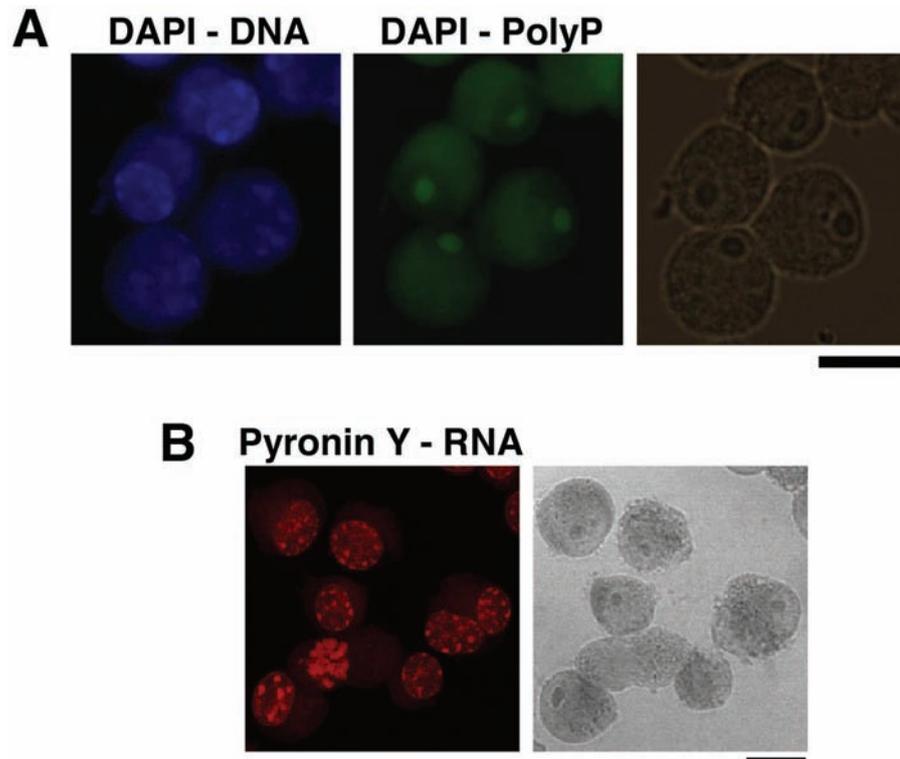


Myeloma cells contain high levels of inorganic polyphosphate which is associated with nucleolar transcription

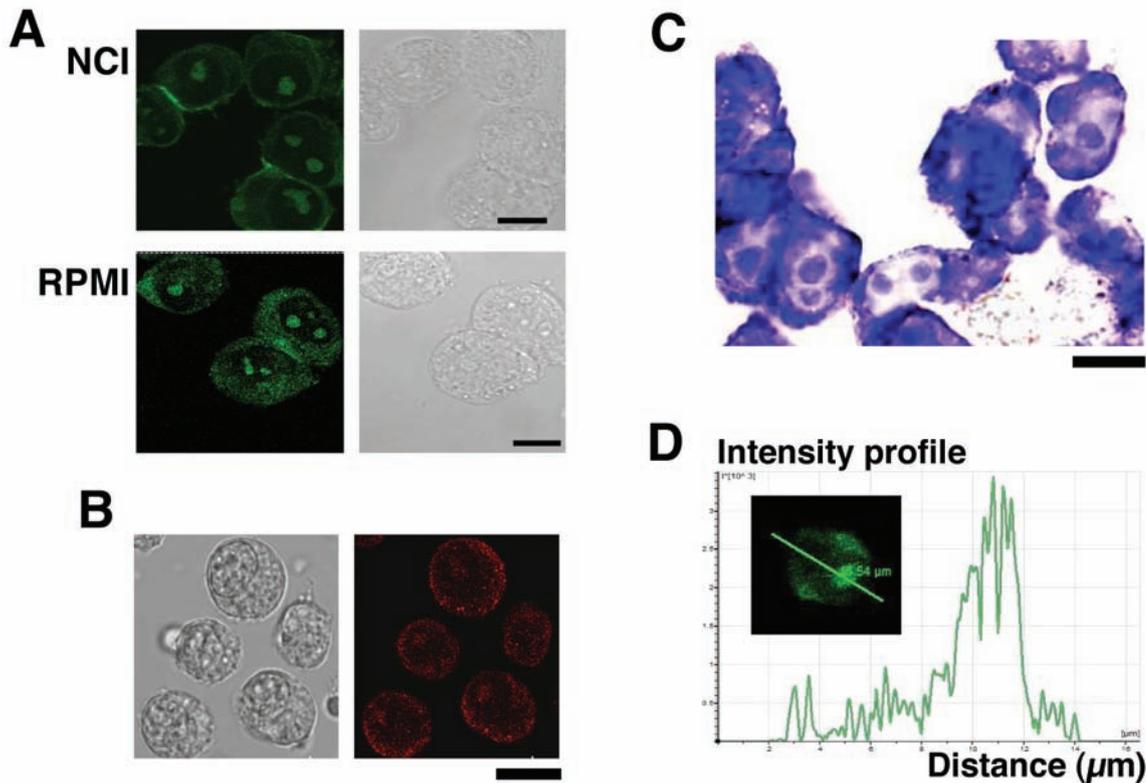
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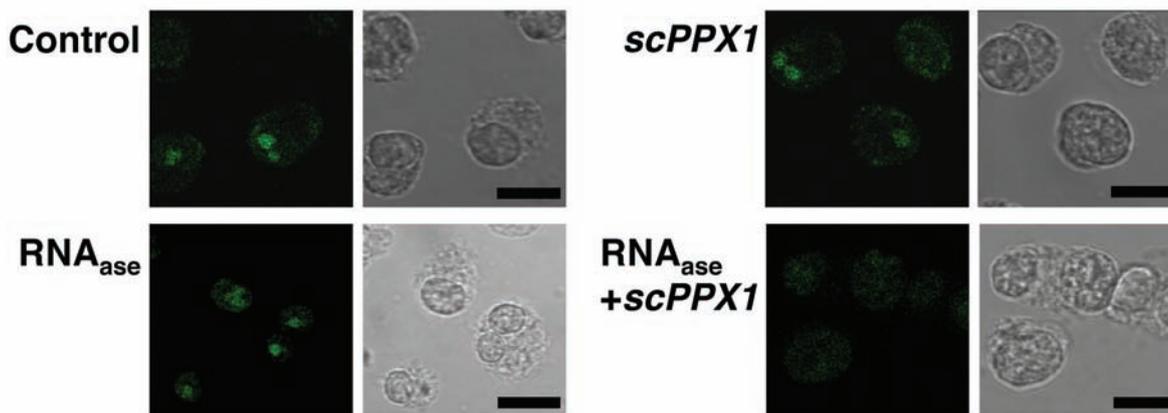
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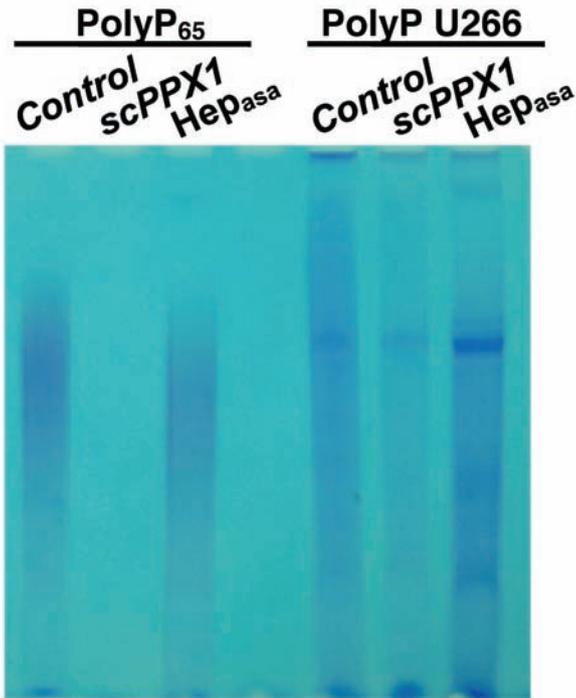
Online Supplementary Figure S1. Fluorescence microscopy for DNA, PolyP or RNA localization in the U266 human myeloma cell line. (A) Fluorescence microscopy with settings for localization of DNA (blue) or polyP (green) in fixed U266 MC using DAPI. Differential detections were performed with an Olympus BX-40 epifluorescence microscope with U-MNUA2 (excitation 360-370nm, emission: 420-460nm), or UMWIBA (excitation 460-490nm, emission: 515-550nm) fluorescence filters. (B) Fluorescence microscopy showing the localization of RNA (red) in fixed U266 MC using Pyronin Y. Corresponding bright field images are shown on the left. Bars: 10 μm. Representative experiments are shown (n=3).



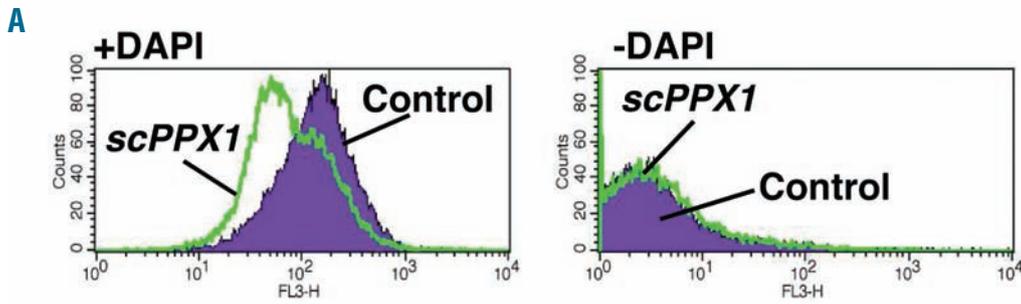
Online Supplementary Figure S2. PolyP distribution in the human myeloma cell lines (HMCL) U266, NCI-H929 and RPMI-8226. (A) Localization of cellular polyP was determined by specific polyP labeling using DAPI and confocal fluorescence microscopy as described in the *Design and Methods* section. Fluorescent images (left) and their corresponding bright field images (right) are shown. (B) Confocal microscopy of polyP localization in U266 MC using the recombinant PPBD of *E. coli* PPX linked to an Xpress epitope tag (Saito *et al.*, Appl Environ Microbiol. 2005;71:5692). Briefly, washed HMCL U266 were bound to polylysine-coated coverslips, fixed in 4% paraformaldehyde, and permeabilized with 0.2% Triton X-100 for 5 min. The coverslips were then blocked with 3% bovine serum albumin for 30 min. Staining was done by incubation for 1 h with: (a) PPBD (8 $\mu\text{g}/\text{mL}$) and anti-Xpress epitope monoclonal antibody (10 $\mu\text{g}/\text{mL}$); and (b) 1/500 diluted Alexa647-labeled anti-mouse IgG as a secondary antibody. All dilutions were done with Tris-buffered saline (100 mM Tris/HCl (pH 7.2) and 150 mM NaCl), and we added 1% bovine serum albumin to the media in both incubations with antibodies. A fluorescent image (right) and its corresponding bright field image (left) are shown. (C) U266 MC labeling using toluidine blue. Bars: 10 μm . (D) Fluorescence intensity profiles of U266 MC labeled for PolyP as described in (A). Relative intensity was collected along a line that crosses an entire cell, using Leica Confocal Software (Leica Microsystems). Results from representative experiments are shown (n=5).



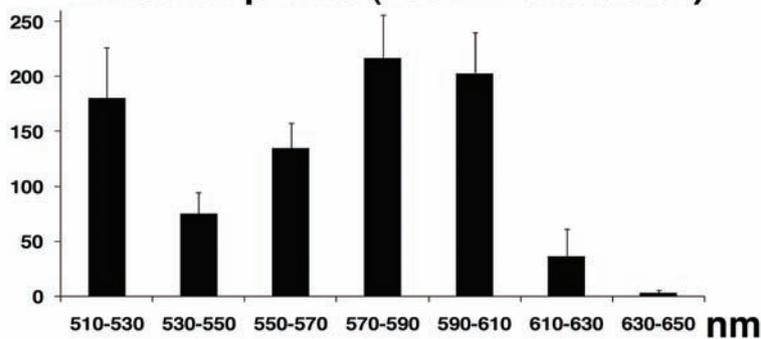
Online Supplementary Figure S3. PolyP in permeabilized MC treated with yeast exopolyphosphatase (scPPX1). U266 human myeloma cell line were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100 in PBS. Samples were then treated with RNA_{ase} and/or scPPX1 in the presence of 30 mM MgCl₂ at 37°C for 30 min. Cellular polyP was determined by specific polyP labeling with DAPI, using confocal fluorescence microscopy as described in the *Design and Methods* section. Fluorescent images (left) and their corresponding bright field images (right) are shown. Bars: 10 μm . Representative experiments are shown (n=3).



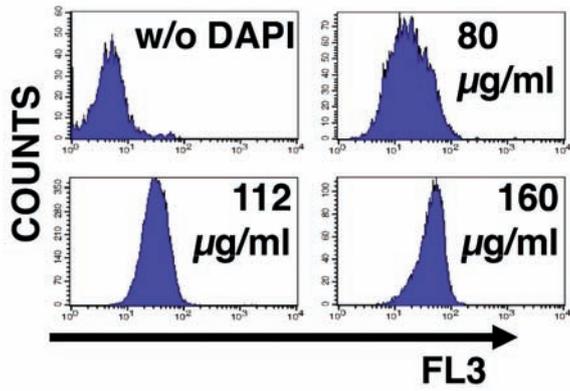
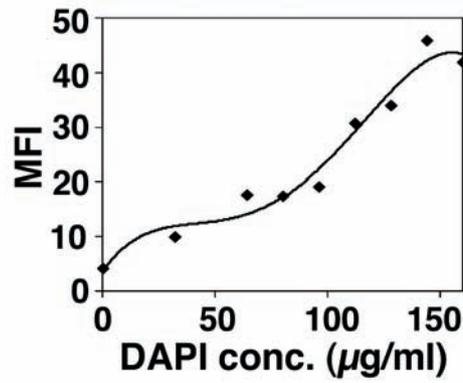
[Online Supplementary Figure S4](#). Enzymatic analysis of polyP extracted from MC. Size analysis of a polyP₆₅ commercial standard (PolyP₆₅) and total polyP extracted from the U266 human myeloma cell line (PolyP U266). Samples (approx. 10 µg) were treated for 1 h at 37°C with Tris-HCl buffer (Control), scPPX1 (scPPX1), or Heparinase III (Hep_{asa}, Sigma 0.3 units), in the presence of 30 mM MgCl₂. Extracted polyP was separated by 6% urea- polyacrylamide gel electrophoresis (U266) and the gel was stained with toluidine blue to visualize polyP. A representative experiment is shown (n=3).



B Emission profile (488 nm excitation)



[Online Supplementary Figure S5](#). Validation of the flow cytometry method. (A) Enzymatic analysis of DAPI fluorescence, measured by flow cytometry. Cells from the U266 human myeloma cell line (HMCL) were fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS) and permeabilized with 0.1% Triton X-100 in PBS. Samples were then incubated in the absence (Control) or presence of scPPX1 (scPPX1), for 30 min at 37°C adding 30 mM MgCl₂. Flow cytometry analysis in the presence (+DAPI) and absence (-DAPI) of DAPI was performed as described in the *Design and Methods* section. Cell fluorescence of CD138 positive cells in the FL3 channel was determined. A representative experiment is shown (n=3). (B) Fluorescence emission profile of DAPI labeled U266 HMCL, excitation wavelength, 488 nm. The nucleolar intensity of fluorescence was determined for ten cells by confocal fluorescence microscopy at the indicated emission ranges using Leica Confocal Software (Leica Microsystems). These profiles resemble the emissions previously reported for pure polyP (J Fluoresc. 2008 18(5):859-66; J Biol Chem. 2010 285(13):9420-8; Can J Microbiol. 1980 26:912; and BBA 1982 721:394). Results represent the mean ± SD of fluorescence intensity in arbitrary units.

A**B**

Online Supplementary Figure S6. Optimization of DAPI concentration for flow cytometry analysis. (A) U266 human myeloma cell line (HMCL) cells, labeled with anti-CD138 phycoerythrin antibodies, were incubated with increasing concentrations of DAPI. Flow cytometry analysis in the FL3 channel was performed as described in the *Design and Methods* section. (B) Quantification of the geometric mean fluorescence intensity (MFI), in the FL3 channel, of U266 HMCL cells after the addition of increasing concentrations of DAPI. Representative experiments are shown (n=3).