The Drosophila RAD21 cohesin persists at the centromere region in mitosis

Citation for published version:

Digital Object Identifier (DOI):
10.1016/S0960-9822(00)00806-X

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Current Biology

Publisher Rights Statement:
cell press open access article

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
The *Drosophila* RAD21 cohesin persists at the centromere region in mitosis

W.D. Warren*, S. Steffensen†, E. Lin*, P. Coelho†, M-L. Loupart‡, N. Cobbe‡, J.Y. Lee§, M.J. McKay*, T. Orr-Weaver§, M.M.S. Heck‡ and C.E. Sunkel†

‘Cohesin’ is a highly conserved multiprotein complex thought to be the primary effector of sister-chromatid cohesion in all eukaryotes. Cohesin complexes in budding yeast hold sister chromatids together from S phase until anaphase, but in metazoans, cohesin proteins dissociate from chromosomes and redistribute into the whole cell volume during prophase, well before sister chromatids separate (reviewed in [1,2]). Here we address this apparent anomaly by investigating the cell-cycle dynamics of RAD21, the *Drosophila* orthologue of the Xenopus XRAD21 and Saccharomyces cerevisiae Scc1p/Mcd1p cohesins [3]. Analysis of RAD21 in S2 *Drosophila* tissue culture cells and live embryos expressing a DRAD21–green fluorescent protein (GFP) fusion revealed the presence of four distinct subcellular pools of DRAD21: a cytoplasmic pool; a chromosome-associated pool which dissociates from chromatin as chromosomes condense in prophase; a short-lived centrosome-associated pool present during metaphase–anaphase; and a centromere-proximal pool which remains bound to condensed chromosomes, is found along the junction of sister chromatids between kinetochores, and persists until the metaphase–anaphase transition. We conclude that in *Drosophila*, and possibly all metazoans, a minor pool of cohesins remains bound to centromere-proximal chromatin after prophase and maintains sister-chromatid cohesion until the metaphase–anaphase transition.

Addresses: *Peter MacCallum Cancer Institute, St Andrews Place, East Melbourne, Victoria 3002, Australia. †Instituto de Biologia Molecular e Celular, Instituto de Ciências, Biomédicas de Abel Salazar, University of Porto, Porto, Portugal. ‡Wellcome Trust Centre for Cell Biology, Institute of Cell and Molecular Biology, Kings Buildings, University of Edinburgh, Edinburgh EH9 3JF, UK. §Whitehead Institute for Biomedical Research and Department of Biology, Massachusetts Institute of Technology, Cambridge, Massachusetts 02142, USA.

Correspondence: William D. Warren
E-mail: w.warren@pmci.unimelb.edu.au

Received: 21 September 2000
Revised: 5 October 2000
Accepted: 5 October 2000
Published: 3 November 2000

Current Biology 2000, 10:1463–1466

0960-9822/00/$ – see front matter © 2000 Elsevier Science Ltd. All rights reserved.

Results and discussion

We raised antisera against a bacterially expressed carboxy-terminal fragment of DRAD21 (see Supplementary material) and used affinity-purified antibodies to examine DRAD21 localization in *Drosophila* S2 tissue culture cells by indirect immunofluorescence. DRAD21 localization was found to vary in a cell-cycle dependent pattern and to be unusually sensitive to fixation conditions. We used a cell preparation method that included Triton X-100 to increase chromatin antigen accessibility and reduced cytoplasmic staining. In interphase S2 cells, we observed the majority of DRAD21 to be localized to the nucleus with the exclusion of the nucleolus (Figure 1a). In comparison to interphase nuclei, nuclei in mitotic prophase showed a lower level of nuclear DRAD21 which was distributed along the condensing chromosomes (Figure 1b). During prometaphase, as chromosomes became fully condensed, DRAD21 was restricted to discrete chromosomal regions (Figure 1c). Cells in metaphase show DRAD21 localized to the centromere-proximal regions of fully condensed chromosomes aligned at the metaphase plate (Figure 1d). After sister-chromatid separation in anaphase, chromatin-associated DRAD21 was undetectable (Figure 1e). Lastly, re-localization of DRAD21 to chromatin was seen to commence in telophase cells (Figure 1f). These data provide clear evidence that, although the majority of DRAD21 disappears from chromosomes during prophase, a sub-pool remains associated with centromere-proximal chromatin until sister-chromatid separation, then rapidly disappears in early anaphase.

To analyse the centromere-proximal pool of DRAD21 in more detail we treated cells with colchicine to disaggregate chromosomes from the metaphase plate. Cells simultaneously stained for DNA with DAPI (Figure 2a), for DRAD21 with affinity-purified antibody (Figure 2b) and with the MP2 monoclonal antibody (Figure 2c) were examined. In *Drosophila* cells, MP2 recognises mitotic phosphoepitopes that predominantly localise to the kinetochore and centromere [4]. Merged images (Figure 2d,e) clearly show chromosomally localized RAD21 restricted to centromere-proximal heterochromatin, which remains joined in standard cytological preparations of *Drosophila* metaphase cells. This metaphase pool of DRAD21 is only found along the junction of paired sister chromatids and in between and perpendiculat to the kinetochores. These data show that DRAD21 persists on chromosomes in metaphase and is appropriately located to mediate sister-chromatid cohesion up until the metaphase–anaphase transition.
As a complement to the immunolocalization studies described above, we investigated the cell-cycle dynamics of a DRAD21–GFP fusion in living cells. Expression of DRAD21–GFP had no detrimental effect on development and adults were fully viable and fertile. Time-lapse observation of a cycle 13 syncytial embryo (Figure 3a, and Supplementary material) revealed DRAD21–GFP to be predominantly nuclear in interphase nuclei (0 min). Nuclei at the anterior and posterior poles of embryos at this stage commence mitosis just before those in the mid-region. As nuclei prepared to divide, DRAD21–GFP started to dissociate from chromosomes and a cytoplasmic cloud of fluorescence formed (3–6 minutes). Close examination revealed the appearance of small brightly fluorescent foci between the nuclei (6 minutes). Telophase nuclei nearest the poles of the embryo showed nuclear DRAD21–GFP fluorescence (9 minutes) followed shortly by nuclei in the middle of the embryo (12 minutes). By 15 minutes, all the nuclei were again in interphase, the bright foci were no longer evident and DRAD21–GFP was again mostly nuclear. Time-lapse observations of DRAD21–GFP dynamics in the asynchronous post-cellularization embryonic divisions (data not shown) were essentially the same as shown in Figure 3a. As the lag between de novo GFP translation and the formation of a fluorescent GFP chromophore is of the order of 40 minutes in Drosophila embryos [5], the observation of nuclear GFP fluorescence commencing in telophase indicates that most DRAD21–GFP molecules are not proteolysed at the end of each embryonic nuclear division.

To follow more closely the intracellular dynamics of DRAD21–GFP, we microinjected rhodamine-labelled tubulin into embryos expressing DRAD21–GFP and examined syncytial mitoses at higher magnification. Figure 3b (and Supplementary material) shows a cycle 11 embryo undergoing a synchronous mitosis. In interphase (0 minutes), DRAD21–GFP was mostly nuclear and rhodamine–tubulin remained excluded from the nucleus. As prophase commenced (2 minutes), DRAD21–GFP began to dissociate from chromatin. When spindles became fully formed in metaphase (4 minutes), DRAD21–GFP was largely cytoplasmic, although some residual chromosomal and/or microtubule-associated DRAD21–GFP remained. Brightly fluorescent foci which colocalised with centrosomes were consistently observed on all high-magnification images of DRAD21–GFP-expressing embryos in metaphase. By anaphase (6 minutes), centrosome fluorescence was slightly more intense and chromosome and microtubule fluorescence diminished. At telophase (8 minutes) the centrosomal foci were no longer visible and by interphase (10 minutes), the DRAD21–GFP was again mostly nuclear. When we fixed DRAD21–GFP-expressing embryos we detected weak microtubule and chromosomal GFP fluorescence at metaphase which was diminished in anaphase (see Supplementary material). Centrosomal

---

**Figure 1**

Cell-cycle localisation of DRAD21 in S2 Drosophila cells. Cells were immunostained to reveal DRAD21 and counterstained for DNA with DAPI. In the merged images DRAD21 is in green and DNA in red. (a) Interphase cell showing strong nuclear staining. (b) Prophase cell in early stages of chromosome condensation showing restriction of DRAD21 to the condensing chromosomes. (c) Prometaphase cell with condensed chromosomes and DRAD21 staining in discrete chromosomal regions. (d) Late prometaphase cell with chromosomes congressed at the metaphase plate. At this stage DRAD21 is highly restricted to the region of the chromosomes aligned at the equator of the spindle. (e) Anaphase cell showing significant reduction of DRAD21 staining following sister-chromatid separation. (f) Telophase cell showing faint chromosomal DRAD21 staining. A faint cytoplasmic signal was observed in all cells irrespective of cell-cycle stage when cells were not Triton-extracted (data not shown). The scale bar represents 5 µm.
and DRAD21 associates transiently with centrosomes during separation, we can only speculate at this point why chromatids and control the timing of sister-chromatid separation, we can only speculate at this point why DRAD21 associates transiently with centrosomes during metaphase–anaphase. Given its transient nature during a time when the S. cerevisiae orthologue of DRAD21 is being degraded, we speculate that we are observing an overloading of the proteasome degradation pathway, causing transient aggresome formation. In mammalian cells, protein overexpression or inhibition of the anaphase-promoting complex (APC/C) has been shown to cause accumulation of intracellular protein aggregates, called 'aggresomes', which form at the microtubule-organising center/centrosome region of cells [6,7]. In Drosophila, APC/C has been shown to be associated with both spindle microtubules and centrosomes, and cyclin B, which is also targeted for degradation at metaphase–anaphase, has been observed to associate transiently with centrosomes immediately before its degradation [8]. Overloading of the proteasome and consequent aggresome formation would also be consistent with accumulation of DRAD21–GFP on spindle microtubules and persistence of low levels of DRAD21–GFP on anaphase chromosomes (Figure 3b, and Supplementary material). However appealing this hypothesis, we cannot rule out the possibility that DRAD21 may have an active role in spindle dynamics or checkpoint monitoring and that its transient association with the centrosome is a reflection of this.

Our analysis of DRAD21 intracellular dynamics extend previous studies of metazoan cohesin complexes by clearly showing that a small percentage of the total cell content of the cohesin subunit RAD21 can be detected on condensed chromosomes at metaphase. These molecules are restricted to where sister chromatids are in contact, suggesting that they are actively participating in the physical maintenance of sister-chromatid cohesion until the metaphase–anaphase transition. Our data are supported by the recent study of the Xenopus XSA1 cohesin subunit [9]. Using XSA1-specific antibodies, Losada et al. [9] showed weak labelling of mitotic chromatin reassembled from Xenopus egg extracts, but were unable to detect XSA1 or any other cohesin subunit on intact metaphase chromosomes. Our data provide strong evidence that cohesin regulates the dissolution of sister-chromatid cohesion in higher eukaryotes via a two-step mechanism, the first step occurring in prophase, to facilitate chromosome condensation, and the second at metaphase–anaphase to separate sister chromatids. As Drosophila and vertebrate homologues of Esp1p, the S. cerevisiae 'separin' protein essential for Scc1p cleavage, have been identified [10,11] it seems likely that the maintenance of sister-chromatid cohesion in metaphase by centromere-proximal cohesin and its dissolution by proteolysis at the onset of anaphase occurs in all metazoans.

As the primary role of the cohesin complex is to link chromatids and control the timing of sister-chromatid separation, we can only speculate at this point why DRAD21 associates transiently with centrosomes during metaphase–anaphase. Given its transient nature during a time when the S. cerevisiae orthologue of DRAD21 is being degraded, we speculate that we are observing an overloading of the proteasome degradation pathway, causing transient aggresome formation. In mammalian cells, protein overexpression or inhibition of the anaphase-promoting complex (APC/C) has been shown to cause accumulation of intracellular protein aggregates, called 'aggresomes', which form at the microtubule-organising center/centrosome region of cells [6,7]. In Drosophila, APC/C has been shown to be associated with both spindle microtubules and centrosomes, and cyclin B, which is also targeted for degradation at metaphase–anaphase, has been observed to associate transiently with centrosomes immediately before its degradation [8]. Overloading of the proteasome and consequent aggresome formation would also be consistent with accumulation of DRAD21–GFP on spindle microtubules and persistence of low levels of DRAD21–GFP on anaphase chromosomes (Figure 3b, and Supplementary material). However appealing this hypothesis, we cannot rule out the possibility that DRAD21 may have an active role in spindle dynamics or checkpoint monitoring and that its transient association with the centrosome is a reflection of this.

Our analysis of DRAD21 intracellular dynamics extend previous studies of metazoan cohesin complexes by clearly showing that a small percentage of the total cell content of the cohesin subunit RAD21 can be detected on condensed chromosomes at metaphase. These molecules are restricted to where sister chromatids are in contact, suggesting that they are actively participating in the physical maintenance of sister-chromatid cohesion until the metaphase–anaphase transition. Our data are supported by the recent study of the Xenopus XSA1 cohesin subunit [9]. Using XSA1-specific antibodies, Losada et al. [9] showed weak labelling of mitotic chromatin reassembled from Xenopus egg extracts, but were unable to detect XSA1 or any other cohesin subunit on intact metaphase chromosomes. Our data provide strong evidence that cohesin regulates the dissolution of sister-chromatid cohesion in higher eukaryotes via a two-step mechanism, the first step occurring in prophase, to facilitate chromosome condensation, and the second at metaphase–anaphase to separate sister chromatids. As Drosophila and vertebrate homologues of Esp1p, the S. cerevisiae ‘separin’ protein essential for Scc1p cleavage, have been identified [10,11] it seems likely that the maintenance of sister-chromatid cohesion in metaphase by centromere-proximal cohesin and its dissolution by proteolysis at the onset of anaphase occurs in all metazoans.

Supplementary material
Acknowledgements
We thank R. Rutkowski and M. Costa for expert technical assistance. W.D.W. is supported by a grant from the Australian Research Council. S.S. is supported by a grant from the TMR program of the European Union and the Danish Research Academy. Research in the Heck laboratory is funded by a Wellcome Trust Senior Research Fellowship in the Basic Biomedical Sciences. The Sunkel laboratory is supported by grants from the Fundaçao para a Ciência e a Tecnologia of Portugal. Research in the Orr-Weaver laboratory is supported by grant MCB-9604135 from the National Science Foundation.

References

Figure 3

Time-lapse confocal fluorescence micrographs of DRAD21–GFP in living syncytial embryos. (a) Low-magnification time-series of the anterior end of a cycle-13 embryo as it undergoes a quasi-synchronous mitosis. Time in minutes is shown in the upper left corner of each image. The scale bar represents 25 µm. (b) High-magnification time-lapse images of a rhodamine–tubulin-injected embryo expressing DRAD21–GFP. Tubulin is shown in the left, DRAD21–GFP in the centre and the merged image on the right with tubulin in red and DRAD21 in green. The scale bar represents 10 µm. See text for full description and Supplementary material for time-lapse movies of these data.