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DNA-Dependent Protein Kinase Defects are Linked to Deficiencies in DNA Repair and V(D)J Recombination [and Discussion]

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DNA-dependent protein kinase defects are linked to deficiencies in DNA repair and V(D)J recombination

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SUMMARY

DNA-dependent protein kinase is a nuclear serine/threonine kinase whose catalytic properties are expressed only when the enzyme is bound to DNA ends or other discontinuities in the DNA. DNA-PK comprises two components: one mediates binding to DNA and corresponds to the heterodimeric human autoimmune antigen Ku; the other, DNA-PK catalytic subunit (DNA-PK_{cs}), is a polypeptide of approximately 450 kDa. DNA-PK deficiencies are associated with certain mutant rodent cell lines that display defects in DNA double strand break repair and V(D)J recombination. Specifically, hamster *xrs-6* cells lack Ku function, whereas murine scid and hamster V3 cells lack functional DNA-PK_{cs}. Furthermore, the phenotypes of *xrs-6* and V3 cells can be corrected by the expression of the genes encoding the 80 kDa component of Ku or DNA-PK_{cs}, respectively. These results imply that DNA-PK is an important component of the DNA double strand break repair/recombination apparatus. Possible roles for DNA-PK in these processes are discussed.

1. INTRODUCTION

In recent years, several protein kinases have been shown to exist within the cell nucleus, suggesting that these enzymes may play important roles in modulating nuclear events. One such enzyme is DNA-dependent protein kinase (DNA-PK), a serine/threonine kinase that has been detected in organisms ranging from man to *Drosophila* (for a review see Finnie *et al.* 1995, also see references in Anderson & Lees-Miller 1992; Gottlieb & Jackson 1994). An important feature of DNA-PK is that it binds to DNA directly and needs to be DNA-bound to be active (Walker *et al.* 1985; Carter & Vancurova 1990; Jackson *et al.* 1990; Lees-Miller & Chen 1990). Biochemical fractionation studies have revealed that DNA-PK is a multiprotein complex comprising a large catalytic subunit (DNA-PK_{cs}) and a DNA binding component called Ku (Dvir *et al.* 1993; Finnie *et al.* 1993; Gottlieb & Jackson 1993). Ku is an antigen recognized by antisera derived from patients with various autoimmune diseases and consists of two polypeptides of approximately 70 and 80 kDa (Ku70 and Ku80, respectively) in a tightly associated 1:1 complex.

Though physiological targets for DNA-PK have not been defined unequivocally, effective substrates for DNA-PK *in vitro* include the 34 kDa subunit of DNA replication/repair factor RPA (Brush *et al.* 1994; Pan *et al.* 1994; G. Smith & S. P. Jackson, unpublished data), and transcription factors Sp1, c-Jun and p53 (Lees-Miller 1992; Bannister *et al.* 1993; Finnie *et al.* 1993; Jackson *et al.* 1993). DNA competition studies indicate that DNA-PK must bind *in cis* to the same DNA molecule as its protein substrate in order to function effectively (Gottlieb & Jackson 1993). The ability of DNA-PK to recognize several transcription factors suggests that it might play a role in transcriptional regulation. Consistent with this, DNA-PK has recently been shown to be a potent repressor of transcription by RNA polymerase I (Kuhn *et al.* 1995; Labhart 1995).

An intriguing feature of Ku is that it binds to DNA termini or other discontinuities in the DNA structure (for example, Paillard & Strauss 1991; Falzon *et al.* 1993). Consequently, DNA-PK is activated *in vitro* by linear but not by closed circular or supercoiled DNA (Carter & Vancurova 1990; Jackson *et al.* 1990, 1993; Gottlieb & Jackson 1993; Morozov *et al.* 1994). These properties suggest that a physiological function for DNA-PK is to recognize DNA strand interruptions in the genome such as occur in recombination intermediates and at the sites of DNA damage (Anderson 1993; Gottlieb & Jackson 1994). This has led ourselves and others to test whether DNA-PK/Ku defects exist in a set of radiation hypersensitive mutant rodent cell lines that share the common phenotype of being impaired specifically in DNA double-strand break (DSB) repair (Kemp *et al.* 1984; Giaccia *et al.* 1985; Whitmore *et al.* 1989; Fulop & Phillips 1990; Biedermann *et al.* 1991; Hendrickson *et al.* 1991). These

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cell lines fall into three distinct complementation groups. Ionizing radiation complementation group 4 (IR4) contains the XR-1 cell line, IR5 includes the Chinese hamster ovary (CHO)-derived cell line *xrs-6*, and IR7 contains the hamster cell line V3 and Scid cells that derived from the murine severe combined immune deficient (Scid) mouse. Scid mice lack a functional immune system due to an inability to perform V(D)J recombination, the site-specific genomic rearrangement process employed by developing B and T lymphocytes to generate the vast diversity of functional antigen receptor genes (for example, Schuler *et al.* 1986). Cell transfection approaches have established that cells of IR groups 4 and 5 are also defective in V(D)J recombination and that, as in Scid cells, this defect is in the effective ligation of recombination intermediates (Pergola *et al.* 1993; Taccioli *et al.* 1993). Taken together, these results imply that the ubiquitous DNA DSB repair apparatus is employed by the cell type-specific V(D)J recombination machinery and suggest that the gene products defined by the mutations in XR-1, *xrs-6* and Scid cells operate in the same biochemical pathway. Here, we review recent work demonstrating that cells of IR groups 5 and 7 are defective in Ku80 and DNA-PK$_{cs}$, respectively, and assess the possible mechanisms by which DNA-PK functions in DNA repair and V(D)J recombination.

2. KU DNA BINDING ACTIVITY IS ABSENT FROM *XRS-6* CELLS

One approach to investigate the status of Ku in rodent cells has been to measure its non-specific DNA binding activity using the electrophoretic mobility shift assay. As shown in figure 1*a*, several DNA-protein complexes are formed when hamster cell extracts are incubated with a radiolabelled dsDNA oligonucleotide probe. Strikingly, one of these complexes (indicated by an arrow) is absent in *xrs-6* extracts but present in extracts from the parental cell line K1. From analysing the results of extract titration experiments, we estimate that the DNA binding activity is at least 50-fold more abundant in K1 cells than in *xrs-6* cells (see figure 1). The *xrs-6* defect appears to be linked to the mutation causing the DNA repair and recombination deficiencies of this cell line rather than some other mutation, because the DNA binding activity is also undetectable in extracts of the independently derived radiosensitive cell line XR-V15B, which falls into the same complementation group as *xrs-6* (see figure 3; compare XR-V15B and its parental line V79). Importantly, mixing *xrs-6* and K1 extracts does not result in the DNA binding activity of K1 cells becoming extinguished (data not shown), implying that the *xrs-6* defect is due to the lack of this factor rather than the presence of an inhibitory activity.

Several lines of evidence indicate that the DNA binding activity that is absent from *xrs-6* extracts is Ku. First, and consistent with the fact that Ku binds DNA ends, this complex is competed effectively by an oligonucleotide but not by supercoiled DNA (see figure 2*a*). Second, this complex is recognized specifically by anti-Ku monoclonal antibody N3H10 (see figure 2*b*).

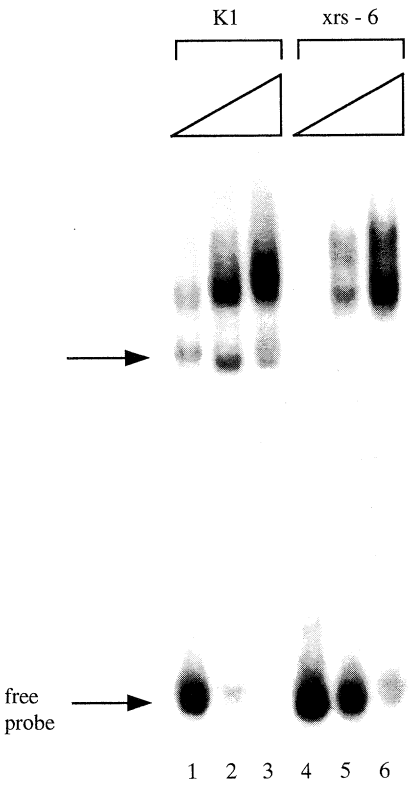

Figure 1. A DNA binding activity is defective in extracts of *xrs-6* cells. Electrophoretic mobility shift assays (EMSAs) were performed as described previously using 10 fmol [α-^{32}P]-labelled ds oligonucleotide M1/M2 (Taccioli *et al.* 1994; Blunt *et al.* 1995). Reactions contained 0.5 μg (lanes 1 and 4), 5 μg (lanes 2 and 5), or 50 μg (lanes 3 and 6) of whole cell extract (WCE) derived from K1 (lanes 1–3) or *xrs-6* (lanes 4–6) cells. The protein-DNA complex formed in K1 but not *xrs-6* extracts is indicated by an arrow.

Third, UV protein-DNA crosslinking reveals that an abundant DNA-binding protein of approximately 70 kDa is depleted in extracts of *xrs-6* cells (see figure 2*c*). This polypeptide comigrates with the major crosslinked protein in human HeLa cell extracts and with Ku70 present in a purified DNA-PK preparation. Fourth, this crosslinked polypeptide is restored in *xrs-6* derived hybrid cell lines '38' and 'H22' that have had their radiosensitivity and V(D)J recombination defects complemented by fragments of human chromosome 2 (see figure 2*c*; and for a description of cell hybrids see Finnie *et al.* 1995). Finally, *xrs-6* cells have been shown to be complemented by expression of the Ku80 cDNA (Taccioli *et al.* 1994; Smider *et al.* 1994; Boubnov *et al.* 1995). The human Ku80 gene has thus been designated as *XRCC5* (X-ray cross complementing gene for IR group 5). These results, taken together with those presented in other recent reports (Getts & Stamato 1994; Rathmell & Chu 1994; Taccioli *et al.* 1994), imply that Ku plays an important role in DNA DSB repair and V(D)J recombination.

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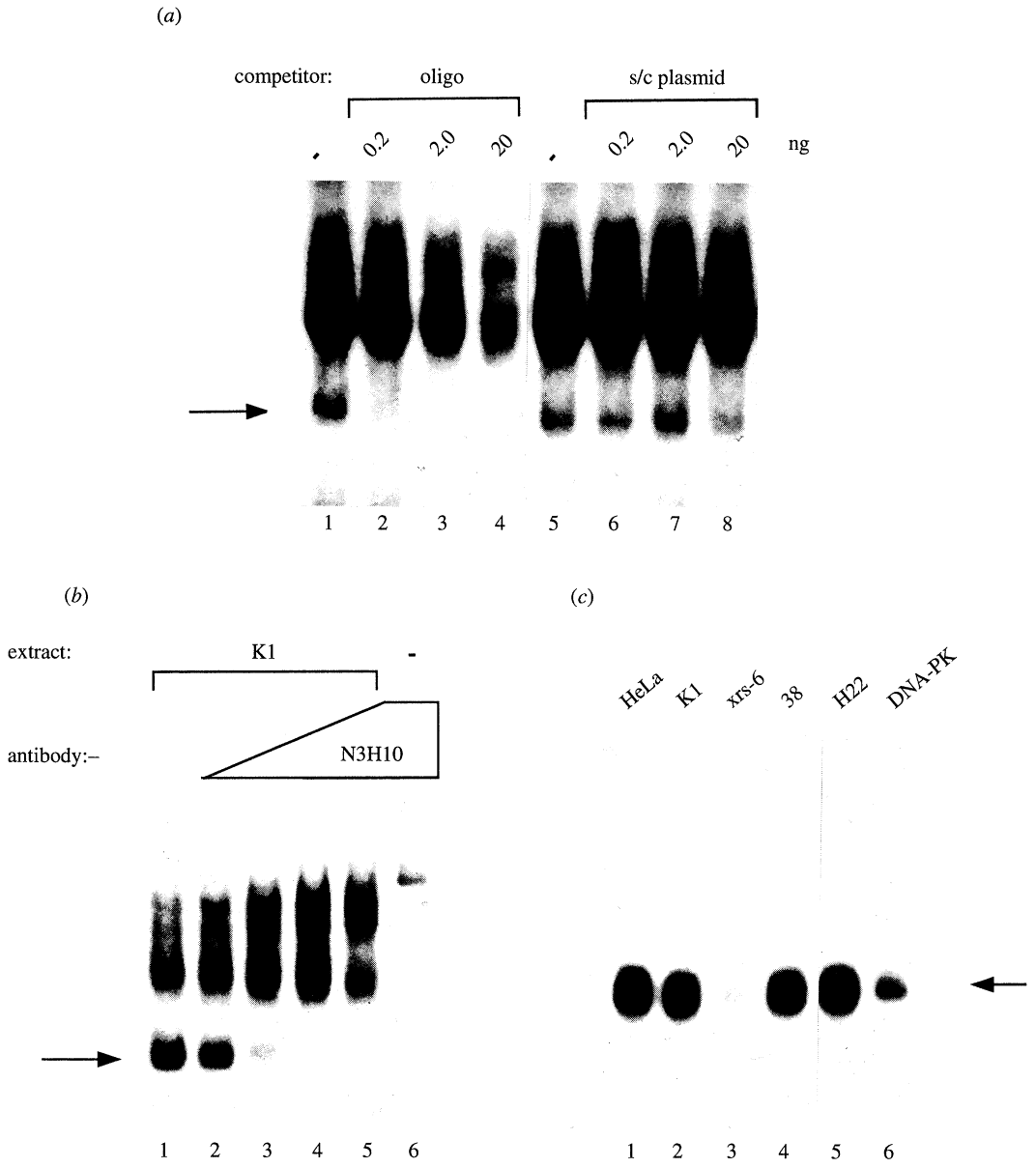

Figure 2. The DNA binding activity absent from *xrs-6* extracts corresponds to Ku. (*a*) The DNA binding activity binds to DNA ends. Whole cell extract (5 μg) derived from K1 cells was preincubated in the absence (−; lanes 1 and 5) or presence (lanes 2–4 and 6–8) of non radiolabeled competitor DNA at 30 °C for 10 min. [α-^{32}P]-radiolabelled ds oligonucleotide M1/M2 (0.2 ng) was then added and, after a further incubation for 10 min at 30 °C, DNA-protein complexes were detected by the EMSA. Reactions 2, 3, and 4, contained 0.2, 2, and 20 ng, respectively, of ds oligonucleotide M3/M4. Reactions 6, 7, and 8, contained 0.2, 2 and 20 ng, respectively, of closed circular plasmid DNA. The protein/DNA complex indicated is that which forms using K1 but not *xrs-6* extracts. (*b*) The protein/DNA complex that correlates with radiation resistance contains Ku70. K1 extract (10 μg) was preincubated at 4 °C for 10 min followed by room temperature (RT) for 10 min with no antibody (lane 1), or 0.02 μl, 0.1 μl, 0.5 μl , or 2.5 μl of anti-Ku70 monoclonal antibody N3H10 (lanes 2, 3, 4 and 5, respectively). Preincubation reaction 6 contained 2.5 μl of N3H10 alone. Next, 20 fmol radiolabelled M1/M2 was added and, after a further 10 min incubation at 30 °C, DNA-protein complexes were analysed by the EMSA. The protein/DNA complex indicated by the arrow is that which is absent in *xrs-6* extracts. (*c*) A 70 kDa DNA-binding polypeptide is deficient in *xrs-6* extracts. Purified DNA-PK (lane 6) or whole cell extract (lanes 1–5) was incubated at 30 °C for 10 min with 20 fmol [α-^{32}P]-end-labelled oligonucleotide M1/M2. Reactions were then subjected to UV-crosslinking for 10 min in a Stratalinker (Stratagene) and analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Extracts employed were: HeLa (lane 1); K1 (lane 2); *xrs-6* (lane 3); hybrid *xrs-6* derived cell line 38 that contains a complementing fragment of human chromosome 2 (lane 4); or hybrid *xrs-6* derived cell line H22 that contains a complementing fragment of human chromosome 2 (lane 5). The major polypeptide labelled in HeLa and K1 extracts is deficient in extracts of non-complemented *xrs-6* extracts and comigrates with Ku70 in the DNA-PK preparation (indicated by an arrow).

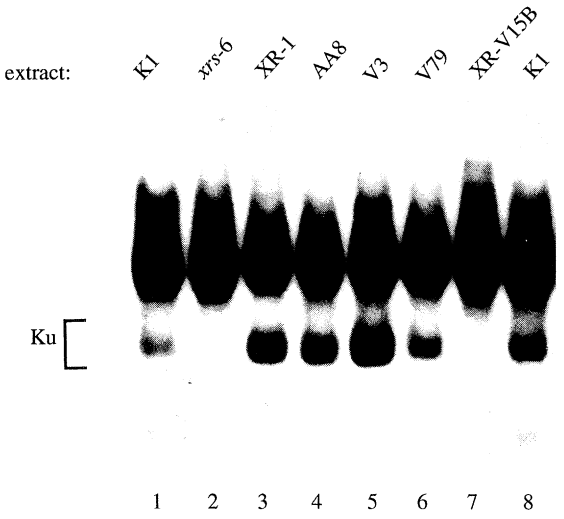

Figure 3. Cells of IR groups 4 and 7 possess Ku DNA end binding activity. EMSAs were conducted as described in the legend for figure 1 using 10 μg of whole cell extract (WCE) derived from the following cells: parental hamster K1 (lanes 1 and 8), hamster *xrs-6* mutant falling into IR5 (lane 2), hamster XR-1 mutant falling into IR4 (lane 3), parental hamster AA8 (lane 4), hamster V3 mutant falling into IR7 (lane 5), parental hamster cell line V79 (lane 6), and radiosensitive mutant cell line XR-V15B that is derived from V79 and whose mutation falls into IR group 5 (lane 7). The bracket indicates the position of the Ku/DNA complex.

3. CELLS OF IR GROUPS 4 AND 7 POSSESS KU DNA BINDING ACTIVITY

The results described above suggest that IR groups 4 and 7 might also lack Ku activity. To investigate this possibility, the Ku status of these cells was assessed by the electrophoretic mobility shift assay. In stark contrast to cells of IR group 5, Ku DNA binding activity is present at wild-type levels in extracts of all other mutant rodent cell lines so far tested (see figure 3, and also see Getts & Stamato 1994; Rathmell & Chu 1994). These include extracts from the hamster XR-1 cell line that falls into IR group 4, and extracts of hamster V3 and murine Scid cells that both fall into IR group 7.

4. CELLS OF IR GROUPS 4 AND 7 LACK DNA-PK ACTIVITY

The presence of Ku DNA binding activity in extracts of IR group 4 and 7 cells does not necessarily indicate that DNA-PK is functional: it is possible that these cells are defective in DNA-PK$_{cs}$, the ability of Ku to activate DNA-PK$_{cs}$, or in factors that regulate DNA-PK activity. As an approach to investigate these possibilities, we developed an assay system that is capable of detecting DNA-PK in extracts of rodent cells (Finnie *et al.* 1995). As shown in figure 4, although XR-1 extracts contain DNA-PK activity, *xrs-6* extracts essentially lack detectable DNA-PK. Furthermore, DNA-PK activity is absent from extracts of hamster V3 cells and murine Scid cells. Several lines of evidence

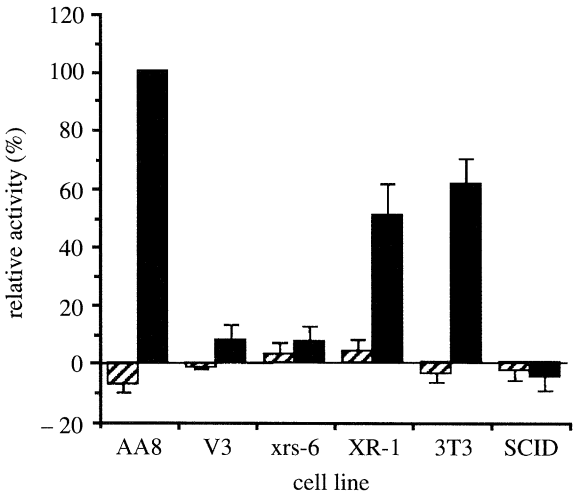

Figure 4. Cells falling into IR groups 5 and 7 lack DNA-PK activity. Whole cell extracts (100 μg) derived from the hamster or mouse cell lines indicated were tested for DNA-PK activity using assay conditions described previously (Finnie *et al.* 1995). Reactions were performed either with wild-type p53 peptide (solid columns) that is recognized effectively by DNA-PK, or with a mutated peptide (hatched columns) that is an ineffective DNA-PK substrate. Counts incorporated into peptide are plotted as a percentage of the value obtained with wild-type AA8 extract using the wt peptide.

indicate that this phenotype of V3 and scid cells is due to defects in DNA-PK$_{cs}$. First, addition of purified DNA-PK$_{cs}$ but not Ku complements V3 and Scid extracts for DNA-PK activity (Blunt *et al.* 1995). Second, introduction of the DNA-PK$_{cs}$ gene into V3 cells results in complementation in terms of DNA-PK activity, DNA repair capacity, and ability to mediate V(D)J recombination (Blunt *et al.* 1995). Third, the DNA-PK$_{cs}$ gene maps to the region of human chromosome 8 that contains the gene *XRCC7* that complements the *scid* mutation (Kirchgessner *et al.* 1995; Sipley *et al.* 1995). Finally, the gene encoding the mouse homologue of DNA-PK$_{cs}$ has been shown to co-segregate with the *scid* mutation in mouse genetic crosses (Miller *et al.* 1995).

5. HAMSTER V3 AND MURINE SCID CELLS CONTAIN DIMINISHED LEVELS OF DNA-PK$_{cs}$

The above data suggest strongly that the mutations present in scid and V3 cells lie within the gene for DNA-PK$_{cs}$. Potentially, these mutations could lead to decreased expression of DNA-PK$_{cs}$, or could cause the production of a mutated polypeptide with reduced specific activity. To determine whether the V3 and Scid mutations affect DNA-PK$_{cs}$ expression, we employed anti-DNA-PK$_{cs}$ antibodies in Western blotting studies. As shown in figure 5, antisera raised against human DNA-PK$_{cs}$ recognize polypeptides in extracts of mouse 3T3 and hamster AA8 cells that comigrate on SDS-polyacrylamide gels with human DNA-PK$_{cs}$. This polypeptide, however, is apparently present in much lower levels in rodent extracts compared to extracts of human cells (compare lanes 1 and 3 with lane 5),

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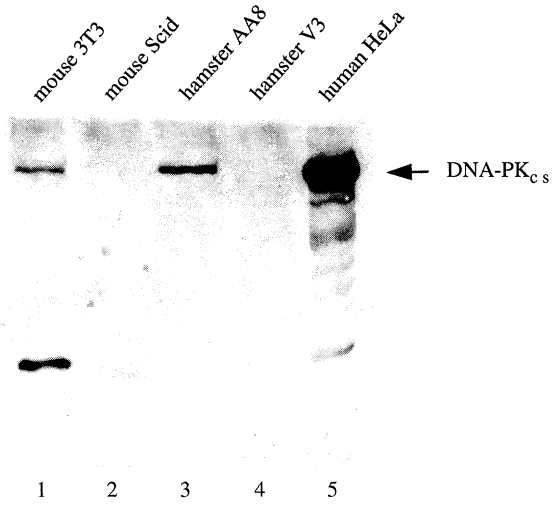

Figure 5. Hamster V3 and murine scid cells lack detectable DNA-PK$_{cs}$. Whole cell extract derived from mouse 3T3 cell (lane 1), murine scid cells (lane 2), parental hamster cell line AA8 (lane 3), V3 mutant cells (lane 4), or human HeLa cells (lane 5) were electrophoresed on a 5% SDS-polyacrylamide gel together with a sample of human DNA-PK (lane 6). The samples were then subjected to Western immunoblot analysis using a rabbit polyclonal antiserum raised against human DNA-PK$_{cs}$. The position of human DNA-PK$_{cs}$ and the crossreactive polypeptide present in wild-type rodent extracts is indicated by an arrow.

which is consistent with our previous finding that rodent cells possess 20- to 50-fold lower DNA-PK activity than human cells (Blunt *et al.* 1995; Finnie *et al.* 1995). Significantly, Western blot assays fail to detect a DNA-PK$_{cs}$ crossreactive protein in extracts of Scid or V3 cells (see figure 5). These results are consistent with the findings of Kirchgessner *et al.* (1995) and imply that the mutations in Scid and V3 cells either abolish DNA-PK$_{cs}$ synthesis, or result in the generation of an unstable protein. It will obviously be of great interest to analyse the DNA-PK$_{cs}$ genes in these two cell lines to define the precise nature of their mutations.

6. POSSIBLE MECHANISMS FOR DNA-PK ACTION *IN VIVO*

The data discussed above are most simply interpreted by concluding that the DNA-PK holoenzyme, comprising DNA-PK$_{cs}$ and Ku plays a central role in DNA DSB repair. DNA-PK activity also appears to be necessary for the DNA end joining steps of V(D)J recombination. There are a several ways in which DNA-PK may be envisaged to operate in these two processes. First and foremost, it is likely that the recognition of DNA DSBs by the Ku component of DNA-PK is central to its mode of action. For example, interactions between the DNA-PK holoenzyme and DNA termini might prevent exonucleases from destroying important genetic information before ligation events are able to be completed. Another way in which DNA-PK might function in DNA repair is by phosphorylating and activating other components of the DNA repair apparatus. Indeed, the restriction of kinase activity in the vicinity of DNA ends could ensure that the repair machinery is only activated in the site of a DNA DSB. Alternatively, or in addition, DNA-PK could recruit other DNA repair components to the site of DNA damage by protein-protein interactions. Because DNA-PK$_{cs}$ is over 450 kDa in size (Hartley *et al.* 1995), the possibility exists that it fulfils a scaffolding role, mediating contacts with several other factors and orienting these appropriately with respect to one another to facilitate effective DNA repair. Polypeptides that might bind to DNA-PK$_{cs}$ include the products of the V(D)J recombination activating genes *RAG1* and *RAG2* (Oettinger *et al.* 1990), the product that is mutated in the XR-1 cell line, DNA ligases, and enzymes such as nucleases and terminal deoxynucleotidyl transferase that function in V(D)J recombination by mediating the controlled modification of coding ends prior to their joining.

A final possible role for DNA-PK is that of a DNA end sensor to alert the cell to the fact that it has sustained injury. Indeed, it is tempting to speculate that DNA-PK activation by DNA damage might trigger a protein phosphorylation cascade that results in pleiotropic effects on cellular metabolism. These effects potentially include the transcriptional or post-transcriptional induction of components that directly or indirectly mediate DNA repair, and the activation of cell cycle checkpoint controls. Although there is at present no evidence to suggest that checkpoint control mechanisms are defective in cells that lack DNA-PK function, it is possible that multiple, partially redundant pathways for detecting DNA DSBs exist in higher eukaryotic cells and that DNA-PK constitutes just one of these. Clearly, the availability of clones for DNA-PK components and DNA-PK defective cell lines will prove invaluable in addressing the various possibilities discussed above and in defining the precise physiological roles of DNA-PK.

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REFERENCES

Anderson, C. W. 1993 DNA damage and the DNA-activated protein kinase. *Trends biochem. Sci.* **18**, 433–437.

Anderson, C. W. & Lees-Miller, S. P. 1992 The nuclear serine/threonine protein kinase DNA-PK. *Crit. Rev. Eukary. Gene Express.* **2**, 283–314.

Bannister, A. J., Gottlieb, T. M., Kouzarides, T. & Jackson, S. P. 1993 c-Jun is phosphorylated by the DNA-dependent protein kinase in vitro; definition of the minimal kinase recognition motif. *Nucl. Acids Res.* **21**, 1289–1295.

Biedermann, K. A., Sun, J., Giaccia, A. J., Tosto, L. M. & Brown, J. M. 1991 scid mutation in mice confers hypersensitivity to ionizing radiation and a deficiency in DNA double-strand break repair. *Proc. natn Acad. Sci. U.S.A.* **88**, 1394–1397.

Blunt, T., Finnie, N. J., Taccioli, G. E., Smith, G. C. M., Demengeot, J., Gottlieb, T. M., Mizuta, R., Varghese, A. J., Alt, F. W., Jeggo, P. A. & Jackson, S. P. 1995

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Defective DNA-dependent protein kinase activity is linked to V(D)J recombination and DNA repair defects associated with the murine *scid* mutation. *Cell* **80**, 813–823.

Boubnov, N. V., Hall, K. T., Wills, Z., Lee, S. E., He, D. M., Benjamin, D. M., Pulaski, C. R., Pulaski, B., Pulaski, H., Reeves, W., Hendrickson, E. A. & Weaver, D. 1995 Complementation of ionizing radiation sensitivity, DNA end binding, and V(D)J recombination defects of double-strand break repair mutants of the p86 Ku autoantigen. *Proc. natn Acad. Sci. U.S.A.* **92**, 890–894.

Brush, G. S., Anderson, C. W. & Kelly, T. J. 1994 The DNA-activated protein kinase is required for the phosphorylation of replication protein A during simian virus 40 DNA replication. *Proc. natn Acad. Sci. U.S.A.* **91**, 12520–12524.

Carter, T., Vancurova, I., Sun, I., Lou, W. & DeLeon, S. 1990 A DNA-activated protein kinase from HeLa cell nuclei. *Molec. Cell. Biol.* **10**, 6460–6471.

Dvir, A., Stein, L. Y., Calore, B. L. & Dynan, W. S. 1993 Purification and characterization of a template-associated protein kinase that phosphorylates RNA polymerase II. *J. biol. Chem.* **268**, 10440–10447.

Falzon, M., Fewell, J. W. & Kuff, E. L. 1993 EBP-80, a transcription factor closely resembling the human autoantigen Ku, recognizes single-to double-strand transition in DNA. *J. biol. Chem.* **268**, 10546–10552.

Finnie, N., Gottlieb, T., Hartley, K. & Jackson, S. P. 1993 Transcription factor phosphorylation by the DNA-dependent protein kinase. *Biochem. Soc. Trans.* **21**, 930–935.

Finnie, N. J., Gottlieb, T. M., Blunt, T., Jeggo, P. & Jackson, S. P. 1995 DNA-PK activity is absent in xrs-6 cells; implications for site-specific recombination and DNA double-strand break repair. *Proc. natn Acad. Sci. U.S.A.* **92**, 320–324.

Fulop, G. M. & Phillips, R. A. 1990 The scid mutation in mice causes a general defect in DNA repair. *Nature, Lond.* **347**, 479–482.

Getts, R. C. & Stamato, T. D. 1994 Absence of a Ku-like DNA end-binding activity in the xrs double-strand DNA repair deficient mutant. *J. biol. Chem.* **269**, 15981–15984.

Giaccia, A., Weinstein, R., Hu, K. J. & Stamato, T. D. 1985 Cell cycle-dependent repair of double-stranded DNA breaks in a gamma-ray sensitive Chinese hamster cell. *Somat. Cell. molec. Genet.* **11**, 485–491.

Gottlieb, T. M. & Jackson, S. P. 1993 The DNA-dependent protein kinase: requirement for DNA ends and association with Ku antigen. *Cell* **72**, 131–142.

Gottlieb, T. M. & Jackson, S. P. 1994 Protein kinases and DNA damage. *Trends biochem. Sci.* **19**, 500–503.

Hartley, K. O., Gell, D., Smith, G. C. M., Zhang, H., Divecha, N., Connelly, M. A., Admon, A., Lees-Miller, S. P., Anderson, C. W. & Jackson, S. P. 1995 DNA-dependent protein kinase catalytic subunit: a relative of phosphatidylinositol 3-kinase and the ataxia telangiectasia gene product. *Cell* **82**, 849–856.

Hendrickson, E. A., Qin, X. -Q., Bump, E. A., Schatz, D. G., Oettinger, M. & Weaver, D. T. 1991 A link between double-strand break-related repair and V(D)J recombination: the scid mutation. *Proc. natn Acad. Sci. U.S.A.* **88**, 4061–4065.

Jackson, S. P., Gottlieb, T. & Hartley, K. 1993 Phosphorylation of transcription factor Sp1 by the DNA-dependent protein kinase. *Adv. Sec. Messeng. Phosphopro. Res.* **28**, 279–286.

Jackson, S. P., MacDonald, J. J., Lees-Miller, S. & Tjian, R. 1990 GC box binding induces phosphorylation of Sp1 by a DNA-dependent protein kinase. *Cell* **63**, 155–165.

Kemp, L. M., Sedgwick, S. G. & Jeggo, P. A. 1984 X-ray sensitive mutants of Chinese hamster ovary cells defective in double-stranded break rejoining. *Mut. Res.* **132**, 189–196.

Kirchgessner, C. U., Patil, C. K., Evans, J. W., Carter, T. & Brown, J. M. 1995 DNA dependent kinase (p350)is a candidate gene for murine scid defect. *Science, Wash.* **267**, 1178–1182.

Kuhn, A., Gottlieb, T. M., Jackson, S. P. & Grummt, I. 1995 DNA-dependent protein kinase: a potent inhibitor of transcription by RNA polymerase I. *Genes Dev.* **9**, 193–203.

Labhart, P. 1995 DNA-dependent protein kinase specifically represses promoter-directed transcription initiation by RNA polymerase I. *Proc. natn Acad. Sci. U.S.A.* **92**, 2934–2938.

Lees-Miller, S. P., Chen, Y. -R. & Anderson, C. W. 1990 Human cells contain a DNA-activated protein kinase that phosphorylates simian virus 40 T antigen, mouse p53 and the human Ku autoantigen. *Molec. Cell. Biol.* **10**, 6472–6481.

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

Miller, R. D., Hogg, J., Ozaki, J. H., Gell, D., Jackson, S. P. & Riblet, R. 1995 The gene for the catalytic subunit of mouse DNA-dependent protein kinase (DNA-PK_{cs}) maps to the *scid* locus. *Proc. natn. Acad. Sci. U.S.A.* **92**. (In the press.)

Morozov, V. E., Falzon, M., Anderson, C. W. & Kuff, E. L. 1994 DNA-dependent protein kinase is activated by nicks and larger single-stranded gaps. *J. biol. Chem.* **269**, 16684–16688.

Oettinger, M. A., Schatz, D. G., Gorka, C. & Baltimore, D. 1990 RAG 1 and RAG 2, adjacent genes that synergistically activate V(D)J recombination. *Science, Wash.* **248**, 1517–1523.

Paillard, S. & Strauss, F. 1991 Analysis of the mechanism of interaction of simian Ku protein with DNA. *Nucl. Acids. Res.* **19**, 5619–5624.

Pan, Z.-Q., Amin, A. A., Gibbs, E., Niu, H. & Hurwitz, J. 1994 Phosphorylation of the p34 subunit of human single-stranded-DNA-binding protein in cyclin A-activated G1 extracts is catalysed by cdk-cyclin 1 complex and DNA-dependent protein kinase. *Proc. natn Acad. Sci. U.S.A.* **91**, 8343–8347.

Pergola, F., Zdzienicka, M. Z. & Lieber, M. R. 1993 V(D)J recombination in mammalian cell mutants defective in DNA double-strand break repair. *Molec. Cell. Biol.* **13**, 3464–3471.

Rathmell, W. K. & Chu, G. 1994 Involvement of the Ku autoantigen in the cellular response to DNA double-strand breaks. *Proc. natn Acad. Sci. U.S.A.* **91**, 7623–7627.

Schuler, W., Weiler, I. J., Schuler, A., Phillips, R. A., Rosenberg, N., Mak, T. W., Kearney, J. F., Perry, R. P. & Bosma, J. J. 1986 Rearrangement of antigen receptor genes is defective in mice with severe combined immune deficiency. *Cell* **46**, 963–972.

Sipley, J. D., Mennenger, J., Hartley, K. O., Ward, D., Jackson, S. P. & Anderson, C. W. 1995 The gene for the catalytic subunit of the human DNA-activated protein kinase maps to the site of the *XRCC7* gene on chromosome 8. *Proc. natn Acad. Sci. U.S.A.* **92**, 7515–7519.

Smider, V., Rathmell, W. K., Lieber, M. R. & Chu, G. 1994 Restoration of X-ray resistance and V(D)J recombination in mutant cells by Ku cDNA. *Science, Wash.* **266**, 288–291.

Taccioli, G. E., Gottlieb, T. M., Blunt, T., Priestley, A., Demengeot, J., Mizuta, R., Lehmann, A. R., Alt, F. W.,

PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B BIOLOGICAL SCIENCES

Jackson, S. P. & Jeggo, P. A. 1994 Ku80: Product of the *XRCC5* gene and its role in DNA repair and V(D)J recombination. *Science, Wash.* **265**, 1442–1445.

Taccioli, G. E., Rathbun, G., Oltz, E., Stamato, T., Jeggo, P. A. & Alt, F. W. 1993 Impairment of V(D)J recombination in double-stranded break repair mutants. *Science, Wash.* **260**, 207–210.

Walker, A. I., Hunt, T., Jackson, R. J. & Anderson, C. W. 1985 Double-stranded DNA induces the phosphorylation of several proteins including the 90000 mol. wt. heat-shock protein in animal cell extracts. *EMBO J.* **4**, 139–145.

Whitmore, G. F., Varghese, A. J. & Gulyas, S. 1989 Cell cycle responses of two X-ray sensitive mutants defective in DNA repair. *Int. J. radiat. Biol.* **56**, 657–665.

Discussion

Y. A. Hannun (*Department of Medicine & Cell Biology, Duke Univeristy Medical Center, North Carolina, U.S.A.*). Does DNA-PK phosphorylate nucleic acid?

S. P. Jackson. We have never done that experiment.

M. Karin (*Department of Pharmacology, University of California, San Diego, U.S.A.*). Have you ever tried to link Ku's kinase recognition sequence onto different DNA-binding sequences, to see whether you can recruit DNA-PK$_{cs}$ to regions other than double-stranded breaks.

S. P. Jackson. We do not yet know the answer to that, but there is evidence that DNA-PK$_{cs}$ may itself need to contact DNA: if it is targeted to DNA-bound Ku, it can itself get UV cross-linked to the DNA. DNA-PK may even have itself to contact DNA ends. We are now trying to do yeast two-hybrid studies to try and narrow down the interaction domain between the kinase and Ku.

M. Karin. How does DNA-PK influence PolI transcription?

S. P. Jackson. Our work on this was published earlier this year (Kuhn *et al.* 1995). DNA-PK inhibits transcription, as a result of phosphorylating some elements of the PolI transcriptional apparatus. It certainly inhibits elongation, but it is not clear whether it also inhibits initiation. It also inhibits transcription by PolIII.

Reference

Kuhn, A., Gottlieb, T. M., Jackson, S. P. & Grummt, I. 1995 DNA-dependent protein kinase: a potent inhibitor of transcription by RNA polymerase I. *Genes Dev.* **9**, 193–203.

Question. Is DNA-PK ubiquitous, or is its abundance regulated in relation to cell proliferation or tumorigenesis?

S. P. Jackson. There is some evidence that the Ku concentration increases in rapidly proliferating cells. However, this effect is not large, and DNA-PK and Ku are abundant proteins. Human cells have about half a million copies of Ku and not many less of the DNA-PK$_{cs}$. For some reason, rodents have about 50-fold less DNA-PK than humans. My current hypothesis is that this may be related to longevity, in that mice may not need to repair DNA as effectively as humans. There is no evidence that DNA-PK levels change in response to radiation or in cell-lines. However, there are derived cell-lines that have reduced DNA-PK levels, and these should prove very useful in analysing its function. Carl Anderson, with whom we have been collaborating on the cloning of DNA-PK, has now got to the promoter region, which looks like the promoter of a housekeeping gene.

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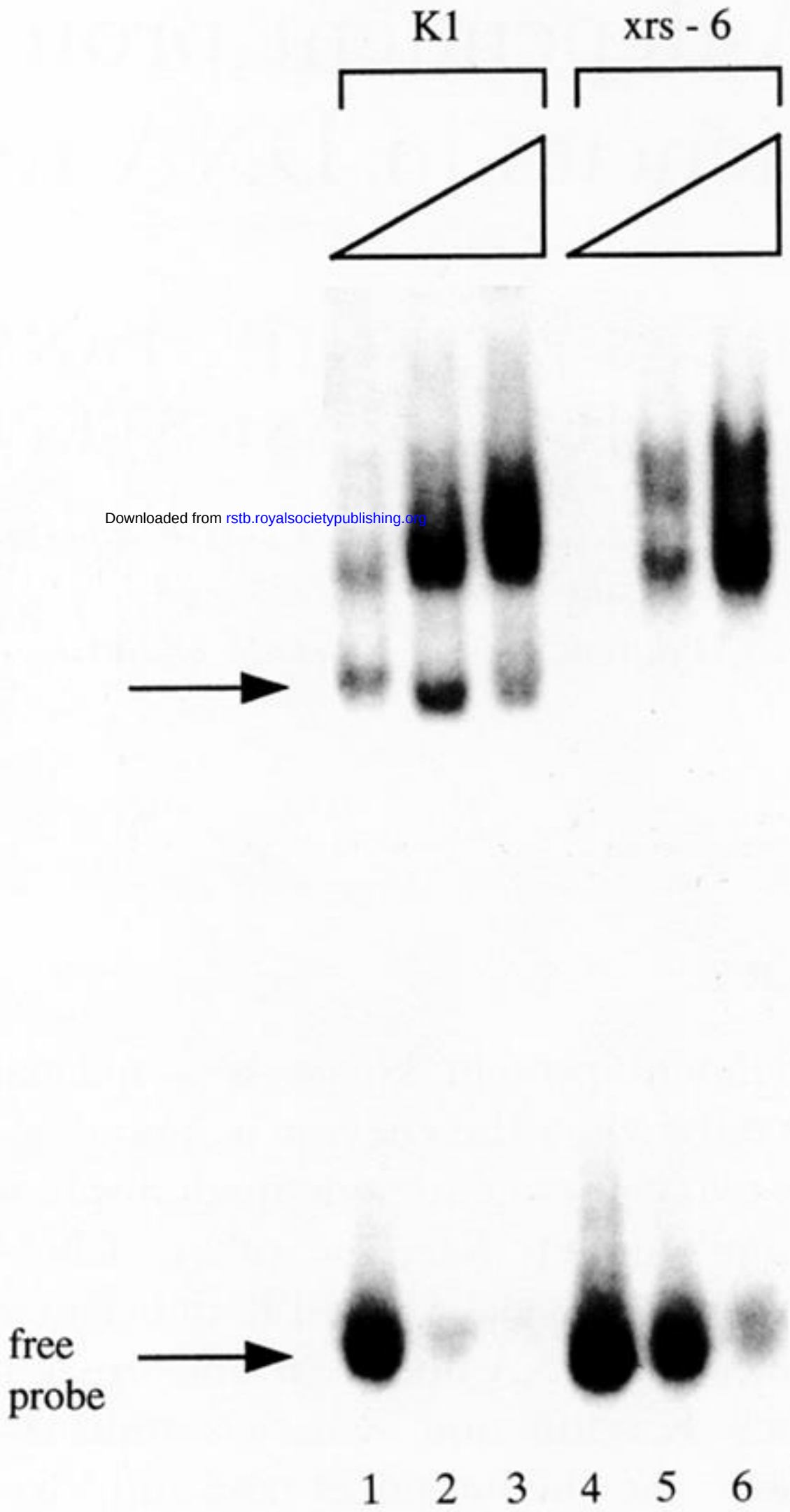
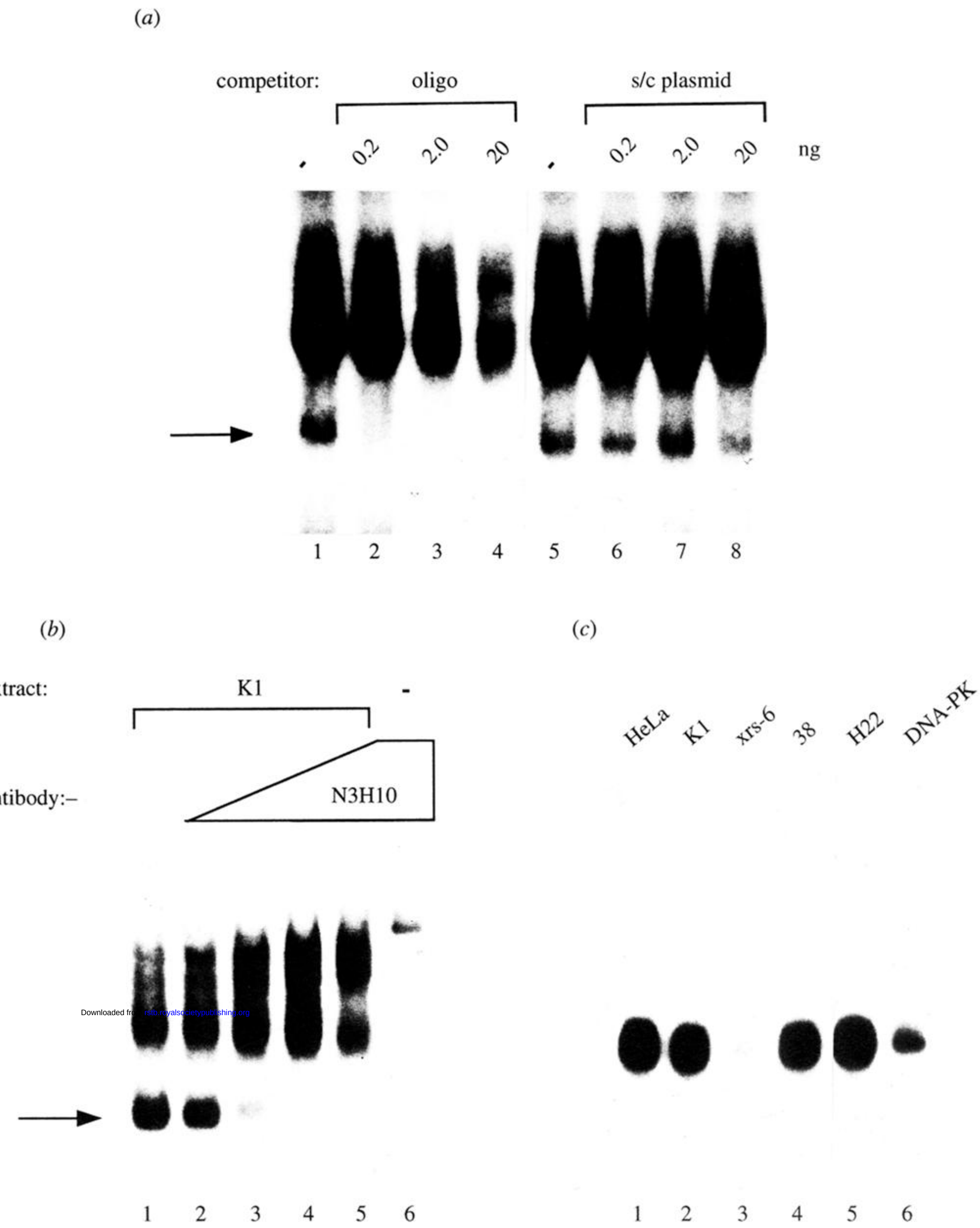
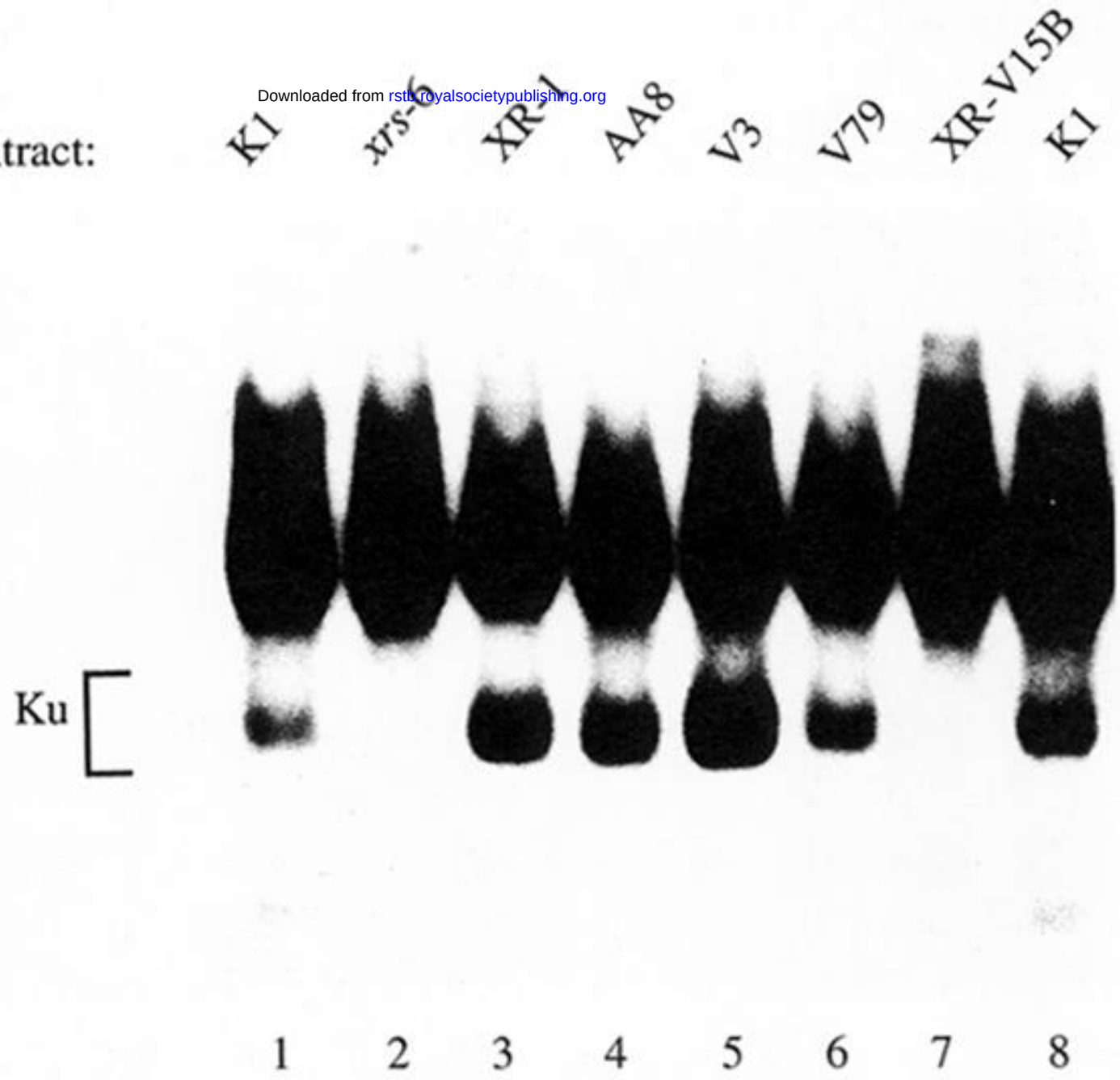


gure 1. A DNA binding activity is defective in extracts of *s-6* cells. Electrophoretic mobility shift assays (EMSAs) were rformed as described previously using 10 fmol [α-^{32}P]-belled ds oligonucleotide M1/M2 (Taccioli *et al.* 1994; unt *et al.* 1995). Reactions contained 0.5 µg (lanes 1 and 4), µg (lanes 2 and 5), or 50 µg (lanes 3 and 6) of whole cell tract (WCE) derived from K1 (lanes 1–3) or *xrs-6* (lanes -6) cells. The protein-DNA complex formed in K1 but not *s-6* extracts is indicated by an arrow.



gure 2. The DNA binding activity absent from *xrs-6* extracts corresponds to Ku. (*a*) The DNA binding activity nds to DNA ends. Whole cell extract (5 μg) derived from K1 cells was preincubated in the absence (−; lanes 1 and or presence (lanes 2–4 and 6–8) of non radiolabeled competitor DNA at 30 °C for 10 min. [α-^{32}P]-radiolabelled oligonucleotide M1/M2 (0.2 ng) was then added and, after a further incubation for 10 min at 30 °C, DNA-protein mplexes were detected by the EMSA. Reactions 2, 3, and 4, contained 0.2, 2, and 20 ng, respectively, of ds gonucleotide M3/M4. Reactions 6, 7, and 8, contained 0.2, 2 and 20 ng, respectively, of closed circular plasmid NA. The protein/DNA complex indicated is that which forms using K1 but not *xrs-6* extracts. (*b*) The protein/DNA mplex that correlates with radiation resistance contains Ku70. K1 extract (10 μg) was preincubated at 4 °C for min followed by room temperature (RT) for 10 min with no antibody (lane 1), or 0.02 μl, 0.1 μl, 0.5 μl , or 2.5 μl anti-Ku70 monoclonal antibody N3H10 (lanes 2, 3, 4 and 5, respectively). Preincubation reaction 6 contained 5 μl of N3H10 alone. Next, 20 fmol radiolabelled M1/M2 was added and, after a further 10 min incubation at °C, DNA-protein complexes were analysed by the EMSA. The protein/DNA complex indicated by the arrow is that hich is absent in *xrs-6* extracts. (*c*) A 70 kDa DNA-binding polypeptide is deficient in *xrs-6* extracts. Purified DNA-ζ (lane 6) or whole cell extract (lanes 1–5) was incubated at 30 °C for 10 min with 20 fmol [α-^{32}P]-end-labelled gonucleotide M1/M2. Reactions were then subjected to UV-crosslinking for 10 min in a Stratalinker (Stratagene) d analysed by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and autoradiography. Extracts employed were: eLa (lane 1); K1 (lane 2); *xrs-6* (lane 3); hybrid *xrs-6* derived cell line 38 that contains a complementing fragment human chromosome 2 (lane 4); or hybrid *xrs-6* derived cell line H22 that contains a complementing fragment of man chromosome 2 (lane 5). The major polypeptide labelled in HeLa and K1 extracts is deficient in extracts of n-complemented *xrs-6* extracts and comigrates with Ku70 in the DNA-PK preparation (indicated by an arrow).



gure 3. Cells of IR groups 4 and 7 possess Ku DNA end nding activity. EMSAs were conducted as described in the gend for figure 1 using 10 µg of whole cell extract (WCE) rived from the following cells: parental hamster K1 (lanes and 8), hamster *xrs-6* mutant falling into IR5 (lane 2), mster XR-1 mutant falling into IR4 (lane 3), parental mster AA8 (lane 4), hamster V3 mutant falling into IR7 ne 5), parental hamster cell line V79 (lane 6), and diosensitive mutant cell line XR-V15B that is derived from 79 and whose mutation falls into IR group 5 (lane 7). The acket indicates the position of the Ku/DNA complex.

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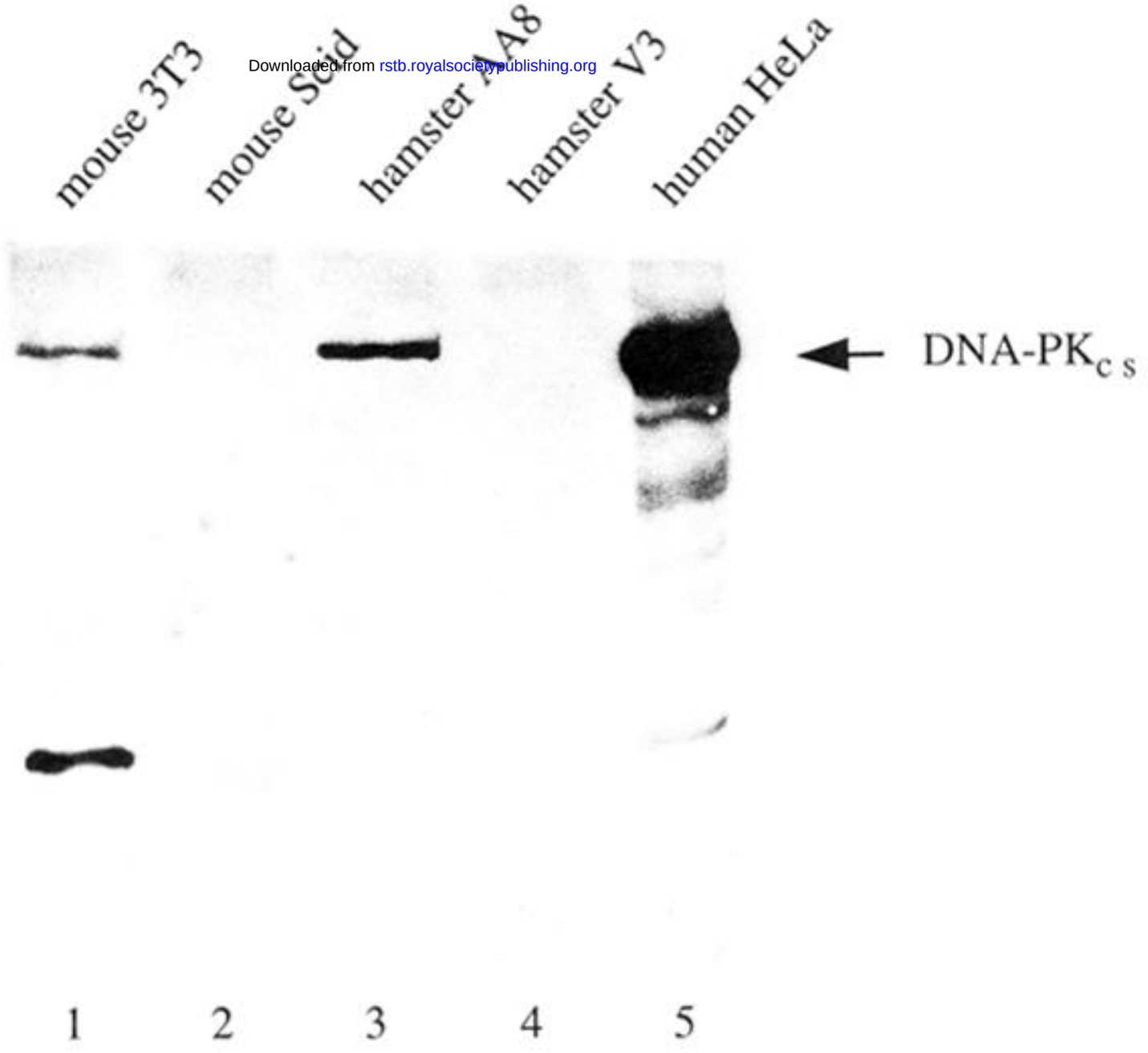

gure 5. Hamster V3 and murine scid cells lack detectable NA-PK$_{cs}$. Whole cell extract derived from mouse 3T3 cell ane 1), murine scid cells (lane 2), parental hamster cell line A8 (lane 3), V3 mutant cells (lane 4), or human HeLa cells ane 5) were electrophoresed on a 5% SDS-polyacrylamide l together with a sample of human DNA-PK (lane 6). The mples were then subjected to Western immunoblot analysis ing a rabbit polyclonal antiserum raised against human NA-PK$_{cs}$. The position of human DNA-PK$_{cs}$ and the ossreactive polypeptide present in wild-type rodent extracts indicated by an arrow.