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Evolutionary Pathways of the Calcitonin (CALC) Genes

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Recombinant DNA techniques have made it possible to establish the structure of various genes encoding polypeptide hormones. Comparison of nucleotide sequences of the calcitonin (CALC) genes in man has revealed surprising similarities and variations. These findings and the homologies among the sequences in different species offered an opportunity for speculation about relationships between these genes and about their evolutionary origin. The first gene (CALC-I) directing the synthesis of calcitonin (CT) or CT gene-related peptide (CGRP) comprises six exons and gives rise to two mRNAs by an alternative RNA-processing mechanism. The homology between CGRP and CT reflects their common origin. The human genome contains a second gene (CALC-II) that is structurally related to the CALC-I gene. The CALC-II RNA transcripts do not appear to be differentially processed, as only preproCGRP-II mRNA and not preproCT-II is detected. The first and second CT/CGRP genes probably have evolved from a common ancestor gene early in evolution. Meanwhile, a third genomic locus containing nucleotide sequences highly homologous to exons 2 and 3 of both CALC genes was detected and probably generated by duplication of a part of CALC-II. This locus is not likely to encode a CT- or CGRP-related polypeptide hormone. The CALC genes and this last (pseudo) gene are located on the short arm of chromosome 11. Recently, islet- or insulinoma-amyloid polypeptide (IAPP) was isolated as a major constituent of amyloid present in human insulinoma and in pancreatic islet amyloid in noninsulin-dependent diabetes mellitus. IAPP shows 46% amino acid sequence homology with human CGRP-II. In contrast to the CALC-genes, the human IAPP gene is located on chromosome 12. All these findings have provided insight into the mechanisms underlying the increasing diversity of polypeptide hormones. (Henry Ford Hosp Med J 1989;37:201-3)

Over one century ago Charles Darwin (1809-1882) proposed a hypothesis on the relationships among animals by comparing their phenotype in his speculation on the descent of man. Today we can study evolution on a molecular level by using tools like restriction enzymes and recombinant DNA techniques to compare nucleotide and amino acid sequences.

In Utrecht, The Netherlands, we have been interested in calcitonin (CT) since 1974 because of the families we see with the multiple endocrine neoplasia type 2 (MEN 2) syndrome with the predisposition to medullary thyroid carcinoma (MTC) and pheochromocytoma.

MTC has its origin in the thyroid gland in the CT-producing neuroectodermal C-cell. CT is an excellent tumor marker. Patients and their closely related family members are screened periodically by CT procedures and have surgery if any indication of C-cell proliferation is evident.

The CT-encoding gene family (CALC genes) offers an excellent opportunity for speculation about its evolutionary origin and the relationship of its products.

The first gene (CALC-I) encodes a primary RNA-transcript that comprises six exons and gives rise to two messenger RNAs (mRNAs) (Fig 1). It directs the synthesis of CT or CT gene-related peptide (CGRP) by an alternative RNA-processing mechanism (1). In normal subjects CT is predominantly produced by the C-cell in the thyroid gland, whereas CGRP is produced by the central and peripheral nervous system. The 22% to 31% homology between CGRP and CT reflects their common origin in a primordial gene. The close structural relationship between salmon and chicken CT (84%) suggests that in these species CT plays the same important role in calcium preservation. The low degree of homology between salmon (or chicken) and human CT (50%) suggests that human CT plays a less important role in calcium metabolism and is perhaps more important as a neuropeptide. The high degree of homology between chicken and human CGRP suggests that CGRP is an important old and well conserved polypeptide.

From a cDNA library derived from a metastasis of a sporadic human MTC, a second CGRP-mRNA having a high degree of homology with the first CGRP-encoding mRNA was detected in 1985. This human genome appeared to contain a second gene (CALC-II) (Fig 1), which is structurally related to the CALC-I gene (2). The CALC-II RNA transcripts do not appear to be differentially processed since only preproCGRP-II mRNA and not preproCT-II mRNA is detected. There appeared to be stop codons in the CT-encoding region so that only CGRP can be produced by this gene (3). Expression of this second gene occurs in

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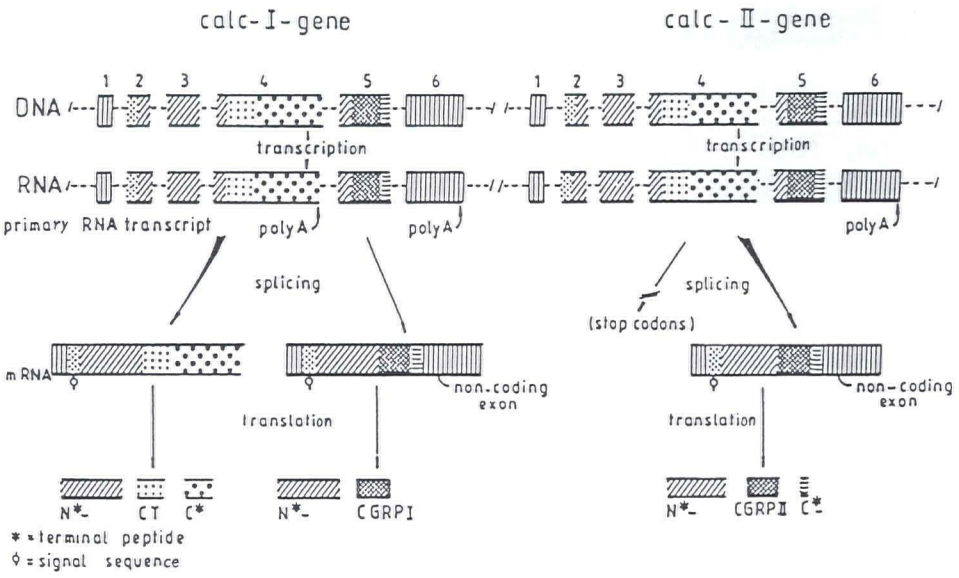

Fig 1—Alternative processing of the primary RNA transcript generates different mature mRNAs from the first CALC gene. From the second gene only CGRP-RNA is produced. Both genes are located on chromosome 11.

the central nervous system, in MTC, and in Ewing sarcoma, a malignant bone tumor in children (4). The first and second CT/CGRP gene probably have evolved from a common ancestor gene (5).

The function of the products of the first two human CALC genes has not been completely clarified, although in the thyroid gland it may have a paracrine function.

From a genomic DNA library, a third locus containing nucleotide sequences highly homologous to exons 2 and 3 of both CALC genes was discovered in 1987 (Fig 2). This third gene probably is generated by duplication of a part of CALC-II. It is not likely to encode a CT- or CGRP-related polypeptide hormone. Since the significance of this third gene is not known, it may be a pseudogene (6). The CALC genes and this last (pseudo) gene are located on the short arm of chromosome 11 (3,6).

In 1986 Westermark et al (7) identified a peptide related to amyloid deposits in the islets of Langerhans, the most characteristic morphologic abnormality in patients with type 2 noninsulin-dependent diabetes mellitus (NIDDM). This novel 37 amino-acid peptide was characterized and termed insulinoma amyloid polypeptide or islet amyloid polypeptide (IAPP) when extracted from insulinomas or pancreatic islets and diabetes associated peptide (DAP) when extracted from the diabetic pancreas.

The human sequence of IAPP shows a 46% homology with CGRP-II and may be considered as another CALC gene (CALC-IV) (Fig 2). This amyloid peptide (IAPP) is produced by the beta cell where it is probably cosecreted with insulin, and at present only speculations can be made regarding its function. It might have a paracrine role within the pancreatic islets or a hormonal role at the periphery where it inhibits the actions of insulin on glyconeogenesis.

Investigation of a cDNA library derived from insulinoma tissue indicated that this peptide is a normal processing product of an 89 amino-acid protein precursor and is released by the beta cell (8). The amyloidogenic properties of human IAPP appear to reside in a region spanning amino-acid residues 20-29 of the mature molecule. A synthetic peptide representing this region of IAPP spontaneously aggregates as amyloid fibrils in vitro. Age-associated spontaneous-onset NIDDM probably only occurs in species that develop this islet amyloid.

The IAPP gene has been identified and, in contrast to the earlier described CALC genes, is not localized to chromosome 11 but to chromosome 12 (9). An evolutionary relationship between chromosomes 11 and 12 has been suggested (10). Probably the IAPP gene arose by duplication and translocation to chromosome 12 of a progenitor CALC gene and subsequent divergence. Other polypeptide hormone families have members on chromosome 11 as well as chromosome 12 (eg, PTH on 11, PTH-related peptide on 12, IGF-II on 11, IGF-I on 12).

The molecular mechanisms by which CALC genes were duplicated are not yet known but probably unequal crossover is involved (11). Unequal crossover leaves one chromatid with less DNA, with the other having acquired more. In most known families of duplicated genes, such as the CALC genes, the duplicated genes lie relatively close to one another on the same chromosome. However, exchanges of genetic material among chromosomes can occur either by unequal crossover between chromatids of different chromosomes or by direct DNA trans-

position. The duplicate genes usually diverge independently with one copy retaining its original structure and function so that the organism would not be handicapped by the lack of an essential protein. The redundant copy would then be free to mutate without constraints from natural selection. Most mutations generate nonfunctional proteins but an occasional advantageous change can create either an improved version of the original protein or a protein with an entirely new function.

The body adapts to its environment by the occurrence of new mutations. Its attitude has to be opportunistic to take advantage of the fortuitous evolvement of a new peptide that may be used as a signal or a receptor. Unfavorable mutations, however, may lead to an acute defect and the extinction of the subject and/or its offspring may follow. Ecologic and cultural developments provide a continuous selection pressure restricting unlimited divergence. The evolution of humans is directed by its environment, or, euphemistically, the environment is a challenge for new developments. We believe more CALC-gene family members may be discovered in the future.

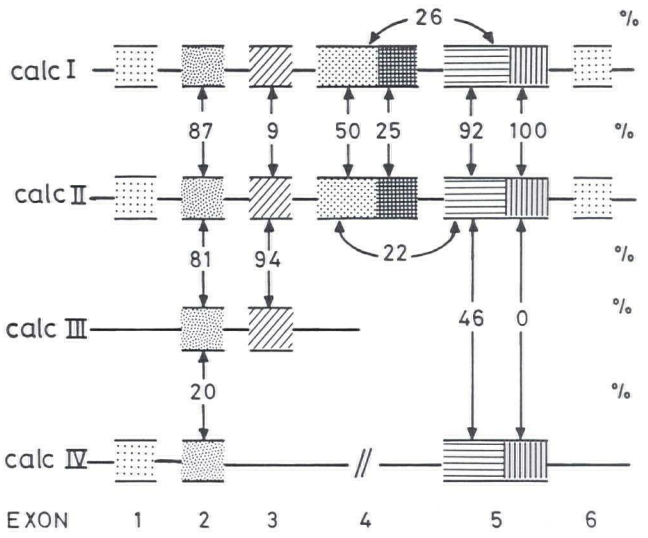

Fig 2—Homology of the CALC genes with a comparison of the amino-acid sequences. Exons 1 and 6 are noncoding exons. From CALC-II only CGRP is produced; CALC-III probably has a pseudogene structure. Probably CGRP-II is the oldest structure which has been "tandem" duplicated to CT to form the complete CALC-II gene. Tandem duplication of the CALC-II gene may have resulted in the CALC-I gene, and translocation of CGRP-II to chromosome 12 may have resulted in IAPP.

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