assay as above (data not shown). This confirms that the specific binding component of HAP is protein.

Specific HAP binding to *hsp82* gene chromatin is almost totally inhibited by pretreating active extracts with a short *hsp82* DNA fragment containing the binding site (Fig. 4, lane *b*). No inhibition on pretreatment is observed using further upstream flanking sequences or sequences in the *hsp82* coding region, nor when other DNAs are used (lanes *d-i*). On the other hand, purified DNA from the 5' region of the other heat shock genes: *hsp70*, *hsp68*, *hsp26*, *hsp23*, *hsp22* do inhibit to a partial extent the binding of HAP factor to *hsp82* gene chromatin (lanes *j, l, o-q*), whereas purified DNA from the coding sequences of *hsp70*, *hsp68*, *hsp22* and *hsp26* do not (lanes *k, m, r, s*). To demonstrate that the HAP activity we have measured in the extract is a primary DNA binding activity and not a secondary enzymatic activity which converts an ineffective HAP to its specific DNA binding mode, we pretreated active extract with target DNA and digested excess DNA with micrococcal nuclease. The resultant extract has no HAP binding activity towards the *hsp82* gene chromatin (Fig. 4, lanes *c, n*); the activity should have been retained were the active component secondary to DNA binding. (Effective removal of excess DNA by micrococcal nuclease is demonstrated by the recovery of HAP binding activity to chromatin on readdition of active extract; data not shown.)

We infer from these inhibition experiments that HAP factor for the *hsp82* gene is able to bind to purified DNA, not only to its own target sequence but also to the upstream consensus sequence³ shared by other heat shock genes. The simplest model consistent with the data is that HAP, in concert with bound TAB, can coordinately activate the whole heat shock gene family by binding to each upstream element, and that the variation in the kinetics of activation among these genes reflects differential binding affinities of HAP for individual upstream elements that are similar but not identical in sequence. Such a model would predict a direct relationship between decreasing HAP binding affinities and the increasing order of transcriptional activation. The available evidence supports this prediction. The *hsp82* gene is most easily activated^{6,9-11,12}, whilst HAP binding strength *in vivo* (ref. 2 and unpublished data), or *in vitro* (Fig. 4) is greater for the *hsp82* gene than for other heat shock genes. It will be of interest to investigate further the correlation between binding strength and activation kinetics.

We re-examined the sequence of the 36 bp HAP binding site² for *hsp82* and found that it covers a central 28 base sequence with almost perfect dyad symmetry (24 bases out of 28): (–81) GAAGCCTCTAGAAG|TTTCTAGAGACTTC (–54). None of the upstream elements of the other heat shock genes have this high degree of symmetry. The exceptionally strong binding affinity of HAP to this symmetrical dyad suggests that it may be the canonical recognition sequence for HAP binding (as a protein dimer or tetramer like prokaryotic DNA binding proteins).

We do not believe that HAP is synthesized *de novo* upon heat shock, because the response can be brought on within minutes after stimulation, and can occur in the absence of protein synthesis¹³. It seems that HAP should either be decompartmentalized upon heat shock or more likely, be converted from a pre-existing inactive form to a specific DNA binding form. Our finding that HAP activity in the crude extract binds DNA directly does not preclude the presence of secondary modifying activities in the extract. We are currently devising appropriate assays to retrace the physiological pathway from the terminal events of heat shock.

Others have shown the presence of eukaryotic sequence-specific DNA binding proteins^{14,15}, and activators of heat shock genes have been reported in studies employing different methods¹⁶⁻¹⁹. HAP factor appears to be unrelated to these activities. During the preparation of this manuscript, Parker and Topol²⁰ published a report on a *Drosophila* transcription factor designated HSTF that is specific for the *hsp70* gene; partially purified preparations of this factor bind to a 55 bp region

upstream of the TATA box. HAP and HSTF bind roughly to the same DNA sequence, but their binding activities in non-heat-shocked cells appear to be very different; further work should illuminate the relationship between the two factors.

We believe that the *in vitro* chromatin binding, exonuclease protection assay reported here is a powerful method for analysing eukaryotic DNA binding proteins for the following reasons: (1) precision in delimiting the binding site, (2) confidence in the physiological relevance of the binding provided by *in vivo* comparison, and (3) exceptional sensitivity, as seen by detection of binding activity in crude extracts with no fractionation whatsoever. This feature permits binding activity to be tracked from the start of the purification; at later stages, more traditional assays could be used to increase experimental efficiency.

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Turbulence in an oceanic convective mixed layer—Corrigendum

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In the above letter it was stated that the mixed layer mean value of ϵ/J_b^0 in the ocean was a factor of two lower than that in the atmosphere, and that difference was left unexplained. The difference was the result of a computational error. When that error is corrected, the two cases are in much better agreement, as seen in the figure. For the region $0.4 < z/D < 1.0$, the mean value of ϵ/J_b^0 in both the ocean and atmosphere is 0.45. The difference in scatter may be due to more restrictive editing of the atmospheric data as well as the influence of lateral processes in the ocean.