Heat shock transcription factor 1 (HSF1) functions as the master regulator of the heat shock response in eukaryotes. We have previously shown that, in addition to its role as a transcription factor, HSF1 stimulates the activity of the DNA-dependent protein kinase (DNA-PK). DNA-PK is composed of two components: a 460-kDa catalytic subunit and a 70- and 86-kDa heterodimeric regulatory component, also known as the Ku protein. We report here that HSF1 binds specifically to each of the two components of DNA-PK. Binding occurs in the absence of DNA. The complex with the Ku protein is stable and forms at a stoichiometry close to unity between the Ku protein heterodimer and the active HSF1 trimer. The binding is blocked by antibodies against HSF1. Our results show that HSF1 also binds directly, but more weakly, to the catalytic subunit of DNA-PK. Both interactions are dependent on a specific region within the HSF1 regulatory domain. This sequence is necessary but not sufficient for HSF1 stimulation of DNA-PK activity. The ability of HSF1 to interact with both components of DNA-PK provides a potential mechanism for the activation of DNA-PK in response to heat and other forms of stress.

Prior to stress, heat shock transcription factor 1 (HSF1) is present in human and other metazoan cells in a latent, monomeric form (1–4). In response to heat and certain other forms of stress, HSF1 undergoes a conformational change to form an active trimer (5–11). The trimeric form of HSF1 binds to heat shock elements in DNA and activates transcription of heat shock genes by RNA polymerase II. The functional domains of HSF1 have been delineated by mutagenesis. A sequence near the N terminus forms the DNA binding domain (12). Adjacent to this is a 4/3 hydrophobic repeat or "leucine zipper" that mediates trimerization (9, 13, 14). The central part of the molecule contains several elements that maintain HSF1 in its latent form (5, 9) or that regulate the activity of transcriptional activation domains in response to stress (15–18). Sequences within the regulatory domain undergo specific phosphorylation and dephosphorylation in response to stress (19–21). The C-terminal portion of HSF1 contains the main transcriptional activation regions (15–18).

HSF1 interacts extensively with the cellular signal transduction machinery. The temperature at which HSF1 is activated is modified in response to the inflammatory mediator, arachidonate, which also induces changes in HSF1 phosphorylation (22). HSF1 is a target of mitogen-activated protein kinases, and its activity is down-regulated when the ras signaling cascade is active (19–21, 23–25). HSF1 also interacts with the DNA-dependent protein kinase (26). In vitro experiments show that HSF1 is both a target of DNA-PK phosphorylation and an activator of DNA-PK, inducing DNA-PK to phosphorylate other substrates.

DNA-PK has a well-established role in the repair of DNA damage but is suspected to have other functions as well (reviewed in Refs. 27 and 28). DNA-PK consists of two components, a 460-kDa catalytic subunit (DNA-PKcs) and a 70- and 86-kDa heterodimeric regulatory component, the Ku protein (29, 30). HSF1 activates preparations of the DNA-PK catalytic subunit containing little or no Ku protein, suggesting that there is a direct functional interaction between HSF1 and the catalytic subunit (26). However, unlike the Ku protein, it does not appear that HSF1 recruits DNA-PKcs into a stable, DNA-bound complex. Thus, HSF1 does not replace Ku protein in the reaction but instead works by a different mechanism. Consistent with this, HSF1 cooperates with Ku protein in vitro to give a multiplicative activation of DNA-PK activity (26). A truncated form of HSF1 containing only the DNA binding domain does not activate DNA-PK, suggesting that the interaction between these proteins is dependent on specific amino acid sequences within HSF1 (26).

In vivo experiments support the idea that DNA-PK is involved in the heat shock response, although the results are somewhat complex. Overexpression of the 70-kDa subunit of human Ku protein in rat cells suppresses expression of heat shock protein 70 but not other heat shock proteins (31, 32), while expression of the 86-kDa subunit of human Ku protein does not have this effect (31). It has been proposed that the Ku protein binds to heat shock elements (HSEs) and displaces HSF1, resulting in repression of hsp70 gene expression (33). Consistent with this, Ku protein binds competitively with HSF1 to HSE-containing oligonucleotides (33). However, this may reflect nonspecific binding of Ku protein to DNA ends, since the sequence specificity of the Ku protein-HSE interaction has not been established, and other workers have recently shown that Ku protein does not bind to HSE sequences in DNA.
lacking double strand breaks (33). These findings suggest that the mechanism of Ku-mediated repression of heat shock gene expression may be different than originally proposed. One possibility, for example, is that overexpression of the Ku70 subunit interferes with the assembly of Ku protein into functional complexes with other components of the cellular signal transduction apparatus.

In the present study, we have further characterized the functional interaction between HSF1 and DNA-PK. We have developed an antigen capture assay that allows measurement of protein-protein interactions between HSF1 and DNA-PK components. We find that HSF1 forms a specific, stable complex with Ku protein in the absence of DNA. HSF1 also appears to interact more weakly with the catalytic subunit of DNA-PK.

We suggest that these interactions may provide a mechanism for the activation of DNA-PK in vivo under stress conditions.

MATERIALS AND METHODS

Construction of HSF1-(1–450) and Mutant Derivatives—HSF1-(1–450) consists of the first 450 of the 529 amino acids of HSF1, with a His6 tag at the carboxyl terminus to facilitate purification (25). The HSF1-(1–450) protein was indistinguishable from wild-type HSF1 in its DNA binding properties and in its ability to activate DNA-PK. In-frame deletion mutants were derived from HSF1-(1–450) as diagrammed in Fig. 1. Details of the plasmid construction have been previously published (25).

Protein Purification—Native DNA-PKcs and Ku protein were purified from HeLa cell nuclear extracts as described previously, except that the phenyl-Superose and Mono S steps were omitted (35).

To produce recombinant Ku protein, a 100-ml suspension culture of SB cells was grown to a density of 1.5 × 10^8 cells/ml and was co-infected with VBB2–86Ku and VBB270tH6 (36). After 4 days, cells were collected by centrifugation, lysed by freeze-thawing twice, resuspended in 10 ml of ice-cold Buffer A (50 mM phosphate buffer, pH 8.0, 500 mM NaCl, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride, 20 μM dithiothreitol, and 10% glycerol), and centrifuged for 30 min at 12,000 × g. The pellet was dissolved in 10 ml of Buffer A with 10% glycerol, insoluble material was removed by centrifugation for 10 min at 12,000 × g, and the supernatant was mixed with 2 ml of Ni2+-nitrilotriacetic acid-agarose (Quiagen 30230) at 4 °C. After 1 h of incubation, the mix was poured through the column, washed sequentially with 10 ml of Buffer A, 20 ml of Buffer B (50 mM phosphate buffer, pH 7.0, 300 mM NaCl, 5 mM benzamidine, 1 mM phenylmethylsulfonyl fluoride), and 20 ml of Buffer B containing 10 mM imidazole. Ku protein was eluted with a linear gradient of Buffer B containing 10–500 mM imidazole. Ku-containing fractions were concentrated by ultracentrifugation (Amicon YM10 membrane) and subjected to size exclusion chromatography using a Superdex-200 column (Pharmacia Biotech Inc., HR16/60) equilibrated in Buffer CB (50 mM Tris, pH 7.9, 1 mM EDTA, 5% glycerol, 0.1% Tween 20, 1 mM dithiothreitol, 0.1 mM EDTA, and washed three times with PBST. Absorbance was read at 655 nm. The amount of bound HSF1 was calculated by comparison to standards using Microplate Manager III software (Bio-Rad).

GCTD Phosphorylation Assay—Phosphorylation assays were performed as described (26, 35) with minor modifications. Reactions contained 0.2 nm of a 309-base pair BglI-EspI fragment of pHSpA50HSE1, containing a single binding site for an HSF1 trimer, which is sufficient to allow DNA-PK stimulation, provided that it is not located at the extreme end of the fragment. Reactions also contained 70 mM GCTD fusion protein, 0.65 mM DNA-PKcs, 1.3 μM Ku protein, 12.5 μM [γ-32P]ATP (8 Ci/mmol), 25 mM Tris-HCl, pH 7.9, 50 mM KCl, 6.25 mM MgCl2, 0.5 mM EDTA, 0.5 mM dithiothreitol, 10% glycerol, and HSF1 as described in the figure legends, in a 30-μl reaction volume. To assemble the reactions, HSF1 was incubated with DNA and GCTD for 20 min at 30 °C, Ku protein was added, and incubation was continued for 10 min. Then DNA-PKcs was added, immediately followed by ATP. Reactions were incubated for 40 min at 30 °C and analyzed by 7.5% SDS-PAGE.

RESULTS

Construction and Expression of HSF1 Mutants—In a previous study, we showed that human HSF1 stimulated the activity of purified DNA-PK in an in vitro reaction. In contrast to the wild-type protein, a mutant protein containing only the minimal DNA binding and trimerization domains (HSF1 M3), failed to stimulate DNA-PK activity, suggesting that sequences in the regulatory or transcriptional activation domains of HSF1 were required for interaction with DNA-PK (26).

We wished to delineate these sequences more precisely as well as to better define the mechanism of interaction between HSF1 and DNA-PK. To accomplish this, a new series of HSF1 mutants was expressed and purified. All constructs were based on HSF1-(1–450), which contains the first 450 amino acids of HSF1 fused to a C-terminal His6 tag. The mutants contained specific deletions as diagrammed in Fig. 1A. These proteins were expressed in E. coli and purified by Ni2+ affinity and Superdex S-200 chromatography (Fig. 1B). As expected for bacteriaally expressed HSF1, the proteins were constitutively active for DNA binding (Fig. 1C). All of the proteins appeared to form heterotrimers, as judged by gel filtration chromatography (not shown). Fig. 1B also shows the Ku protein, DNA-PKcs, and GCTD substrate used in these studies. All proteins appeared homogeneous as judged by SDS-PAGE analysis.

Development of an Antigen Capture Assay for HSF1—To measure the ability of various HSF1 mutants to interact with DNA-PK components, we developed an antigen capture assay based on ELISA technology. Preliminary experiments (not shown) using a nitrocellulose filter dot-blot technique suggested that there was a stable interaction between HSF1 and

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the Ku protein regulatory component of DNA-PK. Therefore, our initial attempts to develop the ELISA assay focused on Ku protein as the target for HSF1 binding.

Different amounts of purified Ku protein were added to individual wells of a 96-well microtiter plate and incubated to allow protein adsorption (see “Materials and Methods”). The plate was then blocked with excess bovine serum albumin, and HSF1 was added. After a further period of incubation, the plate was washed, and HSF1 capture was measured by ELISA using an anti-HSF1 antibody. This assay permitted direct measurement of HSF1-Ku protein interaction in the absence of DNA. Moreover, the 96-well ELISA format allowed us to vary a large number of reaction parameters and to obtain quantitative results.

Representative data using HSF1-(1–450) are shown in Fig. 2. One of the most important assay variables proved to be the pH in the binding reaction. At a pH of 7.2, HSF1 was captured on the plate in a reproducible, Ku-dependent manner. HSF1 capture increased as a function of the amount of Ku protein added to the well, approaching a maximum when 10–20 ng of Ku protein was present. In contrast to the results at pH 7.2, very little binding was apparent at pH 6.7, 7.9, or 8.5. It should be noted, however, that the background of HSF1 bound in the absence of Ku protein was much higher at these pH values (see legend to Fig. 2). This background has been subtracted in all panels of Fig. 2. The high background may have obscured specific binding at pH values other than 7.2.

The concentration of monovalent and divalent cations in the binding reaction also had a large effect on the assay results. The binding of HSF1 to Ku protein was inhibited by KCl concentrations above 100 mM and by MgCl₂ concentrations above 12.5 mM (Fig. 2, B and C). These results are suggestive of a significant electrostatic contribution to the free energy of HSF1-Ku protein interaction. Low concentrations of monovalent and divalent cations in the binding reaction generally led to somewhat higher backgrounds of HSF1 bound in the absence of Ku protein, although the effects were smaller than with pH (see Fig. 2 legend). Based on the results of our experiments, we adopted standard binding conditions of pH 7.2, 50 mM KCl, and 6.25 mM MgCl₂, which allowed high efficiency HSF1 capture with minimal background.

The complexes formed between HSF1 and Ku protein are stable to extensive washing and incubation following the binding. The interaction appears to be highly specific. There is little binding of HSF1 to the bovine serum albumin that is present in all wells as a blocking agent. There is also little binding to proteins in nonfat milk, an alternative blocking agent (data not shown).

As an additional test of specificity, we performed the binding assay in the presence of polyclonal anti-HSF1 antiserum. The presence of antibody blocked the capture of HSF1 on the plate. The effect was dependent on the concentration of antibody (Fig. 3). Control nonimmune serum had no effect on HSF1 binding. These results suggest that interaction between HSF1 and Ku protein was dependent on the accessibility of specific epitopes that could be blocked by preincubation with antibody.

Binding of HSF1-(1–450) and Mutant Derivatives to Ku Protein—To identify the specific sequences within HSF1 that are required for binding to Ku protein, we performed studies with mutant derivatives of HSF1. In these experiments, it was important to take into account potential differences in antibody reactivity of the various mutants. To minimize these differences, in most experiments we used an antiseraum directed against an invariant peptide at the C terminus of HSF1-(1–450). To correct for residual differences in reactivity, each set of assays included standards, where known amounts of HSF1-(1–450) were bound directly to the plate prior to blocking with bovine serum albumin and detected with anti-HSF1 antibody. Separate standard curves were constructed for each mutant as described under “Materials and Methods.” Quantitation of HSF1 by this procedure was considered reliable in the range of 0–20 ng.

The results of quantitative binding studies using HSF1-(1–450) and various derivatives are shown in Fig. 4. The binding of HSF1-(1–450) and Δ24 was approximately equal, whereas the binding of HSF1 Δ01 and Δ12 was severely reduced (Fig. 4A). In a separate experiment, which used a different antibody capable of detecting HSF1 Δ36, binding of this mutant was also approximately equal to HSF1-(1–450). These results show that sequences missing from Δ24 and Δ36, i.e. sequences carboxyl to position 280, are not required for interaction of HSF1 with Ku protein. By contrast, sequences missing from Δ01 or Δ12, i.e.
sequences between positions 203 and 280, are essential for interaction with Ku protein. These sequences lie within the previously defined regulatory domain of HSF1 (15–18).

An experiment was also performed where the amount of Ku protein was held constant and the concentration of HSF1 in the assay was varied. In this experiment, HSF1-(1–450) and D
24 bound equivalently, and binding of HSF1
D01 and D12 was severely reduced (Fig. 4C). These results are consistent with the results in Fig. 4A.

An estimate of binding stoichiometry can be made from the data in Fig. 4C. The amount of bound HSF1-(1–450) approached a plateau as increasing amounts of this protein were added to the reaction. Saturation occurs with 10–15 ng of HSF1 bound to 4.8 ng of Ku protein, which is an apparent stoichiometry of approximately 2:1 (mol of HSF1 trimer:mol of Ku heterodimer). There are a number of potential sources of inaccuracy in this value, such that it may not be possible to distinguish a stoichiometry of 2:1 from 1:1. Nevertheless, the finding that binding is saturable with an apparent stoichiometry close to unity is consistent with a physiological interaction between HSF1 and Ku protein.

HSF1 Binds to Ku Protein and DNA-PKcs—To further explore the interaction between HSF1 and DNA-PK, antigen capture assays were performed using purified DNA-PKcs as the target. We observed capture of HSF1 on the plate that increased as the amount of DNA-PKcs was increased (Fig. 5A). The level of binding was much lower than with Ku protein, however (note difference in scale between Figs. 4 and 5). This indicates that there is a direct, although apparently weak, interaction between HSF1 and DNA-PKcs.

The amount of binding was severely decreased with HSF1 \( \Delta 01 \) and \( \Delta 12 \), the same mutants that are defective for binding to Ku.
protein. HSF1 Δ24 bound equivalently to HSF1-(1–450) wild type. Qualitatively similar results were obtained in experiments where the amount of Ku protein was held constant and the amount of HSF1 was varied (Fig. 5B), one difference being that in these experiments, HSF1 Δ24 bound even more avidly than HSF1-(1–450).

To confirm the results of these experiments and to demonstrate that binding was not attributable to contamination of
DNA-PKcs with Ku protein, we performed antigen capture assays using chromatographic fractions obtained in the final step of purification of DNA-PK from HeLa cells. These results show a distinct peak of HSF1 binding in fractions 28 and 29, corresponding to the peak of DNA-PKcs polypeptide (Fig. 5, compare closed symbols, panel C, with SDS-PAGE analysis, inset). The presence of this peak, which elutes at a position distinct from the peak of the Ku protein, shows that binding of HSF1 to DNA-PKcs cannot be attributed simply to contaminating Ku protein. This experiment also shows that HSF1 binds to native Ku protein about as well as to the recombinant Ku protein used in other experiments. When the HSF1 binding is normalized to the amount of protein present in the various chromatographic fractions, it can be seen that the amount of HSF1 bound per mol of DNA-PKcs was lower than for Ku protein, consistent with earlier results (compare open symbols in Fig. 5C with Fig. 4).

The maximum stoichiometry of binding of HSF1 to DNA-PKcs that we have observed in the experiments presented here is in the range of 1:2 to 1:6 (mol of HSF1 trimer:mol of DNA-PKcs). These values, which do not necessarily represent saturation, are 4–12-fold lower than the observed stoichiometry of binding of HSF1 to Ku protein. It may be that HSF1 binds to DNA-PKcs less stably, causing a loss of HSF1 during subsequent incubation and washing. Alternatively, it may be that only a fraction of the DNA-PKcs is active for HSF1 binding or that HSF1 that is in a complex with DNA-PKcs is less available for reaction with the antibody used to detect binding.

Stimulation of DNA-PK Activity by HSF1-(1–450) and Mutant Derivatives—The HSF1 derivatives were next tested for their ability to stimulate DNA-PK activity. In these assays, we incubated various amounts of each HSF1 derivative with DNA-PK in a reaction mix containing a reporter substrate. The reporter has multiple DNA-PK phosphorylation sites. We measured the increase in reporter phosphorylation as a function of HSF1 concentration. In previous studies, we have shown that the effect of HSF1 on DNA-PK activity is similar with any of several different reporter substrates (26). In the present study, we used a reporter substrate, GCTD, that contains sequences from the C-terminal domain of RNA polymerase II, which is phosphorylated processively at multiple sites to yield a product, GCTD0, that migrates markedly more slowly in SDS-PAGE than the starting material.

The results of these assays are shown in Fig. 6. In the absence of HSF1, there was some phosphorylation of Ku protein but very little phosphorylation of the GCTD reporter substrate (Fig. 6). The addition of HSF1-(1–450) to the reaction caused a progressive increase in the amount of the GCTD0 product. With 33 nM HSF1-(1–450), there was an approximately 20-fold increase in GCTD0 phosphorylation. This is similar to the results in previous experiments with recombinant wild-type HSF1 (26). HSF1-(1–450) differs slightly from the wild-type because of the C-terminal histidine tag and because of a deletion in the extreme C terminus, but neither of these modifications has a significant effect in this assay.

The mutants, HSF1 Δ01 and Δ12, had a reduced ability to stimulate DNA-PK. Stimulation was 30 and 50%, respectively, of the stimulation seen with HSF1-(1–450). The residual activity seen with these mutants was higher than expected, given that they had almost no binding activity in the antigen capture assay. It may be that colocalization of HSF1 and DNA-PK components on a fragment of DNA promotes weak functional interactions that cannot be detected in the direct binding assay.

Interestingly, the other mutants, HSF1 Δ24 and Δ36, also had a reduced activity relative to HSF1-(1–450), despite the fact that these mutants bind to Ku and DNA-PKcs at least as well as wild type HSF1-(1–450). We conclude from this that the HSF1 sequences that are required for binding to Ku protein and DNA-PKcs are necessary, but not sufficient, for stimulation of DNA-PK activity.

All of the HSF1 derivatives were themselves substrates for DNA-PK. Phosphorylation of HSF1-(1–450) caused a substantial decrease in electrophoretic mobility. Nonphosphorylated HSF1-(1–450) migrates at 62 kDa (Fig. 1B), whereas the fully phosphorylated form migrates at about 80 kDa (Fig. 6). The magnitude of the shift is suggestive of phosphorylation at multiple sites, and consistent with this, partially phosphorylated intermediates can be seen. The HSF1 mutants are also phosphorylated, although with different efficiencies. Notably, HSF1 Δ24 is phosphorylated much more efficiently than any of the other derivatives, although it is unable to stimulate DNA-PK to phosphorylate the GCTD reporter. These data show that the ability of an HSF1 derivative to serve as a phosphorylation substrate does not necessarily confer an ability to stimulate DNA-PK activity, and these two properties may even be inversely correlated.

The ability of DNA-PK to phosphorylate HSF1 complicates the interpretation of the DNA-PKcs-HSF1 binding data. For example, the enhanced binding of HSF1 Δ24, which is a better substrate than even wild-type HSF1-(1–450), could be due an avid interaction with the DNA-PKcs kinase active site. However, the failure of HSF Δ01 and Δ12 to bind, despite the fact that they are good substrates, suggests that substrate activity

![Fig. 6. Stimulation of DNA-PK activity by HSF1-(1–450) and mutant derivatives. In vitro phosphorylation reactions were performed using purified DNA-PK components, HSE-containing DNA fragment, and GCTD substrate as described under “Materials and Methods.” Reactions contained various amounts of HSF1-(1–450) and mutant derivatives in the indicated concentrations (nM). Radiolabeled products were analyzed by 7.5% SDS-PAGE and visualized by Molecular Dynamics PhosphorImager analysis. The position of phosphorylated Ku 70, phosphorylated Ku 86, and hyperphosphorylated GCTD0, are indicated. Singly phosphorylated GCTD0 which migrates near Ku 86, did not appear to be present. Phosphorylated HSF1 migrated as a series of bands in the range indicated, depending on the amount of phosphorylation and the size of each deletion mutant. The lower panel shows a quantitation of relative GCTD0 phosphorylation, normalized to the amount of product formed with 33 nM HSF1-(1–450).](image)
alone is not sufficient for formation of a stable complex in the antigen capture assay.

**Stimulation of DNA-PK Activity by HSF1 in the Absence of Ku Protein**—HSF1 derivatives were also tested for their ability to stimulate DNA-PKcs separately in the absence of Ku protein. Although DNA-PK activity is 5-fold lower in the absence of Ku protein, there is still substantial stimulation by HSF1 (Fig. 7A), consistent with the results of earlier work (26). It is difficult to be certain that no trace of Ku protein remains in these preparations of DNA-PKcs. No Ku protein autophosphorylation was detected, however. In addition, the DNA-PKcs fractions are from the leading edge of the chromatographic peak and are thus unlikely to be contaminated with Ku protein. (fraction 28; Fig. 5C, inset).

We next tested individual HSF1 mutant derivatives for their ability to stimulate DNA-PKcs in the absence of Ku protein. In general, the same pattern of relative activities was obtained in the absence of Ku protein as in its presence (compare Fig. 7B with Fig. 6). Thus, HSF1 Δ01 and Δ12 are still observed to be defective for DNA-PKcs stimulation even in the apparent absence of Ku protein. Close inspection of the results in Fig. 7 shows slight differences in the relative behavior of the different mutants in the absence of Ku protein. HSF1 Δ24 has almost no detectable activity, whereas HSF1 Δ01 is slightly more active than other mutants in the regulatory domain. These results suggest that the determinants of HSF1-stimulatory activity in the presence and absence of Ku protein may be slightly separable. The overall level of phosphorylation is very low in these experiments, however, making this interpretation tentative.

**DISCUSSION**

Previous work showed that the transcription factor HSF1, which regulates the heat shock response in eukaryotes, is capable of stimulating the activity of purified DNA-PK in an *in vitro* reaction (26). In these earlier studies, we were not able to define the mechanism of stimulation. We have now shown that HSF1 binds directly to each component of DNA-PK. HSF1 forms a stable complex with Ku protein, the regulatory component of DNA-PK, and to a lesser extent, forms a complex with DNA-PKcs. The complexes appear to be specific by biochemical criteria. Capture of HSF1 increases as increasing amounts of target protein are coated on the ELISA plate. There is little or no binding to the bovine serum albumin that is used to block the plate. Binding to Ku protein is inhibited when surface epitopes of HSF1 are blocked by preincubation with polyclonal antibody. Binding to Ku protein occurs with a fixed stoichiometry that is close to unity. Finally, binding to both Ku protein and DNA-PKcs is dependent on a sharply delineated sequence within the conserved regulatory domain of HSF1.

The HSF1 sequences that are required for binding to Ku protein and DNA-PKcs are also required for stimulation of DNA-PK activity. Two internal deletion mutants, HSF1 Δ01 and Δ12, are defective in both binding and stimulation. However, the correlation between binding and stimulation of activity is not absolute, since several other mutants bind well to Ku protein and DNA-PKcs but do not give fully wild-type levels of stimulation. It is possible that these mutants perturb the overall structure of HSF1, such that HSF1 retains the ability to bind to individual DNA-PK components but is unable to assume the correct geometry in a larger complex containing both protein components and DNA.

It appears that the same small region of HSF1, defined by HSF1 Δ01 and Δ12, is critically important for binding to both Ku protein and DNA-PKcs. This does not imply that the contacts made by Ku protein and DNA-PKcs in this 76-amino acid region are necessarily identical. For example, each component could interact with different surfaces of the same domain. The ability of HSF1 to cooperate with Ku protein to give a synergistic effect on DNA-PK activity suggests that all of these proteins can probably interact with each other simultaneously. Technical limitations of the antigen capture assay, including very high levels of background binding when DNA-PKcs is present in the soluble phase, have prevented us from testing this directly. We hope to develop alternative methods of meas-
uring binding, however, that will allow us to address this question in the future.

The finding that HSF1 stimulates DNA-PK in the apparent absence of the Ku protein suggests that the interaction of HSF1 with the catalytic subunit is the critical determinant of activation under the conditions used for the phosphorylation assay. The conditions of this assay are quite different from those that are present in the cell nucleus, however. In particular, the in vitro phosphorylation assay system contains linear DNA, which is, in itself, capable of inducing the formation of active Ku protein-DNA-PKcs complexes at fragment ends. The addition of HSF1, in essence, is superactivating the DNA-PK. By contrast, under conditions of heat shock in vitro, there is very little DNA fragmentation, and hence no opportunity for formation of complexes at DNA ends. Under these conditions, the protein-protein interaction of HSF1 with both Ku protein and DNA-PKcs may be critical. Ku protein and DNA-PKcs do not ordinarily bind one another in the absence of DNA breaks (35, 38). Because HSF1 is capable of binding both components in the absence of DNA, it may provide a mechanism for assembling the two components of DNA-PK into an active complex in the absence of DNA damage.

HSF1 has a well established function as a transcription factor. It is of interest whether the interaction of HSF1 with DNA-PK is important for this function as a transcriptional activator or whether it reflects an additional, nontranscriptional function for HSF1. Our present results show that the region of HSF1 that is required for binding to Ku protein and DNA-PKcs is separate from the HSF1 transcriptional activation domains that have been mapped in previous studies (15–18), which suggests that the interaction with DNA-PK is not directly related to the transcriptional activation function of HSF1. Consistent with this, when cells with a genetic deficiency in DNA-PKcs are subjected to heat shock, they synthesize an initial burst of RNA from the hsp70 promoter with normal kinetics. However, these cells are much more sensitive to heat-induced apoptosis. Taken together, these data strongly suggest that the interaction of HSF1 with DNA-PK affects a process that is distinct from the immediate effects of HSF1 on transcription. It is of interest whether the interaction of HSF1 with DNA-PK effects some other property of HSF1, however, including the interaction with DNA-PK itself.

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