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NOTES

Nonexpression of a Major Heat Shock Gene in Mouse Plasmacytoma MPC-11

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The major heat shock gene coding for a 68,000-dalton protein was found to be silent in mouse plasmacytoma MPC-11 in both control and stressed cells. The block appears to be at the level of transcription, although RNA processing or instability has not been ruled out. It is not caused by a major deletion or rearrangement of the gene.

Deregulation of c-*myc* oncogene expression has been associated with a variety of human, murine, and avian tumors (10). Recently, the c-*myc* gene was found to be homologous to the adenovirus E1A gene (31), a known regulator of viral gene expression (20, 27) which also induces the expression of certain host genes, including the human heat shock gene hsp 70 (28). Both gene products are found in the nucleus (1, 9, 12). Either of these genes is capable of immortalizing primary cells when complemented by the c-Ha-*ras* gene (19). Kington et al. (17) showed that the mouse c-*myc* gene product could stimulate the expression of chimeric genes containing the *Drosophila* hsp 70 promoter. This promoter had previously been shown to respond to heat shock in mammalian cells (8, 30). These observations point to a regulatory role for the c-*myc* gene product (17, 29). It remains to be determined whether *myc* also regulates the expression of endogenous genes, possibly through sequences similar to that of the *Drosophila* hsp 70 promoter. Likely candidates are the heat shock genes. Although high constitutive levels of heat shock proteins have been observed in some transformed cells (13), a direct correlation between *myc* and heat shock genes remains to be demonstrated. We have begun an investigation of this problem by determining the expression of the major mouse heat shock gene hsp 68 in transformed cells displaying high *myc* expression. Mouse plasmacytoma MPC-11 possesses a characteristic translocation that breaks the c-*myc* gene (34). This is accompanied by abundant production of a truncated but functional c-*myc* transcript (23).

Protein synthesis in MPC-11 cells (obtained from K. B. Marcu) under control and stressed conditions was examined by gel electrophoresis and compared with that in a known heat-responsive mouse cell line, the F9 embryonal carcinoma (5, 25) (Fig. 1). Cells cultured in minimum essential medium (MEM) were either preincubated for 1 h in methionine-free MEM (lanes 1 and 3) or heat shocked at the indicated temperatures in the same medium for 10 min, left to recover for 1 h at 37°C, and labeled for 1 h with 30 μCi of [^{35}S]methionine per ml at 37°C. In our experience and in that of others (5, 12, 24, 25), mouse cells respond to a 44°C, 10- to 20-min heat shock by synthesis of a limited number of heat shock proteins. The most prominent is a 68,000-dalton protein (hsp 68). After heat shock, a band corresponding to this protein is clearly visible in F9 cells (lane 2), but it is absent in MPC-11 at all temperatures tested (41 to 46°C) (lanes 4 to 8), as well as in control F9 cells (lane 1).

Heat shock has a significantly greater negative effect on total protein synthesis in MPC-11 than in F9 cells. After a 44°C exposure, total incorporation of [^{35}S]methionine was reduced to 10 to 15% of that in control cell levels, and after 46° it was almost totally abolished (Fig. 1, lane 8). Translational recovery was restored by 3 h after a 10-min heat shock at 44°C (lane 10), and the restored pattern was identical to that for control cells. Reducing the severity of the shock (5 min or less at 44°C) did not alter the banding pattern (lane 9). Prolonged exposure (30 to 60 min) had no effect other than to reduce total amino acid incorporation (not shown).

Although MPC-11 cells stressed at 42°C showed no hsp 68 induction, they did display increased synthesis of 89- and 70-kilodalton (kDa) proteins (Fig. 1, lane 5), which was abolished by the addition of 1 μg of actinomycin D per ml during the heat shock and recovery period (compare lanes 11 and 12). At 43°C, there also appeared to be increased synthesis of a 74-kDa protein (lane 6). Densitometric scanning of the autoradiograph indicated an approximately fivefold increase in expression of the 70-kDa protein and two- to threefold increases of the 89- and 74-kDa bands at 43°C. Proteins of these sizes have been identified previously as heat shock products in mammalian cells (5, 15, 16, 22, 24, 25). In responsive cells, such as F9 (data not shown) or mouse L cells (M. D. Perry, D. G. Lowe, W. D. Fulford, and L. A. Moran, submitted for publication), hsp 68 is induced at 42°C. The induction of at least the 89- and 70-kDa proteins at this temperature suggests that transcriptional heat shock regulation does take place in MPC-11 cells. The absence of hsp 68 expression cannot, therefore, be explained by incapacity for heat shock response. We also determined whether MPC-11 cells respond to sodium arsenite, which is known to induce heat shock proteins in mouse cells (5). Cells were incubated for 1 h with 150 μM sodium arsenite, washed with α-MEM, left to recover for 1 h in α-MEM supplemented with 10% fetal calf serum, and then left for another hour in methionine-free MEM before being labeled with [^{35}S]methionine. Incubation of MPC-11 cells in 150 μM sodium arsenite did not lead to the expression of hsp 68 (Fig 1, lane 13), although parallel incubation of F9 cells did

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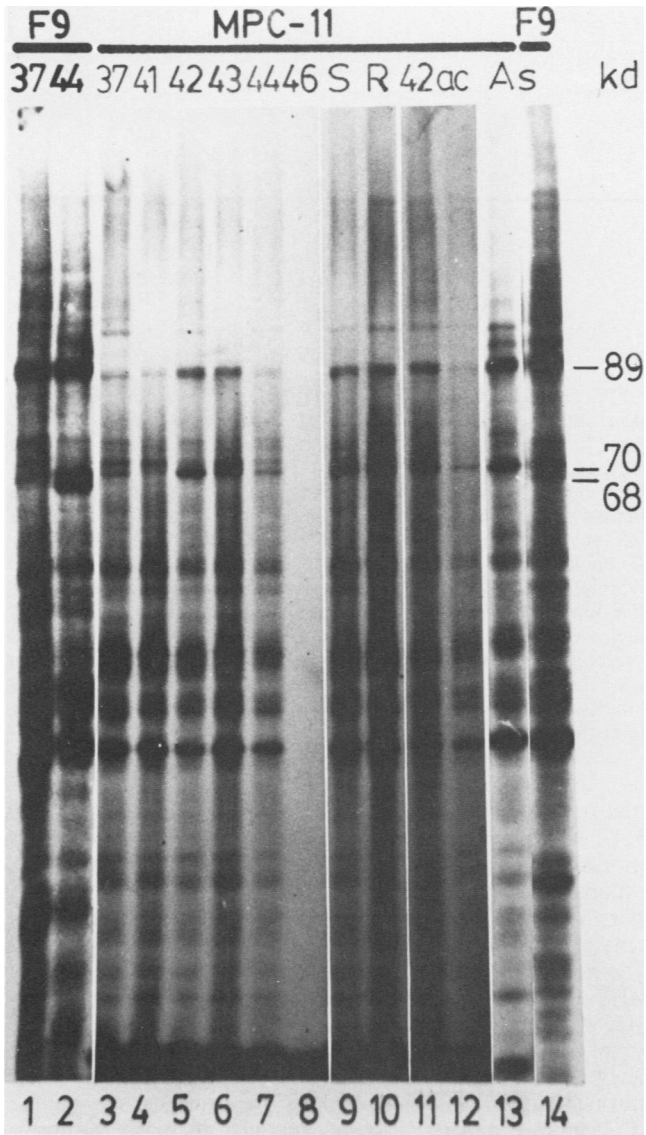


FIG. 1. Protein synthesis in control and heat-shocked MPC-11 and F9 cells. Lanes 1, 2, and 14: F9; 3 to 13: MPC-11; 1 and 3: 37°C control; 2: 44°C heat shock; 4: 41°C heat shock; 5: 42°C heat shock; 6: 43°C heat shock; 7: 44°C heat shock; 8: 46°C heat shock; 9: 44°C, 5-min heat shock; 10: 44°C, 10-min heat shock, 3-h recovery; 11: 42°C heat shock; 12: 42°C heat shock plus 1 μg of actinomycin D per ml; 13 to 14: plus 150 μM sodium arsenite. Labeling conditions are described in the text. Labeled cells were washed with phosphate-buffered saline and solubilized in 100 μl of lysis buffer (62.5 mM Tris hydrochloride [pH 6.8], 3% sodium dodecyl sulfate, 10% glycerol, 5% 2-mercaptoethanol). All samples were boiled for 2 to 3 min before being applied to a 7.5% acrylamide gel (18). The same number of counts (100,000 cpm) was applied per lane (except in lanes 7 and 8). The gel was treated with salicylic acid (7) before exposure to Cronex-Plus X-ray film. The positions of the 89-, 70-, and 68-kDa heat shock polypeptides are indicated.

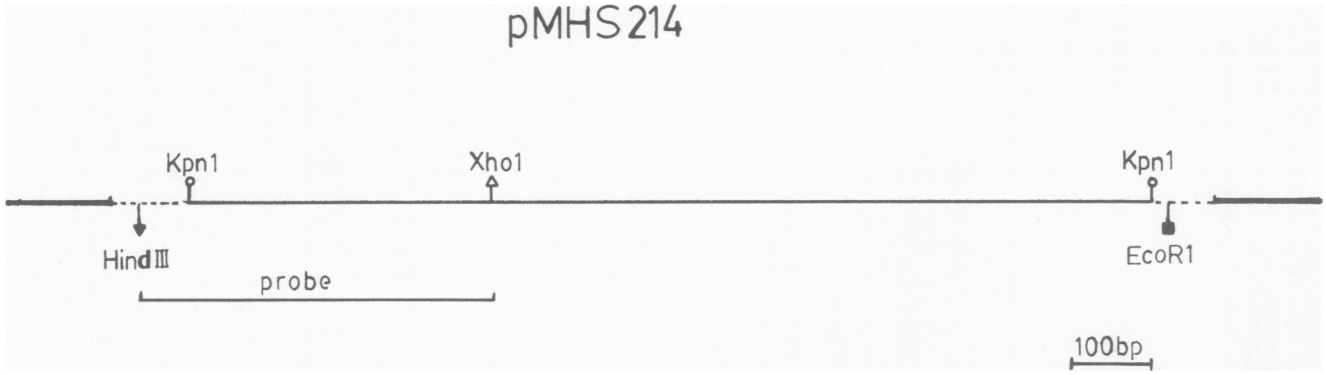
induce this product (lane 14). An increase of the 89- and 70-kDa bands is evident for both cell lines.

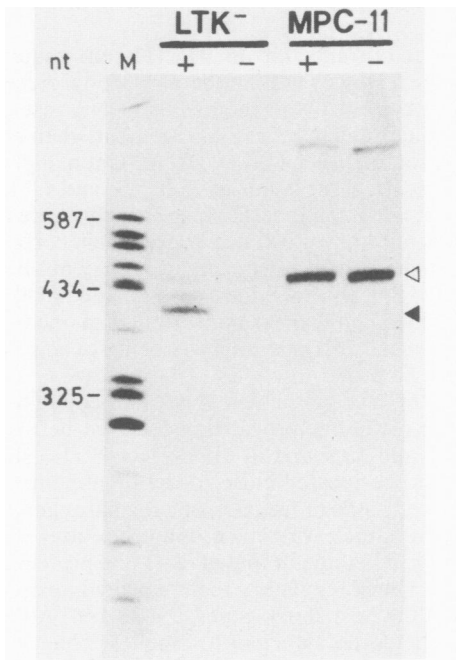
The absence of hsp 68 synthesis in MPC-11 might be the result of a block at the level of translation, with the transcriptional response being normal. To check this possibility we assessed the presence of hsp 68 mRNA in MPC-11 cells by using an S1 nuclease (Bethesda Research Laboratories, Inc.) assay (6). Plasmid pMHS214 (from L. Moran) contains a 1.5-kilobase hsp 68 cDNA insert in the *Kpn*I site of vector pDPL13 (see Fig. 2A). This is flanked by linker segments containing, among others, a *Hin*dIII and an *Eco*RI site on either side. An *Xho*I site in the left third of the insert allows the [γ-^{32}P]ATP end labeling of a ≃450-nucleotide-long *Hin*dIII-*Xho*I fragment, of which ≃400 nucleotides are protected against S1 nuclease digestion by hybridization to hsp 68 mRNA. No band corresponding to such a protected fragment is visible in either control or shocked (44°C) MPC-11 cells (Fig. 2B). In contrast, a major protected fragment of ≃400 nucleotides is found in a control heat-shocked mouse L(TK$^-$) RNA preparation. The same result was obtained with RNA from cells shocked at 42°C, and the result was confirmed by Northern blot hybridization (not shown).

The absence of transcripts in MPC-11 cells could reflect a gene deletion. However, Southern blot hybridization of genomic DNA probed with the hsp 68 cDNA insert did not reveal any major deletion or rearrangement when compared with a control mouse L(TK$^-$) DNA. Upon digestion by *Eco*RI or *Hin*dIII, three bands of 15.0, 5.3, and 4.7 kilobases and 21.0, 7.5, and 6.0 kilobases, respectively, were obtained (Fig. 3). It is not known whether these bands correspond to distinct heat shock genes (which may or may not be active). Furthermore, this analysis does not exclude small or point mutations which could affect transcription of one or more of the hsp 68 genes. This possibility is being examined with a genomic hsp 68 probe.

The lack of hsp 68 gene expression could also be a consequence of the reciprocal translocation between chromosomes 12 and 15 found in MPC-11 cells (34) should the hsp 68 gene(s) be located close to the breakpoint. Alternatively, the expression or function of a regulator gene controlling the heat shock response may be altered. In *D. melanogaster*, it is thought that at least one protein interacts with the heat shock regulatory region located upstream from the 5′ end of the heat shock genes (29, 38, 39). Alteration of this factor should be sufficient to block or change the heat shock response. Such a possibility has already been suggested for a *Dictyostelium* mutant (21) in which none of eight low-molecular-weight heat shock proteins could be induced, implying that the mutation may have affected a component required for their common induction. Alteration of a regulatory factor in MPC-11 cells is further suggested by the observation that a chemical inducer, sodium arsenite, does not induce hsp 68 either. This seems to rule out the possibility that the lack of heat shock response at the hsp 68 locus is caused by inceased sensitivity of MPC-11 cells to hyperthermia, which by disrupting chromatin structure would prevent response to an otherwise normal modulator.

Differential expression of certain heat shock proteins in various tissues or cell types has already been described. For example, heat shock proteins cannot be induced in mouse embryonic cells (4). In chicken embryos, on the other hand, there is expression of a small heat shock protein (35), while in chicken embryo fibroblasts (15, 37) hsp 70 is induced specifically by amino acid analogs or arsenite. Morange et al. (25) noted the absence of a normal heat shock response in mouse teratocarcinoma PCC4-Aza-RI cells in the undifferentiated state, with recovery of induction upon retinoic acid-induced differentiation. More recently, Singh and Yu (33) reported accumulation of hsp 70 during hemin-induced differentiation of the human erythroid cell line K 562. These and other studies (2) clearly establish that expression of heat shock genes may depend on the developmental or differenti-





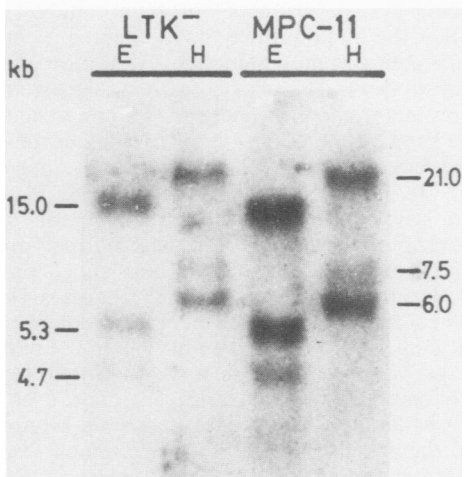


FIG. 3. Southern blot analysis of mouse L(TK⁻) and MPC-11 DNA. Phenol-extracted DNA (11) was digested with either *Eco*RI (E) or *Hin*dIII (H) and electrophoresed through a 1% agarose gel before transfer to a Gene-Screen-Plus (New England Nuclear Corp.) membrane filter. Hybridization to the nick-translated (32) probe, consisting of the entire insert from plasmid pMHS214 (Fig. 2) recovered as a 1.5-kilobase *Hin*dIII-*Eco*RI fragment, was as described previously (38). Blots were washed at 65°C with 2× SSC–0.1% sodium dodecyl sulfate (1× SSC is 0.15 M NaCl plus 0.015 M sodium citrate) and then with 0.2× SSC–0.1% sodium dodecyl sulfate before exposure. Differences in the amount of DNA per lane (≈10 μg) account for the variations in band intensities between the two samples.

FIG. 2. (A) Plasmid pMHS214 with a 1.5-kilobase hsp 68 cDNA insert into the *Kpn*I site of vector pDPL13. See text and legend to Fig. 3 for discussion. (B) S1 nuclease analysis of RNA isolated from MPC-11 and mouse L(TK⁻) cells. Cytoplasmic RNA was isolated from control (−) and heat-shocked (+) cells by a modification of the method of Jeffrey et al. (14). Cells were heat shocked for 10 min at 44°C and left to recover for 1 h at 37° before RNA was isolated. For S1 nuclease analysis (6), 30 μg of total RNA was hybridized overnight at 55°C to 10^5 cpm of a [γ-^{32}P]ATP end-labeled probe and then underwent a 1-h digestion at 37°C with 100 to 200 U of S1 nuclease, phenol extraction, and precipitation. Samples were analyzed on 8 M urea–5% acrylamide sequencing gels. The marker (M) is γ-^{32}P-end-labeled pSV1 (3) cut with *Hae*III. The solid arrowhead points to the 400-nucleotide (nt) protected fragment; the open arrow points to the residual probe.

ated state of cells. Analysis of avian (26; B. G. Atkinson, *in* B. G. Atkinson and D. B. Walden, ed., *The Effects of Stress on Gene Expression*, in press) and mouse lymphocytes (Atkinson, in press; L. Aujame, unpublished data) shows that these cells express the normal set of their respective heat shock proteins. Thus, it remains to be determined whether the observation reported here is unique to the MPC-11 cell line or whether it is a more general characteristic of either plasmacytomas or other transformed murine lymphoid cells. Preliminary data on two other independently derived mouse plasmacytoma cell lines as well as other murine lymphoid lines suggest that the observation is unique to plasmacytomas (L. Aujame, manuscript in preparation). Furthermore, recent evidence indicates that, in contrast, human myelomas (B. G. Atkinson, personal communication) and lymphoblastoid cells (Aujame, unpublished data) express inducible heat shock proteins in large amounts in the absence of stress. We cannot reach any conclusion about any direct correlation between deregulated c-*myc* expression and the absence of heat shock protein induction. However, it is possible that the c-*myc* gene product exerts its function by interacting with a heat shock regulatory factor which could be altered in this cell line. Such a model would not be inconsistent with the results of Kingston et al. (17), who showed that the c-*myc* product could regulate the expression of the *Drosophila* hsp 70 gene but did not demonstrate whether this was the result of a direct interaction with the

promoter or through such a factor. This cell line, which has been used extensively for analysis of oncogene expression (34), should also prove useful for studies on the heat shock transcriptional response.

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