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# Antibody supershift assay is inadequate for determining HSF stoichiometry in HSE complexes

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Sir,

Prompted by the discrepancies concerning activation of the human heat shock transcription factors 1 and 2 (HSF1 and HSF2) (Sistonen et al. 1992, Yoshima et al. 1998), we wish to bring to attention of the heat shock field the caveats of using the antibody supershift assay for revealing the composition of HSF-HSE complexes.

Using antibody supershift technique, we have noticed marked differences in the ability of antibodies raised against human, mouse, and chicken HSFs to detect the human HSF1 and HSF2 in the heat shock or hemin-induced HSF-HSE complexes in K562 erythroleukemia cells. As seen in Figure 1A, αm2 (anti-mouse HSF2, Sarge et al. 1993) did not interfere with the heat shock-induced HSF-HSE complex, whereas αcHSF2δ (anti-chicken HSF2, Nakai et al. 1995) slightly affected the complex formation when using 1:10 dilution. As expected, αm1 (anti-mouse HSF1; Sarge et al. 1993) supershifted and almost completely abolished the complex formation at 1:10 dilution. The antibody against chicken HSF1, αcHSF1γ (Nakai et al. 1995), partially reduced the mobility of the complex at 1:10 dilution. Similar to the αm2 antibody, another anti-mouse HSF2 antibody (αmHSF2; Alastalo et al. 1998), had no effect on the heat shock-induced HSF-HSE complex. Our new anti-human HSF1 antibody (αhHSF1; C.I. Holmberg and L. Sistonen, unpublished data) retained the complex in the well at 1:10 dilution. Upon hemin-induced HSE-binding, as shown in Figure 1B, the antibody against mouse HSF2, αm2, inhibited migration of the HSF-HSE complex into the gel, and the αcHSF2δ antibody against chicken HSF2 interferred with the complex formation at 1:10 dilution. It is noteworthy that both αm1 and αhHSF1 slightly affected the HSF-HSE complex at 1:10 dilution, as did, although to a lesser extent, the αcHSF1γ antibody against chicken HSF1.

While the antibody supershift assay is an important qualitative method, multiple pitfalls restrict its use in quantitative analysis of protein-DNA complexes. Regarding antibodies raised against HSF1 and HSF2, as exemplified in Figure 1, distinct antibodies differently affect the formation and mobility of the protein-DNA complexes in the gel. In addition, an antibody might be specific for a particular protein, but still require high concentrations in order to supershift the DNA-binding complex. This could be due to low binding affinity, or due to inaccessibility of an epitope to a specific antibody, which might explain the finding that the other antibody against mouse HSF2 (αmHSF2; Fig. 1B) did not affect the hemin-induced HSF-HSE complex although it has previously shown highly specific reactivity against HSF2 in both immunohistochemistry and in non-native state in Western blotting (Alastalo et al. 1998). Furthermore, antibodies raised against distinct HSFs might have different on- and off-rates, as well as stabilities during gel electrophoresis. Therefore, no quantitative conclusions concerning the composition of the HSF-HSE complexes can be made solely based on antibody supershift assays, and additional methods are needed to confirm and quantitate the results obtained using the supershift technique. For example, studies using the gene knock-out technique should be very revealing. In this respect, by using cells derived from HSF1-deficient mouse, it has been shown that HSF2 is not able to compensate the lack of HSF1 and heat stress-inducible Hsp70 accumulation in *HSF1*$^{-/-}$ cells (McMillan et al. 1998). Similar analyses should elucidate the specific role of HSF2 once the knock-out model is available. In conclusion, there is an urgent need for further dissecting the cooperativity of HSF1 and HSF2, and

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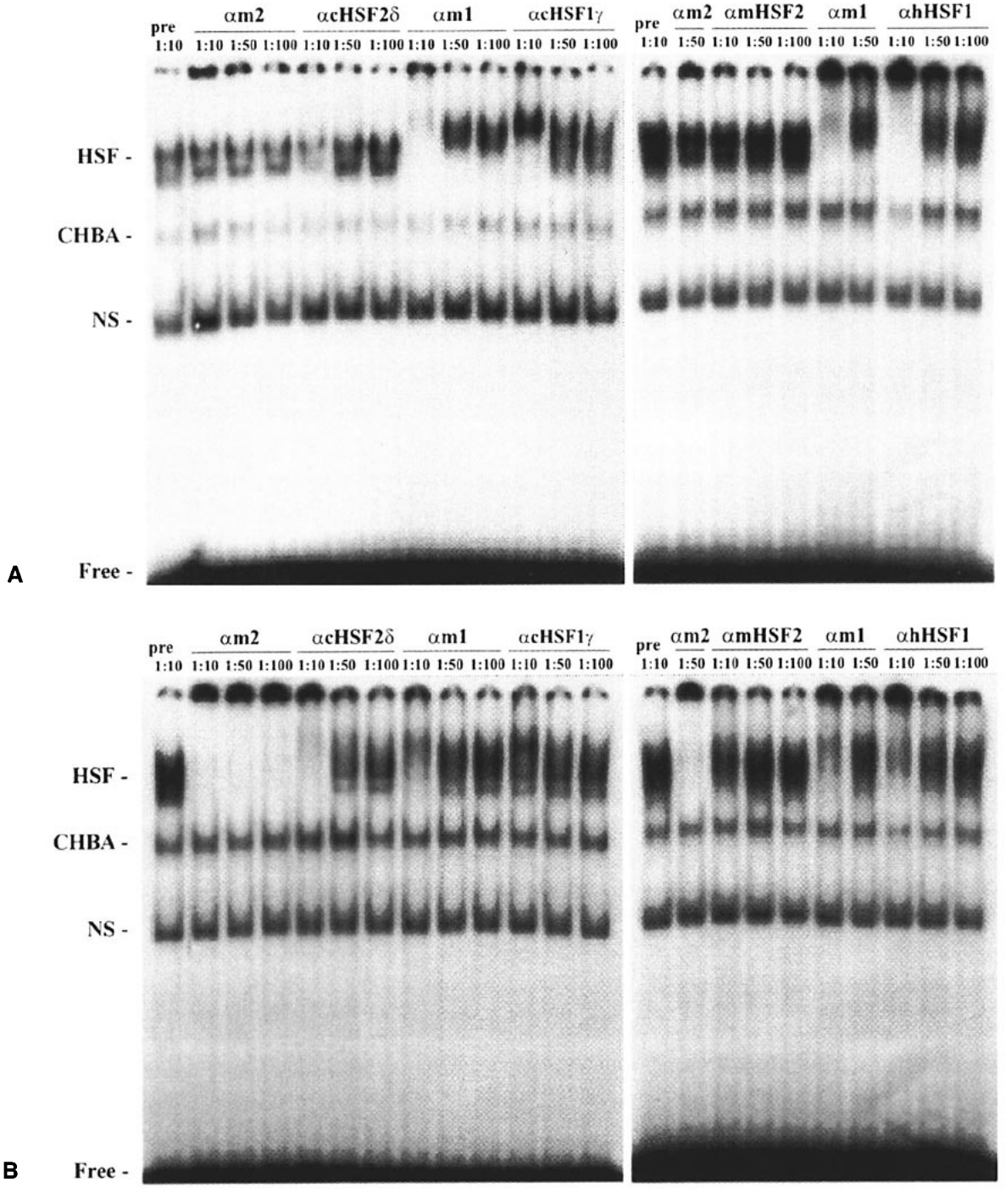


**Fig. 1** Human K562 erythroleukemia cells were heat-shocked at 42°C in a waterbath for 1 h (A) or treated with 40 μM hemin (Aldrich) for 18 h at 37°C (B). Whole cell extracts were incubated in the presence of a $^{32}$P-labeled oligonucleotide representing the proximal HSE of the human hsp70 promoter (Mosser et al. 1988). For antibody supershift experiments to analyze HSF1 and HSF2 in the HSE-binding complex in K562 cells, indicated dilutions of rabbit polyclonal antibodies raised against human HSF1 (αhHSF1; C.I. Holmberg and L. Sistonen, unpublished data), mouse HSF1 and mouse HSF2 (αm1 and αm2, respectively; Sarge et al. 1993; kindly provided by Dr R.I. Morimoto), mouse HSF2 (αmHSF2; Alastalo et al. 1998), and chicken HSF1 and chicken HSF2 (αcHSF1γ and αcHSF2δ, respectively; Nakai et al. 1995; kindly provided by Dr A. Nakai), were added to whole cell extracts and incubated at 25°C for 15 min prior to the gel mobility shift analysis. HSF indicates the specific inducible HSF-HSE complex, CHBA indicates the constitutive HSE-binding activity reported previously (Mosser et al. 1988), NS denotes non-specific protein-DNA interaction, and Free indicates free probe.

the contribution of each of these factors in regulation of heat shock genes, or other possible target genes, in response to different stimuli.

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