A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor

Shun Sawatsubashi,1,2 Takuya Murata,1 Jinseon Lim,1 Ryoji Fujiki,1 Saya Ito,1,2 Eriko Suzuki,1,2 Masahiko Tanabe,1,2 Yue Zhao,1 Shuhei Kimura,1 Sally Fujiyama,1,2 Yue Zhao,1 Takashi Ueda,1 Daiki Umetsu,1 Takashi Ito,3 Ken-ichi Takeyama,1 and Shigeaki Kato1,2,4

1Institute of Molecular and Cellular Biosciences, University of Tokyo, Tokyo 113-0032, Japan; 2Exploratory Research for Advanced Technology, Japan Science and Technology Agency, Saitama 332-0012, Japan; 3Department of Biochemistry, Nagasaki University School of Medicine, Nagasaki 852-8523, Japan

Chromatin reorganization is essential for transcriptional control by sequence-specific transcription factors. However, the molecular link between transcriptional control and chromatin reconfiguration remains unclear. By colocalization of the nuclear ecdysone receptor (EcR) on the ecdysone-induced puff in the salivary gland, Drosophila DEK (dDEK) was genetically identified as a coactivator of EcR in both insect cells and intact flies. Biochemical purification and characterization of the complexes containing fly and human DEKs revealed that DEKs serve as histone chaperones via phosphorylation by forming complexes with casein kinase 2. Consistent with the preferential association of the DEK complex with histones enriched in active epigenetic marks, dDEK facilitated H3.3 assembly during puff formation. In some human myeloid leukemia patients, DEK was fused to CAN by chromosomal translocation. This mutation significantly reduced formation of the DEK complex, which is required for histone chaperone activity. Thus, the present study suggests that at least one histone chaperone can be categorized as a type of transcriptional coactivator for nuclear receptors.

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Chromatin structure is reorganized during gene activation through chromatin remodeling and epigenetic modification [Henikoff 2008; Jiang and Pugh 2009]. Through these processes, nucleosomal DNA becomes accessible to sequence-specific transcription factors, facilitating their stable binding at specific sites in target gene promoters [Cairns 2009]. Chromatin reconfiguration supporting transcriptional initiation and subsequent pre-mRNA elongation requires a number of functionally distinct regulatory complexes [Narlikar et al. 2002; Roeder 2005; Rosenfeld et al. 2006]. One such class is an ATP-dependent chromatin remodeling complex. This class of complexes uses ATP hydrolysis to directly rearrange nucleosomal arrays in a noncovalent manner by sliding and transferring histone octamers associating with chromosomal DNA [Kouzarides 2007; Morrison and Shen 2009]. Another class of regulatory complexes can be categorized as histone-modifying enzymes, which epigenetically modify histone proteins [Borrelli et al. 2008; Sims and Reinberg 2008]. These enzymes covalently supply reversible epigenetic marks [acetyl groups, methyl groups, and phosphates] at specific residues on histone tails. Combinations of histone modifications generate a "histone code" to direct chromatin configuration in surrounding chromatin areas [Strahl and Allis 2000; Kouzarides 2007]. Moreover, the eviction and reassembly of histone octamers at transcribing gene loci are evident during chromatin reconfiguration, and require a third class of nucleosomal regulators: histone chaperones [De Koning et al. 2007; Park and Luger 2008]. In this regard, histone chaperones are believed to transcriptionally coordinate the function of sequence-specific regulators because core histones are mobilized during the transcription and elongation process [Adkins et al. 2004; Adkins and Tyler 2006]. Specific deposition of histone variants like histone H3.3 is also well documented in transcriptionally active loci in Drosophila cells [Mito et al. 2005; Schwartz and Ahmad 2005; Henikoff 2008], but the histone chaperones involved in this deposition have not been identified. The exact form of histone chaperone units and their mode of function appear diverse. However,
the role of each histone chaperone in the processes of transcriptional control by sequence-specific regulators is poorly understood.

An insect steroid hormone, ecdysone, induces metamorphosis [Thummel 1996]. Like mammalian nuclear receptors [NRs] [Evans 1988; Green and Chambon 1988], nuclear ecdysone receptor [EcR] has been characterized as a ligand-dependent and sequence-specific transcriptional activator, and heterodimerizes with ultraspiracle [USP] to control target gene expression in an ecdysone-dependent manner [Koelle et al. 1991; King-Jones and Thummel 2005]. Not surprisingly, key transcriptional coregulators are functionally and structurally conserved from insects to mammals [Bai et al. 2000; Takeyama et al. 2002; Sedkov et al. 2003]. This observation suggests that chromatin reconfiguration might be essential for EcR-mediated transcriptional control, as observed previously for mammalian NRs. In this respect, ecdysone-induced puff formation in the salivary gland of the fly [Ashburner 1990; Thummel 2002] is a readily observed example of chromatin reorganization induced by NRs. Although the prominent morphological alteration of chromatin structure was initially described decades ago [Ashburner 1967], the molecular basis and the associated regulatory factors are scarcely known. In the present study, we used Drosophila genetic screening to identify regulators supporting chromatin reorganization induced by liganded EcR. We found that a chaperone, Drosophila DEK [dDEK], is colococalized with EcR at the ecdysone-induced puff, and acts as a transcriptional EcR coactivator. Biochemical purification and characterization of fly and human DEK [hDEK] complexes revealed that phosphorylated DEKs associating with casein kinase 2 [CK2] serve as a histone chaperone. Moreover, in a group of acute myeloid leukemia [AML] patients, a mutant hDEK protein is known to be fused with CAN [Soekarman et al. 1992; von Lindern et al. 1992]. We found the mutant to be defective in chaperone activity. Thus, the present study suggests that a specific class of histone chaperones serves as a NR coactivator.

Results

Genetic screening identified dDEK as an ecdysone-inducible puff-localized factor

To identify a regulator involved in ecdysone-induced puff formation in the salivary gland of Drosophila, we genetically screened candidates from EGFP protein trap library lines [Morin et al. 2001; Buszczak et al. 2007] treated with a synthetic ecdysone: Muristerone A (Mur) [Supplemental Fig. S1A]. In the induced puff, several candidate lines colococalizing with EcR were identified. Among them, two lines—C00131 [Fig. 1A] and CA06616 [Supplemental Fig. S1B; Morin et al. 2001; Buszczak et al. 2007]—were selected for further analysis. The sequence of the flanking genomic DNA and the transcripts from these two lines led us to identify the same gene [Fig. 1B], the Drosophila ortholog [dDEK] of the hDEK oncogene [von Lindern et al. 1992]. To characterize endogenous dDEK expression in the salivary gland, we generated a polyclonal antibody against dDEK [Supplemental Fig. S1C]. Staining of polytene chromosomes from wild-type larvae with the antibody showed that dDEK and EcR overlapped on puffs [Supplemental Fig. S2A]. Based on the immunofluorescence of polytene chromosomes with anti-Ser5-phosphorylated RNA polymerase II [Pol II] [Weeks et al. 1993], dDEK appeared to be associated with transcriptionally active loci [Fig. 1C]. dDEK was seen in the less-compact chromatin interbands (estimated as weak DAPI staining), and its location was the converse of that of histone H1, a marker of condensed chromatin [Fig. 1D; Supplemental Fig. S2B, Kim et al. 2004]. These findings suggested that dDEK was localized in regions of transcriptionally active chromatin.

dDEK is an EcR coactivator

To determine if dDEK was functionally involved in ecdysone-induced chromatin reorganization, we tested the impact of dDEK in ecdysone-induced gene expression in intact flies. We used an RNAi approach with the GAL4-UAS binary system to knock down endogenous dDEK [Pili-Floury et al. 2004]. We established UAS inverted repeat [IR] transgenic strains, carrying fragments of the dDEK gene to form dsRNA to target the dDEK transcript under control of a salivary gland-specific GAL4 driver [sgs3-GAL4] [Stabell et al. 2007]. Likewise, either EcR or Taiman [Tai] was knocked down in the salivary glands [Supplemental Fig. S3A]. As anticipated, in vitro treatment of wild-type flies’ salivary glands with Mur induced expression of EcR target genes [Eip74EF, Eip75B, and BR-C] located within puff regions [Fig. 2C; Supplemental Fig. S4A; Burtis et al. 1990; Karim and Thummel 1992]. However, knockdown of either dDEK [IR-dDEK] or EcR [IR-EcR] resulted in the loss of the Mur response, which was also observed following knockdown of Tai [IR-Tai] [Fig. 2C, Supplemental Fig. S4A]. Taiman, a Drosophila homolog of human AIB1 histone acetyltransferase [HAT], is known to coactivate EcR [Bai et al. 2000]. Thus, it appeared that dDEK was a transcriptional EcR coactivator. Using the same knockdown approach with Mur-treated S2 cells [Supplemental Fig. S3B], we verified a significant role of dDEK in EcR-mediated gene induction [Supplemental Fig. S4B].

To directly test if dDEK coactivated the transcriptional function of EcR, the coregulator role of dDEK was tested in a transient luciferase reporter assay [Sawatsubashi et al. 2004]. Overexpression of dDEK coactivated EcR in the presence of Mur [Fig. 2D, lanes 3,4], without potentiation of the basal promoter activity [Fig. 2D, lanes 1,2], consistent
with knockdown assays using dsRNA [Fig. 2D, lanes 5,7]. Similarly, transactivation was attenuated when the known EcR coactivator Tai was knocked down (Fig. 2D, lane 6).

To determine if the observed association of EcR with dDEK also occurred at the EcR target gene promoter, the endogenous promoter of the \textit{Eip75B} gene in S2 cells was subjected to chromatin immunoprecipitation (ChIP) analysis. Although the EcR-binding sites remain to be mapped in this promoter, an EcR-binding site was found in the first intron region designated as position E, after testing regions A–J (see Supplemental Fig. S4C). EcR was recruited upon Mur treatment, and recruitment of dDEK was also inducible by Mur at site E (Fig. 2E). When EcR was knocked down in S2 cells, recruitment of dDEK was abolished. In contrast, dDEK was not indispensable for ligand-induced EcR recruitment (Fig. 2E).

**Biochemical identification of a dDEK complex**

Regulators reorganizing chromatin’s configuration often form nuclear multisubunit complexes. Thus, a dDEK-containing complex was biochemically purified from the nuclear extracts (NEs) of a newly established stable S2 transformant expressing Flag-tagged dDEK (e-dDEK). A dDEK-containing complex was purified through several chromatographic steps [Fig. 3A], and the components were analyzed using MALDI-TOF/mass spectrometry (MS) [Ohtake et al. 2007; Fujiki et al. 2009]. The \(\alpha\) and \(\beta\) subunits of \textit{Drosophila} CK2 (dCK2) were copurified and identified together with dDEK (Fig. 3B, top panel), and were further verified by Western blot (Fig. 3B, bottom panel). The purified dDEK–CK2 complex [dDEK (NE) com] exhibited a size of \(\sim 670\) kDa by gel filtration, and dDEK was phosphorylated (Fig. 3C, bottom panel). Consistent with the isolation of the dDEK–CK2 complex, colocalization of dCK2\(\alpha\) with dDEK and EcR was detected in the puff regions on polytene chromosome [Supplemental Fig. S5A,B].

Functional kinase activity of CK2 is achieved through formation of a heterotetramer containing the catalytic \(\alpha\) subunit and the regulatory \(\beta\) subunit [\(\sim 130\) kDa] [Litchfield 2003]. As bacterially expressed dDEK recombinant proteins
also appeared to form a tetramer by Superose 6 gel filtration analysis (Supplemental Fig. S6A), glycerol gradient sedimentation (Supplemental Fig. S6B), and Blue Native PAGE (Supplemental Fig. S6C; Wittig et al. 2006), we assumed that this complex was composed of the two tetramers.

Association of dDEK with the CK2α subunit was tested in a pull-down assay with dDEK mutants (Fig. 4A; Supplemental Fig. S7). The dDEK SAP domain (282–316 amino acids), a putative DNA-binding motif, was mapped as a CK2α-interacting domain, and this association was potentiated by the presence of ATP (Fig. 4B), suggesting phosphorylation dependency in the interaction between dDEK and CK2α. Furthermore, formation of the dDEK–CK2α and dDEK–CK2β complex was detected as shifted-up bands only in the presence of ATP (Fig. 4C).

We then asked whether CK2 phosphorylated dDEK. The dDEK protein was phosphorylated by affinity-purified dCK2α/β from S2 cells as well as human CK2α/β, and this phosphorylation was clearly blocked by a CK2 inhibitor: DMAT (Fig. 4D). The triple mutant (G303A/S304A/K305A; GSK > AAA), which is clearly defective in its interaction with CK2α, was significantly less phosphorylated by CK2 than dDEK(WT) (Fig. 4E). Thus, dDEK appeared to form a complex with CK2 through the phosphorylated dDEK.
dDEK is a CK2-dependent nucleosome assembly factor

During characterization of the dDEK–CK2 complex, dDEK was found to tightly associate with chromatin, and it copurified with core histones from the soluble chromatin fraction of micrococcal nuclease (MNase)-digested nuclear pellets (Supplemental Fig. S8A,B). Immunoprecipitation of dDEK from the chromatin fraction from S2 cells revealed that phosphorylated dDEK associated with core histones (Fig. 5A, left panel), and knockdown of CK2α in S2 cells resulted in the loss of dDEK complex formation with histones (Fig. 5A, right panel). In a pull-down assay using S-tagged dDEK beads, dDEK interacted directly with native core histones purified from S2 cells, but CK2α/β was not needed for this association in vitro (Supplemental Fig. S9). However, when the histones were reconstituted with plasmid DNA, histone association with dDEK–CK2s was abolished (Supplemental Fig. S9). These findings led us to suggest that dDEK serves as a histone chaperone to assemble histones into chromatin.

To test this idea, we asked if dDEK was capable of forming a nucleosomal array. Using a reconstitution assay with native core histones purified from S2 cells and supercoiled plasmid DNA [Supplemental Fig. S10A; Ito et al. 1997], dDEK was found to transfer histones to DNA, resulting in the formation of a histone–DNA complex that was evident in the bottom fraction of the glycerol density gradient [Supplemental Fig. S10B]. Furthermore, MNase digestion analysis of this bottom fraction detected formation of mono- and dinucleosomes [Supplemental Fig. S10C]. Then, we asked if dDEK could replace a well-known histone chaperone: Drosophila NAP-1 (dNAP-1). An in vitro nucleosome assembly assay was performed with Drosophila ACF and dNAP-1 proteins as well as core histones [Supplemental Fig. S11] in the presence of plasmid DNA [Ito et al. 1997; Kitagawa et al. 2003]. Reconstitution of the nucleosomal array by dNAP-1 histone chaperone and dACF was confirmed by MNase digestion analysis [Fig. 5B, lanes 3,4]. Under these conditions, the purified dDEK–CK2 complex [dDEK (NE) com] transferred core histones to DNA [Fig. 5B, lanes 5,6]. Importantly, the chaperone activity of this complex was abolished in the presence of a CK2 inhibitor: DMAT (Fig. 5B, lanes 7,8). The recombinant dDEK protein alone showed only weak nucleosome assembly activity (Fig. 5C, lanes 5,6), but, as expected, its activity was potentiated by the presence of recombinant human CK2 (hCK2) (Fig. 5C, lanes 7,8). As anticipated, the GSK > AAA mutant had no CK2-dependent histone chaperone activity (Fig. 5D, lanes 7,8). Thus, these findings suggest that dDEK requires CK2 for histone chaperone activity, achieved through formation of a functional complex via phosphorylation of dDEK.

dDEK assembles transcriptionally active chromatin

We then asked if dDEK preferentially associated with transcriptionally active or inactive histone modifications using a pull-down assay with S-tagged dDEK and the chromatin fraction from S2 cells [Supplemental Fig. S12A]. Consistent with dDEK localization in transcriptionally active regions on polytene chromosomes [Fig. 1C], histones pulled down with dDEK were marked with transcriptionally active modifications such as histone H3K4 methylation [Supplemental Fig. S12B]. As dDEK appeared to associate with histones in the transcriptionally active chromatin, we then asked if dDEK also interacted with the histone H3 variant H3.3. When Myc-tagged histones were overexpressed in S2 cells, dDEK was efficiently coimmunoprecipitated with H3.3, but much less with H3 [Fig. 6A]. By in vitro binding assays, dDEK exhibited affinity for H2A–H2B dimers, but the association with the H3.3–H4 tetramer appeared to be more stable [Fig. 6B]. To address this point under physiological conditions, H3.3’s association with dDEK was tested in intact flies. dDEK’s localization pattern in the salivary gland overlapped with that of H3.3, rather than that of H3 [Fig. 6C]. As dDEK physically interacts with
In vitro kinase assays using 32P-blotting using indicated antibodies. Bound proteins were analyzed by SDS-PAGE and Western blotting using anti-CK2 antibody. hCK2-dependent formation of the dDEK–hCK2 complex. Recombinant hCK2 subunit-binding region in dDEK. For each protein, the location of N-terminal conserved region containing SAP (pfam 02037) or DEK-C (pfam 08766) domains. For each protein, the location of N-terminal S-tag is shown. Triple-alanine mutated residues are shown as closed asterisks (G303A/S304A/K305A; GSK > AAA mutant) or opened asterisks (G303A/S304A/K305A; GS > AAA). Determination of the human CK2 subunit-binding region in dDEK. For pull-down assays, recombinant hCK2α (1 μg) was incubated with S-tagged dDEK (5 μg)-immobilized beads in the absence or presence of 100 μM ATP. The pull-downs were analyzed by Western blotting using anti-CK2α antibody. (C) Phosphorylation-dependent formation of the dDEK–hCK2 complex. Recombinant hCK2α (1 μg) and/or hCK2β (0.6 μg) proteins were pulled down by S-tagged FL and mutant dDEK proteins with or without ATP. Bound proteins were analyzed by SDS-PAGE and Western blotting using indicated antibodies. (D, E) Phosphorylation of dDEK by CK2. In vitro kinase assays using 32P-γ-ATP were performed using purified dDEK [NE] complex or indicated recombinant proteins in the absence or presence of 5 μM DMAT as CK2 inhibitor (D), and dDEK mutant variants (E). The phosphorylated products were visualized by autoradiography.

Figure 4. CK2-mediated phosphorylation of dDEK regulates formation of the dDEK–CK2 complex. (A) Schematic representation of dDEK domain organization of full-length (FL) and mutant variants. The gray boxes represent the evolutionarily conserved region containing SAP (pFam 02037) or DEK-C (pFam 08766) domains. For each protein, the location of N-terminal S-tag is shown. Triple-alanine mutated residues are shown as closed asterisks (G303A/S304A/K305A; GSK > AAA mutant) or opened asterisks (G303A/S304A/K305A; GS > AAA). (B) Determination of the human CK2 subunit-binding region in dDEK. For pull-down assays, recombinant hCK2α (1 μg) was incubated with S-tagged dDEK (5 μg)-immobilized beads in the absence or presence of 100 μM ATP. The pull-downs were analyzed by Western blotting using anti-CK2α antibody. (C) Phosphorylation-dependent formation of the dDEK–hCK2 complex. Recombinant hCK2α (1 μg) and/or hCK2β (0.6 μg) proteins were pulled down by S-tagged FL and mutant dDEK proteins with or without ATP. Bound proteins were analyzed by SDS-PAGE and Western blotting using indicated antibodies. (D, E) Phosphorylation of dDEK by CK2. In vitro kinase assays using 32P-γ-ATP were performed using purified dDEK [NE] complex or indicated recombinant proteins in the absence or presence of 5 μM DMAT as CK2 inhibitor (D), and dDEK mutant variants (E). The phosphorylated products were visualized by autoradiography.

EcR, H3.3’s localization was then examined in edcysine-induced puff loci in the salivary glands (Schwartz and Ahmad 2005). As a result, H3.3 accumulation and colocalization with dDEK were observed in a Mur-dependent manner on puffs. In a group of AML patients, the human DEK gene (located on chromosome 9q34 by chromosomal translocation (von Lindern et al. 1992). In such AML patients, the C-terminal domain of hDEK is spliced into the N-terminal truncated CAN (Fig. 7A), and malfunction of hDEK has been assumed. To address this issue, the histone chaperone function of hDEK-CAN was assessed. When the hDEK-CAN fusion protein was expressed in HEK 293T cells, the association of hDEK with either CK2α, CK2β, or histones was abolished (Fig. 7F). In EcR-mediated activation of the endogenous EcR target genes in intact flies, hDEK-CAN acted as a dominant-negative mutant (Fig. 7G; Supplemental Fig. S15). We attribute the disruption of functional complex formed by endogenous DEK–CK2 to hDEK/hDEK-CAN oligomerization (Fig. 7F). Thus, it appears that hDEK fusion to CAN impairs histone chaperone activity and contributes to the onset of this type of AML.

Discussion

Genetic identification of an EcR coregulator by an EGFP protein trap approach

Puff formation has long been recognized as reconfiguration of chromatin, a change that plays an important
physiologic role at the molecular level (Ashburner 1967, 1990). However, factors responsible for the reconfiguration have remained largely unidentified. The steroid hormone ecdysone induces puff formation, and we genetically screened for EcR coregulators. For this purpose, an EGFP protein trap approach was applied to screen for factors colocalized with EcR on polytene chromosomes. Several candidate factors encoded known transcriptional coregulators: osa (a SWI/SNF complex component) (Supplemental Fig. S16) and skd (a mediator complex component) (Boube et al. 2000; Mohrmann et al. 2004; data not shown), as well as functionally uncharacterized proteins, including dDEK. The chromosomal localization revealed that dDEK supports the process of gene activation coupled to puff formation.

DEK is a histone chaperone

Two forms of DEK complexes were biochemically purified from insect and human cells, with very similar compositions [Figs. 3B, 7C, 7D; Supplemental Fig. S8B]. We hypothesize that formation of the dDEK–CK2 complex is required for dDEK phosphorylation, rendering histone chaperone activity, while the dDEK–histone complex represents an intermediate during assembly into chromatin. Reflecting dDEK localization [Fig. 1C], histones associating with dDEK harbored epigenetic marks for transcriptional activation [Supplemental Fig. S12B]. The purified complex contained H3.3 [data not shown], and dDEK appears to accommodate more H3.3–H4 tetramers than H3–H4 in S2 cells [Fig. 6A]. Moreover, on polytene chromosomes, we observed that dDEK preferentially colocalized with H3.3 rather than with H3 [Fig. 6C]. It is unclear at this stage if dDEK is also capable of disassembling histones from chromatin. While HIRA has been characterized as a histone chaperone for H3.3 [Tagami et al. 2004; Henikoff 2008], H3.3 deposition on chromosome has been observed in flies deficient of HIRA [Bonnefoy et al. 2007]. dDEK may be functionally similar to HIRA in H3.3 assembly into chromatin. Together with the previous findings that nucleosomes containing H3.3 tend to be less stable than those containing H3 [Jin and Felsenfeld 2007], it is conceivable that dDEK remodels nucleosomal histone octamers into more transcriptionally active octamers through its histone chaperone activity, in cooperation with chromatin remodelers (Henikoff and Ahmad 2005; De Koning et al. 2007; Henikoff 2008).

The histone chaperone activity of DEK depends on its phosphorylation by CK2

In the in vitro chromatin assembly system, DEK function required CK2, and a CK2 kinase inhibitor abrogated chromatin assembly. The dDEK mutant (GSK > AAA) was unable to stably associate with CK2α [Fig. 4B,C] and was defective in assembling histones on DNA [Fig. 5D]. Thus, CK2 requires the phosphorylation of dDEK to form a dDEK–CK2 complex as a histone chaperone. Like CK2, the DEK–CK2 functional complex appears to be composed of two units: One is a phosphorylated dDEK tetramer functioning as a histone chaperone, and the other is a CK2 tetramer functioning as a regulatory unit. In this respect, DEK appears to be an atypical histone chaperone in terms of phosphorylation dependency as well as subunit composition, although a number of histone chaperones have been described with diverse forms of oligomerization (Park and Luger 2008).
DEK is a transcriptional coactivator for EcR

It is believed that histone chaperones coregulate transcription; however, no clear evidence, particularly in intact animals, supports a histone chaperone serving as a transcriptional coregulator for a given transcriptional activator. Here, we show that endogenous dDEK co-activates endogenous EcR in the chromosomal regions of ecdysone-induced puffs in intact flies (Fig. 2C). Its co-activator function is likely attributable to its histone chaperone activity (Fig. 6E; Supplemental Fig. S14), leading to assembly of active histones in chromatin [Fig. 6A; Supplemental Fig. S12B]. As dDEK and EcR were detected together with CK2α at the EcR-binding sites in EcR target genes [Fig. 2E; Supplemental Fig. S1C], they likely contribute to ecdysone-dependent nucleosomal reorganization. In this respect, dDEK may be unique among histone chaperones in terms of its selective and physical interaction with EcR [Fig. 2A,B]. However, other histone chaperones may also assist in the assembly and disassembly of histone octamers on promoter/enhancer sequences where EcR regulates gene expression. This concept is supported by data showing ecdysone-induced puff formation was not abrogated in a transgenic fly line overexpressing a dominant-negative form of dDEK.
regardless of aberrant expression of the tested EcR target genes (Fig. 6E).

dDEK localization on polytene chromosomes was also seen in regions other than the puff sites, and the localization pattern was not significantly affected by knockdown of EcR (data not shown). Since histone chaperone activity supports both eviction and assembly of histone octamers on chromatin, we speculate that the dDEK histone chaperone might serve as an inhibitor for transcriptional events, depending on dDEK localization on chromosomes. These facets of dDEK function may account for bidirectional coregulatory roles in transcriptional control; i.e., as an activator in the present study versus a repressor in a previous report (Gamble and Fisher 2007).

A class of AML is attributed to impaired histone chaperone activity of hDEK by fusion with CAN

The similarities of motif organizations in human and fly DEKs suggest that DEKs serve as a class of histone chaperone. Thus, it is feasible that this hDEK function is compromised by its fusion with CAN in AML patients [Fig. 7A; Soekarman et al. 1992; von Lindern et al. 1992]. In the present study, we observed that the hDEK-CAN fusion protein is unable to associate with CK2 (Fig. 7F). As phosphorylation of DEK by CK2 was required for histone chaperone activity, it is most likely that histone chaperone function is impaired in the fusion protein. As this stage, the global roles of hDEK function as a histone chaperone in chromosomal changes remain to be defined. However, from the present observations, the hDEK-CAN fusion protein appears defective, at least in part, in assisting transcriptional regulation of certain genes that might be essential for normal differentiation of hematopoietic cells.

Materials and methods

For a detailed description of the Materials and Methods, see the Supplemental Material.
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Screening

Salivary glands of third instar larvae of an EGFP protein trap line from 5-d-old cultures were dissected in phosphate-buffered saline [PBS]. After removing as much fat as possible, the organ was cultured for 1 h at 22°C in 300 μL of Drosophila SFM [Invitrogen] containing 2.5 × 10⁻⁷ M Mur [Wako]. After incubation, the glands were washed with PBS and immediately fixed for 20 min in 4% paraformaldehyde at room temperature. For immunofluorescence staining, they were incubated with anti-EcR primary antibodies [Ag10.2 and DDA2.7 from Developmental Studies Hybridoma Bank], then with Cy3-conjugated anti-mouse secondary antibody [Jackson ImmunoResearch] and DAPI [Roche] as a counterstain for 1 h at room temperature. Confocal microscopy was performed on a Zeiss Confocal Laser Scanning System 510, and images were assessed using Adobe Photoshop 7.0 [Adobe].

Immunostaining of polytene chromosome squashes

Immunostaining of polytene chromosome squashes from third instar larvae were performed as described in Zhao et al. (2009) with some modifications. Detailed information is available in the Supplemental Material.

Fly strains

Fly stocks were maintained at 22°C on standard cornmeal–agar yeast food. The yw strain was used as wild-type in all experiments. G00131 and CA06616 (kind gifts from L. Cooley and A. Spradling) were generated by mobilizing the EGFP-trapping P-element as described [Morin et al. 2001; Buszczak et al. 2007]. The fly lines expressing Flag-tagged or monomeric RFP (mRFP)–tagged dDEK, hDEK, hDEK-CAN, and dCK2α were created by transforming Drosophila germline cells [BestGene, Inc.] using the pUAST vectors. Several independent lines were established and crossed with sgs3-GAL4 driver lines [sgs3-G4] expressed in salivary glands. RNAi transgenic fly lines of dDEK were obtained by crossing with sgs3-G4 flies, sgs3-G4, and dCK2α were created by transforming Drosophila germline cells [BestGene, Inc.] using the pUAST vectors. Several independent lines were established and crossed with sgs3-GAL4 driver lines [sgs3-G4] expressed in salivary glands. RNAi transgenic fly lines of dDEK were obtained using the inducible RNAi method. A 661-base-pair (bp) cDNA fragment (nucleotide position 1–661 of the coding sequence) was used for transformation. Drosophila UAS-IR-EcR was provided from R. Ueda.

Purification of dDEK and hDEK complexes

Drosophila S2 cells stably expressing N-terminal Flag epitope-tagged DEK [c-dDEK] were established with a Drosophila Expression System [Invitrogen]. The dDEK complex was immunoprecipitated from NEs [0.1 g] with anti-Flag M2 resin in buffer D [20 mM HEPES-KOH, 0.2 mM EDTA, 5 mM MgCl₂, 150 mM KCl, 0.05% [v/v] NP-40, 10% [v/v] glycerol, 0.5 mM DTT, 0.2 mM PMSF, protease inhibitor cocktail [Roche] at pH 7.9]. The bound polypeptides were eluted with the Flag peptide (0.2 mg/mL) and were subjected to Mono Q chromatography using AKTA explorer 105 (GE Healthcare) [Fujiki et al. 2009]. The components were identified by peptide mass fingerprint analysis using MALDI-TOF/MS [Bruker Daltonics] [Ohtake et al. 2007; Fujiki et al. 2009]. For gel filtration, the fractions from the anti-Flag purification were loaded onto a Superose 6 10/300 GL column and fractionated with buffer D in 1-mL fractions using AKTA explorer 105.

Nucleosome assembly and MNase digestion assays

Nucleosome assembly and MNase digestion assays were performed as described previously [Ito et al. 1997; Kitagawa et al. 2003]. A standard reaction contained supercoiled plasmid DNA, purified core histones from S2 cells, purified recombinant dDEK, purified recombinant dACF, ATP (3 mM), and the ATP-regenerating system.

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Erratum

Genes & Development 24: 159–170 [2010]

A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor
Shun Sawatsubashi, Takuya Murata, Jinseon Lim, Ryoji Fujiki, Saya Ito, Eriko Suzuki, Masahiko Tanabe, Yue Zhao, Shuhei Kimura, Sally Fujiyama, Takashi Ueda, Daiki Umetsu, Takashi Ito, Ken-ichi Takeyama, and Shigeaki Kato

Due to an error during figure preparation for the above-mentioned article, one of the images in Figure 2A was accidentally used such that the two top panels, showing negative controls represented as “Mock” (for αFlag), were duplicated. A corrected version of the two panels is shown below, where “IP:αFlag” remains as originally presented and “Input” is replaced by the correct, nonduplicated image. This correction does not alter the conclusions of the study. The authors apologize for the error.
A histone chaperone, DEK, transcriptionally coactivates a nuclear receptor

Shun Sawatsubashi, Takuya Murata, Jinseon Lim, et al.

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