SHORT COMMUNICATION

MECHANISMS OF INITIATION OF SKELETAL MINERALIZATION: THE ROLE OF PHOSPHATASES

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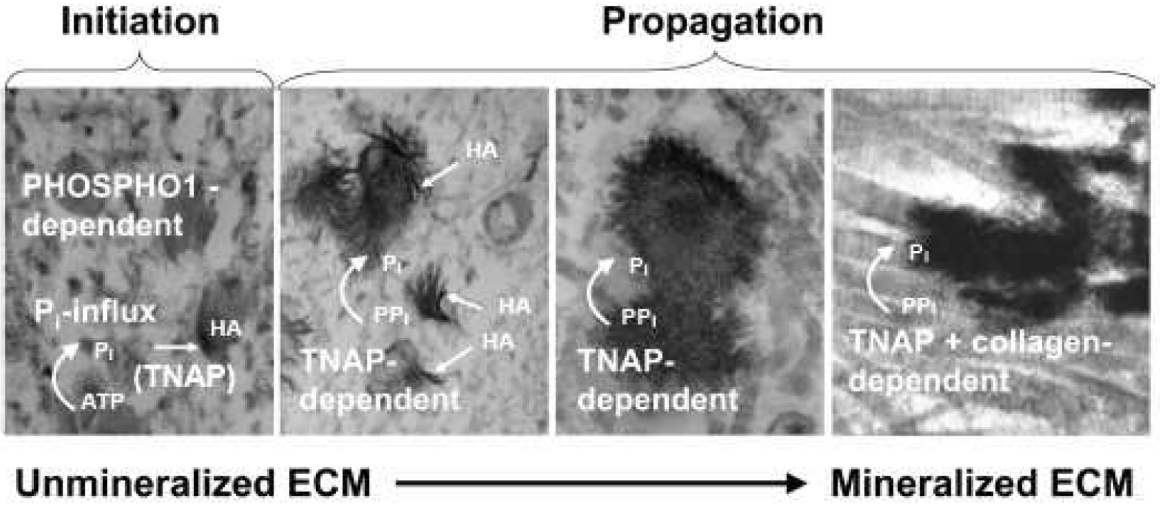
Endochondral ossification is a carefully orchestrated process mediated by promoters and inhibitors of mineralization. Phosphatases are implicated, but their identities and functions remain unclear. Alkaline phosphatase (TNAP) plays a crucial role promoting mineralization of the extracellular matrix by restricting the concentration of the calcification inhibitor inorganic pyrophosphate (PPi) and by hydrolyzing ATP helping drive mineralization forward. Mutations in the TNAP gene cause hypophosphatasia, a heritable form of rickets and osteomalacia.

PHOSPHO1, a phosphatase with specificity for phosphoethanolamine and phosphocholine, plays a functional role in the initiation of calcification and the simultaneous ablation of PHOSPHO1 and TNAP prevents skeletal mineralization. ENPP1, an enzyme responsible for PPi production on the surface of chondrocytes and osteoblasts can also function as an ATPase and a PPiase at the level of matrix vesicles (MVs) acting as a backup enzyme in the absence of TNAP. Integrating these data, we propose an inclusive model (Figure) for the initiation of skeletal mineralization that unifies a number of concepts and enzymatic functions that have been considered contradictory in the past.

Our unified model starts with the MVs as the site of initiation of mineralization. Experimental evidence suggests that the initial formation of apatite crystals inside the MVs is favored by Pi accumulation through two mechanisms, PHOSPHO1-mediated intra-vesicular production and transporter-mediated influx of extravesicular Pi produced primarily by the ATPase activity of either TNAP or ENPP1, in the absence of TNAP. Organophosphate compounds (ATP), polyphosphates (polyPs) and perhaps also pyrophosphate (PPi), appear to be the source of Pi for this initial step of calcification.



TNAP and ENPP1 also support the next phase of collagenmediated extra-vesicular calcification, although it is the PPiase, rather than ATPase or polyphosphatase, activity of these enzymes that is predominant in this step. Extra-vesicular calcification is driven by the availability of inorganic Pi, the control of extracellular PPi concentrations and the presence of a collagenous fibrillar scaffold, and is aided by other ECM non-collagenous mineral-binding proteins. This model takes into account the roles of both organic and inorganic phosphates in skeletal calcification and unifies the MV-mediated and collagenmediated models of calcification as two separate but linked steps during endochondral ossification.

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