Heat shock protein hsp70 protects cells from thermal stress even after deletion of its ATP-binding domain

(stable expression/thermal resistance/ATP binding)

GLORIA C. LI*†, LIGENG LI*, RICHARD Y. LIU*, MUHAMMED REHMAN‡, AND WILLIAM M. F. LEE‡

*Departments of Medical Physics and Radiation Oncology, Memorial Sloan–Kettering Cancer Center, New York, NY 10021; and ‡Hematology–Oncology, Department of Medicine and the Cancer Center, Philadelphia, PA 19104

ABSTRACT   Retroviral-mediated gene transfer experiments show that rodent cells become heat resistant when stably and constitutively expressing a cloned human gene encoding an intact human 70-kDa heat shock protein (hsp70). Cells expressing higher levels of the hsp70 protein generally tolerate thermal stress better, whereas cells expressing either of two mutated hsp70-encoding genes, one with a 4-base pair out-of-frame deletion and one with an in-frame deletion of codons 438–618, are heat sensitive. These results provide strong evidence that expression of hsp70 leads directly to thermal tolerance. Surprisingly, cells expressing a mutant hsp70 of a human gene missing codons 120–428 are, nevertheless, heat resistant. Because the deleted region of this mutant contains the ATP-binding domain of human hsp70, this domain appears dispensable in the hsp70-mediated protection of cells from thermal stress.

When exposed to a nonlethal heat shock, cells acquire thermotolerance—i.e., a transient resistance to subsequent heat exposures (1–4). Studies suggest that the induction of heat shock proteins (hsps)—in particular hsp70—may be involved (5–10). However, evidence is largely circumstantial and rests on a correlation of cellular levels of hsp70 with thermotolerance. Overexpression of hsp70 has been achieved only by experiments that could alter other cellular components, which, in turn, might affect cellular response to thermal stress (4, 6, 11–13). Similarly, correlation of permanent heat resistance with elevated hsp70 expression in different cell lines naturally expressing various levels of the protein may be attributable to other cellular differences (14–16). Experimental manipulations that depress the hsp70 level, such as microinjection of anti-hsp70 antibodies (17), show that hsp70 may participate in protecting cells against thermal stress but do not show that hsp70 expression alone is sufficient for thermal protection.

We have shown (10) that rat cells expressing a cloned gene for human hsp70 via DNA-mediated gene transfer become thermal resistant. However, because of the low incidence of thermal-resistant transfectants, their identification involved a heat-selection protocol in which pooled populations of transfected cells were subject to six cycles of heating (10). To rule out the possibility that this protocol biased the outcome, we have used a retroviral gene-transfer system to efficiently deliver and express human hsp70 in rodent cells and, thus, obviated heat selection. We show here that the constitutive and stable expression of this gene confers heat resistance, which implies a direct link between expression of functional mammalian hsp70 and cell survival at elevated temperatures. Furthermore, we find that cells expressing a mutant human hsp70 that deletes the region required for ATP binding (18) are equally heat resistant. This surprising finding is poten-

tially important in view of the widely held notion that ATP binding and hydrolysis modulate interactions between hsp70 and its putative targets, including nascent polypeptides and denatured proteins (19, 20).

MATERIALS AND METHODS

Construction of Human hsp70 Retroviral Expression Vector and hsp70 Deletion and Fusion Genes. To construct the pMVH human hsp70 proviral vector, the 2.3-kilobase (kb) *Bam*HI–*Hin*dIII fragment of the cloned gene for human hsp70 (21, 22), which contains the intact coding region, was subcloned between these same sites in a pGem3 vector (Promega). Most of the hsp70 3′ untranslated region of this subclone (including the polyadenylylation signal) was removed with Bal-31, and the truncated hsp70 fragment was inserted between the *Bam*HI and *Hinc*II sites of pGem3. Subsequently, the hsp70 fragment was excised from pGem3 with *Eco*RI and *Hin*dIII and subcloned into the pMV6 proviral vector (23, 24), generating pMVH (Fig. 1*A*).

The mutant hsp70 gene was derived from the intact gene in pGem3. The translation initiation ATG codon was deleted by Bal-31 digestion from the 5′ end. The deleted hsp70 fragments were subcloned into pGem3 cut with *Sma* I and *Hin*dIII. We sequenced several deletion mutants and selected for further study a mutant in which the ATG and the guanine nucleotide of the second hsp70 codon were deleted (clone Δ21). The intact human hsp70-encoding gene contains two in-frame *Bgl* II sites and two in-frame *Sma* I sites, which allow in-frame deletion of the intervening sequences and yielded the ΔBg (deleting codons 120–428) and the ΔSm (deleting codons 438–618) mutants (Fig. 1*B*). All these mutants were cloned into the pMV6 proviral vector to generate pMVHΔ21, pMVHΔBg, and pMVHΔSm; the last two were also engineered into a hsp70–substance P fusion gene (see below) before being subcloned into pMV6 and generating pMVHPΔBg and pMVHPΔSm.

To express substance P-tagged human hsp70, nucleotides encoding this immunogenic peptide were introduced between the penultimate and termination codons of human hsp70 gene using the PCR and synthetic oligonucleotide primers: 5′-TTCGACATCGATGCCAACGGC-3′, which specifies the sequence surrounding the *Cla* I site of human hsp70 gene near codon 481, and 5′-CGCAAGCTTTTACATGAGCCCGAA-GAACTGATCTACCTCCTCAATGGTGGGGCC-3′, which specifies a *Hin*dIII site followed in succession by sequences complementary to a termination codon, codons for the substance P hexapeptide, and the last seven coding codons of human hsp70. After PCR amplification with the cloned gene
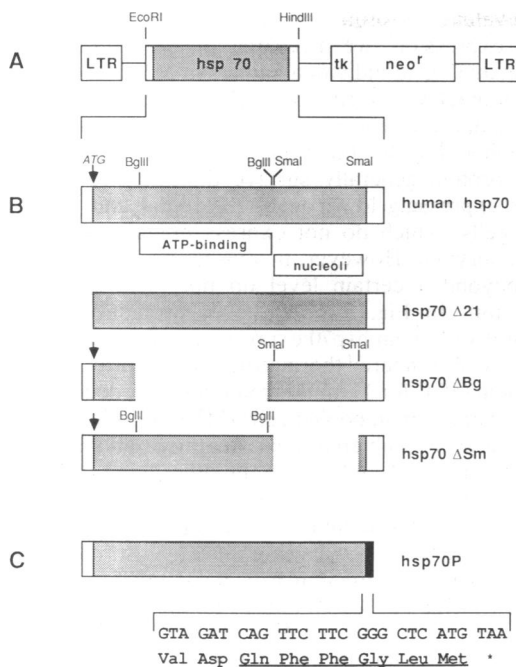

FIG. 1. Human hsp70 retroviral expression vector and hsp70 deletion and fusion genes. (*A*) pMVH human hsp70 proviral vector. This provirus contains a human hsp70-encoding gene (21) with an intact coding domain (shaded box) and a G418-resistance gene (neo^r) driven by a thymidine kinase promoter (tk) between retroviral long terminal repeats (LTR). (*B*) Mutant hsp70 genes. Intact human hsp70 gene is shown with relevant restriction sites and the ATG initiation codon. Hsp70Δ21 is an out-of-frame deletion mutant. Hsp70ΔBg (deletion of codons 120–428) and hsp70ΔSm (deletion of codons 438–618) are two in-frame deletion mutants (deleted regions indicated by spaces). (*C*) Substance P-tagged human hsp70 gene. Substance P C-terminal hexapeptide-encoding nucleotides are represented by black box; nucleotides encoding this immunogenic peptide are underlined.

as template, the product was digested with *Cla* I and *Hind*III and used to replace the *Cla* I to *Hind*III section of the normal intact gene. After verifying the absence of unintended mutations, this modified human hsp70 gene was introduced into pMV6, generating pMVHP (Fig. 1*C*).

Cell Cultures and Retroviral Gene Transfer System. Cultures of rat fibroblasts, designated Rat-1, were grown in Dulbecco's modified medium (DME-H21)/10% fetal bovine serum with appropriate antibiotics (10). The pMV6-derived proviral vectors were transfected into φ2 packaging cells (25, 26). The helper-free, replication-defective, ecotropic recombinant retroviruses obtained were used to infect Rat-1 cells. Selection of the infected cells with G418 (400 μg/ml) generated hundreds of drug-resistant colonies that were isolated both as single colonies (designated MVH-1-1 to MVH-1-24 and MVH-2-1 to MVH-2-26; the 1 and 2 after MVH distinguish colonies from separate infection experiments) and as pooled populations of a minimum of 200 individual colonies (designated MVH-1-p1, MVH-1-p2, MVH-2-p1, and MVH-2-p2, etc.). As controls, Rat-1 cells were infected with (*i*) the parental MV6 retrovirus bearing the G418-resistance gene but not the human hsp70 gene (MV6-p cells), or (*ii*) one of several recombinant retroviruses bearing mutant genes for human hsp70 (Fig. 1*B*).

All transfected cell lines were routinely maintained in medium/G418 at 200 μg/ml. For cell survival studies, protein labeling, and immunoblotting analysis, monolayers of cells were plated on day 0 in medium with no G418, grown exponentially, and used on day 3. All cell lines were stable; plating efficiencies were 80–90%, 80–90%, and 35–65%, and

doubling times were ≈16, 16, and 24 hr for Rat-1, MV6-infected Rat-1, and MVH-infected Rat-1 cells, respectively. Heating and cell survival studies were done as described (6, 8, 10).

Protein Labeling and Gel Electrophoresis. Protein labeling and two-dimensional gel electrophoresis was done as described (10).

Antibodies, Preparation of Cell Lysates, and Immunoblot. Monoclonal antibodies (mAbs) against hsp70 used in these studies were from commercial sources (Amersham or StressGen Biotechnologies, Sidney, Canada). Antisera against substance P (RAS 7451N) were purchased from Peninsula Laboratories. Second antibodies and reagents for antibody-enzyme-coupled detection of antigens were from Vector Laboratories. Cell lysates were prepared as described (10). Immunoblotting was done as described by Towbin *et al.* (27).

Flow Cytometric Analysis of hsp70. Fluorescein isothiocyanate (FITC)-conjugated anti-hsp70 antibodies and flow cytometry were used to quantify hsp70 level. Cells were fixed in 70% ethanol and stained with anti-hsp70 mAbs (Amersham, 1:500 dilution). FITC-conjugated anti-mouse immunoglobulin (Amersham, 1:100 dilution) was then added to the cell samples, and immunofluorescence was analyzed by flow cytometry (Becton Dickinson, FACS 440). hsp70 level was estimated from the mean FITC fluorescence intensity of the cell population.

RESULTS

Human hsp70 Can Be Stably Expressed via Retroviral-Mediated Gene Transfer. Analysis of control Rat-1 or MV6-p cellular extracts reveals that these cells produce two major proteins in the 70-kDa size range in response to heat shock (Fig. 2*A*, *a* and *b*): synthesis of the 72-kDa protein occurs when these cells are grown at 37°C (Fig. 2*Aa*, arrowhead) but is significantly enhanced after heat shock treatment (Fig. 2*Ab*); synthesis of the 70-kDa protein, the inducible form of endogenous rat hsp70, is not detectable until after heat shock (Fig. 2*Ab*, arrowhead on right). In MVH-1-p1 cells, which were infected with retroviruses containing the human hsp70 gene, an additional protein of ≈70-kDa is synthesized under normal growth conditions at 37°C and after heat shock (Fig. 2*A*, *c* and *d*, arrow labeled 70$_h$). This protein (70 kDa, pI = 5.9) clearly separated from constitutive rat hsp70 (72 kDa, pI = 5.6) and heat-inducible rat hsp70 (70 kDa, pI = 6.1) and has an electrophoretic mobility and isoelectric point identical to the human hsp70 protein expressed in human 293 cells (data not shown). To further confirm that this protein constitutively synthesized in MVH-1-p1 (designated as hsp70$_h$), but not in Rat-1 or MV6-p cells, represents the product of the human hsp70 gene introduced by retroviral infection, cellular proteins resolved by two-dimensional gel electrophoresis were transferred to nitrocellulose filters and probed with a mixture of two mAbs, C92F3A-5 (specific against inducible form) and N27F3-4 (specific against constitutive and inducible form) (StressGen, ref. 28). Use of this mAb mixture allows detection of all hsp70 proteins in our system. Immunoblot analysis of proteins separated on two-dimensional gels (Fig. 2*B*) clearly shows immunologically that this additional 70-kDa protein, constitutively synthesized and accumulated in MVH-infected Rat-1 cells, is the human hsp70 gene product. Human hsp70 is expressed only in MVH-infected Rat-1 cells (Fig. 2*Bc*) and not in control or heat-shocked Rat-1 cells (Fig. 2*B*, *a* and *b*). Fig. 2*C* shows that almost all clones derived from Rat-1 cells infected with the MVH retrovirus (15 of 17 randomly picked and examined) express readily detectable human hsp70 protein. Thus, retroviral delivery consistently results in significant constitutive human hsp70 gene expression in rat fibroblasts and allows us to study the effect
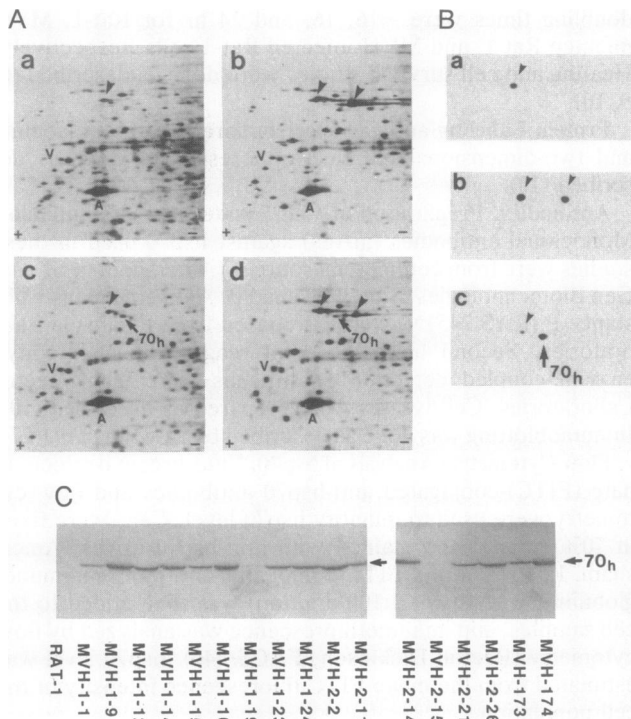
FIG. 2. hsps synthesized in Rat-1 cells and Rat-1 cells infected with retroviruses bearing human hsp70 gene. (A) Autoradiograms showing expression of human hsp70 protein in infected rat MVH-1-p cells (-p means pooled population). (a) MV6-1-p cells at 37°C. (b) MV6-1-p cells at 45°C for 15 min. (c) MVH-1-p cells at 37°C. (d) MVH-1-p cells at 45°C for 15 min. All groups were labeled for 6 hr at 37°C. Arrowhead, endogenous rat hsp70s; arrows, human hsp70 expressed in MVH-1-p cells (70ₕ) in c and d; A, actin; V, vimentin. (B) Immunoblot analysis. (a) MV6-1-p cells at 37°C. (b) MV6-1-p cells heat shocked at 45°C for 15 min and then incubated at 37°C for 16 hr. (c) MVH-2-1 cells at 37°C. MVH-2-1 is an individual clone derived from Rat-1 cells infected with MVH retroviruses. Arrowheads, endogenous rat hsp70s; arrow, human hsp70 expressed in MVH-2-1 cells (70ₕ). (C) Expression of human hsp70 among different individual clones of infected Rat-1 cells. Cells grown at 37°C were lysed in Nonidet P-40 lysis buffer (10). Equal amounts of proteins were separated by one-dimensional gel electrophoresis, transferred to nitrocellulose membranes, and probed with mAb C92F3A-5. Human hsp70 is indicated by arrow at right. Rat-1, control uninfected Rat-1 cells. MVH-1-1- and MVH-2-2- etc.; clones derived from MVH-infected Rat-1 cells.

of selective hsp70 gene expression on cell survival after heat shock.

The retroviral-mediated expression of human hsp70 does not induce endogenous rat hsp70 proteins (Fig. 2B) or other rat hsps—e.g., hsp27 (data not shown). Similar conclusions can be drawn by comparing the two-dimensional protein patterns in Fig. 2A, a and b: other than the presence of hsp70ₕ in c, no significant difference between autoradiograms is seen.

Constitutive Expression of Human hsp70 Protects Cells from Thermal Stress. We used both pooled populations and individually cloned lines of MVH-infected cells to examine the effect of exogenous human hsp70 expression on cell survival after heat shock. Fig. 3A shows that MV6-infected Rat-1 cells exhibited similar clonogenic survival compared to parental Rat-1 cells after 45, 60, or 75 min of heat shock at 45°C. In contrast, six independent pools of MVH-infected Rat-1 cells exhibited ≈100-fold higher survival after 60 and 75 min of 45°C heat treatment. Similar results were obtained when the 45°C cell survival experiments were done with individually cloned lines of MVH-infected Rat-1 cells (Fig. 3B). These results strongly suggest that expression of the human hsp70 gene protects cells from thermal stress.

To evaluate the protective effect of different levels of human hsp70 expression, we measured the level of human hsp70 protein at 37°C in individual clones of MVH-infected cells by flow cytometry (10). In a parallel experiment, the clonogenic survival of these cells after 75 min of 45°C heat shock was also determined. Fig. 3C shows that clones expressing more human hsp70 protein generally survive thermal stress better than clones expressing lower levels. Parental and MV6-infected Rat-1 cells, which do not express human hsp70, have the lowest survival. However, increases in human hsp70 expression beyond a certain level do not yield greater thermal protection. In Fig. 3B, degree of protection afforded by constitutive human hsp70 expression is compared to survival of Rat-1 cells rendered thermotolerant by a prior sublethal heat treatment (45°C for 15 min). Protection afforded by exogenous hsp70 expression in pooled cells (MVH-p) is less than protection offered by sublethal heat treatment given 24 hr earlier (compare Fig. 3 A and B). On the other hand, MVH-infected clones with high constitutive human hsp70 expression appear almost equally protected when compared to the maximally thermotolerant Rat-1 cells (Fig. 3B).

Expression of Mutant hsp70 and Its Effects on Thermal Sensitivity. We constructed three mutant human hsp70 genes in the retroviral vector pMV6. The MVHΔ21 is an out-of-frame deletion mutant, in which 4 base pairs (bp) beginning with the initiation ATG were deleted. Cells infected with MVHΔ21 produced no exogenous protein detectable by our hsp70-specific antibodies (Fig. 4A, lane 3). In the two in-frame deletion mutants MVHΔBg and MVHΔSm, segments encoding amino acids 120–428 and 438–618 of human hsp70 were, respectively, deleted (see Fig. 1B for details). Fig. 4A, lanes 4–6, show that in cells infected with MVHΔBg, but not in cells infected with MVHΔSm, a protein smaller than hsp70 was readily detectable with mAbs against human hsp70. The molecular mass of this protein in MVHΔBg-infected cells (lanes 5 and 6), ≈40 kDa, is that of the expected mutant hsp70 protein with a deletion of 309 amino acids.

To test whether the absence of the expected protein in MVHΔSm-infected cells (Fig. 4A, lane 4) was due to removal of epitopes required for recognition by the mAbs, vector pMVHP (see Fig. 1C) was used to add codons encoding the C-terminal hexapeptide of neuropeptide substance P to the 3' end of the hsp70-coding region of MVHΔSm (29). The heterologous epitopes in hsp fusion proteins should allow anti-substance P antibodies to detect mutant fusion proteins that hsp70-specific antibodies cannot. Cells infected with the immunotagged construct MVHPΔSm produced a 50-kDa protein recognized by substance P-specific antibodies (Fig. 4A, lanes 12 and 13), suggesting that cells infected with MVHΔSm virus probably expressed the truncated protein as well. For comparisons, vector pMVHP was also used to express substance P-tagged intact human hsp70 (MVHP, Fig. 4A, lanes 10 and 11) and to construct a clone for expressing immunotagged ΔBg deletion protein (MVHPΔBg, Fig. 4A, lane 14). Fig. 4A (lanes 10, 11, and 14) clearly shows that these tagged proteins were readily detectable in cells infected with the corresponding constructs. We also examined the protein patterns of Rat-1 cells and Rat-1 cells expressing intact or mutant human hsp70 by labeling cells with [¹⁴C]leucine followed by one-dimensional gel electrophoresis; other than the presence of intact or mutant hsp70ₕ, no significant difference was seen (data not shown).

In contrast to the heat-resistant phenotype of rodent cells expressing intact human hsp70, cell survival experiments with pools of MVHΔ21- or MVHΔSm-infected cells showed no change in thermal sensitivity relative to control MV6-infected or uninfected Rat-1 cells (Fig. 4B). Cells expressing human hsp70 with the substance P hexapeptide immunotag at its C terminus exhibited significantly higher thermal resistance when compared to the parent rodent cells, although
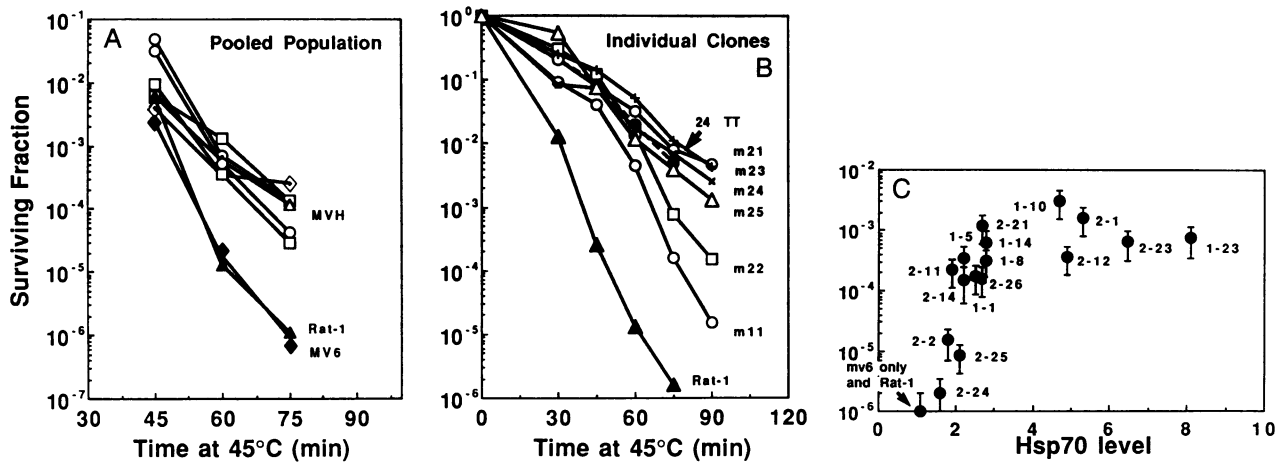
Fig. 3. Expression of human hsp70 gene confers thermal resistance to Rat-1 cells. (*A*) Survival at 45°C of pooled MVH-infected, MV6-infected, and uninfected Rat-1 cells. Survivals from six pooled populations of MVH-infected cells independently derived from separate infection experiments were shown (open symbols). Each pool was derived by pooling 200–600 colonies. Survival values after 30-min heating at 45°C were clustered around 10% for all cells and were, therefore, omitted for clarity. (*B*) Survival at 45°C of individually cloned lines derived from MVH-infected cells. Each cell line expresses human hsp70 in the following order: m21 (highest) ≥ m23, m24 > m25 > m22, m11 (lowest). Survival data for 24 hr-thermotolerant Rat-1 cells (24 TT, ●) were shown for comparison. Thermotolerance was induced by a heat treatment at 45°C for 15 min, followed by 24-hr incubation at 37°C. (*C*) Monolayers of exponentially growing individual clones of MVH-infected cells (1–2 × 10^6 cells in 60-mm Petri dish) were exposed to 45°C for 75 min, and survival was determined. In parallel experiments, relative levels of human hsp70 in these MVH clones were measured by flow cytometry (Becton Dickinson, FACS 440), using mAb C92F3A-5 specifically against human hsp70. Relative levels of hsp70 were estimated by mean FITC fluorescence intensity of cell population. At least 20,000 cells were analyzed for each flow cytometry measurement. Thermal survivals of various infected cell lines were plotted against the relative level of human hsp70. Experiments were done at least twice and yielded consistent results.

slightly less resistance when compared with cells expressing the intact human hsp70 without the immunotag (Fig. 4*B*). Cells expressing ΔSm-truncated human hsp70 with the same immunotag did not increase in survival relative to parent

rodent cells; however, whether pools of MVHPΔSm-infected cells or individual clones of cells expressing high levels of tagged mutant human hsp70 protein (hsp70ΔSm) were used (Figs. 4 *B* and *C*).
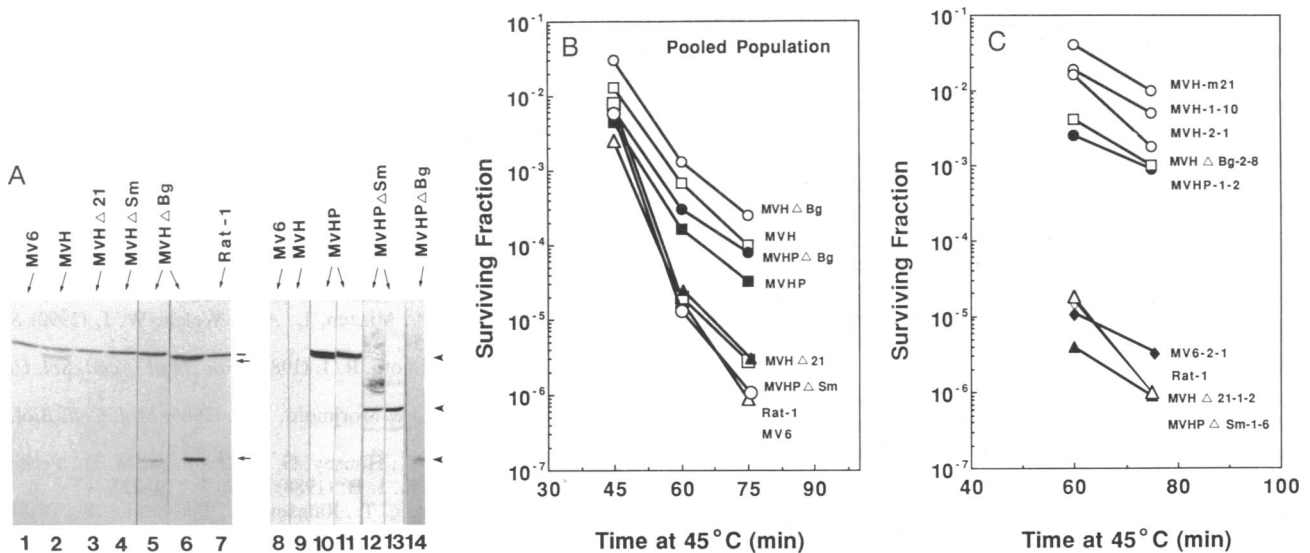

Fig. 4. Expression of and thermal protection offered by mutant hsp70. (*A*) Immunoblot analysis. Expression of intact or mutant hsp70 proteins was established by using mAb N27F3-4 against hsp70 (lanes 1–7) or antisera against substance P (lanes 8–14), whenever appropriate. Pooled populations from at least 600 colonies were used. Experiments were repeated at least twice and yielded identical results. Proteins were extracted from the following cells: control MV6 pool (lane 1); MVH pool, expressing intact untagged human hsp70 (lane 2); MVHΔ21 pool (lane 3); MVHΔSm pool, untagged (lane 4); MVHΔBg pool, untagged (lane 5); MVHΔBg clone (lane 6); Rat-1 cells (lane 7); MV6 pool (lane 8); MVH pool expressing intact untagged hsp70 (lane 9); MVHP pool expressing tagged hsp70 (lane 10); MVHP clone (lane 11); MVHPΔSm pool (lane 12); MVHPΔSm clone (lane 13); MVHPΔBg pool (lane 14). Substance P-tagged human hsp70 is indicated by upper arrowhead on far right, untagged human hsp70 and its deletion mutant are indicated by arrows, and the endogenous rat hsp71 is indicated by the bar above arrows. Middle and lower arrowheads indicate the 50-kDa and 40-kDa mutant proteins recognized by substance P antibodies, respectively. (*B*) Survival after 45°C heat shock of pooled MVHΔBg-, MVHPΔSm-, and MVHΔ21-infected cells. (*C*) Survival after 45°C heat shock of individual clones of MVH-, MVHP-, MVHΔBg-, and MVHPΔSm-infected Rat-1 cells. Note significant thermal resistance in cells expressing ΔBg mutant protein (MVHΔBg-2-8). On the other hand, thermal survival for cells expressing ΔSm is almost indistinguishable from uninfected Rat-1, MV6-2-1 (Rat-1 cells infected with MV6 containing only the neomycin-resistance gene) or MVHΔ21 cells. MVH-m21, MVH-1-10, and MVH-2-1 clones express intact, untagged human hsp70; MVHP-1-2 cells express tagged human hsp70.

Human hsp70 Lacking ATP-Binding Domain Can Still Protect Cells Against Thermal Stress. Interestingly, cells infected with MVHΔBg are more resistant to 45°C heating when compared to parental lines (Fig. 4B). When individual clones of cells expressing high levels of intact hsp70 or mutant hsp70ΔBg protein are selected, the enhanced thermal resistance due to expression of this mutant hsp70ΔBg protein is even more striking (Fig. 4C).

DISCUSSION

Our data provide direct evidence for a causal relation between expression of a functional form of mammalian hsp70 and survival of cells at elevated temperatures. Constitutive expression of human hsp70 protein, by itself, confers thermal resistance. Production of hsp70 is only part of the program of protein biosynthesis initiated after heat shock, and other components of this response might also enhance cell survival. This hypothesis is supported by the observation that Rat-1 cells rendered thermotolerant by a prior exposure to sublethal heat treatment and expressing the full panoply of hsps can better withstand heat shock than pooled cells only expressing exogenous human hsp70. However, the survival difference between thermotolerant and heterogeneous populations of recombinant Rat-1 cells may also be from a quantitative difference in the level (or concentration) of rat and human hsp70 expressed in the respective cells before heat shock, because clones of recombinant cells expressing the highest human hsp70 levels are nearly as resistant to heat shock as the thermotolerant cells.

The molecular and biochemical basis underlying the protective effect of hsp70 expression is unknown, although hypotheses have been advanced (19, 30). To study the mechanisms involved, hsp70 has been characterized, and several properties have been described. Among these, the ability of hsp70 to bind ATP and to localize to the nucleolus after heat shock are well established (18, 31). Although the functional significance of these properties is unknown, the regions of the protein responsible have been delineated. Thus, sequences in the C-terminal third of the human protein have been shown necessary for nucleolar localization and dispensable for ATP binding. Conversely, sequences in the N-terminal two-thirds of the protein appear necessary for ATP binding and dispensable for nucleolar localization. That removal of amino acids 438–618 from human hsp70 (in ΔSm mutants) abrogates both its nucleolar localization after heat shock and thermal protective effect suggests that the former may be important for the latter. Whether the two properties are linked or separable will require examination of smaller mutations.

Our observation that removal of amino acids 120–428 from human hsp70 (in ΔBg mutants), which deletes nearly half of the protein (all from its N-terminal two-thirds), does not affect its protection of cells from heat shock suggests that ATP binding is unnecessary for its thermal protective function. This finding is surprising in view of the widely held idea that ATP binding and hydrolysis modulate interactions between hsp70 and its putative targets, including nascent polypeptides and denatured proteins (19, 20). The binding of ATP to hsp70 has been suggested to affect its interaction with cellular proteins, which may, in turn, prevent detrimental aggregation of these proteins; ATP hydrolysis presumably allows hsp70 to dissociate from its targets and, thus, to turn over (19, 20); or alternatively, ATP binding and/or hydrolysis by hsp70 may facilitate dissociation of protein aggregates already formed (19). Our results suggest that ATP binding

and/or hydrolysis by hsp70 are dispensable in its protection of cells from thermal killing. Perhaps hsp70 lacking the ATP-binding domain can still bind to cellular proteins and prevent their aggregation at elevated temperature; rather, ATP binding and/or hydrolysis may be used to enable hsp70 to dissociate from its substrates or to facilitate the dissociation of aggregated proteins.

1. Gerner, E. W. & Schneider, M. J. (1975) *Nature (London)* **256**, 500–502.
2. Henle, K. J. & Leeper, D. B. (1976) *Radiat. Res.* **66**, 505–518.
3. Henle, K. J. & Dethlefsen, L. A. (1978) *Cancer Res.* **38**, 1843–1851.
4. Lindquist, S. & Craig, E. A. (1988) *Annu. Rev. Genet.* **22**, 631–677.
5. Landry, S., Bremier, D., Chretien, P., Nicole, L. M., Tanquay, R. M. & Marceau, N. (1982) *Cancer Res.* **42**, 2457–2461.
6. Li, G. C. & Werb, Z. (1982) *Proc. Natl. Acad. Sci. USA* **79**, 3219–3222.
7. Subjeck, J. R., Sciandra, J. J. & Johnson, R. J. (1982) *Br. J. Radiol.* **55**, 579–584.
8. Li, G. C. (1985) *Int. J. Radiat. Oncol. Biol. Phys.* **11**, 165–177.
9. Laszlo, A. & Li, G. C. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 8029–8033.
10. Li, G. C., Li, L., Liu, Y. K., Mak, J. Y., Chen, L. & Lee, W. M. F. (1991) *Proc. Natl. Acad. Sci. USA* **88**, 1681–1685.
11. Ashburner, M. & Bonner, J. J. (1979) *Cell* **17**, 241–254.
12. Lindquist, S. L. (1986) *Annu. Rev. Biochem.* **55**, 1151–1191.
13. Edington, B. V., Whenlan, S. A. & Hightower, L. E. (1989) *J. Cell. Physiol.* **139**, 219–228.
14. Aiello, L., Guiloyle, R., Huebner, K. & Weinmann, R. (1979) *Virology* **94**, 460–469.
15. Anderson, R. L., Tao, T. W. & Hahn, G. M. (1988) *Int. J. Radiat. Biol.* **54**, 813–823.
16. Landry, J., Chretien, P., Lambert, H., Hickey, E. & Weber, L. A. (1989) *J. Cell Biol.* **109**, 7–15.
17. Riabowol, K. T., Mizzen, L. A. & Welch, W. J. (1988) *Science* **242**, 433–436.
18. Milarski, K. L. & Morimoto, R. I. (1989) *J. Cell Biol.* **109**, 1947–1962.
19. Pelham, H. R. B. (1986) *Cell* **46**, 959–961.
20. Beckmann, R. P., Mizzen, L. A. & Welch, W. J. (1990) *Science* **248**, 850–854.
21. Hunt, C. & Morimoto, R. I. (1985) *Proc. Natl. Acad. Sci. USA* **82**, 6455–6459.
22. Wu, B., Hunt, C. & Morimoto, R. I. (1985) *Mol. Cell. Biol.* **5**, 330–341.
23. Kirschmeier, P. T., Housey, G. M., Johnson, M. D., Perkins, A. S. & Weinstein, I. B. (1988) *DNA* **7**, 219–225.
24. Stone, J., de Lange, T., Ramsey, G., Jakobovits, E., Bishop, J. M., Varmus, H. E. & Lee, W. (1987) *Mol. Cell. Biol.* **7**, 1697–1709.
25. Mann, R., Mulligan, C. & Baltimore, D. (1983) *Cell* **33**, 153–159.
26. Cepko, C. L., Roberts, B. E. & Mulligan, R. C. (1984) *Cell* **37**, 1053–1062.
27. Towbin, H., Staehelin, T. & Gordon, J. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 4350–4354.
28. Welch, W. J. & Suhan, J. P. (1986) *J. Cell Biol.* **103**, 2035–2052.
29. Murno, S. & Pelham, H. R. B. (1984) *EMBO J.* **3**, 3087–3093.
30. Pelham, H. R. B. (1984) *EMBO J.* **3**, 3095–3100.
31. Lewis, M. J. & Pelham, H. R. B. (1985) *EMBO J.* **4**, 3137–3143.