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C-Nap1 mutation affects centriole cohesion and is associated with a Seckel-like syndrome in cattle

Sandrine Floriot, Christine Vesque, Sabrina Rodriguez, Florence Bourgain-Guglielmetti, Anthi Karaïskou, Mathieu Gautier, Amandine Duchesne, Sarah Barbey, Sébastien Fritz, Alexandre Vasilescu, Maud Bertaud, Mohammed Moudjou, Sophie Halliez, Valérie Cormier-Daire, Joyce E.L. Hokayem, Erich A. Nigg, Luc Manciaux, Raphaël Guatteo, Nora Cesbron, Geraldine Toutirais, André Eggen, Sylvie Schneider-Maunoury, Didier Boichard, Joelle Sobczak-Thépot✉ & Laurent Schibler✉

Easier and Safer Biological Staining: High Contrast UranylLess Staining of TEM Grids using mPrep/g Capsules

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DOI: <https://doi.org/10.1017/S1431927615004407> Published online: 23 September 2015



Export citation

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Abstract

721

doi:10.1017/S1431927615004407

Paper No. 0361

Microsc. Microanal. 21 (Suppl 3), 2015

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Easier and Safer Biological Staining: High Contrast UranylLess Staining of TEM Grids using mPrep/g Capsules

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Uranyl acetate (UA) has been used for decades in life science electron microscopy as a positive and negative stain [1, 2]. But due to recent regulations (especially in Europe and Japan) there has been considerable effort to find less toxic and non-radioactive replacements, including stains based on Oolong tea extracts [3], platinum blue [4], and gadolinium [5]. This report demonstrates a safe and non-radioactive stain that provide the contrast, broad utility, rapid staining, and ease of use of UA.



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☐ C-Nap1 mutation affects centriole cohesion and is associated with a Seckel-like syndrome in cattle.

(PMID:25902731 PMID:PMC4423223)

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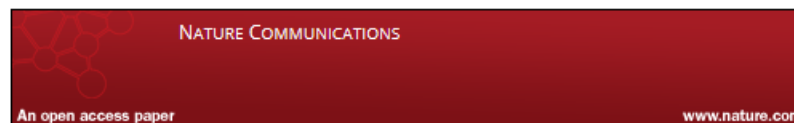
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Nat Commun. 2015 April 23; 6: 6894.

doi: [10.1038/ncomms7894](https://doi.org/10.1038/ncomms7894)

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C-Nap1 mutation affects centriole cohesion and is associated with a Seckel-like syndrome in cattle

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Received 2014 July 27; Accepted 2015 March 11.

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Chapter 6 OPEN ACCESS

Transmission Electron Microscopy for the Characterization of Cellulose Nanocrystals

By Madhu Kaushik, Carole Fraschini, Grégory Chauve, Jean-Luc Putaux and Audrey Moores

DOI: 10.5772/60985

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Article

Overview

1. Introduction

1.1. Native cellulose and the production of cellulose nanocrystals



Figure 1. (a) Structural hierarchy

Transmission Electron Microscopy for the Characterization of Cellulose Nanocrystals

Madhu Kaushik¹, Carole Fraschini², Grégory Chauve², Jean-Luc Putaux^{3, 4} and Audrey Moores¹

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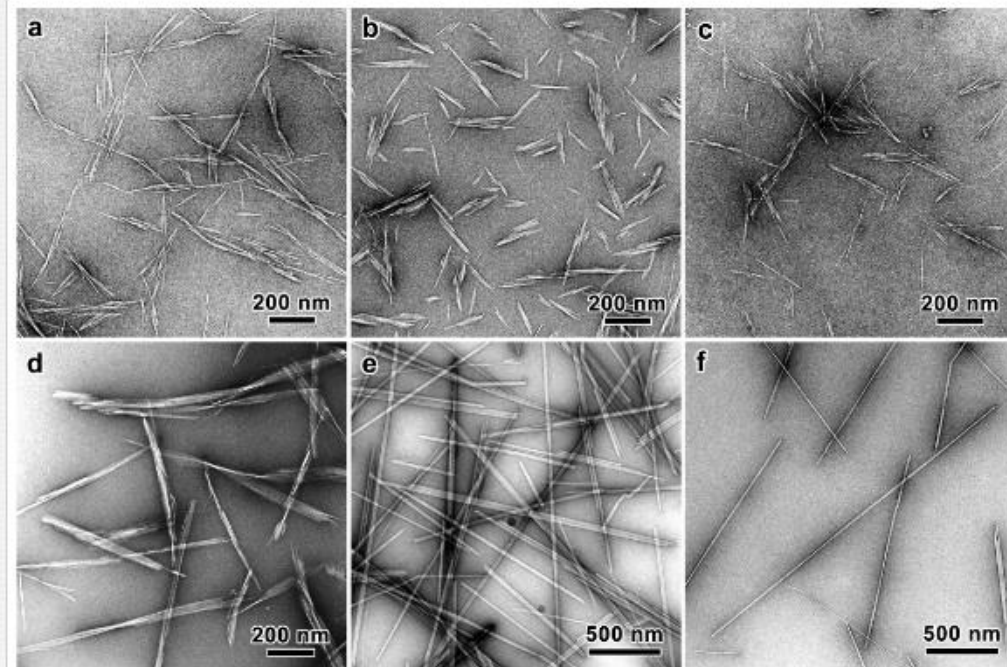


FIGURE 2.

TEM images of negatively stained preparations of CNCs of various origins: a) wood (courtesy of G. Chauve, FPInnovations); b) cotton (courtesy of F. Azzam, CERMAV); c) bamboo (courtesy B. Jean, CERMAV); d) *Gluconacetobacter xylinus* (courtesy of H. Bizot, INRA); e) *Glaucocystis* (courtesy of Y. Nishiyama, CERMAV); f) *Halocynthia papillosa* (courtesy of A. Osorio-Madrado, A. Ludwig

Article

Upregulated Autophagy in Sertoli Cells of Ethanol-Treated Rats Is Associated with Induction of Inducible Nitric Oxide Synthase (iNOS), Androgen Receptor Suppression and Germ Cell Apoptosis

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Academic Editor: Anthony Lemarié

Received: 23 February 2017; Accepted: 10 May 2017; Published: 15 May 2017

Abstract: This study was conducted to investigate the autophagic response of Sertoli cells (SCs) to acute ethanol toxicity using in vivo and in vitro models. Adult Wistar rats were intraperitoneally injected with either 5 g/kg ethanol or phosphate-buffered saline (for the control group) and sacrificed 0, 3, 6 and 24 h after injection. Compared to the control group, enhanced germ cell apoptosis was observed in the ethanol-treated rats (ETRs) in association with upregulation of iNOS and reduced expression of androgen receptor protein levels in SCs, which were resistant to apoptosis. Meanwhile, autophagy was upregulated in ETR SCs (peaking at 24 h) compared to the control group, as evidenced by transcription factor EB (TFEB) nuclear translocation, enhanced expression of microtubule-associated protein 1 light chain3-II (LC3-II), lysosome-associated membrane protein-2 (LAMP-2), pan cathepsin protein levels and reduced expression of p62. This upregulation of SC autophagy was confirmed ultrastructurally by enhanced formation of autophagic vacuoles and by immunofluorescent double labelling of autophagosomal and lysosomal markers. Study of cultured SCs confirmed enhanced autophagic response to ethanol toxicity, which was cytoprotective based on decreased viability of SCs upon blocking autophagy with 3-methyladenine (3-MA). The results highlighted the molecular mechanisms of prosurvival autophagy in ETR SCs for the first time, and may have significant implications for male fertility.

4.11. TEM and Quantitative Analysis

Ultra-thin sections (70 nm) from blocks embedded in epoxy resin were cut out with a diamond knife, stained first with **Uranyless** stain (Delta Microscopy, Mauressac, France) [60,61] and then with lead citrate, and examined using an H-7650 transmission electron microscope (Hitachi, Japan). For quantification of autophagic vacuoles in SCs, 10 lower-magnification photomicrographs from the testes of controls and ETRs ($\times 2500$ – 3000) were selected (each image containing at least one SC nucleus) [31,62,63].

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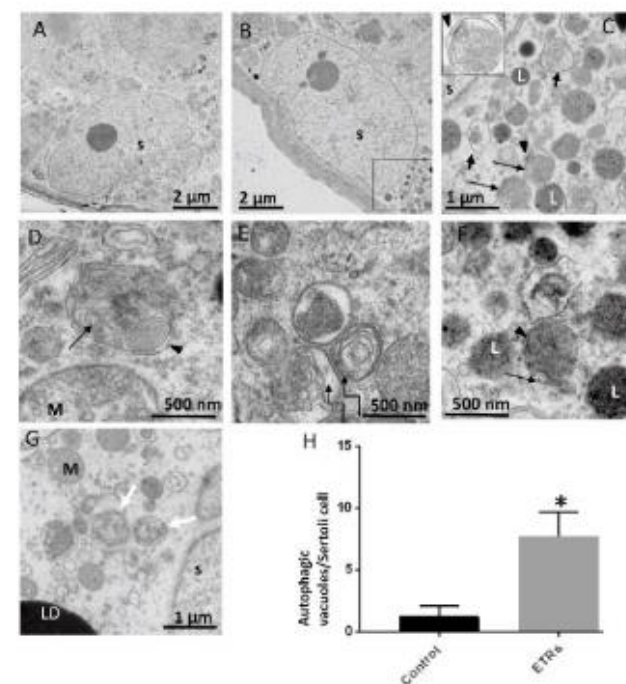


Figure 5. Ultrastructural features of upregulated autophagy in ETR SCs. TEM of control (A) and ETRs (B–G). The histogram (H) shows a significant increase in the number of autophagic vacuoles (AVs) in ETR SCs. The long black arrows mark autophagosomes with a double limiting membrane (arrowheads) (magnified in the inset in (C)). The short arrows indicate autolysosomes. The broken arrows in (E) show multilamellar bodies, while the white arrows in (G) show autophagosomes containing fragmented mitochondria. S: SC nucleus; L: lysosome; M: mitochondria; LD: lipid droplet. * $p < 0.05$.

ORIGINAL ARTICLE

PNPLA1 defects in patients with autosomal recessive congenital ichthyosis and KO mice sustain PNPLA1 irreplaceable function in epidermal omega-O-acylceramide synthesis and skin permeability barrier

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Electron microscopy

The skin was cut in pieces of $\approx 1\text{mm}^2$, fixed in 2% glutaraldehyde in Cacodylate buffer (0.1 M, pH 7.2, EMS, Hatfield, PA) for 24 h at 4°C and post-fixed with 1% OsO₄ in Cacodylate buffer (Cacodylate 0.1 M, OsO₄ 1%, EMS) for 1 h at 4°C. For lamellae visualization, 50 μM skin cryosections were fixed in 2% glutaraldehyde in Cacodylate buffer (0.1 M, pH 7.2, EMS, Hatfield, PA) for 24 h at 4°C and post-fixed with 1% OsO₄ in Cacodylate buffer (Cacodylate 0.1 M, OsO₄ 1%, EMS) for 1 h at 4°C followed by two post-fixation with 0.2% RuO₄, 0.25% K₃Fe (Cn)₆ in Cacodylate buffer for 1 h at 4°C.

Samples were then dehydrated in a graded acetone series and embedded in Spurr's resin. After 48 h of polymerization at 60°C, ultrathin sections (80 nm thick) were mounted on 75 mesh formvar-carbon coated copper grids. Sections were stained with Uranylless (Delta Microscopies) and lead citrate. Grids were examined with a TEM (Jeol JEM-1400, JEOL Inc) at 80 kV. Images were acquired using a digital camera (Gatan Orius, Gatan Inc).