CHD1 Motor Protein Is Required for Deposition of Histone Variant H3.3 into Chromatin in Vivo

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The organization of chromatin affects all aspects of nuclear DNA metabolism in eukaryotes. H3.3 is an evolutionarily conserved histone variant that is deposited by a set of ATP-dependent factors (1) during DNA replication and cell division. H3.3 associates with a subset of developmental genes, including those involved in tissue-specific gene expression and differentiation. In vitro, chromatin assembly requires the action of histone chaperones and adenosine triphosphate (ATP)-utilizing factors (3). Histone chaperones may specialize for certain histone variants. For example, H3.3 associates with a complex containing HIRA, whereas canonical H3 is in a complex with CAF-1 (chromatin assembly factor 1) (4). The molecular motors known to assemble nucleosomes are ACF (ATP-utilizing chromatin assembly and remodeling factor), CHRAC (chromatin accessibility complex), and RSF (nucleosome-remodeling and spacing factor), which contain the Snf2 family member ISWI as the catalytic subunit (5–7), and CHD1, which belongs to the CHD subfamily of Snf2-like adenine triphosphatases (ATPases) (8). These factors have not been shown to mediate deposition of histones in vivo. We previously demonstrated that CHD1, together with the chaperone NAP-1, assembles nucleosome arrays from DNA and histones in vitro (9). Here, we investigated the role of CHD1 in chromatin assembly in vivo in Drosophila.

We generated Chd1 alleles by P element-mediated mutagenesis (Fig. 1A) (10). Two excisions, Df(2L)Chd1[1] and Df(2L)Chd1[2], deleted fragments of the Chd1 gene and fragments of unrelated adjacent genes. Heterozygous combinations, however, of Chd1[1] or Chd1[2] with Df(2L)Exel7014 affect both copies of the Chd1 gene only (Fig. 1B). We also identified a single point mutation that results in premature translation termination of Chd1 [Q1394*] in a previously described lethal allele, l(2)23Cd[A7-4] (11). Hence, l(2)23Cd[A7-4] was renamed Chd1[3].

Analysis of Western blots of embryos from heterozygous Chd1[3] fruit flies revealed the presence of a truncated polypeptide besides full-length CHD1 (Fig. 1C). No truncated polypeptides were detected in heterozygous Chd1[1] or Chd1[2] embryos. Therefore, the corresponding deficiencies result in null mutations of Chd1. Crosses of heterozygous Chd1 mutant alleles with Df(2L)JS17/CyO or Df(2L)Exel7014/CyO produced subviable adult homozygous mutant progeny (Fig. S1). Both males and females were sterile. Homozygous null females mated to wild-type males laid fertilized eggs that died before hatching. Therefore, maternal CHD1 is essential for embryonic development. When we examined the chromosome structure of 0- to 4-hour-old embryos laid by Chd1-null females, we observed that, during syncytial mitoses (cycles 3 to 13), the nuclei appeared to be abnormally small. The observed numbers of anaphase chromosomes suggested that they were

![Fig. 1. Characterization of Chd1 mutant alleles.](http://science.sciencemag.org/content/vol317/issue41)/
haploid (Fig. 2A). To confirm this observation, we mated wild-type or Chd1-null females with males that carried a green fluorescent protein (GFP) transgene. Embryonic DNA was amplified with primers detecting male-specific GFP and a reference gene, Asf1. In wild-type embryos, both primer pairs produced polymerase chain reaction (PCR)–amplified products, whereas only the Asf1 fragment was amplified in the mutants (Fig. 2B). Thus, Chd1 embryos develop with haploid, maternally derived chromosome content.

To investigate the causes of haploidy in mutant animals, we compared distributions of various developmental stages in samples of wild-type and Chd1-null embryos (table S1). The lack of maternal CHD1 dramatically changed this distribution. Most notably, at 0 to 4 hours after egg deposition, the majority of Chd1 embryos (56%) remained at a very early stage of development in contrast to the wild type (24%) (table S1).

In Drosophila eggs, meiosis gives rise to four haploid nuclei. When the egg is fertilized, one of them is selected as a female pronucleus; the remaining three form the polar body. After breakdown of the sperm nuclear envelope, the compacted sperm chromatin is decondensed, and sperm-specific protamines are replaced with maternal histones. The male and female pronuclei juxtapose in the middle of the embryo and undergo one round of separate haploid mitoses. The resulting products fuse with their counterparts to give two diploid nuclei (12). In the majority of Chd1 embryos, we observed partial decondensation of the sperm chromatin and normal apposition of parental pronuclei. Then, however, one pronucleus underwent mitosis; the other one did not (Fig. 2C). Considering the subsequent loss of paternal DNA (Fig. 2B), we conclude that mitotic progression of the male pronucleus is hindered in Chd1 embryos.

Because CHD1 can assemble nucleosomes in vitro, we asked whether the absence of CHD1 affects histone incorporation into the male pronucleus. Embryos from wild-type or Chd1-null females were stained with an antibody against histone H3. In wild-type embryos, we observed uniform staining in both parental pronuclei (Fig. 3A). In contrast, in Chd1-null embryos only the

![Fig. 2. Embryos from homozygous Chd1 mutant females are haploid. (A) Propidium iodide (PI) staining reveals the haploid chromosome content in Chd1-null embryos (right). Cycle 10 embryos are shown. (B) Propagation of only the maternal genome is detected by PCR in embryos from Chd1 females that have been mated with males carrying a GFP transgene. Primers for GFP recognize the paternal DNA; primers for Asf1 amplify sequences from both male and female genomes. (C) The absence of maternal CHD1 results in the inability of one pronucleus (arrows) to enter the first mitosis. The other pronucleus (arrowheads) continues with divisions (left, prophase to metaphase; right, post anaphase). Labeling above the panels refers to genotypes of mothers. Scale bars, 10 μm.](http://science.sciencemag.org/content/f10378/fig-2)

![Fig. 3. CHD1 is required for incorporation of histones into decondensing sperm chromatin. (A) H3 colocalizes with DNA in both parental pronuclei of wild-type embryos (left, interphase or prophase; right, metaphase). (B) In Chd1 mutant embryos, the male pronucleus fails to undergo mitosis and accumulates abnormally little H3. (C) H3.3-FLAG is incorporated into chromosomes of the male pronucleus in wild-type embryos. Panels show the first metaphase. (D) In Chd1 mutant eggs, H3.3-FLAG accumulates in the periphery of the male pronucleus. The female pronucleus proceeds with mitosis (left, prophase; right, anaphase). (B, C, and D) Arrows, male pronuclei (m); arrowheads, female pronuclei (f). Labeling above the panels refers to genotype of mothers. Red, PI; green, H3 (A and B) or H3.3-FLAG (C and D). Scale bars, 10 μm.](http://science.sciencemag.org/content/f10378/fig-3)
female chromatin was brightly stained. The male pronucleus contained considerably less histone H3 (Fig. 3B). These observations indicate that CHD1 is necessary for nucleosome assembly during sperm decondensation.

Sperm DNA does not replicate during decondensation, and histones are deposited by replication-independent assembly mechanisms, which involve the variant histone H3.3 but not canonical H3 (13). It has been shown in Drosophila and mice that H3.3 is specifically present in the male pronucleus (14, 15). We analyzed the distribution of H3.3 in embryos derived from Chd1-null females that carry a FLAG-tagged H3.3 transgene. In wild-type embryos, we observed colocalization of the H3.3-FLAG signal with male pronuclear DNA during migration and apposition. No H3.3-FLAG was detectable in the maternal pronucleus (Fig. 3C). In Chd1-null embryos, the male pronucleus showed altered H3.3-FLAG staining. The signal did not co-localize with the DNA but remained constrained to the nuclear periphery in a saclike pattern (Fig. 3D).

These findings suggest that in the earliest phases of Drosophila development CHD1 is essential for the incorporation of H3.3 and normal assembly of paternal chromatin. In contrast, CHD1 does not appear to affect the organization of maternal chromatin. We conclude that CHD1 is required for replication-independent nucleosome assembly in the decondensing male pronucleus, but is dispensable for replication-coupled incorporation of H3.

It was shown recently that the sesame (ssm) mutation of Drosophila histone chaperone HIRA caused the development of haploid embryos and abolished H3.3 deposition into the male pronucleus (14). Chd1 and ssm mutants, however, differ profoundly in the manifestation of this phenotype. In ssm embryos, H3.3 is absent from the male pronucleus. In contrast, in Chd1-null embryos, H3.3 delivery to the male pronucleus appears to be unaffected. Thus, our observations allow us to mechanistically discern the roles of CHD1 and HIRA. Whereas HIRA is essential for histone delivery to the sites of nucleosome assembly, CHD1 directly facilitates histone deposition (fig. S3). Our findings are consistent with observations in vitro that histone chaperones either do not assemble nucleosomes or assemble them at a greatly reduced rate in the absence of ATP-utilizing factors. Our data provide evidence that histone deposition in vivo also transpires through an ATP-dependent mechanism.

CHD1 has been implicated in transcription elongation–related chromatin remodeling (16). We demonstrate that CHD1 functions in nucleosome assembly in the early Drosophila embryo, which is transcriptionally silent. The biological role of CHD1, therefore, is not confined to transcription-related processes. The Schizosaccharomyces pombe homolog of CHD1, Hrp1, has been shown to function in loading of the centromere-specific H3 variant CENP-A (17). Similarly to H3.3, incorporation of CENP-A into chromatin is not restricted to S phase. Therefore, CHD1 may have a general role in replication-independent nucleosome assembly.

Sperm decondensation involves not only histone incorporation, but also eviction of protamines. To discern whether CHD1 functions in this process, we analyzed the fate of protamine B (Mst35Bb) in Chd1-null embryos. Although we detected GFP-tagged Mst35Bb in the sperm head immediately upon fertilization, we did not observe Mst35Bb-GFP signal in the male pronucleus (fig. S2). Thus, like HIRA (18), CHD1 is dispensable for protamine removal. We have shown that the male pronucleus in Chd1-null embryos contains very low amounts of histones (Fig. 3), yet the DNA is not packaged with protamines. It remains an open question whether other DNA–protein complexes exist in the male pronucleus.

Drosophila eggs contain stores of both known chromatin assembly factors CHD1 and ISWI (fig. S4A) (19). Nevertheless, ISWI is unable to substitute for CHD1 in the deposition of H3.3. To examine whether CHD1 and ISWI differ in their ability to interact with the H3.3 chaperone HIRA, we performed coimmunoprecipitation experiments with extracts from embryos expressing FLAG-HIRA. CHD1 signal was readily detected in FLAG-specific immunoprecipitates, whereas ISWI did not coimmunoprecipitate with HIRA (fig. S4B). Thus, a fraction of CHD1, but not ISWI, physically associates with HIRA. This property of CHD1 may account for its unique function in the H3.3 deposition process.

A subpopulation of Chd1 mutant haploid embryos survives beyond apposition stage (table S1). Therefore, we asked whether H3.3 deposition is altered in Chd1 mutant embryos during later developmental stages. In wild-type nuclei, the H3.3-FLAG signal originating from the male pronucleus becomes undetectable after 2 to 3 divisions. Most maternal H3.3 remains distributed diffusely throughout the syncytium. After cycle 11 (roughly correlating with the onset of zygotic transcription) H3.3-FLAG is redistributed into the nuclei, where it colocalizes with the DNA (Fig. 4A). In contrast, incorporation of H3.3 into Chd1 mutant nuclei was impaired. H3.3-FLAG produced a speckled staining with numerous bright dots that poorly overlapped with the maxima of DNA staining (Fig. 4B). It is important to note that, in the ssm (HIRA) mutant, H3.3 incorporation defects in tissues or developmental stages other than the apposition stage were not observed. This result is consistent with the idea that misincorporation of H3.3 in Chd1 embryos is a direct effect of CHD1 deletion rather than a consequence of haploid development. We also conclude that CHD1 functions in H3.3 deposition during later stages of embryonic development, possibly in a HIRA-independent fashion.

This study provides evidence that ATP-dependent mechanisms are used for histone deposition during chromatin assembly in vivo. Thus, molecular motor proteins, such as CHD1, function not only in remodeling of existing nucleosomes but also in de novo nucleosome assembly from DNA and histones. Finally, our work identifies CHD1 as a specific factor in the assembly of nucleosomes that contain variant histone H3.3.

References and Notes

Blue-Light–Activated Histidine Kinases: Two-Component Sensors in Bacteria

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Histidine kinases, used for environmental sensing by bacterial two-component systems, are involved in regulation of bacterial gene expression, chemotaxis, phototaxis, and virulence. Flavin-containing domains function as light-sensor modules in plant and algal phototropins and in fungal blue-light receptors. We have discovered that the prokaryotes Brucella melitensis, Brucella abortus, Erythrobacter litoralis, and Pseudomonas syringae contain light-activated histidine kinases that bind a flavin chromophore and undergo photochemistry indicative of cysteinyl-flavin adduct formation. Infection of macrophages by B. abortus was stimulated by light in the wild type but was limited in photochemically inactive and null mutants, indicating that the flavin-containing histidine kinase functions as a photoreceptor regulating B. abortus virulence.

LOV (light, oxygen, or voltage) domains are distributed in the three kingdoms of life (Eukarya, Archaea, and Bacteria) (1, 2). In most cases, the LOV domain is the primary sensory module that conveys a signal to protein domains with known or putative functions as diverse as regulation of gene expression, regulation of protein catabolism, and activation of serine/threonine kinases in eukaryotes and histidine kinases in prokaryotes (3, 4). The only two LOV-domain proteins from bacteria that have been studied are YtvA—a LOV-STATS (LOV–sulfate transporter and anti-sigma factor antagonist) protein from Bacillus subtilis—and a LOV protein (containing no other known domains) from Pseudomonas putida (3–8).

Fig. 1. Domain alignment of LOV histidine kinase proteins (LOV-HK).
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Science 317 (5841), 1087-1090.
DOI: 10.1126/science.1145339