Regulation of Heat Shock Gene Expression by RNA Polymerase II Elongation Factor, Elongin A*

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The elongation stage of transcription by RNA polymerase II (Pol II) has emerged as an essential regulated step. Elongin A (EloA) is the largest subunit of the Elongin complex that can increase the catalytic rate of mRNA synthesis by Pol II. We recently demonstrated that the Elongin A homologue in Drosophila, dEloA, is essential and has properties consistent with those of a Pol II elongation factor in vitro. The goal of this study was to test whether dEloA is required for heat shock gene transcription, since heat shock gene expression is thought to be controlled at the level of Pol II elongation. Here, we demonstrate that dEloA is rapidly recruited to heat shock loci with Pol II in response to heat shock. Furthermore, through the use of RNA interference in vivo, we show that dEloA is required for the proper expression of one of these genes, HSP70, and that its requirement for heat shock gene expression is exerted after the initiation of transcription at heat shock loci. Our data represent the first demonstration of an essential role for an RNA polymerase II elongation factor in the regulation of heat shock gene expression in an animal model.

The Elongin complex was originally purified based on its ability to stimulate Pol II1 elongation in an in vitro transcription assay (1). Elongin is a heterotrimeric complex, consisting of A, B, and C subunits, which are ~110, 18, and 15 kilodaltons in size, respectively. Elongin A is the transcriptionally active subunit of the complex, and the addition of this protein alone to in vitro transcription reactions is sufficient to stimulate transcription elongation by Pol II (1–3). In the absence of Elongin A, Elongin BC is unable to enhance Pol II processivity.

A number of Elongin A family members have been identified. They include three mammalian homologues (Elongin A, A2, and A3) and an Elongin A homologue in Caenorhabditis elegans (1, 4, 5). Each of these proteins is capable of stimulating Pol II elongation in vitro and interacting with the Elongin BC complex.

Recently, we identified and characterized the Drosophila melanogaster homologue of Elongin A, named dEloA (14). Like the other Elongin A family members, dEloA stimulates Pol II elongation activity in vitro. Additionally, dEloA is concentrated at transcriptionally active loci on Drosophila polytene chromosomes, and it colocalizes extensively with transcriptionally active isoforms of Pol II. Consistent with this observation, dEloA interacts with Pol II in Drosophila extracts. Collectively, these observations are consistent with the hypothesis that the Elongin proteins are Pol II-associated factors in vivo.

Heat shock gene expression is thought to be controlled at the level of promoter clearance (16). Therefore, the expression of heat shock genes should be particularly sensitive to the cellular level of Pol II elongation factors. A variety of Pol II elongation factors are recruited to sites of heat shock gene expression during heat shock (6–9, 15). However, very little is known about the role(s) of these factors in the regulation of heat shock gene transcription. To examine the role of EloA in the regulation of specific gene expression, we tested the requirement for this Pol II elongation factor during the heat shock response in an animal model system. In this study, we show that dEloA is rapidly recruited to heat shock loci in response to heat shock and strongly colocalizes with phosphorylated Pol II at these sites. Using RNA interference (RNAi)-mediated knockdown of dEloA, we demonstrate that organisms with reduced levels of dEloA exhibit a marked reduction in HSP70 mRNA levels following heat shock. The data presented here provide strong evidence that dEloA is required for efficient expression of the HSP70 genes in vivo and are the first demonstration that a member of this class of elongation factors plays a critical role in heat shock gene expression in an animal model system.

MATERIALS AND METHODS

Characterization of dEloA Polyclonal Antiserum—Recombinant dEloA was prepared as described previously (14) and was used for Western blot analysis with either preimmune serum (1:5000 dilution in 1× Tris-buffered saline-Tween) or dEloA polyclonal antiserum (same dilution). Blots were then incubated with anti-rabbit secondary antibody with a peroxidase conjugate (Sigma) and developed via chemiluminescent detection (Western Lightning, PerkinElmer Life Sciences).

Immunofluorescence Staining—Salivary glands from late third-instar larvae were fixed for 30 s in 2% formaldehyde in gland buffer, then in 45% acetic acid 2% formaldehyde for 3 min as described (9), before storage in 67% glycerol/33% PBS at −20 °C. For optimal staining, dEloA polyclonal antibody (1:300 dilution) was incubated with polytene chromosomes overnight at 4 °C. Pol II monoclonal antibodies (H5 and H14, Covance) were used at 1:1000 dilution and incubated overnight at 4 °C. Appropriate secondary antibodies (The Jackson Laboratory, anti-rabbit–Alexa 488 conjugate and anti-mouse–Cy3 conjugate) were used at 1:1000 dilution. Fluorescence detection was by epifluorescence using an Olympus BX60 fluorescence microscope with an NB barrier filter for fluorescein and Cy3 detection and a WG barrier filter for rhodamine and Cy3. Images were recorded with a SPOT CCD camera (Diagnostic

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1 The abbreviations used are: Pol II, polymerase II; RNAi, RNA interference; MOPS, 4-morpholinopropanesulfonic acid.

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Isoforms of RNA Pol II that are phosphorylated at Ser2 (P) and Ser5 (P) are concentrated at heat shock puff sites on the right arm of chromosome 3 (Fig. 2D). Using phase-contrast microscopy, we mapped the presence of dEloA on heat shock loci 87A and 87C before (phase contrast shown in left panel) and after (phase contrast shown in right panel) heat shock induction.

RESULTS AND DISCUSSION

Elongin A Associates with Heat Shock Puff Sites in Response to Environmental Stress—In Drosophila, heat shock results in a dramatic relocalization of Pol II from a widely distributed collection of transcriptionally active loci to a select few vigorously transcribed heat shock gene loci (11). Previous reports have demonstrated that several Drosophila elongation factors, including P-TEFb, Spt4 and -5, Spt6, FACT, and dELL, are mobilized to heat shock loci (6–9). Using a specific anti-dEloA polyclonal antibody that we have developed (Fig. 1), we compared the immunolocalization pattern of dEloA under heat shock and non-heat shock conditions. Prior to heat shock, dEloA is widely distributed on polytene chromosomes. Following a 20-min heat shock at 37 °C, dEloA is rapidly and dramatically relocalized to a small number of induced puff sites (compare Fig. 2, A and B). Using phase-contrast microscopy, we mapped these loci and identified them as heat shock puffs (Fig. 2C). To determine that dEloA is not associated with heat shock loci prior to heat shock, polytene chromosomes isolated from animals before and after heat shock were mapped for the recruitment of dEloA. As shown in Fig. 2, D and E, dEloA is recruited to 87A and 87C loci upon heat shock and is not found to be associated with these loci before heat shock induction.

dEloA Colocalizes with Phosphorylated Pol II at Heat Shock Puffs—Isosforms of RNA Pol II that are phosphorylated at Ser2 of the large subunit heptapeptide repeat are thought to be the elongating isoforms of polymerase in vivo (13). To test whether dEloA is colocalized with elongating Pol II, we compared the

FIG. 1. Characterization of dEloA polyclonal antibody. A, Western blot analysis of recombinant dEloA (rdeLoA) protein with preimmune serum (left panel) and dEloA polyclonal antibody (right panel). The amount of protein loaded in each lane is indicated. dEloA polyclonal yields a robust signal at the expected molecular weight, while preimmune serum yields a background signal. Both preimmune serum and dEloA antiserum were used at a 1:5000 dilution. B, Western blot analysis of total protein extracts from Drosophila third-instar larvae with preimmune serum and dEloA polyclonal antibody. The amount of total protein loaded is indicated for each lane.
dEloA is required for heat shock gene expression—Given the strong recruitment of dEloA to heat shock loci upon induction, we examined the potential requirement for dEloA in the transcription of heat shock genes following heat shock. We utilized RNAi to reduce the levels of dEloA in developing flies. As shown in Fig. 4A, RNAi targeting of dEloA transcript dramatically reduces dEloA levels on polytene chromosomes. In contrast, the levels of phosphorylated Pol II appear very similar to wild-type levels, suggesting that loss of dEloA does not affect global RNA Pol II protein levels, phosphorylation levels, or distribution. Also, the level of dEloA protein tested by Western analysis in the same animals indicated that the induction of dEloA RNAi results in the reduction of dEloA protein level (Fig. 4B).

To further characterize the role of dEloA in heat shock gene expression, adult males carrying an X-linked Sym-pUAST-dEloA transgene were crossed to virgin females carrying the daGal4 driver. In the progeny obtained from this cross, the RNAi was activated against dEloA only in females; therefore, the majority of females are unable to survive to adulthood, because the expression of Elongin A is required for viability (14). We tested whether the expression of HSP70 in response to heat-shock differs between male and female progeny from this cross. As predicted, HSP70 transcription in heat-shocked females with RNAi-mediated depletion of dEloA is markedly reduced in comparison to their heat-shocked male siblings lacking the Sym-pUAST-dEloA transgene necessary for RNAi (Fig. 4C).

These data, taken together with the localization studies, strongly suggest that dEloA is required for efficient expression of HSP70 during heat shock response. While these observations are consistent with previous reports that mammalian Elongin A is limiting for the expression of specific genes (12), our data also suggest a direct effect of dEloA levels on the efficiency of HSP70 expression, since dEloA is specifically recruited to the loci where transcription of HSP70 occurs during heat shock.

The exact role of Elongin A in the regulation of transcriptional elongation in vivo has remained elusive. In vitro transcriptional studies have suggested that Elongin functions by increasing the catalytic rate of transcription elongation and altering the $V_{\text{max}}$ of transcription (14). This transcriptional elongation effect is exerted on Pol II after the synthesis of a message of about 10–20 nucleotides in length (14). Our in vivo studies support a role for this elongation factor with the elongating form of Pol II. To begin to understand the function of Elongin A in the transcription cycle in an in vivo model system, we performed immunostaining to detect Serine phosphorylated Pol II on heat-shocked chromosomes from wild-type control
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In this paper, we have demonstrated that (a) the Pol II elongation factor dEloA is rapidly recruited to heat shock puff sites with Pol II in response to heat shock; (b) reduction of dEloA levels via RNAi results in reduction of heat shock gene expression, indicating the requirement of dEloA for proper heat shock gene expression in a developing organism; and (c) in vivo, dEloA plays a role in the regulation of heat shock gene expression after initiation of transcription at the heat shock loci. Collectively, our data demonstrate, for the first time, the requirement of any Pol II elongation factor for proper heat shock gene expression in a developing organism.

Future comparative studies on the role of Elongin A and other Pol II elongation factors identified at the heat shock puff sites should clarify the specific in vivo role of each of these factors in proper heat shock gene expression and why they are recruited to heat shock loci upon stimulation. Such studies will also determine whether Pol II elongation factors can also be involved in processes such as transcriptional termination, RNA processing, and transport in higher metazoan.

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