Recent studies have implicated heat shock proteins (HSP) and heat shock transcription factor 1 (HSF1) in tumor progression. We have examined the role of HSF1 in the malignant phenotype of PC-3 prostate carcinoma cells. We have developed a dominant negative construct of HSF1 that antagonizes transcription from HSP promoters and results in the depletion of intracellular HSP 70. Our studies indicate that expression of DN-HSF1 dramatically alters the DNA content of PC-3 cells (derived from p53 null prostatic carcinoma) and inhibits aneuploidy in these cells. This effect is due to prolonged expression of DN-HSF1, and transient expression of the dominant negative factor from an inducible promoter failed to cause the effect. Inhibition of aneuploidy in p53 null PC-3 cells by DN-HSF1 expression was recapitulated by expression within the cells of wild type p53. Furthermore, cells expressing DN-HSF1 showed a profound inhibition in the development of aneuploidy when exposed to chemical agents that disrupt the mitotic spindle and prevent progression through metaphase. Inhibition of aneuploidy in PC-3 cells expressing DN-HSF1 was associated with delayed breakdown of cyclin B1 compared with controls, consistent with a role for wild type HSF1 in the regulation of cyclin B1 degradation, a key step in the control of mitosis. Our experiments therefore demonstrate that HSF1 plays a functional role in cancer cells under nonstress conditions and influences cell cycle behavior and progression through mitosis and promotes the development of the aneuploid state.

Exposure of cells to elevated temperatures leads to expression of the heat shock response, which involves the induction of a cohort of heat shock proteins (HSPs) by stress and is accompanied by the expression of heat resistance (1, 2). HSPs accumulate to high levels in stressed cells and remain elevated for a prolonged period (1, 2). Accumulation of HSPs is due to activation of HSP gene expression at the levels of transcription, mRNA stability, translation, and protein stability (1–3). In mammalian cells, heat shock genes are transcriptionally regulated by heat shock factor-1 (HSF1), a sequence-specific transcription factor that binds to the heat shock elements (HSE) in their promoters (3–5). The mechanisms involved in HSF1 activation are post-translational and involve the conversion of HSF1 from a latent cytoplasmic form to an active nuclear protein (6–8). HSF family members are unique among transcription factors in binding to DNA as homotrimers (6–11). Trimerization is governed by arrays of amphipathic α-helical residues in the amino-terminal domain of HSF family proteins that interact to form coiled coiled, and trimerization is negatively regulated under nonstress conditions by a fourth region of amphipathic α-helix in the carboxyl-terminal domain (6–10). Most evidence indicates a model for HSF1 regulation in which the protein exists as a monomer in the cytoplasm associated with molecular chaperones that act as repressors of activation (3, 10, 12, 13).

Recent studies suggest that the heat shock response becomes deregulated in cancer and that HSPs become expressed to a high level and play a role in carcinogenesis (14) HSP70 overexpression for instance leads to malignant transformation in fibroblasts, and this may be related to the antiapoptotic effects of HSP70 (15); transformation was ascribed to a switch in the apoptotic index, with HSP70 overexpression favoring cell survival and the balance between cell birth and death being altered (15). Our studies show that the expression of HSP70 antisense RNA in PC-3 prostate carcinoma cells leads to spontaneous apoptosis.2 In addition, many tumor types contain high concentrations of HSP of the HSP27, HSP70, and HSP90 families (16–21). There is a molecular link between HSP expression and tumor progression in prostate cancer in that HSP56, -70, and -90 regulate the function of the androgen receptor (22, 23). Escape from androgen receptor dependence during tumorigenesis may involve altered HSP-androgen receptor interactions (22). The role of HSF in tumor development may also be related to their function in the development of tolerance to stress (24). We have therefore examined the potential role of HSF1 in the malignant phenotype of PC-3 prostate carcinoma cells using a dominant negative hsf1 construct (DN-HSF1) that antagonizes the transcription from heat shock promoters and depletes intracellular HSP concentrations. We show that expression of DN-HSF1 dramatically alters DNA content in PC-3 cell populations and inhibits aneuploidy. Our experiments suggest that HSF1 is active in malignant cells under nonstress conditions and influences cell cycle behavior and ploidy regulation.

MATERIALS AND METHODS

Cell Culture, Synchrony, and Measurement of Cell Cycle Parameters—PC-3 cells from the American Type Tissue Culture Collection

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3 The abbreviations used are: HSP, heat shock protein; HSF1, heat shock factor 1; HSE, heat shock element(s); DN, dominant negative; EMSA, electrophoretic mobility shift assay; wtHSF1, wild type HSF1.
were maintained in F-12 medium supplemented with 10% fetal calf serum in a 5% CO₂/air atmosphere. Cells were arrested in G₂ by serum deprivation. Cultures were washed once in 0.5% fetal calf serum in F-12 medium and maintained in this medium for 72 h before release from the block with 10% fetal calf serum.

To monitor cell cycle progression after release from serum starvation, thymidine block, or nocodazole block, cultures were pulsed with 0.5 μCi of [3H]thymidine per 2×10⁵ cells at 48-h intervals and 6-h pulses were performed. Cells were harvested and analyzed by flow cytometry.

Radioactive incorporation of [3H]thymidine was determined. To examine the effect of spindle disruption on cell cycle parameters, cells were treated with 0.1 mg/ml colchicines for a duration of one-half hour. To release cells from the block, nocodazole was added to the cultures, and nocodazole treatment and release from the block were monitored by flow cytometry.

The C-terminal truncation mutant DN-HSF1 was generated by the polymerase chain reaction using plasmids designed to give rise to a fragment containing codons 1–379 from HSF1. DN-HSF1 was then inserted into pCDNA3.1 as described above.

Conditionally inducible expression of wild type and dominant negative HSF1 was achieved using the regulated edesmycine-inducible system (Invitrogen). The HSF1 constructs were cloned into the pMDV5-His edesmycine-inducible expression vector. Prior to transfection with these vectors, PC-3 cells were transfected with the pVγRXR vector, which constitutively expresses the edesmycine receptor and selected for incorporation of plasmid with the antibiotic zeomycin. The HSF1 constructs were then inserted into pCDNA3.1 (–4) and isolated nuclei were assayed for HSF binding.

**RESULTS**

**Generation of Dominant Negative HSF1: C-terminal Truncation Mutants of HSF1 Repress Activation of the HSP70B Promoter by HSF1 and Prevent HSP72 Expression during Heat Shock** —We first attempted to generate HSF1 constructs that would act as specific inhibitors of HSP transcription when overexpressed in cells. Two molecular strategies were employed to design such negatively acting constructs. The first strategy included the use of HSF1 containing a point mutation (HSF1-L22) that we have shown to prevent its binding to HSE in target promoters (31); the rationale behind this approach was that such a mutant would form trimetric complexes with wild type HSF1 (wtHSF1) and thus reduce the affinity of HSF1 for HSE elements in target promoters. High affinity HSF1–HSE binding requires contact between each member of the HSF1 trimer and the triple inverted nGAAn repeat that comprises the canonical HSE (3). The second strategy employed the use of an HSF1 construct HSF1 1–379 with a 3’ truncation of 150 codons, removing the N-terminal trans-activation domains and leaving DNA binding and trimization domains (12, 32, 33). This approach has the added advantage of producing an HSF1 constitutively competent to form intracellular trimers due to the deletion of the inhibitory C-terminal leucine zipper (4). For these experiments, the HSF1 vector was inserted into the pMDV5-His vector and co-transfected with pGL.HSP70B, and the potential inhibitory HSF1 constructs were examined at a range of plasmid concentrations. The first strategy using pHSF1 (L22) proved ineffective, and the HSF1 point mutant, although defective in HSE binding, did not inhibit wtHSF1 from activating the HSP70B promoter (pGL.HSP70B) by overexpression of wtHSF1. Our previous experiments have shown that the HSP70B promoter is activated by HSF1 overexpression (34, 35). When wtHSF1 was co-transfected with pGL.HSP70B, and the potential inhibitory HSF1 constructs were examined at a range of plasmid concentrations. The first strategy using pHSF1 (L22) proved ineffective, and the HSF1 point mutant, although defective in HSE binding, did not inhibit wtHSF1 from activating the HSP70B promoter even when transfected at 50 times excess over the wtHSF1 in co-transfection experiments (data not shown). The second strategy was highly effective and led to a construct (HSF1 1–379) that antagonized wtHSF1 in a dose-dependent manner, causing significant repression even at a mutant/wild type plasmid ratio of 1:1 (Fig. 1A). As predicted, part of the effectiveness of this construct may be due to enhanced ability to bind to HSP promoters. The EMSA experiment in Fig. 1B shows that HSF1 1–379 in nuclear extracts from HSF1 1–379 transfected cells binds HSE effectively, whereas transfected wtHSF1 or pHSF1 (L22) was less effective. The identity of the HSE binding band is indicated by the fact that the construct was not supershifted by anti-HSF1 Ab68-3 antibody, which
Fig. 1. Selection of a dominant negative hsf1 construct. A, a dominant negative mutant of HSF1 competitively inhibits the ability of wild type HSF1 to trans-activate the HSP70B promoter. PC-3 cells were transiently co-transfected with the luciferase-based HSP70B promoter-reporter construct pGL.HSP70B and HSF1 expression plasmid pHSF1. Cells were then co-transfected with HSF1 truncation mutant plasmid pHSF1 1–379 at a range of plasmid doses and incubated at 37 °C. Cells were then lysed and assayed for protein content and luciferase activity containing no HSF1 encoding sequence (lanes 10) or empty plasmid containing no HSF1 encoding sequence (lanes 1–9) (Fig. 1C). The intracellular expression of HSF1 at the protein level, of a strongly heat-inducible member of the HSF family, HSP72, in cells stably expressing either wtHSF1 or HSF1 1–379. The immunoblot experiments in Fig. 1C show that the HSP72 protein is expressed at 37 °C, that HSP72 levels are increased when wtHSF1 is overexpressed, and that HSP72 expression is reduced when dominant negative construct HSF1 1–379 is expressed. Heat shock led to a marked increase in HSP72 concentrations in PC-3 cells, and this increase was effectively inhibited by HSF1 1–379 overexpression (Fig. 1C). Fig. 1D shows the relative levels of HSF1 and HSF1 1–379 protein in cells stably expressing either wtHSF1 or the dominant negative construct. We therefore utilized the HSF1 1–379 construct in subsequent experiments (DN-HSF1) as a specific inhibitor of HSF1 function to study the role of HSF1 in the malignant phenotype of prostate carcinoma cells. Extracts were also probed for relative levels of β-actin to show similar loading (Fig. 1E).

Cell Cycle Distribution in PC-3 Cells Stably Expressing either wtHSF1 or DN-HSF1—Since HSF1 and relative HSP expression have been shown to affect cell cycle distribution (28) and the rate of apoptosis (37), we next examined cell cycle distribution in populations of cells stably transfected with either empty vector alone, pHSF1 or the HSF1 1–379 construct (Fig. 2 and Table I). Each of the transfected cell types grew in continuous culture, although the cells expressing wtHSF1 grew markedly more slowly than wild type cells in the earlier passages after selection (data not shown). Subsequent analysis of the rate of growth of wild type PC-3, vector alone cell lines, wtHSF1, or DN-HSF1 indicated essentially similar rates of growth in each of the cell types (data not shown). Analysis of populations of wild type (data not shown) and empty vector transfectants by flow cytometric analysis of DNA content indicated that PC-3 cells are near triploid (Fig. 2). Expression of wtHSF1 caused a relative increase in the aneuploid fraction, whereas expression of DN-HSF1 led to a marked diminishment in aneuploidy (Fig. 2). These experiments therefore suggest that the specific inhibition of HSF1 activity in PC-3 cells either leads directly to reversal of the aneuploid DNA distribution that occurs spontaneously in these cells or that HSF1 favors the gradual formation of aneuploidy. In order to determine whether the effects of HSF1 were due to acute effects of the overexpressed proteins on the PC-3 phenotype, we next examined cell lines stably transfected with inducible vectors that express either HSF1 or DN-HSF1 from the Drosophila ecdysone promoter.

37 °C (left panel) or heat-shocked for 30 min at 43 °C and allowed to recover for 6 h (right panel) prior to lysis, 10% SDS-PAGE, and immunoblot analysis with anti-HSF1 antibodies. D, expression of wtHSF1 and dominant negative mutant HSF1 1–379 in stably transfected cell lines. Cell lines stably expressing wtHSF1 (F1) or the DN fragment HSF1 1–379 (D) or empty plasmid containing no HSF1 encoding sequence (V) were selected as described under "Materials and Methods." Cells were then either maintained at 37 °C (left panel) or prior to lysis, electrophoresis as above, and immunoblot analysis with anti-HSF1 antibodies. Cells were then either maintained at 37 °C (left panel) or heat-shocked for 30 min at 43 °C and allowed to recover for 6 h (right panel) prior to lysis. 10% SDS gel electrophoresis, and immunoblot analysis with anti-HSF72 antibodies. E, β-actin loading control derived from reprobing the blot used in D with anti-β-actin antibodies.
Cell Cycle Distribution in PC-3 Cells Acutely Expressing either wtHSF1 or HSF1 1–379 from an Inducible Expression Vector—Fig. 3A and Table II show the relative distributions in DNA content of PC-3 cell populations derived from cells stably co-expressing the ecdysone receptor and either DN-HSF1 or wtHSF1 from the ecdysone regulated promoter. As can be seen, identical aneuploid PC-3 populations were observed in cells induced for 24 h with the ecdysone receptor agonist ponasterone, suggesting that the effects of the HSF1 constructs on the PC-3 cell populations are more likely due to long term DN-HSF1 expression rather than such acute effects of protein expression as the killing of selected cell subpopulations or the acute regulation of cellular DNA content. Fig. 3B shows that despite the lack of effect of the inducer on cell ploidy, both HSF1 and DN-HSF1 1–379 are expressed in the cell populations induced with ponasterone.

Stable Transfection with either DN-HSF1 or Wild Type p53 Leads to a Reduction in DNA Content in PC-3 Cell Populations—Since previous studies show that inactivation or mutation of the p53 tumor antigen can lead to the induction of aneuploidy, we examined whether stable overexpression of p53 in the p53 null PC-3 cells (Fig. 4B and Table III) would lead to the reduction of aneuploidy. This turned out to be the case in our experiments, and both p53 and DN-HSF1 expression pro-

TABLE I

| Condition | M1 | M2 | M3 |
|-----------|----|----|----|
| Vector    | 43.6 ± 3.4 | 29.1 ± 3.1 | 18.2 ± 2.5 |
| pHSF1     | 27 ± 2.5 | 47 ± 4.1 | 25 ± 1.7 |
| pDN-HSF1  | 67 ± 5.9 | 22 ± 1.8 | |

TABLE II

| Condition | M1 | M2 | M3 |
|-----------|----|----|----|
| pDN-HSF1  | 22.8 ± 2.6 | 41 ± 3.9 | 21 ± 2.3 |
| pDN-HSF1 + ponasterone | 26 ± 4.8 | 40 ± 5.2 | 19 ± 2.4 |
| pHSF1     | 24 ± 3.7 | 41 ± 5.3 | 19 ± 3.6 |
| pHSF1 + ponasterone | 20 ± 3.2 | 36 ± 4.7 | 28 ± 3.1 |
duced PC-3 cell lines in which the occurrence of aneuploid cells was markedly reduced (Fig. 4A).

Overexpression of DN-HSF1 Inhibits the Generation of Aneuploidy in Cells Exposed to Colcemid—The deregulation of ploidy in malignant cells is associated with a defect in mitotic checkpoints (38). These include the spindle assembly checkpoint and the G\textsubscript{1} tetraploidy checkpoint, the cell cycle regulatory mechanism in which DNA replication is linked to the completion of the prior mitosis (39–42). The G\textsubscript{1} tetraploidy checkpoint appears to require the expression of wild type p53, and aneuploidy is associated with loss of functional p53 and the uncoupling of DNA replication from the completion of mitosis (41, 43, 44). The spindle assembly checkpoint does not require p53 (42). PC-3 cells are null for p53 expression (45–47), and this p53 null phenotype may contribute to the spontaneous generation of aneuploidy in PC-3 cells (Fig. 2). In fact, as shown above, stable transfection of these cells with a vector that leads to the constitutive expression of wild type p53 (\textit{wtp53}) protein leads to a reduction in aneuploidy (Fig. 4). The properties of DN-HSF1 thus resemble those of wild type p53 in ability to prevent the formation of aneuploid cell population in the PC-3 cell line, apparently by the activation of mitotic checkpoints. Therefore, we next examined the effect of microtubule active agents that block spindle assembly/progression through mitosis on cell cycle distribution in the PC-3 cell lines. Cells were synchronized in G\textsubscript{0} by serum deprivation, and the cohort of synchronized cells was then released by 10% serum addition in the presence of colcemid for one-half, 1, 2, and 4 population-doubling times (Fig. 5A and Tables IV and V). In the vector control cells, exposure to colcemid led to the formation of cell populations with increasing concentrations of aneuploid cells with up to 16N DNA detectable after 4 PDL (Fig. 5A). An increase in DNA content of colcemid-treated cells is consistent with colcemid preventing cellular segregation, whereas several rounds of DNA replication continue (48). This effect was exaggerated in cells overexpressing HSF1 (Fig. 5A). In populations expressing DN-HSF1, however, cells became synchronized with a 4N content of DNA, consistent with the majority of cells in the population arresting in metaphase (Fig. 5A). Similar findings were observed in previous studies of cells expressing wt p53 (41, 48). Colcemid-treated cells retained a 4N DNA content for up to two population-doubling times (Fig. 5A). By the time corresponding to four population doublings, a fraction of the cells appeared to escape the block, although the majority of cells still retained a 4N DNA content (Fig. 5A). These data are consistent with the hypothesis that HSF1 is needed for cells to effectively bypass the spindle assembly checkpoint and proceed into replication with unsegregated chromosomes. We also carried out similar experiments in PC-3 cells stably expressing wild type p53 (Fig. 5B). The results were essentially similar to those obtained with DN-HSF1, indicating a potent ability of p53 to prevent the generation of aneuploidy in cells in which spindle assembly is inhibited (Fig. 5B). This is probably due to the engagement of the p53-dependent G\textsubscript{1} tetraploidy checkpoint (49). It seems unlikely that DN-HSF1 acts by engaging the G\textsubscript{1} tetraploidy checkpoint as PC-3 cells lack p53, and we therefore investigated a potential role for the spindle assembly checkpoint in the effects of DN-HSF1. Since this checkpoint is mediated through the regulation of cyclin B protein expression, we next examined cyclin B1 levels in the stably transfected PC-3 cell lines (50).

Potential Role of Cyclin B1 Destabilization in the Ability of DN-HSF1 to Prevent the Expression of Aneuploidy in PC-3 Cell Populations—We measured cyclin B1 levels by immunoblot analysis in the stably transfected PC-3 cell lines at various stages after release from arrest in G\textsubscript{0} (Fig. 6). In the upper part of the figure, we show that cyclin B1 accumulates in cells expressing DN-HSF1 to a greater extent than in the other cell types stably expressing either empty vector or wtHSF1 (Fig. 6A). In similar experiments carried out in synchronized cells exposed to colcemid, cyclin B1 levels increased in all cell lines but decayed faster in the control cells and in the HSF1-overexpressing cells (Fig. 6B).
FIG. 5. Development of aneuploidy in synchronous PC-3 cell lines exposed to colcemid is antagonized by DN-HSF1 and p53 expression. A, cells stably transfected with either empty vector (Vector; left panel), wild type HSF1 (HSF1; middle panel), or DN-HSF1 (right panel) were synchronized in G0 by serum deprivation, released from G0 block by the addition of 10% fetal calf serum, and simultaneously exposed...
Expression of DN-HSF1 appears to inhibit destruction of cyclin B1 decay, and levels remained high at 7 days (corresponding to ~4 PDL), when levels were declining in the other cell lines (Fig. 6B). These data are consistent with a mechanism for regulation of DNA content involving enhanced decay of cyclin B1, release from mitotic control, and escape from the mitotic spindle assembly checkpoint regulated by HSF1 or a product downstream of HSF1 transcription. However, other mechanisms could contribute to the effects of DN-HSF1, including a direct effect on the expression of cyclin/cdk-dependent kinase inhibitor p21. p21 is regulated transcriptionally by p53 and thus contributes to p53 dependent checkpoint regulation (42). However, p21 can also be regulated independently of p53 in some circumstances (51). We found, however, in our system that both HSF1 and DN-HSF1 expression lead to a similar slight increase in p21 levels, which are undetectable in wild type PC-3 (Fig. 6C). Such a mechanism thus cannot explain the opposite effects of wtHSF1 and DN-HSF1 expression on aneuploidy in PC-3 cells (Fig. 2). A role for a mechanism involving p21 in HSF1-DN-dependent effects on cell cycle regulation therefore seems unlikely.

**DISCUSSION**

Our experiments indicate that treatment of PC-3 cells with a construct derived from HSF1 that acts in a dominant negative manner to inhibit HSF1 activity and deplete cellular HSP70 levels has a profound effect on cell cycle regulation in these malignant cells. Analysis of the DNA distribution data suggests that prior to treatment with the HSF1 constructs, PC-3 cell populations are aneuploid (Fig. 2). Overexpression in these cells of DN-HSF1 inhibits such aneuploidy (Fig. 2). A number of mechanisms have been explored to account for these findings. One possible explanation is that expression of DN-HSF1 is toxic to the aneuploid population of cells. We tested this hypothesis in cell lines that are conditional for DN-HSF1 expression, under the control of the edeysone-inducible promoter (Fig. 3). High level induction of HSF1 for 24 h failed to alter the DNA distribution of the PC-3 cell line, suggesting that the changes in DNA distribution observed are not due to acute toxicity of DN-HSF1 but reflect a longer term effect on DNA distribution in the cell population. A further hypothesis that might account for such findings is that aneuploid cells arise continuously in the PC-3 population due to a defect in the regulation of chromosome number and that cells also die continuously. An agent that prevents the generation of aneuploid cells would thus tend to reduce the levels of aneuploidy in the cell population. Other hypotheses could also be suggested, such as the reversal of polyploidy due to a round of cell division without replication, although these seem less plausible. Polyploidy is observed widely in malignant cell populations, and its incidence has been correlated with loss or mutation of the p53 tumor antigen (41, 43). The wild type p53 gene has been implicated in mitotic checkpoints that couple DNA replication to the completion of mitosis; thus, replication cannot commence until cell division is complete, and cells retain a normal diploid DNA distribution (39–41, 48). Our experiments are consistent with this hypothesis, and we find that stable transfection of the p53 null PC-3 cell line with a vector that leads to wt-p53 overexpression inhibits aneuploidy in PC-3 cell populations (Fig. 4). Expression of DN-HSF1 seems to have a similar effect as wild type p53 in its influence over cell cycle regulation in PC-3 cells, and DN-HSF1 overexpression leads to a similar inhibition of aneuploidy (Figs. 2 and 4). However, the molecular mechanisms involved in DN-HSF1- and p53-dependent regulation of ploidy appear to be different. The effects of p53 are probably mediated through p21 expression and activation of the G1 tetraploidy checkpoint (42). The effects of DH-HSF1 appear largely independent of p53 and p21 but do seem to involve cyclin B stabilization, all hallmarks of the spindle assembly checkpoint (42). Indeed, overexpression of DN-HSF1 led to delayed degradation of cyclin B1 (Fig. 6A). Additionally, in experiments in which cell division is blocked with a chemical inhibitor and the PC-3 cells, lacking in mitotic spindle checkpoint activity, progress to a polyploidy DNA distribution, expression of DN-HSF1 prevented polyploidy and delayed the decay in cyclin B1 levels (Figs. 5 and 6). Thus, our data support the hypothesis that HSF1 is a positively acting factor in the mechanisms involved in overriding the spindle assembly checkpoint in cancer cells. The detailed mechanisms involved, however, are not clear at this stage. It has been shown that the decay in cyclin B1 levels in cells progressing from M to G1 phase is associated with its targeted degradation through the anaphase promoting complex, a large complex of ubiquitin-targeted proteases (52, 53). Expression of DN-HSF1 appears to inhibit destruction of cyclin B1 in cells progressing through mitosis, and this may at least partially explain its role in the control of ploidy in PC-3 cells

| TABLE IV | Cells in M1, M2, and M3 in Fig. 5A |
|---|---|---|---|---|
| Vector | M1 | M2 | M3 | M4 |
| 1/2 | 44 ± 4.7 | 44 ± 4.4 | 12 ± 1.5 | |
| 1 | 13 ± 2.2 | 38 ± 3.3 | 39 ± 4.1 | 11 ± 2.2 |
| 2 | 16 ± 2.1 | 46 ± 3.8 | 18 ± 2.0 | 20 ± 3.1 |
| 4 | 6 ± 1.8 | 34 ± 3.5 | 36 ± 2.2 | 34 ± 3.2 |
| HSF1 | 1/2 | 38 ± 4.2 | 38 ± 3.8 | 24 ± 2.9 |
| 1 | 8 ± 2.4 | 44 ± 3.9 | 40 ± 2.5 | 10 ± 3.6 |
| 2 | 7 ± 1.9 | 36 ± 2.9 | 28 ± 4.0 | 29 ± 5.4 |
| 4 | 8 ± 1.9 | 25 ± 2.6 | 24 ± 2.6 | 44 ± 3.4 |
| pDN-HSF1 | 1/2 | 71 ± 5.1 | 29 ± 2.4 |
| 1 | 20 ± 4.4 | 78 ± 7.9 | 3 ± 1.9 |
| 4 | 3 ± 1 | 92 ± 6.5 | 5 ± 2.7 |
| 4 | 11 ± 1.8 | 46 ± 3.4 | 39 ± 6.2 | 4 ± 2.3 |

| TABLE V | Cells in M1, M2, and M3 in Fig. 5B |
|---|---|---|---|---|
| Vector | M1 | M2 | M3 | M4 |
| 1 | 25 | 48 | 20 | 6 |
| 2 | 12 | 59 | 22 | 3 |
| 4 | 10 | 55 | 27 | 5 |
| p-p53 | 1 | 43 | 46 | 9 | 1 |
| 2 | 12 | 66 | 17 | 3 |
| 4 | 14 | 61 | 22 | 2 |
Fig. 6. Cyclin B1 expression in synchronous cultures of PC-3 cells at various times after release from G0 block. In A, cells were released from synchrony in G0 by serum (10%) refeeding; quenched in Laemmli sample buffer at 12, 24, 36, and 48 h after release; and cell lysates were subjected to 10% SDS-PAGE and immunoblot analysis with anti-human cyclin B1 antibodies. Cells in B, after being released from synchrony, were exposed to colcemid as in Fig. 5 and then quenched at 1–7 days after treatment and subjected to immunoblot analysis with anti-human cyclin B1 antibodies as above. In C, we have examined the relative expression of p21 in wild type PC-3 cells (lane 1), in empty vector controls (lane 2), and in HSF1 DN-expressing (lane 3) and in HSF1 (lane 4) wild-type expressing PC-3 cell lines. Positive controls for p21 expression were extracts from MCF– breast carcinoma cells (lane 5). Results are representative of two independently performed experiments with similar results.

HSF1 and Cancer Cell Ploidy

(FIG. 6, lane 1). HSF1 may affect cell cycle progression by enhancing a step in cyclin B1 breakdown through the proteosomal pathway. Recent studies have indeed shown that proteosomal activity is down-regulated in HSF1 null cells, further suggesting a role for the proteosome in this phenomenon (52–54). In addition, HSP70 and HSP90, both products downstream of HSF1 activity, have been shown to be physically associated with the proteosome and may play a functional role in proteosomal activity (55, 56). Moreover, both HSP70 and the proteosomal components are localized to the centrosome (56–58). Since centrosome amplification has been shown to accompany aneuploidy in p53 mutant cells, a mechanism involving HSP70 and the proteosome could be involved in cyclin B1 degradation at the centrosomes of tumor cells (45). However, the mechanisms involved in HSF1 in the regulation of ploidy are still unclear, and much remains to be learned regarding whether a specific product of HSF1 transcription is involved and the exact role of cyclin B degradation or other potential mechanisms.

These findings suggest that HSF1 exerts regulatory effects on cell processes in addition to control of the heat shock response. The best characterized function of the HSF1 is as a stress-activated transcription factor that controls HSP molecular chaperone expression (3). The transcriptional function of HSF1 is fairly weak in unstressed tissue culture cells compared with its potent activity in stressed cells, although malignant cells such as PC-3 appear to have enhanced HSF1 activity (59).3 Indeed, in our studies, the properties of DN-HSF1 in PC-3 cells correlate with its ability to block transcription of the HSP70B gene and reduce HSP70 synthesis (Fig. 1). It is also possible, however, that HSF1 may play a nontranscriptional role in cancer cells. HSF1 has been shown, for instance, to bind both subunits of DNA-dependent protein kinase, to activate the enzyme to auto-phosphorylation, and to phosphorylate exogenous substrates (60).

The consequences of aneuploidy for malignant transformation are also not yet clear. However, polyplody is associated with genomic instability and may thus play a permissive role in malignant progression (45, 61–63). HSF1 may contribute to the malignant progression through a permissive role in the dysregulation of ploidy, particularly in p53 null cells. It may be significant in this respect that recent studies have implicated a role for HSF1 overexpression in the development of a metastatic variant of LnCap cells (14).

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