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Spodoptera frugiperda FKBP-46 Is a Consensus p53 Motif Binding Protein

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ABSTRACT

p53 protein, the central molecule of the apoptosis pathway, is mutated in 50% of the human cancers. Of late, p53 homologues have been identified from different invertebrates including *Drosophila melanogaster*, *Caenorhabditis elegans*, Squid, and Clams. We report the identification of a p53-like protein in *Spodoptera frugiperda* (*Sf*9) insect cells, which is activated during oxidative stress, caused by exposure to UV-B or H_2O_2, and binds to p53 consensus DNA binding motifs as well as other p53 cognate motifs. *Sf*9 p53 motif-binding protein is similar to murine and Drosophila p53 in terms of molecular size, which is around 50–60 kDa, as evident from UV cross-linking, and displays DNA binding characteristics similar to both insect and vertebrate p53 as seen from electrophoretic mobility shift assays. The N-terminal sequencing of the purified *Sf*9 p53 motif-binding protein reveals extensive homology to the pro-apoptotic FK-506 binding protein (FKBP-46), earlier identified in *Sf*9 cells as a factor which interacts with murine casein kinase. FKBP, an evolutionarily conserved protein of mammalian origin functions as a pro-apoptotic factor. Identification of FKBP-46 as a novel p53 motif-binding protein in insect cells adds a new facet to our understanding of the mechanisms of apoptosis under oxidative stress in the absence of a typical p53 homologue. J. Cell. Biochem. 114: 899–907, 2013. © 2012 Wiley Periodicals, Inc.

KEY WORDS: *Spodoptera frugiperda*; OXIDATIVE STRESS; *Sf*9 p53-LIKE FACTOR; FKBP-46

p53, a tumor suppressor protein and the molecular guardian of the cell, is a sequence specific transcription factor [Kern et al., 1991] activated by various cellular stresses including DNA damaging agents [Zhan et al., 1993], heat shock [Ohnishi et al., 1996], nutrient stress [Schumacher et al., 2001], growth factor deprivation [Levine et al., 2006], nucleotide depletion [Myers et al., 2009], oncogene activation [Hogan et al., 2008], etc. DNA damage immediately activates several damage sensors, which in turn activate p53 resulting in the activation of DNA repair pathway, cell cycle arrest or apoptosis [Ko and Prives, 1996; Levine, 1997; Giaccia and Kastan, 1998; Kamijo et al., 1998]. p53 exerts a pro-apoptotic function by both transcription dependent and independent processes [Fields and Jang, 1990; O'Rourke et al., 1990; Leu et al., 2004]. As a transcription factor, it activates the expression of genes involved in the response pathway including *p21* (cdk inhibitor—cell cycle arrest [Johnson et al., 1994]), *GADD 45* (DNA repair [Zhan et al., 1994]), *bax*, *caspase* (apoptosis [Gupta et al., 2002]), etc. p53 has been reported to repress the transcription of several genes including *bcl2* [Ginsberg et al., 1991; Miyashita et al., 1994]. The DNA binding domain of p53 recognizes a sequence of four repeats of a consensus DNA sequence 5′-PuPuPuC(A/T)-3′ [Kern et al., 1991], and the DNA binding domain is the most conserved domain in p53. Earlier reports of p53 homologs from invertebrates like *Drosophila melanogaster* [Jin et al., 2000], *Caenorhabditis elegans* [Schmale and Bamberger, 1997], clams [Barker et al., 1997], squid [Van Beneden et al., 1997], etc. suggest that p53 homologs possess less sequence identity but have conserved DNA binding domain.

We earlier established *Spodoptera frugiperda* (*Sf*) cells as a model to study oxidative stress induced apoptosis [Hasnain et al., 1999]. With a view to understand the pro- and anti-apoptotic events in *Sf*9 cells, we provide evidence of the presence of a p53-like protein from *Sf* cells, which is activated during oxidative stress in a time

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dependent manner. Using electrophoretic mobility shift assays, we characterized the DNA binding activity of this *Sf*9 p53-like protein. The purification and sequencing of this protein revealed its identity as FK-506 binding protein (FKBP-46). FKBP are a family of proteins which bind to immune-suppressor drug FK-506. In addition to their drug binding activity, their precise function in the cellular physiology is not well understood. However, FKBP family proteins have been reported to contribute to several functions in the cell such as protein folding, regulation of cytokines, transport of steroid receptor complexes, nucleic acid binding, histone assembly, and modulation of apoptosis which are mediated by specific domains that adopt distinct tertiary conformations [Alnemri et al., 1994]. FKBP-46, a member of the family, is a nuclear protein with DNA binding activity [Somarelli et al., 2008]. The identification of FKBP as a p53-like factor in *Sf*9, adds a new facet to our understanding of the function of FKBP and its role in *S. frugiperda*.

MATERIALS AND METHODS

CELL CULTURE

*Sf*9 cells were cultured in vitro in complete medium [TNM-FH, supplemented with 10% fetal bovine serum, 1% antibiotic–antimycotic (GIBCO-BRL, USA)] and was maintained at 27°C [Hasnain et al., 1999]. For large scale growth, cells were grown in spinner bottle on a magnetic rotator (Techne, USA) at room temperature till an OD of 2 million cells per ml was obtained.

OXIDATIVE STRESS TREATMENT

UV-B (302 nm) exposure was given essentially as described [Sah et al., 1999; Sahdev et al., 2003] using Transilluminator (UVP, USA) for 90 s equivalent to 720 J (sufficient to cause 70% apoptosis when left overnight) and incubated further in dark at 27°C. *Sf*9 cells were treated with H_2O_2 at a final concentration of 750 μM (EMSA) or 1 mM for 6 h (protein purification), in dark and the cells were further incubated at 27°C overnight.

ELECTROPHORETIC MOBILITY SHIFT ASSAY

Electrophoretic Mobility Shift Assays (EMSA) were carried out as described earlier [Ramachandran et al., 2001]. Briefly, 1 ng of labeled p53 oligonucleotide with specific activity of 10^4 cpm was mixed with 3–5 μg of *Sf*9 nuclear extract in a binding buffer (10 mM Tris pH 7.5, 50 mM NaCl, 1 mM EDTA, 10% glycerol, 1 μg BSA, 1 μg pdIdC, 1 mM DTT, 1 mM PMSF, 50 mM magnesium chloride) to a final volume of 20 μl and incubated at room temperature for 30 min (Hasnain et al., 1996). The binding reaction was terminated on ice by adding 2 μl of loading dye (Promega). The DNA–protein complexes were resolved in a native 4–6% polyacrylamide gel (PAGE; 29:1) at 4°C in 1× TBE at 350 V/50 min. After electrophoresis, the gel was transferred onto a piece of 3 mm Whatman, covered with Saran Wrap and dried at 80°C in a vacuum drier. The DNA–protein complexes were visualized by autoradiography at −70°C or on a phosphor-imaging system (Biorad). p53 oligonucleotides were commercially obtained (Santacruz Biotech, USA) carrying consensus p53 binding sequence 5′ TAC AGA ACA TGT CTA AGC ATG CTG GGG ACT 3′. The mutant p53 5′ TAC AGA A**TC GC**T CTA AGC ATG CTG GGG ACT 3′ oligonucleotide had a mutation in the cognate motif (shown in bold).

DNA–PROTEIN CROSS LINKING ASSAY

The DNA–protein complex formation was carried out as described for EMSA. The reaction mix was subjected to UV-cross linking for 2 min at 1,200 J and then fractionated on 10% SDS–PAGE after addition of equal volume of PAGE sample loading dye (Mukherjee et al., 1995). The gel was dried and further exposed to phosphor imager and further image analysis was carried out using phosphor imaging system.

PURIFICATION OF p53 LIKE PROTEIN FROM *S. frugiperda* CELLS

Following oxidative stress treatment (1 mM H_2O_2) for 6 h, 500 ml culture of *Sf*9 cells at a density of 2 million/ml were harvested and nuclear extract was isolated and subjected to 20% ammonium sulfate precipitation and the resulting protein pellet was discarded. The resultant supernatant protein solution was further subjected to 60% ammonium sulfate precipitation. The precipitate was resuspended in 20 mM Tris pH 8.0 and 100 mM NaCl (binding buffer). The resuspended proteins were then subjected to Q-sepharose chromatography and the bound proteins were eluted by a gradient of NaCl (100–500 mM) in binding buffer. The 400 and 500 mM NaCl eluates of Q-sepharose chromatography (containing DNA binding active fractions) were diluted to 100 mM NaCl containing 20 mM Tris pH 8.0 and further subjected to Heparin sepharose chromatography. Bound proteins were eluted again with sodium chloride gradient of 100–1,000 mM in binding buffer. The eluates were further fractionated on 10% SDS–PAGE and visualized by Coomassie blue staining and also monitored for DNA binding activity.

RESULTS

*Sf*9 NUCLEAR EXTRACT FORMS A SPECIFIC COMPLEX WITH CONSENSUS P53 MOTIF

Given the established role of p53 in apoptosis, the existence of such factor in *Sf*9 nuclear extract was investigated. Electrophoretic mobility shift assay (EMSA) was carried out using synthetic oligonucleotides carrying the human consensus p53 motif and 3 μg of *Sf*9 nuclear extract. The formation of DNA–protein complex is indicative of the presence of a p53-like factor in these cells (Fig. 1). Homologous cold competition using oligonucleotide (TACAGAACATGTCTAAGCATGCTGGGGACT) carrying consensus p53 motif critical for DNA binding significantly abolished complex formation (Fig. 1, lane 3). However, when oligonucleotide (TACAGAA**TCGC**TCTAAGCATGCTGGGGACT) carrying mutation in the bases critical for p53 binding shown in bold were used these could not compete for binding (Fig. 1, lane 4). These results pointed to the existence of a p53-like factor in *S. frugiperda*.

*Sf*9 p53-LIKE PROTEIN IS ACTIVATED DURING OXIDATIVE STRESS

Mammalian p53 is known to be activated by oxidative stress. We therefore designed experiments to ascertain whether the p53-like factor, present in insect cells, is also modulated by oxidative stress. Nuclear extracts were prepared from *Sf*9 cells exposed to UV-B or treated with H_2O_2 (750 μM) for different times postexposure. EMSA

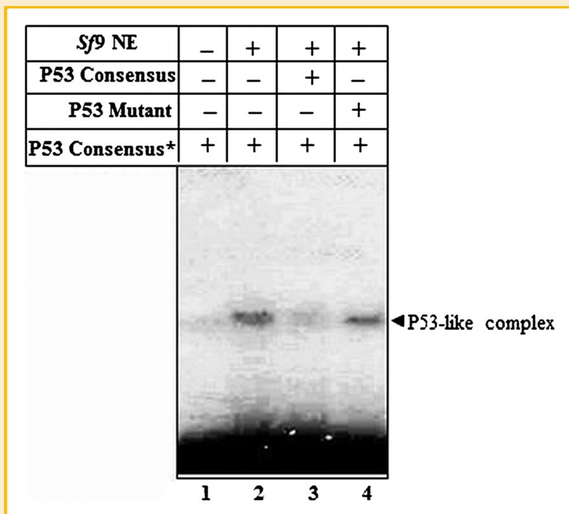


Fig. 1. *Sf*9 nuclear extract forms a specific complex with p53 consensus DNA binding motif. EMSA with 3 μg of *Sf*9 nuclear extract using consensus p53 probe shows a specific DNA–protein complex (lane 2), which is competed out by 100× unlabeled consensus p53 element (lane 3) but not by an oligodeoxyribonucleotide carrying a mutation in the p53 binding motif (lane 4). *Radio-labeling of the oligonucleotide under study.

analysis using radio-labeled consensus p53 probe and equal amount of nuclear extracts (5 μg in UV-B; 3 μg in H_2O_2) from the cells at different times postoxidative stress clearly reveal increasing intensity of complex formation as a function of time of oxidative stress (Fig. 2). This was irrespective of whether the extract was prepared from cells exposed to varying periods of UV-B irradiation (Fig. 2a) or H_2O_2 treatment (Fig. 2b). A maximum intensity of the DNA–protein complex could be seen after 6–8 h postoxidative stress, whether caused by UV exposure (Fig. 2a, lane 8) or by H_2O_2 treatment (Fig. 2b, lane 6) after which the cells showed morphological symptoms of apoptosis (data not shown). The increasing intensity of the DNA–protein complex as a function of density of UV-B irradiation or H_2O_2 treatment is indicative of the activation of the *Sf*9 p53-motif binding protein by oxidative stress.

*Sf*9 p53-LIKE PROTEIN IS SIMILAR IN TERMS OF SEQUENCE SPECIFICITY AND MOLECULAR SIZE TO THE VERTEBRATE p53

Having established the presence of a *Sf*9 p53-like protein showing specific binding to putative p53 cognate motifs and activated by oxidative stress, we attempted to investigate similarities between *Sf*p53 motif-binding protein and other known p53 proteins. EMSA was carried out using radio-labeled consensus p53 probe and 5 μg nuclear extract prepared from *Sf*9, Drosophila, Bombyx, and Human (U937) cell lines (Fig. 3a). Apparently, the different extracts (*Sf*9-A, Drososphila-B, Bombyx-C, and Human U937-D) form a specific complex with p53 consensus cognate motif (Fig. 3a, lanes 1, 4, 6, and 9) as evident from competition experiments using homologous p53 consensus oligonucleotide (Fig. 3a, lanes 2, 5, 7, and 10) but is unaffected when mutant oligonucleotide carrying mutation in the consensus p53 sequence motif critical for p53 DNA binding are used

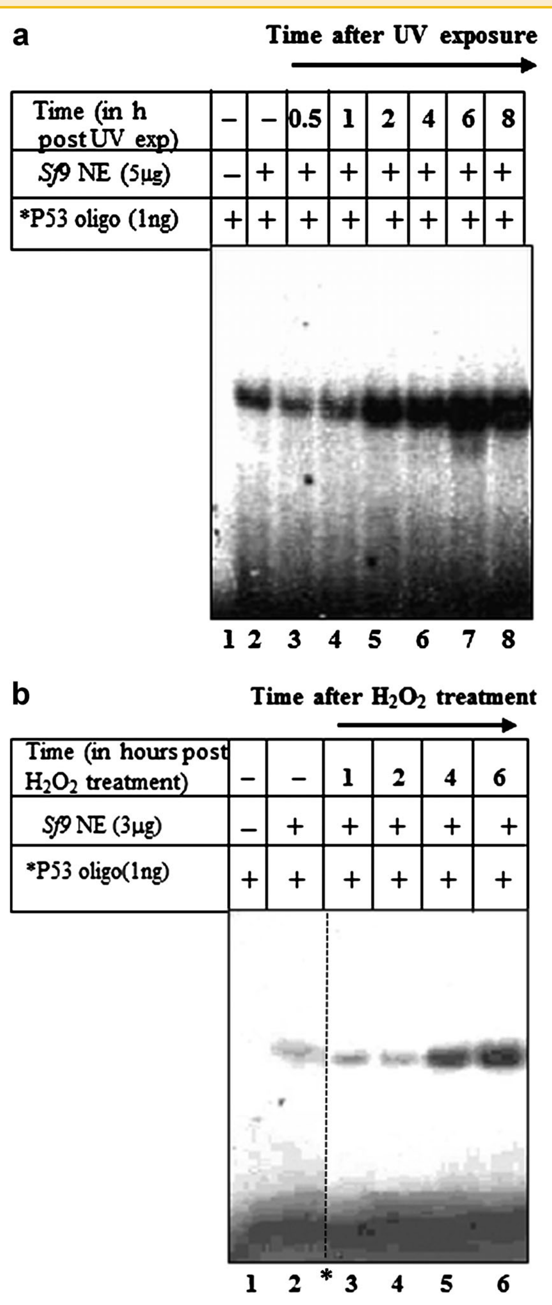


Fig. 2. *Sf*9 p53-like protein appears as a function of oxidative stress. EMSA using p53 consensus probe was carried out using 5 μg of nuclear extract prepared from *Sf*9 cells undergoing oxidative stress caused by UV-B irradiation (a), or exposure to H_2O_2 (b), for different times. The p53-like factor accumulates as a function of time of oxidative stress caused by either UV-B irradiation (a) or H_2O_2 (750 μM) treatment (b). *The dotted line indicates that these bands (1–2 and 3–6) were cropped and re-aligned from the same gel.

in competition assays (Fig. 3a, lanes 3, 8, and 11). These results categorically demonstrate that the consensus p53 recognition motif specifically binds to p53-like factor from *Sf*9 and this is similar to other species including Bombyx, Drosophila, and Human.

In order to determine the molecular size of the p53-like factor present in *Sf*9 nuclear extract, radio-labeled p53 consensus

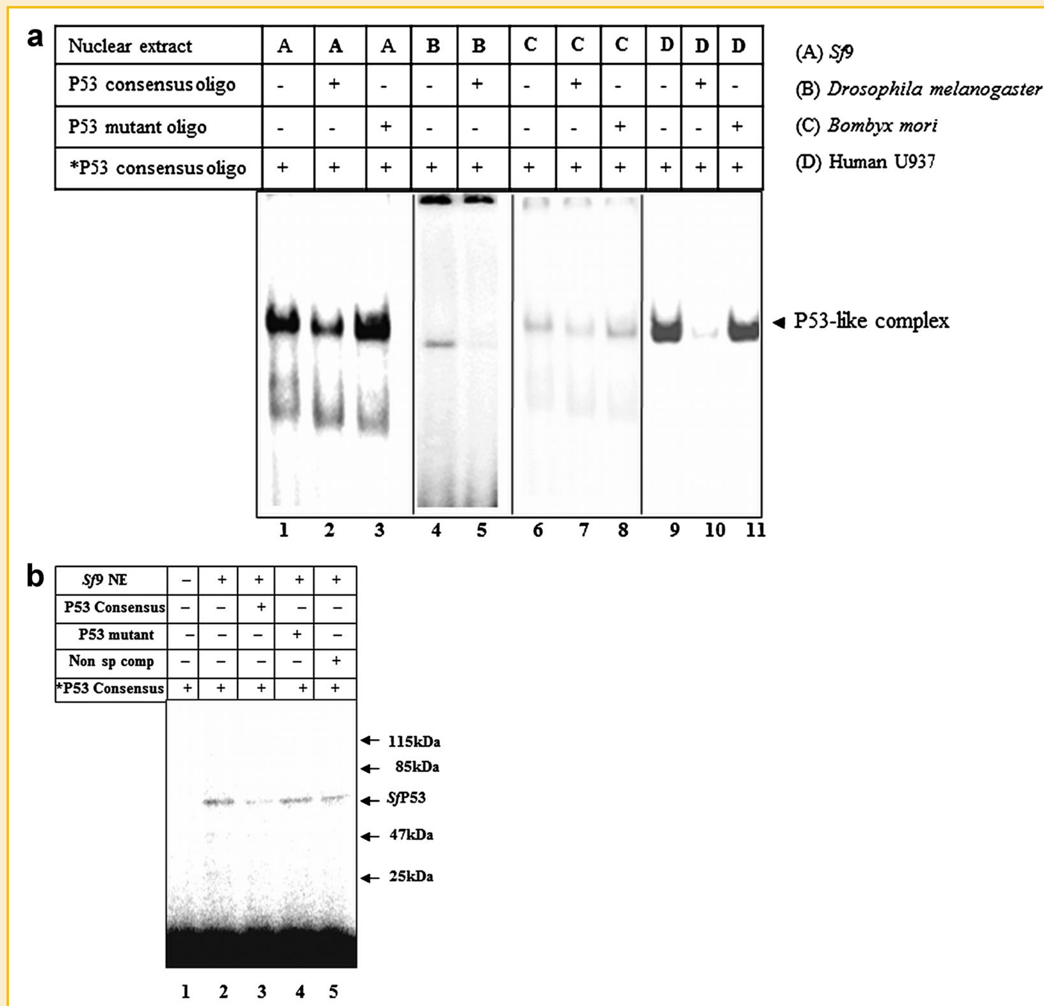


Fig. 3. *Sf*9 p53-like protein is comparable to insect and mammalian p53 in sequence specificity and molecular size. (a) EMSA was carried out using nuclear extract derived from *Sf*9 (5 μg, lanes 1–3) or Drosophila (3 μg, lanes 4 and 5) or *Bombyx mori* (3 μg, lanes 6–8), or human promonocytic U937 cells (5 μg, lanes 9–11) and consensus p53 probe. The different lanes are: 1, 4, 6, and 9, DNA–protein complex with the respective nuclear extracts; 2, 5, 7, 10, complex in the presence of homologous p53 competitor; 3, 8, 11, competition by an oligodeoxyribonucleotide carrying a mutation in the p53 binding motif. b: The *Sf*9 p53-motif binding protein complex was allowed to form as described in materials and methods, UV cross-linked, fractionated on a 10% denaturing polyacrylamide gel dried and finally exposed in phosphor imager cassette. The different lanes are: 1, no nuclear extract; 2, *Sf*9 protein complex; 3, complex in the presence of homologous p53 competitor; 4, complex in presence p53 binding motif mutant oligo; 5, complex in the presence of non-specific competitor. A protein complex in the size range of 50–60 kDa is visible in the autoradiograph.

oligonucleotide was incubated with 5 μg of *Sf*9 nuclear extract followed by UV cross-linking of the DNA–protein complex. The reaction product was then fractionated by electrophoresis on a 10% PAGE (Fig. 3b). The p53 motif-binding protein from *Sf*9 appears to be around 50 kDa (Fig. 3b, lane 2) which is competed out in presence of cold competitor (Fig. 3b, lane 3) but not when mutant oligonucleotide carrying a mutation in the p53 cognate motif was used (Fig. 3b, lane 4) or when non-specific competitor was used (Fig. 3b, lane 5). Together, these results point to the apparent similarity of *Sf*p53-like protein to that of mammalian systems.

Sfp53 MOTIF BINDING PROTEIN BINDS TO DIFFERENT p53 COGNATE MOTIFS

In order to check whether the p53-like protein present in *Sf*9 cells interacts with various other p53 cognate motifs, EMSA was carried out using different p53 cognate sequence elements as probes (Fig. 4). DNA–protein complex formed using oligodeoxyribonucleotides corresponding to the consensus p53 cognate motif (p53Con); a predicted p53 cognate motif present within the regulatory sequence of the baculovirus *Ac*NPV anti-apoptotic *p35* gene (*p35*–p53) [Mohareer et al., 2011], and p53 cognate motif within the regulatory sequence of Drosophila *decay* gene were compared. The *decay* gene is a caspase analogue of Drosophila. The *Sf*p53-like protein forms a complex (shown by arrow head) when oligonucleotides corresponding to Drosophila *decay* element (Fig. 4, lanes 14–18), which is comparable to that obtained using consensus p53 motif (Fig. 4, lanes 2–6). Also, when the p53 element identified in the viral genome (*Ac*MNPV), upstream of *p35* gene at −1,915 (GACATGCGTTTAAA-CATGTT) with respect to *p35* translation start site was used, a similar complex was evident (Fig. 4, compare lanes 2–6 with lanes 8–12). Inter element competition shows different affinities of the *Sf*p53-like factor to these elements, based on the band intensities. The

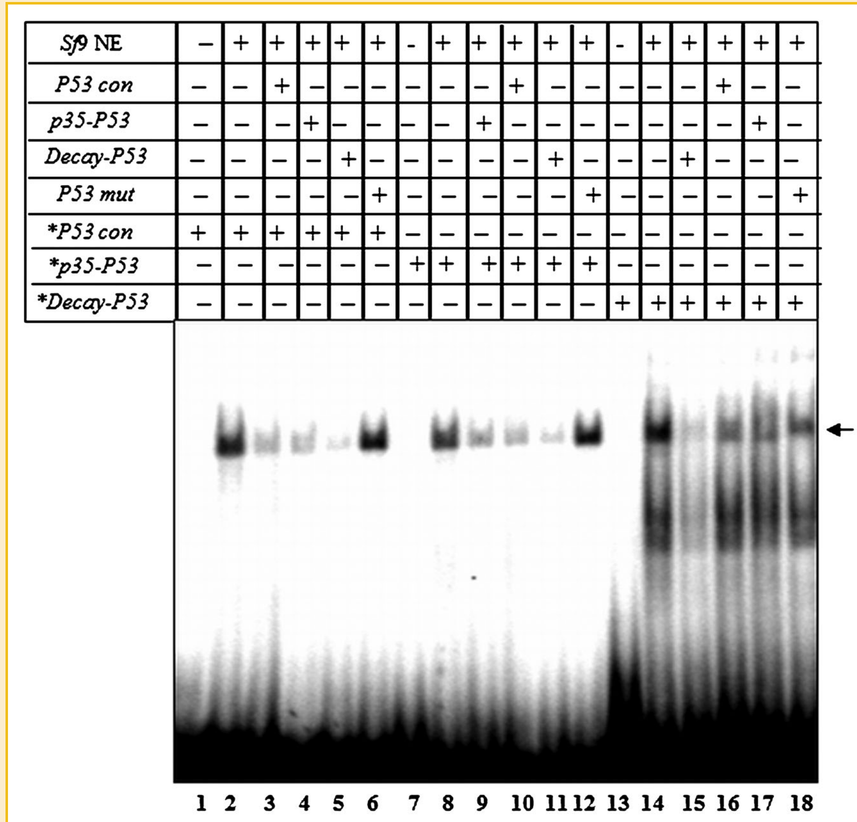


Fig. 4. *Sf*p53-like protein binds to different cognate elements. The *Sf*–p53 motif binding protein forms a complex with different intensities to native and heterologous p53 elements. EMSA with homologous and heterologous competitors was carried out using 5 μg of *Sf*9 nuclear extract. Different labeled oligonucleotides used for complex formation include consensus p53 (lanes 1–6), *p35*–p53 (lanes 7–12) and Drosophila *decay*–p53 (lanes 13–18). Both homologous (lanes 3, 9, and 15) and heterologous (lanes 4–6, 10–12, and 16–18) competitions were carried out using the corresponding oligonucleotide to demonstrate the specificity of the complex. When oligonucleotide carrying mutation at the conserved position critical for binding was used, no competition was observed (lanes 6, 12, and 18) pointing to the specificity of the p53–DNA complexes derived from different p53 cognate elements.

*Sf*p53-like factor shows maximum affinity to Drosophila *decay* (it almost completely competes out p53 consensus (lane 5) and *p35*–p53 (lane 11) in addition to itself (lane 15). *p35*–p53 almost competes p53 consensus (lane 4) and itself (lane 9) but competes with *decay* p53 element to a much less extent (lane 17). Consensus p53 motif also competes efficiently with *p35*–p53 (compare lane 10 with lane 8) and itself (compare lane 3 with lane 2) but competes with *decay* p53 element to a much less extent (lane 16). However, mutant p53 element does not compete with any of these elements (lanes 6, 12, and 18); showing that this complex represents an authentic p53-like factor. Taken together, these results demonstrate that *Sf*9 nuclear extract contains a p53-like factor, which binds to the cognate motifs represented by the consensus sequence or those derived from Drosophila or the Baculoviral *p35* gene upstream element.

Sfp53-LIKE PROTEIN BINDS STRONGLY TO COGNATE BINDING MOTIFS AND REQUIRES BOTH MONOVALENT AND DIVALENT CATIONS

To assess the stability of the *Sf*p53-like protein:DNA complex under varying salt (NaCl and KCl) conditions, EMSA with *Sf*9 nuclear extract was carried out in the presence of increasing concentration of NaCl (0.5–2 M). The DNA-*Sf*p53-like factor complex remains unaffected even up to a concentration of 2 M NaCl (Fig. 5a, lanes 1–3). Similarly when EMSA was carried out in the presence of increasing concentration of KCl up to 2 M (Fig. 5b, lanes 1–6), the binding was stable and in fact increased up to 0.5 M after which the intensity of the complex gradually decreased. To investigate the requirement of a divalent metal ion in the DNA binding activity of *Sf*9 p53 motif-binding protein, EMSA was carried out using 5 μg of *Sf*9 nuclear extract in the presence of increasing concentration (up to 100 mM) of divalent metal ion chelator, EDTA. At concentrations of 25 mM EDTA and above, the binding of *Sf*p53 motif-binding protein to cognate motif was completely abolished (Fig. 5c, lanes 4–6), demonstrating that divalent cations are essential for DNA binding activity.

Sfp53-LIKE PROTEIN HAS A K_D OF 7.5 NMOL

To determine the dissociation constant of *Sf*p53 DNA binding to consensus p53, EMSA was carried out with 5 μg of *Sf*9 nuclear extract and decreasing concentration of probe (1–0.625 ng). The dissociation constant of a ligand is defined as half the concentration

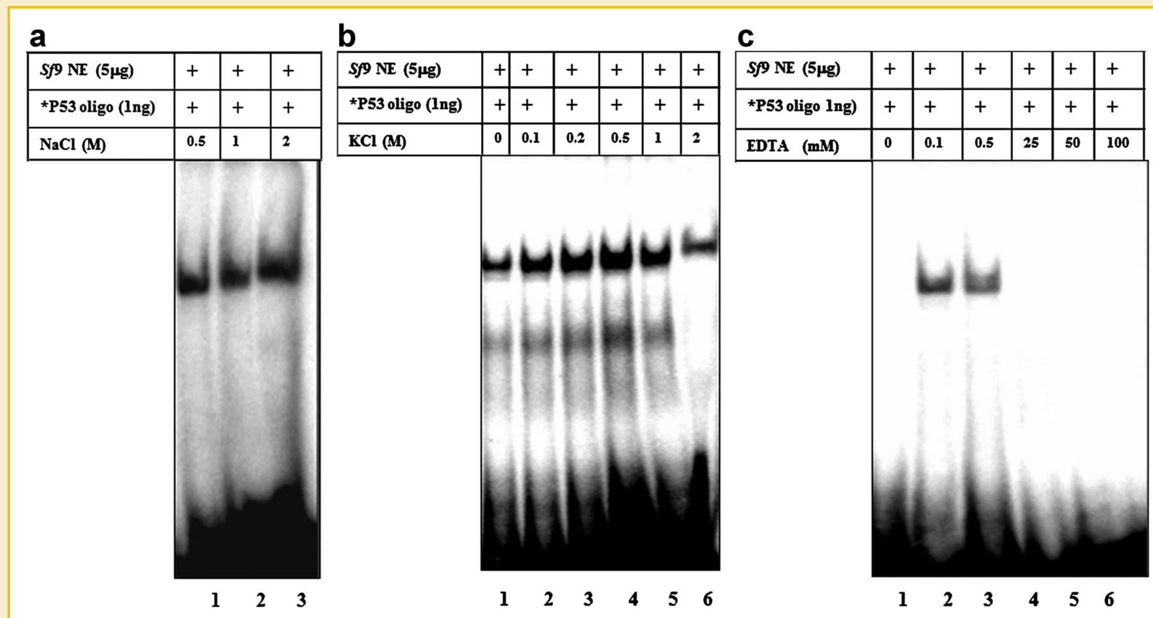


Fig. 5. *Sf*p53-like protein binds strongly to cognate motifs and requires both monovalent and divalent ions. EMSA was carried out with 5 µg of *Sf*9 nuclear extract and consensus p53 probe in the presence of increasing concentration of sodium chloride (panel a, lanes 1–3), or KCl (panel b, lanes 1–6), or EDTA (panel c, lanes 1–6).

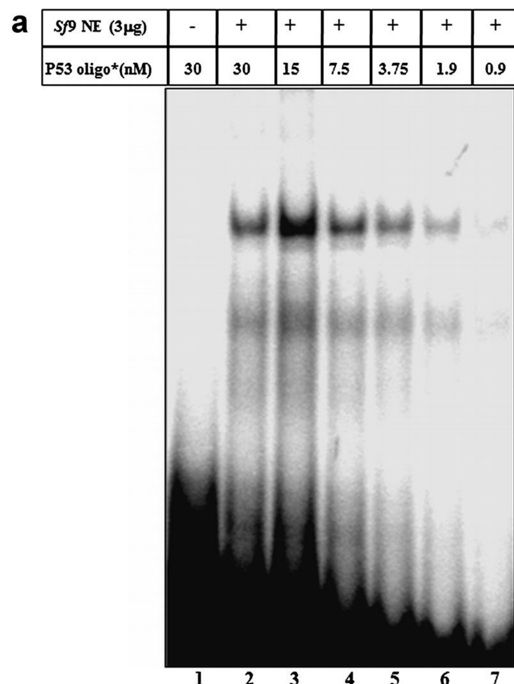
at which it reaches saturation. The complex intensity decreases as a function of probe concentration (Fig. 6a, lanes 2–7). Plotting free versus bound/free (Fig. 6b), reveals that the complex saturates at 0.5 ng equivalent to 7.5 nmol. Hence the K_d for *Sf*p53-like protein–DNA is 7.5 nmol. Together these data suggest a strong binding of *Sf*p53-like protein to the cognate DNA motif displaying a K_d value similar to p53 of higher vertebrates and requiring both monovalent and divalent ions for DNA binding.

Sfp53-LIKE PROTEIN APPEARS TO BE IDENTICAL TO FK-506 BINDING PROTEIN

Having biochemically characterized the p53 motif binding protein in *Sf* cells; we sought to identify the protein by purification and subsequent N-terminal sequencing. For this, *Sf*9 cells were grown in large scale in suspension culture as described [O'Reilly et al., 1992]. Since there is enhanced protein translocation into nucleus upon oxidative stress induction (Fig. 2), the p53-like binding protein was purified from *Sf*9 cells exposed to either UV-B irradiation for 90 s or treated with 1 mM H_2O_2 for 6 h so as to achieve maximum yield. The cells were harvested at 2,000 rpm at 4°C for 10 min and processed for protein purification as described in Materials and Methods Section. The 400 and 500 mM Q-sepharose fractions (Fig. 7) were diluted to 100 mM NaCl and further subjected to Heparin-sepharose chromatography. Again, the 400 and 500 mM NaCl fractions containing the p53-like protein, as evident from EMSA, were collected and the major band at around 50 kDa in 400 and 500 mM NaCl elution fractions which corresponded to DNA binding active fraction was excised out and sequenced at the Australian Proteomics Facility (APAF). The N-terminal sequence (MFWGLIMEPNKR) of the protein revealed its identity as FK-506 binding protein as *Sf*9 FKBP-46 upon NCBI BLAST analysis (with a score of 47.3), which had no other homologous proteins.

DISCUSSION

Having earlier demonstrated the utility of *S. frugiperda* insect cells as a model for studying oxidative stress mediated cell death, we investigated the presence of a p53-like factor in *Sf*9 cells. In the present work, we report the existence, preliminary characterization and activation of a p53-like factor in *Sf*9 cells based on electrophoretic mobility shift assays and proteomics approach. The presence of p53-like factor was suggestive from the work of Bae et al. [1995] and Suman et al. [2009] where the induction of a p53-like factor was shown by Western analyses based on cross reactivity of *Sf*9 insect cell extracts with anti-human p53 polyclonal antibodies. Identification of p53-like factor in insect cells was authenticated by the appearance of specific DNA:protein complex using consensus human P53 cognate motif by EMSA (Fig. 1). The DNA–protein complex appeared to be similar both in size and specificity to that of Drosophila, Bombyx, and human p53 as evident from EMSA (Fig. 3A) and UV cross-linking (Fig. 3B). The *Sf*9 p53 motif-binding protein also appears to be similar in molecular size to that of murine p53 (data not shown). The *Sf*p53-like protein also binds to various p53 cognate elements derived from Drosophila and *Ac*MNPV specifically (Fig. 4), although with different affinities. Different DNA binding parameters including salt tolerance (Fig. 5a,b), metal ion requirement (Fig. 5c), dissociation constant (Fig. 6a,b), role of cysteine and histidine residues (Supplementary Fig. 1) and groove binding preferences (data not shown) of the *Spodoptera* p53 motif binding protein were carried out which collectively demonstrated a strong resemblance of *Sf*p53-like protein to the vertebrate p53 in several respects. The *Sf*p53-like protein:DNA complex showed a high tolerance to salt even up to 2 M NaCl and 2 M KCl. Vertebrate p53 DNA binding domain involves



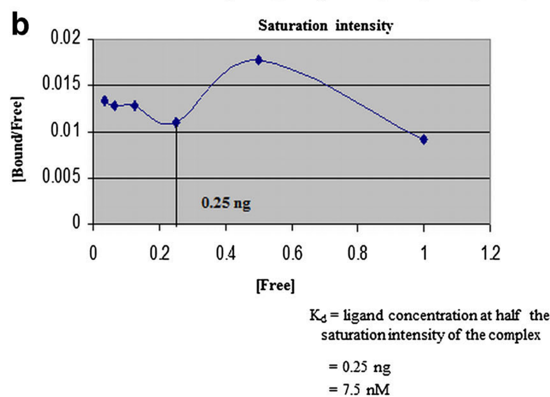


Fig. 6. *Sf*p53-like protein has a K_d value of 7.5 nmol. EMSA was carried out with 5 μg of *Sf*9 nuclear extract in the presence of decreasing amount of p53 consensus probe (panel a, lanes 1–7). Plot of free versus bound/free shows a sigmoid curve with peak saturation at 0.5 ng of probe (panel b).

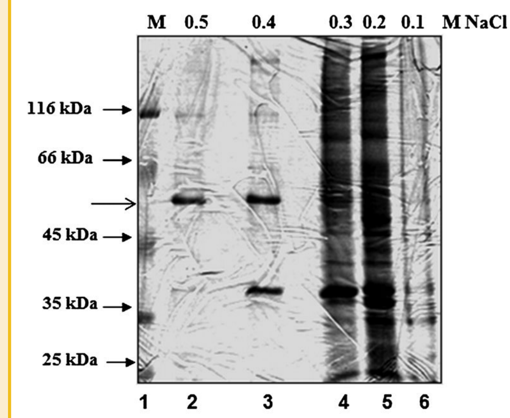


Fig. 7. Purification of *Sf*p53-like protein and N-terminal sequencing revealed identity to FKBP-506 binding protein. The p53 motif-binding protein from *Sf*9 nuclear extract was purified and eluted (lanes 1–6) as described in experimental procedures. The 400 and 500 mM fractions containing the protein were further purified by Heparin-sepharose chromatography. The proteins were subjected to N-terminal sequencing at APAF. The N-terminal sequence of *Sf*p53-like protein was found to be MFWGLIMEPNKR, which is homologous to *Spodoptera frugiperda* FKBP-46.

co-ordination of Zn^{2+} ion with two amino acid residues each of cysteine and histidine to bind to DNA. By modification of either free cysteine (iodoacetamide) or histidine (DEPC) residues or by chelation of Zn^{2+} (EDTA), the DNA binding activity of *Sf*p53-like factor was abolished as a function of its concentration (Fig. 5c), suggesting the role of both cysteine and histidine residues and Zn^{2+} ions in binding to DNA. Also most p53 homologues differ in sequence identity but show similar recognition motifs.

The protein upon purification and sequencing revealed its N-terminal identity to FKBP-46, which was earlier identified in *Sf*9 cells as co-purificant with casein kinase [Alnemri et al., 1994]. FKBP shares sequence homology with DNA binding domain of p53 [Siemion et al., 1998], so there is great likelihood of FKBP binding to similar recognition motif as p53 as shown in the study. FKBP has also been reported as the target of *Sf* caspase, which cleaves it into 25 and 21 kDa fragments during apoptosis [Ahmad et al., 1997]. The interaction of FKBP with casein kinase points to the role of phosphorylation in regulation of the activity of this protein. FKBPs are evolutionarily conserved proteins which catalyze the inter-conversion of *cis* and *trans* rotamers of peptidyl-prolyl amide bonds of peptide substrates [Göthel and Marahiel, 1999].

FKBPs from the mammalian counterparts function in a wide range of cellular activities. The origin of its name however comes from its ability to bind to the drug FK-506 which is an immune-suppressant and inhibits T-cell activation. These proteins act as molecular chaperones and assist in protein folding and also inhibit cell cycle progression [Somarelli et al., 2007]. FKBP-12 (neuroimmunophilin) has been shown to be protective against neuronal degeneration and is increased in experimental model of Parkinsons disease [Nilsson et al., 2007]. FKBPs also possess nucleic acid binding ability (both DNA and RNA) [Somarelli et al., 2007]. Interestingly, FKBP from Chinese cabbage, which has striking degree of identity with human, mouse, and yeast FKBP, has anti-fungal properties [Park et al., 2007].

FKBP is also being considered as a new member of the histone deacetylase (HDAC) family based on its partial sequence homology to HDAC family proteins, pointing to a new role of this protein in transcription [Aravind, 1998]. It has also been shown to physically interact with HDAC through transcription factor YY1 [Wen-Ming et al., 2001]. FKBP-25 of human origin is inhibited by hepatitis C virus protein NS5A and thereby evades apoptosis induced by FKBP [Wang et al., 2006]. Based on the scattered evidences of its role in transcription and apoptosis, and with the present evidence of its sequence specific binding to p53 cognate motif, we suggest a novel role for this protein as a p53 functional analogue in insects.

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