The Hsp70 chaperone is a major player in stress-induced transposable element activation

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Previous studies have shown that heat shock stress may activate transposable elements (TEs) in *Drosophila* and other organisms. Such an effect depends on the disruption of a chaperone complex that is normally involved in biogenesis of Piwi-interacting RNAs (piRNAs), the largest class of germline-enriched small noncoding RNAs implicated in the epigenetic silencing of TEs. However, a satisfying picture of how chaperones could be involved in repressing TEs in germ cells is still unknown. Here we show that, in *Drosophila*, heat shock stress increases the expression of TEs at a posttranscriptional level by affecting piRNA biogenesis through the action of the inducible chaperone Hsp70. We found that stress-induced TE activation is triggered by an interaction of Hsp70 with the Hsc70–Hsp90 complex and other factors all involved in piRNA biogenesis in both ovaries and testes. Such interaction induces a displacement of all such factors to the lysosomes, resulting in a functional collapse of piRNA biogenesis. This mechanism has clear evolutionary implications. In the presence of drastic environmental changes, Hsp70 plays a key dual role in increasing both the survival probability of individuals and the genetic variability in their germ cells. The consequent increase of genetic variation in a population potentiates evolutionary plasticity and evolvability.

transposable elements | Hsp70 | evolution

Previous studies in *Drosophila* and other organisms have shown that heat shock stress may increase transcription levels of certain transposable elements (TEs), leading to bursts of transposition (1–4). Most recently, it has been also shown that heat shock treatment of different *Drosophila* strains at the pupal stage can produce mutations by transposable element insertions (5). Hints for the mechanism that underlies this phenomenon come from the demonstration that functional alterations of the heat shock protein Hsp90, known as Hsp83 in *Drosophila*, cause the activation of transposable elements in germ cells of *Drosophila* (6), including a class of repetitive sequences called *Stellate* in the male germline, due to the involvement of the Hsp83 chaperone in the Piwi-interacting RNA (piRNA) biogenesis (7–9). This class of small interfering RNAs is located in the nuage, a specific electron-dense region around the nucleus of germ cells, where ribonucleoprotein complexes called RNA-induced silencing complexes (RISCs) are formed. RISCs are involved in a posttranscriptional mechanism that maintains TEs and repeated sequences in a repressed state (10–13). In addition to the piRNAs, 2 other classes of small RNAs, the siRNAs and microRNAs (miRNAs), mediate posttranscriptional silencing by generating RISCs in somatic cells (10, 14, 15). A fundamental component of the RISCs is represented by proteins of the Argonaute (Ago) family; Ago1 and Ago2 proteins have been reported to bind miRNAs and siRNAs, respectively (16, 17), while Piwi, Aub, and Ago3, belonging to the Piwi subclade of Argonaute proteins, specifically bind piRNAs (18–21).

The involvement of additional heat shock-related chaperones in RISC function is demonstrated by the observation that both siRNA and miRNA duplexes require the aid of Hsc70/Hsp90 chaperone machinery to load onto Ago1 or Ago2 (22–25), similar to the Hsp90-dependent loading of ligand onto steroid hormone receptors which leads to their activation (26–28). In *Drosophila*, it has been shown that the Hsc70/Hsp90 machinery used for the loading of siRNAs and miRNAs to Ago1 and Ago2 may also include cochaperones such as Hop (Hsp70/Hsp90 organizing protein homolog) (29), Hsc70-4, and Droj2 (DnaJ-like-2) (25). This last factor belongs to the Hsp40 cochaperones family which is essential in the Hsp70 cycle. These results suggest that a similar chaperone machinery is probably also required for loading piRNAs onto Ago3, and further suggest a causal correlation between heat shock-induced TE activity and the functional destabilization of the Hsc70/Hsp90 chaperone machinery. We tested this hypothesis, and found that heat shock activates transposable elements in germ cells by affecting the Hsc70/Hsp90 chaperone machinery, and that the heat-inducible Hsp70 chaperone is required for transposable element derepression upon heat shock.

Results and Discussion

Transposable Element Induction after Heat Shock Is Mainly Due to Disruption of Posttranscriptional Silencing. We first asked how heat shock would impact transposable element expression in our *Drosophila Oregon-R (Ore-R)* laboratory stock. Adult male and

Significance

We have identified, by genetic and cytological analyses, the minimal components of a chaperone complex involved in transposon (TE) silencing in *Drosophila* germ cells. We found that, after heat shock, the stress-inducible Hsp70 chaperone interacts with the chaperone complex and factors involved in piRNAs biogenesis in both ovaries and testes. Hsp70 induces displacement of these factors to the lysosomes. Concomitantly, we observed a significant activation of TEs at the posttranscriptional level, suggesting an involvement of Hsp70 in TE activation after stress. We propose that such a mechanism has evolutionary implications for the genome's response to environmental stress.

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The authors declare no conflict of interest.

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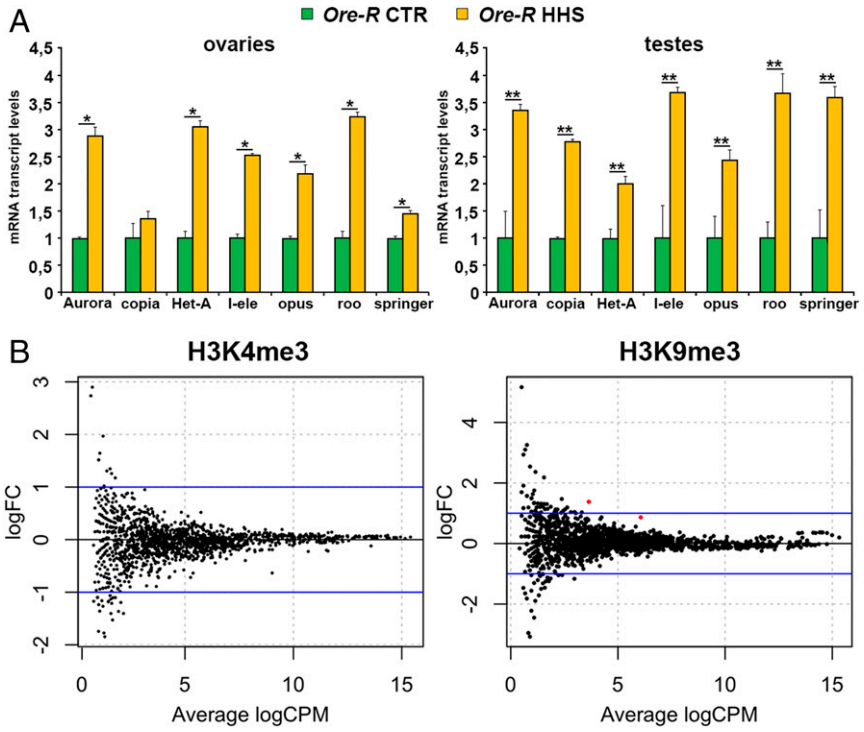

Fig. 1. Heat shock treatment impairs posttranscriptional silencing of TEs. (*A*) TE expression profiles in ovaries and testes from control and heat-stressed flies analyzed 2 d after the heat treatment. Changes in the levels of TE transcripts were determined by quantitative RT-PCR relative to *Rp49* expression. Data are expressed as the mean ± SEM of 3 biological replicates and are normalized to the no heat shock controls (*$P < 0.05$; **$P < 0.01$). (*B*) MA plot for H3K4me3 and H3K9me3 marks on all of the TEs between the 2 conditions (heat-stressed versus control). Each point represents one TE, with the corresponding $\log_2$ fold change and the average $\log_2$ counts per million (cpm). Red dots highlight TEs with statistically significant changes (FDR < 0.05). Horizontal blue lines correspond to a fold change (FC) of 2.

female flies were exposed to a heavy heat shock (HHS; 37 °C for 1 h followed by 4 °C for 1 h, with the cycle repeated 3 times), and their germinal tissues were analyzed by qRT-PCR using oligonucleotides specific to different families of *Drosophila* retrotransposable elements. The results showed a significant increase of the transcripts of all TEs in both ovaries and testes, except for copia in ovaries, where the difference did not reach the 5% level of significance (Fig. 1*A*).

To test a possible effect of heat shock stress on transcriptional control of TEs, we performed chromatin immunoprecipitation sequencing (ChIP-seq) experiments on ovary extracts from heat-treated and untreated females using antibodies against histone H3K9me3 and H3K4me3, 2 specific epigenetic marks for transcriptionally inactive and active chromatin, respectively. We did not find a significant difference in the H3K4me3 mark on any family of TEs when comparing before and after heat shock treatment; in the H3K9me3 experiment, we found only 2 elements (FBti0060728 and FBti0063005) differentially enriched [false discovery rate (FDR) = 0.009], but with only small changes after heat shock ($\log_2$ fold change = 1.4 and 0.9, respectively) (Fig. 1*B*). This result strongly suggests that the derepression of TEs after stress is mainly due to alterations of the posttranscriptional silencing mechanism.

A Chaperone Complex Mediates Posttranscriptional Silencing of Transposable Elements. Since it has been shown that Hsp90 and Hop are involved in piRNA biogenesis (6, 29), we tested a possible

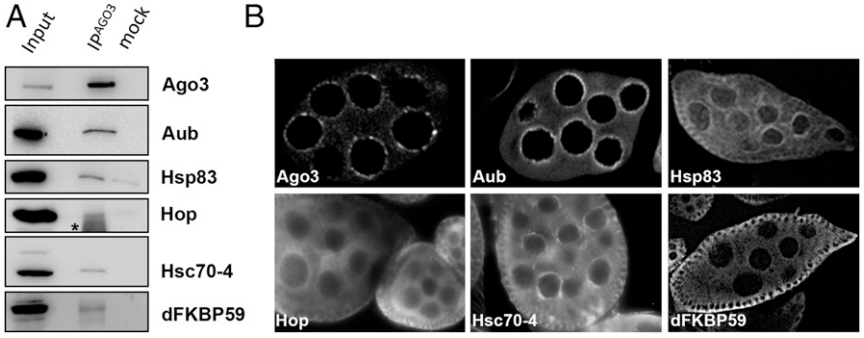

Fig. 2. Interaction of Ago3 with components of the Hsc70/Hsp90 chaperone machinery. (*A*) Ago3 immunoprecipitates from wild-type ovaries subjected to Western blotting analysis are probed with antibodies against the indicated cochaperones. Coimmunoprecipitation experiments clearly show that all of the proteins tested are direct interactors of Ago3; the asterisk indicates the IgG heavy chain. (*B*) Immunofluorescence analysis confirms the colocalization of Ago3 with the same cochaperones in the nuage. Images were obtained using a 40× magnification.

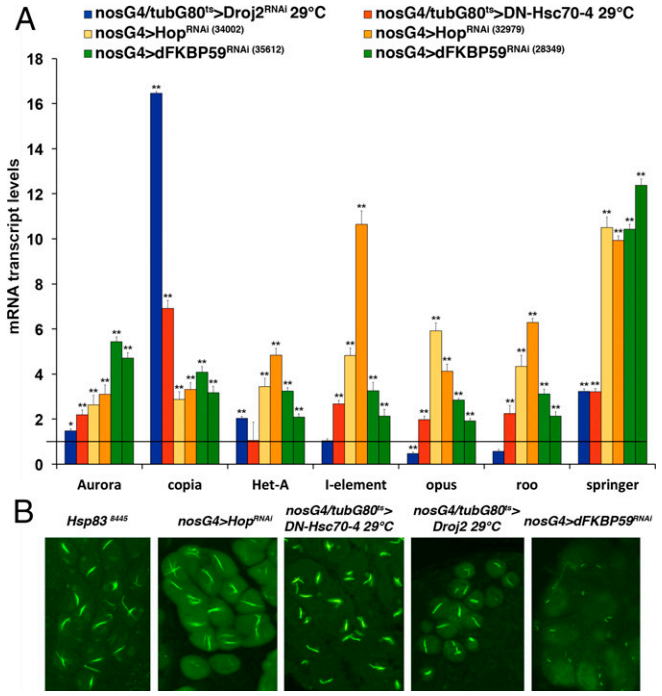

Fig. 3. Components of the Hsc70/Hsp90 chaperone machinery are required for TE silencing. (*A*) Activation of various TEs by silencing cochaperone genes *Droj2*, *Hsc70-4*, *Hop*, and *dFKBP59*. Note that, for both *Hop* and *dFKBP59*, we analyzed 2 independent RNAi transgenic lines, while, for *Hsc70-4*, we tested the dominant negative variant *DN-Hsc70-4*. Germline expression was driven with *nos-Gal4* (*nosG4*). To temporally control the expression of *DN-Hsc70-4* and *Droj2* RNAi knockdown, the *tub-Gal80^{ts}* system was used; the black line indicates the control value. TE expression levels from *Hop* and *dFKBP* RNAi samples were compared to *nosG4* sample. For *Droj2* RNAi and *DN-HSC70-4*, the same genotypes raised to the permissive temperature for *GAL80^{ts}* (18 °C) were used as controls. Results are presented as mean ± SEM (**P* < 0.05; ***P* < 0.01). (*B*) Silencing of cochaperones in the male germline derepresses *Stellate* sequences, as visualized by the presence of crystals in primary spermatocytes. Images of squashed testes were acquired using a 40× magnification.

involvement of Hsc70-4 and other cochaperones such as Droj2 and dFKBP59, an Hsp90-associated cochaperone belonging to the class of immunophillins, which are known key components of the molecular Hsc70/Hsp90 chaperone machinery (25, 27, 30). To this end, we performed coimmunoprecipitation experiments on ovaries from nonstressed flies using a specific antibody against Ago3, and subsequent Western blot analysis with antibodies specific for each candidate. The results clearly demonstrated a binding interaction of Ago3 with Aub, Hsp83, Hop, Hsc70-4, and dFKBP59 (Fig. 2*A*). We also performed immunolocalization experiments and found that each of these cochaperones colocalizes in ovaries to the nuage where Aub and Ago3 are also found (Fig. 2*B*). We were unable to check for the presence of Droj2 in both coimmunoprecipitation and immunofluorescence assays because of the lack of a specific antibody. However, as reported below, we were able to analyze its functional involvement in piRNA biogenesis by RNA interference (RNAi) silencing.

To test whether the physical interactions and colocalization of these factors correspond to a functional requirement in transposable element silencing, we analyzed TE expression profiles from ovaries where Droj2, Hsc70-4, and dFKBP59 were depleted by in vivo RNAi using the nanos-Gal4 driver (nosG4). Both nanos-Gal4–mediated Droj2 and Hsc70-4 knockdown cause a complete ovarian and testes degeneration, as shown below, thus complicating the molecular analyses of TE transcripts; to bypass these developmental defects, we used the tub-Gal80^{ts} (tubG80^{ts}) system to temporally control the expression of the dominant negative Hsc70-4^{7TS} variant (DN-Hsc70-4) and Droj2 knockdown driven by nanos-Gal4. NosG4/tubG80^{ts} > Droj2^{RNAi} and nosG4/tubG80^{ts} > DN-Hsc70-4 females were aged for 6 d at the permissive temperature (18 °C) and then shifted to the restrictive temperature (29 °C) for 5 d before dissecting the ovaries for RNA purification. As shown in Fig. 3, we found that functional inhibition of Hsc70-4, dFKBP59, or Droj2 in gonads of nonstressed flies derepressed various classes of TEs in ovaries (Fig. 3*A*), as previously shown for Hsp90 and Hop (6, 29).

To determine whether these chaperone components affected piRNA silencing in the male germline, we examined testes for the presence of Stellate (Ste) crystals, which are normally repressed by piRNA-mediated silencing. Inhibition of Hsc70-4, dFKBP59, or Droj2 in testes resulted in the generation of Ste

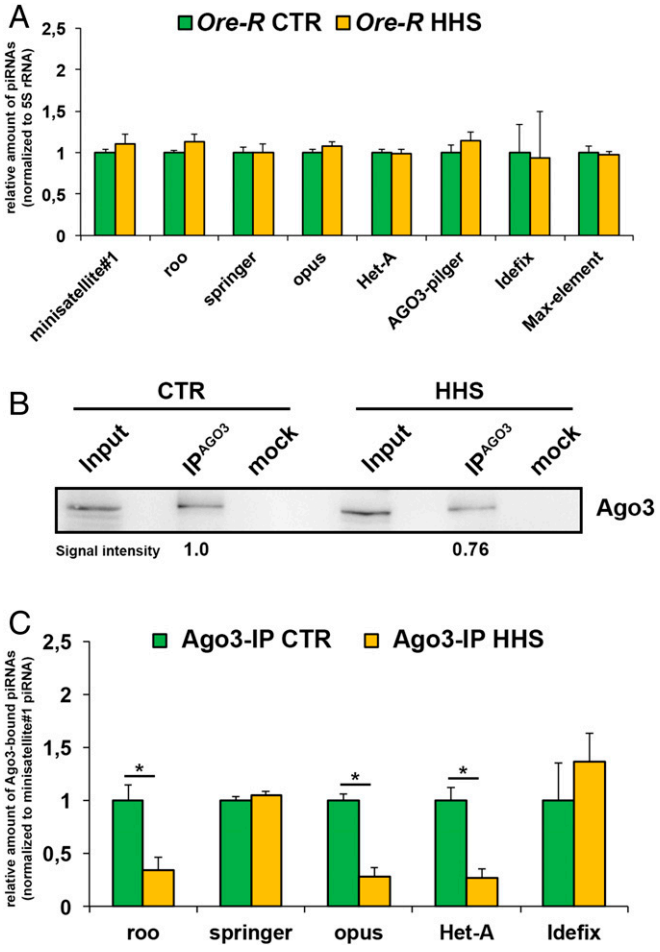

Fig. 4. Heat shock affects piRNA loading onto Ago3. (*A*) qRT-PCR analysis of a panel of ovary-enriched piRNAs in ovary lysates from HHS and control (CTR) samples. Relative abundance of each piRNA was determined by the $2^{-\Delta\Delta Ct}$ method using 5S piRNA as an internal control. (*B*) Western blot assay showing Ago3-IP efficiency in both stressed and control samples. Ago3 signal intensity in each IP sample was performed to equalize the Ago3 protein in HHS and control immunoprecipitates. (*C*) Quantification by qRT-PCR of a specific set of piRNAs targeting different stress-induced TEs in Ago3 immunoprecipitates from HHS and control ovary lysates. Relative abundance of each piRNA was determined by the $2^{-\Delta\Delta Ct}$ method using, as internal control, Minisatellite#1 piRNA that is not modulated by HHS treatment. Data were expressed as the mean values ± SEM of 2 biological replicates (**P* < 0.05).

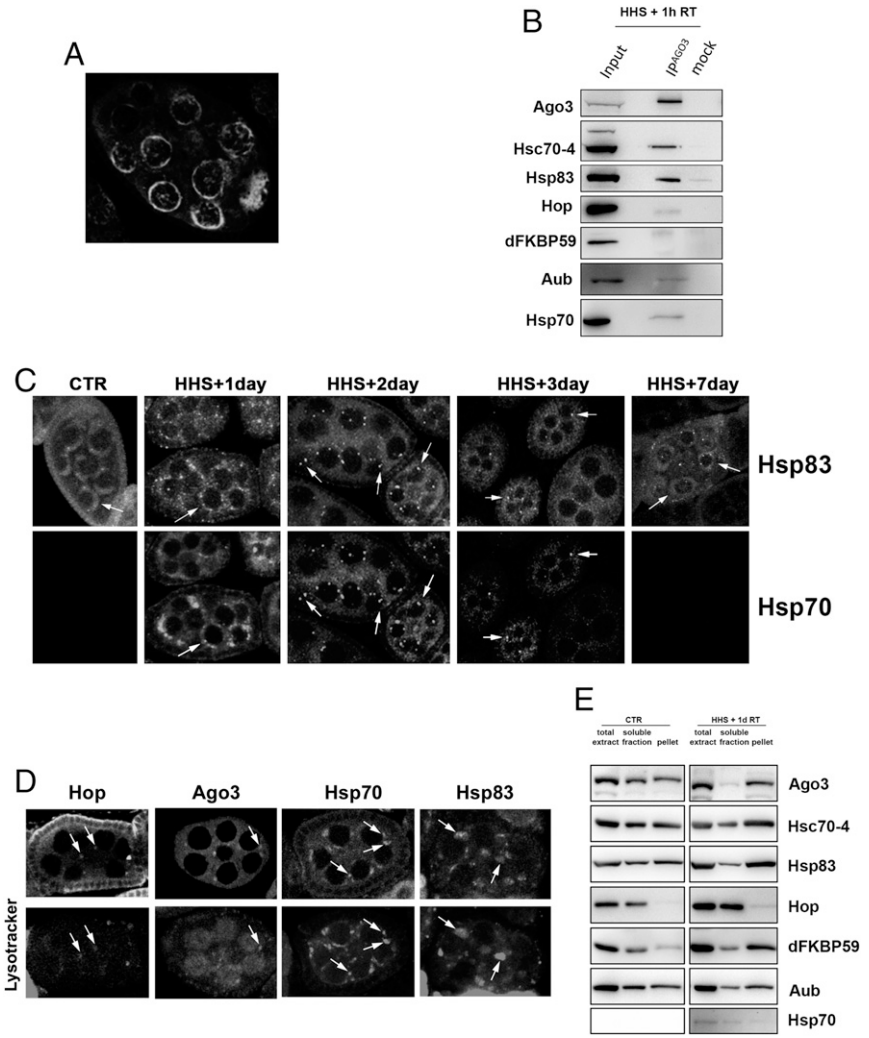

Fig. 5. Hsp70 interacts with Ago3 and the components of the chaperone machinery after heat shock. (*A*) One hour after heat shock, Hsp70 localizes in the nuage and (*B*) interacts with Ago3 and other chaperones, except dFKBP59. (*C*) Two days after heat shock, Hsp70 interacts with Hsp90 in forming cytoplasmic bodies outside the nuage (see arrows). Seven days after heat shock, Hsp83 is again localized in the nuage, while Hsp70 signals are absent. (*D*) Hsp830–Hsp70 cytoplasmic aggregates also include Ago3 and Hop and colocalize to lysosomes, as shown by their colocalization with the specific lysosomal marker LysoTracker (see arrows). (*E*) Solubility assay. Western blot of fractionated proteins obtained from adult ovaries after 1 d of recovery from HHS treatment. Image in *A* was obtained using a 63× magnification; images in *C* and *D* were acquired using a 40× magnification.

crystalline aggregates, confirming that these chaperone components are also involved in the piRNA pathway in males (Fig. 3*B*). The knockdown efficiency of each RNAi line used in this study is summarized in *SI Appendix*, Fig. S1.

Heat Shock Reduces Loading of piRNAs onto Ago3. To understand how heat shock stress affects transposable element derepression, we asked whether high temperature affects piRNA production generally. We used qRT-PCR to quantify the levels of a panel of 8 ovary-enriched piRNAs (20, 21, 31) in ovary lysates from control and HHS samples collected after 1-d recovery following heat stress. We found that heat shock does not significantly affect production of these piRNAs (Fig. 4*A*).

We then asked whether heat stress would impair piRNA loading onto Ago3. For this purpose, we compared the amounts of 4 Ago3-bound piRNAs targeting different stress-induced TEs, in Ago3 immunoprecipitates from HHS and control ovary lysates. To equalize the amount of Ago3 in Ago3 immunoprecipitates, we quantified, by Western blot analysis, Ago3 signal intensity in each IP sample and diluted Ago3 immunoprecipitates from control sample accordingly (Fig. 4*B*). All measured piRNAs, with the exception of *springer* piRNA, are reduced in the Ago3 immunoprecipitates from HHS sample compared with those from the control sample (Fig. 4*C*). As a negative internal control, we used *Idefix* piRNA, a Piwi-bound somatic piRNA (32). These results indicate that heat shock treatment impairs TE silencing by partially affecting piRNA loading onto Ago3 (Fig. 4).

Hsp70 Chaperone as a Major Player in Transposable Element Induction after Heat Shock. Our results show that, at normal temperature, TEs are largely repressed by an RNAi mechanism in which the Hsc70/Hsp90 chaperone machinery plays an important role. Impaired function of any one of several chaperones or cochaperones abrogates TE silencing (Fig. 3). Paradoxically, however, even though heat shock results in an enormous increase in chaperone production, it also derepresses transposable elements. This seeming contradiction could be resolved if, as a response to heat shock, the chaperone complex is reassigned from its normal functions in piRNA biogenesis to instead deal with the effects of heat stress. We theorized that shifting chaperone machinery away from the repression of TE activity after heat shock might involve the major heat shock protein Hsp70. In

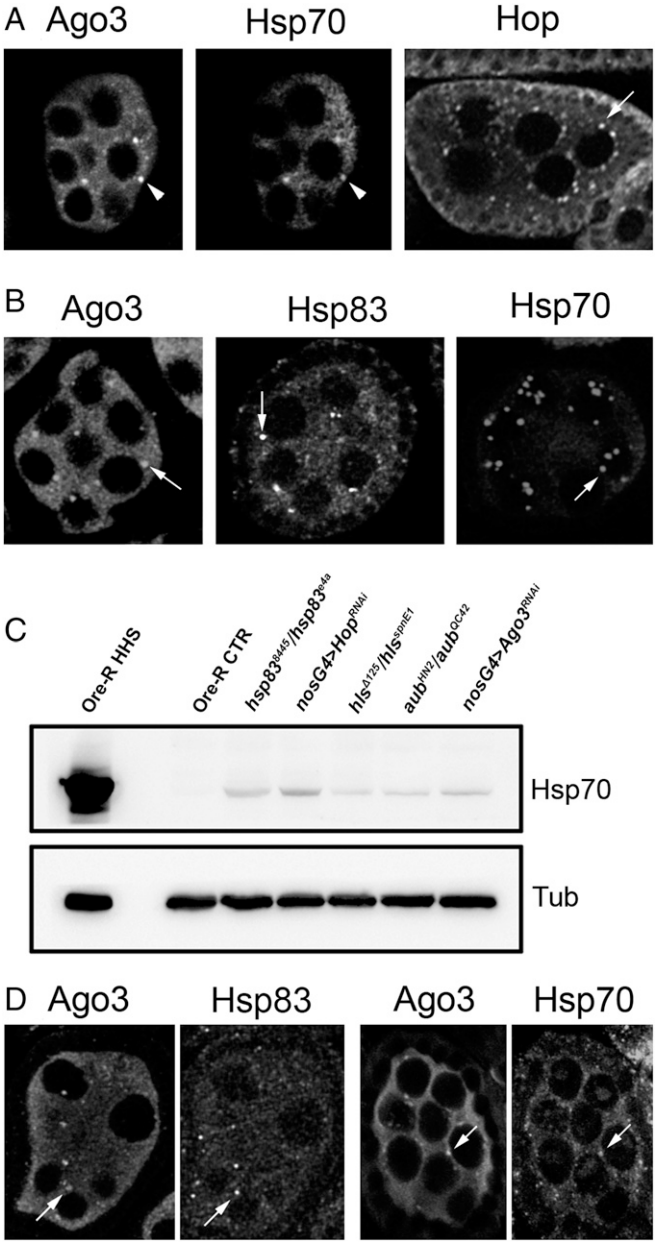

Fig. 6. Hsp70 activation and colocalization with piRNA components to cytoplasmic bodies. Functional inactivation of chaperones induces Hsp70 expression at normal temperatures, and it colocalizes with Ago3 and the other chaperones to cytoplasmic bodies outside the nuage. (*A*) Ovaries from Hsp83 mutants ($hsp83^{8445}/hsp83^{e4A}$) showing cytoplasmic bodies outside the nuage (see arrows for examples) in which Ago3 colocalizes with Hop and Hsp70 (see arrowheads for an example). (*B*) Hop-depleted ovaries [nosG4 > $Hop^{RNAi\ (34002)}$] showing Ago3 and Hsp83 in cytoplasmic bodies with Hsp70 (arrows for examples). (*C*) The quantitative reduction of chaperones Hsp83 or Hop, or other factors involved in piRNA biogenesis such as Spindle-E ($hls^{\Delta 125}/hls^{spnE1}$), Ago3 (nosG4 > $Ago3^{RNAi}$), and Aubergine (aub^{HN2}/aub^{QC42}) induces activation of Hsp70 at normal temperature. (*D*) The expression of UAS-Hsp70 transgenic construct driven by nos-G4 at normal temperatures induces the formation of cytoplasmic bodies containing Ago3 and Hsp83 (arrows for examples). Images in *A*, *B*, and *D* were acquired using a 40× magnification.

Drosophila, Hsp70 is induced by heat shock and establishes an interaction with Hsp90 which is mediated by the cochaperone Hop (Hsp70/Hsp90 organizing protein). Hsp70 might also interact with other components of the chaperone machinery that are involved in piRNA biogenesis, forming new aggregates that migrate outside the nuage for probable degradation. Removal of chaperone machinery from the nuage would then decrease the efficacy of TE repression.

To get insight into the potential role of Hsp70 in the impairment of the piRNA pathway, we analyzed the relationship of Hsp70 with the Hsc70/Hsp90 machinery after heat shock in ovaries by immunostaining and immunoprecipitation experiments using specific antibodies. As shown in Fig. 5*A*, after heat shock, induced Hsp70 localizes to the nuage. Furthermore, Hsp70 coimmunoprecipitates with Hop, Hsc70-4, Hsp83, Ago3, and Aub following stress, but not with dFKBP59, which lacks a physical interaction with Ago3 (Fig. 5*B*). These results strongly suggest that all these factors physically interact in the nuage. To follow the fate of the interaction among these factors, we examined the localization of Hsp83 and Hsp70 at different times during recovery from heat stress on cytological preparations of ovaries. In control ovaries, Hsp83 shows a broad perinuclear distribution characteristic of the nuage. One day after heat shock, Hsp83 and Hsp70 start to colocalize to a small number of cytoplasmic foci, many of which are adjacent to the nucleus. These brightly stained foci are clearly distinct from the mostly uniform perinuclear distribution that Hsp83 showed prior to heat shock. After 2 d, Hsp83 is present primarily in these cytoplasmic foci, where it colocalizes with Hsp70; after 3 d, these foci, and Hsp70, are greatly reduced; after 7 d, Hsp83 again exhibits the broad perinuclear distribution it showed prior to heat shock (Fig. 5*C*). These results acquire special significance in light of the fact that the increase of transposable element transcripts is also seen 2 d after recovery from heat shock, when Hsp83 shows the peak of its relocation (Fig. 1*A*).

Immunofluorescence examination of ovaries 1 d after heat shock showed that Ago3 and Hop also localize in cytoplasmic bodies outside the nuage (Fig. 5*D*). This suggests that Hsp83,

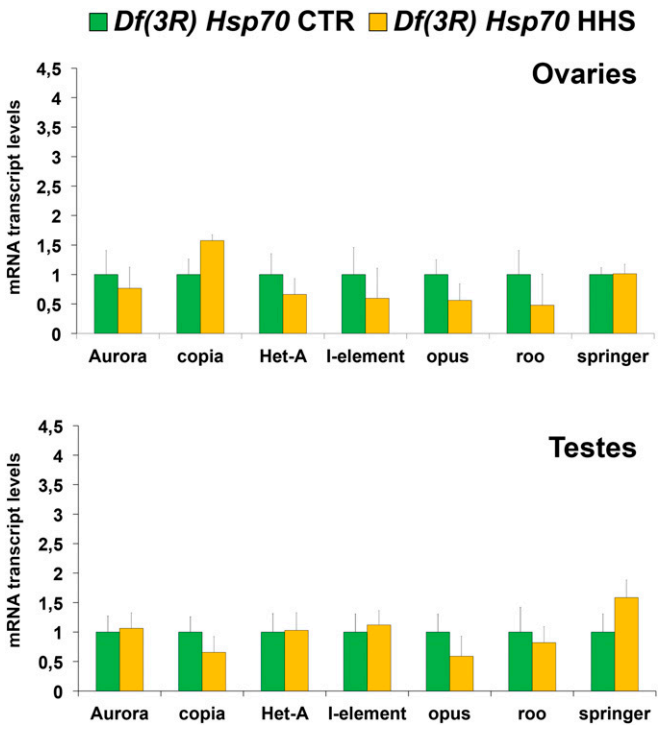

Fig. 7. Hsp70 is required for transposable element derepression after heat shock. TE transcript levels were analyzed in germline tissue of flies carrying a complete deletion of Hsp70 genes cluster [*Df(3R)Hsp70A, Df(3R)Hsp70B*] after 2 d of recovery from HHS. Data are represented as the mean ± SEM of 3 biological replicates quantified by qRT-PCR. Heat shock had no significant effect on TE transcripts.

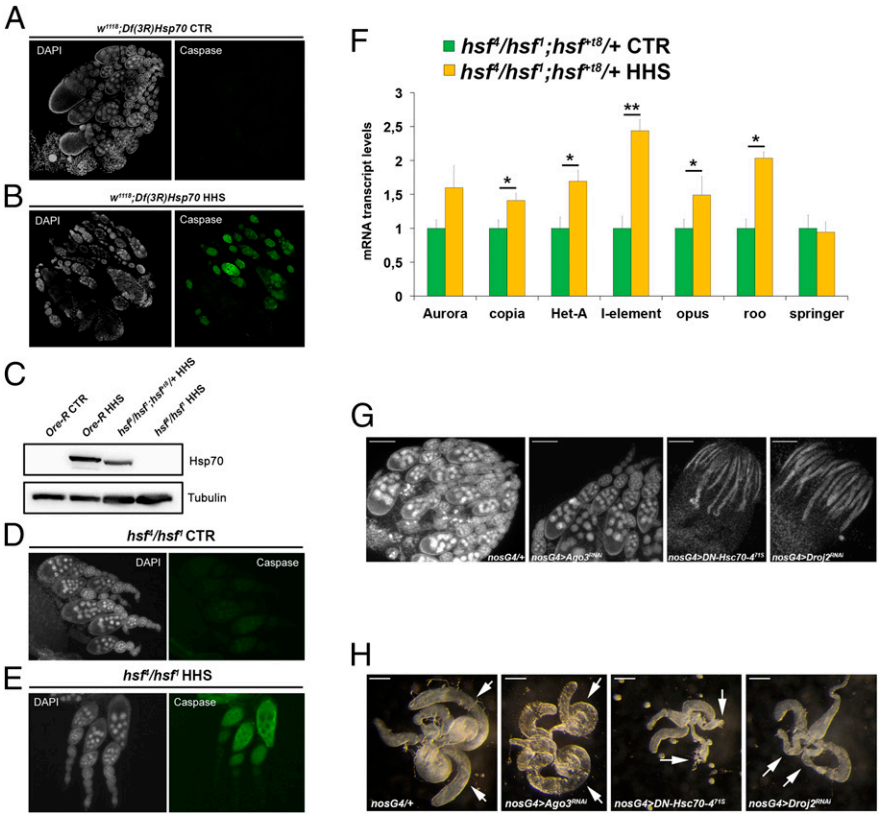

Fig. 8. Heat shock stress causes degeneration of ovaries in flies that lack *Hsp70*. (*A*) The ovaries from Hsp70-deficient flies appear normal without heat shock. (*B*) Twenty-four hours after HHS treatment, the ovaries contain degenerating egg chambers that are heavily stained by antibody against cleaved Caspase-3 (in green). (*C*) Western blot analysis shows that Hsp70 is not induced by heat shock in ovaries of females lacking the Hsf. Hsp70 can be induced by heat shock in *hsf* mutant females carrying a transgenic wild-type copy (hsf^{+t8}) of *hsf* gene. (*D*) No degeneration is observed without heat shock in ovaries from *hsf* transheterozygous mutants (hsf^{4}/hsf^{1}), while (*E*) strong degeneration 24 h after heat shock is clearly visible as indicated by Caspase-3 staining. (*F*) In ovaries from *hsf* mutant females that also carry a transgenic wild-type copy of *hsf,* an increased amount of TE transcript is detectable after heat shock; results are presented as mean ± SEM (*$P < 0.05$; **$P < 0.01$). (*G*) *Hsc70-4* (*nosG4 > DN-Hsc70-4*^{715}) and *Droj2* (*nosG4 > Droj2*^{RNAi}) mutant females show DAPI-stained ovaries with empty germaria arrested in stage 2, while *Ago3* (*nosG4 > Ago3*^{RNAi}) mutant females show normal ovaries. (Scale bar, 100 μm.) (*H*) Bright-field images of freshly dissected whole testes from *Hsc70-4*, *Droj2*, and *Ago3* mutant adult males show testes (arrows) that are abnormal compared to those of wild-type males and lack spermatocytes. (Scale bar, 200 μm.) Ovary images were acquired using a 10× objective. Whole testes were photographed using a stereomicroscope equipped with a NIKON D5000 camera (16× magnification).

Hsp70, Hop, and Ago3 colocalize to lysosomes, probably for degradation. To test this, we analyzed ovary preparations obtained after 1 d of recovery from heat shock, using LysoTracker, a highly specific lysosomal marker, and immunofluorescence. We observed that all 4 proteins localized to lysosomes (Fig. 5*D*), confirming that these factors are carried to lysosomes for degradation. At the same time point, we also found that most nuage components were highly enriched in the insoluble fractions of ovary cell lysates, consistent with their occurrence in large aggregates. Hop and Hsp70 signals were still more intense in the soluble fraction (Fig. 5*E*).

To understand whether the cytoplasmic bodies observed in ovaries of stressed flies are specifically related to the dysfunction of Hsc70–Hsp90 machinery in the piRNA pathway, we also looked for the presence of these aggregates in Hsp83- or Hop-depleted ovaries in the absence of stress (Fig. 6 *A* and *B*) We observed numerous discrete cytoplasmic bodies where Ago3 localizes with Hop (Fig. 6*A*) or Hsp83 (Fig. 6*B*). Intriguingly, when either Hsp83 or Hop is silenced, Hsp70 is also expressed and localizes to these cytoplasmic bodies. Furthermore, at normal temperature, Hsp70 was expressed in ovaries of flies in which other RISC components were silenced (Fig. 6*C*). These data strongly suggest that the formation of cytoplasmic bodies observed after stress or in chaperone mutants could be related to the presence of Hsp70. We confirmed this by using transgenic flies harboring an extra copy of *Hsp70Ab* gene under the

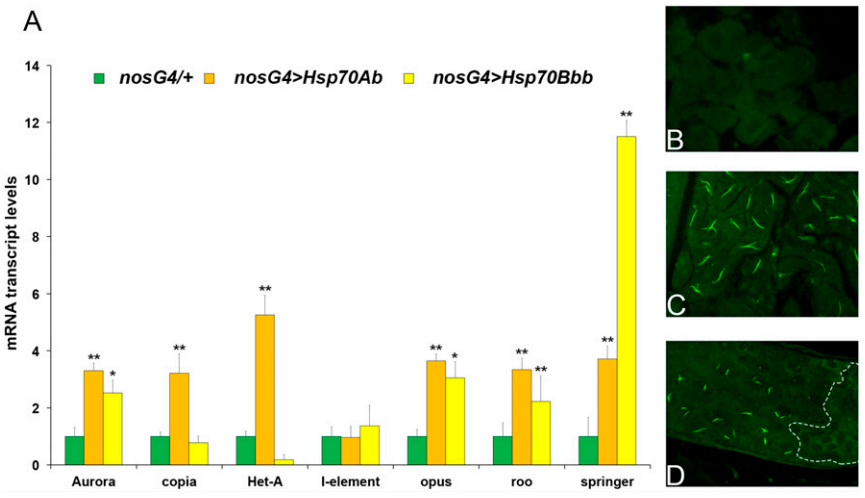

Fig. 9. Hsp70 transgene expression driven by *nosG4* is able to induce TE and *Stellate (Ste)* activation in absence of stress. (*A*) qRT-PCR analysis shows a significant increase of TE transcripts in ovaries from transgenic females expressing 2 genes of the *Hsp70* genes cluster (Hsp70Ab and Hsp70Bbb). Results presented as mean ± SEM (*$P < 0.05$; **$P < 0.01$). (*B*) Testes from control males stained by a specific anti-Stellate antibody. No signal is evident. (*C* and *D*) Testes from males expressing the (*C*) *Hsp70Ab* or (*D*) *Hsp70Bbb* transgenes show the presence of crystalline aggregates in spermatocytes after staining by anti-Ste antibody. The dashed line delimits spermatogonia. Images of squashed testes were acquired using a 40× magnification.

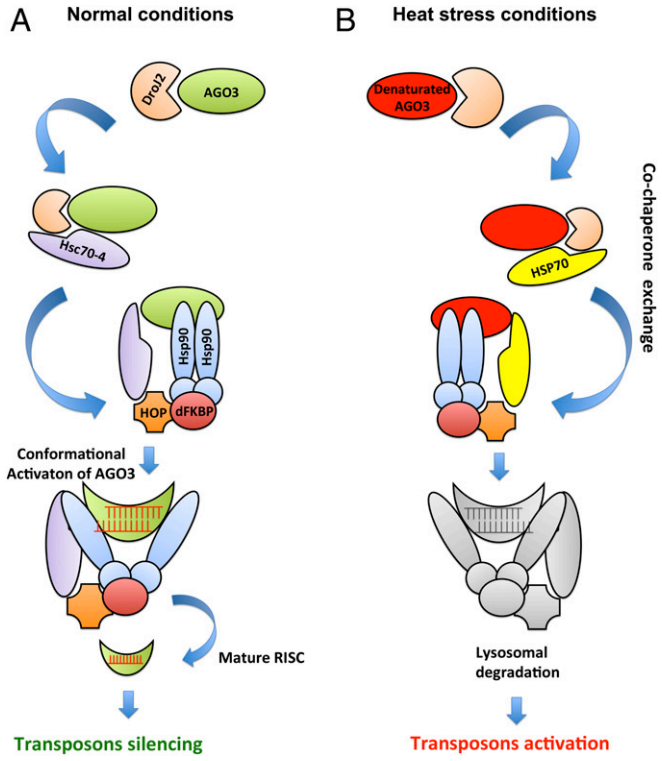

Fig. 10. Model of cochaperone complex involved in piRNA biogenesis and TE silencing. (*A*) In normal conditions, the chaperone complex and Ago3 protein cooperate in mature RISC formation. Since, in normal conditions, Hsp70 gene cluster is completely repressed, the model is applicable also in case of flies carrying a complete deletion of such a cluster. (*B*) During heat shock, the chaperone–Ago3 complex is disrupted, and all of the factors are targeted to the lysosomes for degradation by the involvement of inducible Hsp70 chaperone. As a consequence, TEs are activated.

control of upstream activation sequences (UAS) (33). When transgenic Hsp70Ab was ectopically expressed in the germline by nanos-Gal4 at normal temperature, we saw the formation of cytoplasmic bodies containing Ago3, Hsp83, and Hsp70 in ovaries (Fig. 6*D*).

To assess the functional relevance of Hsp70 in transposable element activation after stress, we examined TE activity in stressed flies carrying a complete deletion of the *Hsp70* gene cluster (34). We found that, in testes or ovaries from heat-shocked flies lacking the Hsp70 cluster, there was negligible increase of TE transcripts compared with that seen in heat-shocked wild-type flies (compare Figs. 1*A* and 7). This suggests an active role of Hsp70 in stress induction of TE expression. However, this conclusion should be qualified with respect to ovaries, because, while the testes of heat-shocked males appear normal (*SI Appendix*, Fig. S2), the ovaries from heat-shocked females lacking the Hsp70 cluster exhibit an early strong degeneration as confirmed by caspase-3 activation (Fig. 8 *A* and *B*). We also found that flies mutant for the heat shock factor (Hsf), a transcription factor necessary for Hsp70 activation after stress (35–38), showed ovary degeneration after heat shock (Fig. 8 *C*–*E*). However, we did observe a significant increase of TE transcripts after heat shock in ovaries of *hsf* mutant females expressing an hsf^{+t8} transgene that permits the Hsp70 induction (39) (Fig. 8*F*). Thus, Hsp70 is required not only for TE expression after stress but also to avoid ovary degeneration. A point of interest is that, at normal temperature, silencing of Hsc70-4 or Droj2 also induces strong degeneration of ovaries (Fig. 8*G*) and testes (Fig. 8*H*). We conclude that Hsc70-4 and Droj2 play a central role in ovary and testis development at normal temperature, while Hsp70 is necessary for the maintenance of ovaries after heat shock. Since we also observed that the silencing of Ago3 has no effect on the development of ovaries or testes (Fig. 8 *G* and *H*), the perturbation of Hsc70-4 or Droj2 produces in both male and female gonads 2 unrelated effects—namely, strong degeneration and TE activation.

The experiments described above show that Hsp70 is necessary for transposable element derepression after heat shock. To determine whether expression of Hsp70 is sufficient to derepress TE, we analyzed the amount of TE transcript in the germ lines of nonstressed transgenic flies expressing 2 different Hsp70 transgenes under UAS control. We found (Fig. 9*A* and *SI Appendix*, Fig. S3) a significant activation of different transposable elements in ovaries. Additionally, in testes, we observed numerous Ste crystalline aggregates in spermatocytes, indicating derepression of the *Stellate* locus (Fig. 9 *B*–*D*). These results demonstrate that ectopically expressed Hsp70 at normal temperature is sufficient to derepress TEs. Our results suggest that Hsp70 is the main regulator of transposable element activity after stress. We propose that the increase of TE expression after heat shock results from the active role of Hsp70 in moving factors critical for piRNA-mediated repression from the nuage to lysosomes, preventing their function in transposable element repression (Fig. 10).

Protection against Stress and Induction of Genetic Variability: 2 Sides of the Same Hsp70 Coin. In conclusion, our results demonstrate that heat shock stress increases the expression of TEs mainly at posttranscriptional level by affecting piRNA biogenesis through the action of the inducible Hsp70 chaperone. Since several types of biotic and abiotic stress are able to trigger or reinforce Hsp70 activity in both plants and animals (40), we think that such a mechanism contains relevant evolutionary implications. In the presence of drastic environmental changes, Hsp70 could play a key role not only in protecting the survival of individuals (34) but also in increasing the frequency of mutations in their germ cells. This, in turn, should translate to an increase in genetic variation in a population, thus potentiating environmental adaptability and evolvability (41).

Materials and Methods

A detailed description of *Drosophila* strains, HHS treatment, coimmunoprecipitation assays, Western blot analyses, preparation of insoluble and soluble fractions, ChIP, ChIP-seq analysis, qRT-PCR, and *Drosophila* ovaries and testes immunofluorescence can be found in *SI Appendix, Supplementary Information Materials and Methods*.

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