Current Biology *17*, 237–243, February 6, 2007 ©2007 Elsevier Ltd All rights reserved DOI 10.1016/j.cub.2006.11.051

Report

Incorporation of *Drosophila* CID/CENP-A and CENP-C into Centromeres during Early Embryonic Anaphase

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Summary

The centromere/kinetochore complex is indispensable for accurate segregation of chromosomes during cell divisions when it serves as the attachment site for spindle microtubules. Centromere identity in metazoans is believed to be governed by epigenetic mechanisms, because the highly repetitive centromeric DNA is neither sufficient nor required for specifying the assembly site of the kinetochore [1–4]. A candidate for an epigenetic mark is the centromere-specific histone H3 variant CENP-A that replaces H3 in alternating blocks of chromatin exclusively in active centromeres [1, 2, 5, 6]. CENP-A acts as an initiator of kinetochore assembly, but the detailed dynamics of the deposition of metazoan CENP-A and of other constitutive kinetochore components are largely unknown [1, 2, 7–10]. Here we show by quantitative fluorescence measurements in living early embryos that functional fluorescent fusion proteins of the *Drosophila* CENP-A and CENP-C homologs are rapidly incorporated into centromeres during anaphase. This incorporation is independent of ongoing DNA synthesis and pulling forces generated by the mitotic spindle, but strictly coupled to mitotic progression. Thus, our findings uncover a strikingly dynamic behavior of centromere components in anaphase.

Results and Discussion

We have analyzed the incorporation dynamics of the two constitutive centromere/kinetochore complex components described so far in *Drosophila*, the CENP-A homolog centromere identifier (CID) and the recently discovered, highly diverged CENP-C [11, 12]. CID and CENP-C were fused with the enhanced green fluorescent protein (EGFP) and enhanced yellow fluorescent protein (EYFP), respectively. The dynamics of the two fusion proteins were monitored during the syncytial nuclear divisions of the early *Drosophila* blastoderm embryo. These extremely rapid and synchronous cycles occur on the surface of the embryo, allowing simultaneous data acquisition of multiple nuclei arranged in the same optical plane [13].

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EGFP-CID and EYFP-CENP-C Are Specifically Incorporated into Centromeres during Anaphase

We measured the cumulative centromere-localized fluorescence intensity of EGFP-CID and EYFP-CENP-C per nucleus throughout the cell cycle. While all sister centromeres are still grouped within one chromatin plate in metaphase, they are separated into two chromosome groups in anaphase. After the metaphase-to-anaphase transition, the fluorescence intensity per chromosome group is therefore expected to drop to 50% of the value observed in the metaphase plate. Subsequently, the fluorescence intensity is expected to increase to the initial value before the next mitosis, reflecting deposition of new EGFP-CID or EYFP-CENP-C. To reliably assign the cell-cycle phase when this intensity increase occurs, we constructed strains coexpressing the green/yellow fluorescent centromere protein variants with a red fluorescent chromatin protein. For this purpose, we established a transgene expressing a functional histone 2A variant fused to the monomeric red fluorescent protein [14] (His2Av-mRFP1). The expected sharp drop in fluorescence intensity was indeed recorded at anaphase onset, when the sister centromere groups of early anaphase figures were assigned to separate nuclei (Figures 1A and 1C). Surprisingly, we detected immediately afterwards a steep increase in fluorescence intensity for both EGFP-CID and EYFP-CENP-C. This increase occurred during anaphase (Figures 1A and 1C). The fluorescence increase during anaphase was also found for embryos expressing exclusively EGFP-CID in a *cid* mutant background (see Figure S1 in the Supplemental Data available online), ruling out the possibility that the mitotic deposition of EGFP-CID is an artifact resulting from competition with endogenous, untagged CID. We point out that both EGFP-CID and EYFP-CENP-C are fully functional, as shown by the fact they rescue the phenotypic consequences associated with *cid* and *Cenp-C* mutations, respectively (see Supplemental Experimental Procedures and [11]). To exclude the possibility that the recorded intensity increase in anaphase results from a clustering of centromeres within the chromatin, we also analyzed the behavior of single centromeres (Figure 1B, Movie S1). Centromere fluorescence intensity was constant during interphase, but sharply dropped in late prophase/early metaphase, when individual sister centromeres are separated sufficiently by the pull of the mitotic spindle to allow their distinct quantification. During metaphase, the centromere fluorescence intensity stayed low. However, centromere fluorescence intensities strongly increased again during anaphase up to the level observed before sister centromere separation, thus confirming our quantification results of centromeric fluorescence per nucleus.

To assess whether the mitotic fluorescence intensity increase observed for centromeric chromatin components reflects a general behavior of chromatin proteins during the rapid syncytial divisions, we also monitored the fluorescence of His2Av-mRFP1. In this case,

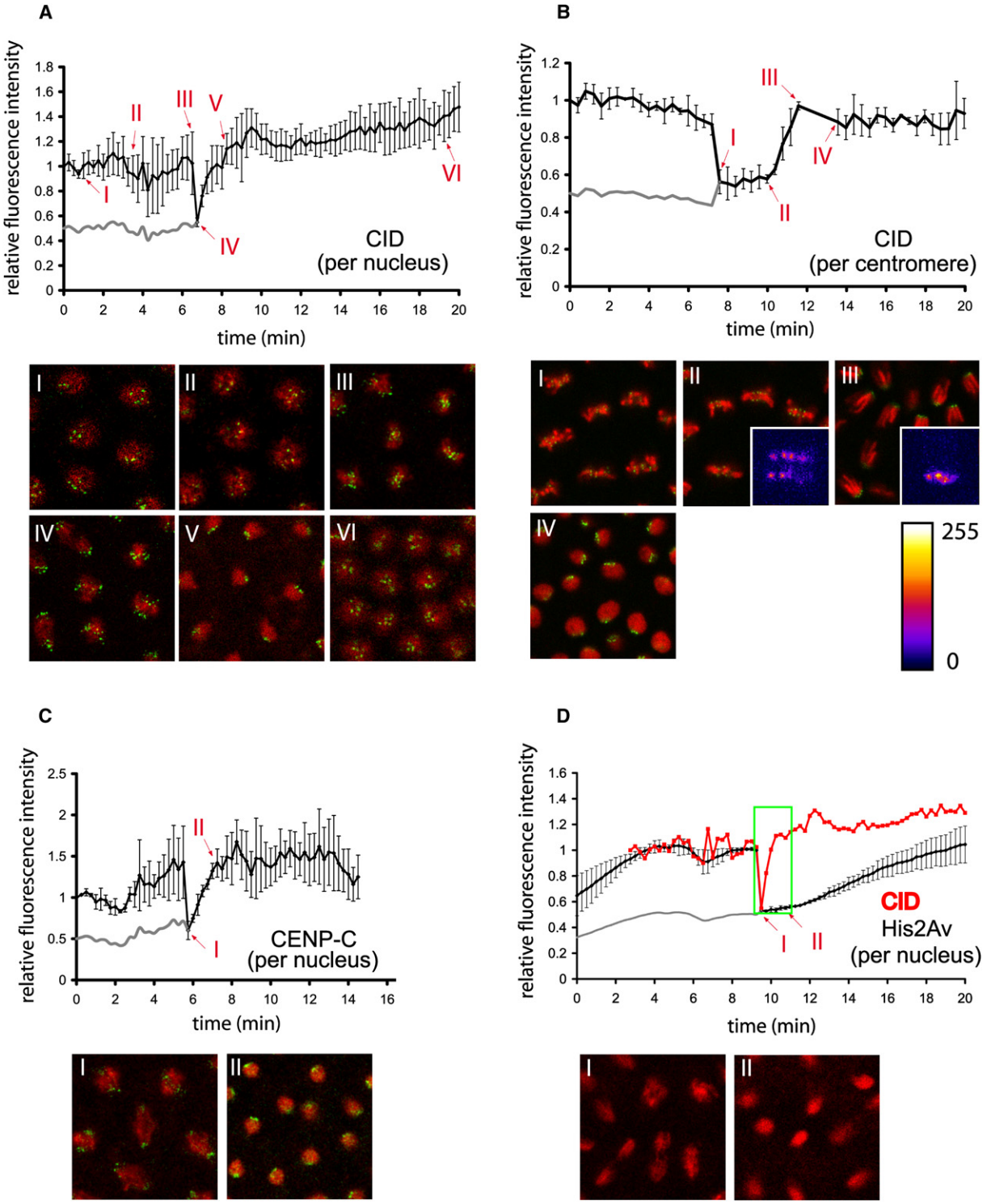

Figure 1. *Drosophila* CID and CENP-C Are Incorporated into Centromeres during Anaphase

Living embryos expressing *EGFP-cid* (A, B) or *EYFP-Cenp-C* (C) together with *His2Av-mRFP1* or *His2Av-mRFP1* alone (D) were observed while progressing through mitosis 12. Fluorescence intensities of EGFP-CID (A), EYFP-CENP-C (C), and His2Av-mRFP1 (D) were determined for selected nuclei in each frame and are plotted as relative intensities per nucleus. Beginning at the onset of anaphase, segregating sister centromere groups or chromatin masses were evaluated separately, resulting in an approximately 50% intensity drop (labeled IV in [A] and I in [C] and [D]). Data sets from a total of 25 to 30 nuclei from three embryos were aligned. EGFP-CID signal intensities of isolated single centromeres were evaluated and plotted per centromere (B). In this case, a 50% intensity drop occurs at the beginning of metaphase because the prominent poleward stretching of sister centromeres allowed a distinct evaluation of individual sister centromeres already at this stage. The subsequent fluorescence intensity increase during anaphase of individual centromeres is illustrated with a false color representation ([B], insets in II and III, Look Up Table shown below). Images corresponding to selected time points (indicated by arrows numbered with roman numerals) of the series are displayed below the graphs. His2Av-mRFP1 is shown in red and EGFP-CID (A, B) and EYFP-CENP-C (C) in green. For comparison, the EGFP-CID graph