60 YEARS OF POMC

POMC: an evolutionary perspective

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Abstract

Proopiomelanocortin (POMC) is a complex precursor that comprises several peptidic hormones, including melanocyte-stimulating hormones (MSHs), adrenocorticotropic hormone (ACTH), and β-endorphin. POMC belongs to the opioid/orphanin gene family, whose precursors include either opioid (YGGF) or the orphanin/nociceptin core sequences (FGGF). This gene family diversified during early tetraploidizations of the vertebrate genome to generate four different precursors: proenkephalin (PENK), prodynorphin (PDYN), and nociceptin/proorphanin (PNOC) as well as POMC, although both PNOC and POMC seem to have arisen due to a local duplication event. POMC underwent complex evolutionary processes, including internal tandem duplications and putative coevolutionary events. Controversial and conflicting hypotheses have emerged concerning the sequenced genomes. In this article, we summarize the different evolutionary hypotheses proposed for POMC evolution.

Key Words
- proopiomelanocortin
- MSH
- ACTH
- β-endorphin
- opioid
- evolution

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Introduction

Proopiomelanocortin (*POMC*) gene encodes a protein precursor whose posttranslational processing yields the melanocortins among other biologically active peptides. In tetrapods, this precursor integrates three main domains: the N-terminal pro-γ-melanocyte-stimulating hormone (MSH), the central adrenocorticotropic hormone (ACTH), and the C-terminal β-lipotropin (Eipper & Mains 1980). Each domain contains one MSH peptide easily identified by a core sequence HFRW: γ-MSH in pro-γ-MSH, α-MSH as the N-terminal sequence of ACTH, and β-MSH in the middle of the β-lipotropin domain. The C-terminal of the latter also includes β-endorphin, an endogenous opioid peptide (Nakanishi *et al.* 1979) (Fig. 1). POMC is mainly produced in the pituitary gland and its posttranslational processing occurs in a tissue-specific manner. The proteolytic cleavage of POMC by prohormone convertase 1 (PC1) generates pro-γ-MSH, ACTH, and β-lipotropin in the corticotrophs of the anterior pituitary, whereas the cleavage by PC1 and PC2 produces α-MSH and β-endorphin in the melanotrophs of the pars intermedia (Castro & Morrison 1997).

Evolution of opioid/orphanin family

POMC gene belongs to the opioid/orphanin gene family, in which all genes encode at least one opioid core sequence, YGGF, or the PNOC core sequence, FGGF (Dores *et al.* 2002, Dores & Lecaude 2005). The gene

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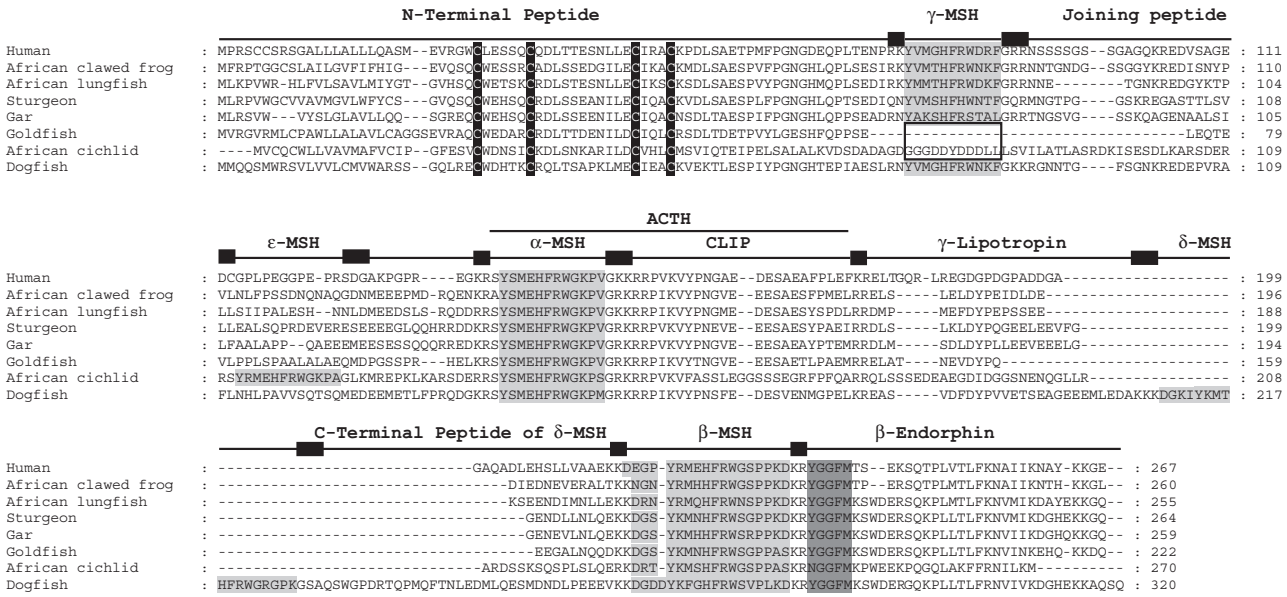

Figure 1

Alignment of POMC sequences from human (*Homo sapiens*), African clawed frog form B (*Xenopus laevis*) (Deen *et al.* 1991), African lungfish (*Protopterus annectens*) (Amemiya *et al.* 1999*a*), white sturgeon form B (*Acipenser transmontanus*) (Amemiya *et al.* 1997), gar (*Lepisosteus osseus*) (Dores *et al.* 1997), goldfish (*Carassius auratus*) (Cerdá-Reverter *et al.* 2003), African cichlid fish (*Haplochromis burtoni*) (Harris *et al.* 2013), and dogfish (*Squalus acanthias*) (Amemiya et al. 1999*b*). White letters on black background indicate fully conserved cysteine residues in all POMC sequences. Light and dark grey boxes show MSH peptides and endorphin core, respectively. The boxed area demarcates γ-MSH segment in species lacking γ-MSH. Black boxes indicate endoproteolytic cleavage sites (data from Cerdá-Reverter *et al.* 2003).

family includes proenkephalin (*PENK*), prodynorphin (*PDYN*), and nociceptin/proorphanin (*PNOC*), as well as *POMC* (Danielson & Dores 1999). Besides this core motif, members of the peptide family differ substantially among themselves and between species, but they all have retained a set of conserved six cysteine residues at the N-terminal region of the molecule. Six residues are found in PENK, PDYN, and PNOC, and only four in POMC. In addition, all family genes exhibit a single intron shortly after the region encoding the signal peptide (Larhammar *et al.* 2015). These unifying factors suggest that they are all derived from a common ancestral opioid gene that probably appeared early in the cordate evolution (Dores & Baron 2011). Neither opioid/orphanin-related genes nor melanocortin receptor-related genes have been described in the genome of cephalochordates (amphioxus and tunicates). Subsequently, the gene family grew concomitantly with the two duplication rounds of the vertebrate genome (1R and 2R), and the 'extra' duplication occurred in the ancestor of teleost fish (3R) (Sundström *et al.* 2008).

Synteny studies have demonstrated that all four opioid peptide genes are only located on three chromosomes of the vertebrate genome. In the human genome, *PDYN* and *POMC* are located on chromosomes 20 and 2, respectively, whereas chromosome 8 hosts both *PENK* and *PNOC* genes. The chicken genome exhibits a different organization because *PENK* is found on chromosome 2, whereas both *POMC* and *PNOC* occur on chromosome 3. *PDYN* is absent from the chicken genome. Therefore, *PNOC* is located together with *POMC* on chicken chromosome 3, but is found with *PENK* on human chromosome 8 apparently as a result of a translocation in the ancestor of placental mammals. The ancestral structure seems to involve the association between *POMC* and *PNOC* because both genes share the same chromosome in the opossum as well as in the genomes of all teleost fish that have been studied (Sundström *et al.* 2010; reviewed in Larhammar *et al.* 2015). It suggests that the last opioid peptide arose by an event of local duplication resulting in *PNOC* and *POMC*. Both genes are close together in several species, including teleost fish. In fact, in the softshell turtle, they are just around 1 Mb apart. However, we cannot exclude the possibility that *PNOC* and *POMC* were on separate chromosomes after 2R and that they were brought together by a translocation event (Larhammar *et al.* 2015). Therefore, the expansion of the opioid peptide system seems to be the result of two complete genome duplications (1R and 2R) and one event of local duplication.

Dating when local duplication took place is complicated. There are three alternative scenarios for the

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evolutionary scheme of opioid peptides, which differ in the timing of the local duplication that generated both *PNOC* and *POMC*. This local duplication could have taken place before 1R (scenario 1), after 1R but before 2R (scenario 2), or after 2R (scenario 3, Fig. 2). The lamprey genome could help solve such dating ambiguities because cyclostomes have two *POMC* sequences. The proopiocortin (*POC*) gene encodes an ACTH sequence, a β-MSH-related sequence, and a β-endorphin sequence, whereas the proopiomelanotropin (*POM*) encodes MSH-B (an α-MSH-related peptide), MSH-A (a β-MSH-related peptide), and a β-endorphin sequence (Takahashi *et al.* 1995*a*,*b*). The fact that lampreys have *POMC* sequences means that the duplication generating *PNOC* and *POMC* probably occurred before the split of cyclostomes form other chordates. It is impossible to say that *POMC* emerged before the second genome duplication because it is unknown whether genome lampreys double one or twice (Sundström *et al.* 2010). In fact, Dores (2013) reported a new model that accepts that lampreys are 2R organisms, which provides considerable support for the existence of two *POMC* orthologs in lampreys. This model predicts unidentified *PENK* and *PDYN* genes in lampreys. Enkephalin-like peptides have been characterized in the lamprey brain (Dores 2013), but final corroboration will depend on the conclusion of the lamprey genome sequencing project. Ongoing results of the genome sequencing project has provided sound evidence that lampreys have undergone 2R (Smith *et al.* 2013) but probably also a third independent genome duplication (Mehta *et al.* 2013). Therefore, it is possible that POC and POM arose in this specific lamprey duplication event.

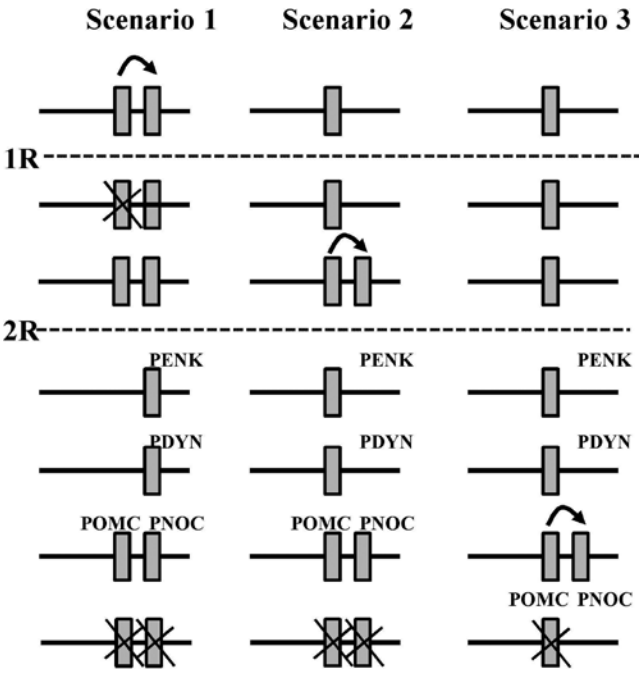

Figure 2
Proposed evolutionary histories for the opioid/orphanin family by genome and local duplications. The timing of the duplication of the common ancestor leading to PNOC and POMC is uncertain, and three different scenarios have been suggested. Local duplication generating both PNOC and POMC could take place before 1R (scenario 1), after 1R but before 2R (scenario 2), or after 2R (scenario 3). PENK, preproenkephalin; PDYN, preprodynorphin; PNOC, preproorphanin; POMC, proopioimelanocortin (Data from Sundström *et al.* 2010).

The genome of teleost fish experienced a third genome duplication round (3R), and the emerging duplicated genes have often been conserved (Meyer & Van de Peer 2005). These new gene copies can experience neofunctionalization (by the new copy), subfunctionalization (both copies share the function of the original copy), or pseudofunctionalization (the sequence of the new copy degenerates and loses its function). *POMC* duplicates have been described in all teleost fish in which the genome is sequenced already (Sundström *et al.* 2010, for references) and subfunctionalization has been demonstrated in *Tetraodon* (de Souza *et al.* 2005). Two different *POMC* orthologs have been characterized in *Tetraodon*. *POMCα* is expressed in the nucleus lateralis tuberis of the hypothalamus, the homolog of the arcuate nucleus in fish, as well as in the rostral pars distalis and pars intermedia of the pituitary gland, whereas *POMCβ* is expressed in the preoptic area of the brain and weakly in pars intermedia of the pituitary gland. *POMCβ* genes have a β-endorphin segment that lacks the consensus opioid signal and seems to be under neutral evolution in tetraodontids, whereas *POMCα* genes possess well-conserved peptide regions. Thus, *POMC* paralogs have experienced subfunctionalization of both expression and peptide domains during teleost evolution. Three different genes have been found in some species such as barfin flounder (*Verasper moseri*) (Takahashi *et al.* 2005), Burton's mouthbrooder (*Haplochromis burtoni*), and medaka (*Oryzias latipes*) (Harris *et al.* 2013), but they seem to be the consequence of lineage-specific gene duplication events other rather than genome duplication (Sundström *et al.* 2010).

Evolution of POMC structure

POMC is the most complex of the four opioid precursors due probably to the insertion of the melanocortin sequences (ACTH and other melanocortin motifs). This complex precursor probably arose when a DNA segment encoding an MSH sequence was inserted into the preproendorphin gene as both N-terminal region and carboxyterminal

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peptide maintain structural/sequence identities with the three other opioid prepropeptides. POMC keeps the four cysteine residues in the aminoterminal region and encodes an endorphin peptide in the C-terminal region, although it could also be the result of accumulative mutations in the ancestral gene that lead to multiple melanocortin sequences as a result of unequal crossing-over events (Dores & Baron 2011). Consequently, POMC has coevolved together with two different receptor families: opioid and melanocortin receptors. One of the key questions about the POMC evolution is when the segment encoding melanocortins or a larger segment encoding multiple melanocortin copies was inserted. Sundström *et al.* (2010) provided evidence that this segment was inserted after the two vertebrate tetraploidizations because it was then when a distinct preproendorphin gene arose. However, it cannot be excluded that a gene encoding ACTH/melanocortin arose independently much earlier as genes encoding melanocortin receptors have also suggested arising as a result of tetraploidizations from a single gene (Cortés *et al.* 2014).

As mentioned previously, the structural plan of the POMC precursors differs between species. Although the organization plan of the agnathan POMC before tetraploidization is doubtful, there is greater certainty regarding the structure of the ancestral gnathostome POMC. The presence of three MSH core sequences in tetrapod POMC sequences (namely, α-MSH, β-MSH, and γ-MSH) suggests POMC evolved through intragenic duplication of an ancestral *MSH* gene (Nakanishi *et al.* 1979). POMC precursor in sarcopterygian fish (lobe-finned fish) shows the same three MSH domains (Amemiya *et al.* 1999*a*, Dores *et al.* 1999). The γ-MSH domain appears only as a vestige in non-teleost fish, including sturgeons (Amemiya *et al.* 1997, Alrubaian *et al.* 1999), and is not present in teleosts (Salbert *et al.* 1992, Cerdá-Reverter *et al.* 2003). Cartilaginous fish have an additional fourth MSH domain termed δ-MSH (Amemiya *et al.* 1999*b*). A novel melanocortin peptide, termed δ-MSH, has been described in cichlid and Pomacentridae species as a result of a putative tandem duplication of the segment α-MSH-ACTH (Harris *et al.* 2013) (Fig. 3).

This suggests that the POMC precursor of the ancestral gnathostome exhibited three melanocortin domains (γ-, α-, and β-MSH), and, following the divergence of the ancestral gnathostome into cartilaginous, ray-, and lobe-finned fish, three structural planes emerged. Lobe-finned fish, including lungfishes (*Protopterus annectens* and *Neoceratodus forsteri*), coelacanth (*Latimeria chalumnae*), and subsequently tetrapods, kept the ancestral organization, i.e. three melanocortin domains (γ-, α-, and β-MSH). The cartilaginous lineage added a fourth melanocortin domain (γ-MSH) to the POMC structure, which probably arose as duplication from the β-MSH–β-endorphin segment. The new δ-MSH sequence is positioned between ACTH/α-MSH and β-MSH, but its function is unknown as its binding to melanocortin receptors is poor (Amemiya *et al.* 1999*b*). This duplication process seems to be unique

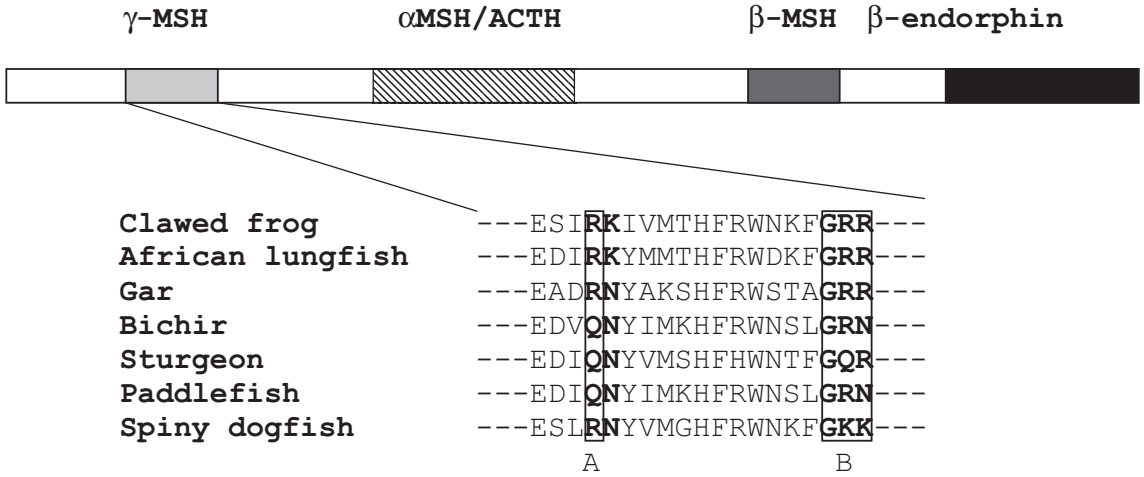

Figure 3
Comparison of γ-MSH sequences. The γ-MSH sequence and the flanked proposed endoproteolytic cleavage sites for five ray-finned fish, one lobe-finned fish, and two tetrapods are presented. The N-terminal cleavage site (A) and the C-terminal cleavage and α-amidation site (B) are boxed. Bichir (*Polypterus senegalus*) (Bagrosky *et al.* 2003); paddlefish (*Polyodon spathula*) (Danielson *et al.* 1999). Figure 1 for additional references (Data from Dores & Baron 2011).

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to the cartilaginous lineage and may have occurred after divergence from the gnathostome of the osteichthyan lineage. This genetic rearrangement took place early after the divergence of cartilaginous fish, as indicated by the presence of the fourth MSH peptide in both elasmobranch and holocephalan lineages (Amemiya *et al.* 1999*b*).

The third organizational plan is found in ray-finned fish that currently include Chondrostei and Neopterygii lineages. Chondostrei includes bichirs (Polypteriformes) and sturgeons and paddlefish (Acipenseriformes), whereas Neopterygii integrates gars (Semiontiformes), bowfin (Amiiformes), and teleost fish. The endpoint in ray-finned fish is the complete deletion of the γ-MSH domain (Cerdá-Reverter *et al.* 2003). The meaning of the γ-MSH peptide is one of the most intriguing and challenging questions of POMC evolution. γ-MSH is the main ligand of melanocortin 3 receptor (MC3R), but the levels of processed peptide are low in any tested species (Roselli-Rehfuss *et al.* 1993). In addition, the search for potent specific full agonist has been unfertile (Hruby *et al.* 2007). It suggests that the N-terminal part could play a role in the binding to the receptor, and by extension, it suggests the alternative processing of the N-terminal region and the existence of N-extended forms much more active at the MC3R. Experiments during the 1990s by Robert Dores and coworkers suggest that this complete deletion of γ-MSH domain in teleost fish took place gradually during the evolutionary process. Therefore, chondrostean kept the ancient organizational plan, i.e. three MSH domains (γ-MSH, α-MSH/ACTH, and β-MSH/β-endorphin); however, the N-terminal γ-MSH domain exhibits some distinctive features. Lobe-finned fish and tetrapod species show an endoproteolytic cleavage site just prior to the γ-MSH peptide, which is characterized by the presence of an arginine basic residue (R) while maintaining a lysine residue (K) at the N-terminal extreme of γ-MSH processed peptide (Dores *et al.* 1999). Both endoproteolytic cleavage and amidation signals also occur at the C-terminal region of the γ-MSH peptide (Fig. 3). This basic arginine residue is still present in gar but absent in bichir, sturgeon, and paddlefish POMC precursors. However, the gar sequence does not exhibit a basic lysine residue C-terminal to the arginine residue. It is not clear whether the RN motif present in the gar precursor can work as a dibasic cleavage site. Interestingly, spiny dogfish also exhibits the same dibasic pair. However, it is clear that this cleavage motif is absent in polypteryformes and acipensiformes precursors, which makes γ-MSH processing and release from these POMC precursors improbable. The C-terminal cleavage site is also absent in bichir, sturgeon, and paddlefish POMC precursors but present in gar sequences. In addition, the sturgeon precursor also exhibits a substitution in the core sequence HFRW, which is crucial for binding to melanocortin receptors. All these mutations suggest a gradual degeneration of POMC precursors in ray-finned fish, leading to the complete deletion observed in teleost fish. In this last lineage, γ-MSH domain is absent and the deletion even affects the spacer region between γ-MSH and α-MSH/ACTH. However, this massive modification in the N-terminal region did not affect POMC processing in the teleost pituitary. Interestingly, most teleost fish lack MC3R, which has been reported as the γ-MSH receptor in mammalian species. The absence of both MC3R and γ-MSH in the POMC precursor suggests a coevolutionary process of the peptide/receptor system.

Summary

In summary, the POMC is a vertebrate hormonal precursor belonging to the opioid/orphanin family. It probably emerged prior to the rise of jawless vertebrates, over 500 million years ago. The family expanded thanks to the two genome duplication rounds, generating PENK, PDYN, PNOC, and POMC precursors. The complex evolution of POMC also includes internal tandem duplications within the *POMC* gene to generate to up to five different MSH sequence cores or local duplications to generate POMC and PNOC. The lack of MC3R and the main receptor ligand γ-MSH in teleost fish POMC also suggest the presence of putative coevolutionary processes during the POMC evolution.

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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