| AKT                           | Positive               | Cell survival during HIV infection | [111,112]    |
| ATM                           | Positive               | Post-integrational repair (indirect evidence) | [101,106,108,113] |
| DCLRE1C (Artemis)             | Positive               | No data                  | [114]        |
| DHX9                         | Positive               | Reverse transcription     | [115,116]    |
| EIF4A1                       | Positive               | Reverse transcription     | [117]        |
| FUS                          | Negative               | LTR-dependent transcription | [118]       |
| H2AFX (H2AX)                 | Dispensable            | H2AFX is phosphorylated during integration, but not essential for HIV replication | [119]    |
| HNRNPA1                      | Complex                | HIV transcription, viral mRNA splicing, mRNA transport | [120,121]  |
| HSP90AA1                     | Positive               | Transcription, capsid core stability | [122]        |
| JUN                          | Complex                | Transcription. c-Jun enhances Tat-mediated LTR transcription but suppresses basal LTR transcription without Tat | [123]    |
Table 2. Cont.

| Gene Name (Common Protein Name) | Role in HIV Life Cycle | Comments/Life Cycle Step | Publications |
|---------------------------------|------------------------|--------------------------|--------------|
| MDM2 Complex                    | Positive regulator of early replicative stages in macrophages by inhibition of p53 activity; Negative regulation of Vif stability, removes its counteracting effect on the APOBEC3G restriction factor; Positive regulation of Tat activity |                          | [124–126]    |
| MRE11                            | Controversial          | Integration, pre-integration steps, post-integrational DNA repair (indirect and controversial evidences) | [106,110]    |
| MYC Complex                      | Complex                | 1. Positive regulation of cDNA nuclear transport; 2. c-Myc and Sp1 contribute to proviral latency. Negative regulation of transcription from LTR promoter | [127,128]    |
| NBN (Nibrin) Complex             | Controversial          | Integration, pre-integration steps, post-integrational DNA repair (indirect and controversial evidences) | [106,110]    |
| NFKBIA (IκBα)                   | Negative               | IκBα but not IκBβ suppress latent-active transcription transition | [129]         |
| PARP1                            | Controversial          | 1. Early replicative steps (integration and/or post-integrational DNA-repair (indirect evidence) 2. LTR-dependent transcription | [130–138]    |
| POU2F1 (Oct-1)                   | Negative               | Repress LTR-mediated transcription | [139]         |
| RBBP7                            | Negative               | LTR-mediated transcription | [140]         |
| SP1                              | Positive               | c-Myc and Sp1 contribute to proviral latency | [141]         |
| TBP                              | Positive               | LTR-mediated transcription | [142,143]    |
| TP53 (p53)                       | Negative               | 1. Reverse transcription 2. LTR-mediated transcription 3. Cell survival during HIV-infection | [144–148]    |
| TRIM28                           | Negative               | Promotes HIV-1 Latency. DNA-PKcs dependent phosphorylation reactivates LTR mediated transcription | [14,149]      |
| VIM                              | Positive               | No data | [150]         |
| WRN                              | Positive               | LTR-mediated transcription | [151,152]    |
| XRCC4                            | Positive               | early replicative stages | [103]         |
| XRCC5 (Ku80) Complex             | Positive               | 1. LTR-mediated transcription 2. Integration, post-integrational DNA repair (direct evidence) | [10,101–107] |
| XRCC6 (Ku70)                     | Positive               | Post-integrational DNA repair (direct evidence) | [10]          |

PARP-1 is another DNA-PK target, the role of which in HIV-1 replication has been actively studied, but, to date, the results are rather controversial [153]. Some studies [130–133] show that PARP-1 plays an important role in HIV-1 integration. At the same time, the importance of PARP-1 for retroviral
replication is doubted in other studies [134-136]. More recent research shows that PARP-1 is involved in HIV-1 replication in the step of repression of transcription from the provirus, but it does not affect the integration level [138,154]. Nevertheless, various studies describe the beneficial effect of PARP-1 on the transcription from the HIV-1 promoter [130,137,155], as well as its negative effect [138,154,156]. However, the data on the negative effect of PARP-1 on transcription were obtained in a chicken B lymphoblastoid cell system, whereas the beneficial effect was observed in human cells (HeLa, J111 and human primary monocyte-derived macrophages), which is a more relevant model for studying HIV-1.

Histone H2AX (H2AFX gene), another DNA-PK target, is phosphorylated upon retroviral integration; however, this event is not likely to be necessary for efficient replication [119], but can be used as a marker for PIR research.

Most proteins in Table 2 regulate transcription from the LTR promoter, i.e., one of late stages of the replication cycle. HIV-1 genes are transcribed by RNAP II (POLR2A is a part of this complex) from a viral promoter in the 5′LTR (long terminal repeat). Besides the promoter, the 5′LTR contains a modulator sequence and an enhancer, interacting with a number of transcription factors regulating the transcription of HIV genes. The provirus may be latent or actively transcribed. The latent state may be characterized by the absence of transcription from the LTR promoter or by synthesis of short abortive fragments of 60–80 nucleotides, forming a stable RNA hairpin TAR (trans-activation response element) [157]. After TAR RNA synthesis, RNAP II stops, being bound to factors repressing transcription elongation, namely NELF (negative elongation factor) and DSIF (5,6-dichloro-1-β-D-ribofuranosylbenzimidazole sensitivity-inducing factor) [158]. For the transition into the state of active transcription, the C-terminal domain of RNAP II needs to be hyperphosphorylated on S2. The key participant of this process is P-TEFb complex (positive transcription elongation factor), recruited to HIV-1 promoter by direct interaction with Tat viral protein bound to TAR RNA [159]. However, there is increasing evidence of DNA-PK involvement in this process.

In particular, it is known that DNA-PK may phosphorylate the C-terminal domain of RNAP II [74], and, along with the Ku70/Ku80 heterodimer, DNA-PKcs may be precipitated from human cells together with the RNAP II holoenzyme [160]. It has also been shown that the Ku70/Ku80 heterodimer may interact with hairpin RNA involved in transcription performed by RNAP II, HIV-1 TAR PHK and 7SK PHK [159,161,162], whereas the catalytic subunit of DNA-PKcs forms a complex with HIV-1 Tat [163] and may phosphorylate it [164]. These various data indicate the possible involvement of the DNA-PK DNA repair complex in the regulation of transcription from the HIV-1 promoter. Of note, S. Tyagi et al. demonstrated the parallel distribution of DNA-PKcs with RNAP II along the HIV-1 provirus before and after transcription activation with tumor necrosis factor alpha. Interestingly, when the provirus changes its state from latent to active, the levels of both DNA-PKcs and RNAP II associated with the HIV-1 promoter increase dramatically [164]. Finally, it has recently been shown that, in different cell lines, DNA-PK increases the phosphorylation of RNAP II C-terminal domain at S5 and S2 by directly catalyzing phosphorylation and by augmenting the recruitment of P-TEFb at HIV LTR. Thus, the DNA-PK-dependent phosphorylation of RNAP II likely plays an important role in both transcription initiation and elongation [14]. Considering the role of Top II and DNA-PKcs in pause release of RNAP II through the DSB formation in cellular stimuli induced genes, one may assume that LTR-driven transcription may also be regulated in the same manner. Although this idea is more speculative and needs further testing, TRIM28, which is main maintainer of paused RNAP II, has been demonstrated to participate in HIV latency by SUMOylating CDK9 and inhibiting P-TEFb [149]. Moreover, DNA-PK utilizes the mechanism of the TRIM28 recruitment at LTR and its phosphorylation to release paused RNAP II, thus influencing different steps of transcription from the LTR promoter [14].

LTR is an intricate regulatory sequence of the HIV-1 genome. It contains binding sites of such transcription regulators as AP-1 complex (JUN), c-MYB, NFAT, GR (NR3C1), USF1, ETS1, LEF1, CEBP, NF-κB, SP1, TBP, UBPI, UBPII, CTF/NF1, and IRF [141]. Some of these factors, such as JUN, SP1, and TBP, may be phosphorylated by DNA-PK in response to various stimuli (Table 1). The phosphorylated TBP and TFIIB synergistically stimulate RNAP II basal transcription from adenovirus major late
promoter, which means that DNA-PK can positively regulate the RNAP II basal transcription by phosphorylating TBP and TFII B [52]. It has been shown that Sp1 forms a tight protein–protein complex with viral Tat, and both proteins in this complex are phosphorylated by DNA-PK. Importantly, it is the phosphorylation status of Sp1 and not the levels of LTR-promoter-bound Sp1 that affects HIV-1 transcription [165]. It was previously shown that DNA-PK-dependent phosphorylation of NF-κB inhibitors IκBα (NFKBIA) and IκBβ (NFKBIB) promotes the association of NF-κB with DNA [68]. Therefore, DNA-PK associated with the LTR promoter may favor LTR-dependent HIV-1 transcription by promoting the binding of NF-κB with LTR.

5. Conclusions

In this work, we tried to systematize the literature data on proteins phosphorylated by DNA-PK, having two goals: to understand in which cellular processes the phosphorylating activity of this kinase may be important and to find potential participants of PIR among DNA-PK targets. The results of our work undoubtedly show the variety of DNA-PK’s functions, not limited by participation in DNA double-strand break repair by NHEJ pathway. In the case of HIV-1, it is involved in at least two stages of the replication cycle of the virus: post-integrational DNA repair and regulation of transcription from the LTR promoter. Unfortunately, the analysis of the targets of DNA-PK related to HIV-1 replication cycle (Table 2) does not clearly reveal the participants of the PIR process exposed to phosphorylation. Assuming PIR may involve proteins from other repair processes, the most likely candidates are ATM, Artemis (DCLRE1C), MRE11, NBN, and XRCC4. However, further research is clearly needed to exactly define the events of HIV-1 PIR occurring upon DNA-PKcs activation.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4409/9/8/1907/s1, Table S1: DAVID functional annotation of DNA-PKcs targets, Table S2: Enriched biological processes and molecular functions in DNA-PKcs targets list.

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References

1. Blackford, A.N.; Jackson, S.P. ATM, ATR, and DNA-PK: The Trinity at the Heart of the DNA Damage Response. Mol. Cell 2017, 66, 801–817. [CrossRef] [PubMed]
2. Goodwin, J.F.; Knudsen, K.E. Beyond DNA Repair: DNA-PK Function in Cancer. Cancer Discov. 2014, 4, 1126–1139. [CrossRef] [PubMed]
3. Chung, J.H. The role of DNA-PK in aging and energy metabolism. FEBS J. 2018, 285, 1959–1972. [CrossRef] [PubMed]
4. Goodwin, J.F.; Kothari, V.; Drake, J.M.; Zhao, S.; Dylgjeri, E.; Dean, J.L.; Schiewer, M.J.; McNair, C.; Jones, J.K.; Aytes, A.; et al. DNA-PKcs-Mediated Transcriptional Regulation Drives Prostate Cancer Progression and Metastasis. Cancer Cell 2015, 28, 97–113. [CrossRef]
5. Ting, N.S.Y.; Pohorelic, B.; Yu, Y.; Lees-Miller, S.P.; Beatie, T.L. The human telomerase RNA component, hTR, activates the DNA-dependent protein kinase to phosphorylate heterogeneous nuclear ribonucleoprotein A1. Nucleic Acids Res. 2009, 37, 6105–6115. [CrossRef]
6. Sui, J.; Zhang, S.; Chen, B.P. DNA-dependent protein kinase in telomere maintenance and protection. Cell. Mol. Biol. Lett. 2020, 25, 1–14. [CrossRef]
7. Ferguson, B.J.; Mansur, D.S.; Peters, N.S.; Ren, H.; Smith, G.L. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. eLife 2012, 1, e00047. [CrossRef]
8. Malu, S.; Malshetty, V.; Francis, D.; Cortes, P. Role of non-homologous end joining in V(D)J recombination. *Immunol. Res.* 2012, 54, 233–246. [CrossRef]

9. Zhang, S.; Schlott, B.; Görlich, M.; Grosse, F. DNA-dependent protein kinase (DNA-PK) phosphorylates nuclear DNA helicase II/rDNA helicase A and hnRNPs proteins in an RNA-dependent manner. *Nucleic Acids Res.* 2004, 32, 1–10. [CrossRef]

10. Knyazhanskaya, E.S.; Anisenko, A.; Shadrina, O.; Kalinina, A.; Zatsepin, T.; Zalyskoy, A.; Mazurov, D.; Gottikh, M. NHEJ pathway is involved in post-integrational DNA repair due to Ku70 binding to HIV-1 integrase. *Retrovirology* 2019, 16, 30. [CrossRef]

11. Skalka, A.M.; Katz, R.A. Retroviral DNA integration and the DNA damage response. *Cell Death Differ.* 2005, 12, 971–978. [CrossRef] [PubMed]

12. Anisenko, A.N.; Knyazhanskaya, E.S.; Isaguliants, M.G.; Gottikh, M.B. A qPCR assay for measuring the post-integrational DNA repair in HIV-1 replication. *J. Virol. Methods* 2018, 262, 12–19. [CrossRef] [PubMed]

13. Bunch, H.; Zheng, X.; Burkholder, A.; Dillon, S.T.; Motola, S.; Birrane, G.; Ebmeier, C.C.; Levine, S.S.; Fargo, D.; Hu, G.; et al. TRIM28 regulates RNA polymerase II promoter-proximal pausing and pause release. *Nat. Struct. Mol. Biol.* 2014, 21, 876–883. [CrossRef] [PubMed]

14. Zicari, S.; Sharma, A.L.; Sahu, G.; Dubrovsky, I.; Sun, L.; Yue, H.; Jada, T.; Ochem, A.; Simon, G.; Bukrinsky, M.; et al. DNA dependent protein kinase (DNA-PK) enhances HIV transcription by promoting RNA polymerase II activity and recruitment of transcription machinery at HIV LTR. *Oncotarget* 2020, 11, 699–726. [CrossRef] [PubMed]

15. Dynan, W.S.; Yoo, S. Interaction of Ku protein and DNA-dependent protein kinase catalytic subunit with nucleic acids. *Nucleic Acids Res.* 1998, 26, 1551–1559. [CrossRef] [PubMed]

16. Kotula, E.; Faigle, W.; Berthault, N.; Dingli, F.; Loew, D.; Sun, J.-S.; Dutreix, M.; Quanz, M. DNA-PK Target Identification Reveals Novel Links between DNA Repair Signaling and Cytoskeletal Regulation. *PLoS ONE* 2013, 8, e80313. [CrossRef]

17. Shrivastav, M.; De Haro, L.P.; Nickoloff, J.A. Regulation of DNA double-strand break repair pathway choice. *Cell Res.* 2008, 18, 134–147. [CrossRef]

18. Neal, J.A.; Dang, V.; Douglas, P.; Wold, M.S.; Lees-Miller, S.P.; Meek, K. Inhibition of Homologous Recombination by DNA-Dependent Protein Kinase Requires Kinase Activity, Is Titratable, and Is Modulated by Autophosphorylation. *Mol. Cell. Biol.* 2011, 31, 1719–1733. [CrossRef]

19. Zhou, Y.; Lee, J.-H.; Jiang, W.; Crowe, J.L.; Zha, S.; Paull, T.T. Regulation of the DNA Damage Response by DNA-PKcs Inhibitory Phosphorylation of ATM. *Mol. Cell* 2017, 65, 91–104. [CrossRef]

20. Shao, Z.; Davis, A.J.; Fattah, K.R.; So, S.; Sun, J.; Lee, K.-J.; Harrison, L.; Yang, J.; Chen, D.J. Persistently bound Ku at DNA ends attenuates DNA end resection and homologous recombination. *DNA Repair* 2012, 11, 310–316. [CrossRef]

21. Britton, S.; Coates, J.; Jackson, S.P. A new method for high-resolution imaging of Ku foci to decipher mechanisms of DNA double-strand break repair. *J. Cell Biol.* 2013, 202, 579–595. [CrossRef] [PubMed]

22. West, R.B.; Yaneva, M.; Lieber, M.R. Productive and Nonproductive Complexes of Ku and DNA-Dependent Protein Kinase at DNA Termini. *Mol. Cell. Biol.* 1998, 18, 5908–5920. [CrossRef] [PubMed]

23. Davis, A.J.; Chen, D.J. DNA double strand break repair via non-homologous end-joining. *Transl. Cancer Res.* 2013, 2, 130–143. [PubMed]

24. Scully, R.; Xie, A. Double strand break repair functions of histone H2AX. *Mutat. Res. Fundam. Mol. Mech. Mutagen.* 2013, 750, 5–14. [CrossRef] [PubMed]

25. Wu, Q. Structural mechanism of DNA-end synapsis in the non-homologous end joining pathway for repairing double-strand breaks: Bridge over troubled ends. *Biochem. Soc. Trans.* 2019, 47, 1609–1619. [CrossRef] [PubMed]

26. Jette, N.; Lees-Miller, S.P. The DNA-dependent protein kinase: A multifunctional protein kinase with roles in DNA double strand break repair and mitosis. *Prog. Biophys. Mol. Biol.* 2015, 117, 194–205. [CrossRef]

27. Chang, H.H.Y.; Pannunzio, N.R.; Adachi, N.; Lieber, M.R. Non-homologous DNA end joining and alternative pathways to double-strand break repair. *Nat. Rev. Mol. Cell Biol.* 2017, 18, 495–506. [CrossRef]

28. Dempsey, A.; Bowie, A.G. Innate immune recognition of DNA: A recent history. *Virology* 2015, 146–152. [CrossRef]
29. Scuttis, S.R.; Ember, S.W.; Ren, H.; Ye, C.; Lovejoy, C.A.; Mazzon, M.; Veyer, D.L.; Sumner, R.P.; Smith, G.L. DNA-PK Is Targeted by Multiple Vaccinia Virus Proteins to Inhibit DNA Sensing. Cell Rep. 2018, 25, 1953–1965. [CrossRef]

30. Burleigh, K.; Maltbaek, J.H.; Cambier, S.; Green, R.; Gale, M.; James, R.G.; Stetson, D.B. Human DNA-PK activates a STING-independent DNA sensing pathway. Sci. Immunol. 2020, 5, eaba4219. [CrossRef]

31. Lamaa, A.; Le Bras, M.; Skuli, N.; Britton, S.; Frit, P.; Calsou, P.; Prats, H.; Cammas, A.; Millevoi, S. A novel cytoprotective function for the DNA repair protein Ku in regulating p53 mRNA translation and function. EMBO Rep. 2016, 17, 508–518. [CrossRef] [PubMed]

32. Mohiuddin, I.S.; Kang, M.H. DNA-PK as an Emerging Therapeutic Target in Cancer. Front. Oncol. 2019, 9, 635. [CrossRef] [PubMed]

33. Douglas, P.; Sapkota, G.P.; Morrice, N.; Yu, Y.; Goodarzi, A.A.; Merkle, D.; Meek, K.; Alessi, D.R.; Lees-Miller, S.P. DNA-Dependent Protein Kinase Phosphorylation Sites in XRCC4 are not required for survival after radiation or for V(D)J recombination. FEBS Lett. 2000, 478, 67–71. [CrossRef]

34. Chan, D.W.; Ye, R.; Veillette, C.J.; Lees-Miller, S.P. DNA-Dependent Protein Kinase Phosphorylation Sites in Ku 70/80 Heterodimer. Biochemistry 1999, 38, 1819–1828. [CrossRef] [PubMed]

35. Chen, B.P.; Chan, D.W.; Kobayashi, J.; Burma, S.; Asaithamby, A.; Morotomi-Yano, K.; Botvinick, E.; Qin, J.; Chen, D.J. Cell Cycle Dependence of DNA-dependent Protein Kinase Phosphorylation in Response to DNA Double Strand Breaks. J. Biol. Chem. 2005, 280, 14709–14715. [CrossRef]

36. Ma, Y.; Pannicke, U.; Lu, H.; Niwollik, D.; Schwarz, K.; Lieber, M.R. The DNA-dependent Protein Kinase Catalytic Subunit Phosphorylation Sites in Human Artemis. J. Biol. Chem. 2005, 280, 33839–33846. [CrossRef]

37. Karmakar, P.; Piotrowski, J.; Brosh, R.M.; Sommers, J.A.; Miller, S.P.L.; Cheng, W.H.; Snowden, C.M.; Ramsden, D.A.; Bohr, V.A. Werner Protein Is a Target of DNA-dependent Protein Kinase In Vivo and In Vitro, and Its Catalytic Activities Are Regulated by Phosphorylation. J. Biol. Chem. 2002, 277, 18291–18302. [CrossRef]

38. Kusumoto-Matsuo, R.; Ghosh, D.; Karmakar, P.; May, A.; Ramsden, D.; Bohr, V.A. Serines 440 and 467 in the Werner syndrome protein are phosphorylated by DNA-PK and a Ku 70 heterodimer. J. Biol. Chem. 2002, 277, 508–518. [CrossRef] [PubMed]

39. Leber, R.; Wise, T.W.; Mizuta, R.; Meek, K. The XRCC4 Gene Product Is a Target for and Interacts with the DNA-dependent Protein Kinase. J. Biol. Chem. 1998, 273, 1794–1801. [CrossRef] [PubMed]

40. Emberton, R.J.G.; Artemis Repair Protein. J. Biol. Chem. 2005, 280, 779–789. [CrossRef] [PubMed]

41. Critchlow, S.E.; Bowater, R.P.; Jackson, S.P. Mammalian DNA double-strand break repair protein XRCC4 interacts with DNA ligase IV. Curr. Biol. 1997, 7, 588–598. [CrossRef]

42. Moghani, A.R.A.; Jackson, S.P. DNA-dependent Protein Kinase Catalytic Subunit Phosphorylation Sites in Human Artemis. J. Biol. Chem. 1999, 274, 1819–1828. [CrossRef] [PubMed]

43. Dragoi, A.M.; Fu, X.; Zhang, P.; Sheng, L.; Wu, D.; Li, G.C.; Chu, W.M. DNA-PKcs, but not TLR9, is required for activation of Akt by CpG-DNA. EMBO J. 2005, 24, 779–789. [CrossRef]

44. Tu, Y.; Ji, C.; Yang, B.; Yang, Z.; Gu, H.; Lu, C.C.; Wang, R.; Su, Z.L.; Chen, B.; Sun, W.L.; et al. DNA-dependent protein kinase catalytic subunit (DNA-PKcs)-SIN1 association mediates ultraviolet B (UVB)-induced Akt Ser-473 phosphorylation and skin cell survival. Mol. Cancer 2013, 12, 1–12. [CrossRef] [PubMed]

45. Yavuzer, U.; Smith, G.C.; Bliss, T.; Werner, D.; Jackson, S.P. DNA end-independent activation of DNA-PK mediated via association with the DNA-binding protein C1D. Genes Dev. 1998, 12, 2188–2199. [CrossRef] [PubMed]

46. Goodarzi, A.A.; Yu, Y.; Riballo, E.; Douglas, P.; Walker, S.A.; Ye, R.; Härer, C.; Marchetti, C.; Morrice, N.; Jeggo, P.A.; et al. DNA-PK autophosphorylation facilitates Artemis endonuclease activity. EMBO J. 2006, 25, 3880–3889. [CrossRef]

47. Soubeyrand, S.; Pope, L.; De Chasseval, R.; Gosselin, D.; Dong, F.; De Villartay, J.-P.; Haché, R.J.G. Artemis Phosphorylated by DNA-dependent Protein Kinase Associates Preferentially with Discrete Regions of Chromatin. J. Mol. Biol. 2006, 358, 1200–1211. [CrossRef]
49. Ting, N.S.Y.; Kao, P.N.; Chan, D.W.; Lintott, L.G.; Lees-Miller, S.P. DNA-dependent protein kinase interacts with antigen receptor response element binding proteins NP90 and NF45. J. Biol. Chem. 1998, 273, 2136–2145. [CrossRef]
50. Deng, Q.; Holler, C.J.; Taylor, G.; Hudson, K.F.; Watkins, W.; Gearing, M.; Ito, D.; Murray, M.E.; Dickson, D.W.; Seyfried, N.T.; et al. FUS is Phosphorylated by DNA-PK and Accumulates in the Cytoplasm after DNA Damage. J. Neurosci. 2014, 34, 7802–7813. [CrossRef]
51. Farber-Katz, S.E.; Dippold, H.C.; Buschman, M.D.; Peterman, M.C.; Xing, M.; Noakes, C.J.; Tat, J.; Ng, M.M.; Rahajeng, J.; Cowan, D.M.; et al. DNA Damage Triggers Golgi Dispersal via DNA-PK and GOLPH3. Cell 2014, 156, 413–427. [CrossRef] [PubMed]
52. Chibazakura, T.; Watanabe, F.; Kitajima, S.; Tsukada, K.; Yasukochi, Y.; Teraoka, H. Phosphorylation of human general transcription factors TATA-binding protein and transcription factor IIB by DNA-dependent protein kinase—Synergistic stimulation of RNA polymerase II basal transcription in vitro. JBC J. Biol. Inorg. Chem. 1997, 247, 1166–1173. [CrossRef] [PubMed]
53. Park, E.-J.; Chan, D.W.; Park, J.-H.; Oettinger, M.A.; Kwon, J. DNA-PK is activated by nucleosomes and phosphorylates H2AX within the nucleosomes in an acetylation-dependent manner. Nucleic Acids Res. 2003, 31, 6819–6827. [CrossRef] [PubMed]
54. Rogakou, E.P.; Pilch, D.R.; Orr, A.H.; Ivanova, V.S.; Bonner, W.M. DNA Double-stranded Breaks Induce Histone H2AX Phosphorylation on Serine 139. J. Biol. Chem. 1998, 273, 5858–5868. [CrossRef] [PubMed]
55. An, J.; Huang, Y.C.; Xu, Q.Z.; Zhou, L.J.; Shang, Z.F.; Huang, B.; Wang, Y.; Liu, X.D.; Wu, D.C.; Zhou, P.K. DNA-PKcs plays a dominant role in the regulation of H2AX phosphorylation in response to DNA damage and cell cycle progression. BMC Mol. Biol. 2010, 11, 18. [CrossRef]
56. Koike, M.; Sugawara, J.; Yasuda, M.; Koike, A. Tissue-specific DNA-PK-dependent H2AX phosphorylation and γ-H2AX elimination after X-irradiation in vivo. Biochem. Biophys. Res. Commun. 2008, 367, 52–55. [CrossRef]
57. Wang, H.; Wang, M.; Wang, H.; Böcker, W.; Iliakis, G. Complex H2AX phosphorylation patterns by multiple kinases including ATM and DNA-PK in human cells exposed to ionizing radiation and treated with kinase inhibitors. J. Cell. Physiol. 2005, 202, 492–502. [CrossRef]
58. Reitsma, T.; Klokov, D.; Banáth, J.P.; Olive, P.L. DNA-PK is responsible for enhanced phosphorylation of histone H2AX under hypertonic conditions. DNA Repair 2005, 4, 1172–1181. [CrossRef]
59. Sui, J.; Lin, Y.-F.; Xu, K.; Lee, K.-J.; Wang, D.; Chen, B.P. DNA-PKcs phosphorylates hnRNP-A1 to facilitate the RPA-to-POT1 switch and telomere capping after replication. Nucleic Acids Res. 2015, 43, 5971–5983. [CrossRef]
60. Berglund, F.M.; Clarke, P.R. hnRNP-U is a specific DNA-dependent protein kinase substrate phosphorylated in response to DNA double-strand breaks. Biochem. Biophys. Res. Commun. 2009, 381, 59–64. [CrossRef]
61. Britton, S.; Froment, C.; Frit, P.; Monsarrat, B.; Salles, B.; Calsou, P. Cell nonhomologous end joining capacity
62. Walker, A.; Hunt, T.; Jackson, R.; Anderson, C. Double-stranded DNA induces the phosphorylation of several proteins including the 90 000 mol. wt. heat-shock protein in animal cell extracts. EMBO J. 1985, 4, 139–145. [CrossRef] [PubMed]
63. Quanz, M.; Herbette, A.; Sayarath, M.; De Koninck, L.; Dubois, T.; Sun, J.-S.; Dutreix, M. Heat Shock Protein 90α (Hsp90α) Is Phosphorylated in Response to DNA Damage and Accumulates in Repair Foci. J. Biol. Chem. 2012, 287, 8803–8815. [CrossRef] [PubMed]
64. Lees-Miller, S.P.; Anderson, C.W. The human double-stranded DNA-activated protein kinase phosphorylates the 90-kDa heat-shock protein, hsp90 alpha at two NH2-terminal threonine residues. J. Biol. Chem. 1989, 264, 17275–17280.
65. Bannister, A.J.; Gottlieb, T.M.; Kouzarides, T.; Jackson, S.P. c-Jun is phosphorylated by the DNA-dependent protein kinase in vitro; definition of the minimal kinase recognition motif. Nucleic Acids Res. 1993, 21, 1289–1295. [CrossRef]
66. Mayo, L.D.; Turchi, J.J.; Berberich, S.J. Mdm-2 phosphorylation by DNA-dependent protein kinase prevents interaction with p53. Cancer Res. 1997, 57, 5013–5016.
67. Iijima, S.; Teraoka, H.; Date, T.; Tsukada, K. DNA-activated protein kinase in Raji Burkitt’s lymphoma cells. Eur. J. Biochem. 1992, 206, 595–603. [CrossRef]
68. Liu, L.; Kwak, Y.-T.; Bex, F.; Garcia-Martinez, L.F.; Li, X.-H.; Meek, K.; Lane, W.S.; Gaynor, R.B. DNA-Dependent Protein Kinase Phosphorylation of IxBal and IxBβ Regulates NF-xB DNA Binding Properties. *Mol. Cell. Biol.* 1998, 18, 4221-4234. [CrossRef]

69. Yu, Y.; Mahaney, B.L.; Yano, K.-I.; Ye, R.; Fang, S.; Douglas, P.; Chen, D.J.; Lees-Miller, S.P. DNA-PK and ATM phosphorylation sites in XLF/Cernunnos are not required for repair of DNA double strand breaks. *DNA Repair* 2008, 7, 1680-1692. [CrossRef]

70. Malewicz, M.; Kadkhodaei, B.; Lee, D.; Myung, K. DNA-PK-Dependent RPA2 Hyperphosphorylation Facilitates DNA Repair. *Analyst* 2009, 134, 999-1003. [CrossRef] [PubMed]

71. Sastre-Moreno, G.; Pryor, J.M.; Moreno-Oñate, M.; Herrero-Ruiz, A.M.; Cortés-Ledesma, F.; Blanco, L.; Ramsden, D.A.; Ruiz, J.F. Regulation of human polα by ATM-mediated phosphorylation during non-homologous end joining. *DNA Repair* 2017, 51, 31-45. [CrossRef] [PubMed]

72. Block, W.D.; Yu, Y.; Lees-Miller, S.P. Phosphatidyl inositol 3-kinase-like serine/threonine protein kinases (PIKKs) are required for DNA damage-induced phosphorylation of the 32 kDa subunit of replication protein A at threonine 21. *Nucleic Acids Res.* 2004, 32, 997-1005. [CrossRef]

73. Schild-Poulter, C.; Shih, A.; Yarymowich, N.C.; Hachè, R.J.G. Down-regulation of histone H2B by DNA-dependent protein kinase in response to DNA damage through modulation of octamer transcription factor 1. *Cancer Res.* 2003, 63, 7197-7205. [PubMed]

74. Liu, S.H.; Ma, J.T.; Yueh, A.Y.; Lees-Miller, S.P.; Anderson, C.W.; Ng, S.Y. The carboxyl-terminal transactivation domain of human serum response factor contains DNA-activated protein kinase phosphorylation sites. *EMBO J.* 1992, 11, 325-334. [CrossRef]

75. Komiyama, S.; Taniguchi, S.; Matsumoto, Y.; Tsunoda, E.; Ohto, T.; Suzuki, Y.; Yin, H.L.; Tomita, M.; Lewensohn, R.; Van Gent, D.C.; et al. Essential role for DNA-PK-mediated phosphorylation of NR4A nuclear orphan receptors in DNA double-strand break repair. *Genes Dev.* 2011, 25, 2031–2040. [CrossRef]

76. Zolner, A.E.; Abdou, I.; Ye, R.; Mani, R.S.; Fanta, M.; Yu, Y.; Douglas, P.; Tahbaz, N.; Fang, S.; Dobbs, T.; et al. Phosphorylation of polynucleotide kinase/phosphatase by DNA-dependent protein kinase and ataxia-telangiectasia mutated regulates its association with sites of DNA damage. *Nucleic Acids Res.* 2011, 39, 9224–9237. [CrossRef] [PubMed]

77. Lees-Miller, S.P.; Sakaguchi, K.; Ullrich, S.J.; Appella, E.; Anderson, C.W. Human DNA-activated protein kinase phosphorylates serines 15 and 37 in the amino-terminal transactivation domain of human p53. *Mol. Cell. Biol.* 1992, 12, 5041–5049. [CrossRef] [PubMed]

78. Jackson, S.P.; Macdonald, J.J.; Lees-Miller, S.; Tjian, R. GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. *Cell* 1990, 63, 155–165. [CrossRef]

79. Liu, S.H.; Ma, J.T.; Yueh, A.Y.; Lees-Miller, S.P.; Anderson, C.W.; Ng, S.Y. The carboxyl-terminal transactivation domain of human serum response factor contains DNA-activated protein kinase phosphorylation sites. *J. Biol. Chem.* 1993, 268, 21147–21154. [PubMed]

80. Komiyama, S.; Taniguchi, S.; Matsumoto, Y.; Tsunoda, E.; Ohto, T.; Suzuki, Y.; Yin, H.L.; Tomita, M.; Enomoto, A.; Morita, A.; et al. Potentiality of DNA-dependent protein kinase to phosphorylate Ser46 of human p53. *Biochem. Biophys. Res. Commun.* 2004, 323, 816–822. [CrossRef] [PubMed]

81. Komiyama, S.; Taniguchi, S.; Matsumoto, Y.; Tsunoda, E.; Ohto, T.; Suzuki, Y.; Yin, H.L.; Tomita, M.; Enomoto, A.; Morita, A.; et al. Potentiality of DNA-dependent protein kinase to phosphorylate Ser46 of human p53. *Biochem. Biophys. Res. Commun.* 2004, 323, 816–822. [CrossRef] [PubMed]

82. Malewicz, M.; Kadkhodaei, B.; Lee, D.; Myung, K. DNA-PK-Dependent RPA2 Hyperphosphorylation Facilitates DNA Repair and Suppresses Sister Chromatid Exchange. *Proc. Natl. Acad. Sci. USA* 1993, 90, 4221–4234. [CrossRef]

83. Sastre-Moreno, G.; Pryor, J.M.; Moreno-Oñate, M.; Herrero-Ruiz, A.M.; Cortés-Ledesma, F.; Blanco, L.; Ramsden, D.A.; Ruiz, J.F. Regulation of human polα by ATM-mediated phosphorylation during non-homologous end joining. *DNA Repair* 2017, 51, 31-45. [CrossRef] [PubMed]

84. Shieh, S.-Y.; Ikeda, M.; Taya, Y.; Prives, C. DNA Damage-Induced Phosphorylation of p53 Alleviates Inhibition of site-specific phosphorylation of p53 protein and its interaction with MDM2 protein. *Analyst* 2019, 144, 6033–6040. [CrossRef]

85. Shieh, S.-Y.; Ikeda, M.; Taya, Y.; Prives, C. DNA Damage-Induced Phosphorylation of p53 Alleviates Inhibition of site-specific phosphorylation of p53 protein and its interaction with MDM2 protein. *Analyst* 2019, 144, 6033–6040. [CrossRef]

86. Tomimatsu, N.; Mukherjee, B.; Burma, S. Distinct roles of ATR and DNA-PKcs in triggering DNA damage responses in ATM-deficient cells. *EMBO Rep.* 2009, 10, 629–635. [CrossRef]
87. Bunch, H.; Lawney, B.P.; Lin, Y.-F.; Asaithamby, A.; Murshid, A.; Wang, Y.E.; Chen, B.P.C.; Calderwood, S.K. Transcriptional elongation requires DNA break-induced signalling. Nat. Commun. 2015, 6, 1–12. [CrossRef]

88. Yannone, S.M.; Roy, S.; Chan, D.W.; Murphy, M.B.; Huang, S.; Campisi, J.; Chen, D.J. Werner syndrome protein is regulated and phosphorylated by DNA-dependent protein kinase. J. Biol. Chem. 2001, 276, 38242–38248. [CrossRef]

89. Douglas, P.; Gupta, S.; Morrice, N.; Meek, K.; Lees-Miller, S.P. DNA-PK-dependent phosphorylation of Ku70/80 is not required for non-homologous end joining. DNA Repair 2005, 4, 1006–1018. [CrossRef] [PubMed]

90. Podhorecka, M.; Skladanowski, A.; Bozko, P. H2AX Phosphorylation: Its Role in DNA Damage Response and Cancer Therapy. J. Nucleic Acids 2010, 2010, 920161. [CrossRef]

91. DAVID Functional Annotation Bioinformatics Microarray Analysis. Available online: https://david.ncifcrf.gov (accessed on 28 March 2020).

92. STRING: Functional Protein Association Networks. Available online: https://string-db.org (accessed on 28 March 2020).

93. Ju, B.-G.; Lunyak, V.V.; Perissi, V.; Garcia-Bassets, I.; Rose, D.W.; Glass, C.K.; Rosenfeld, M.G. A Topoisomerase IIβ-Mediated dsDNA Break Required for Regulated Transcription. Science 2006, 312, 1798–1802. [CrossRef] [PubMed]

94. Bunch, H. Role of genome guardian proteins in transcriptional elongation. FEBS Lett. 2016, 590, 1064–1075. [CrossRef]

95. Rahl, P.B.; Lin, C.Y.; Seila, A.C.; Flynn, R.A.; McCuine, S.; Burge, C.B.; Sharp, P.A.; Young, R.A. c-Myc Regulates Transcriptional Pause Release. Cell 2010, 141, 432–445. [CrossRef] [PubMed]

96. Haffner, M.C.; De Marzo, A.M.; Meeker, A.K.; Nelson, W.G.; Yegnasubramanian, S. Transcription-induced DNA double strand breaks: Both oncogenic force and potential therapeutic target? Clin. Cancer Res. 2011, 17, 3858–3864. [CrossRef] [PubMed]

97. Pankotai, T.; Bonhomme, C.; Chen, D.; Soutoglou, E. DNAPKcs-dependent arrest of RNA polymerase II transcription in the presence of DNA breaks. Nat. Struct. Mol. Biol. 2012, 19, 276–282. [CrossRef]

98. Iannelli, F.; Galbiati, A.; Capozzo, I.; Nguyen, Q.; Magnuson, B.; Michelini, F.; D’Alessandro, G.; Cabrini, M.; Roncador, M.; Francia, S.; et al. A damaged genome’s transcriptional landscape through multilayered expression profiling around in situ-mapped DNA double-strand breaks. Nat. Commun. 2017, 8, 15656. [CrossRef]

99. Janssens, S.; Tschopp, J. Signals from within: The DNA-damage-induced NF-kappaB response. Cell Death Diﬀer. 2006, 13, 773–784. [CrossRef]

100. Sutton, J.; Dotson, D.; Dong, X. Molecular Events in Late Stages of HIV-1 Replication. JSM Microbiol. 2013, 1, 1004.

101. Daniel, R.; Katz, R.A.; Merkel, G.; Hittle, J.C.; Yen, T.J.; Skalka, A.M. Wortmannin Potentiates Integrase-Mediated Killing of Lymphocytes and Reduces the Efficiency of Stable Transduction by Retroviruses. Mol. Cell. Biol. 2001, 21, 1164–1172. [CrossRef]

102. Daniel, R.; Katz, R.A.; Skalka, A.M. A Role for DNA-PK in Retroviral DNA Integration. Science 1999, 284, 644–647. [CrossRef]

103. Daniel, R.; Greger, J.G.; Katz, R.A.; Taganov, K.D.; Wu, X.; Kappes, J.C.; Skalka, A.M. Evidence that Stable Retroviral Transduction and Cell Survival following DNA Integration Depend on Components of the Nonhomologous End Joining Repair Pathway. J. Virol. 2004, 78, 8573–8581. [CrossRef] [PubMed]

104. Jeanson, L.; Subra, F.; Vaganay, S.; Hervy, M.; Marangoni, E.; Bourhis, J.; Mouscadet, J.F. Effect of Ku80 Depletion on the Preintegrative Steps of HIV-1 Replication in Human Cells. Virology 2002, 300, 100–108. [CrossRef]

105. Sakurai, Y.; Komatsu, K.; Agematsu, K.; Matsuoka, M. DNA double strand break repair enzymes function at multiple steps in retroviral infection. Retrovirology 2009, 6, 114. [CrossRef] [PubMed]

106. Manic, G.; Maurin-Marlin, A.; Laurent, F.; Vitale, I.; Thierry, S.; Delelis, O.; Dessen, P.; Vincendeau, M.; Leib-Mösch, C.; Hazan, U.; et al. Impact of the Ku Complex on HIV-1 Expression and Latency. PLoS ONE 2013, 8, e69691. [CrossRef] [PubMed]
108. Lau, A.; Swinbank, K.M.; Ahmed, P.S.; Taylor, D.L.; Jackson, S.P.; Smith, G.C.M.; O'Connor, M.J. Suppression of HIV-1 infection by a small molecule inhibitor of the ATM kinase. Nat. Cell Biol. 2005, 7, 493–500. [CrossRef]

109. Yang, J.; Yu, Y.; Hamrick, H.E.; Duersken-Hughes, P.J. ATM, ATR and DNA-PK: Initiators of the cellular genotoxic stress responses. Carcinogenesis 2003, 24, 1571–1580. [CrossRef]

110. Smith, J.A.; Wang, F.X.; Zhang, H.; Wu, K.J.; Williams, K.J.; Daniel, R. Evidence that the Nijmegen breakage syndrome protein, an early sensor of double-strand DNA breaks (DSB), is involved in HIV-1 post-integration repair by recruiting the ataxia telangiectasia-mutated kinase in a process similar to, but distinct from, cellular DSB repair. Virol. J. 2008, 5, 11. [CrossRef]

111. Borgatti, P.; Zauli, G.; Colamussi, M.L.; Gibellini, D.; Previati, M.; Capitani, S. Extracellular HIV-1 Tat protein activates phosphatidylinositol 3- and Akt/PKB kinases in CD4+ T lymphoblastoid Jurkat cells. Eur. J. Immunol. 1997, 27, 2805–2811. [CrossRef]

112. Zhou, H.; Xu, M.; Huang, Q.; Gates, A.T.; Zhang, X.D.; Castle, J.C.; Stec, E.; Ferrer, M.; Strulovici, B.; Hazuda, D.J.; et al. Genome-Scale RNAi Screen for Host Factors Required for HIV Replication. Cell Host Microbe 2008, 4, 495–504. [CrossRef]

113. Dehart, J.L.; Andersen, J.L.; Zimmerman, E.S.; Ardon, O.; An, D.S.; Blackett, J.; Kim, B.; Pianelles, V. The Ataxia Telangiectasia-Mutated and Rad3-Related Protein Is Dispensable for Retroviral Integration. J. Virol. 2005, 79, 1389–1396. [CrossRef] [PubMed]

114. Espeseth, A.S.; Fishef, R.; Hazuda, D.; Huang, Q.; Xu, M.; Yoder, K.; Zhou, H. siRNA Screening of a Targeted Library of DNA Repair Factors in HIV Infection Reveals a Role for Base Excision Repair in HIV Integration. PLoS ONE 2011, 6, e17612. [CrossRef] [PubMed]

115. Brady, S.; Singh, G.; Bolinger, C.; Song, Z.; Boeras, I.; Weng, K.; Trent, B.; Brown, W.C.; Singh, K.; Boris-Lawrie, K.; et al. Virion-associated, host-derived DHX9/RNA helicase A enhances the processivity of HIV-1 reverse transcriptase on genomic RNA. J. Biol. Chem. 2019, 294, 11473–11485. [CrossRef] [PubMed]

116. Boeras, I.; Song, Z.; Moran, A.; Franklin, J.; Brown, W.C.; Johnson, M.; Boris-Lawrie, K.; Heng, X. DHX9/RHA Binding to the PBS-Segment of the Genomic RNA during HIV-1 Assembly Bolsters Virion Infectivity. J. Mol. Biol. 2016, 428, 2418–2429. [CrossRef]

117. Ndzinu, J.K.; Takeuchi, H.; Saito, H.; Yoshida, T.; Yamaoka, S. eIF4A2 is a host factor required for efficient HIV-1 replication. Microbes Infect. 2018, 20, 346–352. [CrossRef] [PubMed]

118. Krasnopol’sky, S.; Marom, L.; Victor, R.A.; Fujinaga, K.; Taube, R. Fused in sarcoma silences HIV gene transcription and maintains viral latency through suppressing AFF4 gene activation. Retrovirology 2019, 16, 16. [CrossRef]

119. Daniel, R.; Ramcharan, J.; Rogakou, E.; Taganov, K.D.; Greger, J.G.; Bonner, W.; Nussenweig, A.; Katz, R.A.; Skalka, A.M. Histone H2AX Is Phosphorylated at Sites of Retroviral DNA Integration but Is Dispensable for Postintegration Repair. J. Biol. Chem. 2004, 279, 45810–45814. [CrossRef]

120. Monette, A.; Ajamian, L.; López-Lastra, M.; Mouland, A.J. Human Immunodeficiency Virus Type 1 (HIV-1) Induces the Cytoplasmic Retention of Heterogeneous Nuclear Ribonucleoprotein A1 by Disrupting Nuclear Import: Implications for HIV-1 gene expression. J. Biol. Chem. 2009, 284, 31350–31362. [CrossRef]

121. Mizutani, T.; Ishizaka, A.; Suzuki, Y.; Iba, H. 7SK small nuclear ribonucleoprotein complex is recruited to the HIV-1 promoter via short viral transcripts. FEBS Lett. 2014, 588, 1630–1636. [CrossRef]

122. Low, J.S.; Fassati, A. Hsp90: A chaperone for HIV-1. Parasitology 2014, 141, 1192–1202. [CrossRef]

123. Van Der Sluis, R.M.; Derking, R.; Breidel, S.; Speijer, D.; Berkhout, B.; Jeeninga, R.E. Interplay between viral Tat protein and c-Jun transcription factor in controlling LTR promoter activity in different human immunodeficiency virus type I subtypes. J. Gen. Virol. 2014, 95, 968–979. [CrossRef] [PubMed]

124. Breton, Y.; Desrosiers, V.; Ouellet, M.; Deshiere, A.; Torresilla, C.; Cohen, É.A.; Tremblay, M.J. Expression of MDM2 in Macrophages Promotes the Early Postentry Steps of HIV-1 Infection through Inhibition of p53. J. Virol. 2009, 83, 6176–6185. [CrossRef] [PubMed]

125. Izumi, T.; Takaori-Kondo, A.; Shirakawa, K.; Higashitsuji, H.; Itoh, K.; Io, K.; Matsui, M.; Iwai, K.; Kondoh, H.; Sato, T.; et al. MDM2 is a novel E3 ligase for HIV-1 Vif. Retrovirology 2009, 6, 1. [CrossRef] [PubMed]

126. Bros, V.; Kiernan, R.E.; Linares, L.K.; Chable-Bessia, C.; Plechakova, O.; Treand, C.; Emiliani, S.; Peloponese, J.-M.; Jeang, K.-T.; Coux, O.; et al. A non-proteolytic role for ubiquitin in Tat-mediated transactivation of the HIV-1 promoter. Nat. Cell Biol. 2003, 5, 754–761. [CrossRef]

127. Sun, Y.; Clark, E.C. Expression of the c-myc Proto-oncogene Is Essential for HIV-1 Infection in Activated T Cells. J. Exp. Med. 1999, 189, 1391–1398. [CrossRef]
128. Jiang, G.; Espeseth, A.; Hazuda, D.J.; Margolis, D.M. c-Myc and Sp1 Contribute to Proviral Latency by Recruiting Histone Deacetylase 1 to the Human Immunodeficiency Virus Type 1 Promoter. *J. Virol.* 2007, 81, 10914–10923. [CrossRef]

129. Fernandez, G.; Zaikos, T.D.; Khan, S.Z.; Jacobi, A.M.; Behlke, M.A.; Zeichner, S.L. Targeting IxB Proteins for HIV Latency Activation: The Role of Individual IxB and NF-xB Proteins. *J. Virol.* 2013, 87, 3966–3978. [CrossRef]

130. Kameoka, M.; Nukuzuma, S.; Itaya, A.; Tanaka, Y.; Ota, K.; Ikuta, K.; Yoshikawa, K. RNA Interference Directed against Poly(ADP-Ribose) Polymerase 1 Efficiently Suppresses Human Immunodeficiency Virus Type 1 Replication in Human Cells. *J. Virol.* 2004, 78, 8931–8934. [CrossRef]

131. Kameoka, M.; Nukuzuma, S.; Itaya, A.; Tanaka, Y.; Ota, K.; Inada, Y.; Ikuta, K.; Yoshikawa, K. Poly(ADP-ribose)polymerase-1 is required for integration of the human immunodeficiency virus type 1 genome near centromeric alphoid DNA in human and murine cells. *Biochem. Biophys. Res. Commun.* 2005, 334, 412–417. [CrossRef]

132. Gäken, J.A.; Tavassoli, M.; Gan, S.U.; Vallian, S.; Giddings, I.; Darling, D.C.; Galea-Lauri, J.; Thomas, M.G.; Abedi, H.; Schreiber, V.; et al. Efficient retroviral infection of mammalian cells is blocked by inhibition of poly(ADP-ribose) polymerase activity. *J. Virol.* 1996, 70, 3992–4000. [CrossRef]

133. Ha, H.C.; Juluri, K.; Zhou, Y.; Leung, S.; Hermankova, M.; Snyder, S.H. Poly(ADP-ribose) polymerase-1 is required for efficient HIV-1 integration. *Proc. Natl. Acad. Sci. USA* 2001, 98, 3364–3368. [CrossRef] [PubMed]

134. Siva, A.C.; Bushman, F.D. Poly(ADP-Ribose) Polymerase 1 Is Not Strictly Required for Infection of Murine Cells by Retroviruses. *J. Virol.* 2002, 76, 11904–11910. [CrossRef] [PubMed]

135. Ariumi, Y.; Turelli, P.; Masutani, M.; Trono, D. DNA Damage Sensors ATM, ATR, DNA-PKcs, and PARP-1 Are Required for e cient Retroviral Infection of Mammalian Cells. *Cell. Mol. Biol.* 2003, 49, 87, 383–390. [CrossRef] [PubMed]

136. Baekelandt, V.; Claeys, A.; Cherepanov, P.; De Clercq, E.; De Strooper, B.; Nuttin, B.; Debyser, Z.; Brown, A.J.L.; Precious, H.M.; Whitcomb, J.M.; et al. DNA-Dependent Protein Kinase Is Not Required for Efficient Lentivirus Integration. *J. Virol.* 2000, 74, 11278–11285. [CrossRef]

137. Yu, D.; Liu, R.; Yang, G.; Zhou, Q. The PARP1-Siah1 Axis Controls HIV-1 Transcription and Expression of Siah1 Substrates. *Cell Rep.* 2018, 23, 3741–3749. [CrossRef]

138. Bueno, M.T.D.; Reyes, D.; Valdes, L.; Saheba, A.; Urias, E.; Mendoza, C.; Fregoso, O.I.; Llano, M. Poly(ADP-Ribose) Polymerase 1 Promotes Transcriptional Repression of Integrated Retroviruses. *J. Virol.* 2013, 87, 2496–2507. [CrossRef]

139. Liu, Y.-Z.; Latchman, D.S. The octamer-binding proteins Oct-1 and Oct-2 repress the HIV long terminal repeat promoter and its transactivation by Tat. *Biochem. J.* 2013, 450, 297–307. [CrossRef]

140. Khan, S.; Iqbal, M.; Tariq, M.; Baig, S.M.; Abbas, W. Epigenetic regulation of HIV-1 latency: Focus on polycomb group (PcG) proteins. *Clin. Epigenetics* 2018, 10, 14. [CrossRef]

141. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

142. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

143. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

144. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

145. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

146. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

147. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]

148. Majello, B.; Napolitano, G.; Lania, L. Recruitment of the TATA-binding protein to the HIV-1 promoter is a limiting step for Tat transactivation. *AIDS* 1998, 12, 1957–1964. [CrossRef]
149. Ma, X.; Yang, T.; Luo, Y.; Wu, L.; Jiang, Y.; Song, Z.; Pan, T.; Liu, B.; Liu, G.; Liu, J.; et al. TRIM28 promotes HIV-1 latency by SUMOylating CDK9 and inhibiting P-TEFb. *eLife* 2019, *8*, e42426. [CrossRef]

150. Fernández-Ortega, C.; Ramirez, A.; Casillas, D.; Paneque, T.; Ubieta, R.; Dubed, M.; Navea, L.; Castellanos-Serra, L.; Duarte, C.A.; Falcón-Cama, V.; et al. Identification of Vimentin as a Potential Therapeutic Target against HIV Infection. *Viruses* 2016, *8*, 98. [CrossRef]

151. Sharma, A.; Awasthi, S.; Harrod, C.K.; Matlock, E.F.; Khan, S.; Xu, L.; Chan, S.; Yang, H.; Thammavaram, C.K.; Rasor, R.A.; et al. The Werner Syndrome Helicase Is a Cofactor for HIV-1 Long Terminal Repeat Transactivation and Retroviral Replication. *J. Biol. Chem.* 2007, *282*, 12048–12057. [CrossRef]

152. Mizutani, T.; Ishizaka, A.; Furuichi, Y. The Werner Protein Acts as a Coactivator of Nuclear Factor κB (NF-κB) on HIV-1 and Interleukin-8 (IL-8) Promoters. *J. Biol. Chem.* 2015, *290*, 18391–18399. [CrossRef]

153. Anisenko, A.N.; Gottikh, M.B. Role of Cellular DNA Repair Systems in HIV-1 Replication. *Mol. Biol.* 2019, *53*, 313–322. [CrossRef]

154. Gutiérrez, D.A.; Valdes, L.; Serrugera, C.; Llano, M. Poly(ADP-ribose) polymerase-1 silences retroviruses independently of viral DNA integration or heterochromatin formation. *J. Gen. Virol.* 2016, *97*, 1686–1692. [CrossRef] [PubMed]

155. Rom, S.; Reichenbach, N.L.; Dykstra, H.; Persidsky, Y. The dual action of poly(ADP-ribose) polymerase-1 (PARP-1) inhibition in HIV-1 infection: HIV-1 LTR inhibition and diminution in Rho GTPase activity. *Front. Microbiol.* 2015, *6*, 878. [CrossRef] [PubMed]

156. Parent, M.; Yung, T.M.C.; Rancourt, A.; Ho, E.L.Y.; Vispé, S.; Suzuki-Matsuda, E.; Uehara, A.; Wada, T.; Handa, H.; Satoh, M.S.; et al. Poly(ADP-ribose) Polymerase-1 Is a Negative Regulator of HIV-1 Transcription through Competitive Binding to TAR RNA with Tat Positive Transcription Elongation Factor b (p-TEFb) Complex. *J. Biol. Chem.* 2005, *280*, 448–457. [CrossRef]

157. Chun, R.; Semmes, O.J.; Neuveut, C.; Jeang, K.T. Modulation of Sp1 Phosphorylation by Human Immunodeficiency Virus Type 1 Tat. *J. Virol.* 1998, *72*, 2615–2629. [CrossRef] [PubMed]

158. Tyagi, S.; Ochem, A.; Tyagi, M. DNA-dependent protein kinase interacts functionally with the RNA polymerase II complex recruited at the human immunodeficiency virus (HIV) long terminal repeat and plays an important role in HIV gene expression. *J. Gen. Virol.* 2011, *92*, 1710–1720. [CrossRef] [PubMed]

159. Tan, N.Y.; Khachigian, L.M. Sp1 Phosphorylation and Its Regulation of Gene Transcription. *Mol. Cell. Biol.* 2009, *29*, 2483–2488. [CrossRef] [PubMed]

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