coarser bed grains. Supercritical climbing is still difficult to produce without deposition rates of nearly 100% of impacting grains. Further experiment and impact simulation are needed to determine splash dynamics on beds of mixed grain size.

The generic classes of eolian deposits that we have simulated are identifiable in the geologic record. Analytical methods of studying and interpreting such stratigraphy have been discussed by Rubin and Hunter (*15*) and applied mainly to large two-dimensional bedforms. Rubin (*16*) has also described a geometrical model for studying the migration and resulting stratigraphy of three-dimensional bedforms using superpositions of trigonometric functions. The value of the simulations is their basis in the physics of particle behavior after impact. Prevailing wind and sediment conditions at the time of ripple formation and the corresponding bedding structures that are preserved are directly connected through the splash and deposition physics.

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11. It might be argued that flattening of ripple crests by direct fluid forces remains a possibility. Although not strictly ruled out for an undulating surface, for flat surfaces direct entrainment of grains by the wind is thought to be a small effect compared to impact-induced mobilization (*8*). The sharpness of the ripple crests in Fig. 1 is due to the use of a single impact angle in the simulations.
12. Hunter (*10*) recorded such surfaces by periodically sprinkling a small amount of dark magnetite grains on them.
13. In subcritical climbing the ratio of the rate of vertical motion (due to deposition) to the rate of forward motion is less than the upwind slope of the ripple, so that upwind surfaces are eroded. In supercritical climbing this ratio is greater than the upwind slope, so that upwind surfaces can be preserved in the accumulating column of sediment.
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Effect of Sodium Salicylate on the Human Heat Shock Response

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Sodium salicylate, an anti-inflammatory agent, was examined for its effects on the heat shock response in cultured human cells. Salicylate activation of DNA binding by the heat shock transcription factor (HSF) was comparable to activation attained during heat shock. However, sodium salicylate did not induce heat shock gene transcription even though the HSF was bound in vivo to the heat shock elements upstream of the heat shock protein 70 (Hsp 70) gene. These results reveal that activation of the heat shock transcriptional response is a multistep process. Modulation of extracellular pH augments sensitivity to salicylate-induced activation of HSF.

THE ANTI-INFLAMMATORY AGENT SODIUM salicylate (*1*) induces heat shock–responsive chromosomal puffs in *Drosophila* salivary glands and stimulates HSF DNA binding activity in cultured *Drosophila* cells (*2, 3*). Inflammation causes a rise in temperature and is accompanied by tissue injury; at the cellular level during inflammation, heat shock and other forms of physiological stress induce the transcription of heat shock genes (*4*). Chondrocytes isolated from inflamed tissues of arthritic patients exhibit unusually high levels of heat shock proteins (*5*). Furthermore, mediators of inflammatory responses, such as the tumor necrosis factor and complement, map adjacent to the Hsp 70 gene on human chromosome 6 (*6*). Therefore, we examined whether salicylate treatment alters the expression of heat shock–inducible genes in human cells.

HeLa cells were exposed to sodium salicylate and short-term–labeled with [^{35}S]methionine, and cellular proteins were analyzed by SDS–polyacrylamide gel electrophoresis. We did not detect additional synthesis of any heat shock proteins over a range of sodium salicylate concentrations (2 to 30 mM) and exposure times (0 to 6 hours). After treatment of cells with 20 mM salicylate, electrophoretic mobility-shift assays were performed with HeLa cell extracts and a heat shock element (HSE) from a human heat shock protein gene. Salicylate activated HSF DNA binding comparable to that obtained with a 42°C heat shock (Fig. 1A). Competition with oligonucleotides showed that salicylate-activated HSF exhibited the same DNA sequence specificity as heat-activated HSF.

We next examined the effect of salicylate on in vivo transcription rates using nuclear run-on analysis. Despite the large amount of HSF DNA binding activity that was induced by salicylate, we did not observe an increase in the transcription rates of either Hsp 70 or Hsp 90 genes (Fig. 1B). Because of this result, we examined whether salicylate-activated HSF DNA binding activity was nuclear-localized and bound in vivo to the HSEs of the Hsp 70 gene. We compared the in vivo occupancy of the HSEs by genomic footprint analysis of the endogenous Hsp 70 gene promoter in salicylate-treated and heat-shocked cells (*5*). The patterns of dimethyl sulfate (DMS) sensitivity for the naked (deproteinated) DNA and control-cell DNA were indistinguishable (Fig. 2A, lanes 1, 2, 6, and 7). The footprint of heat-shocked cells (lanes 3 and 8) indicated HSF binding to five adjacent HSEs corresponding to sites 1, 3, and 5 on the coding strand as well as sites 2 and 4 on the noncoding strand (*7*). The genomic footprint of the cells treated with 20 mM salicylate (lanes 4 and 9) revealed that the salicylate-activated HSF bound in vivo to all five HSE sites. The combination of salicylate and heat shock (lanes 5 and 10) also strongly protected guanine residues known to

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contact HSF (7). In addition, both salicylate and heat shock resulted in DMS hypersensitivity at guanine residues −116, −95, and −89. Quantitative results were obtained by scanning densitometry (Fig. 2B). The HSEs in control cells were vacant, but HSF DNA binding activity in salicylate-treated cells was equal to or greater than that in heat-shocked cells.

We next examined whether the salicylate-induced HSF, perhaps representing an inert DNA-bound state, interfered with a subsequent heat shock response. HeLa cells were treated with salicylate and heat-shocked at 42°C, and nuclei were harvested for run-on analysis. Salicylate treatment did not inhibit thermal induction of heat shock gene transcription (Fig. 3). These results indicate that salicylate treatment itself does not interfere with overall RNA polymerase II activity. However, these results do not indicate whether the transcriptionally inert, salicylate-activated HSF is exchanged for transcription-

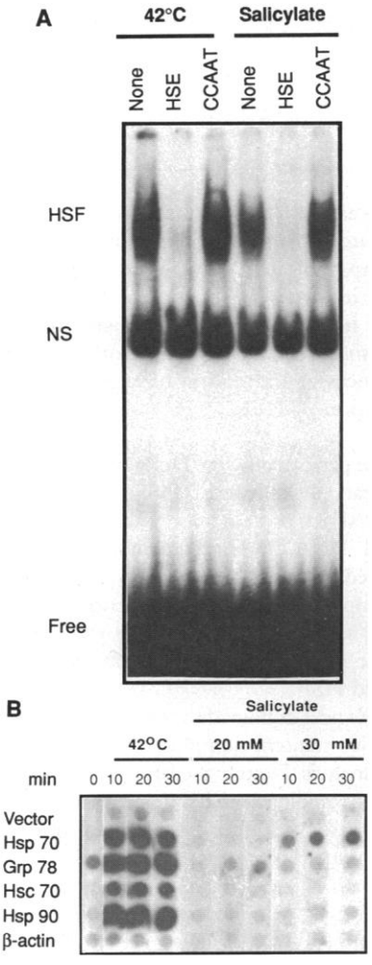

Fig. 1. Effect of sodium salicylate on the heat shock response in HeLa cells. (**A**) Gel mobility-shift analysis (*20*) of whole-cell extracts treated with either 42°C heat shock or 20 mM sodium salicylate at 37°C for 10 min. Analysis was performed as described with a ^{32}P-labeled oligonucleotide containing the heat shock element (HSE) from the human heat shock protein promoter (*15*). Competition with a 100-fold excess of unlabeled HSE oligonucleotide or the CCAAT element oligonucleotide marked above. HSF, heat shock transcription factor; NS, nonspecific protein–DNA binding; Free, unbound HSE oligonucleotide. (**B**) Transcription rates, measured by nuclear run-on assay (*10*), of human heat shock genes Hsp 70 (heat shock protein 70) (pH2.3), Grp 78 (glucose-regulated protein 78) (pHG2), Hsc 70 (heat shock cognate 70) (pHA7.6), Hsp 90 (heat shock protein 90) (pUC 801), and β-actin (pHFβA-1) (*21*). HeLa cells were heat-shocked at 42°C or exposed to 20 mM and 30 mM salicylate at 37°C as indicated. The vector plasmid (pAT 153) was used as a nonspecific hybridization control.

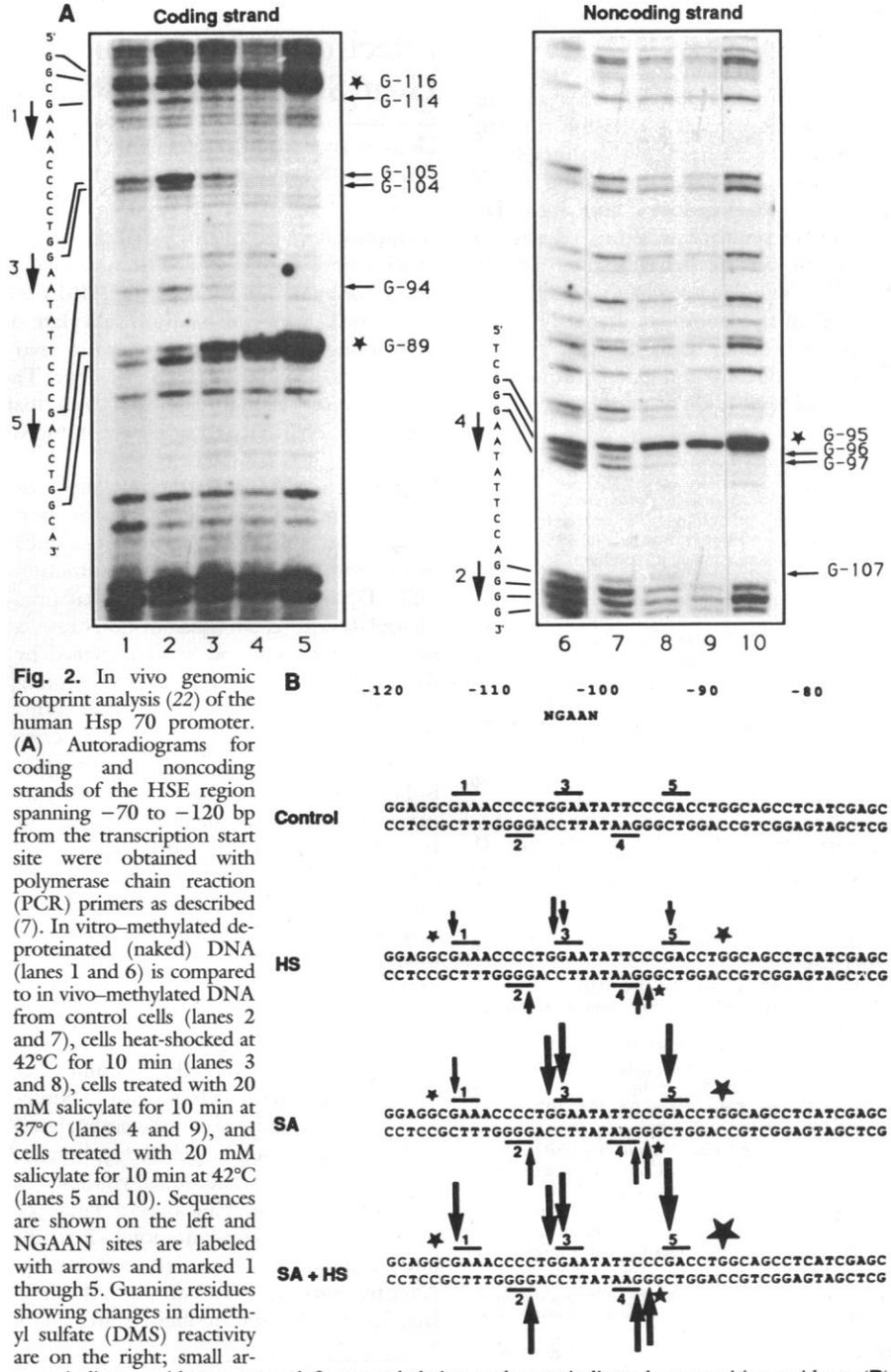

Fig. 2. In vivo genomic footprint analysis (*22*) of the human Hsp 70 promoter. (**A**) Autoradiograms for coding and noncoding strands of the HSE region spanning −70 to −120 bp from the transcription start site were obtained with polymerase chain reaction (PCR) primers as described (7). In vitro–methylated deproteinated (naked) DNA (lanes 1 and 6) is compared to in vivo–methylated DNA from control cells (lanes 2 and 7), cells heat-shocked at 42°C for 10 min (lanes 3 and 8), cells treated with 20 mM salicylate for 10 min at 37°C (lanes 4 and 9), and cells treated with 20 mM salicylate for 10 min at 42°C (lanes 5 and 10). Sequences are shown on the left and NGAAN sites are labeled with arrows and marked 1 through 5. Guanine residues showing changes in dimethyl sulfate (DMS) reactivity are on the right; small arrows indicate residues protected from methylation and stars indicate hypersensitive residues. (**B**) Summary and quantitation of DMS reactivity patterns of guanine residues in the Hsp 70 promoter for each of the treatments in (A). HS, heat shock; SA, salicylate; SA + HS, salicylate and heat shock. Quantitation was done on an LKB densitometer (Bromma, Sweden); arrow size indicates the amount of protection from methylation by protein contacts, and star size indicates relative intensity of hypersensitivity to methylation. Relative to control cells, the arrow and star sizes indicate approximately two-, three-, and fourfold differences in band intensities.

ally competent, heat shock–activated HSF, or whether the salicylate-induced HSF is converted to active heat shock–induced HSF.

The activation of HSF DNA binding by salicylate indicates that this anti-inflammatory drug can signal the first step of the heat shock response, the conversion of HSF from a nonbinding state to a sequence-specific DNA binding factor. However, unlike other inducers of the heat shock response, salicylate does not lead to transcriptional competency, the second step of the heat shock response. These results are important in considering the differences in the heat shock response of yeast and mammalian cells. In *Saccharomyces cerevisiae*, HSF binds constitutively to the HSE and, upon heat shock, becomes phosphorylated and acquires the features of an inducible transcription factor (*8*). In higher eukaryotes, including *Drosophila*, the HSEs are not occupied until heat shock or other stresses have activated HSF (*9, 10*). The effects of salicylate reveal that the mechanism of HSF activation in mammalian cells is a multistep process with activation and in vivo HSF binding representing the first step, which can be uncoupled from transcriptional activation.

The modulation of HSF DNA binding kinetics by salicylate raises the question of whether physiological factors such as temperature or extracellular pH influence the stress response. We compared HSF DNA binding activity and Hsp 70 transcription rates in cells treated with salicylate and then heat-shocked with that in cells heat-shocked alone. Salicylate extended the period during which HSF was maintained in an active state (*11*). This observation suggests that anti-inflammatory agents can potentiate the natural stress response.

We next examined whether factors that influence the availability of salicylate outside cells and its subsequent entry into cells modulate the activation of HSF DNA binding. Pharmacologic activity of salicylate is affected by extracellular pH, which determines the ionization state of the drug (*12*). At low pH, the protonated form of salicylate preferentially crosses the cell membrane. Therefore, we examined the effect of pH on activation of HSF DNA binding by salicylate in cells in which the media were adjusted to pH 6.8 or 7.6 (Fig. 4). Although alteration of extracellular pH did not itself induce HSF DNA binding, in slightly acidic cells (pH 6.8), HSF DNA binding activity was detected after a 10-min exposure to 5 mM salicylate; in cells maintained at a higher pH (7.6), exposure to greater than 20 mM salicylate induced a similar amount of HSF. These results reveal that extracellular pH influences activation of HSF DNA binding activity by salicylate. This conclusion may be relevant in diseases such as rheumatoid arthritis, where the molecular action of anti-inflammatory drugs could depend on conditions like altered pH (*13*) that are associated with damaged tissue and inflammation.

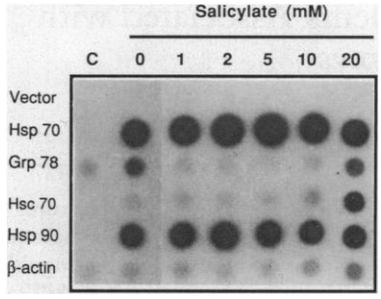

Fig. 3. The effect of salicylate on heat shock transcription rates. Transcription rates for Hsp 70, Grp 78, Hsc 70, Hsp 90, and β-actin were assessed by nuclear run-on assay in cells pretreated with 1 to 20 mM salicylate for 10 min and then heat-shocked for 20 min. C, control cells at 37°C.

Salicylate also acts as a second messenger in plants responding to viral infections (*14*). Second messengers have not been invoked for HSF activation, although calcium acts in vitro to activate HSF (*15*). The protein synthesis inhibitor cycloheximide inhibits the activation of HSF by salicylate (*11*). This suggests that salicylate, like heat shock, may interfere with protein synthesis or lead to the accumulation of aberrant newly synthesized proteins, conditions known to stimulate the heat shock response (*16, 17*). Whether salicylate influences HSF binding by damaging proteins is still unknown; salicylate action in vitro includes inhibition of enzymes associated with arachidonate metabolism (*18*), and in vivo treatment of HeLa cells with sodium arachidonate activates HSF DNA binding (*11*). This observation, together with reports that specific prostaglandins induce expression of heat shock genes (*19*), encourages further studies on the activation of HSF DNA binding via lipid-based signals.

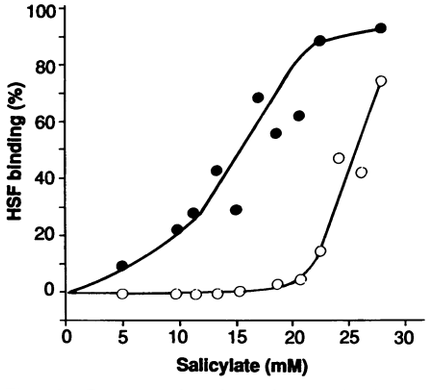

Fig. 4. Concentration-dependent activation of HSF binding in HeLa cells exposed to acidic or alkaline media. Gel shift analysis of HSF binding was evaluated in whole-cell extracts of cells exposed to 0 to 30 mM salicylate for 10 min after a 30-min equilibration period in media containing 20 mM Hepes buffer adjusted to pH 6.8 or 7.6. A curve fitted to multiple points of HSF binding levels as determined by scanning densitometry is shown for each of the concentrations tested at either pH 6.8 (solid circles) or pH 7.6 (hollow circles).

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