

## ANALYSIS OF *SMC1*-RNAi INDUCED CHROMOSOME ABERRATIONS BY ATOMIC FORCE MICROSCOPY

Antonio Musio, Maria Luisa Focarelli, Paolo Vezzoni, Istituto di Tecnologie  
Biomediche, CNR, Segrate, Italy.

Tullio Mariani, Istituto per i Processi Chimico Fisici, CNR, Pisa, Italy.  
tullio.mariani@ipcf.cnr.it

The atomic force microscope (AFM) is a tool, which gives maps of the sample height and allows a 3D reconstruction of the sample surface with a resolution much higher than light microscopy. Thanks to its operating principle, the AFM can give images of unstained, untreated, native specimens and has already shown its usefulness in the analysis of cellular and subcellular structures [1, 2, 3].

In this report, we applied for the first time the AFM to the structural analysis of chromosome aberrations induced by RNA interference (RNAi). RNAi corresponding to *SMC1* mRNA was designed as recommended [4] with two base overhangs. The following gene-specific sequence was used: RNAi -*SMC1* 5'- AUC UCA UGG AUG CCA UCA G dTT-3'. Scrambled RNA was constructed as control. Primary human fibroblasts (at 40-60% confluence) were transfected with RNAi by using siPort Amine, or treated with aphidicolin (0.4  $\mu$ M) for 26 hr, alone or in combination with *SMC1* inhibitor.

The inhibition of *SMC1* and combined treatment (*SMC1*-inhibition plus aphidicolin) induced  $0.26\pm 0.5$  and  $1.79\pm 1.39$  aberrations per cell respectively. It is noteworthy that most of aberrations were gaps, and that their frequency increased after combined treatment. Unstained metaphases were imaged by AFM after individual and combined treatments. Some of the resulting images are reported in Figure 1.

Figure 1A presents a chromosomal exchange induced by aphidicolin in which the regions involved in the aberration are fused, while in Figure 1B a chromosome gap appearing as a decondensed chromatin region can be seen. This gap involves the terminal region of chromosome and further supports the notion that the gap itself originates from decondensed chromatin. In general, thanks to the height sensitivity of the instrument, it is relatively easy to discriminate, in AFM images between gaps and breaks. Finally, Figure 1C shows a chromosome with two aberrations induced by the combined treatment (aphidicolin plus RNAi-*SMC1*). This chromosome shows an isolated fragment, derived from the complete breakage of one chromatid, and a break in the other chromatid. The high resolution of the AFM reveals surface structures that deserve further analysis.

Our results show that Smc1 is required for chromosome stability also after aphidicolin treatment. It might play this role through two different pathways. First, an increase in Smc1 synthesis can stabilize sister chromatid cohesion improving the recruitment of DNA repair enzymes. Second, its phosphorylation may be required for activation of the S phase checkpoint as already shown to occur in irradiated cells [5, 6].

### References

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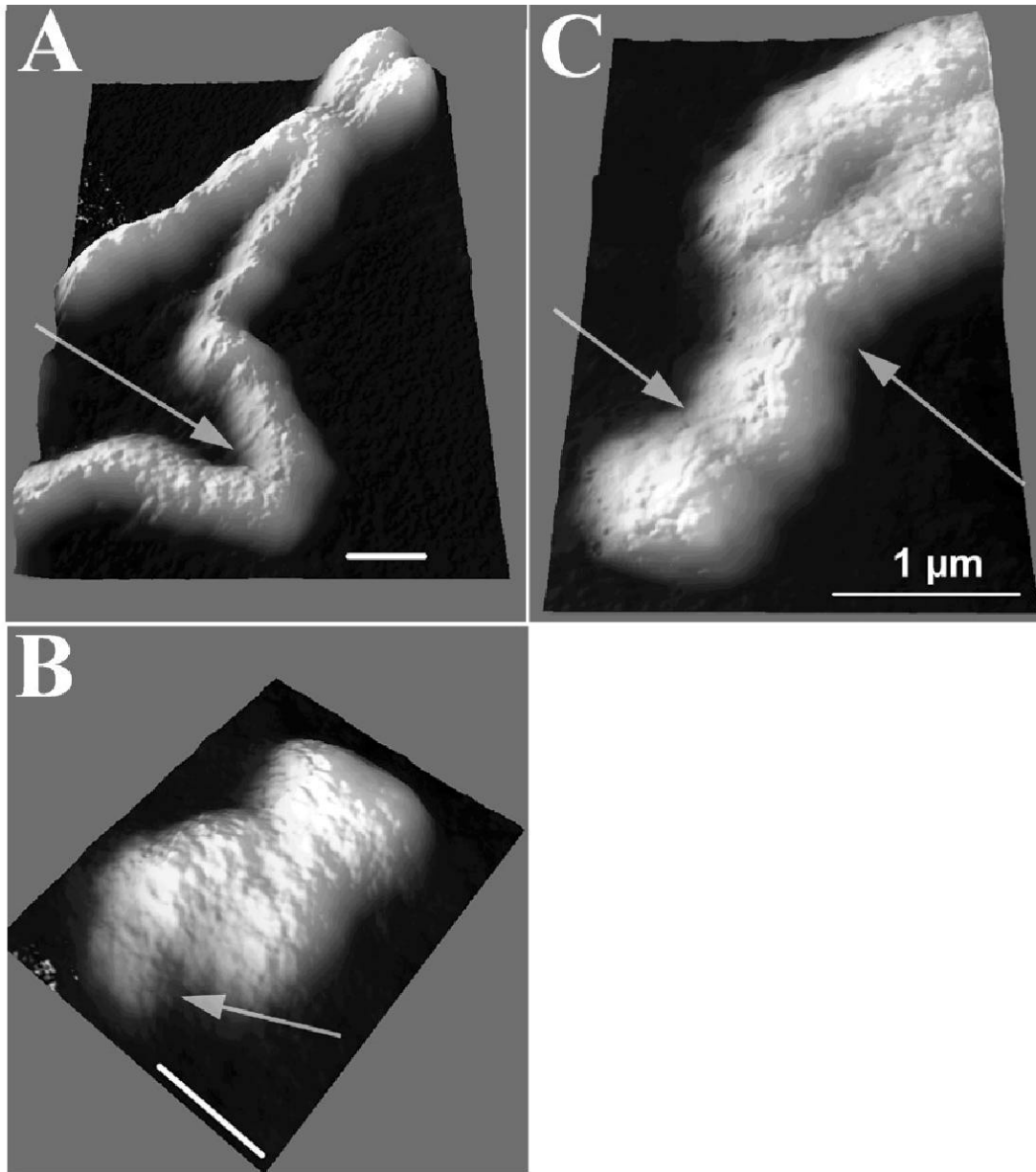


Figure 1 - Human chromosomes showing various aberrations, as imaged by Atomic Force Microscopy. The 3D information obtained by the AFM allows a perspective representation of the objects analysed. Bars correspond to 1  $\mu\text{m}$ .