

HSF1 transcription factor concentrates in nuclear foci during heat shock: relationship with transcription sites

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SUMMARY

In this paper, we show that upon heat shock, HSF1 concentrates in the nucleus of diploid human fibroblasts in two large foci. The relative distribution of HSF1 nuclear foci and active heat shock protein (hsp) genes was investigated by combining fluorescence in situ hybridization (FISH) for the detection of hsp nuclear transcripts and immunofluorescence for the detection of HSF1. We show that the HSF1 foci are distinct from the sites of hsp70 and hsp90 genes transcription. This

is the second report of ploidy-dependent foci of transcription factors that are independent of their specific transcription sites. However, the correlation between the number of HSF1 foci and the ploidy of the cells strongly supports the existence of a specific chromosomal target for HSF1 foci.

Key words: Heat shock, hsp gene, Transcription factor, Fluorescence in situ hybridization, Nuclear transcript, Nuclear structure

INTRODUCTION

It is now well accepted that the cell nucleus has a highly organized internal structure. Thanks to the recent development of methodologies for the in situ detection of nucleic acids and proteins, it is clear today that chromosomes are not randomly distributed within the interphase nucleus but occupy discrete territories, also termed chromosome domains, within the nucleoplasm (Cremer et al., 1993). Recently, several active and inactive genes were found to be preferentially located at the periphery of these chromosome domains (Kurz et al., 1996). Moreover, activities such as replication, transcription, and RNA processing are confined to particular regions within the nucleus (for reviews see Moen et al., 1995; Strouboulis and Wolffe, 1996). Studies on the nuclear distribution of poly(A) RNAs, nascent RNA polymerase II transcripts and RNA polymerase II have revealed that a limited number of specific transcription sites also enriched in splicing components are scattered throughout the nucleoplasm (reviewed by Spector, 1993). Since there appear to be fewer transcription/splicing domains than active genes, each domain is likely to represent the site of transcription and/or RNA splicing activity of several genes. A functional clustering of genes that are regulated by the same transcription factor could be advantageous for the control of transcription. However, except for RNA polymerase I and the associated factors UBF (upstream binding factor) and SL1 which concentrate on ribosomal genes (Roussel et al., 1993), little is known about the relative distribution of co-regulated genes and transcription factors in the nucleus.

Heat shock response has been a valuable system to investigate inducible gene regulation and the target heat shock genes have

been cloned, the promoter regions characterized, and the stress-regulated heat shock transcription factors (HSFs) cloned and analyzed (for reviews see Schlesinger, 1990; Morimoto et al., 1996). This model was of particular interest to study the relative distribution of specific transcription factors and transcription sites, since the heat shock transcription factor 1 (HSF1) has been shown to concentrate in several foci in the nucleus of heat-shocked cells (Sarge et al., 1993; also see companion paper: Cotto et al., 1997a). Upon heat shock, activation of hsp gene transcription by HSF1 involves phosphorylation, trimerization, translocation into the nucleus, and binding of the factor to the promoter of hsp genes (Sarge et al., 1993; reviewed by Wu, 1995; Morimoto et al., 1996). The kinetics of appearance of HSF1 nuclear foci correlates with the biochemical events associated with the complete activation of HSF1 (Cotto et al., 1997a).

Using immunofluorescence, we show that upon heat shock, HSF1 concentrates in the nucleus of normal human fibroblasts in two large foci. These nuclear accumulations could either correspond to the sites of hsp genes transcription or to sites of storage and/or recycling of HSF1. To address this question, the spatial relationship between HSF1 foci and active transcription sites was investigated.

MATERIALS AND METHODS

Cell culture

Normal primary fibroblasts were obtained from a skin biopsy from a healthy female donor. They were grown in RPMI medium (Gibco BRL) supplemented with 10% fetal calf serum. To synchronize the cells in the G₀-G₁ phases, cells were incubated in serum-free medium for 36 hours. Analysis of the cell cycle by flow cytometry confirmed

that more than 95% of the serum-depleted cells were in G₀-G₁ phases. For inhibition of RNA polymerase II transcription, cells were incubated at different times after heat shock for 15 or 60 minutes with actinomycin D at a final concentration of 5 µg/ml (Sigma).

The A431 (epidermoid carcinoma), MCF-7 (breast adenocarcinoma), and T47D (breast ductal carcinoma) cell lines were obtained from the American Type Culture Collection (Rockville, USA) and grown in DMEM medium (TechGen) supplemented with 10% fetal calf serum.

For in situ hybridization and immunofluorescence assays, cells were grown on glass slides. Heat shock was performed by immersion of the Petri dish containing the slides in a waterbath at a temperature varying from 42°C to 45°C. In some experiments, cells were allowed to recover in an incubator at 37°C after heat exposure.

Probes

The genomic probe pH2.3, obtained from Dr R. I. Morimoto (Evanston, USA), covers 2.3 kb of the coding sequence of the hsp70 gene (Wu et al., 1985). cDNA probes specific for hsp90 alpha (pHS 801) and hsp90 beta (pHS 811) genes were obtained from Dr E. Hickey (Reno, USA). pHS 801 and 811 contain, respectively, 1.3 kb and 0.9 kb of the coding region (Hickey et al., 1986). All probes were labeled according to standard nick-translation procedures, using either digoxigenin-11-dUTP (Boehringer Mannheim) or biotin-14-dATP (Gibco BRL).

Antibodies

The rat monoclonal antibodies 10H8 against HSF1 were obtained from Dr J. J. Cotto (Evanston, USA) (see Cotto et al., 1997a). The antiserum was used for immunofluorescence assays at a dilution of 1/300.

Immunofluorescence

Cells were fixed in 4% paraformaldehyde in PBS for 5 minutes. After a one-hour incubation in 10% foetal calf serum/0.3% Triton X-100/PBS to block non-specific binding sites, cells were incubated for one hour at 37°C with the anti-HSF1 antibody. The antibodies were detected using a sheep anti-rabbit antibody coupled to TRITC (Sigma) or a goat anti-rat antibody coupled to FITC (Sigma). Nuclei were counterstained with 4',6-diamidino-2-phenylindole:2HCl (DAPI) 250 ng/ml in an anti-fading medium composed of 90% glycerol and 2.3% diaza-bicyclo-octane (DABCO).

Fluorescence in situ hybridization to RNA

The detection of hsp nuclear transcripts was performed as described elsewhere (Jolly et al., 1998a). Cells were rinsed briefly in PBS and immediately fixed in 4% formaldehyde/PBS for 10 minutes. The accessibility of the probe to the target was improved by a 10 minute incubation in 0.1 M HCl, followed by two successive incubations of 10 minutes each in 0.5% saponin/0.5% Triton X-100/PBS. Cells were subsequently dehydrated in a series of cold ethanol baths. 100 ng of cDNA probes were precipitated together with 10 µg of sonicated

salmon sperm DNA and denatured for 5 minutes at 75°C in 50% formamide/2× SSC/10% dextran sulfate. Hybridization was allowed to run overnight at 37°C in a moisture chamber. After three washes in 60% formamide/2× SSC at 37°C and three washes in 2× SSC at room temperature, detection was performed using either an anti-digoxigenin antibody coupled to TRITC (Boehringer Mannheim) or avidin-FITC (Vector). Nuclei were counterstained with DAPI.

Combined immunofluorescence and FISH

The best results were obtained when in situ hybridization was performed before the immunofluorescence procedure (Jolly et al., 1998a). Specific nuclear transcripts were detected as described above. Detection of probes labelled with biotin was performed using avidin-FITC (Vector). After post-detection washes in 4× SSC/0.1% Tween-20, a 45 minute incubation in 10% FCS/0.3% Triton X-100/PBS was performed, followed by incubation for 90 minutes at 37°C with the rat monoclonal anti-HSF1 antibody. The antibody was revealed using an anti-rat antibody coupled to TRITC (Sigma), and nuclei were counterstained with DAPI.

Image acquisition and calculations

Preparations were observed under an epifluorescence microscope (Zeiss Axiophot) equipped with a 100 W mercury lamp using appropriate filter sets for DAPI, FITC, and TRITC fluorescence. Images were acquired with a cooled CCD camera (C4880 Hamamatsu) using a ×63, 1.25 NA oil immersion objective and an intermediate magnification (optovar) of ×1.25. Images were processed and merged using software developed in our laboratory.

Quantification of cellular viability and apoptotic index

The cellular viability and apoptotic index of non heat-shocked and heat-shocked cell populations was performed by acridine orange/ethidium bromide uptake, according to a procedure described by McGahon et al. (1995). This protocol is based on the differential uptake of these two fluorescent DNA binding dyes to determine viable and nonviable cells in a population. The composition of the dye mix is 100 µg/ml acridine orange, 100 µg/ml ethidium bromide in PBS. Viable cells appear with a uniformly stained green nucleus, due to acridine orange that intercalates into the DNA. Ethidium bromide is only taken up by nonviable cells, which thus appear with an orange nucleus. Apoptotic cells display green nuclei, but chromatin condensation becomes visible as bright green patches in the nucleus. Percentages were determined on the basis of 200 cells.

RESULTS

Nuclear distribution of HSF1

At 37°C, human diploid fibroblasts displayed an homogeneous distribution of HSF1 in the nucleus excluding nucleoli (Fig.

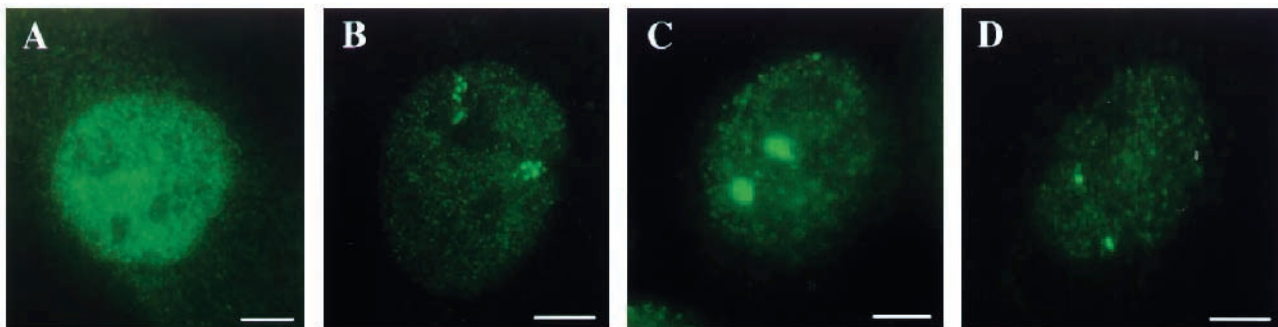


Fig. 1. Intracellular localization of HSF1 transcription factor in (A) non heat-shocked and (B to D) heat-shocked normal fibroblasts using indirect immunofluorescence. (A) At 37°C, the fluorescence is dispersed throughout the nucleoplasm excluding nucleoli. (B,C,D) Under heat shock (42°C to 45°C), HSF1 concentrates in the nucleus in two large foci which can be classified into three groups according to their shape: large clusters of granules (B), highly compact foci (C), and small punctate foci (D). In all cases, a diffuse staining of the nucleoplasm is also observed. Bars, 5 µm.

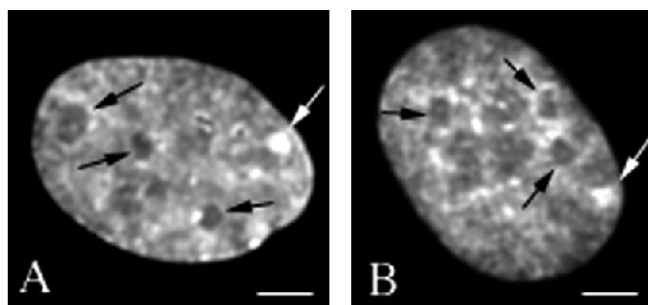


Fig. 2. Chromatin texture in the nuclei of primary fibroblasts (A) before and (B) after a one-hour heat shock. No major changes in the general texture of the chromatin can be seen on the basis of a DAPI staining. In particular, the Barr body (white arrows) as well as the nucleoli (black arrows) are still visible after heat exposure. Bars, 5 μ m.

1A). The extremely low diffuse staining of the cytoplasm which did not significantly differ from the background suggests that the inactive HSF1 factor is essentially present in the nucleus as detected by this antibody. After a one-hour heat shock at 42°C or 43°C, a spatial clustering of the HSF1 factor in two foci was observed in about 10% of the nuclei, in addition to a remaining diffuse staining of the nucleoplasm (Fig. 1B to D). No foci were detected when the anti-HSF1 antibody was omitted. The percentage of labelled nuclei did not significantly increase when cells were submitted to prolonged exposure to 42°C or 43°C (up to four hours). The appearance of HSF1 granules is clearly temperature-dependent. Indeed, after a one-hour heat shock at 44 or 45°C, the two HSF1 foci were detected in 90% of the nuclei.

As also reported by Cotto et al. (1997a) in HeLa cells, the shape of HSF1 nuclear foci varies from one nucleus to another. In normal fibroblasts, HSF1 foci can be classified in three major groups according to their morphology (Fig. 1B to D): 77% of HSF1 foci consisted of a cluster of up to ten granules which sometimes displayed a necklace or ring-like shape (Fig. 1B), 11% of the foci appeared as large foci in which granules could not be distinguished (Fig. 1C), and 12% of HSF1 foci appeared as two unique punctate signals (Fig. 1D). The granular morphology of these foci suggests that they could represent large accumulations of smaller subunits. In addition to these two major sites of accumulation, 2 to 10 tiny foci were also observed in about 25% of the nuclei. Although most HSF1 factors seemed to be concentrated in the nuclear foci at 45°C, the presence of a diffuse staining of the nucleoplasm suggests that a non negligible fraction of HSF1 factors is also widespread in the nuclear volume.

The temperature required for the appearance of HSF1 nuclear foci differs depending on the cell line considered. In diploid primary fibroblasts only a small percentage of nuclei displayed HSF1 foci at 42-43°C while in the several tumor cell lines tested so far, most nuclei displayed HSF1 foci at this temperature (data not shown). The reason for these differences is not clear at present. They do not seem to be directly correlated to thermo-tolerance (Yost and Lindquist, 1986), since the formation of HSF1 foci in normal fibroblasts at 45°C was not prevented by a one-hour pretreatment at 42°C prior to heat shock.

HSF1 foci could represent regions of recycling of the factor or reflect a high local concentration of HSF1 in the vicinity of hsp genes. To test these different hypotheses, we used normal primary human fibroblast cells since the presence of only two HSF1 nuclear foci in these cells greatly facilitated the analysis of the relative distribution between HSF1 foci and other nuclear components. Because of the higher percentage of nuclei displaying HSF1 foci at 44-45°C in normal fibroblasts, these heat shock conditions were chosen for further experiments.

HSF1 foci, cell cycle and apoptosis

To investigate the possible relationship between the presence and shape of HSF1 foci and the cell cycle, immunofluorescence assays were performed on fibroblasts induced to accumulate in the G₀-G₁ phases by serum deprivation (see Materials and Methods). After one hour of heat shock, most nuclei displayed two HSF1 foci, the shape of which varied from one nucleus to another in the same proportion as for cycling fibroblasts. This result clearly indicates that the appearance and the shape of these foci is independent of the phase of the cell cycle.

Other stresses have been shown to induce the formation of HSF1 foci, suggesting that the appearance of these granules is not a mere consequence of protein aggregation in response to heat exposure (Cotto et al., 1997a). However, as heat shock has been shown to induce some changes in the nuclear architecture of rat primary fibroblasts (Welch and Suhan, 1985), it was necessary to first investigate the effect of a 44-45°C heat shock on different features of the cellular and nuclear morphology. On the basis of the DAPI counterstaining, the nucleoli as well as the Barr body could be clearly identified in all nuclei (arrows in Fig. 2). In addition, no major changes in chromatin texture were found after heat shock (compare Fig. 2A and B).

Since severe heat shock has been reported to induce apoptosis and necrosis on several cell types (Barry et al., 1990), the viability of normal primary fibroblasts after a 44-45°C heat shock was investigated using a test combining

Table 1. Cellular viability and apoptotic index of the fibroblast population before and after a one-hour heat shock at 45°C*

| | 37°C | 45°C (%) | 45°C+ 2 hours rec (%) | 45°C+ 4 hours rec (%) | 45°C+ 6 hours rec (%) | 45°C+ 12 hours rec (%) | 45°C+ 24 hours rec (%) |
|-----------------|------|-------------|-----------------------------|-----------------------------|-----------------------------|------------------------------|------------------------------|
| Living cells | 96 | 95 | 93 | 92 | 91 | 89 | 87 |
| Apoptotic cells | 2 | 3 | 4 | 4.5 | 5 | 7 | 10.5 |
| Necrotic cells | 2 | 2 | 3 | 3.5 | 4 | 4 | 2.5 |

*Quantification using acridine orange/ethidium bromide uptake (see Materials and Methods). 200 cells analyzed for each heat shock condition; rec, recovery from heat shock.

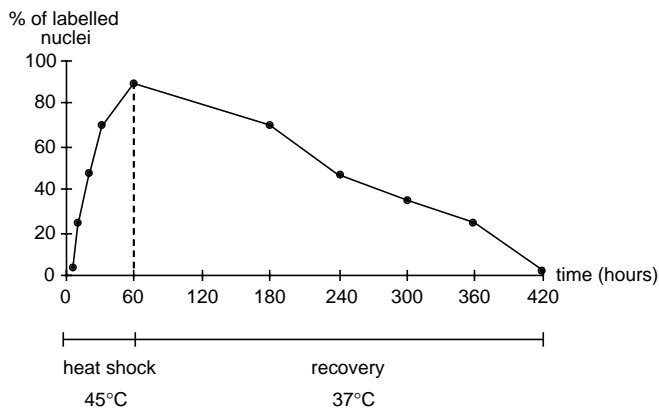


Fig. 3. Kinetics of appearance and disappearance of HSF1 foci during and after heat exposure at 45°C. 200 nuclei were analyzed for each time point.

acridine orange and ethidium bromide staining, which allows discrimination between living cells, apoptotic cells, and dead cells (McGahon et al., 1995; see Materials and Methods). The proportion of apoptotic and necrotic cells was determined after one hour of heat shock and at various times during recovery after heat exposure. As shown in Table 1, normal fibroblasts display little sensitivity to a one-hour heat shock at 45°C as assessed by the low percentage of cell death after heat exposure. Moreover, the cells were not only viable after 24 hours of recovery but also displayed the characteristic HSF1 foci when submitted to a second one-hour heat shock. It is interesting to note that a high percentage of cell death was observed in peripheral blood lymphocytes from the same donor when exposed to heat shock (data not shown). In conclusion, the low percentage of heat-induced cell death confirms the high resistance of the primary fibroblasts to heat shock. In addition, the presence of HSF1 foci is clearly not related to irreversible cellular damages such as those induced during apoptosis. These observations might not be totally surprising since the fibroblasts used in this study were obtained from a skin biopsy, and thus correspond to a cell type commonly exposed to elevated temperatures.

Kinetics of formation of HSF1 foci

To better understand the role of HSF1 foci during heat shock, the kinetics of their appearance as well as their stability during the period of recovery at 37°C were determined. Immunofluorescence assays were initially performed on cells which had been submitted to a heat shock for a period of time varying from 5 to 60 minutes. After five minutes of heat shock, less

than 5% of the nuclei displayed HSF1 foci, while foci were present in 23% of the nuclei after 10 minutes of heat exposure. The percentage of labeled nuclei increased regularly with the time of heat exposure. After one hour of heat shock, 90% of the nuclei displayed the characteristic HSF1 foci (Fig. 3).

The distribution of HSF1 was also investigated in cells which had been allowed to recover for one to six hours at 37°C after a one-hour exposure to heat shock. The proportion of labeled nuclei and the intensity of labelling regularly decreased with time (Fig. 3). After one hour, the foci were still present in most nuclei (87%), whereas only 35% of the nuclei were labeled after four hours. After six hours, only 2.5% of the nuclei displayed HSF1 foci. Whatever the length of the recovery period, the morphology of HSF1 foci was similar to that described above: clusters of granules, highly compact foci, and punctate foci were still present in the same proportion as after a one-hour heat shock. These results show that the formation of HSF1 foci upon heat shock is rapid and that the foci persist for a long period of time during recovery.

The fibroblast cell line used in this study is a primary cell line. Surprisingly, we found that the percentage of nuclei displaying the characteristic HSF1 foci decreased with the number of cell passages (data not shown). This observation suggests a relationship between the ability of cells to form HSF1 foci and *in vitro* aging. This aspect of HSF1 foci formation is currently under investigation.

A specific nuclear target for HSF1 foci?

Relative distribution of HSF1 foci and sites of active transcription

On the basis of DAPI counterstaining, HSF1 foci appeared to colocalize with regions of the nucleus displaying a weak DAPI staining (arrows in Fig. 4B). These regions were distinct from the nucleoli. As DAPI displays a higher affinity for AT-rich regions, this result suggests that HSF1 foci are localized into GC-rich regions of the nucleus which are known to be enriched in active genes. Alternatively, HSF1 foci could also accumulate into regions of lower DNA density.

Due to the essential role of HSF1 in the heat-shock response, these nuclear foci could thus serve as sites of active gene transcription. Such an association should be more pronounced during heat shock when transcription of hsp genes is enhanced. To investigate the possibility that HSF1 foci could correspond to sites of hsp genes transcription, HSF1 was detected together with hsp70 and hsp90 alpha genes, whose thermo-induced expression is known to be regulated by HSF1 (Wu, 1995; Morimoto et al., 1996). The functionality of hsp genes was assessed by an indirect approach based on the visualization of specific nuclear transcripts using fluorescence *in situ* hybridization (FISH). Previous studies have shown that

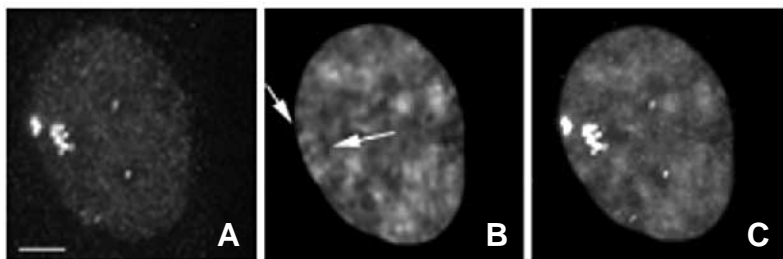


Fig. 4. HSF1 foci are localized in DAPI-negative regions of the nucleus in heat-shocked primary fibroblasts. HSF1 foci were detected by immunofluorescence (A) and nuclear DNA was counterstained with DAPI (B). HSF1 foci are localized within regions with weak DAPI staining (arrows). The merged image (C) was generated by overlaying the DAPI and HSF1 images. Bar, 5 µm.

specific transcripts accumulate in the cell nucleus at their sites of transcription (Lawrence et al., 1989; for a review see Moen et al., 1995). The detection of nuclear transcripts by FISH thus allows an indirect visualization of transcriptionally active genes within the cells nucleus. We found that hsp transcripts appeared as two foci in the nucleus of all cells as early as after five minutes of heat shock (Fig. 5A). No FISH signals were detected when cells were treated with RNase A prior to hybridization, confirming the specificity of hybridization on RNA. When cells were incubated for 15 to 60 minutes immediately after heat shock with actinomycin D at a concentration known to specifically inhibit the activity of RNA polymerase II, hsp nuclear transcripts could no longer be detected by FISH, suggesting that most if not all of the transcripts present in these foci correspond to newly synthesized transcripts. In a previous study, we have shown that hsp70 transcripts are always found in the vicinity of hsp90 beta transcripts (Jolly et al., 1998b). This is most probably due to the physical proximity of hsp70 and hsp90 beta genes, which map to the 6p21.3 (Harrison et al., 1987) and 6p12 loci, respectively (Durkin et al., 1993). In contrast, hsp90 alpha transcripts, whose gene maps to the 14q32.3 region (Vamvakopoulos et al., 1993), displayed a pronounced variability in their intranuclear positioning relative to hsp70 transcripts (Fig. 5A) (Jolly et al., 1998b).

Despite the absence of a functional clustering of hsp70 and hsp90 genes in the nucleus, the possibility that one of these hsp genes would colocalize with HSF1 foci was tested. Indeed, one can imagine a model in which only one of these genes would colocalize with HSF1. To investigate the relative distribution of HSF1 and hsp nuclear transcripts, a methodology combining FISH and immunofluorescence was developed (Jolly et al., 1998a). HSF1 foci were detected together with hsp70, hsp90 alpha, and hsp90 beta transcripts. As shown in Fig. 5B to D, HSF1 foci clearly displayed a pronounced variability in their intranuclear positioning relative to hsp70 (Fig. 5B), hsp90 alpha (Fig. 5C), and hsp90 beta (Fig. 5D) transcripts. The possibility that a functional clustering between HSF1 foci and hsp genes would occur early in the heat shock response was tested. After 10 minutes of heat shock, no co-distribution between HSF1 foci and the transcription sites was observed. At that time, nuclear transcripts could be detected in all nuclei, while only a few nuclei displayed HSF1 foci (see Fig. 3). Similarly, no co-distribution was observed in cells allowed to recover at 37°C for a period of time varying from 1 to 6 hours after one hour of heat shock. The absence of co-localization between HSF1 foci and hsp transcripts during and after heat shock clearly demonstrates that HSF1 foci do not correspond to sites of hsp genes transcription. Moreover, in cells allowed to recover for one hour in the presence of actinomycin D after one hour of heat shock, HSF1 foci were still present in most nuclei. This result clearly shows that the stability of HSF1 foci is not dependent upon transcriptional activity. However, it does not exclude the possibility that HSF1 appearance depends on some transcriptional activity.

Relationship between the number of HSF1 foci and the ploidy of the cell

The presence of two HSF1 foci in the nuclei of diploid cells strongly suggests a correlation between the number of foci

and the ploidy of the cells. To confirm this assumption, HSF1 was detected in a variety of human cells with different levels of ploidy. A small proportion of the fibroblast cells used in this study do not undergo cytodieresis, leading to the appearance of tetraploid cells which can be easily identified on the basis of their larger nucleus. The tetraploidy of these cells was confirmed by the presence of four centromeres of chromosome 6. These cells not only displayed four distinct signals for each hsp nuclear transcript, but also four HSF1 foci (Fig. 6A). The relationship between ploidy and number of HSF1 foci is clearly independent of cell type. In another primary cell line of diploid amniocytes, two HSF1 foci were also found in the nucleus of diploid cells under heat shock, while four HSF1 foci were detected in the nucleus of tetraploid cells (data not shown). HSF1 was also detected in three tumor cell lines displaying a high degree of polyploidy: the A431, MCF7, and T47D cell lines. When these cells were exposed to a heat shock at 44-45°C, HSF1 concentrated in the nucleus in 6 to 12 small foci (see Fig. 6B). These results strongly confirm the correlation between the number of HSF1 foci and the ploidy of the cells, and suggest the existence of a chromosomal target for the HSF1 transcription factor in heat-shocked cells.

DISCUSSION

In this paper, we show that under heat shock (42°C to 45°C), HSF1 concentrates in two large foci in the nucleus of normal human fibroblasts, while nuclei of tetraploid fibroblasts displayed four HSF1 foci. Moreover, tumor cells displayed a number of foci ranging from 6 to 12 per nucleus, suggesting a correlation between the number of nuclear foci and the ploidy of the cells. These findings strongly support the existence of a specific chromosomal target for HSF1 foci. This hypothesis is in good agreement with the observation that the kinetics of formation of HSF1 granules in HeLa cells at 42°C is tightly correlated with the biochemical events associated with the complete activation of HSF1 (Cotto et al., 1997a). This assumption is also consistent with the staining of numerous specific bands observed on *Drosophila* polytene chromosomes labelled with an anti-HSF antiserum (Westwood et al., 1991). However, due to the natural amplification of hsp genes within polytene chromosomes, some of these labelling sites may reflect binding of HSF to the promoter of hsp genes. Surprisingly, we found that the capacity of primary fibroblasts to form HSF1 nuclear foci greatly decreased with the number of cell passages, whereas these cells were still able to respond to heat shock, as assessed by the presence of nuclear hsp transcripts in all nuclei (data not shown). These modifications could be correlated to a decrease of the DNA-binding activity of HSF1 with in vitro aging (Choi et al., 1990), suggesting that the HSF1 nuclear foci could correspond to sites of accumulation of transcriptionally active rather than inactive HSF1. In this context, it is noteworthy that other stressors known to activate HSF1 also induce the appearance of HSF1 foci, excepted for salicylate which is the only stress inducer which uncouples the DNA-binding activity of HSF1 from its ability to activate transcription (Cotto et al., 1997a). This observation is in good agreement with the hypothesis of a role for HSF1 foci in transcription.

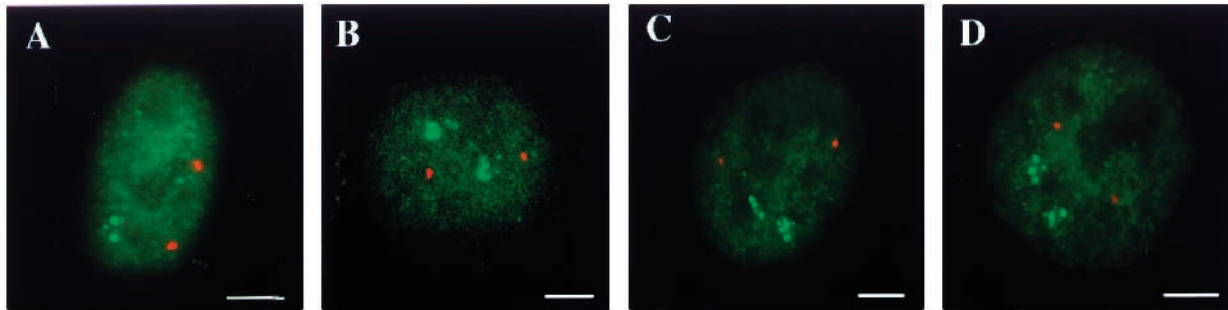


Fig. 5. Detection of hsp nuclear transcripts and HSF1 foci in heat-shocked diploid fibroblasts. (A) Hsp70 (green) and hsp90 alpha (red) nuclear transcripts detected by FISH appear as two bright foci randomly distributed in the nucleus of heat-shocked fibroblasts. (B to D) HSF1 foci (green) do not colocalize with either hsp70 (B), hsp90 alpha (C), or hsp90 beta (D) nuclear transcripts (red). Bars, 5 μ m.

To test the possibility that HSF1 nuclear foci would correspond to sites of hsp genes transcription, we have developed a methodology which combines FISH for the detection of hsp nuclear transcripts and immunofluorescence for the detection of HSF1 foci (Jolly et al., 1998a). The detection of nuclear transcripts was chosen since it allowed a clear distinction between inactive and active hsp genes (Lawrence et al., 1989; for a review see Moen et al., 1995). The absence of co-distribution between HSF1 granules and hsp70 and hsp90 transcripts at any time during and after heat exposure clearly excludes the hypothesis of a higher concentration of HSF1 in the vicinity of the active hsp genes. It is quite obvious that some HSF1 is bound to the promoter of hsp genes under heat shock. However, due to the limited sensitivity of immunofluorescence detection, it is rather unlikely that this interaction could be visualized by *in situ* approaches.

What is the biological significance of these nuclear accumulations of HSF1 specific transcription factor? Several nuclear bodies or domains of unknown function have already been described in the literature (for review see Moen et al., 1995). The most striking examples are the coiled bodies which are known to contain various proteins and RNAs such as fibrillarin, p80-coilin, the U7 snRNA and the U3 small nucleolar RNA (Bohmann et al., 1995). Other transcription factors such as the glucocorticoid and mineralocorticoid receptors (van Steensel et al., 1995, 1996), p53 (Jackson et

al., 1994), the retinoblastoma protein (Mancini et al., 1994), and the GATA transcription factors (Elefanty et al., 1996) have also been shown to display a non-homogeneous nuclear distribution. In the case of steroid receptors and GATA transcription factors, the relative distribution of these factors and sites of transcription was investigated. Co-detection experiments using immunofluorescence combined with either BrUTP incorporation (van Steensel et al., 1996; Elefanty et al., 1996) or DNA detection by FISH (Elefanty et al., 1996) have shown that these nuclear foci do not correspond to accumulations of the factors at the sites of transcription of their target genes. Of particular interest is the observation that GATA-1 concentrates in the nucleus of murine erythroblasts and megakaryocytes in specific foci whose number is related to the ploidy of the cells (Elefanty et al., 1996). So far our observation on HSF1 foci is the second report of ploidy-dependent accumulations of transcription factors which are not directly involved in the transcription of their target genes.

The nature and role of these new nuclear compartments enriched in transcription factors still remain to be determined. The relationship with the ploidy clearly suggests the existence of a specific chromosomal target, and investigations are currently underway to identify this target. It is worth noting that HSF1 factor is involved in the transcriptional repression of the prointerleukin 1 β gene, suggesting unexpected roles for HSFs (Cahill et al., 1996). Alternatively, as has been suggested for coiled bodies (Bohmann et al., 1995), HSF1 nuclear foci may represent sites of storage and/or recycling of transcription factors. The identification of new components present in these nuclear structures may help in the understanding of their function.

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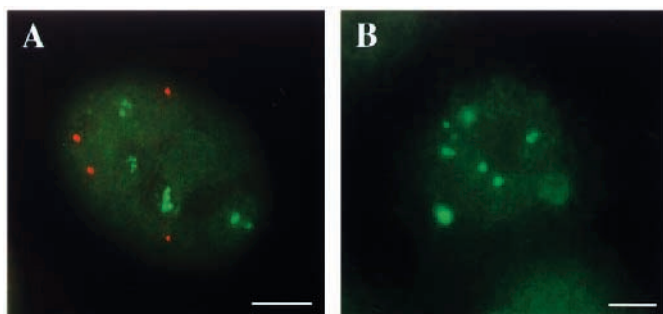


Fig. 6. Relationship between the number of HSF1 foci and the ploidy of the cell. (A) Simultaneous detection of hsp70 transcripts (red) and HSF1 foci (green) in tetraploid fibroblasts. Under heat shock conditions these cells display four nuclear transcripts foci, and four nuclear HSF1 foci. (B) Heat-shocked MCF7 tumor cells display several nuclear HSF1 foci. Bars, 5 μ m.

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