Adaptive Evolution of Cid, a Centromere-Specific Histone in Drosophila

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ABSTRACT

Centromeric DNA is generally composed of large blocks of tandem satellite repeats that change rapidly due to loss of old arrays and expansion of new repeat classes. This extreme heterogeneity of centromeric DNA is difficult to reconcile with the conservation of the eukaryotic chromosome segregation machinery. Histone H3-like proteins, including Cid in Drosophila melanogaster, are a unique chromatin component of centromeres. In comparisons between closely related species of Drosophila, we find an excess of replacement changes that have been fixed since the separation of D. melanogaster and D. simulans, suggesting adaptive evolution. The last adaptive changes appear to have occurred recently, as evident from a reduction in polymorphism in the melanogaster lineage. Adaptive evolution has occurred both in the long N-terminal tail as well as in the histone fold of Cid. In the histone fold, the replacement changes have occurred in the region proposed to mediate binding to DNA. We propose that this rapid evolution of Cid is driven by a response to the changing satellite repeats at centromeres. Thus, centromeric H3-like proteins may act as adaptors between evolutionarily labile centromeric DNA and the conserved kinetochore machinery.

WHAT defines a centromere? In the budding yeast Saccharomyces cerevisiae, centromeric function can be assigned to a distinct 125-bp consensus sequence (Wiens and Sørgard 1998); however, this simplicity turns out to be exceptional. In most complex eukaryotes, there is no such candidate sequence. Rather, centromeres are composed of large blocks of highly repetitive satellite sequences. These satellite sequences, such as α-satellites in primates (Willard 1991; Murphy and Karpen 1998) and 180-bp repeats in Arabidopsis thaliana (Round et al. 1997; Copenhaver et al. 1999), are not always unique to centromeres. This underlying complexity of sequence appears at odds with the extreme conservation of the chromosome segregation apparatus, with many components conserved throughout the history of Eukarya.

What all eukaryotic centromeres have in common is the presence of atypical H3-like proteins packaged into specialized nucleosomes (Palmer et al. 1991; Stoler et al. 1995). These H3-like proteins have been described as CENP-A in humans (Palmer et al. 1991; Shelby et al. 1997), Cse4p in S. cerevisiae (Stoler et al. 1995), Cid in D. melanogaster (Henikoff et al. 2000), HCP3 in Caenorhabditis elegans (Buchwitz et al. 1999), and spCENP-A in Schizosaccharomyces pombe (Takahashi et al. 2000). They share 35–50% identity with histone H3 in the 100-amino-acid core histone fold, compared to >75% identity among the histone H3s themselves (Figure 1B). However, they are distinguished from histone H3 by the presence of an atypical N-terminal tail (Figure 1A), which is conserved in neither sequence nor length, even among the centromeric H3-like molecules. It has been demonstrated that specific subdomains of CENP-A are responsible for centromeric localization (Shelby et al. 1997). Various models have been proposed to account for this localization: by recognition of satellite arrays by the CENP-A containing nucleosomes (Shelby et al. 1997), by recognition of pre-existing CENP-A (Palmer et al. 1990), or by the replication timing of centromeres (Shelby et al. 1997; Csink and Henikoff 1998).

Transfection studies have strongly suggested the competence of α-satellites for centromere formation in humans (Harrington et al. 1997; Masumoto et al. 1998). Three types of studies challenge this view. First is the absence of a universal “centromeric” sequence. In fact, centromeric satellite repeats can differ at different chromosomes, even within the same species (Lohe et al. 1993). Second, many neocentromeres appear to be devoid of α-satellites. In one case, no sequence differences could be found that could distinguish neocentromeric from parental, noncentromeric DNA (Barry et al. 2000). Third, in vitro nucleosome reconstitution experiments with CENP-A fail to show a specificity for α-satellites (Yoda et al. 2000). These observations have led to the prevailing view that centromere formation is not dependent on the particular DNA sequence (Karpen and Allshire 1997).

Is centromeric sequence important at all? We have addressed this issue by investigating the evolution of centromeric H3-like proteins in closely related species of the D. melanogaster subgroup. We find strong evidence for the adaptive evolution of Cid in this subgroup. We propose that this adaptive evolution is driven in re-
response to the rapidly changing satellite sequences at Drosophila centromeres. The evolution of Cid and Drosophila centromeric DNA may also have a direct impact on previously described meiotic drive processes involving chromosome orientation during meiosis (Zwick et al. 1999).

MATERIALS AND METHODS

Fly strains: Various fly strains were obtained from the National Drosophila Species Resource Center (Bowling Green State University). Isofemale lines of D. melanogaster from Zimbabwe were kindly provided by Ying Chen (University of Rochester).

Cloning and sequencing: Flanking genes to Cid were used as queries in a BLASTP search and putative homologs identified in other metazoans. Primers were designed to conserved regions identified in the BLASTP search. PCR was done using primers to the upstream gene 5'-TCCGTGCGAAACCCTCTCGC-3' (Cid-upstream) and to the Cid gene 5'-CTCAGGGACGAGCGCCACGG ACTACGG-3' (CidmidFor) and a primer to the downstream gene, 5'-CTCAGGGACGAGCGCCACGG ACTACGG-3' (Ciddownstream). Both strands of PCR products were sequenced in the Cid coding region, using ABI Big Dye sequencing. The sequences obtained have been deposited in GenBank under accession nos. AF321923–AF321926.

Tests for positive selection: The various sequences were aligned using CLUSTALX (Thompson et al. 1997) and analyzed for deviations from neutrality (McDonald-Kreitman test (McDonald and Kreitman 1991) using DnaSP (Rozas and Rozas 1999). Other tests of deviations from the neutral hypothesis, e.g., Hudson-Kreitman-Aguade test, Tajima’s test, and Fu and Li’s test) were also performed using DnaSP (the latter two tests do not reject neutrality).

RESULTS AND DISCUSSION

Adaptive evolution of Cid in the melanogaster subgroup: The single-copy gene Cid is 678 nucleotides long with no introns with the N-terminal tail encoded by bases 1–375, and the remainder (376–675) encoding the core-histone fold region (Henikoff et al. 2000). We determined the sequence of Cid from 15 strains of D. melanogaster, 8 strains of D. simulans, and 1 strain each from D. mauritiana and D. sechellia by PCR (see MATERIALS AND METHODS) and direct sequencing. We also sequenced Cid from D. teissieri and determined that it encoded a 231-amino-acid protein with no introns. Representative Cid sequences from the five species are shown aligned in Figure 2, along with the histone H3 sequences from D. melanogaster and Entamoeba histolytica, one of the earliest-branching eukaryotes. While the melanogaster subgroup is thought to be 2.3 million years old, the divergence between D. melanogaster and E. histolytica is likely to be greater than a billion years.

The polymorphic sites of the different strains of D. melanogaster and D. simulans (Figure 3) show an excess of replacement changes in the melanogaster-simulans comparison. To investigate whether these replacement changes have resulted from adaptive evolution, we separated all polymorphic sites into either replacement (R) or synonymous (S) sites and into either fixed differences between species or polymorphic within species. If mutations at synonymous and nonsynonymous sites were due to neutral mutations alone, the R:S ratio between species (divergence) would be expected to be the same as the R:S ratio within species (polymorphism). Significant deviations from this expectation are of two types: excess of replacement changes fixed between species is expected in the case of adaptive evolution (positive selec-
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Figure 2.—Alignment of the Cid proteins from five species of the melanogaster subgroup with histone H3 proteins from D. melanogaster and E. histolytica. Various centromeric H3-like proteins are 35–50% identical to histone H3, but even divergent histone H3 show 75% identity to each other. The thin vertical line separates the N-terminal tails of Cid from H3, as these are not similar to each other. A schematic secondary structure is also presented (modified from Luger et al. 1997; Shelby et al. 1997) with the core histone fold starting at Helix N. Loop I is between helices I and II. Fixed replacement changes in the core between D. melanogaster and D. simulans Cid are highlighted with a "!".

Between D. melanogaster and D. simulans, the R:S ratio is 18:10, while the pooled polymorphic sites within the two species have an R:S ratio of 9:28. These ratios are different at highly significant levels ($P < 0.0025$), and demonstrate an excess of replacement changes fixed between species (18 instead of 9/28). These differences and intraspecific polymorphic sites in Table 1.

We compared R:S ratios for interspecific fixed differences and intraspecific polymorphic sites in Table 1.

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tion; McDonald and Kreitman 1991), while an excess of replacement polymorphisms within species indicates the accumulation of deleterious mutations (Nachman et al. 1996).

We compared R:S ratios for interspecific fixed differences and intraspecific polymorphic sites in Table 1.
TABLE 1

McDonald-Kreitman test

<table>
<thead>
<tr>
<th>Fixed (interspecies)</th>
<th>Polymorphic (intraspecies)</th>
<th>Ratios</th>
<th>G-value*</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replacement</td>
<td>14 (T) + 4 (C)</td>
<td>14:5:9:15 (T)</td>
<td>5.58 (T)</td>
<td>0.0185</td>
</tr>
<tr>
<td>Synonymous</td>
<td>5 (T) + 5 (C)</td>
<td>4:5:9:13 (C)</td>
<td>7.48 (C)</td>
<td>0.006</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18:10:9:28 (W)</td>
<td>10.43 (W)</td>
<td>0.0012</td>
</tr>
</tbody>
</table>

* D. melanogaster and D. simulans (pooled)

D. melanogaster lineage alone

| Replacement         | 8                              | 8:4:0:9 (W) | 5.58 (T) | 0.0185  |
| Synonymous          | 4                              | 9:9:9:15 (T) | 7.48 (C) | 0.006   |

D. simulans lineage alone

| Replacement         | 9                              | 9:3:9:20 (W) | 5.58 (T) | 0.0185  |
| Synonymous          | 3                              | 20          | 9:3:9:20 (W) | 0.011   |

The test is applied to the N-terminal tail (T), C-terminal core (C), and the whole cid gene (W) for D. melanogaster and D. simulans. *G-test of independence with Williams’ correction for continuity was carried out (Sokal and Rohlf 1995). Tajima’s and the Fu and Li’s tests were also carried out but do not reject the neutral model (P > 0.1).

expected). Thus, we can reject the neutral mutation hypothesis for the entire Cid gene. Further, when the analysis is partitioned to just the N-terminal tail, or just the C-terminal core histone fold, we can still reject neutrality, implying that both the N-terminal tail and the C-terminal core have undergone positive selection. This is the first instance where positive selection has been documented in a structural protein that is both ubiquitous and essential in all eukaryotes.

All 9 replacement polymorphisms (Table 1) have occurred within strains of D. simulans, none within D. melanogaster (Figure 3). We investigated the possibility of disparate evolutionary pressures on Cid along the two lineages, one leading to D. melanogaster and the other to D. simulans, by comparisons using D. teissieri Cid as an outgroup (Figure 3). There are 28 fixed changes between D. simulans and D. melanogaster, of which 4 cannot be assigned to either lineage using D. teissieri (asterisks in Figure 3). Starting with the most recent common ancestor of D. simulans and D. melanogaster, 8 replacement and 4 synonymous changes are assigned to have taken place in the lineage leading to D. melanogaster (0 replacement and 9 synonymous polymorphisms within species) while 9 replacement and 3 synonymous changes occurred in the lineage leading to D. simulans (9 replacement and 20 synonymous polymorphisms within species). This indicates that adaptive evolution has occurred on both the D. melanogaster (P < 10^{-3}) and the D. simulans (P = 0.01) lineages since their split from a common ancestor.

With each adaptive substitution, polymorphisms are lost from the surrounding region. If such a selective sweep had occurred recently, then we should detect fewer polymorphisms than expected in a region around the adaptive site. We find that in D. melanogaster, the region from nucleotides 121–467 is devoid of any polymorphisms, but has at least five fixed replacement changes, which is consistent with a selective sweep. Using the Hudson-Aguade-Kreitman test, we find θ in region 1–120 and 468–675 = 0.0237 to be significantly different from θ in region 121–467 = 0.01 (χ^2 = 4.227, P = 0.04; Hudson et al. 1987) consistent with a selective sweep recent enough that no synonymous polymorphisms have appeared in this window (121–467) since the sweep.

Where are the targets of positive selection? Nucleotide differences tabulated in Figure 3 are represented graphically in a sliding window analysis in Figure 4 as either polymorphisms within species for D. simulans (represented by π) or divergent sites between species (κ). Two peaks of interspecific divergence are observed, consistent with positive selection in both the N-terminal tail and the core. Cid’s histone core sequence is similar to that of histone H3, and previous studies have suggested an identical topology of the centromeric H3-like proteins within the nucleosomal particle (Shelby et al. 1997; Yoda et al. 2000). We find it compelling that two out of the three amino acid replacements that are fixed between D. melanogaster and D. simulans in the core map to or adjoining the Loop 1 region (Figure 1A). Loop 1 from histone H3 has been shown in previous studies to make direct contacts with the DNA that is wrapped around the nucleosome (Arents et al. 1991; Luger et al. 1997). The Loop 1 region in Cid and other centromeric H3-like molecules is typically two to three amino acids longer and may play a more significant role in the specificity of DNA binding (Shelby et al. 1997). Studies that have swapped the Loop 1 region between the centromeric histone H3-like proteins and histone H3 have confirmed the importance of this region for centromeric localization (Shelby et al. 1997) and chromosome segregation (Keith et al. 1999). Consistent with these previous results, we find that the signal of positive selection we have observed in the Cid core is in the region
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Cid is the first centromere-associated protein whose pattern of variation parallels that of the satellite repeats found at centromeres. The 2.3-million-year evolutionary divergence of Cid is amplified when we compare the heterogeneity of the N-terminal tail of centromeric H3-like proteins in different lineages (Figure 1; Henikoff et al. 2000). Thus, N-terminal tails from Cse4, CENP-A, HCP3, and Cid show no recognizable similarity to each other, despite apparently carrying out analogous functions in their respective genomes.

Are the satellites themselves driven? In female meiosis, an opportunity for competition between centromeres can arise, since only one of the four meiotic products is included as a pronucleus in the oocyte. Any chromosomal element that improves the likelihood of inclusion into the oocyte will thus have a significant advantage (Zwick et al. 1999). Among laboratory stocks of Drosophila, centromere strength can vary, influenced by the amount of flanking heterochromatin (Novitski 1955; Ault and Lyttle 1988). Since a satellite block can have innate centromeric competence (Platero et al. 1999), chromosomes with different satellite expansions may have differential centromeric strength, leading to meiotic competition in their orientation and propagation. Particular satellite variants may be preferred because of preferential binding by a host factor. Cid could be that host factor.

Any drive process is inherently deleterious because it can lead to takeover by unfit alleles as well as to lowered fertility. A satellite-centromere drive can be especially deleterious. Consider a situation in which a satellite sequence that can be bound by Cid expands near a centromere. By a process of stochastic expansion, this satellite could supplement the pre-existing centromeric satellite sequence, leading to an increase in centromere strength, and consequently a preferential retention of the chromosome in female meiosis. Left unchecked, this meiotic advantage could lead to runaway centromere expansions and chromosomal imbalances in mitosis. The host genome would prefer to restore balance among different chromosomes. Cid is the best candidate for alleviating this centromere-drive process by modulating binding to different satellite variants.

The observation that Cid is subject to adaptive evolution provides us the opportunity to test specific models about causes of satellite evolution (Zwick et al. 1999) and Cid’s potential role in mediating isolation between species, as species arrive at different Cid-satellite configurations. The conservation of the kinetochore apparatus and the generality of rapidly evolving centromeric satellites (Csík and Henikoff 1998) predict that similar adaptive evolution will be found for centromeric H3-like proteins in other complex eukaryotes, including humans. Conversely, strong adaptive evolution is not expected for the centromeric H3-like Cse4 protein in

Figure 4.—A sliding window analysis [50-nucleotide (nt) window, steps of 10 nt] of the intraspecific polymorphism (within D. simulans) represented by π and the interspecific divergence (K) for Cid performed using all sites (synonymous and replacement) with the y-axis indicating nucleotide position and the x-axis indicating π or K. A majority of the divergent sites in the first peak are nonsynonymous, while a majority of those in the second peak (but not all) are synonymous changes. The dashed line separates the N-terminal tail region from the C-terminal core.
S. cerevisiae, which has simple centromeres. These possibilities can now be tested using methods that we have applied to the analysis of Cid evolution.

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LITERATURE CITED


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