# Glucocorticoids induce a near-total suppression of hyaluronan synthase mRNA in dermal fibroblasts and in osteoblasts: a molecular mechanism contributing to organ atrophy

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Glucocorticoid (GC) administration induces atrophy of skin, bone, and other organs, partly by reducing tissue content of glycosaminoglycans, particularly hyaluronic acid (HA). We took advantage of the recent cloning of the three human hyaluronan synthase (HAS) enzymes (HAS1, HAS2 and HAS3), to explore the molecular mechanisms of this side effect. Northern and slot blots performed on RNA extracted from cultured dermal fibroblasts and the MG-63 osteoblast-like osteosarcoma cell line indicated that HAS2 is the predominant HAS mRNA in these cells. Incubation of both cell types for 24 h in the presence of $10^{-6}$ M dexamethasone (DEX) resulted in a striking 97–98 % suppression of HAS2 mRNA levels. Time-course studies in fibroblasts demonstrated suppression of HAS2 mRNA to 28 % of control by 1 h, and to 1.2 % of control by 2 h, after addition of DEX. Dose-response studies in fibroblasts indicated that the majority of the suppressive effect required concentrations characteristic of cell-surface GC receptors, a point confirmed by persistent DEX-induced suppression in the presence of RU486, an antagonist of classic cytosolic steroid hormone receptors. Nuclear run-off experiments showed a 70 % suppression of *HAS2* gene transcription in nuclei from DEX-treated fibroblasts, which is unlikely to fully explain the rapid 50–80-fold reduction in message levels. Experiments with actinomycin D (AMD) demonstrated that the message half-life was 25 min in cells without DEX, whereas the combination of AMD with DEX dramatically increased the half-life of HAS2 mRNA, suggesting that DEX acts by inducing a short-lived destabilizer of the HAS2 message. Direct assessment of HAS2 mRNA stability by wash-out of incorporated uridine label established a half-life of 31 min in cells without DEX, which substantially shortened in the presence of DEX. In conclusion, GCs induce a rapid and sustained, near-total suppression of HAS2 message levels, mediated through substantial decreases in both gene transcription and message stability. These effects may contribute to the loss of HA in GC-treated organs.

Key words: extracellular matrix, fibroblasts, hyaluronic acid.

## INTRODUCTION

Hyaluronic acid (HA; also called hyaluronan) is an ancient, highly conserved, extracellular glycosaminoglycan that serves many crucial physiologic functions, including barrier effects, tissue resilience and elasticity, control of cell proliferation and migration, wound healing, and organization of hyaluronan-binding molecules, such as hyalectin-type chondroitin sulphate proteoglycans, fibrinogen, and types I and VI collagen into macromolecular assemblies [1–6]. HA is found in nearly every tissue in vertebrates. Although the amount of HA in extracellular matrix is reduced in several human conditions, including cutaneous atrophy, aging, impaired wound healing, and possibly osteoporosis, the molecular control of tissue HA content is incompletely understood [7–9].

HA is synthesized by monomeric enzymes, called hyaluronic acid synthases (HASs), that use both sugar precursors, UDP-glucuronic acid and UDP-*N*-acetyl glucosamine, to assemble the glycosaminoglycan chain. The mode of synthesis is unique amongst macromolecules since it is proposed to occur at the plasma membrane, and during its synthesis the developing HA molecule is extruded directly into the extracellular space [10]. cDNAs encoding human HAS1, human HAS2, and a partial sequence of human HAS3 have been reported [10–12], and the chromosomal locations of the three human *HAS* genes have been identified [13]. In addition, homologues in many other species have been reported, including full-length sequences encoding the murine Has1, Has2 and Has3 [13–15].

Studies using these molecular tools to examine HAS regulation have only recently begun to appear in the literature. Murine cumulus cell–oocyte complexes isolated immediately after inducing an ovulatory cycle (at which time they do not synthesize HA), showed no HAS2 mRNA by reverse-transcriptase-mediated PCR, but when HA synthesis begins 3–4 h later, HAS2 mRNA was expressed at high levels [16]. Similarly, in cultured human dermal fibroblasts and epidermal keratinocytes, transforming growth factor-$\beta$, a known stimulus for HA synthesis [17], increased the levels of HAS1 and HAS2 message (HAS3 message was not examined) [18]. These studies suggest that HAS message levels, possibly reflecting gene transcription, control HA synthesis. In addition, the promoter sequence for murine *Has1* was recently determined and found to contain several potential binding motifs for transcription factors [19].

In the present study, we sought to determine the effect of glucocorticoids (GCs) on the molecular regulation of HA synthases in skin and bone cells. GCs are widely used therapeutic

Abbreviations used: AMD, actinomycin D; DEX, dexamethasone; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; GAG, glycosaminoglycan; GC, glucocorticoid; HA, hyaluronic acid; HAS, hyaluronan synthase.

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agents with a serious side effect of reducing the tissue content of glycosaminoglycans, especially HA [9,20,21]. The resulting organ atrophy, particularly of skin and skeleton, produces substantial morbidity and mortality [22,23]. We found that the predominant HAS in cultured human dermal fibroblasts and osteoblast-like osteosarcoma cells is HAS2, and that GCs induce a large and rapid suppression of HAS2 expression in these cell types through effects on both transcription and mRNA stability. These molecular effects are likely to contribute to GC-induced atrophy of skin and skeleton *in vivo*.

## EXPERIMENTAL PROCEDURES

### Chemicals

RNAzol B was purchased from Tel-Test (Friendswood, TX, U.S.A.). Dexamethasone (DEX) was obtained from American Regent Lab, Inc. (Shirley, NY, U.S.A.). All other chemicals were obtained from Fisher and Sigma.

### Cultured cells

Normal human dermal fibroblasts and the human osteosarcoma cell line, MG-63, a well-established model of osteoblasts [24], were obtained from the A.T.C.C. (Rockville, MD, U.S.A.). Cells were plated in Petri dishes (100 mm diameter; Corning, NY, U.S.A.) in Dulbecco's modified Eagle medium (DMEM) supplemented with 10% fetal bovine serum (FBS), and grown to 90% confluency, at which point the media were replaced by DMEM/10% FBS, with or without DEX (1 $\mu$M). The DEX dose-response experiment was carried out with doses of DEX ranging from 0.15 pM to 1.5 $\mu$M. Experiments with RU486, a specific antagonist of classic cytosolic steroid hormone receptors [25], were performed by pre-incubating fibroblasts for 14 h in DMEM/10% FBS, without or with this agent, followed by the addition of 1 $\mu$M DEX, and then a further 2 h incubation.

### cDNA probes

The cDNA sequence in GenBank® for each human HAS enzyme was used to design specific PCR primers to amplify a segment near the 3′ end of each coding region. These segments were chosen based on their relatively poor conservation amongst HAS1, HAS2 and HAS3 cDNAs, and the fact that only the 3′ end of the human HAS3 cDNA sequence is currently available. The sequence portion and primers for each human HAS cDNA were as follows: HAS1, from bp 1116–1715 [11] using sense primer 5′-tgctactcagagacgccctc-3′ and antisense primer, 5′-ccagtacagcgtcaacatgg-3′; HAS2, from bp 1445–2016 [12] using sense primer 5′-tttatgggcaaccaatgtagc-3′ and antisense primer 5′-aaaatcacaccacccaggag-3′; and HAS3, from bp 2–490 [15] using sense primer 5′-cctactttggctgtgtgcag-3′ and antisense primer, 5′-agatcatctctgcattgccc-3′. As template for these PCR reactions, we used cDNA that we had synthesized using reverse transcriptase (Superscript II; Gibco BRL, Gaithersburg, MD, U.S.A.), oligo(dT) primers, and total RNA isolated from cultured human umbilical vein endothelial cells. Each PCR reaction generated a single band of the correct molecular mass (approx. 600 bp for each), which was then cloned into the T-tailed vector pCRII (Invitrogen, Carlsbad, CA, U.S.A.), and the identity of each insert was confirmed by sequencing.

To obtain HAS cDNA for labelling, the T-tailed vectors with the appropriate insert either were digested with *Eco*RI, or were used as template for a second PCR reaction that employed the same primers as above. As a housekeeping control, we used a probe for 18 S ribosomal RNA (1.5 kb), which was cloned into the pBluescript SK$^+$ vector, then digested with *Eco*RI to obtain an 18 S cDNA fragment for labelling. All probes were $^{32}$P-labelled by random priming (Strategene, La Jolla, CA, U.S.A.) for use in Northern and slot blotting.

### Northern blotting and slot blotting

Cellular RNA was extracted by adding RNAzolB (Tel-Test) directly to 100 mm Petri dishes, followed by standard RNA isolation procedures using phenol/$CHCl_3$ extraction and isopropanol precipitation. Specific binding was confirmed for each labelled probe by Northern blot. For slot blots, total fibroblast RNA was applied to nylon membranes (Magna Charge 45 $\mu$m nylon transfer membranes; MSI, Westboro, MA, U.S.A.) using a slot-blot apparatus (10 $\mu$g/slot), and then hybridized to labelled cDNA probes [26]. The level of each transcript was quantified by PhosphorImage (Molecular Dynamics, Sunnyvale, CA, U.S.A.) and normalized to 18 S RNA. In our initial experiments to compare levels of the three HAS messages in cultured cells, the mass of each HAS mRNA and of 18 S was calculated based on its PhosphorImage signal, and the specific radioactivity of the labelled cDNA probe used for hybridization.

### Nuclear run-off assay

Cultured cells were treated with or without DEX for 2 h, and then nuclei were isolated, resuspended in storage buffer [50 mM Tris/HCl (pH 8.3), 5 mM $MgCl_2$, 0.1 M EDTA with 40% glycerol], and kept in liquid nitrogen until use. The run-off reaction was performed by incubating thawed nuclei with ATP, CTP, GTP, and [$\alpha$-$^{32}$ P]UTP. Newly completed, labelled RNA was isolated after 30 min, using isopropranol precipitation with Trizol (Gibco BRL). Labelled HAS2 and 18 S RNAs were quantified by hybridization to unlabelled immobilized HAS2 and 18S cDNAs on a slot-blot apparatus, followed by autoradiography on a PhosphorImager, using established procedures [27].

### Assessment of HAS2 message half-life using actinomycin-D

Subconfluent fibroblasts were incubated for 30 min with 1.0 $\mu$M DEX or control media, then the cells were treated with actinomycin D (AMD; 5 $\mu$g/ml) to block further RNA synthesis. RNA was obtained upon addition of AMD, then at 20 min, 40 min, and 90 min thereafter. Quantifications of mRNA for HAS2 by slot blot were normalized to 18 S RNA, which is useful for these kinds of studies owing to its extremely long half-life [28]. Data were displayed by semi-log plot, and $t_{\frac{1}{2}}$ values were computed from linear regression of the ln of normalized mRNA values versus the time after addition of AMD, as previously described [28].

### Direct assessment of HAS2 message half-life by wash-out of incorporated uridine label

Cells were labelled essentially to the steady state by incubation with [$^3$H]uridine for 2 days and then rinsed with PBS. A wash-out was performed by incubation of cells in the presence of excess unlabelled uridine for the rest of the experiment [29]. After 1 h of uridine wash-out, DEX was added to half the cells. At several time points after wells had received DEX (0, 15 and 60 min), residual tritium label in the HAS2 and 18 S RNAs was quantified. The quantification was performed by hybridization of total cellular RNA for 2 days with excess unlabelled HAS2 and 18 S cDNA probes which had been bound to nitrocellulose

membranes. These membranes were washed, and then bound $^{3}$H-labelled RNA was eluted with 0.5 ml of 1 M NaOH for 3 min at 95 °C. The eluate was neutralized with 1 M HCl, and radioactivity quantified using a liquid scintillation counter (Beckman LS 60001C). Data were displayed by semi-log plot, and $t_{\frac{1}{2}}$ values were computed from linear regression of the ln of normalized mRNA values versus the time after addition of DEX.

### Statistics

Unless otherwise indicated, summary statistics are reported as means ± S.E.M.; $n = 3$. Comparisons between two means were performed using the unpaired two-tailed $t$ test. Comparisons of several groups simultaneously were performed by initially using ANOVA. When the ANOVA indicated differences among the groups, pairwise comparisons were performed using the Student–Newman–Keuls $q$ statistic [30]. Standard errors for the differences between means of groups with equal $n$ were calculated as the square root of the sum of the squares of the individual S.E. values. Absent error bars in graphical displays of summary statistics for $n = 3$ indicate S.E.M. values smaller than the drawn symbols.

## RESULTS

### Quantification of HAS messages in cultured human fibroblasts and osteosarcoma cells

Total RNA was isolated from subconfluent cultured human fibroblasts and analysed by Northern blotting using $^{32}$P-labelled HAS1, HAS2 or HAS3 cDNA probes. As expected [18], HAS1 mRNA was detected as a single 2.4 kb band, and HAS2 mRNA was detected as two bands of 3.2 kb and 4.8 kb. No HAS3 message was detected in the RNA from these cells. Quantitative analysis of triplicate slot blots showed that the dominant HAS message in control fibroblasts encodes HAS2 (Figure 1). Virtually identical results were obtained in the MG-63 osteoblast line. Therefore our subsequent studies focused on the HAS2 message.

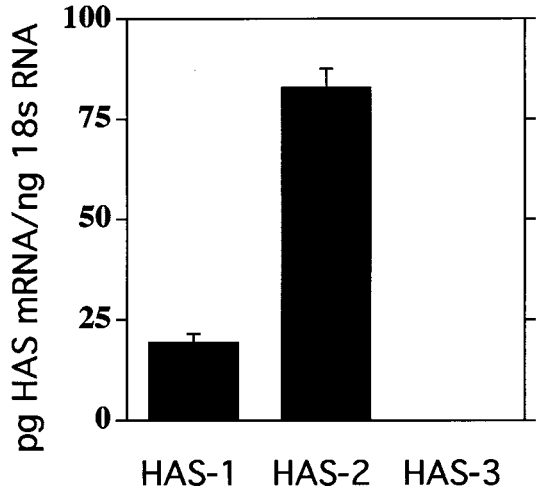


**Figure 1 Quantitative comparison of HAS message levels in the absence of GCs**

RNA was extracted from cultured fibroblast monolayers and analysed by slot blot. The filters were hybridized sequentially with HAS1, HAS2 and HAS3 cDNA probes and then rehybridized with the 18 S probe. The mass of each RNA was computed, based on the radioactive signal and the specific radioactivities of the labelled cDNA probes. Displayed are HAS mRNA mass data, normalized to 18 S mass. ANOVA indicated differences amongst the groups ($P < 0.0001$; Student–Newman–Keuls test indicated that the normalized HAS2 mRNA level was greater than either the HAS1 or the HAS3 signals, and that HAS1 was greater than HAS3; $P < 0.01$ for each of these pairwise comparisons).

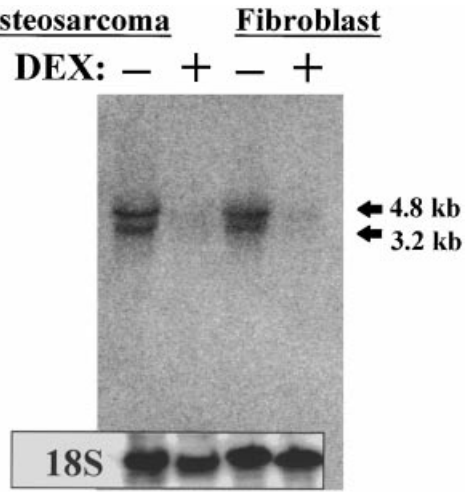


**Figure 2 Effect of DEX on HAS2 mRNA levels in cultured fibroblasts and osteosarcoma MG-63 cells**

90% confluent fibroblasts and osteosarcoma cells were cultured without (−) or with (+) 1 $\mu$M DEX for 24 h. Displayed is a Northern blot of total cellular RNA hybridized with $^{32}$P-labelled HAS2 cDNA probe. The inset (18 S) shows the result from stripping then hybridizing the same blot with a labelled 18 S probe.

### Effect of DEX on HAS2 message levels

Twenty-four hours after addition of 1 $\mu$M DEX or control buffer to cultured cells, total RNA was harvested and analysed by Northern blot. In DEX-treated fibroblasts, the 4.8 kb and 3.2 kb HAS2 mRNA bands, normalized to 18 S, were suppressed by 98 % and 97 % respectively, compared with their levels in control cells (Figure 2, fibroblast lanes, + versus −). Similarly, DEX-treated osteosarcoma cells exhibited a 98 % suppression of both HAS2 bands compared with control cells without DEX (Figure 2, osteosarcoma lanes, + versus −).

The time course of DEX-induced suppression of HAS2 mRNA was examined in fibroblasts in a long-term study, which indicated a sustained effect over 36 h (Figure 3A), and subsequently in a shorter study, which indicated near-total suppression of HAS2 message by 2 h (Figure 3B). HAS1 mRNA suppression was also observed, but to a smaller degree (by 25 % ± 7.6 % at 24 h, $P < 0.03$). Thus GCs induce a rapid, sustained, near-total suppression of HAS2 mRNA, the predominant HAS message in these cells.

### Pathways for DEX signalling

GCs exert effects through three distinct pathways [31]: (i) the classic cytosolic GC hormone receptor, which is activated in more than 30 min by 1 pM–1 nM prednisone equivalents [31], i.e. between 0.14 pM and 0.14 nM DEX (0.75 mg of DEX is equivalent to 5.0 mg of prednisone, and the ratio of molecular mass is 1.09), and is inhibited by the general antagonist of cytosolic steroid hormone receptors, RU-486 [25]; (ii) putative

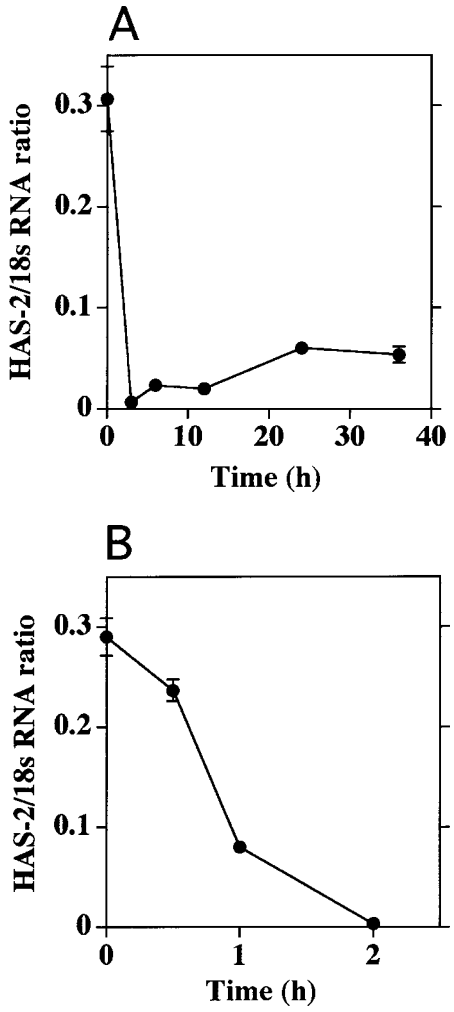


**Figure 3 Time courses for DEX-induced suppression of HAS2 mRNA**

Fibroblasts were incubated with DEX for the indicated times, and then RNA was harvested. Displayed are the results from triplicate slot-blot quantifications of HAS2 mRNA normalized to 18 S RNA in (**A**) long-term and (**B**) short-term incubations.

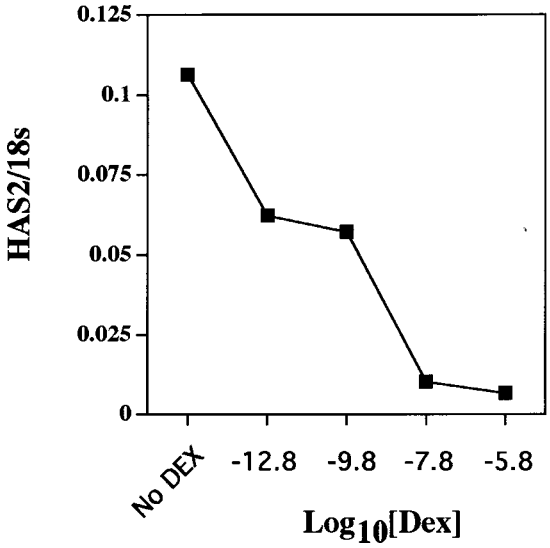


**Figure 4 Dose response for DEX-induced suppression of HAS2 mRNA**

Fibroblasts were incubated with the indicated concentrations of DEX, and RNA was harvested 24 h later. Displayed are the results from duplicate Northern-blot quantifications of HAS2 mRNA normalized to 18S RNA. The non-zero doses of DEX (0.15 pM, 0.15 nM, 15 nM and 1.5 $\mu$M) are indicated on the x-axis as the $base_{10}$ log.

cell-surface steroid hormone receptors, which are activated within seconds to 1–2 min by 0.14 nM or greater DEX; and (iii) direct physical actions on cellular membranes, which require seconds and $> 14\,\mu$M DEX [31]. Thus rapid suppression of HAS2 mRNA by 1 $\mu$M DEX is consistent with the characteristics of cytosolic and cell-surface receptors, but not direct physical effects.

Two methods were used to distinguish the roles of these different classes of receptors. First, we examined the dose response of DEX-induced suppression of HAS2 mRNA in fibroblasts. Figure 4 shows that $\leqslant 45\,\%$ suppression of HAS2 mRNA occurred at 0.15 pM and 0.15 nM DEX, consistent with a limited contribution from cytosolic receptors, but the full suppression required 1.5 nM and 1.5 $\mu$M DEX, consistent with a predominant role for the cell-surface steroid hormone receptors. Secondly, blocking experiments were performed with RU486, an antagonist for cytosolic steroid hormone receptors. In the absence of DEX, RU486 produced approx. $50\,\%$ decreases in HAS2 mRNA (Figure 5), possibly because of blockage of other non-GC steroid hormones [32,33]. Addition of DEX in the absence or presence of RU486, however, produced a near-total suppression of HAS2 mRNA levels, confirming that the majority of DEX-induced suppression is independent of cytosolic steroid hormone receptors. Taken together, these results suggest a major role for the cell-surface receptors.

### Molecular mechanisms of DEX-induced suppression of HAS2 message levels

To explore the molecular basis for the large effect of GCs on HAS2 message levels, we began by examining *HAS2* transcription

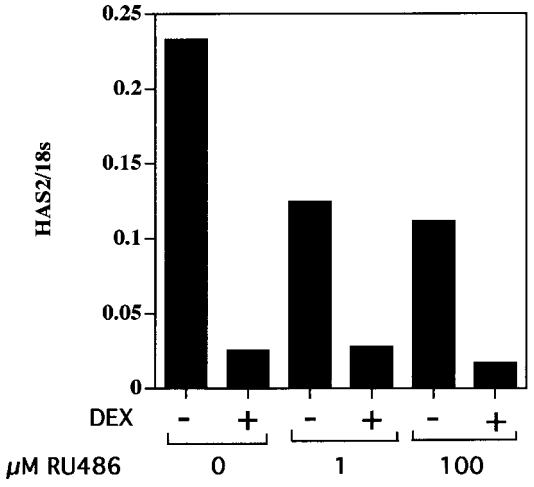


**Figure 5 Lack of effect of RU486 on DEX-induced suppression of HAS2 mRNA**

Fibroblasts were incubated with 1.0 or 100 $\mu$M RU486 overnight, as indicated, followed by a 2 h incubation without (−) or with (+) 1 $\mu$M DEX. RNA was harvested 2 h later. Displayed are the results from duplicate Northern-blot quantifications of HAS2 mRNA normalized to 18 S RNA.

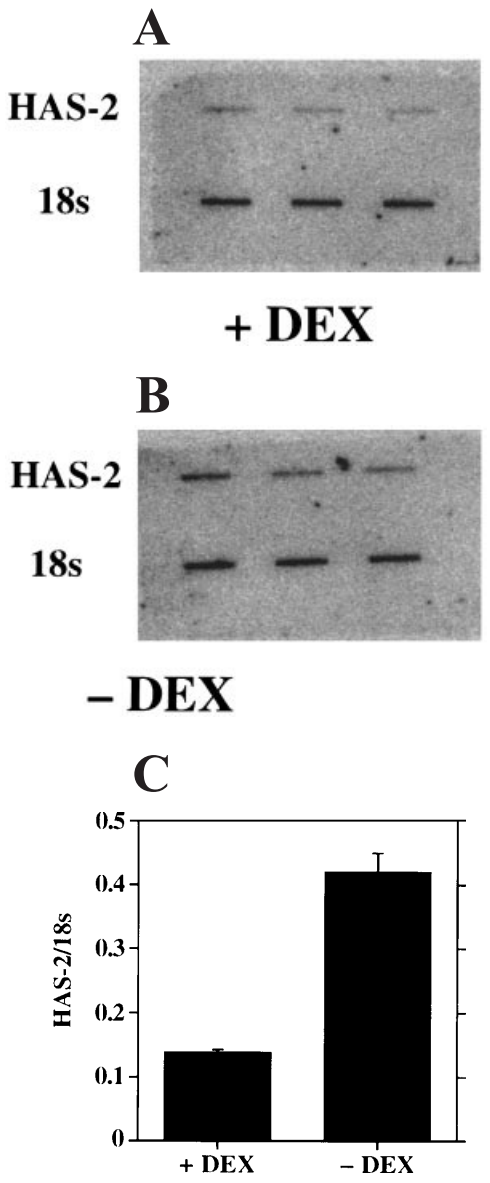


**Figure 6 Effect of DEX on HAS2 gene transcription**

Nuclei isolated from fibroblasts which had been pre-incubated for 2 h in the presence (A) or absence (B) of 1 $\mu$M DEX were allowed to complete the synthesis of RNA molecules during an incubation in the presence of labelled nucleotide precursors. (**A**, **B**) Autoradiograms of newly completed labelled HAS2 and 18 S RNAs, which were captured by hybridization to unlabelled, immobilized HAS2 and 18 S cDNAs on a membrane. (**C**) Graphical representation of the same information quantitatively. DEX caused a significant reduction in the ratio of labelled HAS2 to 18 S RNA made by these nuclei ($P < 0.001$).

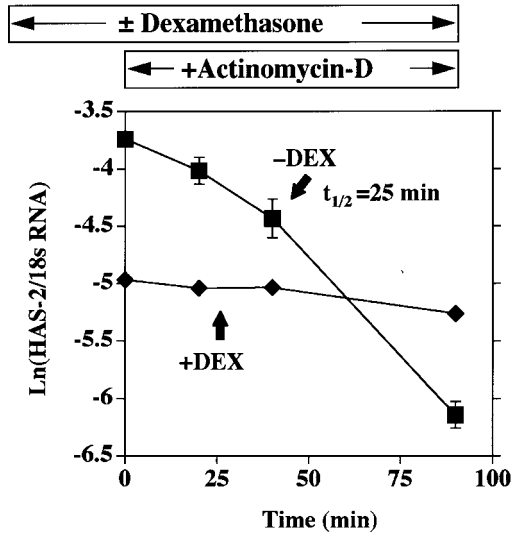


**Figure 7 Assessment of HAS2 message half-life using actinomycin D**

Fibroblasts were placed into media with or without DEX at $t = -30$ min ($\pm$DEX). At t = 0, all cells were given AMD (+AMD). Displayed is a semi-log plot of HAS2 message levels, normalized to 18 S, at the indicated times.

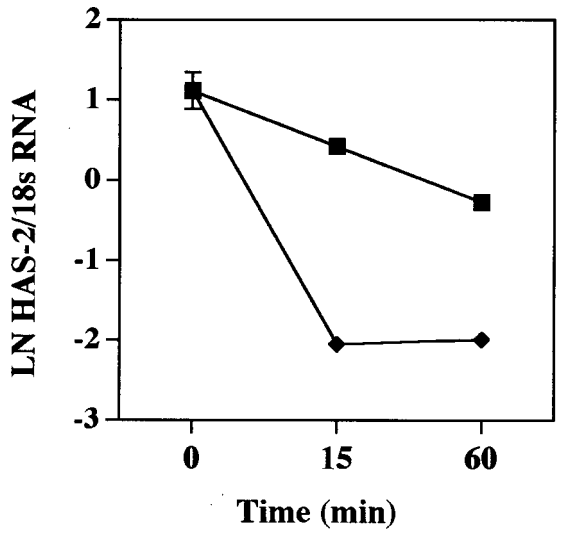


**Figure 8 Direct assessment of HAS2 message half-life by wash-out of incorporated uridine label**

Fibroblasts were incubated with [$^3$H]uridine for two days, and then a wash-out was performed by incubation of cells in excess unlabelled uridine. At 1 h into the wash-out, DEX was added (diamonds) or not (squares). Displayed is a semi-log plot of residual tritium label in HAS2 message, normalized to the residual label in 18S, at the indicated times.

using the nuclear run-off assay. Nuclei isolated from fibroblasts that had been pre-incubated for 2 h in medium supplemented with DEX consistently showed a 70 % reduction in HAS2 mRNA transcription rate compared with control nuclei isolated from cells pre-incubated without DEX (Figure 6). Though substantial, this suppression of transcription is inadequate to explain the rapid and large effect seen on HAS2 mRNA levels.

To examine HAS2 message stability, we used the standard approach of adding AMD, an inhibitor of RNA synthesis, to DEX-treated or control cells, then following the disappearance of specific RNAs (i.e. HAS2 mRNA and 18 S) over time. Using this approach, we determined that the half-life of HAS2 mRNA in cultured human fibroblasts in the absence of DEX was 25 min (Figure 7). Notice that this half-life will not permit 98 % of HAS2 mRNA to disappear in just 2 h (four half-lives), even if transcription completely stops. Addition of DEX 30 min before addition of AMD resulted in an initial fall in HAS2 mRNA (Figure 7; $t = 0$), consistent with our earlier results, whereas

addition of AMD led to a marked decrease in rate of HAS2 mRNA degradation (Figure 7). This prolongation of mRNA half-life in the presence of DEX and AMD suggests that AMD in this circumstance may be blocking the synthesis of a destabilizing factor, such as an RNase [34]. We thus sought a direct method to measure HAS2 mRNA half-life that did not require the use of metabolic inhibitors with potential secondary effects.

Our next approach was steady-state labelling of RNA by prolonged incubation of cells with [$^3$H]uridine, followed by a wash-out in the presence of excess unlabelled uridine, during which we examined the disappearance over time of labelled HAS2 and 18S RNA. In the absence of DEX, uridine-labelled HAS2 mRNA disappeared from cells with a half-life of 31 min (Figure 8), consistent with our earlier results using AMD. In contrast, uridine-labelled HAS2 mRNA in DEX-treated cells exhibited a substantially faster decline, with a half-life of less than 4 min (Figure 8; notice that by 20 min, the labelled HAS2 message was already below the limits of detection of this assay). Additional experimentation confirmed the half-life of HAS2 mRNA of just under 30 min in the absence of DEX, but with some variability in the half-life in the presence of DEX (though always below 16 min).

## DISCUSSION

In this report, we have shown that GCs rapidly induce a sustained, near-total suppression of HAS2 mRNA, which we found to be the predominant HAS message in dermal fibroblasts and osteoblast-like cells. Dose–response studies in fibroblasts indicated that the majority of the suppressive effect required concentrations characteristic of cell-surface GC receptors, a point confirmed by persistent DEX-induced suppression in the presence of RU486, an antagonist of classic cytosolic steroid hormone receptors. The molecular mechanism for GC-induced suppression of HAS2 message involves a combination of decreased gene transcription and decreased message stability. It is likely that these effects contribute to the substantial loss of HA from skin, skeleton and other organs during GC-induced organ atrophy *in vivo*.

HA plays specific, essential roles in skin and skeleton. Skin contains about half of the total body HA [35]; the major hyalectin proteoglycan in skin is versican, which assembles along strands of HA [36]. In normal skin, HA is found in the intercellular spaces of the epidermis, with the exception of the upper granular layer and stratum corneum. In the dermis, staining for HA is diffuse throughout, but is increased below the basement membrane and around the skin appendages [37]. In epidermis and dermis, large networks of HA, with versican and other molecules, form a highly charged lattice that enhances the epidermal barrier as well as tissue resiliency. Regarding wound healing, the absence of cutaneous scarring in fetuses has been related to the high content of HA and cell-surface HA receptors in fetal skin [17,38]. During treatment with GCs, several changes occur in skin that contribute to atrophy of that organ [27]. Histologically, the atrophic changes in skin from GC treatment are characterized by thinning of the epidermis and a large decrease in dermal ground substance [28], which is largely composed of glycosaminoglycans (GAGs), including HA. GC treatment not only reduces the quantity of various GAGs but also changes the distribution, the relative proportion, and the structure of connective-tissue proteoglycans, partly because of the loss of HA, which ordinarily serves as a scaffold for other matrix components.

In bone and cartilage, the major hyalectin proteoglycan is aggrecan, which also assembles along strands of HA [39]. HA and proteoglycans, particularly aggrecan in the osteoid matrix, are involved in mineralization [40]. The distribution of hyaluronan in vitamin D-treated chick bone and the alterations observed in rachitic tissue suggest an essential role for hyaluronan in endochondral bone formation [41].

Tissue content of HA is controlled by the synthesis and degradation of this molecule. HA has a short half-life in many tissues: the $t_{\frac{1}{2}}$ is only 0.5 day in skin and joints [2,35,42]. Thus changes in HA synthesis can lead to rapid changes in tissue HA content. HAS protein has an extremely short half-life of 2–4 h, and enzyme activity is 6.5 times higher in exponential growth phase versus stationary growth phase cells [3]. GCs are already known to suppress HA synthesis in dermal fibroblasts [20,21], cartilage chondrocytes [43], synovial fibroblasts [44] and palate cells [45]. Intra-articular injection of GCs into knee joints reduces the levels of proteoglycans and HA in articular cartilage [46]. Systemic administration of pharmacological doses of GCs causes significant dermal atrophy, leading to thinning of skin, easy bruising, and cutaneous ulceration [22,47]. Systemic GCs also cause atrophy of bone, leading to fractures [23]. In both skin and bone, these changes involve a substantial loss of matrix, which is primarily GAGs and collagen [48]. Our results suggest that a major molecular mechanism for GC-induced organ atrophy is rapid, prolonged and near-total suppression of HAS2 message.

Our dose–response studies and the results of addition of RU486 are consistent with a major role for the putative cell-surface GC receptors (with some contribution from the classic cytosolic GC receptors) in the suppression of HAS2 message. Cell-surface GC receptors appear to act via intracellular second messengers, such as Ins$P_3$, $Ca_2^+$, or protein kinase C [31]. Effects mediated by cytosolic GC receptors can be direct, if there are GC response elements in the promoter of the target gene, or indirect, if the target gene responds to a protein, such as a cytokine, which is regulated by GCs. The *HAS2* promoter has not yet been characterized, but the promoter for *HAS1* contains no known GC response elements, although several motifs associated with cytokine responsiveness are present [19]. It is known that transforming growth factor-$\beta$ increases HAS1 and HAS2 message [18], and that GCs suppress the effects of this cytokine [49]. Several other cytokines, including interleukin-1, tumour necrosis factor-$\alpha$, epidermal growth factor and platelet-derived growth factor, also up-regulate HA synthesis [50,51], and GCs suppress many of these cytokines as well [52]. Thus it is possible that some of the effects of GCs on HAS2 mRNA levels, particularly during prolonged exposure, may involve the classic cytosolic GC receptor acting indirectly through suppression of cytokines or cytokine action. We also found that AMD, an inhibitor of RNA synthesis, stabilizes the HAS2 message in the presence of DEX (Figure 7). Such findings are usually interpreted as implying the existence of a destabilizing factor, such as a specific RNase [34], which in this case would be induced by DEX and required for the effect of the drug on HAS2 message half-life.

The HAS enzymes are attractive targets for pharmacological manipulation. Enhancing levels of HAS messages, HAS enzymes, HA synthesis and tissue HA content could improve cutaneous elasticity and resiliency, skeletal integrity, and perhaps scar-free wound healing. Furthermore, the devastating side effects of GC-induced organ atrophy might be lessened or averted.

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