The EMBO Journal vol.14 no.1 pp.117–123, 1995

Determination of heat-shock transcription factor 2 stoichiometry at looped DNA complexes using scanning force microscopy

Claire Wyman, Eva Grotkopp, Carlos Bustamante[1] and Hillary C.M.Nelson[2]

Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720 and [1]Institute of Molecular Biology and Department of Chemistry, University of Oregon, Eugene, OR 97403, USA

[2]Corresponding author

Communicated by H.Pelham

Gene activation frequently requires an array of proteins bound to sites distal to the transcription start site. The assembly of these protein-bound sites into specialized nucleoprotein complexes is a prerequisite for transcriptional activation. Structural analysis of these higher order complexes will provide crucial information for understanding the mechanisms of gene activation. We have used both electron microscopy and scanning force microscopy to elucidate the structure of complexes formed between DNA and heat-shock transcription factor (HSF) 2, a human heat-shock transcriptional activator that binds DNA as a trimer. Electron microscopy reveals that HSF2 will bring together distant DNA sites to create a loop. We show that this association requires only the DNA binding and trimerization domains of HSF2. Metal shadowing techniques used for electron microscopy obscure details of these nucleoprotein structures. Greatly increased resolution was achieved by directly imaging the complexes in the scanning force microscope, which reveals that at least two trimers are required for the association of HSF2-bound DNA sites.

Key words: DNA looping/electron microscopy/heat-shock transcription factor/protein–DNA interactions/scanning force microscopy

Introduction

Eukaryotic heat-shock genes require the heat-shock transcription factor (HSF) for inducible transcriptional activation (Lis and Wu, 1993). The HSF genes from many organisms have been cloned and show extensive homology within their DNA binding and trimerization domains (Sorger and Pelham, 1988; Wiederrecht *et al.*, 1988; Clos *et al.*, 1990; Scharf *et al.*, 1990; Rabindran *et al.*, 1991; Sarge *et al.*, 1991; Schuetz *et al.*, 1991; Nakai and Morimoto, 1993). Several organisms, including human, have multiple HSF genes with different roles within the stress response (Scharf *et al.*, 1990; Rabindran *et al.*, 1991; Sarge *et al.*, 1991; Schuetz *et al.*, 1991; Nakai and Morimoto, 1993). Human HSF1 is induced to activate transcription in response to heat-shock and other chemical stimuli, while human HSF2 stimulates transcription in response to developmental signals such as hemin-induced differentiation of erythroleukemia cells (Sistonen *et al.*, 1994). Prior to induction, human HSF1 and HSF2 are both found in the cytoplasm in an inactive monomeric or dimeric form. After receiving their specific induction signal, the proteins migrate to the nucleus as functional trimeric DNA binding proteins (Baler *et al.*, 1993; Sarge *et al.*, 1993; Sistonen *et al.*, 1994).

The promoter regions that respond to HSFs contain specific sequences, called heat-shock elements (HSEs), to which HSF binds. An HSE is composed of inverted repeats of the 5 bp nGAAn sequence (Amin *et al.*, 1988; Xiao and Lis, 1988). Typical HSEs range from two to six nGAAn repeats. Although a single HSE of three nGAAn repeats is required for minimal transcriptional activity, most promoters have several HSEs located both proximal and distal to the promoter. The arrangement of HSEs suggests that protein-bound sites co-operate in transcriptional activation. Distal HSEs can function as enhancer elements (Bienz and Pelham, 1986; Riddihough and Pelham, 1986) and, in many cases, they are crucial for transcriptional activity (Simon and Lis, 1987; Chen and Pederson, 1993). Multiple HSEs can have a synergistic effect on transcriptional activity (Riddihough and Pelham, 1986; Cohen and Meselson, 1988; Amin *et al.*, 1994), and this is directly related to the level of occupancy of the HSEs by HSF (Chen and Pederson, 1993; Bonner *et al.*, 1994). Rotational alignment of two HSEs is important for maximal transcriptional activity when the two HSEs are within 80 bp of each other (Cohen and Meselson, 1988; Amin *et al.*, 1994).

HSF is unusual in that it binds DNA as a sequence-specific, homotrimeric protein. In the budding yeasts, HSF is trimeric and capable of high affinity DNA binding whether or not the cells are stressed (Sorger and Pelham, 1987, 1988; Jakobsen and Pelham, 1988, 1991). In other eukaryotes, HSF is monomeric or dimeric with a low affinity for DNA when purified from non-stressed cells (Baler *et al.*, 1993; Sarge *et al.*, 1993; Sistonen *et al.*, 1994). Recombinant HSF or HSF purified from stressed cells is trimeric and has a high affinity for DNA (Rabindran *et al.*, 1991; Sarge *et al.*, 1991, 1993; Schuetz *et al.*, 1991; Baler *et al.*, 1993; Sistonen *et al.*, 1994).

HSF can also bind to an HSE in complexes larger than trimers (Sorger and Pelham, 1987; Perisic *et al.*, 1989; Sorger and Nelson, 1989; Xiao *et al.*, 1991; Westwood and Wu, 1993; Bonner *et al.*, 1994). These larger complexes are composed of multimers of HSF trimers (Rye *et al.*, 1993). Binding of multiple HSF trimers to a single HSE is highly co-operative *in vitro*. For example, binding of one HSF trimer to an HSE with six nGAAn repeats facilitates the binding of a second HSF trimer by >2000-fold (Xiao *et al.*, 1991). HSF can also bind co-operatively to adjacent HSEs *in vivo* (Topol *et al.*, 1985; Amin *et al.*, 1994).

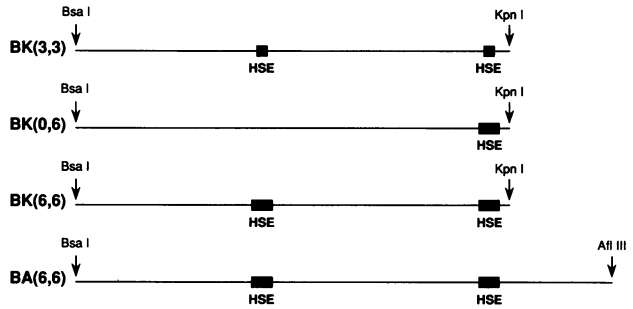

Fig. 1. Diagram of DNA fragments used for HSF2 binding studies. The BK fragments are 1.7 kb long and the BA fragment is 2.1 kb long. The HSEs are separated by ~880 bp. The HSEs are not drawn to scale.

Genomic footprinting has shown that proximal and distal HSEs are occupied by HSF concurrently upon heat-shock (Thomas and Elgin, 1988). Taken together with the results discussed above, these data strongly suggest that interactions between HSFs bound to distant HSEs are involved in transcriptional activation.

We have used electron microscopy to look directly for evidence of such interactions between human HSF2 and distant HSEs. We show that HSF2 will bind to two HSEs to form a DNA loop. The ability to bring together two distant DNA sites requires only the HSF2 DNA binding and trimerization domains and not the transcriptional activation domain. Additional details of the looped nucleoprotein structures were obtained using scanning force microscopy. This method reveals that the loops are formed between at least two DNA-bound HSF trimers.

Results

Electron microscopy of HSF2–HSE complexes

We investigated the ability of HSF2 trimers to form higher order nucleoprotein complexes on DNA fragments containing more than one HSE. Recombinant HSF2 was purified as an active, trimeric protein from a prokaryotic expression system. The purified HSF2 was incubated with DNA fragments containing either one or two HSEs separated by ~900 bp (Figure 1). The resulting complexes were prepared for electron microscopy without fixation or cross-linking. Protein-mediated association of two HSEs, which results in looping out of the intervening DNA, was readily observed on DNA fragments containing two HSEs (Figure 2).

For each fragment tested, the different types of nucleoprotein complex were counted and the percentage of each complex was calculated relative to the total number of DNA fragments (Table I). Looped complexes on fragments BK(3,3) and BK(6,6), with two HSEs of either three or six inverted nGAAn repeats, were 5.2 and 6.4%, respectively, of all observed DNA molecules, resulting in 10–11% of the protein-bound DNA molecules. These percentages of looped complexes are significantly greater than the 1.6% of looped complexes relative to all DNA molecules observed on fragment BK(0,6), containing a single HSE of six inverted nGAAn repeats. The lengths of loops and tails measured from the looped complexes formed with fragments BK(3,3) and BK(6,6) were consistent with the expected sizes, supporting the idea that HSF was bound specifically at the two HSEs. On the other hand, the lengths of the loops and tails measured from complexes formed with fragment BK(0,6) were variable in size, supporting the idea that protein was bound non-specifically at one site. Significantly, for both the BK(3,3) and BK(6,6) DNA fragments, there were more looped complexes (5.2 and 6.4%, respectively) than complexes with protein bound separately at the two sites (3.8 and 4.8%, respectively). These results indicate that distant HSEs associate to form looped nucleoprotein complexes in a co-operative manner.

We studied the ability of HSF2(1–207), which contains only the DNA binding and trimerization domains, to form looped nucleoprotein complexes. Recombinant HSF2(1–207) was purified by a protocol similar to that used for the full-length protein. Both recombinant HSF2 and HSF2(1–207) bind DNA with similar affinities and form multiple shifted species in gel mobility assays (data not shown). We observed that HSF2(1–207) formed looped nucleoprotein complexes co-operatively on fragments of DNA containing two HSEs (Table I, lines 4 and 5). The percentage of looped complexes formed (6.6%) was similar to that seen for the full-length HSF2 protein on the same DNA fragment (6.4%). This indicates that the DNA binding and trimerization domains are sufficient to promote the association of two distant HSEs.

Scanning force microscopy of HSF2–HSE complexes

To determine the number of HSF2 trimers in the looped DNA structures, we exploited the increased resolution possible with scanning force microscopy. DNA–protein complexes were formed in reactions similar to those performed for electron microscopy. The resulting complexes were deposited onto mica in buffer, briefly dried and imaged in air at room humidity. Optimal images of HSF2–DNA complexes were obtained using a NanoScope III in the tapping mode. Tapping mode minimizes distortion of the sample due to sheer forces that otherwise occur during scanning and increases the resolution while imaging in air (Bustamante *et al.*, 1992, 1993). The same proportions of different complexes formed with HSF2 and fragment BK(6,6) were observed in scanning force and electron microscopy. This suggests that the deposition conditions, which differ dramatically for electron and scanning force microscopy, do not bias the observation of different nucleoprotein complexes. One scan set including 12 looped complexes was used for DNA length measurements. In these complexes, the loops represented 52.0 ± 4.5% of the DNA and the tails represented 48.0 ± 4.9% of the DNA which is not significantly different from the expected (55 and 45%, respectively). These measurements indicated that the looped DNA complexes contain protein specifically bound to each HSE.

Strikingly, scanning force microscopy revealed that the protein complexes at the base of the DNA loops displayed a distinct bilobed shape. Of the 35 specific looped nucleoprotein complexes observed, 31 had a bilobed protein structure at the loop junction (for examples see Figure 3A–F) and the other four had even larger protein structures (for example see Figure 3G, upper right). To determine the number of HSF2 trimers at the loop junctions we compared the size of the protein structures bound at single

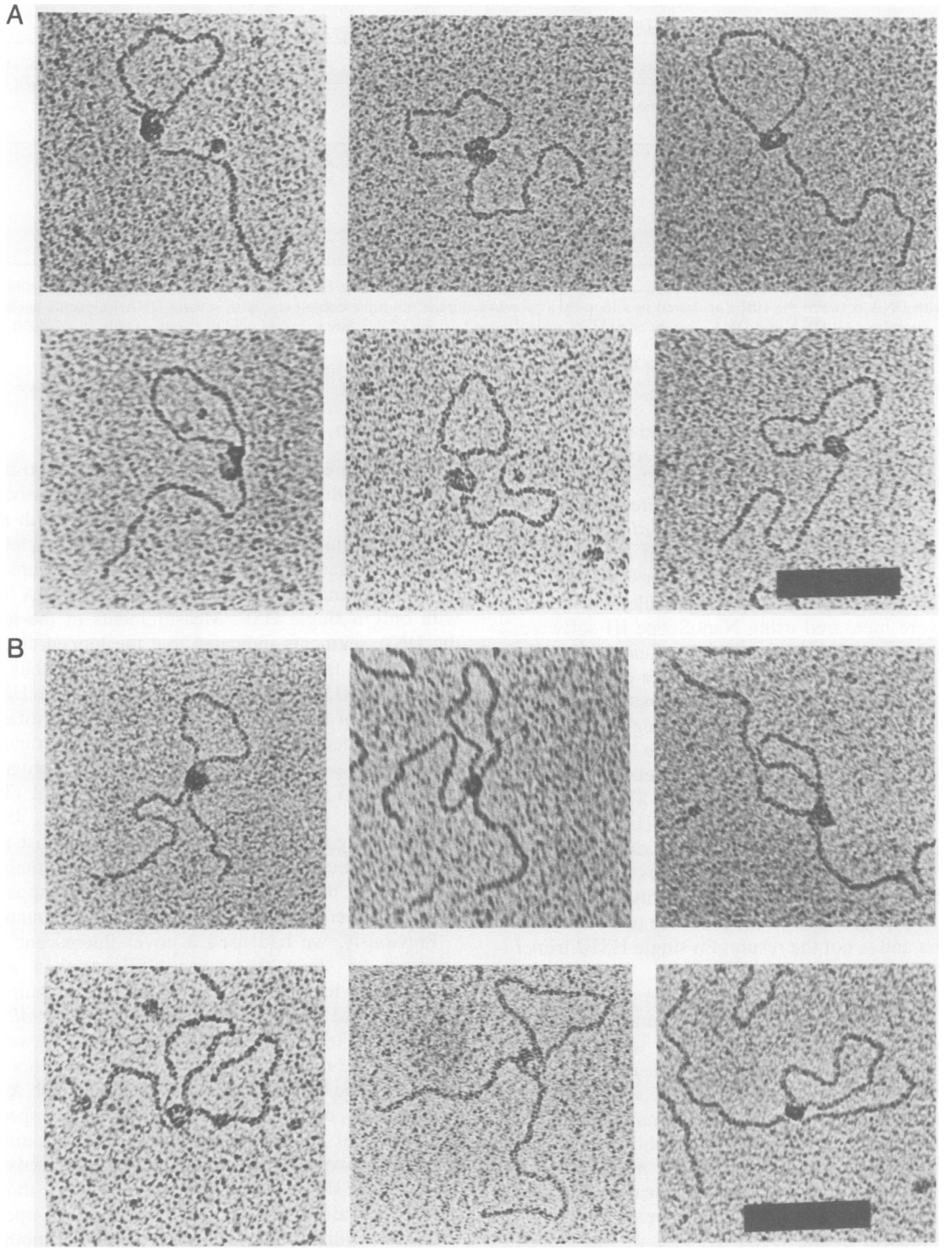

Fig. 2. Examples of HSF2-mediated looped DNA complexes observed by electron microscopy. (**A**) Complexes formed between HSF2 and DNA fragment BK(6,6). (**B**) Complexes formed between HSF2 and DNA fragment BA(6,6). The bar represents ~100 nm.

sites with the size of the protein structures at the loop junctions. The absolute x and y dimensions of the molecules are distorted due to the size and shape of the individual tip used to image them (Bustamante *et al.*, 1992). In addition, for reasons that have yet to be defined, the scanning force microscopy-measured z dimensions of biological molecules are reduced from those expected from crystal structure dimensions. For instance, B-form DNA, which should have a height and width of 2 nm, is typically 10–20 nm wide and only 0.3–1.2 nm high in scanning force microscopy images (see the legend to Figure 3). However, based on the dimensions of different DNA molecules, the $x-y$ and z distortions are constant within a scan or set of scans using the same deposition and the same tip.

We have not attempted to correct for the distortions of

Table I. Quantitative electron microscopy of HSF2–DNA complexes

	Protein	DNA fragment	Percentage of total observed structures (%)					No. of structures counted
			Free DNA	Protein at one site	Protein at two sites	Loop	Multiple complexes	
1	HSF2	BK(0,6)[a]	53	41	1.7	1.6	3.2	627
2	HSF2	BK(3,3)	51	38	3.3	5.2	2.7	777
3	HSF2	BK(6,6)	44	43	4.8	6.4	2.2	1200
4	HSF2(1–207)	BK(0,6)[a]	63	29	2.1	2.1	3.1	800
5	HSF2(1–207)	BK(6,6)	53	34	3.8	6.6	3.3	800

The DNA–protein complexes were classified as: protein at one site, with HSF2 bound at either HSE; protein at two sites, with HSF2 bound to both HSEs; loop, with DNA between the HSEs anchored in a loop by a protein complex; multiple complexes, with several DNA fragments anchored into a large protein complex. All DNA and DNA–protein complexes were counted as one of the listed classes while scanning fields at ×30 000 or ×50 000 magnification.

[a]Protein at two sites and loop complexes on fragment BK(0,6) include one protein bound non-specifically to DNA.

absolute size but rather have compared relative sizes of protein complexes within one scan set. At the protein concentration used to generate the HSF2–DNA complexes, the most common protein structure bound to a single site is a single HSF2 trimer (Rye *et al.*, 1993). The diameter at the base and maximum height of the single HSF2 trimers bound to single sites on non-looped DNA (for examples see Figure 3A, right molecule, and C, left molecule) were measured using NanoScope III software. The base diameter and maximum height of each lobe were measured separately for the bilobed protein complexes at the loop junctions. Single HSF2 trimers had a base diameter of 34.5 ± 6.4 and an average height of 2.01 ± 0.18 nm (average of 20 molecules). The lobes of the bilobed complexes in the same scan set had a base diameter of 33.8 ± 7.8 and a height of 1.87 ± 0.44 nm (average of 26 lobes at 13 loop junctions). These measurements indicate that each individual lobe of the bilobed protein complexes at the looped junctions is about the same size as the single HSF2 trimers. Thus, the bilobed appearance is probably due to the presence of at least two HSF2 trimers and is not the result of a single HSF2 trimer viewed from a different perspective.

In addition, the relative volumes of some of the protein complexes were measured using NIH Image software. The volume of protein complexes bound to single sites on non-looped DNA was assumed to represent the volume of a single HSF2 trimer (for the reasons stated above). The protein complexes at the loop junctions had volumes ranging from 2.19 to 2.62 times the volume of single trimers in the same scan. The bilobed appearance of the complexes taken together with the measurements of dimensions and volume indicate that at least two HSF2 trimers are present at the DNA synapse of the looped structures.

In addition to increased resolution of protein structure, we also observed increased resolution of DNA in the scanning force microscope. The 74 bp of DNA between the HSE and the end of DNA fragment BK(6,6) were clearly visible in all cases where HSF2 bound to this site (for examples see the looped structures in Figure 3A–F and the single site-bound HSF2 in Figure 3A and B). This length of DNA cannot be reliably seen in the type of electron micrographs shown here (Figure 2A), probably because it is buried in the metal coating of the protein complex.

Discussion

We have used quantitative electron microscopy to demonstrate that DNA-bound HSF2 trimers associate co-operatively to form higher order structures with multiple HSEs. A significant percentage of looped nucleoprotein complexes was observed when two HSEs were present on the DNA fragment in comparison with a DNA fragment with only a single HSE. Measurements of the length of the DNA segments indicated that the looped complexes, which were formed on DNA fragments containing two HSEs, had HSF2 specifically bound to each HSE. The percentage of looped complexes formed is similar to that reported in electron microscopy studies of other eukaryotic transcriptional activators, such as the progesterone receptor, Sp1, and bovine papillomavirus E2 (Théveny *et al.*, 1987; Knight *et al.*, 1991; Su *et al.*, 1991). In addition, fragments with two HSEs had almost twice as many looped complexes as complexes with protein bound separately at the two sites. Thus, there is an apparent co-operative interaction between distant DNA-bound HSEs.

Previously, we had used a novel fluorescent labeling approach to show that yeast HSF forms a series of complexes with a single HSE consisting of multimers of trimers bound to one DNA fragment (Rye *et al.*, 1993). This result implies that the looped complexes could contain more than one HSF trimer. On the other hand, a heterotrimeric HSF, with only two functional DNA binding domains, is capable of binding to DNA specifically (B.Drees and H.C.M.Nelson, personal communication). Thus only two DNA binding domains are necessary for binding to a single HSE. Then, in theory, the third DNA binding domain of a trimer would be free to co-operatively associate with a recognition sequence at another site, i.e. a single HSF2 trimer could potentially mediate the association of distant HSE DNA sites. This would be analogous to what is found for AraC: a single dimer of AraC is capable of generating loops (Lobell and Schleif, 1990). It was not possible to accurately determine the stoichiometry of HSF2 trimers at the loop junctions from the electron micrographs we obtained.

Scanning force microscopy allowed us to look at the structure of the proteins at the loop junctions in more detail. For scanning force microscopy, nucleoprotein complexes are deposited onto mica in buffer and imaged at room pressure and humidity, not in a high vacuum as is

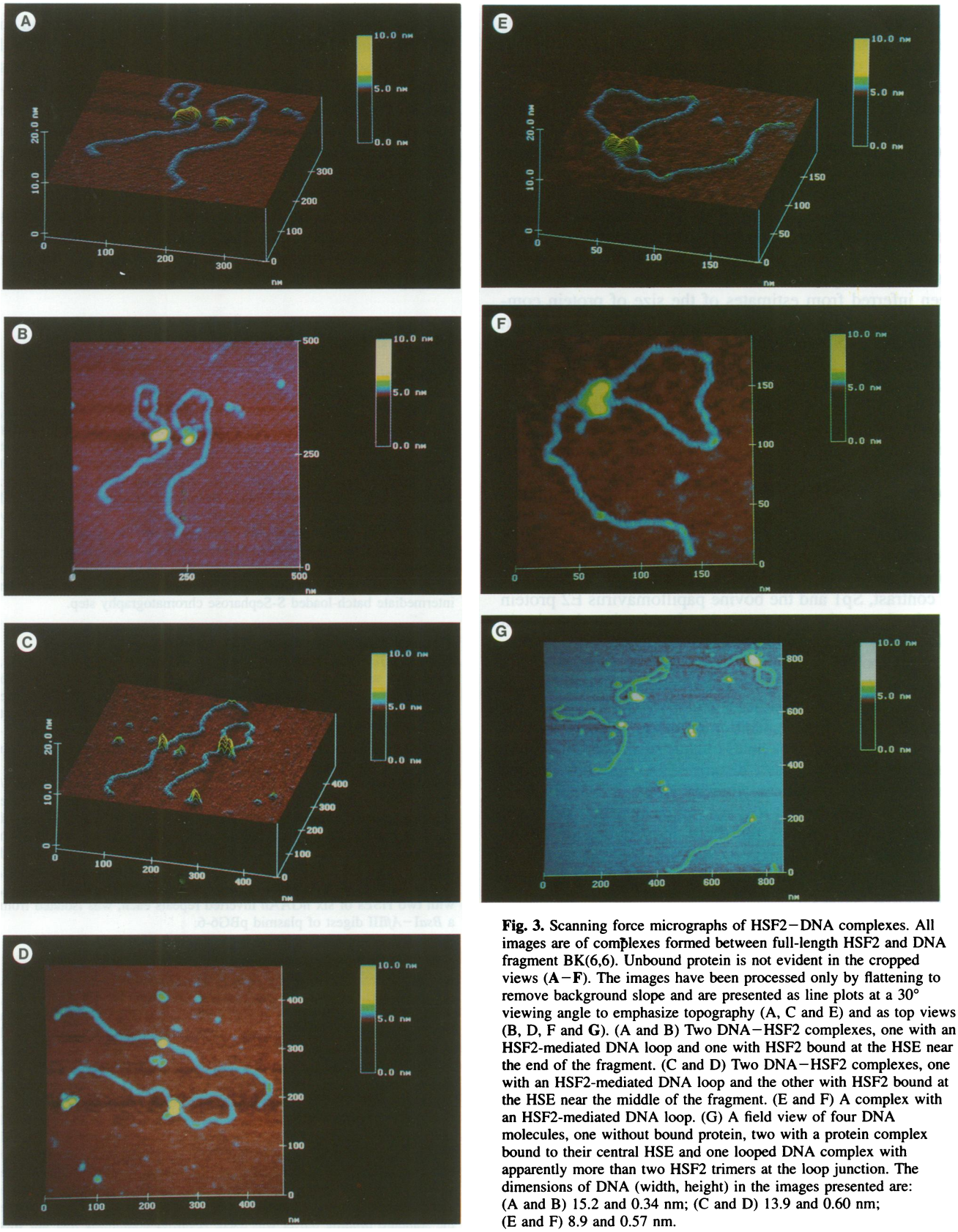

Fig. 3. Scanning force micrographs of HSF2–DNA complexes. All images are of complexes formed between full-length HSF2 and DNA fragment BK(6,6). Unbound protein is not evident in the cropped views (**A**–**F**). The images have been processed only by flattening to remove background slope and are presented as line plots at a 30° viewing angle to emphasize topography (A, C and E) and as top views (B, D, F and G). (A and B) Two DNA–HSF2 complexes, one with an HSF2-mediated DNA loop and one with HSF2 bound at the HSE near the end of the fragment. (C and D) Two DNA–HSF2 complexes, one with an HSF2-mediated DNA loop and the other with HSF2 bound at the HSE near the middle of the fragment. (E and F) A complex with an HSF2-mediated DNA loop. (G) A field view of four DNA molecules, one without bound protein, two with a protein complex bound to their central HSE and one looped DNA complex with apparently more than two HSF2 trimers at the loop junction. The dimensions of DNA (width, height) in the images presented are: (A and B) 15.2 and 0.34 nm; (C and D) 13.9 and 0.60 nm; (E and F) 8.9 and 0.57 nm.

necessary for electron microscopy. Objects are imaged directly in the scanning force microscope without staining and metal shadowing that distort their size and shape. In addition, scanning force microscopy provides a 3-D topographic representation that can be used to determine relative volume of structures without the need for complicated 3-D image reconstruction. We are currently developing internal standards so that scanning force micro-

scopy will be generally useful for determining the sizes and compositions of nucleoprotein complexes.

We have demonstrated that scanning force microscopy can be used to determine the multimer state of a protein bound to DNA. The scanning force microscope images we obtained clearly show that at least two HSF2 trimers are present at the loop junctions. Thus, HSF2-mediated association of distant HSEs does not occur within a single trimer, but instead requires at least two trimers, with one trimer bound to each site. This is consistent with our results from gel retardation assays that indicated that DNA-bound HSFs could associate through protein–protein interactions (Rye *et al.*, 1993). Similar conclusions have been inferred from estimates of the size of protein complexes formed by another eukaryotic transcription factor, the bovine papillomavirus E2 protein, where at least two dimers appear necessary to form looped DNA structures (Knight *et al.*, 1991).

The HSF2 interactions required to form looped nucleoprotein complexes differ from those required of other eukaryotic transcription factors that form similar structures. The ability of HSF2 to bring together distant HSEs requires only the DNA binding and trimerization domains. A truncated HSF2, including only these domains, is similar to the full-length protein in its affinity for HSEs, its ability to form multiple shifted species in a gel mobility assay and its ability to form looped nucleoprotein complexes. In contrast, Sp1 and the bovine papillomavirus E2 protein absolutely require a transcriptional activation domain, in addition to the DNA binding and dimerization domains, to form looped nucleoprotein complexes (Knight *et al.*, 1991; Su *et al.*, 1991). Other eukaryotic regulatory factors, such as the homeoprotein *Ultrabithorax* (Ubx) and the Epstein–Barr virus replication enhancer (EBNA-1), also require amino acid sequences outside of a functional DNA binding unit to associate distant DNA sites into looped nucleoprotein complexes (Beachy *et al.*, 1993; Frappier *et al.*, 1994). Although it is not yet clear which structural motifs are responsible for these interactions of DNA-bound proteins, this suggests that (in contrast to HSF2) SP1, E2, Ubx and EBNA-1 have two independent multimerization surfaces.

Our results on human HSF2 are directly applicable to other HSFs. Because the DNA binding and trimerization domains of all HSFs have been conserved, it is likely that all HSFs have the ability to associate distant DNA sites into looped complexes. Evidence in support of this comes from the observation that a yeast HSF fragment containing just the DNA binding and trimerization domains also shows the same specificity and co-operativity of binding as the full-length protein (Sorger and Pelham, 1987; Sorger and Nelson, 1989; Rye *et al.*, 1993; Flick *et al.*, 1994). We have demonstrated that a human HSF2 truncation with these properties associates distant DNA binding sites into looped structures as well as the full-length protein. Binding experiments that compare human HSF1 with HSF2 have shown that HSF1 is more co-operative than HSF2 when binding to an hsp70 promoter (Kroeger *et al.*, 1993). This suggests that human HSF1 might show an even greater co-operativity in terms of binding to distal HSE sites than HSF2.

Although we do not know how HSF activates transcription, it is likely that the association of DNA-bound trimers is important for this function. Promoters that respond to HSF have multiple HSEs located both proximal and distal to the transcriptional start site. Genomic footprinting has shown that these sites are contacted by HSF upon heat-shock (Thomas and Elgin, 1988; Gross *et al.*, 1990). The transcriptional activity from these promoters is related to the occupancy of the HSEs by HSF (Cohen and Meselson, 1988; Chen and Pederson, 1993; Amin *et al.*, 1994; Bonner *et al.*, 1994). We have shown here that association of DNA-bound HSF is favored if more than one trimer is bound to the same DNA fragment. Thus we believe that the transcriptionally active complex will include the type of looped nucleoprotein complex we have described.

Materials and methods

Protein purification

HSF2 and HSF2(1–207), containing only the first 207 (of 536) amino acids of HSF2, were expressed from plasmids pHN307 and pHN306, respectively. Both plasmids are derivatives of the expression vector pET3-b (Studier *et al.*, 1989). HSF2 protein was expressed from these plasmids in *Escherichia coli* strain BL21(DE3) (Studier *et al.*, 1989) containing a pACYC177 derivative expressing the *lac* repressor. Harvested cells were stored frozen. Before purification, the frozen cell pellets were thawed, lysed by sonication and the lysate cleared by centrifugation. HSF2 protein was purified from the lysate by propyl hydrophobic interaction column chromatography followed by heparin–agarose ion-exchange chromatography. The HSF2(1–207) purification protocol was similar to the HSF2 protocol with the addition of an intermediate batch-loaded S-Sepharose chromatography step.

Plasmid construction and DNA purification

Plasmid pBL6 was created by inserting the *Bam*HI fragments of pI6, containing six nGAAn inverted repeats (Perisic *et al.*, 1989), into pBluescript KS$^+$ (Stratagene). Plasmids pBG3-3 and pBG6-6 are pBluescript KS$^+$ derivatives with either a three or six nGAAn repeat HSE, created by site-directed mutagenesis at the *Ssp*I site near the β-lactamase promoter, and another three or six nGAAn repeat HSE within a *Bam*HI insert obtained from pI3 or pI6 (Perisic *et al.*, 1989).

Restriction fragments were isolated from 0.8% agarose gels by phenol/chloroform extraction. Fragment BK(3,3), with two HSEs of three nGAAn inverted repeats, was isolated from a *Bsa*I–*Kpn*I digest of plasmid pBG3-3. Fragment BK(0,6), with one HSE of six nGAAn inverted repeats, was isolated from a *Bsa*I–*Kpn*I digest of plasmid pBL6. Fragment BK(6,6), with two HSEs of six nGAAn inverted repeats, was isolated from a *Bsa*I–*Kpn*I digest of pBG6-6. Fragment BA(6,6), with two HSEs of six nGAAn inverted repeats each, was isolated from a *Bsa*I–*Afl*III digest of plasmid pBG6-6.

HSF2–DNA binding reactions

Binding reactions using full-length HSF2 included 3–4 nM DNA fragment, 400–500 nM HSF2 as trimer, 50 mM HEPES–KOH (pH 8.0), 200 mM NaCl, 1 mM EDTA and 10% glycerol in 20 μl. After incubation for 15–20 min at room temperature, the reaction mixtures were diluted to 100 μl in the binding buffer and 8 μl were immediately deposited on carbon-coated grids by the polylysine method (Williams, 1977). To prevent protein aggregation and non-specific DNA binding, reactions using the HSF2(1–207) truncation included 6–9 nM DNA fragment, 600–900 nM HSF2(1–207) as trimer, 1.6 nM of an unrelated 6 kb plasmid as non-specific competitor DNA, 50 mM HEPES–KOH (pH 8.0), 10 mM $MgCl_2$, 400 mM NaCl and 0.2% NP-40 in 10 μl. As determined by quantitative electron microscopy, binding reactions performed with the full-length protein in these conditions resulted in essentially the same percentages of different complexes reported in line 3 of Table I where the standard binding buffer was used. A buffer containing less salt was required for scanning force microscopy experiments. In this case, binding reactions included 20 nM DNA fragment, 200 nM HSF2 as trimer, 25 mM HEPES–KOH (pH 7.4), 20 mM KCl, 50 mM NaCl, 0.2% NP-40 and 10 mM $MgCl_2$ in 10–20 μl. As determined by quantitative electron microscopy (data not shown), these conditions resulted in essentially the same percentages of different complexes reported in line 3 of Table I where the standard binding buffer was used.

Electron microscopy

The protein–DNA complexes were not cross-linked or fixed before deposition onto grids. After incubation, diluted binding reaction mixtures were immediately deposited on carbon-coated grids by the polylysine method (Williams, 1977). The samples were stained with uranyl acetate and rotary shadowed with tungsten before observation in a JEOL EX1200 electron microscope operated at 80 kV. Photographs were taken at a magnification of ×50 000. DNA length measurements were obtained from electron micrograph negatives projected onto a Numonics digitizing tablet. Loop and tail percentage lengths were based on the total length of protein-free DNA fragments on the same grid.

Scanning force microscopy

After incubation for 15–20 min at room temperature, 2 μl of the binding reaction mixture were diluted into 20 μl of distilled water and immediately deposited onto freshly cleaved ruby mica. After ~1 min, the mica disk was washed with 30 drops of distilled water and dried briefly in a stream of N_2. The nucleoprotein complexes were imaged in air at room temperature and humidity in a NanoScope III (Digital instruments, Santa Barbara, CA) operating in the tapping mode using commercial silicon nitride tips. The resolution of the images obtained depends critically on the shape of the tip (Bustamante *et al.*, 1993). As is evident from the width of the DNA (see legend to Figure 3), the tip used to produce the image shown in Figure 3E and F was much sharper than the tip used to produce the image in Figure 3A and B, with Figure 3C and D being produced by a tip with intermediate sharpness. The NanoScope III images were imported into NIH Image software to obtain the area and average height measurements, from which the volumes of the protein complexes were calculated. DNA segment length was also measured using NIH Image software. Loop and tail length percentages were calculated relative to the total length of DNA molecules within the same scan. Values obtained in NIH Image were expressed in arbitrary pixel units and were not converted to nm or other absolute units.

Acknowledgements

This work is dedicated to the memory of Hatch Echols, whose ideas on the importance of specialized nucleoprotein structures inspired us to undertake this project. We thank R.Kingston for the HSF2 cDNA plasmid. Chip Walker provided invaluable instructions in scanning force microscopy. R.Kanaar and members of the Nelson laboratory provided valuable comments on the manuscript. This work was partially supported by an award from the Pew Scholars Program in Biomedical Sciences (H.C.M.N.), grants from the National Institutes of Health (separately to H.C.M.N., H.Echols and C.B.), a National Science Foundation grant (C.B.), and a grant from the Lucille P.Markey Charitable Trust to the Institute of Molecular Biology (C.B.). C.W. was supported by a National Cancer Institute Postdoctoral Fellowship.

References

Amin,J., Anathan,J. and Voellmy,R. (1988) *Mol. Cell. Biol.*, **8**, 3761–3769.

Amin,J., Fernandez,M., Ananthan,J., Lis,J.T. and Voellmy,R. (1994) *J. Biol. Chem.*, **269**, 4804–4811.

Baler,R., Dahl,G. and Voellmy,R. (1993) *Mol. Cell. Biol.*, **13**, 2486–2496.

Beachy,P.A., Varkey,J., Young,K.E., von Kessler,D.P., Sun,B.I. and Ekker,S.C. (1993) *Mol. Cell Biol.*, **13**, 6941–6956.

Bienz,M. and Pelham,H.R. (1986) *Cell*, **45**, 753–760.

Bonner,J.J., Ballou,C. and Fackenthal,D.L. (1994) *Mol. Cell. Biol.*, **14**, 501–508.

Bustamante,C., Vesenka,J., Tang,C.L., Rees,W., Guthold,M. and Keller,R. (1992) *Biochemistry*, **31**, 22–26.

Bustamante,C., Keller,D. and Yang,G. (1993) *Curr. Opin. Struct. Biol.*, **3**, 363–372.

Chen,J. and Pederson,D.S. (1993) *J. Biol. Chem.*, **268**, 7442–7448.

Clos,J., Westwood,J.T., Becker,P.B., Wilson,S., Lambert,K. and Wu,C. (1990) *Cell*, **63**, 1085–1097.

Cohen,R.S. and Meselson,M. (1988) *Nature*, **332**, 856–858.

Flick,K.E., Gonzalez,L., Harrison,C.J. and Nelson,H.C.M. (1994) *J. Biol. Chem.*, **269**, 12475–12481.

Frappier,L., Goldsmith,K. and Bendell,L. (1994) *J. Biol. Chem.*, **269**, 1057–1062.

Gross,D.S., English,K.E., Collins,K.W. and Lee,S.W. (1990) *J. Mol. Biol.*, **216**, 611–631.

Jakobsen,B.K. and Pelham,H.R. (1988) *Mol. Cell. Biol.*, **8**, 5040–5042.

Jakobsen,B.K. and Pelham,H.R. (1991) *EMBO J.*, **10**, 369–375.

Knight,J.D., Li,R. and Botchan,M. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 3204–3208.

Kroeger,P.E., Sarge,K.D. and Morimoto,R.I. (1993) *Mol. Cell. Biol.*, **13**, 3370–3383.

Lis,J. and Wu,C. (1993) *Cell*, **74**, 1–4.

Lobell,R.B. and Schleif,R.F. (1990) *Science*, **250**, 528–532.

Nakai,A. and Morimoto,R.I. (1993) *Mol. Cell. Biol.*, **13**, 1983–1997.

Perisic,O., Xiao,H. and Lis,J.T. (1989) *Cell*, **59**, 797–806.

Rabindran,S.K., Giorgi,G., Clos,J. and Wu,C. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 6906–6910.

Riddihough,G. and Pelham,H.R. (1986) *EMBO J.*, **5**, 1653–1658.

Rye,H.S., Drees,B.L., Nelson,H.C.M. and Glazer,A.N. (1993) *J. Biol. Chem.*, **268**, 25229–25238.

Sarge,K.D., Zimarino,V., Holm,K., Wu,C. and Morimoto,R.I. (1991) *Genes Dev.*, **5**, 1902–1911.

Sarge,K.D., Murphy,S.P. and Morimoto,R.I. (1993) *Mol. Cell. Biol.*, **13**, 1392–1407.

Scharf,K.D., Rose,S., Zott,W., Schoffl,F., Nover,L. and Schoffl,F. (1990) *EMBO J.*, **9**, 4495–4501.

Schuetz,T.J., Gallo,G.J., Sheldon,L., Tempst,P. and Kingston,R.E. (1991) *Proc. Natl Acad. Sci. USA*, **88**, 6911–6915.

Simon,J.A. and Lis,J.T. (1987) *Nucleic Acids Res.*, **15**, 2971–2988.

Sistonen,L., Sarge,K.D. and Morimoto,R.I. (1994) *Mol. Cell. Biol.*, **14**, 2087–2099.

Sorger,P.K. and Nelson,H.C.M. (1989) *Cell*, **59**, 807–813.

Sorger,P.K. and Pelham,H.R. (1987) *EMBO J.*, **6**, 3035–3041.

Sorger,P.K. and Pelham,H.R. (1988) *Cell*, **54**, 855–864.

Studier,F.W., Rosenberg,A.H., Dunn,J.J. and Dubendorff,J.W. (1989) *Methods Enzymol.*, **185**, 60–89.

Su,W., Jackson,S., Tjian,R. and Echols,H. (1991) *Genes Dev.*, **5**, 820–826.

Théveny,B., Bailly,A., Rauch,C., Rauch,M., Delain,E. and Milgrom,E. (1987) *Nature*, **329**, 79–81.

Thomas,G.H. and Elgin,S.C. (1988) *EMBO J.*, **7**, 2191–2201.

Topol,J., Ruden,D.M. and Parker,C.S. (1985) *Cell*, **42**, 527–537.

Westwood,J.T. and Wu,C. (1993) *Mol. Cell. Biol.*, **13**, 3481–3486.

Wiederrecht,G., Seto,D. and Parker,C.S. (1988) *Cell*, **54**, 841–853.

Williams,R.C. (1977) *Proc. Natl Acad. Sci. USA*, **74**, 2311–2315.

Xiao,H. and Lis,J.T. (1988) *Science*, **239**, 1139–1142.

Xiao,H., Perisic,O. and Lis,J.T. (1991) *Cell*, **64**, 585–593.

Received on June 23, 1994; revised on September 26, 1994