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Expression of Heat Shock Genes During Differentiation of Mammalian Osteoblasts and Promyelocytic Leukemia Cells

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Abstract The progressive differentiation of both normal rat osteoblasts and HL-60 promyelocytic leukemia cells involves the sequential expression of specific genes encoding proteins that are characteristic of their respective developing cellular phenotypes. In addition to the selective expression of various phenotype marker genes, several members of the heat shock gene family exhibit differential expression throughout the developmental sequence of these two cell types. As determined by steady state mRNA levels, in both osteoblasts and HL-60 cells expression of hsp27, hsp60, hsp70, hsp89α, and hsp89β may be associated with the modifications in gene expression and cellular architecture that occur during differentiation.

In both differentiation systems, the expression of hsp27 mRNA shows a 2.5-fold increase with the down-regulation of proliferation while hsp60 mRNA levels are maximal during active proliferation and subsequently decline post-proliferatively. mRNA expression of two members of the hsp90 family decreases with the shutdown of proliferation, with a parallel relationship between hsp89α mRNA levels and proliferation in osteoblasts and a delay in down-regulation of hsp89α mRNA levels in HL-60 cells and of hsp89β mRNA in both systems. Hsp70 mRNA rapidly increases, almost twofold, as proliferation decreases in HL-60 cells but during osteoblast growth and differentiation was only minimally detectable and showed no significant changes. Although the presence of the various hsp mRNA species is maintained at some level throughout the developmental sequence of both osteoblasts and HL-60 cells, changes in the extent to which the heat shock genes are expressed occur primarily in association with the decline of proliferative activity. The observed differences in patterns of expression for the various heat shock genes are consistent with involvement in mediating a series of regulatory events functionally related to the control of both cell growth and differentiation.

Key words: HL-60 cells, bone, proliferation, gene regulation, hsp27, hsp60, hsp70, hsp89α, hsp89β

The progressive differentiation of cells and tissues is often associated with a sequence of events characterized by an initial period of active proliferation. Subsequently, a series of steps occurs, each involving the selective expression of specific genes encoding proteins that are characteristic of the developing cellular phenotype. When fetal rat calvarial osteoblasts are isolated and placed in culture, the cells undergo an ordered series of events over a 30-day period which leads to the formation of mineralized nodules that have a bone-like structure (Aronow et al., 1989; Owen et al., 1990). Following isolation, the cells proliferate and express growth-associated genes, such as the core histone genes whose expression is tightly coupled to DNA synthesis (Owen et al., 1990; Shalhoub et al., 1989). During the proliferative period, expression of genes for the biosynthesis of the bone extracellular matrix (e.g., Type I collagen, fibronectin, and TGF-B) is already ongoing. Upon cessation of proliferation, the expression of the histone genes is down-regulated and genes involved with the maturation and organization of the mature extracellular matrix, such as alkaline phosphatase, are expressed. A third developmental period involves expression of genes including osteocalcin and osteopontin which are up-regulated in conjunction with the ordered deposition of hydroxyapatite within the bone extracellular matrix (Owen et al., 1990).

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Similarly, when proliferating HL-60 promyelocytic leukemia cells are induced to differentiate into monocyte-like cells over a 4–5-day period following addition of phorbol esters (Rovera et al., 1979; Huberman and Callahan 1979), the expression of proliferation specific genes, such as H3 and H4 histone, is down-regulated (Stein et al., 1989). In a reciprocal manner, a differentiation-related polyadenylated H2b histone variant mRNA becomes transiently expressed (Collart et al., 1991). Subsequently, genes associated with cell attachment and the monocyte phenotype are up-regulated (Harris and Ralph, 1985).

One family of genes whose expression occurs in a broad spectrum of cells and tissues under a variety of biological conditions encodes the heat shock proteins (Lindquist, 1985). Initially, this highly conserved group of proteins, varying in size from 27 kd to 110 kd (Lindquist, 1985; Welch and Feramiso, 1982), was observed to exhibit modifications in levels of expression in response to a variety of physiological perturbations which include elevated temperatures ("heat shock"; Lindquist, 1985; Schlesinger et al., 1982; Hickey and Weber, 1982), exposure to ethanol (Li, 1983), accumulation of abnormal proteins (Schlesinger et al., 1982; Ananthan et al., 1986), incorporation of amino acid analogs (Schlesinger et al., 1982), and viral infection (Schlesinger et al., 1982). On the basis of such observations, it has been postulated that increased expression of heat shock proteins is a component of a cellular mechanism operative in the protection of cells from thermal injury and trauma (Lindquist and Craig, 1988).

Recent studies have shown that several heat shock proteins and their constitutively synthesized homologs (heat shock cognate proteins) have essential roles in normal cellular physiology. The hsp90, hsp70, and hsp60 classes of proteins are abundant in the absence of stress and function as molecular chaperons (Ellis and van der Vies, 1991). Members of the hsp70 protein family bind to newly synthesized polypeptides and prevent folding until appropriate molecular interactions are established with the "correct" partners in the assembly of multisubunit protein complexes. Hsp70 proteins are also required for import of proteins into the endoplasmic reticulum and mitochondria (Chirico et al., 1988; Deshaies et al., 1988), and gene inactivation studies in yeast have shown that hsp70 proteins are required for protein secretion (Vogel et al., 1990). One member of the hsp70 family also functions in the uncoating of clathrin-coated vesicles (Chappell et al., 1986). The hsp90 family has two distinct members in all vertebrates (known as hsp89α and hsp89β in human cells). Although a functional distinction between the forms has not been established, hsp90 proteins have been shown to associate with a specific subset of cellular proteins involved in signal transduction, including steroid hormone receptors (Catelli et al., 1985), and cellular and viral protein kinases (Opperman et al., 1981; Ziemiecki et al., 1986; Rose et al., 1989). Hsp60 is a mitochondrial protein that is encoded by the nuclear genome. While the function of hsp60 in facilitating correct folding of other proteins within the mitochondrial compartment is well established, mutants of hsp60 also affect assembly of cytoplasmic microtubules (Jindal et al., 1989). The function of hsp27 is unknown. However, the protein is found in many types of cells in the absence of stress and accumulates in stationary phase cell cultures (Gaestel et al., 1989). Hsp27 is rapidly phosphorylated in response to a variety of growth factors and cytokines and it has been proposed that the protein may have a role in signal transduction pathways (Landry et al., 1991).

Consistent with their essential roles as molecular chaperons, genes encoding members of the hsp70 and hsp89 families contain serum response elements and are induced by growth factors during normal cell proliferation (Wu and Morimoto, 1985; Wu et al., 1987; Hickey et al., 1989). The same classes of proteins are also induced during activation of macrophages (Joslin et al., 1991) and lymphocytes (Ferris et al., 1988; Haire et al., 1988; Hansen et al., 1991). The question arises as to whether the modifications in gene expression and cellular architecture that occur during differentiation also involve changes in the expression of heat shock proteins. To address this question we measured the level of mRNA encoding five human heat shock proteins during induced differentiation of rat osteoblasts and HL-60 promyelocytic leukemia cells. Our results indicate that in both systems, specific heat shock protein mRNA levels undergo substantial regulation during initial inhibition of proliferation and during later periods characterized by progressive expression of genes encoding products associated with terminal differentiation.

MATERIALS AND METHODS

Rat Osteoblast Cell Growth and Differentiation

Osteoblasts were isolated from the calvaria of 21 day gestation fetal rats by trypsin/collagenase digestion as described (Aronow et al., 1989; Owen et al., 1990). Osteoblasts were plated in minimal essential medium (MEM, GIBCO) supplemented with 10% fetal calf serum (FCS) in 100-mm dishes at a density of 5×10^5 cells/dish. To enhance osteoblast differentiation, the following cell feeding schedule was used. On day 4 after plating, the cells were fed with MEM/FCS supplemented with ascorbic acid (25 μg/ml). Beginning seven days after plating and on every second day throughout a 30-day differentiation time course, cells were fed with BGJb medium (GIBCO) supplemented with 50 μg/ml ascorbic acid, 10 mM β-glycerol phosphate (Sigma, St. Louis), and 10% FCS.

HL-60 Cell Growth and Differentiation

HL-60 human acute promyelocytic leukemia cells were grown in suspension culture in RPMI 1640 Medium (Gibco, Grand Island, N.Y.), supplemented with 5% FCS (GIBCO) in T-25 flasks in a 37°C incubator with a humidified atmosphere containing 10% CO_2. Cells were maintained in logarithmic growth by serial subcultivation to 10^5 cells/ml at three-day intervals. HL-60 cells (1×10^6 cells/ml) were induced to differentiate along the monocyte/macrophage lineage by the addition of 12-0-tetradecanoylphorbol 13-acetate (TPA) (Sigma, St. Louis) from a 100 μg/ml stock in acetone to a final concentration of 100 ng/ml. The down-regulation of proliferation following TPA addition was monitored by pulse labeling triplicate samples of 10^6 cells with 10 μCi of ^{3}H-thymidine (20 Ci/mmol, Amersham, Arlington Heights, IL) for 0.5 h. Incorporated ^{3}H-thymidine was measured as trichloroacetic acid precipitable radioactivity.

Preparation and Analysis of RNA

Total cellular RNA was extracted from 2.5×10^7 HL-60 cells at each time point by lysis in guanidinium isothiocyanate followed by cesium chloride centrifugation (Chirgwin et al., 1979). Cytoplasmic RNA was prepared from 5×10^7 rat osteoblasts at each time point by the LiCl/urea precipitation method after removal of the nuclei (Tushinski et al., 1977). RNA (10–20 μg) was size fractionated by electrophoresis in 1.2% (w/v) agarose-formaldehyde gels. The integrity and quantitation of RNA was confirmed by visualization of ethidium bromide staining prior to transfer to ZetaProbe membrane (BioRad, Richmond, CA) in 20 × SSC.

Hybridization Conditions

The probes used were cDNAs encoding human heat shock sequences of hsp27 (pHS208), hsp60 (pHS601), hsp70 (pHS709), hsp89α (pHS801), hsp89β (pHS811) (Hickey et al., 1986), or rat hsp70 (provided by Dr. Rubin Mestril, University of California, San Diego). Other probes used included human histone H2b (Collart et al., 1991), rat alkaline phosphatase (Noda et al., 1987), and rat osteocalcin (Lian et al., 1989). DNA probes were labelled with [α-^{32}P] dCTP (3,000 Ci/mmol; New England Nuclear, Boston, MA) by the random primer technique (Feinberg and Vogelstein, 1983). The blots were prehybridized in 50% formamide, 5 × SSC, 5 × Denhardt's solution, 50 mM phosphate buffer (pH 6.5), 1% w/v SDS, 250 μg/ml salmon sperm DNA at 43°C for 2 h. For hybridization, 10^6 cpm/ml of denatured DNA probe was added and incubated at 43°C for 18 h. Following hybridization, blots were washed three times in 2 × SSC/0.1% SDS at room temperature for 15 min each and twice in 1 × SSC/0.1% SDS at 65°C, for 30 min each. Blots were exposed to Kodak XAR film at −70°C and the resulting autoradiographs were quantitated with an LKB Ultrascan XL Laser Densitometer. Results were normalized to 28S ribosomal RNA to account for any variations in RNA quantitation. Each time point represents the average of three assays from at least two independent experiments.

RESULTS

The differentiation of both osteoblasts and HL-60 cells involves the sequential expression of various phenotype marker genes both initially while the cells are proliferating and during the post-proliferative period. Figure 1A illustrates expression of cell growth and tissue related genes during osteoblast differentiation. Following isolation of osteoblasts from 21 day fetal rat calvaria, the cells undergo a period of proliferation as shown by high levels of H2b histone gene expression (Collart et al., 1991). As proliferation is down-regulated, alkaline phosphatase, an initial marker of the differentiated osteoblast phenotype, is expressed. This is in turn followed by increased expression of osteocalcin with the

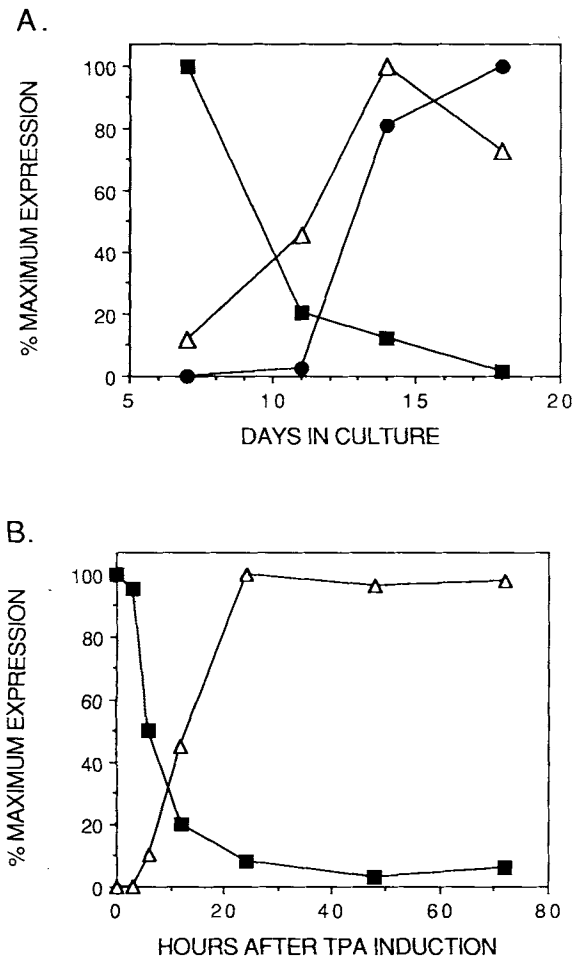

Fig. 1. Expression of rat osteoblast and HL-60 cell proliferation and differentiation parameters. **A:** Expression of H2b histone (■), alkaline phosphatase (△), and osteocalcin (●) mRNAs was analyzed by Northern blot on the indicated days following isolation of normal diploid osteoblasts from fetal rat calvaria. Values are presented as percent of the maximal expression of each transcript as determined by densitometry. **B:** Expression of H2b histone (■) mRNA during HL-60 cell differentiation was analyzed as in A. Differentiation of the cells following TPA induction was monitored by reduction of nitroblue tetrazolium (NBT) and expressed as percent of maximal activity.

onset of mineralization of the extracellular matrix. Similarly, the differentiation of HL-60 promyelocytic leukemia cells into monocytes following the addition of phorbol ester exhibits a reciprocal relationship of proliferation to differentiation. In this case, a more rapid pattern of expression of growth and phenotype-specific genes is observed. Figure 1B demonstrates that proliferation ceases in HL-60 within 24 h of TPA addition as shown by H2b histone gene expression. Accompanying this, one finds the induction of differentiation as measured by reduction of nitroblue tetrazolium. It has also been reported elsewhere that this cessation of cell growth is accompanied by induction of expression of a variant H2b histone gene, associated with cellular differentiation (Collart et al., 1991).

Expression of hsp genes was monitored during the differentiation of rat osteoblasts and HL-60 cells by Northern blot analysis of RNA harvested at time points which represent various stages of cell proliferation and differentiation in both cell types. Figure 2 shows representative autoradiographs of Northern blot analysis measuring expression of hsp27 during rat osteoblast differentiation (Fig. 2B) and hsp60 during HL-60 cell differentiation (Fig. 2A). The autoradiographs of these blots, as well as those hybridized with the other hsp probes, were quantitated by scanning laser densitometry and the results are presented graphically in subsequent figures.

Changes in the expression of hsp27 during osteoblast and HL-60 differentiation are reported in Figure 3. In both differentiation systems the down-regulation of proliferation is accompanied by a transient 2.5-fold increase in the expression of hsp27 mRNA. Throughout subsequent differentiation, levels of hsp27 mRNA decline to 20% of maximum in osteoblasts (Fig. 3A) and to 20–50% of maximal levels in HL-60 cells (Fig. 3B).

In contrast, hsp60 mRNA levels are maximal in both osteoblasts and HL-60 cells during active cell division, and decrease with the down-regulation of proliferation (Fig. 4). Hsp60 mRNA is maintained at approximately 70% of maximal levels throughout the osteoblast developmental sequence (Fig. 4A), while expression declines to low (10–20% of maximal, Fig. 4B) levels in differentiated HL-60 cells.

In both cell culture systems, during development of the osteoblast and monocytic phenotypes, the two members of the hsp90 family, hsp89α and hsp89β, also exhibit maximal mRNA levels in proliferating cells (Figs. 5, 6). Hsp89α and β mRNA levels declined post-proliferatively in osteoblasts and HL-60 cells. However, the decrease in cellular levels of hsp89α mRNA closely parallels the shutdown of proliferation in osteoblasts, while the decline in hsp89α mRNA levels in HL-60 cells and hsp89β in osteoblasts as well as in HL-60 cells exhibits a delay in down-regulation. With the post-proliferative de-

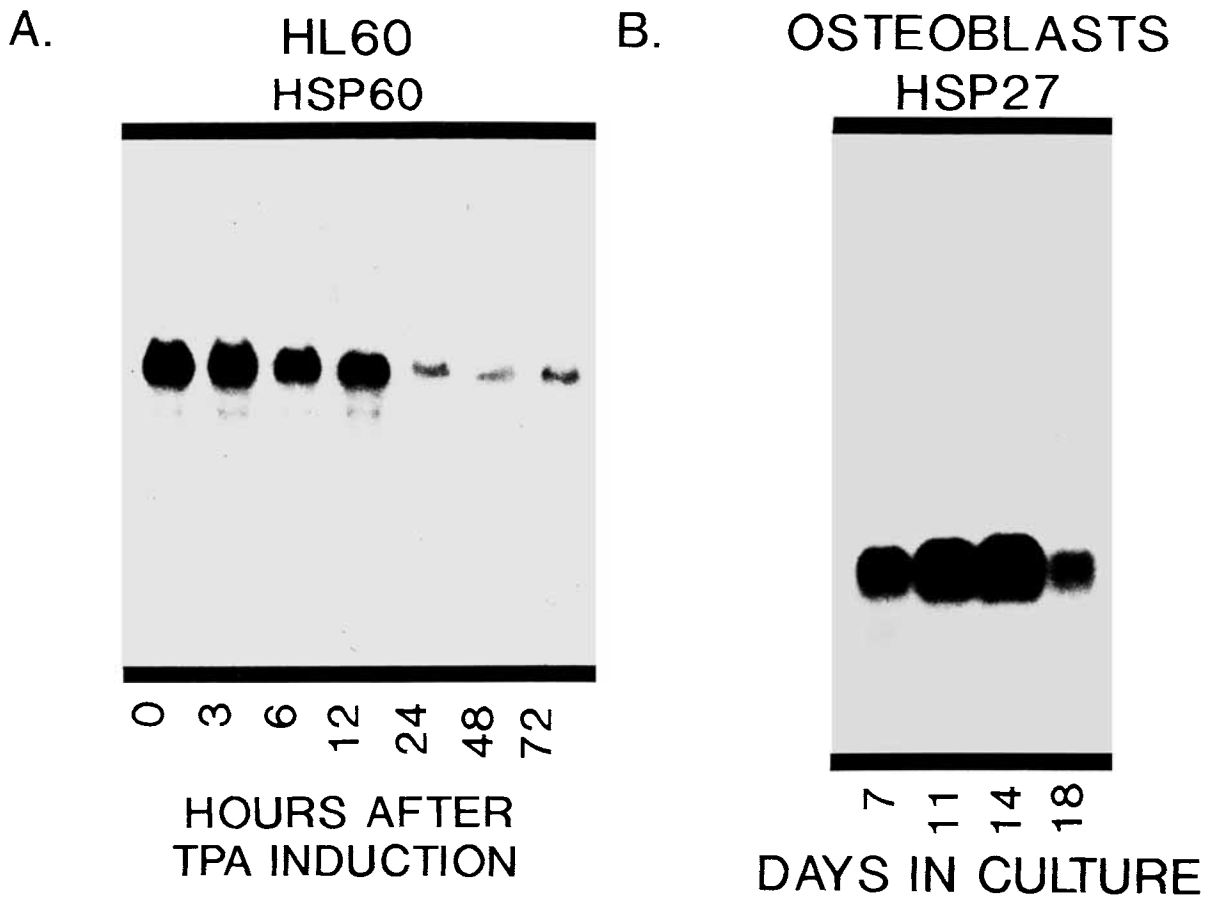

Fig. 2. Representative Northern blots of hsp gene expression during the differentiation of rat osteoblasts and HL-60 cells. **A:** Hsp60 expression following induction of HL-60 cell differentiation with TPA. **B:** Hsp27 expression during osteoblast differentiation.

velopment of the monocytic phenotype, hsp89α and hsp89β mRNAs persist at reduced levels, 20% and 40% of maximum, respectively (Figs. 5A, 6A). Later during monocytic differentiation, 70 h following phorbol ester induction, hsp89α mRNA levels exhibit a greater than twofold increase and levels of hsp89β mRNA are further up-regulated, returning to levels observed during proliferation. This biphasic modulation in representation of hsp89α and 89β mRNAs does not occur during the post-proliferative development of the osteoblast phenotype. Hsp89α gene transcripts are reduced to levels that are not detectable during extracellular matrix mineralization (day 18) and hsp89β mRNA transcripts remain at 40% of proliferative levels throughout the osteoblast developmental sequence.

Hsp70 mRNA rapidly increases almost twofold during the shutdown of proliferation following the addition of TPA to HL-60 cells (Fig. 7). The maximal levels observed at the end of the proliferative period decrease to 70% of maximum during the remainder of the differentiation time course. In cultured rat osteoblasts, hsp70 mRNA was not detectable using a human cDNA probe and minimally detectable with a rat cDNA probe that represents the inducible form of hsp70. Significant changes in hsp70 mRNA were not observed during the osteoblast developmental sequence.

DISCUSSION

In both cell systems examined, inhibition of proliferation corresponds temporally with an approximate 2.5-fold increase in hsp27 mRNA followed by a gradual decline during differentiation. In contrast, the abundance of hsp60, hsp89α, and hsp89β mRNA generally declined with inhibition of proliferation and the onset of differentiation. Characteristic patterns of abundance of these mRNAs were observed in each cell type. During osteoblast differentiation, hsp60

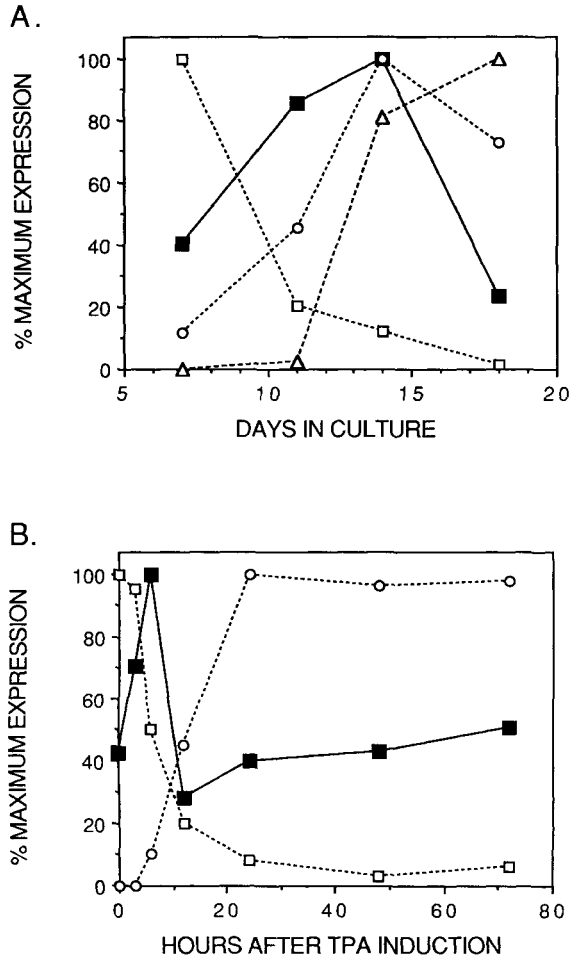

Fig. 3. Expression of hsp27 during the differentiation of rat osteoblasts and HL-60 cells. **A:** Levels of expression of hsp27 (■) mRNA were analyzed by Northern blot and presented as percent of the maximal expression observed during the osteoblast differentiation time course as determined by densitometry. Cell growth and differentiation markers H2b histone (□), alkaline phosphatase (○), and osteocalcin (△) are those presented in Figure 1A. **B:** Levels of expression of hsp27 (■) mRNA were analyzed as in Figure 3A during HL-60 differentiation time course. HL-60 cell growth and differentiation parameters are those presented in Figure 1B.

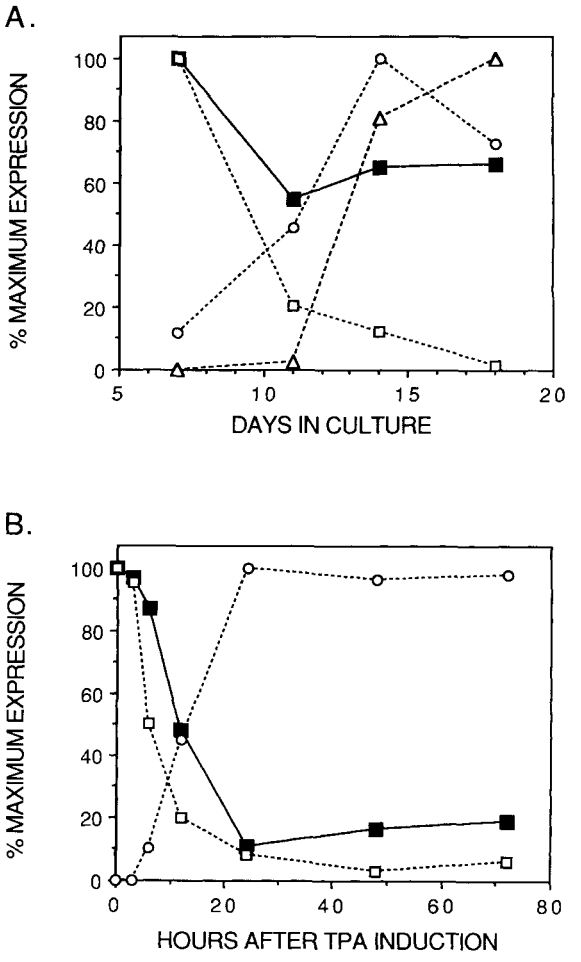

Fig. 4. Expression of hsp60 during the differentiation of rat osteoblasts and HL-60 cells. **A:** Levels of expression of hsp60 (■) mRNA were analyzed by Northern blot and presented as percent of the maximal expression observed during the osteoblast differentiation time course as determined by densitometry. Cell growth and differentiation markers H2b histone (□), alkaline phosphatase (○), and osteocalcin (△) are those presented in Figure 1A. **B:** Levels of expression of hsp60 (■) mRNA were analyzed as in Figure 3A during HL-60 differentiation time course. HL-60 cell growth and differentiation parameters are those presented in Figure 1B.

levels were maintained at about 70% of that found in proliferating cells while in monocyte differentiation the abundance of hsp60 mRNA declined to only 10–20% of that found during proliferation. Hsp89α mRNA is rapidly lost from differentiating osteoblasts with kinetics similar to the loss of histone mRNA. A similar decline takes place in HL-60 cells as proliferation is inhibited, but in these cells hsp89α mRNA recovers to 40% of the proliferating level as monocyte differentiation continues. Hsp89β mRNA declines with inhibition of proliferation in both cell types, but this mRNA is maintained at 40% of the proliferating level in differentiated osteoblasts and recovered to the level found during proliferation in differentiated monocytes. Hsp70 mRNA levels increased twofold during inhibition of proliferation in HL-60 cells and declined only slightly during differentiation. Surprisingly, changes in hsp70 mRNA abundance were not detected during bone differentiation.

Most studies on heat shock proteins and cell proliferation have focused on the hsp70 and hsp90 protein families. Basal hsp70 gene expression has been reported to be cell cycle dependent

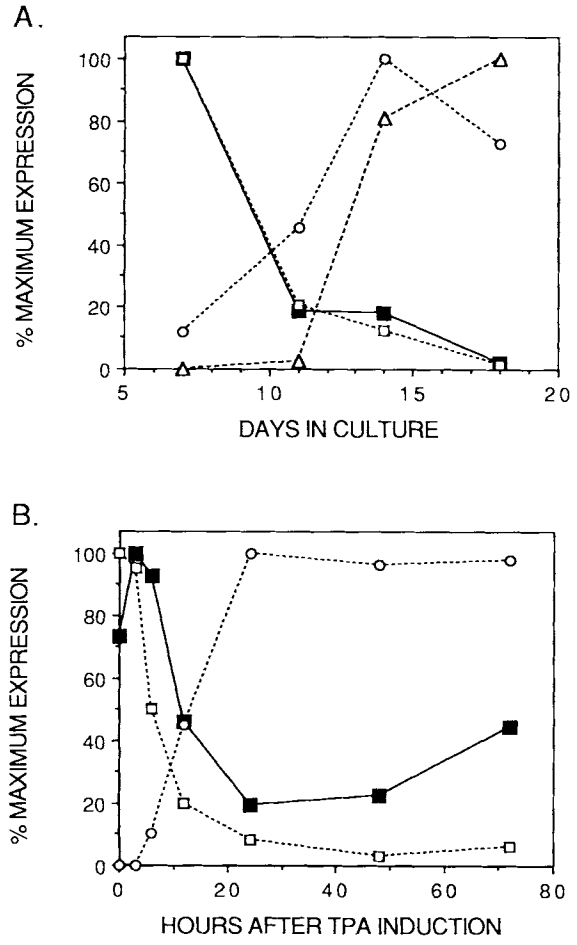

Fig. 5. Expression of hsp89α during the differentiation of rat osteoblasts and HL-60 cells. **A:** Levels of expression of hsp89α (■) mRNA were analyzed by Northern blot and presented as percent of the maximal expression observed during the osteoblast differentiation time course as determined by densitometry. Cell growth and differentiation markers H2b histone (□), alkaline phosphatase (○), and osteocalcin (△) are those presented in Figure 1A. **B:** Levels of expression of hsp89α (■) mRNA were analyzed as in Figure 3A during HL-60 differentiation time course. HL-60 cell growth and differentiation parameters are those presented in Figure 1B.

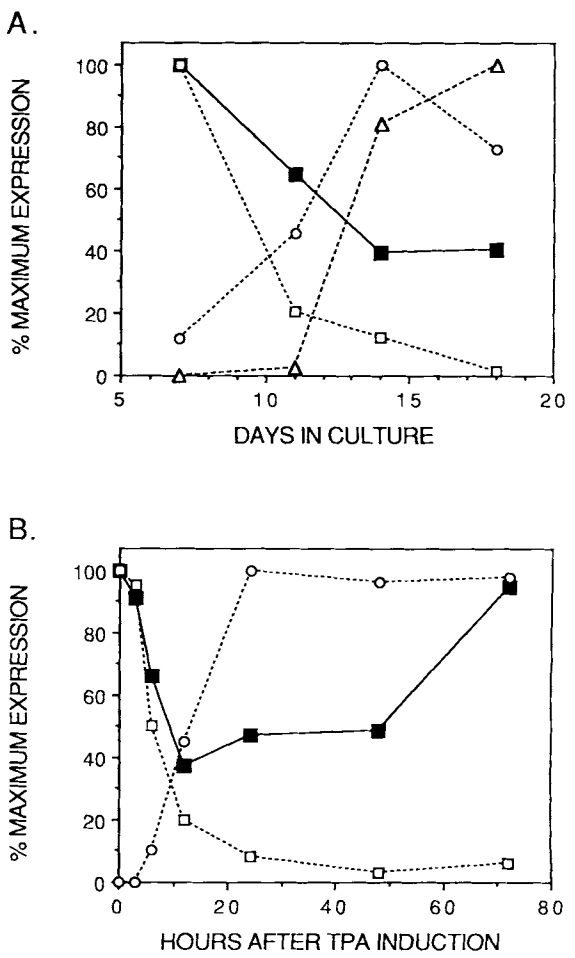

Fig. 6. Expression of hsp89β during the differentiation of rat osteoblasts and HL-60 cells. A: Levels of expression of hsp89β (■) mRNA were analyzed by Northern blot and presented as percent of the maximal expression observed during the osteoblast differentiation time course as determined by densitometry. Cell growth and differentiation markers H2b histone (□), alkaline phosphatase (○), and osteocalcin (△) are those presented in Figure 1A. **B:** Levels of expression of hsp89β (■) mRNA were analyzed as in Figure 3A during HL-60 differentiation time course. HL-60 cell growth and differentiation parameters are those presented in Figure 1B.

in transformed mammalian cells (Kao et al., 1985; Milarski and Morimoto, 1986). It is still controversial whether transcription is restricted to a specific stage of the cell cycle or is a more general consequence of mitogen stimulation (Hickey et al., 1989; Hansen et al., 1991). Nonetheless, at least some members of the hsp70 gene family have complex promoters which include functional serum response elements (SRE) which are activated by growth factor stimulation (Wu et al., 1987). In HeLa cells, transcription of the hsp89α gene is also stimulated by serum in the absence of stress (Hickey et al., 1989).

Previous studies have generally found a positive correlation between cell proliferation and hsp70/hsp90 expression. In regenerating rat liver, hsp70 mRNA increases fourfold following hepatectomy and peaks after c-fos and c-myc mRNA levels in the general proliferative response (Ohmori et al., 1990). EGF stimulation of cultured hepatocytes also brings about an increase in hsp70 mRNA (Ohmori et al., 1990). Conversely, inhibition of neuroblastoma proliferation and stimulation of differentiation with

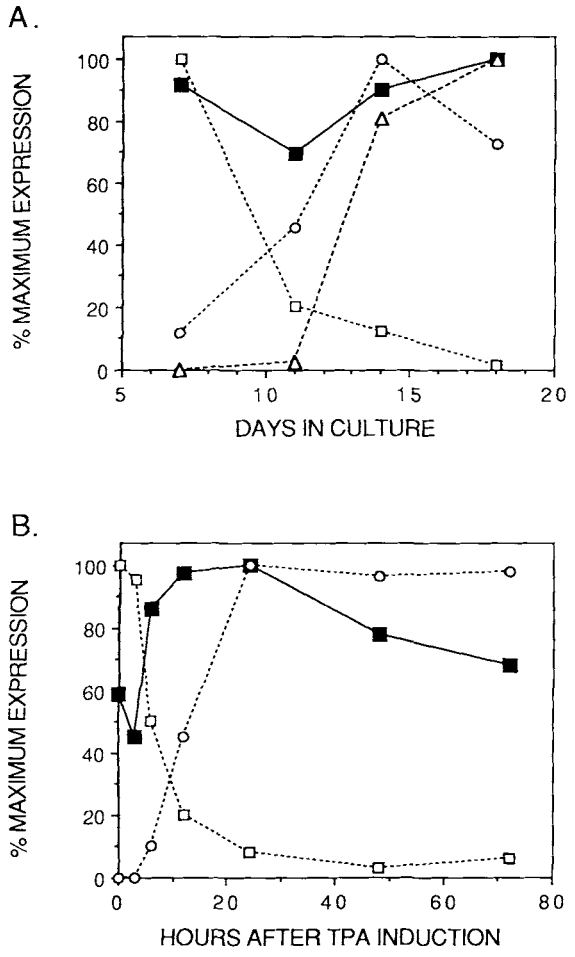

Fig. 7. Expression of hsp70 during the differentiation of rat osteoblasts and HL-60 cells. **A:** Levels of expression of hsp70 (■) mRNA were analyzed by Northern blot and presented as percent of the maximal expression observed during the osteoblast differentiation time course as determined by densitometry. Cell growth and differentiation markers H2b histone (□), alkaline phosphatase (○), and osteocalcin (△) are those presented in Figure 1A. **B:** Levels of expression of hsp70 (■) mRNA were analyzed as in Figure 3A during HL-60 differentiation time course. HL-60 cell growth and differentiation parameters are those presented in Figure 1B.

dibutyryl cyclic AMP and/or retinoic acid causes a decline in hsp70 mRNA (Murakami et al., 1991). Peripheral blood mononuclear cells stimulated to proliferate by PHA treatment show large increases in the abundance of hsp70, hsp89α, and hsp89β mRNA (Hansen et al., 1991). Interestingly, the constitutive hsp70 cognate rather than the heat inducible form of hsp70 was the major mitogen activated species detected in that study. Mitogen stimulation generally brings about an increase in both protein and ribosome synthesis. Since hsp70 functions in the assembly of newly synthesized proteins (Beckmann et al., 1990) and has a likely role in ribosome biogenesis (Pelham, 1984), the requirement for hsp70 might be expected to increase with the rate of cell division and diminish in quiescent cells.

The results of the present study show an increase in hsp70 mRNA accumulation immediately upon inhibition of cell proliferation in HL-60 cells treated with TPA. The processes of differentiation, unlike quiescence, may require additional synthesis of hsp70 protein. Biosynthesis and secretion of type I collagen extracellular matrix continues throughout osteoblast growth and differentiation. In mature osteocytes, the biosynthesis, modification, and secretion of extracellular proteins are important aspects of the differentiated phenotype. Since it is reasonable to expect that these processes require hsp70, it is not surprising that the level of hsp70 mRNA does not decline during the differentiation process. Although no large changes in hsp70 mRNA abundance were detected, we cannot be certain that they did not occur. While we are confident that the human hsp70 cDNA used to probe HL-60 RNA hybridizes with both the constitutive and inducible forms of hsp70 (E. Hickey, unpublished), the ability of the rat cDNA to detect the constitutive form has not been established (R. Mestril, personal communication). Thus, we cannot rule out regulation of the hsp70 cognate gene during rat osteoblast differentiation.

Hsp60 is a constitutive mitochondrial protein that is synthesized at elevated rates in response to stress (McMullin and Hallberg, 1987). The protein is a molecular chaperon that has an essential role in the assembly and continued function of mitochondria (Cheng et al., 1989). Thus, the significance of the large decline in hsp60 mRNA that takes place during monocyte differentiation is not understood. Possibly monocyte differentiation is associated with decreased metabolic activity. Alternatively, proteins involved with mitochondrial activity may be stabilized, thereby reducing the requirement for hsp60 expression. In contrast, the post-proliferative decline in hsp60 gene expression in osteoblasts is modest (only a 30% decline) and consistent with high metabolic activity exhibited by osteoblasts during the biosynthesis and maintenance of function of the mineralizing bone extracellular matrix.

The general decline in hsp89α and β mRNA that was observed with inhibition of prolifera-

tion suggests an important function of this class of proteins in actively dividing cells. This would be consistent with the proposed role of the hsp89 proteins as cytoplasmic shuttles for growth related macromolecules such as steroid hormone receptors, tyrosine protein kinases, and regulators of protein synthesis. The dramatic decline in hsp89α mRNA and the persistence of a lower level of hsp89β mRNA may reflect a restricted responsiveness of differentiated osteoblasts to regulatory effector molecules relative to proliferating cells. Similar declines in mRNA levels and the synthesis of the hsp90 class of proteins have been observed with HL-60 cells induced to differentiate into granulocytes by DMSO treatment (Yufu et al., 1989). The recovery of hsp89 mRNA levels in monocytes following an initial decline may be a consequence of the continued responsiveness of this cell type to steroid hormones and cytokines during the differentiated state.

We have observed a reciprocal relationship between the down-regulation of proliferation and a transient accumulation of hsp27 mRNA during development of both the osteocyte and monocyte phenotype. Very little is known about the normal physiological function of this protein, but overexpression of hsp27 is sufficient to protect cells from heat-induced injury as well as the toxic effect of several drugs (Landry et al., 1989; Huot et al., 1991). The small heat shock proteins show a complex pattern of developmental regulation and become abundant in only certain tissues (Pauli et al., 1990; Haass et al., 1990; Miron et al., 1991). These proteins show similarity to lens α-crystallin (Ingolia and Craig, 1982), form high MW aggregates (Behlke et al., 1991), and are in several ways more like structural proteins than molecular chaperons. Recent evidence suggests that hsp27, the single mammalian small heat shock protein, may function in cell growth signal transduction. Hsp27 becomes phosphorylated at specific serine residues within 1 min following treatment of cells with whole serum, phorbol esters, tumor necrosis factor, bradykinin, calcium chelators and ionophores, thrombin, interleukin 1, and platelet-derived growth factor (Welch, 1985; Saklatvala et al., 1991; Landry et al., 1991). The amino acid sequence motif surrounding the phosphorylation sites is identical to that recognized by a protein kinase that phosphorylates ribosomal protein S6 in response to mitogenic signals (Landry et al., 1991). The increase in hsp27 mRNA and presumably hsp27 protein when cell division becomes inhibited may reflect a signaling mechanism for a key transition point in both osteocyte and monocyte differentiation.

The heat shock protein families are among the most abundant proteins found in all cells during normal growth and differentiation. That they are synthesized in greater amounts during periods of stress is a further indication of the critical roles they have in cell physiology. It remains to be established how each of the individual classes of heat shock proteins functions throughout the complex alterations in cellular structure and function that accompany differentiation. However, the variations we observed in the patterns of expression for the different heat shock proteins that are associated with the growth and phenotype expression periods of monocyte and osteoblast differentiation provide a series of biological circumstances that may increase our understanding of the functional properties of these proteins.

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