Developmentally programmed cell death is regulated by a balance between pro- and anti-death signaling. During *Drosophila* metamorphosis, the removal of larval tissues is dependent on the steroid hormone ecdysone, which controls the levels of pro- and anti-death molecules. Ecdysone binds to its heterodimeric receptor ecdysone receptor/ultraspiracle to mediate transcription of primary response genes. Here we show that CARMER, an arginine-histone methyltransferase, is critical in coordinating ecdysone-induced expression of *Drosophila* cell death genes. Ablation of CARMER blocks ecdysone-induced cell death in *Drosophila* cells, but not apoptosis induced by cell stress. We demonstrate that CARMER associates with the ecdysone receptor complex and modulates the ecdysone-induced transcription of a number of apoptotic genes. Thus, the chromatin-modifying protein, CARMER, modulates cell death by controlling the hormone-dependent expression of the core cell death machinery.

The members of the nuclear hormone receptor (NHR) family bind their cognate ligands to regulate diverse physiological processes by modulating transcription of a distinct array of genes (1). NHRs act via recruiting coactivators. In mammals, the coactivator-associated arginine methyltransferase 1 (CARM1) binds the p160 family of steroid receptor coactivators to enhance transcriptional activation by nuclear receptors (2, 3). CARM1 can methylate histone H3, and a mutation in the S-adenosylmethionine binding domain of CARM1 substantially reduces both methyltransferase and coactivator activities (4). Mouse embryos with disrupted *carm1* are small in size, die perinatally, and show defective estrogen-responsive gene expression (5), suggesting that CARM1 is essential for developmental processes and NHR-mediated transcriptional activation.

In *Drosophila melanogaster* the steroid hormone ecdysone binds its heterodimeric receptor EcR/Usp and regulates molting and metamorphosis (6). Ecdysone also regulates programmed cell death (PCD) during metamorphosis (7–9). An ecdysone pulse toward the end of the larval stage signals pro- and metamorphosis (6). Ecdysone also regulates pro-molt-mediated transcriptional activation.

EXPERIMENTAL PROCEDURES

**CARMER Expression Constructs and GST Fusion Proteins—**The carmer cDNA was cloned by RT-PCR using *Drosophila* embryo mRNA. A methyltransferase-deficient CARMER mutant was generated by replacing the conserved Val-Leu-Asp motif (Fig. 1A) with Ala residues. The open reading frames of wild-type and mutant carmer were amplified with BamHI/EcoRI sites in the primers and cloned into the insect expression vector pIE1-4 (Novagen) and the GST fusion vector pGEX2TK (Amersham Biosciences). For the production of GST fusion proteins, the pGEX2TK construct was transformed into *Escherichia coli* BL21 Star and GST fusion proteins purified by affinity chromatography.

**In Vitro Methylation Assays—**Methylation assays were performed by incubating 3 μg of calf thymus histones (Roche Applied Science) with either 1 μg of purified GST, GST-CARMER, or GST-CARMER mutant in the presence of 7 μM S-adenosyl-l-3H[methyl]-3H[methionine in 30 μl of HMT buffer (20 mM Tris-HCl, pH 8.0, 200 mM NaCl, 0.4 mM EDTA) for 90 min (13). A portion of the mouse voltage-gated sodium channel 1 fused to GST (GST-Na,1,1) (provided by A. Fotia) was used as an unrelated protein control in methylation studies. Reactions were stopped by addition of SDS loading buffer and analyzed by 15% SDS-PAGE. Samples were electrophoresed in duplicate. One gel was stained with Coomassie Blue to localize the positions of various histones, and the other was autoradiographed. Gels were fixed, soaked in Amplify (Amersham Biosciences) for 20 min, dried, and exposed to Kodak film overnight at −70 °C. The autoradiograph was overlaid with the Coomassie-stained gel to identify the methylated histone species. **Cells and Caspase Assays—**2.5 mbn cells (kind gift from Dr A. Dorn) (14) were grown in Schneider’s medium supplemented with 10% fetal bovine serum. Cells, 1.5 × 10^6/well, were seeded in 6-well plates in triplicate. Where necessary, ecdysone (10 μg/ml) (Sigma) was added for the desired times. Cell viability was assessed by trypan blue exclusion. Cell lysates were prepared by freeze/thawing and clarified by centrifugation at 13,000 rpm for 5 min. 50 μg of lysate was assayed for caspase activity using Ac-Asp-Glu-Val-Asp-7-amino-4-methylcoumaride (DEVD-amino-
RNA Extraction from Cells and Flies and Semiquantitative RT-PCR—Total RNA was extracted from cells, dissected tissues, and whole animals using Trizol reagent (Invitrogen) essentially as described (12, 17). Up to 2 μg of total RNA was used as template for cDNA synthesis in a 15-μl reaction with 200 ng of oligo(dT)18 primer using a First Strand Synthesis Kit (Amersham Biosciences) according to the manufacturer’s protocol. By using 2 μl of cDNA template, PCR amplification was performed using appropriate primers in a 50-μl reaction employing 27 cycles. Drosophila rp49 was used as a control. 5 μl of PCR was electrophoresed on a 1.5% agarose gel for analysis. Where indicated, band intensities were determined using ImageQuant software (Amersham Biosciences).

Immunoprecipitation—3 × 10^5 h2mbn cells were transfected with either pE1-4 vector alone, pE1-4 vector containing the FLAG-tagged Broad Complex Z2 isoform (BRC-Z2-FLAG), or pE1-carmer-FLAG. BRC-Z2 is an ecdysone-induced transcription factor with a size similar to carmer (12). BRC-Z2 does not bind EcR/Usp. After 48 h, cells were treated with 10 μM edeysone for 6 h where indicated and harvested in lysis buffer (50 mM Tris-HCl, pH 8.0, 120 mM NaCl, 0.1% Nonidet P-40, and complete protease inhibitor mixture). Cell lysates were prepared by freeze/thawing and clarified by centrifugation. 40 μl of anti-FLAG M2 affinity gel (Sigma) was added to the lysates and allowed to rotate on a wheel overnight at 4 °C. Immunoprecipitates were washed three times in lysis buffer with 2% Tween 20 and analyzed by immunoblotting using the anti-FLAG (Sigma) or anti-EcRB1 antibody (AD4.4, from the Hybridoma Bank). Bands were visualized by ECL (Amersham Biosciences).

Transient Transfections and Apoptosis Assays—2 × 10^5 h2mbn cells were transfected in triplicate with 1 μg of hsp EcR-luciferase vector and either 800 ng pE1-4 vector or 400–800 ng of pE1-carmer or pE1-carmer mutant expression vector. Where needed, pE1-4 was added to make up the DNA to 800 ng. Cells were allowed to recover for 24 h and then treated with 10 μM edeysone for 12 h. Cells were lysed in 100 mM phosphate buffer by freeze-thawing. Equal amounts of protein lysate were used in luciferase assays as described previously (12). For cell death experiments, 2 × 10^5 h2mbn cells were cotransfected with either 2 μg of pE1-4 vector, pE1-carmer, or pE1-carmer mutant together with 500 ng of pE1-LacZ. Cells were allowed to recover for 24 h and then treated with 10 μM edeysone for 24 and 48 h. Cell survival/apoptosis was scored as described previously (12, 18).

RNA Interference (RNAi)—A region of carmer open reading frame

4-methylcoumaride) (Enzyme System Products, CA) substrate as described previously (15, 16).

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RNA Interference (RNAi)—A region of carmer open reading frame

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not treated (none) or treated with 10 μg of agarose gel. dsRNA (10 μg) was annealed to generate dsRNA, and the quality of RNA was analyzed on an agarose gel. Sense and antisense strands were used (16). Plasmids were linearized and RNA synthesized using T7 and SP6 Megascript kits (Ambion). Sense and antisense strands were used (16). Plasmids were linearized and RNA synthesized using T7 and SP6 Megascript kits (Ambion). Sense and antisense strands were used (16). Plasmids were linearized and RNA synthesized using T7 and SP6 Megascript kits (Ambion). Sense and antisense strands were used (16). Plasmids were linearized and RNA synthesized using T7 and SP6 Megascript kits (Ambion). Sense and antisense strands were used (16).

RESULTS AND DISCUSSION

A CARM1 Homologue in Drosophila—The role of coactivators in mediating EcR-induced transcription in Drosophila is not well understood. To understand how ecdysone temporally and spatially regulates gene expression, it is pivotal to identify cofactors that are recruited by the EcR. Given the remarkable conservation in nuclear hormone receptors and apoptotic machinery between invertebrates and mammals, we set out to identify the arginine-histone methyltransferase CARM1 homologue in Drosophila and study its role in PCD. Blast analysis of the Drosophila database using murine CARM1 retrieved a protein (CG5358) with 65% homology to CARM1 and 62% homology to human PRMT4 (Fig. 1A). Although there are a number of other potential protein-arginine methyltransferases in the fly genome (results not shown), CG5358 shares the highest degree of identity with CARM1/PRMT4. Given the role of this protein in EcR/Usp-mediated gene expression, we have named it CARMER (coactivator Arg methyltransferase for EcR/Usp).

The role of coactivators in mediating EcR-induced transcription in Drosophila is not well understood. To understand how ecdysone temporally and spatially regulates gene expression, it is pivotal to identify cofactors that are recruited by the EcR. Given the remarkable conservation in nuclear hormone receptors and apoptotic machinery between invertebrates and mammals, we set out to identify the arginine-histone methyltransferase CARM1 homologue in Drosophila and study its role in PCD. Blast analysis of the Drosophila database using murine CARM1 retrieved a protein (CG5358) with 65% homology to CARM1 and 62% homology to human PRMT4 (Fig. 1A). Although there are a number of other potential protein-arginine methyltransferases in the fly genome (results not shown), CG5358 shares the highest degree of identity with CARM1/PRMT4. Given the role of this protein in EcR/Usp-mediated gene expression, we have named it CARMER (coactivator Arg methyltransferase for EcR/Usp). CARMER is a 530-residue protein containing a conserved central region that harbors the putative binding site for the p160 coactivators and the adomethyl-binding pocket. To assess the catalytic function of CARMER, we performed in vitro histone methylation assays by using either GST-fused purified recombinant CARMER protein or a mutant version where conserved

FIG. 2. CARMER interacts with the ecdysone receptor and potentiates transcription and ecdysone-mediated PCD. A, l(2)mbn cells were transfected with either pE1-4 vector alone (vector), pIE-BRC-Z2-FLAG (BRC-Z2), or pIE-carmer-FLAG (carmer) and either not treated (−) or treated with 10 μg ecdysone (Ecd) (+) for 6 h. Lysates were immunoblotted (WB) with EcR-B1 antibody or anti-FLAG antibody. Where indicated, immunoprecipitations (IP) were carried out using anti-FLAG antibody prior to Western blot with murine EcR-B1 antibody. EcR-B1, the FLAG-tagged CARMER, and BRC-Z2 proteins are indicated. B, l(2)mbn cells were transfected in triplicate with 1 μg of hsp68 EcR-luciferase vector and either 800 ng of pIE empty vector, 400 or 800 ng of pIE-carmer, or pIE-carmer mutant (mutant carmer) expression vector. Where necessary, the amount of DNA was equalized with pIE vector. Cells were treated with ecdysone (+) or left untreated (−), as indicated. Lysates were assayed for luciferase activity which is represented as CPM. C, l(2)mbn cells were transfected in triplicate with either 2 μg of pE1-4 empty vector (vector), pIE-carmer (carmer), or pIE-carmer mutant (Mutant carmer) together with 500 ng of pIE-LacZ. Cells were treated with ecdysone for the indicated time. The β-galactosidase-positive cells were counted as live transfected cells, and the untreated cells for each transfectant were graphed as 100%. Error bars represent S.D.

FIG. 3. Knockdown of carmer inhibits ecdysone-mediated PCD and caspase activation. A, l(2)mbn cells were treated in triplicate with dsRNA to carmer, murine N4WBPA5A (control), or without dsRNA (none). Cells were either left untreated (−) or treated (+) with ecdysone (Ecd) for 24 h where indicated. Cell survival was scored, and untreated cells were graphed as 100%. Error bars represent S.D. B, cells were treated as in A except cycloheximide (CHX) was used as the apoptotic agent. C, whole cell lysates were prepared from the same cells as in A, and equal amounts of protein were used in caspase assays using DEVD-amino-4-methylcoumaride as the caspase substrate. Assays were performed in triplicate, and error bars represent S.D.

Results and Discussion

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CARMER efficiently methylated histone H3. In longer exposures, some methylation of histone H4 was also seen (data not shown). Mutation of the conserved catalytic residues abolished the ability of CARMER to methylate histones. Histone specificity was demonstrated as a non-related substrate was not methylated. These data indicate that CARMER is an authentic homologue of CARM1.

**CARMER Binds EcR and Augments Ecdysone-mediated Transcription**—To test the ability of CARMER to bind EcR/Usp, FLAG-tagged CARMER was transfected into *Drosophila* l(2)mbn cells that express functional EcR/Usp and treated with ecdysone. FLAG immunoprecipitates were analyzed by SDS-PAGE and blotted with an EcR-B1 isoform-specific antibody. As shown in Fig. 2A, CARMER clearly coimmunoprecipitated with EcR-B1, whereas the ecdysone-inducible transcription factor, BRC-Z2, showed no significant association with EcR-B1 over the vector control background. Similar results were obtained in three independent experiments. We then analyzed whether CARMER could transactivate expression of an EcR/Usp-driven reporter. We transfected l(2)mbn cells with a luciferase reporter driven by the *Drosophila* Hsp90 promoter containing EcR/Usp-binding elements with increasing amounts of CARMER or mutant CARMER. In the presence of ecdysone the reporter was significantly activated (Fig. 2B). Transfection of 400–800 ng of carmer expression vector enhanced both the basal level and ecdysone-induced reporter activity. However, increasing the levels of CARMER did not enhance transcription. Cotransfection of the methylase-deficient mutant CARMER showed reduction, rather than an absence, of enhancement of the reporter activity (Fig. 2B). This suggests that in addition to the methylase activity of CARMER, other functions of the protein may contribute to transcriptional activation. This is supported by the fact that mammalian CARM1 can recruit additional cofactors and stimulate their activities (13, 20).

**CARMER Augments Ecdysone-induced Cell Death**—Given its ability to interact with EcR/Usp and transactivation of ecdysone-mediated transcription, we assessed the role of CARMER in enhancing a biological outcome mediated by ecdysone. The *Drosophila* larval cell line, l(2)mbn, underwent apoptosis in response to ecdysone treatment (12, 14) thus making these cells ideal to assess the role of CARMER in ecdysone-mediated PCD. l(2)mbn cells were transfected with either CARMER or CARMER mutant and treated with ecdysone. As shown in Fig. 2C, ectopic expression of CARMER significantly enhanced ecdysone-mediated cell death, whereas the mutation in the adenosyl-binding pocket of CARMER eliminated this ability. The effects seen in Fig. 2, B and C, suggest that other cofactors (such as P160) may be limiting in these experiments.

**CARMER Is Required for Ecdysone-mediated Cell Death**—To test the *in vivo* role of CARMER in ecdysone-induced cell death, we used RNAi to knock down expression of carmer. Whereas addition of ecdysone to l(2)mbn cells or cells transfected with a control dsRNA resulted in apoptosis, knockdown of carmer resulted in a near complete inhibition of cell death (Fig. 3A). Although CARMER was required for hormone-mediated cell death, carmer RNAi had no effect on cycloheximide-induced apoptosis of l(2)mbn cells (Fig. 3B). These results demonstrate for the first time that ecdysone-mediated apoptosis is dependent on CARMER. We have shown previously that ecdysone-mediated apoptosis is caspase-dependent (12). We therefore examined whether the inhibition of ecdysone-induced cell death in l(2)mbn cells resulting from the knockdown of carmer was due to a decrease in caspase activity. As shown in Fig. 3C, untreated cells had low caspase activity, which was elevated following ecdysone addition. Although the treatment of cells with a control dsRNA showed no significant difference in caspase activation, knockdown of carmer resulted in a near complete inhibition of caspase activation (Fig. 3C).

**CARMER Coordinates EcR-mediated Transcription of Death Genes**—The importance of CARMER in ecdysone-mediated cell death prompted us to investigate the downstream target genes affected in the knockdown experiments. Given the marked inhibition of caspase activity in these experiments, we examined the effect of knocking down carmer on the expression of apoptotic genes. carmer transcript was evident in untreated l(2)mbn cells, and ecdysone treatment had no effect on its levels (Fig. 4), suggesting that CARMER itself is not regulated by ecdysone. Whereas the treatment of cells with a control dsRNA had no effect, dsRNA directed to carmer effectively eliminated most of the carmer transcript (Fig. 4). Among the seven caspases in *Drosophila*, DRONC is the key initiator caspase, whereas DRICE and DCP-1 are important downstream caspases (16, 21). Expression analysis showed that the low basal levels of all three caspase transcripts were up-regulated ~5–6-fold in response to ecdysone treatment for 24 h as assessed by band intensities (Fig. 4). The knockdown of carmer reduced the expression levels of all three caspases in response to ecdysone. Whereas ecdysone-induced drice and dcp-1 up-regulation was almost completely abolished, dronc expression.

**Fig. 4. Knockdown of carmer inhibits ecdysone-mediated transcription of apoptotic genes.** l(2)mbn cells were left untreated (none) or incubated with dsRNA to carmer or murine N4WB5A (control). Cells were either left untreated (−) or treated (+) with ecdysone (EcO) for 24 h where indicated. Total RNA was extracted and analyzed by RT-PCR using gene-specific primers. rpl49 was used as a RNA/cDNA control.

**Fig. 5. carmer is expressed throughout *Drosophila* development.** RNA was isolated from *Drosophila* at different stages of development and analyzed by RT-PCR. A, embryonic stages 0–8 and 8–16 represent hours after egg laying (h AEL). Early pupae (E) correspond to the time of salivary gland histolysis. carmer as well as rpl49 control transcripts are shown. B, salivary glands and midguts were dissected from staged larvae. 128–132 h after egg laying represents early pupal stages where the salivary gland is undergoing histolysis (132 h), and 120 h corresponds to the initiation of midgut histolysis. L, late.
was reduced to ~2-fold (from ~5-fold) in the absence of carmer. DARK, an Apaf-1-like adaptor protein required for DRONC activation, is also known to be up-regulated by ecdysone (8, 11). In l2mbn cells, a 24-h ecdysone treatment resulted in ~3.5-fold induction of dark transcript, which was abolished upon the knockdown of carmer (Fig. 4). The upstream proapoptotic proteins RPR and HID have been shown to be up-regulated by ecdysone during salivary gland and midgut cell death (8, 11). rpr and hid transcripts were also up-regulated 2–3-fold by ecdysone in l2mbn cells, and knockdown of carmer almost entirely abolished the levels of ecdysone-mediated up-regulation of these genes (Fig. 4). grim transcript, however, was not significantly induced by ecdysone, and knockdown of carmer had no effect on its expression levels. These data suggest that CARIMER coordinates the EcR/Usp-mediated regulation of several core cell death molecules.

CARIMER Expression during Drosophila Development—RT-PCR analysis of carmer expression throughout fly development showed that carmer transcript is abundant in early embryos; however, its expression is dramatically reduced in late embryonic and larval stages before being up-regulated at the early prepupal stage. This is followed by down-regulation of the transcript until the adult stage where it is again up-regulated (Fig. 5A). Most interesting, the up-regulation of carmer at the early prepupal stage correlates with the histolysis of larval tissues during metamorphosis. This was analyzed more closely in both salivary glands and midguts at specific stages, which showed that in salivary glands carmer levels were up-regulated at a time when this tissue undergoes histolysis (Fig. 5B). carmer transcript was also present in midgut but was not up-regulated (Fig. 5B). These results suggest that CARIMER may be required for ecdysone-mediated deletion of these tissues.

Conclusions—The Drosophila protein that we describe here has a high degree of homology with CARM1, a methyltransferase that can methylate histone H3, and associates with the EcR-B1 isoform. Although a carmer fly mutant is currently unavailable, we would predict such a mutation to be lethal. With the use of RNAi, we have established an essential role for CARIMER in ecdysone-mediated apoptosis of a well-characterized ecdysone-responsive cell line l2mbn (12, 14). The expression of all known ecdysone-responsive cell death genes tested was affected by CARIMER ablation suggesting that CARIMER coordinates ecdysone-mediated gene transcription. Given that CARIMER associates with EcR (probably indirectly through p160 coactivators), we speculate that gene expression by CARIMER may be mediated by its direct recruitment to the EcR/Usp sites on target gene promoters as well as on the promoters of ecdysone-induced transcription factors (e.g. BR-C and E93) that are known to regulate many apoptotic genes (8, 9). Given its dynamic expression throughout Drosophila development, as well as its up-regulation in the salivary gland at the time corresponding to PCD, it would be interesting to determine whether CARIMER is important for all ecdysone-mediated events of metamorphosis or just specific biological events.

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REFERENCES
1. Evans, R. M. (1998) Science 280, 889–985
2. Stallcup, M. R., Kim, J. H., Teyssier, C., Lee, Y. H., Ma, H., and Chen, D. (2003) J. Steroid Biochem. Mol. Biol. 85, 139–145
3. Chen, D., Huang, S. M., and Stallcup, M. R. (2000) J. Biol. Chem. 275, 46810–46816
4. Chen, D., Ma, H., Hong, H., Koh, S. S., Huang, S. M., Schurter, B. T., Aswad, D. W., and Stallcup, M. R. (1999) Science 284, 2174–2177
5. Yadav, N., Lee, J., Kim, J., Shen, J., Hu, M. C. T., Aldar, C. M., and Bedford, M. T. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 6464–6468
6. Truman, J. W., and Reddick, L. M. (2002) Annu. Rev. Entomol. 47, 487–506
7. Jiang, C., Baehrecke, E. H., and Thummel, C. S. (1997) Development 124, 4673–4683
8. Baehrecke, E. H. (2000) Cell Death Differ. 7, 1057–1062
9. Kumar, S., and Cakouros, D. (2004) Trends Biochem. Sci., in press
10. Jiang, C., Lamblin, A.-F. J., Steller, H., and Thummel, C. S. (2000) Mol. Cell 5, 445–455
11. Lee, C.-Y., Wendel, D. P., Reid, P., Lam, G., Thummel, C. S., and Baehrecke, E. H. (2000) Mol. Cell 6, 433–443
12. Cakouros, D., Daish, T., Martin, D., Baehrecke, E. H., and Kumar, S. (2002) J. Cell Biol. 157, 985–995
13. Teyssier, C., Chen, D., and Stallcup, M. R. (2002) J. Biol. Chem. 277, 46066–46072
14. Ress, C., Hoffmann, M., Maas, U., Solsky, J., and Dorn, A. (2000) Tissue & Cell 32, 464–477
15. Dorstyn, L., Colussi, P. A., Quinn, L. M., Richardson, H., and Kumar, S. (1999) Proc. Natl. Acad. Sci. U. S. A. 96, 4307–4312
16. Dorstyn, L., Read, S., Cakouros, D., Huh, J. R., Hay, B. A., and Kumar, S. (2002) J. Cell Biol. 156, 1089–1098
17. Daish, T. J., Cakouros, D., and Kumar, S. (2003) Cell Death Differ. 10, 1348–1356
18. Colussi, P. A., Quinn, L. M., Huang, D. C. S., Coombe, M., Read, S. H., Richardson, H., and Kumar, S. (2000) J. Cell Biol. 148, 703–710
19. Harvey, K. F., Shearwin-Whyatt, L. M., Fotia, A., Parton, R. G., and Kumar, S. (2002) J. Biol. Chem. 277, 9027–9037
20. Xu, W., Cho, H., Kadam, S., Banayo, E. M., Anderson, S., Yates, J. R., III, Emerson, B. M., and Evans, R. M. (2004) Genes Dev. 18, 144–156
21. Kumar, S., and Doymazis, J. (2000) Cell Death Differ. 7, 1039–1044
