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## **Uncovering functions of long-chain polyphosphate in higher eukaryotes - storage and synthesis of polyphosphate in matrix vesicles during mineralization**

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Inorganic polyphosphate (polyP) is a fundamental molecule that occurs throughout life. PolyP has been found in mammalian tissues with particularly high levels of polyP in bone and cartilage, however its metabolism and functions remain poorly understood. Here, we initially demonstrate the highest concentrations of polyP yet observed in a higher eukaryote in matrix vesicles (MVs) secreted from mineralized osteoblasts. We also observed that MVs isolated from both osteoblasts and chondrocytes have a remarkable ability to synthesize very long-chain polyP under physiological condition. PolyP could be hydrolysed to  $P_i$  by one of the critical enzymes of mineralization, tissue non-specific alkaline phosphatase (TNAP). PolyP was consumed during mineralization induced by MVs. Extravesicular PolyP acted as an efficient inhibitor to suppress MV-induced HA formation. Biochemical properties of polyP synthesis were consistent with membrane-bound enzyme activity within the MV. Taken together, our work introduces a new fundamental macromolecule that could be a critical modulator of mineralization in the extracellular matrix.