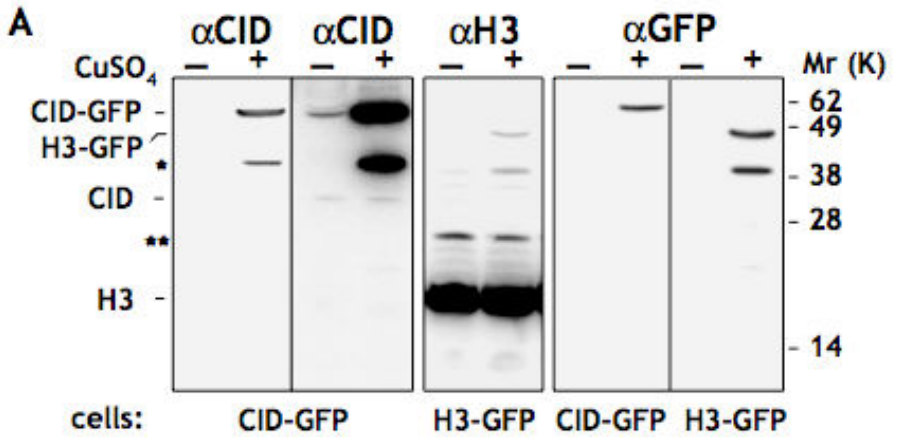
Supplemental Data

Mislocalization of the *Drosophila* Centromere-Specific Histone CID Promotes Formation of Functional Ectopic Kinetochores

Patrick Heun, Sylvia Erhardt, Michael D. Blower, Samara Weiss, Andrew D. Skora, and Gary H. Karpen



B

	- $CuSO_4$	+ $CuSO_4$	Induction
CID-GFP : CID	2:1	70:1	35 fold
H3-GFP : H3	1:350	1:13	27 fold

Figure S1. Induction of CID-GFP Results in Significantly Elevated Protein Levels in Comparison to Endogenous CID

(A) Western blot analysis of total cell extracts from S2 cells stably transfected with CID-GFP or H3-GFP, either induced with $CuSO_4$ (+) or uninduced (-). Expression was determined using anti-CID (short and long exposure), anti-Histone H3, and anti-GFP antibodies. One asterisk indicates degradation products of CID-GFP or H3-GFP respectively; two asterisks indicate low levels of a potential H3/H4-heterodimer. **(B)** Quantitative analysis indicated that the ratio of CID-GFP to endogenous CID was 2-fold without induction and 70-fold after induction; thus, overall induction of CID-GFP was 35-fold. H3-GFP levels without induction were negligible compared to endogenous H3 (1:350), due to the repeated nature and high expression levels of endogenous H3 genes; induction increased H3-GFP levels 27-fold, which resulted in a H3-GFP:H3 ratio of 1:13.

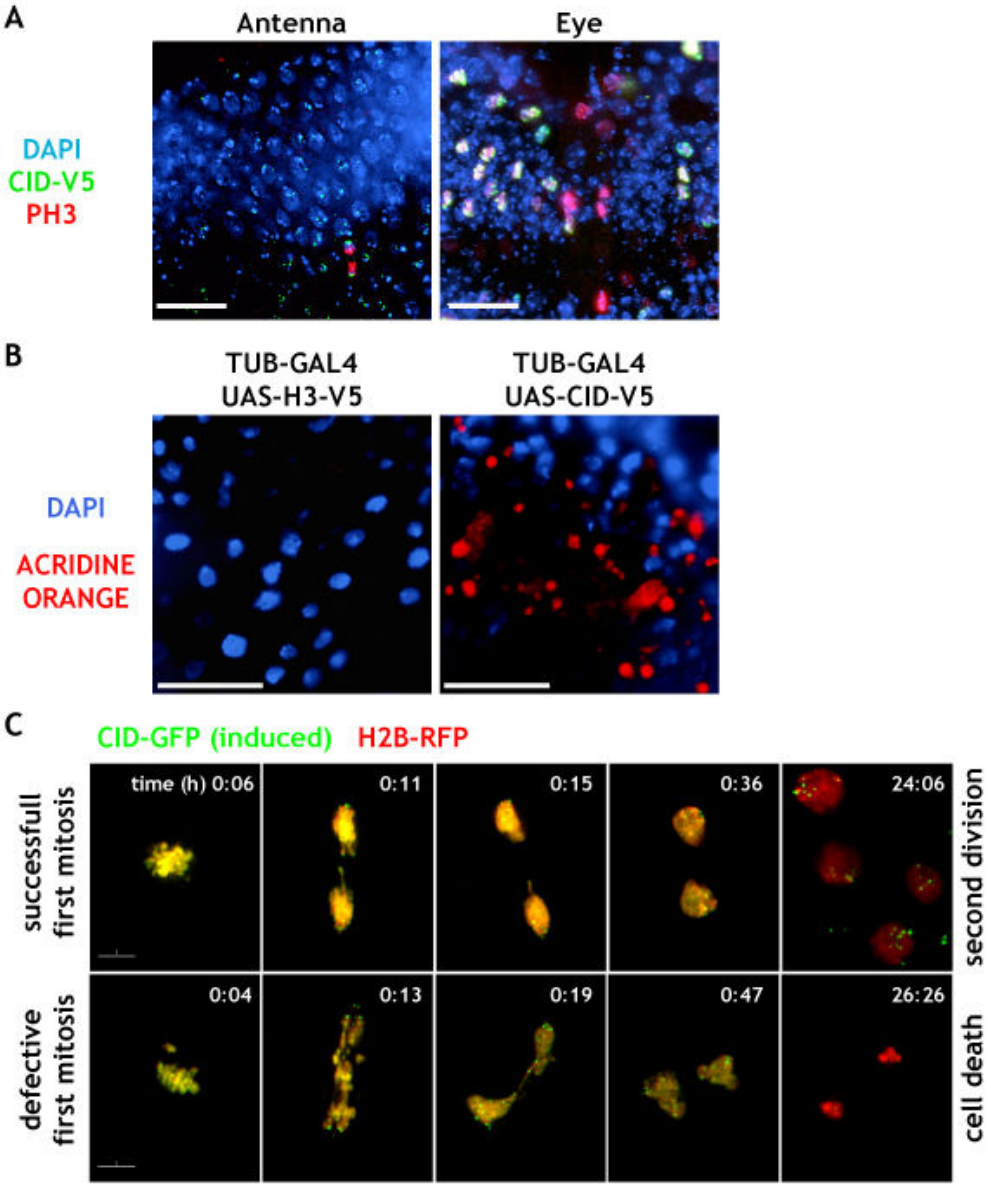


Figure S2. CID Overexpression Elevates the Mitotic Index and Cell Death

(A) Eye-antenna imaginal discs from EY GAL4/ UAS-CID-V5 larvae were stained for CID (green) and a marker for mitotic cells (H3 phospho-Serine10, red). The percentage of cells in mitosis in the eye disc (10.5%) was 3.5-fold higher than in the antenna disc (3%), where CID levels are normal (n=1000). Interestingly, very strong CID overexpression was more likely to be found in mitotic cells, which suggests that these cells were arrested or severely delayed in mitosis. **(B)** Whole discs were stained with acridine orange (AO), a vital dye that stains dying but not living cells. Control discs (H3-V5) did not stain for AO, whereas induced CID-V5 cells frequently stained for AO. These observations suggest that CID overexpression triggers apoptosis, most likely due to the observed chromosome missegregation phenotypes. **(C)** Extended time-lapse analysis of live S2 cells. Frames from time-lapse microscopy are shown for two different cells with medium levels of CID-GFP (green) co-expressed with H2B-RFP (red; chromosome counter stain). One cell appeared to segregate most chromosomes prior to cytokinesis (row 1), and was able to undergo a second round of division after 24 h. In contrast, the cell in row 2 exhibited extensive mitotic defects in the first division, including chromosome stretching, fragmentation, and the cut phenotype. As a consequence, the two daughter cells did not undergo a second division, and died about 26 h later with strongly condensed chromosomes and severely altered nuclear and cellular shape. Scale bars, 20 μm in **A, B**; 5 μm in **C**.

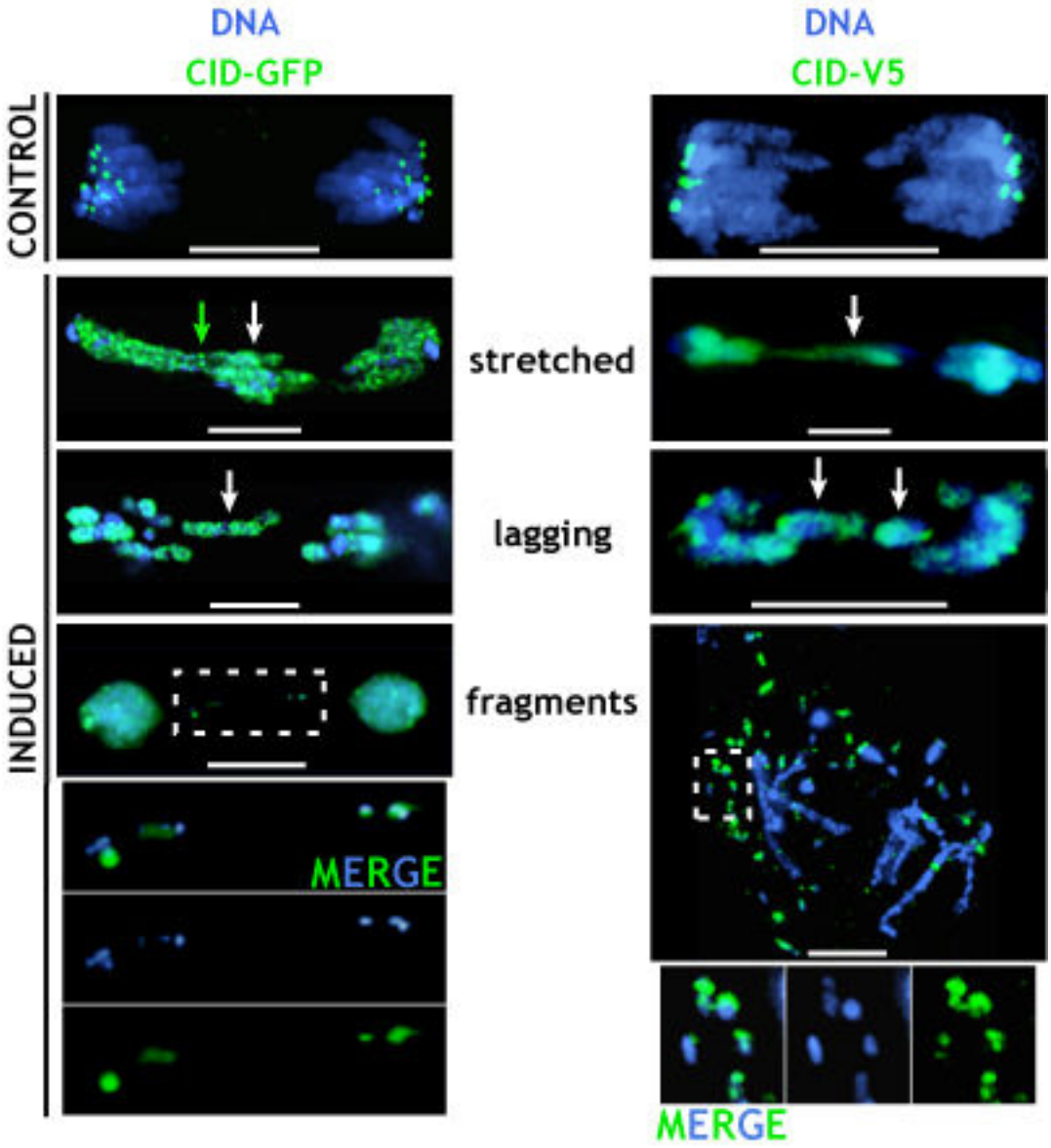


Figure S3. CID Mislocalization Produces Mitotic Defects

Chromosome behavior was assayed in fixed preparations from CID-GFP expressing S2 cells **(A)** and CID-V5 expressing embryos and larval discs and brains, induced by the TUB-GAL4 driver **(B)** (green=CID). Controls show normal progression through mitosis. Induction of CID expression produced stretched, lagging, and fragmented chromosomes at anaphase. The green arrow marks the endogenous centromere, and white arrows mark the corresponding phenotype. Chromosome fragments within the white box are shown at higher magnification below. The fragments contain CID, and in animals are most commonly located close to the spindle poles **(B),** suggesting that they were produced by abnormal spindle attachments. Very few mitotic defects were observed with our control (not shown). Scale bars, 5 µm.

Supplemental Experimental Procedures

Supplemental Figure 1

Total nuclear protein was prepared from stably transfected Schneider S2 cells carrying CID-GFP or H3-GFP, with (24 h) or without $CuSO_4$ induction. Proteins were separated on NuPAGE ™ 12% Bis-Tris gels (Invitrogen) using MES-buffer, pH 7.3, and processed for western blot using the Invitrogen NuPAGE® protocols. Chicken anti-CID (1:1000), rabbit anti-GFP (1:5000) and rabbit anti-H3 (1:5000) were used for primary detection, followed by corresponding secondary-antibodies coupled with horseradish peroxidase (1:5000). Blots were developed using ECL-plus (Pharmacia/Amersham), signals were captured using a Chemidoc XRS (BioRad), and band intensities were quantified with the Quantity One® software (BioRad).

Supplemental Figure 2

Acridine orange staining was performed as described (McCall and Peterson, 2004). To capture consecutive cell cycles by live imaging of the same cell (Supplemental Figure 2C), protein expression was pulse-induced in 1ml of 1x106 cells/ml growing cell culture using 250 mM CuSO4 for 12 hours. Induction medium was than replaced by normal medium (chase). Cells were resuspended and 100 µl was plated into a custom made growth chamber slide, consisting of 6 individual chambers mounted on a regular glass slide with 6 holes and a cover slip bottom. Time-lapse microscopy of <1 hour was performed on cells with low and high levels of CID-GFP in metaphase as described before. After 24 hours and 48 hours the same cells were imaged again with a frame rate of 1 frame/5 min for 4 hours. Cells grew with normal kinetics for at least 3 days under these conditions before they require a medium change.

Supplemental Tables

Table S1. Ectopic Localization of Kinetochore Proteins in S2 Cells

Protein 1	Protein 2	# SPOTS / NUCLEUS (AVG ± SD)								
		1			2			1 + 2		
		C	I	fold □	C	I	fold □	C	I	fold □
CENP-C		26 ±4	71 ±11	2.7	-	-		-	-	
MEI-S332		29 ±4	49 ±9	1.7	-	-		-	-	
BUBR1		26 ±3	44 ±9	1.7	-	-		-	-	
CENP-C	MEI-S332	30 ±4	72 ±6	2.4	32 ±5	61 ±7	1.9	22 ±4	43 ±6	2.0
CENP-C	POLO	30 ±7	75 ±12	2.5	27 ±5	59 ±9	2.2	22 ±4	40 ±4	1.8
ROD	POLO	32 ±5	46 ±8	1.4	30 ±4	51 ±7	1.7	27 ±3	39 ±3	1.4
MEI-S332	BUBR1	34 ±3	66 ±10	1.9	26 ±4	49 ±8	1.9	17 ±2	26 ±8	1.5
DYNEIN	KLP59C	39 ±12	56 ±26	1.4	41 ±13	77 ±35	1.9	16 ±7	36 ±19	2.3

C= control, I=induced

Table S2: Ectopic Localization of Kinetochore Proteins in Disc Cells

Protein 1	Protein 2	# SPOTS / NUCLEUS (AVG ± SD)								
		1			2			1 + 2		
		C	I	fold □	C	I	fold □	C	I	fold □
CENP-C		16 ±1	53 ±10	3.3	-	-		-	-	
MEI-S332		19 ±4	42 ±13	2.2	-	-		-	-	
BUBR1		16 ±2	31 ±5	1.9	-	-		-	-	
CENP-C	MEI-S332	16 ±1	58 ±7	3.6	16 ±1	51 ±7	3.2	16 ±1	45 ±7	2.8
CENP-C	POLO	16 ±1	54 ±10	3.4	16 ±2	42 ±11	2.6	16 ±2	40 ±11	2.5
ROD	POLO	17±3	35±6	2.1	17±5	36±15	2.1	15 ±2	26 ±3	1.7
MEI-S332	BUBR1	16 ±1	43 ±9	2.7	16 ±1	39 ±11	2.4	16 ±1	38 ±12	2.4

C= control, I=induced