The ISWI chromatin remodelling factor NURF is not required for mitotic male X chromosome organisation

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Abstract

The nucleosome remodelling factor (NURF) is an ISWI-class ATP-dependent chromatin remodeling enzyme required both for gene expression and higher order chromatin organisation. NURF binds to histone modifications that decorate the Drosophila polytene male X chromosome and is required to maintain correct organisation of this chromosome. NURF mutants exhibit distorted and decondensed polytene male X chromosomes dependent on the presence of the male-specific lethal (MSL) complex. Here we tested whether mitotic chromosomes similarly require NURF to maintain correct morphology. Surprisingly, although the MSL complex remains associated with mitotic male X chromosomes, NURF is not required to maintain morphology. While the ISWI subunit of NURF is known to remain associated with mitotic chromosomes we show that the NURF specificity subunit Nurf301/BPTF dissociates from chromatin during both Drosophila and human mitosis, further illuminating that NURF is dispensable for mitotic chromosome organisation.

Figure 1. The chromatin remodelling factor NURF is not required for mitotic chromosome cohesion, condensation or X chromosome morphology: A) Mutation of either the NURF specificity subunit Nurf301 or the NURF catalytic subunit Iswi does not disrupt male X chromosome morphology or result in loss of sister chromatid cohesion. Representative neuroblast mitotic chromosome spreads from control (w1118), Nurf3012 homozygous mutant and Iswi1/Iswi2 transheterozygous mutant male and female larval brain squashes are presented. X, X chromosome, Y, Y chromosome. Inset (sg) indicates the bloated male polytene X chromosome phenotype observed in salivary glands from the same animal used for mitotic chromosome preparations. B) Antibody staining using anti-Msl-2 antibodies reveals that components of the dosage compensation complex remain associated with mitotic male X-chromosomes (arrows). C-D) In contrast Nurf301 is not associated with mitotic chromosomes as revealed by double staining with anti-Nurf301 antibodies.
Nurf301 and certain histone modifications and therefore modified chromosome preparation methods to omit acid spreading (Pimpinelli et al., 2002). However, these protocols employed acetic acid treatment to assist chromosome remodeling complexes (Oppikofer et al., 2000). The lack of a mitotic male X chromosome requirement for NURF could be simply due to the dissociation of the MSL complex from mitotic chromosomes. It was shown previously that the bloated NURF mutant male polytene X chromosome phenotype (Bai et al., 2001; Corona et al., 2002).

Mutations in NURF subunits exhibit striking disruption of male polytene X chromosomes. This includes a characteristic decondensation or “bloating” of male polytene X chromosomes, showing that NURF functions to organise extensive regions of the male polytene X chromosome. Polytene chromosomes are the large chromosomes of the giant nuclei of salivary glands from the same mutant animals used for preparation of neuroblast squashes (inset labelled sg in Fig. 1A). Thus, even though components of the MSL complex remain associated with mitotic chromosomes, NURF is dispensable for correct mitotic male X chromosome morphology.

Further inspection of neuroblast squashes indicated no discernible defects in organisation of mitotic autosomes in male or female Nurf301 or Iswi mutant larvae. Mitotic chromosomes were prepared from both male and female larvae. As shown in Fig. 1A no differences in male X chromosome morphology relative to wild-type were detected in either male Nurf301 or Iswi mutant animals. As control the characteristic distorted polytene male X chromosome phenotype could be observed in salivary glands from the same mutant animals used for preparation of neuroblast squashes (inset labelled sg in Fig. 1A).

The lack of a mitotic male X chromosome requirement for NURF could be simply due to the dissociation of the MSL complex from mitotic chromosomes. It was shown previously that the bloated NURF mutant male polytene X chromosome is dependent on the presence of the MSL complex. MSL complex mutants or deletion of high affinity MSL complex binding sites are able to suppress the NURF mutant male polytene X chromosome phenotype (Bai et al., 2007; Corona et al., 2002). However, immunofluorescence microscopy of mitotic chromosomes using anti-Msl-2 antibodies detected continued presence of Msl-2 on mitotic chromosomes (Fig. 1B). Thus, even though components of the MSL complex remain associated with mitotic chromosomes, NURF is dispensable for correct mitotic male X chromosome morphology.

Initial anti-Nurf301 antibody staining of neuroblast squashes prepared using standard methodologies failed to detect Nurf301 at any stage of the cell cycle. However, these protocols employed acetic acid treatment to assist chromosome spreading (Pimpinelli et al., 2010). We observed that acid treatment caused either the loss or masking of signal from Nurf301 and certain histone modifications and therefore modified chromosome preparation methods to omit acid spreading.
treatment, deploying instead an acid-free fixation protocol developed for polytene chromosomes (Di Mario et al., 2006). Interphase nuclei and mitotic chromosomes from larval neuroblasts of control third instar *Drosophila* larvae were double-immunostained with antibodies against Nurf301 as well as antibodies against histone modifications that are enriched on mitotic chromosomes: anti-histone H3 phosphorylated at threonine position 3 (H3T3p) as well as the phosphomethyl mark (H3T3pK4me3). While we could readily detect Nurf301 in interphase neuroblast nuclei, Nurf301 signal was absent from mitotic chromosomes (Fig. 1C,D). This was not due to inability to stain chromatin proteins on mitotic chromosomes as both the H3T3pK4me3 (Fig. 1C) and the H3T3p mark (Fig. 1D) were detected.

These data suggest that NURF is ejected from mitotic chromatin in neuroblasts. To confirm whether this is generally observed in other tissues or organisms we performed immunostaining of *Drosophila* embryos and human HeLa cells using either anti-Nurf301 or anti-BPTF antibodies. Immunostaining of *Drosophila* embryos revealed Nurf301 was predominantly localised to chromatin during interphase (Fig. 1E). During metaphase Nurf301 was delocalized from chromosomes, re-associating with chromatin from anaphase. In a similar manner human BPTF was detected on interphase HeLa cell chromatin but was not localised to chromosomes during mitosis (Fig. 1F).

Cumulatively these data indicate that NURF is ejected from mitotic chromatin and is not required for proper mitotic chromosome condensation or cohesion. Moreover, although *Nurf301* mutant male *polytene* X chromosomes are distorted and decondensed, *Nurf301* mutant male mitotic chromosomes are unaffected. Strikingly, while targeting of the MSL complex to polytene chromosomes is sufficient to trigger aberrant chromosome morphology in *Nurf301* mutants (either normally on the male X chromosome or when targeted to autosomes using roX transgenes (Bai et al., 2007)), the MSL complex is unable to do so on when present on mitotic chromosomes. It is tempting to speculate that this reflects underlying differences in the ability of the MSL complex to promote transcription on polytene versus mitotic chromosomes. Even though recent data indicate that mitotic chromosomes are not transcriptionally silent as has previously been believed, levels of transcription are generally lower (Palozola et al., 2017).

**Methods**

Request a detailed protocol

**Preparation of *Drosophila* neuroblast mitotic chromosomes**

The *Nurf301* strain and isogenic w*1118* control strain are as described (Badenhorst et al. (2002)). *Isw1* and *Isw2* were obtained from John Tamkun and are as described (Deuring et al. (2000)). All strains were raised at 22°C. Mitotic chromosomes were prepared as described by Pimpinelli et al. (2010). In brief 3rd instar larval brains were dissected in saline (0.7% NaCl), washed briefly in saline and transferred to hypotonic solution (0.5% Sodium Citrate) for 10 minutes.Brains were cleared in 45% acetic acid on a siliconized coverslip, fragmented using a tungsten needle and squashed onto slides between blotting paper. Slides were frozen in liquid nitrogen, coverslips removed and slides mounted in Vectashield with DAPI (Vector Laboratories). For anti-Msl-2 staining, the same protocol was followed except after freezing samples were fixed in 1% paraformaldehyde (Polysciences, 18814-10) for 10 minutes prior to two washes in PBTw buffer (1XPBS, 0.1% Tween-20). Primary antibody incubation was performed in blocking buffer (1XPBS, 0.1% Tween-20, 0.1% FCS) containing anti-Msl-2 antibodies (1:1000) overnight. Slides were washed three times in PBTw buffer, followed by secondary antibody incubation in PBTw containing Cy3-conjugated anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 1:400) for 2 hours. Slides were washed in PBTw as above, mounted in Vectashield with DAPI (Vector Laboratories) and imaged using a Zeiss LSM780 confocal microscope.

Immunostaining of mitotic chromosomes for Nurf301 and histone modifications followed the same procedure with the exception that after hypotonic treatment brains were processed using an acid-free protocol as described by DiMario et al. (2006). Briefly, brains were fixed in Brower’s fixation buffer (150mM PIPES, 3mM MgSO4, 1.5mM EGTA, 1.5% NP-40 (pH 6.9) containing 2% formaldehyde (Polysciences, 18814-10)) for 3 minutes, washed in PBT (1XPBS, 0.1% Triton-X100) for 2.5 minutes, transferred to 50% Glycerol for 5 minutes, fragmented and squashed onto Poly-L-lysine (Sigma, P8920) coated slides. Slides were frozen in liquid nitrogen then processed for double-immunostaining. Slides were incubated with anti-Nurf301 antibodies (1:200 in blocking buffer) overnight at 4°C then washed with PBTw three times for 10 minutes. Secondary antibody incubation was conducted using Cy3-conjugated anti-Rabbit IgG (H+L) (Jackson ImmunoResearch, 1:400) for 2 hours. Slides were washed in PBTw as above, mounted in Vectashield with DAPI (Vector Laboratories) and imaged using a Zeiss LSM780 confocal microscope.
Fixation and permeabilization of *Drosophila* embryos

*Drosophila* embryos were collected on apple agar collection plates and rinsed with water onto a Nitex mesh sieve to remove residual yeast. Embryos were dechorionated in 5% sodium hypochlorite solution (Fluka, FL71696) for three minutes. The embryos were transferred to a 1.5ml Eppendorf tube containing 50% n-heptane (Sigma) and 50% PEM-formaldehyde solution (0.1 M PIPES (pH 6.9), 2 mM EGTA, 1 mM MgSO4, 4% formaldehyde (Pierce)). Embryos were fixed for 25 minutes at room temperature on a rotator. The aqueous layer was removed, 1 volume of methanol was added, and the tube was vortexed for one minute to remove the vitelline membrane. The heptane/methanol phases were allowed to separate and upper phases were removed. Two washes were performed with 1 ml methanol and then with 1:1 dilution of methanol and PBTr (0.1% Tween 20 in 1XPBS). Embryos were washed with 1ml PBTr three times for five minutes on a rotator and immunostaining performed as described above.

Huma cell culture and immunofluorescence microscopy

HeLa cells (CCL2 isolate, RRID: CVCL_0030) were obtained from the ATTC and STR profile verified (Amelogenin: X, CSF1PO: 9,10; D13S317: 12,13,3; D16S539: 9,10; D5S818: 11,12; D7S820: 8,12; THO1: 7; TPOX: 8,12; vWA: 16,18). HeLa Cells were incubated at 37°C with 5% CO2 and cultured in Dulbecco’s Modified Eagle’s Medium (DMEM) containing 10% FBS, 100U Penicillin-Streptomycin (Sigma), 1mM Glutamine (GIBCO) and 1x non-essential amino acids (Sigma). Slides were prepared for immunofluorescence microscopy as described in Kwon *et al.* (2020), blocked overnight at 4°C in blocking buffer (1XPBS, 0.1% Tween-20, 0.1% FCS). Primary antibody incubation using anti-BPTF/FALZ antibodies (1:200) was performed in blocking buffer for 2 hours followed by three washes in PBTr buffer (1XPBS, 0.1% Tween-20). Secondary antibody incubation was performed in PBTr at room temperature for 1 hours using Cy3-conjugated AffiniPure goat anti-rabbit antibody (Jackson ImmunoResearch Cat. 111-167-003) at 1:400 dilution. Slides were washed as above, mounted using Vectashield with DAPI (Vector Laboratories) and imaged using a Zeiss LSM780 confocal microscope.

Reagents

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References


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