Molecular Evidence for Precambrian Origin of Amelogenin, the Major Protein of Vertebrate Enamel

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Although molecular dating of cladogenetic events is possible, no molecular method has been described to date the acquisition of various tissues. Taking into account the specificity of the major protein in enamel in formation (amelogenin), we were able to develop such a method for enamel. Indeed, because the amelogenin protein is exclusively involved in enamel formation and mineralization and because it lacks pleiotropic effects, this protein is a good candidate to estimate the date of acquisition of this highly mineralized tissue. We searched DNA banks for similarities between the amelogenin sequence and other sequences. Similarities were found only to exon 2 of SPARC (osteonectin) in two protostomians and in eight deuterostomians, and to exon 2 of three SPARC-related deuterostomian genes (SC1, hevin, and QR1). The other amelogenin exons did not reveal significant similarities to other sequences. In these proteins, exon 2 mainly encodes the peptide signal that plays the essential role in enabling the protein to be ultimately localized in the extracellular matrix. We tested the significance of the exon 2 similarities. The observed values were always significantly higher than the expected randomly generated similarities. This demonstrates a common evolutionary origin of this exon. The phylogenetic analyses of exon 2 sequences indicated that exon 2 was duplicated to amelogenin from an ancestral SPARC sequence in the deuterostomian lineage before the duplication of deuterostomian SPARC and SC1/hevin/QR1. We were able to date the origin of the latter duplication at approximately 630 MYA. Therefore, amelogenin exon 2 was acquired before this date, in the Proterozoic, long before the so-called “Cambrian explosion,” the sudden appearance of several bilateralian phyla in the fossil record at the Proterozoic-Phanerozoic transition. This sudden appearance has been often suggested to reflect intensive cladogenesis during this period. However, molecular dating of protostomian-deuterostomian divergence and of the cladogenesis among several major clades of Bilateria lead to a different conclusion: many bilateralian clades were already present during the late Proterozoic. It has previously been proposed that these bilateralians were not mineralized and that they had low fossilization potential. Our results strongly suggest that late Proterozoic fossils possessing a mineralized tissue homologous to enamel might be found in the future.