60 YEARS OF POMC

From the prohormone theory to pro-opiomelanocortin and to proprotein convertases (PCSK1 to PCSK9)

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Abstract

Pro-opiomelanocortin (POMC), is a polyprotein expressed in the pituitary and the brain where it is proteolytically processed into peptide hormones and neuropeptides with distinct biological activities. It is the prototype of multipotent prohormones. The prohormone theory was first suggested in 1967 when Chrétien and Li discovered γ-lipotropin and observed that (i) it was part of β-lipotropin (β-LPH), a larger polypeptide characterized 2 years earlier and (ii) its C-terminus was β-melanocyte-stimulating hormone (β-MSH). This discovery led them to propose that the lipotropins might be related biosynthetically to the biologically active β-MSH in a precursor to end product relationship. The theory was widely confirmed in subsequent years. As we celebrate the 50th anniversary of the sequencing of β-LPH, we reflect over the lessons learned from the sequencing of those proteins; we explain their extension to the larger POMC precursor; we examine how the theory of precursor endoproteolysis they inspired became relevant for vast fields in biology; and how it led, after a long and arduous search, to the novel proteolytic enzymes called proprotein convertases. This family of nine enzymes plays multifaceted functions in growth, development, metabolism, endocrine, and brain functions. Their genetics has provided many insights into health and disease. Their therapeutic targeting is foreseeable in the near future. Thus, what started five decades ago as a theory based on POMC fragments, has opened up novel and productive avenues of biological and medical research, including, for our own current interest, a highly intriguing hypocholesterolemic Gln152His PCSK9 mutation in French-Canadian families.

Introduction

The prohormone theory was based on the observation that β-lipotropin (β-LPH) and γ-lipotropin (γ-LPH) (Chrétien & Li 1967) contained the entire sequence of β-melanocyte-stimulating hormone (β-MSH), a pituitary octadecapeptide discovered 10 years earlier (Geschwind et al. 1956, Harris & Roos 1956).

In this review, we describe how the LPH model unfolded into the pro-opiomelanocortin (POMC) story and how the prohormone theory that was derived from it extended to many other proteins and became a new tenet of biology. The ‘fil d’Ariane’ that made it possible was simply the knowledge of the primary structure of a few key pituitary
peptides. We owed the capacity to draw such conclusions to the double revolution of protein (Sanger 1959) and DNA sequencing (Maxam & Gilbert 1977, Sanger et al. 1977).

Retroactively, the prohormone theory could have been formulated one decade earlier when Li and co-workers, adopting the Sanger's fluorodinitrobenzene (FDNB) method (Sanger 1945) and the Edman's phenyl thiohydantoins (PTH) procedure (Edman 1950), sequenced several pituitary peptides including adrenocorticotrophic hormone (ACTH) (Li et al. 1955), MSHs (Geschwind et al. 1956, Harris & Lerner 1957), β-LPH (Li et al. 1965), and γ-LPH (Chrétién & Li 1967). In the early 1970s, different groups provided evidence hinting that all these molecules were linked to one another. The final proof showing that they were really pieces of a single molecule, namely POMC, was provided when Nakanishi et al. (1979) cloned and sequenced its cDNA.

This article is divided in seven sections: the first reviews the chronological discoveries of POMC peptides; the second describes how the prohormone theory was born and how the POMC denomination evolved; the third deals with the expansion of the theory to neuropeptides and other important biological molecules; the fourth summarizes the long and diligent efforts to identify and characterize the proprotein convertases (PCs); the fifth illustrates the experimental evidence that PC1/3 and PC2 are the primary POMC convertases, it expands to the importance of endoproteolysis with examples on how a single amino acid substitution at the cleavage site of known precursors profoundly alters its functions; the sixth briefly summarizes the proteolytic cascade of PC activation and presents the novel nonenzymatic role of PCSK9 as an escort protein, which is invalidated in humans by a Gln152His (Q152H) mutation; and the seventh provides a short overview about the importance of the PCs in human biology.

The pieces of the POMC puzzle

This puzzle is made of peptides discovered and sequenced over a period of 24 years, from 1955 to 1979 (Fig. 1).

The first peptide is ACTH. It was characterized by Li et al. (1955). To this day, the sequencing of this 39 amino acid long peptide is remembered as an outstanding achievement: it required 300 mg of pure ACTH and took years to complete. As seen in Fig. 2, a series of enzymatic digestions (pepsin, trypsin, and chymotrypsin) and partial acid hydrolysis were necessary in order to obtain a sufficient number of fragments and, using a combination of Sanger’s and Edman’s methods, to decipher the sequence, one residue every 2–3 days.

The second peptide is β-MSH (Geschwind et al. 1956, Harris & Roos 1956). The sequence of this octadecapeptide revealed that residues 7–13 were similar to amino acid 4–10 of ACTH, a structural homology that was eventually found to explain the MSH-like activity of ACTH.

The third peptide is α-MSH. Made of 13 amino acids, it was identical to the N-terminal tridecapeptide of ACTH (Harris & Lerner 1957). Although not realized at the time, this homology indicated for the first time that ACTH may give rise to a second bioactive molecule. It was later shown that, compared with β-MSH and ACTH, α-MSH is the most potent melanophore-stimulating hormone (Hofmann 1962).

The fourth peptide is β-LPH. It was discovered by Birk and Li (1964), who found it to be a novel substance exhibiting mild lipolytic activity; they named it lipotropin. They also observed that it had minimal MSH-like activity. Being more than twice the size of ACTH, it was far more difficult to sequence (Li et al. 1965). The presence of two methionine residues allowed partial chemical cleavage with cyanogen bromide, yielding five fragments. Each fragment was submitted to trypsin or chymotrypsin digestion, resulting in dozens of peptides that were sequenced by the Sanger’s FDNB and the Edman’s methods. The results were surprising: β-LPH was a novel peptide whose middle fragment contained the
entire sequence of β-MSH sandwiched between pairs of basic amino acids (Fig. 3). The number of amino acids, initially determined to be 90, was corrected to 91 when it was found that sheep β-endorphin contained one more isoleucine residue, which had been missed by manual sequencing (Chrétien et al. 1976a).

Figure 2
Peptide fragmentation of ACTH for sequencing purposes. (Data from Li et al. 1955). Residues 25–32 (underlined) were later corrected by Riniker et al. (1972) to Asn.Gly.Ala.Glu. 

Figure 3
Sequence of β-LPH. Residues are indicated in color-coded circles. Those that were targeted for cleavage to generate smaller peptides for sequencing (Arg and Lys for trypsin digestion; Met for cyanogen bromide treatment) are presented in circles with wiggly borders. The pairs of basic residues flanking the sequences of β-MSH are boxed. (Data from Chrétien & Li 1967 and corrected by addition of Ile83).
The fifth peptide is γ-LPH. While sequencing of β-LPH, Chrétien and Li (1967) discovered another peptide with similar biological properties; they named it γ-LPH. Its sequence corresponded to residues 1–58 of β-LPH, ending with the β-MSH sequence.

The sixth peptide is corticotrophin-like intermediate peptide (CLIP). It was isolated and characterized by Scott et al. (1972). As its sequence turned out to be identical to the C-terminal moiety of ACTH, the authors rightfully proposed that ‘ACTH may be not only a hormone in its own right, but also the precursor of other biologically active peptides’ (Lowry et al. 1977).

The seventh peptide is β-endorphin. In mid-1970s, Hughes et al. (1975) serendipitously noted that the met-enkephalin decapeptide was identical to residues 61–65 of β-LPH. This led to the speculation that the β-LPH C-terminal fragment could also be an opioid peptide. In a matter of months, this hypothesis was confirmed by the isolation and sequencing of β-endorphin from different species (Guillemin 1978, a review), including human (Chrétien et al. 1976a).

The eighth peptide is the N-POMC fragment. Its existence was suspected in the early 1970s with the characterization of big ACTH (Yalow & Berson 1973, Mains & Eipper 1975). Its complete sequence became available with the cloning of POMC cDNA (Nakanishi 1979).

The prohormone theory

That a peptide hormone could be derived from the cleavage of a larger polypeptide (the prohormone theory) was deduced by Chrétien and Li (1967) after determining the sequence of γ-LPH and noticing that it was a truncated fragment of β-LPH containing β-MSH as its C-terminus. They also observed that β-MSH was the most active of the three molecules for their lipolytic and melanophore-stimulating activities (Fig. 4A), supporting a precursor–product relationship among them. This activity correlation between a precursor and its end product held true for proinsulin also: it was shown to possess minimal insulin potency (Kitabchi 1970) (Fig. 4B). Chrétien and Li (1967) proposed that β-MSH could be generated following proteolytic processing. They highlighted the pairs of basic amino acid residues flanking this peptide in the β-LPH/γ-LPH molecules as potential cleavage sites (Fig. 3).

This proinsulin model was simultaneously proposed by Donald Steiner when he discovered that a human insulinoma produced insulin from a larger molecule (Steiner et al. 1967). In 1968, Ronald Chance sequenced proinsulin and confirmed that the cleavages producing the two insulin chains occurred at pairs of basic amino acid residues as in the β-LPH model (Chance et al. 1968).

In subsequent years, many more hormones and neural peptides, including β-endorphin, were shown to be produced through the same mechanism (Chrétien et al. 1984), giving an almost universal support to the theory.

The β-LPH biosynthetic cascade

In the early 1970s, many groups joined the field using various methodological approaches. The rules governing these approaches were best spelled out by Tager et al. (1975): (i) immunoprecipitation experiments with well-characterized antibodies raised against the hormone must specifically precipitate the higher as well as the lower molecular weight forms of the hormones; (ii) peptide mapping of the precursor must demonstrate...
the existence within the larger molecular form of peptides characteristic of the active hormone together with additional fragments; (iii) pulse-labeling and pulse-chase experiments must be carefully conducted to establish the precursor-product relationship between high and low molecular weight forms of the proteins; (iv) sequence analysis of the putative precursor must reveal the existence of additional peptide(s) covalently linked to the hormone.

The first two criteria constituted suggestive evidence for the existence of a precursor molecule. The pulse-chase experiments were more indicative of this fact. More conclusive evidence was the demonstration that the end products were chemically similar to their natural equivalents by, whenever possible, radioactive microsequencing of end products after biosynthetic labeling of the precursor with key amino acid residues. However, the ultimate proof was the sequencing of the precursor itself, showing (an) additional fragment(s) covalently linked to the correct sequence of the biologically active peptide.

Chrétien and collaborators showed that β-LPH and γ-LPH biosynthesized in vitro were chemically indistinguishable from their natural counterparts in bovine pituitary glands (Bertagna et al. 1974, Chrétien et al. 1976b). Soon after, they isolated sheep and human β-endorphin (Chrétien et al. 1976a) and they studied its biosynthesis in vitro (Crine et al. 1977). Not only did they found the radioactive biosynthetic β-endorphin to have similar chemical characteristics with unlabeled β-endorphin, but they confirmed by microsequencing the methionine residue at its fifth position as expected. Soon after, using double labeling with [35S]methionine and [3H]methionine residue at its fifth position as expected. Soon after, they confirmed by microsequencing the β-to have similar chemical characteristics with unlabeled β-endorphin (Crine 1976). Not only did they found the radioactive biosynthetic β-endorphin to have similar chemical characteristics with unlabeled β-endorphin, but they confirmed by microsequencing the methionine residue at its fifth position as expected. Soon after, the isolated sheep and human β-endorphin (Chrétien et al. 1976a) and they studied its biosynthesis in vitro (Crine et al. 1977). Not only did they found the radioactive biosynthetic β-endorphin to have similar chemical characteristics with unlabeled β-endorphin, but they confirmed by microsequencing the methionine residue at its fifth position as expected. Soon after, using double labeling with [35S]methionine and [3H]lysine, they showed that after a 3 h pulse, β-endorphin, γ-LPH, and α-MSH were the major secretory products. They also observed a fourth large molecular weight protein (Crine et al. 1978), which was later identified as the prosegment of POMC.

**Extension of the β-LPH models to POMC**

The POMC model resulted from numerous parallel studies involving many groups. There was: (i) the observation that the pituitary cells containing the LPH/β-MSH/endorphin and ACTH/α-MSH were the same; (ii) the presence of ‘big’ ACTH in pituitary extracts and in plasma; (iii) the biosynthetic studies of ACTH and related peptides in AtT-20 tumor cells, and (iv) in the mid-1970s, the progressive recognition of POMC as the common precursor to β-LPH and ACTH.

**Co-segregation of β-LPH, ACTH, α-MSH, and β-endorphin in pituitary cells**

**β-LPH-containing cells** The pituitary is made of specialized endocrine cell types, each producing specific hormones. These types include somatotrophs, lactotrophs, gonadotrophs, thyrotrophs, and corticotrophs producing, respectively, growth hormone, prolactin, gonadotropins, thyroid-stimulating hormone (TSH), and ACTH. With the discoveries of LPHs, it became necessary to identify which pituitary cell type produced them. Using specific β-LPH polyclonal antibodies, Dessy et al. (1973) were the first to show that β-LPH was present in the corticotroph/melanotroph cells described earlier by Herlant and Pasteels (1967). A few years later, Pelletier and coworkers described at the light and electron microscopic levels that β-LPH is stored in the same granules as ACTH, and they rightfully suggested that both molecules could be released together during granule extrusion (Pelletier et al. 1977).

**ACTH-containing cells** In the early 1930s, Harvey Cushing observed that the disease named after him was associated with pituitary adenoma suspected to produce ACTH (Cushing 1932). The definite proof that those adenoma were really secreting ACTH came in 1958 when Don Nelson identified a patient who, after bilateral adrenalectomy for the treatment of Cushing’s syndrome, developed a large pituitary tumor, became pigmented, and had large amounts of ACTH and MSH bioactivities in his urine. Following the removal of the tumor, the pigmentation markedly decreased along with the urinary ACTH and MSH (Nelson et al. 1958). It took another decade before Herlant and Pasteels (1967) described the ACTH-containing cells (corticotrophs). Phifer et al. (1970) unequivocally confirmed the presence ACTH in these cells. Soon after, using specific antibodies against ACTH, α-MSH, and β-MSH, they showed that the three hormones co-segregated in the same cells (Phifer et al. 1974).

**Endorphin-producing cells** When β-endorphin was discovered in 1976, it came as no surprise that it was found in the LPH/ACTH/MSH cells (Weber et al. 1978).

**Molecular forms of ACTH and LPH** Indications that ACTH is biosynthesized from a larger precursor came initially from radioimmunooassays of gel filtration fractions. Orth et al. (1970) showed that a mouse pituitary adenocarcinoma cell line (AT-T20/D-I) had two immunologically indistinguishable forms of ACTH differing in molecular weights. Soon after, Yalow and Berson (1973) identified three forms of ACTH in human plasma and extracts of pituitary glands. Lowry et al. (1977)
also noted some heterogeneity in the ACTH molecular forms and observed that the larger forms also contained LPH immunoreactivity, which suggested a common precursor for the two molecules.

**Biosynthesis of ACTH and related peptides** Although the sequences of ACTH and of α-MSH had been known for almost 15 years, their structural relationship during biosynthesis did not attract much attention until two additional facts were published: (i) the existence of high molecular forms of ACTH and (ii) the discovery of CLIP by Scott et al. (1972, 1974). The most important in vitro results came in the mid-1970s from Ed Herbert's laboratory in Portland, Oregon. In their first series of experiments, carried out by the Eipper and Mains in tandem (Eipper & Mains 1976), involved a double-immunoprecipitation technique to isolate labeled proteins from cells incubated in media containing radioactive amino acids. The second series of experiments, carried out by Roberts and Herbert (1977) and Roberts et al. (1978), used a cell-free translation of mRNA from AtT-20 cells. Both studies confirmed the hypothesis that ACTH is biosynthesized as a large precursor form of 28,000–31,000 kDa, which is later transformed into 13- and 4.5-kDa forms. Nakanishi et al. (1976, 1977) made similar observations using mRNA from bovine pituitary glands. In pulse-chase experiments carried out in rat pars intermedia, Crine et al. (1978) showed, with microsequencing characterization, the sequential biosynthesis of β-LPH and β-endorphin from a large precursor molecule.

**The sequence of POMC cDNA** Although flanking pairs of basic residues had been identified early on as cleavage motifs for the release of β-MSH and β-endorphin from β-LPH (Chrétien & Li 1967) and α-MSH and CLIP from ACTH (Scott et al. 1974), the cleavage motif between ACTH and β-LPH could not be established for lack of sufficient amounts of the POMC molecule. The difficulty was circumvented when DNA sequencing came about. Roberts et al. (1979) and Nakanishi et al. (1979) cloned the cDNA of mouse and bovine ACTH/βLPH precursor, respectively. The first group reported a partial sequence of the cDNA; the second group a complete one. Quite evidently, pairs of basic residues identified in the sequence were proteolytic signal motifs for the release of ACTH, β-LPH, β-MSH, and β-endorphin. The rat gene was cloned and sequenced by Drouin and Goodman (1980), confirming the cDNA results. Later, Herbert's group was able to define the length of the signal peptide (Policastro et al. 1981). The common precursors were variably called ‘big ACTH’, ‘31K-precursor’, ‘precursor to ACTH and β-endorphin’, ‘proopiocortin’, ‘pro-lipocortin’, and ‘pro-ACTH/endorphin’ (Eipper & Mains 1980). Taking into account the biological activities of its three main end products, the name POMC was proposed (Chrétien et al. 1979, Herbert 1981) and was widely accepted.

**The expansion of the prohormone theory**

The early 1970s saw the identification of numerous hypothalamic factors and other important neuropeptides (Guillemin 1978, Watson et al. 1982, Douglass et al. 1984). The question rapidly arose whether all those neuropeptides followed similar biosynthetic pathways as β-endorphin (Lazarus et al. 1976, Crine et al. 1977, 1978). When the corresponding full-length cDNAs of known neuropeptides were cloned and sequenced, the amino acid sequences deduced from their open reading frames revealed that their active peptide was contained within larger polypeptides and flanked by basic residues (Douglass et al. 1984). Thus, the prohormone theory, which initially applied to peptide hormones, held true for neuropeptides and neurotrophins as well. It implied that the brain could produce many active substances from a limited number of genes. For example, combining partial and total cleavages, POMC, with its 10 pairs of basic residues, could produce up to 65 fragments, while pro-enkephalin, with 12 such pairs, could give rise to 90 different peptides (Chrétien et al. 1989).

The following formula was proposed:

\[
\sum_{i=1}^{n} (i+1)^{i+2+3+\ldots+n+1}
\]

in which \(i\) varies from 1 by integers to \(n\) and \(n\) represents the number of potential cleavage sites.

Through cDNA cloning and sequencing, other proteins were identified as products of larger polypeptides and their number has increased exponentially. According to the CutDB database, there are to this date 6435 documented proteolytic events in the human proteome (http://cutdb.burnham.org/). For hundreds of precursor proteins in the proteome, these events involve endoproteolysis at basic motifs.

**The discovery of PCs**

The prohormone theory entailed the existence of specific proteases that could mediate the proteolytic
processing of the precursor, the so-called PCs. Lazure et al. (1983) reviewed the minimal criteria that had to be met for any enzyme to qualify as PC. Their search by classical biochemical methods of purification from tissues combined with enzyme assays proved to be a long road, paved with many false leads and dead ends, including our own (Chrétien 2012, a review). Although the search for PC was ongoing, the cDNA of 7B2, a pituitary peptide that we characterized in the early 1980s, was cloned in human and mouse; it later turned out to be a specific chaperone and inhibitor of one of the PCs, namely PC2 (Mbikay et al. 2001, a review).

The first experimental evidence by these methods of the existence of an authentic mammalian PC came in 1987, when Davidson and coworkers identified a calcium-dependent endoproteinase from extracts of rat insulinoma (Davidson et al. 1987). In 1980s, it came to be known that Saccharomyces cerevisiae carried a PC, the KEX2 gene product or Kexin, with cleavage specificity for pairs of basic residues (Mizuno & Matsuo 1984), the same metal dependence (Fuller et al. 1989a), and the ability of correctly processing a mammalian prohormone, namely POMC, when expressed into mammalian cells (Thomas et al. 1988b). The turning point in the search of the mammalian homologs was an insightful observation by Fuller et al. (1989b) of a cDNA in the GenBank whose deduced sequence, like Kexin, carried the telltale signatures of the catalytic domain of serine proteases of the subtilase family. The cDNA, a product of the fur gene (upstream region of the fes/fps gene), encoded a type 1 transmembrane protein named furin believed to be a receptor (Roebroek et al. 1986). Unbeknownst to the investigators, the protein was a PC. The observation by Fuller et al. (1989b) launched an intense search for the homologs of this cDNA from endocrine tissue mRNA. The strategy involved the use of PCR with degenerate oligonucleotides designed to overlap the codons corresponding to residues of the active triad found in catalytic pocket of kexin and furin. PC2 cDNA was characterized from a human insulinoma (Smeekens & Steiner 1990) and PC1 and PC2 cDNA from mouse pituitary (Seidah et al. 1990). Soon after, a second cDNA corresponding to PC1, but named PC3, was cloned from the human insulinoma (Smeekens et al. 1991). It is an amazing historical coincidence that the cloning of the cDNAs for these initial PCs was simultaneously obtained by the same two groups which had formulated the prohormone theory 23 years earlier (Seidah & Chrétien 1992, Chrétien 2012).

In the following decade, six other cDNAs encoding enzymes structurally related to furin, PC1/3, and PC2 were identified in various mammalian tissues. They are PC4, PACE4, PC5/6, PC7, S1P/SKI-1, and PCSK9. Overall, these enzymes are biosynthesized aszymogens made of an N-terminal prodomain, a catalytic domain, a P domain, and C-terminal domain (Fig. 5A). Except for PCSK9, which cleaves only itself, these enzymes catalyze the hydrolysis of many precursor proteins (proproteins). Except for S1P/SKI-1 and PCSK9, they all cleave themselves and their substrates after basic motifs with differing stringency. Collectively, they have been named PCs (Seidah & Chrétien 1992, 1999, Seidah 2011, Chrétien 2012).

Figure 5
(A) Biosynthesis of PC1/3. The cascade of proteolytic maturation and activation of the zymogen starts in the ER, and continues in the trans-Golgi network (TGN) and in secretory granules (SG). (B) PC1/3 and PC2 preferred cleavage sites in POMC polypeptide. The sites and the major processing are indicated by color-coded (red for PC1/3 and blue for PC2) arrowheads and lines, respectively.
PC1/3 and PC2 are POMC convertases

Arguments of topology, enzymology, and genetics have concurred to establish that PC1/3 and PC2 are the bona fide POMC converting enzymes.

Both enzymes and the prohormone are localized in the same cells

Early after their discovery, in situ hybridization on rodent brain sections revealed that while PC1/3 and PC2 mRNA as found in both lobes of the pituitary, PC1/3 was most abundant in the anterior lobe and PC2 more abundant in the neurointermediate lobe (Day et al. 1992). Ex vivo studies using mouse AtT-20 corticotrophs showed that PC1/3 was released upon exocytotic stimulation, indicating that, like POMC-derived peptides, it was stored into regulated secretory vesicles (Vindrola & Lindberg 1992), a conclusion that was later confirmed by the detection of its immunoreactive forms at the tips of these cells, where the vesicles containing the peptides accumulate (Hornby et al. 1993). Immunocytochemistry at electron microscopic level corroborated the co-localization of POMC-derived peptides with these enzymes in dense secretory vesicles of the pituitary gland (Takumi et al. 1998).

The enzymes cleave the prohormone into peptides normally found in tissues

Using vaccinia virus vectors, metabolic radioactive labeling, and microsequencing of radiolabeled peptides, Benjannet et al. (1991) showed that POMC was predominantly converted to ACTH when co-expressed with PC1/3 and to MSHs and β-endorphin when co-expressed with PC2, reproducing the pattern of POMC-derived peptides processing previously observed in the anterior and neurointermediary lobes of the pituitary (Fig. 5B).

Spontaneous or induced deficiency of the enzymes causes tissue accumulation of the prohormone or its intermediates processing products

Bloomquist et al. (1991) was the first to demonstrate that antisense RNA inhibition of PC1 mRNA translation in AtT-20 cells resulted in impaired processing of POMC. In humans, Jackson et al. (1997) described the first genetic case of PC1/3 deficiency in which the subject carried POMC in circulation as a consequence of impaired processing. The mutation caused an obesity syndrome. Several other cases were later reported by Philippe et al. (2015).

However, it was the production of a PC2 knockout mouse in 1997 (Furuta et al. 1997) followed by that of PC1/3 (Zhu et al. 2002) 5 years later, which provided the opportunity to evaluate in fine details the molecular consequence of the deficiency of these enzymes on POMC processing. PC1/3-deficient mice exhibited severe impairment of ACTH production and compensatory accumulation of POMC mRNA in the pituitary (Zhu et al. 2002). Using refined immunological techniques with specific antibodies (Miller et al. 2003) showed that the pituitary and hypothalamus of PC2-deficient mice lacked α-MSH, accumulating ACTH, ACTH-containing intermediates, and POMC as well as β-endorphin(1-31). The impaired processing was largely confirmed by mass spectrometry-based peptidomics (Wardman & Fricker 2011). The studies also revealed that processing of POMC by one enzyme or the other showed cleavage site exclusivity, preference, and permissiveness, indicating both specificity and redundancy in their enzymatic functions. Thus, the production of ACTH is dependent on PC1/3, that of MSHs on PC2. Interestingly, the physiopathology of POMC-producing pituitary and nonpituitary tumors and the associated paraneoplastic syndromes can be partially explained by the relative levels of expression of these two convertases, with PC1/3 being more expressed in corticotroph adenoma and PC2 in ectopic tumors (Tateno et al. 2007, Tani et al. 2011).

From proenzyme to active enzyme or escort protein

Maturation and activation of pro-PCs

PCs are themselves products of secretory precursor proteins. Following the basic model defined for furin (Molloy et al. 1994), they are biosynthesized in the endoplasmic reticulum (ER) as inactive zymogens; they get matured by a primary autocatalytic cleavage between the prodomain and the catalytic domains. The propeptide and the mature enzyme navigate as inactive complexes toward more acidic downstream compartments (trans-Golgi network, secretory vesicles), where the propeptide undergoes a secondary cleavage and dissociate from the mature enzyme, which becomes fully active (see Fig. 5A for PC1/3). The primary autocatalytic cleavage site generally corresponds to the cleavage specificity in heterologous substrates.
As shown for furin (Creemers et al. 1995), engineered mutations of this site invariably prevent activation of the proenzyme and causes retention in the ER.

**From proenzymes (PCSK1-8) to PCSK9 as an escort protein**

Unlike all the other PCs, the PCSK9 zymogen, which is primarily produced by the liver, normally undergoes a primary cleavage after Gln152 (F-A-Q\(^{152}\)S-I-P), but not a secondary one. It becomes an enzymatically inactive complex made of the propeptide tightly bound to the mature PCSK9. The complex becomes an escort protein directing LDL receptor into lysosomes for degradation, thus reducing hepatic clearance of LDL-cholesterol (LDL-C) (Mbikay et al. 2013, a review). Therefore, preventing the PCSK9 autocleavage may constitute a strategy of invalidating its escort activity.

We have discovered, in human subjects, a PCSK9 variant that fails to undergo the primary cleavage due to a Gln152His (Q152H) mutation at the P1 residue. First identified in four members of a French-Canadian Quebec family (Mayne et al. 2011), this mutation has been found in two other Quebec families. The families include 51 heterozygous and 3 homozygous carriers. Their mean plasma (LDL-C) is significantly lower than that of noncarriers (Fig. 6). Homozygous carriers have no circulating PCSK9. The Q152H PCSK9 mutation is believed to be strongly cardioprotective. Intriguingly, it has so far been found only in the three previously mentioned French-Canadian families.

The hypocholesterolemic effect of anti-PCSK9 drugs in humans has been widely demonstrated in clinical trials (Page & Watts 2015). Despite the success, there are uncertainties about the metabolic consequences of long-term drastic reduction of plasma cholesterol. In this context, we have shown that a strain of mice globally deficient for PCSK9 exhibited glucose intolerance and prediabetes with age (Mbikay et al. 2010). More recently, we have noted white adipose tissue anomalies in a PCSK9\(^{Q152H}\) female subject (Wassef et al. 2015). It has been recommended that patients treated with anti-PCSK9 drugs be monitored for adverse neurocognitive effects (Swiger & Martin 2015). For our part, we plan to verify whether the remarkable lifelong hypocholesterolemia observed in our PCSK9\(^{Q152H}\) carriers might protect them from cognitive impairment of cerebrovascular origin and/or of Alzheimer’s degeneration.

Our discovery of the PCSK9\(^{Q152H}\) in the Quebec population is a close-to-home illustration of the numerous ramifications of the prohormone theory in various aspects of physiology. It illustrates how a single amino acid substitution in the sequence of a precursor protein can provide an exceptional opportunity to explore novel research avenues. The discovery of flanking pairs of basic amino acids in the β-LPH/γ-LPH/β-MSH model nearly 50 years ago (Chrétien & Li 1967) influenced many research groups into adopting endoproteolysis as a research theme.

**Importance of the PCs in human biology**

In 2001, Gary Thomas summarized the biological importance of the prohormone theory and the PCs in these terms: ‘These studies were as revolutionary as those by Krebs and Fischer, which showed that protein phosphorylation is a universal modification in signal transduction’. He also noted the relationship between the PCs with different pathophysiological conditions (Thomas 2002). Previously, Chrétien et al. (1995) had also predicted a wide range of clinical applications based on the great variety of substrates known to be activated by the PCs. Recent reviews have been written on the subject (Artenstein & Opal 2011, Chrétien 2012, Seidah & Prat 2012). Figure 7 illustrates the
biological ramifications of these enzymes in health and disease. Multiple studies have demonstrated association between genetic variations of PCs and various human health conditions: PC1/3 and PC2 in obesity and diabetes (Zheng et al. 2012, Nead et al. 2015); furin in atherosclerosis (Turpeinen et al. 2011); PC5/6 and mostly PCSK9 in cholesterol metabolism (Iatan et al. 2009, Wu & Li 2014, a review); PC7 in iron metabolism (Oexle et al. 2011). Ex vivo and in vitro as well as animal studies have implicated these enzymes in cancer (Mbikay et al. 1997, Khatib et al. 2002, a review), in vascular remodeling (Stawowy 2015, reviews), and viral diseases (Pasquato et al. 2013, a review). PC-targeted therapies are foreseeable in the near future, most imminent among them being the treatment of hypercholesterolemia and cardiovascular with inhibitors of PCSK9 (Wu et al. 2015). Anti-PCSK9 therapies may also be applicable to infectious diseases as suggested by the resistance to bacterial septic shock of carriers of hypocholesterolemic mutations in its gene (Walley et al. 2014).

Conclusion
Fifty years ago, sequencing of the pituitary peptides related to β-LPH revealed homologies that led to the prohormone theory and, eventually to the POMC precursor model. Who would have predicted that the presence of two key amino acids at the cleavage site would mark the beginning of new chapter in enzymology involving PCSK1 to PCSK9? Five decades later, a mutation at one amino acid of one PC, the Q152H in human PCSK9, in three large families may open up novel avenues of investigation in medical epidemiology and genetics of aging. We owe most of these developments to Frederick Sanger’s lessons in ‘Sequences, sequences, sequences’ (Sanger 1988). What other surprising discoveries are to come of the prohormone theory and its offspring? Only the future will tell.
Declaration of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of this review.

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