Loss of nucleosome remodelers CHRAC/ACF does not sensitize early Drosophila embryos to X-rays

Alessandro Scacchetti\textsuperscript{1} and Peter B. Becker\textsuperscript{1§}

\textsuperscript{1}Molecular Biology Division, Biomedical Center, Ludwig-Maximilians-University, Munich, Germany

\textsuperscript{§}To whom correspondence should be addressed: pbecker@bmc.med.lmu.de

Abstract

Figure 1: A. Effect of X-rays on larval hatching rate. Wild-type and Acf\textsuperscript{C} embryos were irradiated with different X-ray doses (Gy). Each dot represents the % of embryos that hatched in a single experiment (N between 50 and 60 embryos). Horizontal bars represent the mean of the 4 biological replicates. B. Calculated p-values (null hypothesis: difference between means = 0) for the comparisons depicted in panel (A). C. Representative immunofluorescence images of irradiated (1 Gy) wild-type and Acf\textsuperscript{C} embryos 0, 15 and 180 minutes after irradiation. Embryos were stained with DAPI and with antibodies against H2A.V and γH2A.V. Scale bar = 100 μm. D. Quantification of immunofluorescence images of wild-type and Acf\textsuperscript{C} embryos from the time course after X-ray exposure as described in (C). Each dot represents the
normalized γH2A.V signal (see methods) of one experiment (N between 8 and 15 embryos). Horizontal bars represent the mean of 3 biological replicates. E. Calculated p-values (null hypothesis: difference between means = 0) for the comparisons depicted in panel (D).

Description

The ‘Chromatin Accessibility Complex’ (CHRAC) and ‘ATP-utilizing chromatin assembly and remodeling factor’ (ACF) of Drosophila melanogaster are chromatin remodeling complexes that slide nucleosomes (Becker and Horz, 2002). Both originate from the association of the ATPase ISWI and a large subunit ACF1. CHRAC contains two additional histone-fold subunits, CHRAC-14 and CHRAC-16 (Corona et al., 2000), but its nucleosome sliding activity is essentially similar to ACF in vitro (Hartlepp et al., 2005). CHRAC/ACF are implicated in several developmental processes. Mutations of ACF1, which disrupt both complexes, mildly perturb embryogenesis and oogenesis (Boerner et al., 2016; Chioda et al., 2010), and compromise Polycomb silencing and heterochromatin formation (Fyodorov et al., 2004). Through their nucleosome sliding activity CHRAC/ACF contribute to the genome-wide formation of regular nucleosome arrays. Altered nucleosome spacing and regularity in their absence correlates with leakiness of the repressive ground state of chromatin in early embryos (Baldi et al., 2018; Scacchetti et al., 2018). CHRAC/ACF complexes are also conserved in yeast (Iida and Araki, 2004) and mammals (LeRoy et al., 2000; Poot et al., 2000).

CHRAC/ACF may be involved in DNA damage response and repair pathways. The chromatin around a DNA lesion or break must be rendered accessible to the machineries dedicated to the damage recognition and repair (Jeggo et al., 2017). Nucleosome sliders may be involved in freeing the broken DNA from nucleosomes or restoring chromatin after DNA is repaired (Jeggo et al., 2017; Rother and van Attikum, 2017). Human CHRAC/ACF complexes are recruited to DNA breaks and cells depleted of ISWI (SNF2H) or ACF1 (BAZ1A) are more sensitive to DNA damage (Aydin et al., 2014; Klement et al., 2014; Lan et al., 2010; Sanchez-Molina et al., 2011). ACF1 mutant flies show defects in incorporation of the histone variant H2A.V in the Drosophila blastoderm stage (Chioda et al., 2010). As in the case of the mammalian H2A.X variant, H2A.V is a central player during the DNA damage response, as its phosphorylation (referred to as γH2A.V) is a key event within damage-triggered signaling cascades (Baldi and Becker, 2013). Due to the high degree of evolutionary conservation of metazoan CHRAC/ACF complexes it is possible that CHRAC/ACF in Drosophila are also involved in the DNA damage response, but this hypothesis has not been rigorously tested.

We recently generated an ACF1 loss-of-function allele (Acfc) by CRISPR-mediated deletion of the Acf gene (Scacchetti et al., 2018). To test if this deletion alters the sensitivity of early embryos to DNA damage, we exposed 2-3.5 hour old wild-type and Acfc embryos to two different X-ray doses and measured the rate with which they hatched into larvae. For both genotypes, at a dose of 1 Gy most of the embryos successfully completed embryogenesis (average hatching rates of 81.4% and 87.6% for wild-type and Acfc, respectively) while at 10 Gy only very few of them survive (average hatching rates of 1.9% and 8.3% for wild-type and Acfc, respectively) (Figure 1A). We could not measure any significant difference in hatching rates in the Acfc line (Figure 1B) suggesting that ACF1-containing remodelers do not influence the ability of early embryos to cope with DNA breaks.

We also assessed the effect of Acf deletion on the immediate early response of chromatin to DNA breakage, the phosphorylation of H2A.V (γH2A.V), which may reveal kinetic differences in DNA damage signaling and repair. Once again, 2-3.5 hour old embryos were exposed to X-rays and fixed for immunofluorescence microscopy 15 min or 3 hours after irradiation. As expected, upon irradiation with 1 Gy a significant increase in γH2A.V signal was observed after 15 min for both genotypes (average signal increase of 6.7 or 7.0 fold for wild-type and Acfc, respectively) (Figure 1C, D, E). Three hours after X-irradiation, the γH2A.V signal was reduced (average signal increase of 1.9 and 2.6 fold for wild-type and Acfc, respectively) (Figure 1C, D, E), reflecting successful DNA repair. Careful quantification followed by statistical analysis did not show a significant difference of γH2A.V signal between wild-type and Acfc genotypes at both time points, suggesting that the γH2A.V appearance and turnover are unaffected by the absence of ACF1 (Figure 1E).

In conclusion, we did not find an effect of CHRAC/ACF depletion on overall DNA damage signaling or developmental competency upon X-ray irradiation in early fly embryos, where ACF1 expression peaks. During this developmental phase, blastoderm cells divide most rapidly, skipping the G1 phase between mitosis and DNA replication [reviewed in (Farrell and O’Farrell, 2014)]. Given this cell cycle peculiarity, our conclusions may not apply to later developmental stages, specific tissues or other developmental processes, such as oogenesis. It is also possible ACF1-containing remodelers play a role in the DNA damage response, but the effect is not obvious because the deficiency is compensated by other remodelers, such as the related RSF complex, which can also slide nucleosomes and may be involved in H2A.V turnover (Hanai et al., 2008).

Methods

Request a detailed protocol
AcfC mutant flies, backcrossed into OrR (wild-type), were generated and characterized before (Baldi et al., 2018; Scacchetti et al., 2018). The AcfC stock is maintained homozygous and all the embryos were collected from crosses of homozygous AcfC mothers and fathers. For larval hatching analysis, 2-3.5 h old embryos were collected and placed on agar-apple juice plates. Embryos were irradiated with 0 Gy, 1 Gy or 10 Gy using a Faxitron CellRad X-ray source (130 kV, 5 mA) and incubated for additional 23 h at 25°C. Hatching rate was determined by counting the number of embryos that hatched into larvae. Rates were normalized to the mean of the unirradiated (0 Gy) condition. Plots were generated in R using ggplot2. p-values were calculated by fitting a linear model using the lm function in R. For immunofluorescence microscopy, 2-3.5 h old embryos on 10 cm collection plates where irradiated with 1 Gy as above. Unirradiated control embryos (0 Gy) were processed along-side. 15 min after irradiation, approximately half of the embryos were collected (15 min time point) from each plate, dechorionated in 25% bleach for 3-5 min and immediately fixed (see below). The remaining ones where kept at 25°C for additional 3 h (180 min time point) and then processed using the same protocol. Unirradiated embryos of the 15 min time point define the ‘0 min’ time point. After extensive washes with water, embryos were transferred to 1.5 ml tubes. Dechorionated embryos were heat-fixed by adding 500 µl of boiling TN solution (0.03% Triton-X-100, 68 mM NaCl) and immediately cooled down by adding 500 µl of ice-cold TN solution and by incubating them on ice for 5 min. The TN solution was replaced by 500 µl of n-heptane and 500 µl of 100% methanol was added. Tubes were shaken for 15 sec and embryos were allowed to settle on ice for 5 min. Embryos were washed twice with 500 µl of methanol and stored at -20°C in 200 µl of methanol. For immunostaining embryos were transferred into 0.2 ml PCR tubes and rehydrated by three 10 min washes with PBS/0.1% Triton-X-100. Embryos were blocked for 3 h in Blocking Solution [PBS/ 0.3% Triton-X-100/ 5% Normal Donkey Serum (Jackson ImmunoResearch)/ 5% non-fat milk]. After a brief wash with PBS, embryos were incubated overnight at 4°C with primary antibodies diluted in Blocking Solution. Primary antibodies used were rabbit α-H2A.V (1:100) (Börner and Becker, 2016) and mouse α-γH2A.V (1:1000) (UNC93-5.2.1, Developmental Studies Hybridoma Bank) (Lake et al., 2013). Embryos were washed 4 times (15 min each) with PBS/ 0.1% Triton-X-100, then incubated for 3 h at room temperature with secondary antibodies diluted in Blocking Solution (1:300 donkey α-Rabbit,Alexa488, 1:500 donkey α-Mouse.Cy3, both from Jackson ImmunoResearch). Embryos were washed 4 times (15 min each) with PBS/ 0.1% Triton-X-100, then stained with 1:500 DAPI (in PBS) for 10 min. Embryos were washed 2 times (10 min each) with PBS, then mounted using Vectashield (Vector Laboratories).

Pictures were taken using a Leica Sp5 confocal microscope. For quantification, surface pictures of whole embryos were taken using a 20X objective and a 1.27X zoom factor (1024X1024 pixels) maintaining the same settings across all conditions and biological replicates. H2A.V and γH2A.V signals were quantified using a custom script with Fiji (Schindelin et al., 2012). The γH2A.V signal was calculated as follows. For each image the ratio of γH2A.V/H2A.V signal was calculated ("adjusted γH2A.V signal") and averaged across embryos of the same biological replicate. Then, the adjusted γH2A.V signal for each time point was normalized to the mean of the “0 min” time point. Plots were generated in R using ggplot2. p-values were calculated by fitting a linear model using the lm function in R.

Fly strains, antibodies, scripts and raw data are available upon request.

Acknowledgments: We thank T. Schauer for help and feedback in statistical analysis.

References
Aydin, O.Z., Vermeulen, W., and Lans, H. (2014). ISWI chromatin remodeling complexes in the DNA damage response. Cell Cycle 13, 3016-3025. PMID: 25486562.
Baldi, S., and Becker, P.B. (2013). The variant histone H2A.V of Drosophila--three roles, two guises. Chromosoma 122, 245-258. PMID: 23553272.
Baldi, S., Jain, D.S., Harpprecht, L., Zabel, A., Scheibe, M., Butter, F., Straub, T., and Becker, P.B. (2018). Genome-wide Rules of Nucleosome Phasing in Drosophila. Mol Cell 72, 661-672 e664. PMID: 30392927.
Becker, P.B., and Horz, W. (2002). ATP-dependent nucleosome remodeling. Annu Rev Biochem 71, 247-273. PMID: 12045097.
Börner, K., Jain, D., Vazquez-Pianzola, P., Vengadasalam, S., Steffen, N., Fyodorov, D.V., Tomancak, P., Konev, A., Suter, B., and Becker, P.B. (2016). A role for tuned levels of nucleosome remodeler subunit ACF1 during Drosophila oogenesis. Dev Biol 411, 217-230. PMID: 26851213.
Börner, K., and Becker, P.B. (2016). Splice variants of the SWR1-type nucleosome remodeling factor Domino have distinct functions during Drosophila melanogaster oogenesis. Development 143, 3154-3167. PMID: 27578180.
Chioda, M., Vengadasalam, S., Kremmer, E., Eberharder, A., and Becker, P.B. (2010). Developmental role for ACF1-containing nucleosome remodelers in chromatin organisation. Development 137, 3513-3522. PMID: 20843858 .
Corona, D.F.V., Eberharder, A., Budde, A., Deuring, R., Ferrari, S., and Varga-Weisz, P., Becker, P. B. (2000). Two histone fold proteins, CHRAC-14 and CHRAC-16, are developmentally regulated subunits of chromatin accessibility complex
Farrell, J.A., and O'Farrell, P.H. (2014). From egg to gastrula: how the cell cycle is remodeled during the Drosophila mid-blastula transition. Annu Rev Genet 48, 269-294. PMID: 25195504.

Fyodorov, D.V., Blower, M.D., Karpen, G.H., and Kadonaga, J.T. (2004). Acf1 confers unique activities to ACF/CHRAC and promotes the formation rather than disruption of chromatin in vivo. Genes Dev 18, 170-183. PMID: 14752009.

Hanai, K., Furuhashi, H., Yamamoto, T., Akasaka, K., and Hirose, S. (2008). RSF governs silent chromatin formation via histone H2Av replacement. PLoS Genet 4, e1000111. PMID: 18454204.

Hartlepp, K.F., Fernandez-Tornero, C., Eberharter, A., Grune, T., Muller, C.W., and Becker, P.B. (2005). The histone fold subunits of Drosophila CHRAC facilitate nucleosome sliding through dynamic DNA interactions. Molecular and cellular biology 25, 9886-9896. PMID: 16260604.

Iida, T., and Araki, H. (2004). Noncompetitive counteractions of DNA polymerase epsilon and ISW2/yCHRAC for epigenetic inheritance of telomere position effect in Saccharomyces cerevisiae. Mol Cell Biol 24, 217-227. PMID: 14673157.

Jeggo, P.A., Downs, J.A., and Gasser, S.M. (2017). Chromatin modifiers and remodellers in DNA repair and signalling. Philos Trans R Soc Lond B Biol Sci 372. PMID: 28847816.

Klement, K., Luijsterburg, M.S., Pinder, J.B., Cena, C.S., Del Nero, V., Wintersinger, C.M., Dellaire, G., van Attikum, H., and Goodarzi, A.A. (2014). Opposing ISWI- and CHD-class chromatin remodeling activities orchestrate heterochromatic DNA repair. J Cell Biol 207, 717-733. PMID: 25533843.

Lake, C.M., Holsclaw, J.K., Bellendir, S.P., Sekelsky, J., and Hawley, R.S. (2013). The development of a monoclonal antibody recognizing the Drosophila melanogaster phosphorylated histone H2A variant (gamma-H2AV). G3 (Bethesda) 3, 1539-1543. PMID: 23833215.

Lan, L., Ui, A., Nakajima, S., Hatakeyama, K., Hoshi, M., Watanabe, R., Janicki, S.M., Ogigara, H., Kohno, T., Kanno, S., et al. (2010). The ACF1 complex is required for DNA double-strand break repair in human cells. Mol Cell 40, 976-987. PMID: 21172662.

LeRoy, G., Loyola, A., Lane, W.S., and Reinberg, D. (2000). Purification and characterization of a human factor that assembles and remodels chromatin. J Biol Chem 275, 14787-14790. PMID: 10747848.

Poot, R.A., Dellaire, G., Hulsmann, B.B., Grimaldi, M.A., Corona, D.F., Becker, P.B., Bickmore, W.A., and Varga-Weisz, P.D. (2000). HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. EMBO J 19, 3377-3387. PMID: 10880450.

Rother, M.B., and van Attikum, H. (2017). DNA repair goes hip-hop: SMARCA and CHD chromatin remodelers join the break dance. Philos Trans R Soc Lond B Biol Sci 372. PMID: 28847822.

Sanchez-Molina, S., Mortusewicz, O., Bieber, B., Auer, S., Eckey, M., Leonhardt, H., Friedl, A.A., and Becker, P.B. (2011). Role for hACF1 in the G2/M damage checkpoint. Nucleic Acids Res 39, 8445-8456. PMID: 21745822.

Schindelin, J., Arganda-Carreras, I., Frise, E., Kaynig, V., Longair, M., Pietzsch, T., Preibisch, S., Rueden, C., Saalfeld, S., Schmid, B., et al. (2012). Fiji: an open-source platform for biological-image analysis. Nat Methods 9, 676-682. PMID: 22743772.

Funding: This work was founded by European Research Council (ERC), grant MSCA-ITN-2014-ETN No. 642934

Author Contributions: Alessandro Scacchetti: Conceptualization, Investigation, Formal analysis, Writing - original draft, Writing - review and editing, Data curation, Methodology, Visualization. Peter B. Becker: Conceptualization, Project administration, Supervision, Writing - review and editing, Funding acquisition.

Reviewed By: Anonymous

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Citation: Scacchetti, A; Becker, PB (2020). Loss of nucleosome remodelers CHRAC/ACF does not sensitize early Drosophila embryos to X-rays. microPublication Biology. https://doi.org/10.17912/micropub.biology.000287
