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Letter to the Editor

Apolipoprotein(a): A Puzzling Evolutionary Story

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Abstract. Human apolipoprotein(a), a risk factor for heart disease, has over 80% sequence identity to plasminogen. Plasminogen contains five distinct kringle domains plus a catalytic protease subunit. Human apo(a) consists of multiple copies (the number varies in individuals) of a domain resembling kringle 4, a single copy of a domain resembling kringle 5, and a protease-like domain. The recently cloned hedgehog version of apolipoprotein(a), which contains 31 nearly identical copies of plasminogen kringle 3 and lacks a protease domain, has prompted us to investigate the evolutionary history of the apolipoprotein (a) gene in mammals. Our analysis supports the nonfunctionality of the human apolipoprotein(a) protease domain, and a single (or multiple) duplication of plasminogen gene before mammal radiation, which originated apolipoprotein(a) in mammals.

Key words: Apolipoprotein(a) — Plasminogen — Gene duplication — Kringles

The recent report of the independent evolution of an apolipoprotein(a) gene in human and hedgehog lineages, accounting for its phylogenetic distribution in primates, hedgehogs, and, at present, no other species, reveals a novel and fascinating pathway for the evolution of genes (Lawn et al. 1995). Human apolipoprotein(a), a risk factor for heart disease, contains over 80% sequence identity to plasminogen (McLean et al. 1987). One of the insights of modern molecular evolution is the frequent use of duplicated genes and gene segments to provide fertile grounds for the evolution of new proteins. In some sense, the case of apo(a) is an extreme example of this tendency. Plasminogen contains five distinct kringle domains plus a catalytic protease subunit which requires activation. Human apo(a) consists of dozens of nearly identical copies (the number varies in individuals) of a domain closely resembling kringle 4 of human plasminogen, plus a single copy of plasminogen kringle 5 and protease-like domains. Key substitutions render the protease domain inactive, accounting for some of its pathological consequences as an inhibitor of plasminogen activation.

The recently cloned hedgehog version of apo(a) contains 31 nearly identical copies of a modified plasminogen kringle 3 and lacks even a vestigial protease domain. Although clearly distinct from the human gene, the hedgehog version shares the properties of multiple kringles, a single free cysteine (in a different location) al-

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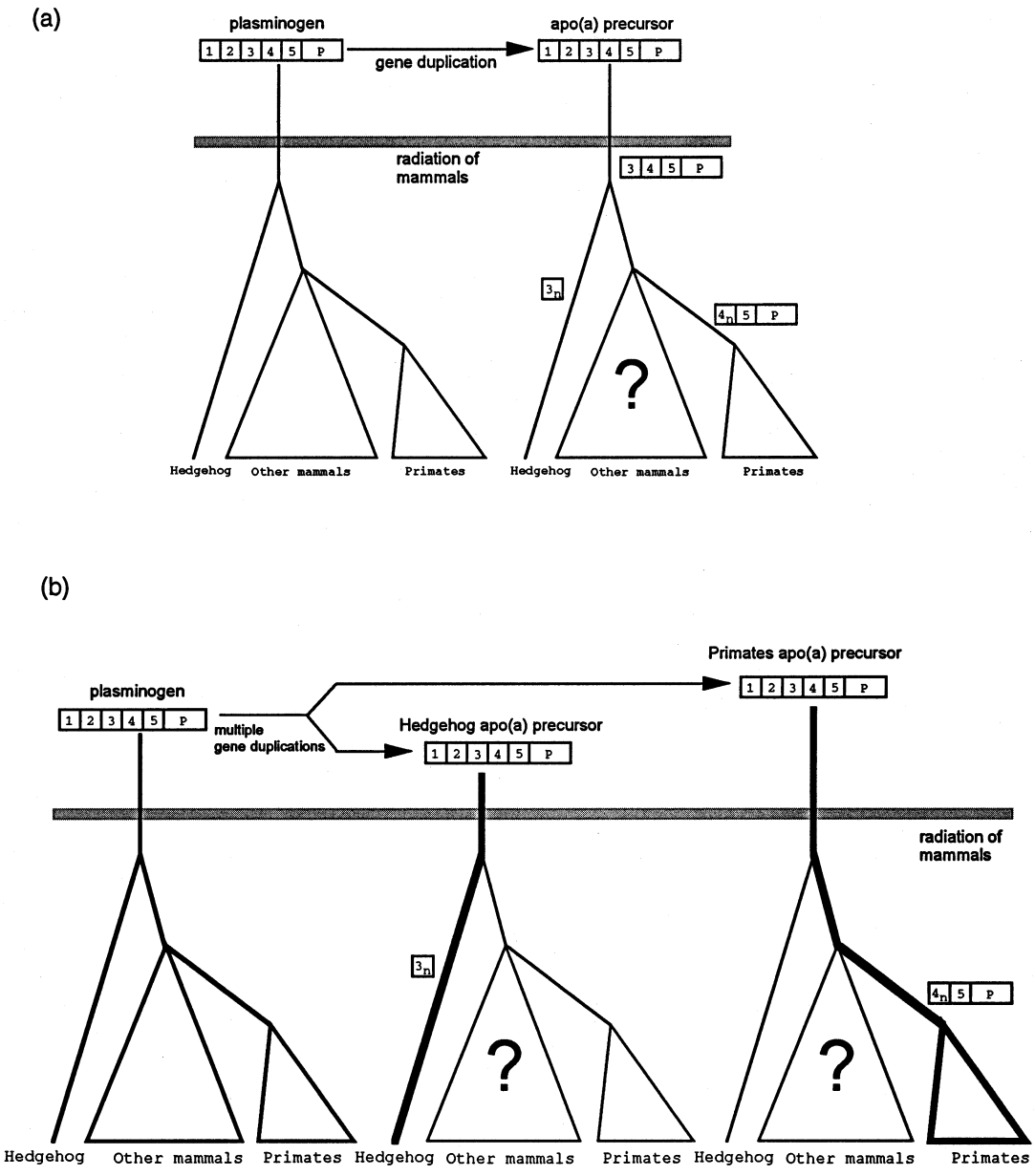

Fig. 1. Alternative schemes for the evolution of apo(a) in mammals implying single **(A)** and multiple **(B)** gene duplications. *Thick lines* represent known actual apo(a) lineages.

lowing covalent linkage to apolipoprotein B, a lysine binding pocket, and lack of proteolytic activity (Lawn et al. 1995). These properties confer on each protein the ability to join a lipoprotein particle and to compete for the binding and activation of plasminogen.

The report gives support to two points raised in an earlier analysis of human and rhesus monkey sequences: the nonfunctionality of the apo(a) protease domain and an early duplication of the plasminogen gene before the radiation of mammals (Pesole et al. 1994). The finding of Lawn et al. (1995) that the hedgehog plasminogen kringle 3 is more closely related to the plasminogen kringle 3 of other mammals than to the hedgehog apo(a) kringle 3 suggests that, as shown for the primate apo(a) (Pesole et al. 1994), the gene duplication which originated the hedgehog apo(a) very likely took place before the mammal radiation. The simplest explanation for these data is that the primate and hedgehog apo(a) originated before this time by a single gene duplication. Then the mammalian apo(a) precursor (containing kringles 3–5 and the protease domain) lost kringles 4 and 5 and the protease domain in the lineage leading to hedgehogs and kringle 3 before the primate radiation (Fig. 1A).

We cannot predict if the protease catalytic activity was already lost before the split between primates and hedgehog, and current data cannot exclude another equally valid scenario, equally as valid as the other one, that convergent evolution occurred and different plasminogen paralogs served as precursors for hedgehog and human apo(a). In fact, numerous plasminogen-like se-

quences exist in the genomes of both species. Some of us recently sequenced two different hedgehog cDNAs which encode seven kringles, and they are more divergent than the plasminogen and apo(a) genes are from one another (G. Lindahl, L.P., R.L., personal communication). At least seven close paralogs of plasminogen exist in the human genome (Byrne et al. 1994). The alternative scheme for the evolution of apo(a) in mammals, implying multiple gene duplications, is shown in Fig. 1B. Clearly, the characterization of other members of this gene family in birds as well as in other mammals is needed to elucidate the most likely scenario for the evolution of the two types of apo(a).

The recurring evolution of apo(a) presents a novel example of gene appearance and evolution and/or convergent evolution. While it is highly improbable that two proteins with global similarity would evolve independently from scratch, there should be other cases where a common problem has been solved by different strategies of modification of a genomic precursor to allow genes of overall similarity to evolve in different ways (Tseng and Green 1988). Finally, we should not lose sight of the fact that the function of apo(a) remains unclear. In addition to the fact that its twin evolution supports the contention that it *does* have a function, it should stimulate some thinking about what hedgehog and primates might have in common to account for this curious phylogenetic distribution. Recent discoveries point out that such proposals should not be limited to roles in thrombosis and lipid transport (O'Reilly et al. 1994).

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