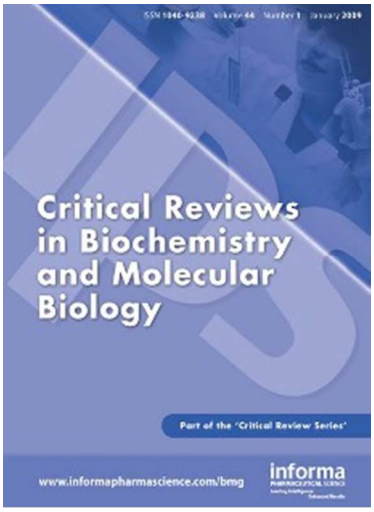

Cryptides: latent peptides everywhere

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Cryptides: latent peptides everywhere

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Cryptides: latent peptides everywhere

Proteomic surveys with top-down platforms are today revealing thousands of naturally occurring fragments of bigger proteins. Some of them have not functional meaning because they derive from pathways responsible for protein degradation, but many have specific functions, often completely different from that one of the parent proteins. These peptides encrypted in the protein sequence are nowadays called cryptides. They are frequent in the animal and plant kingdoms and represent a new interesting -omic field of investigation. To point out how much widespread is their presence, we describe here the most studied cryptides from very common sources such as serum albumin, immunoglobulins, hemoglobin, and from saliva and milk proteins,. Given its vastness, it is unfeasible to cover the topic exhaustively, therefore only several selected examples of cryptides from other sources are thereafter reported. Demanding is the development of new –omic platforms for the functional screening of new cryptides, which could provide suggestion for peptides and peptido-mimetics with variegate fields of application.

Keywords: cryptides; encrypted peptides; latent peptides; hidden peptides: albumin; immunoglobulins; hemoglobin; hemorphins.

1. Introduction

The name of cryptides was firstly introduced by Mukai and colleagues after the discovery of bioactive peptides deriving from mitochondria, which were named mitocryptide-1 and mitocryptide-2 (Mukai et al. 2009). They defined cryptides the fragmented peptides generated during maturation or degradation processes of functional proteins showing various biological activities distinct from those of the parent proteins. Later, cryptides were classified by Autelitano and colleagues in three families: type I cryptides were defined as bioactive peptides detectable *in vivo* with a function different from its precursor; type II cryptides were defined as peptides detectable *in vivo* with activities related but not identical to those of the parent protein; type III cryptides were defined as bioactive peptides generated *in vitro* by digestion of a protein that may or not also exist *in vivo* (Autelitano et al. 2006; Samir and Link 2011). It is well known that the discrimination between a peptide and a protein is conventional, limiting the definition of a peptide to the number of the amino acids in the sequence (commonly not more than 50 residues), however some cryptides might be larger. Moreover, not all the peptide fragments generated by the *in vivo* digestion of a protein have a biologic role and not all the biologically active peptides are cryptides because they might not derive from a parent mature protein. For instance, in the last years it was demonstrated that small peptides are originated from the translation of short upstream RNA open reading frames (Andrews and Rothnagel 2014). However, even if the latter peptides show regulatory activities, they cannot be properly considered cryptic peptides because they are not encrypted in a protein sequence (Fig.1). We limited in this review our description of cryptides to the examples reported in Fig. 1, where their definition is restricted to **functional** sequences encrypted into a **functional** polypeptide of any length which can be released *in vivo* **within a cell** (**intracellular cryptides**) and that can be utilized within the cell and/or outside the cell after their release, as well as to peptides hidden in a **functional** polypeptide sequence of any length generated by proteolytic events occurring **after secretion** (**extracellular cryptides**) regardless they occurred

in vivo, *ex vivo* or *in vitro* (Fig. 1). It is relevant to remark that all cryptides must have a function distinct or related, but not similar to that one of the parent polypeptide.

The first assumption of the existence of these peptides fragments was made around 1960 –'70 by the observation of not-identified proteolytic products from human and mammals milk with antibacterial and antiviral activity (Liepke and Zucht 2001; Oddy 2001; Hosea Blewett et al. 2008;). The possibility that parent proteins can release peptides with variegate activities has been further supported in the 1975-'85 decade by the discovery of active fragments of hemoglobin such as neokyotorphin, a peptide hidden in the α-globin chain (Gomez et al. 2010), and hemorphins, small "non-classical" opioid-like peptides generated by specific enzymatic hydrolysis of the β-type chains of hemoglobin (Zhao et al. 1997; Nyberg et al. 1997). The advent of the modern high throughput proteomic platforms is to date largely expanding the number of putative cryptides identification. The best approach able to characterize with confidence and robustness naturally occurring "latent" peptides is top-down proteomics, that avoiding any proteolytic pre-treatment allows studying directly the complex of the peptides deriving from bigger proteins present in the biological sample (Messana et al. 2013). The aim of this review is to point out the widespread presence of cryptides underlying their important role in several biological pathways selecting interesting examples deriving from the most common and well known proteins, such as serum albumin, immunoglobins, hemoglobin, as well as describing cryptides deriving from bodily fluids as saliva or milk, with particular emphasis to human cryptides. Because the number of cryptides from other sources is huge, the last section is devoted to the description of selected examples, apologizing for relevant omissions.

2. Serum albumin cryptides

Human serum albumin (HSA) is the most abundant protein in blood and cerebrospinal fluid. It controls the plasma oncotic pressure and it is a relevant carrier for endogenous and exogenous

compounds, increases the lifetime of hydrophobic compounds, inactivates toxic compounds, induces chemical modifications of some ligands, and displays antioxidant and enzymatic properties (Ascenzi et al. 2015). Furthermore, HSA can be a source of various cryptides. For instance, the subdomain IIIB of HSA acts as gonadotrophin surge-attenuation factor, an ovarian factor that acts on the pituitary to attenuate the pre-ovulatory LH surge. Tavoulari and colleagues (Tavoulari et al. 2004) have shown that recombinant C-terminal domain of HSA (residues 490-585, subdomain IIIB) reduces the GnRH-induced LH secretion of primary rat pituitary cultures by 50-80%. Interestingly, the recombinant full HSA domain III (residues 381-585) or full-length HSA are inactive demonstrating the specificity of subdomain IIIB. Tryptic and chymotryptic digestion of HSA generates two peptides with cathepsin B inhibitory properties. These peptides correspond to fragments 65-70 (SLHTLF; one letter code) and 403-407 (FQNAL) of HSA and were named cabin-A1 and cabin-A2, respectively (Nakagomi et al. 2002). The trypsin digest of HSA originates peptides with ACE inhibition activity too. One is acein-1 (Nakagomi et al. 1998), a heptapeptide (YLYEIAR, fr. 138-144) acting as non-competitive inhibitor with an IC_{50} value of 16 μmoles/L although, as we are aware, its anti-hypertensive activity was not explored *in vivo*. Another non-competitive ACE-inhibitor is albutensin A (AFKAWAVAR), corresponding to fragment 210-218 of HSA and with an IC_{50} value of 1.7 μmoles/L (Nakagomi et al. 2000). It was demonstrated that albutensin A is able to contract ileum *in vitro* with a contraction profile similar to casoxin C (see section on casein cryptides) and oryzatensin, a cryptide deriving from rice albumin (Takahashi et al. 1998). All these peptides have homology with the C-terminal sequence of complements C3a and C5a and it was demonstrated that, as oryzatensin and casoxin C, albutensin A exhibits its ileum-contracting ability as an agonist for their receptors (Takahashi et al. 1998). Albutensin A is also able to decrease food intake in mice and the effect is always mediated through the complement C3a receptor (Ohinata et al. 2002).

Serorphin (YGFQNA) is a cryptide derived from fragment 399-404 of bovine serum albumin with

opioid-like activity (Takahashi et al. 1998). The study on serorphin stimulated the search of other peptides with opioid-like activity containing the YXF sequence (where X represents a polar amino acid residue) such as historphin (YGFGG, from histone H4), valentorphin (YGFII, from carboxipepdidases A and B) and kapporphin (YSFGG, from immunoglobulin κ-chain) (Takahashi et al. 1998).

During proteomic survey by a top-down platform on different samples from bodily fluids, cell and tissues, various fragments of HSA were detected (Vento et al. 2009). Many of them appeared specifically related to sample under analysis, while others were consistently found in almost all the samples analyzed. In particular the fragment 27-50 (DAHKSEVAHRFKDLGEENFALVL) that is ubiquitous and should be submitted to functional screening to establish potential biological activities.

3. Immunoglobulins cryptides

The biological role of antibodies (Abs), related to humoral immunity, is exerted by their ability to recognize and bind with high affinity and specificity antigens (Ags). The structure of the Abs consists of two identical heavy and light polypeptide chains linked by disulfide bonds characterized by variable and constant regions. Hypervariable domains of variable regions are defined complementarity-determining regions (CDRs), and represent the specific Ag binding site, while a constant part (fragment crystallizable, Fc) acts after the formation of the Ab-Ag complex by recruiting other immune system cells and molecules, and leading to the elimination of Ag.

Several studies performed in recent decades have evidenced that Abs may represent an important source of cryptides not only able to modulate the functions of the immune system, but also exerting anti-infective and antitumor activity. The tetrapeptide tuftsin generated from the Fc-segment of IgGs by the action of splenic endocarboxypeptidase and leucokininase was the first phagocytosis-

stimulating Ab-derived peptide characterized (Najjar and Nishioka 1970), later shown to act also as neurotrophic, immunostimulatory and antitumor agent both *in vitro* and *in vivo* (Siemion 1999). This finding provided the impulse to the search for other bioactive fragments of immunoglobulin origin and led to the characterization of several fragments of the H-chain of IgG generated by enzymatic cleavage of IgG, including rigin, immunorphin, immunocortin, peptide p24 and its fragments, with immunoregulatory properties (Navolotskaya 2014). These peptides have never been detected *in vivo*. Three Fc-peptides derived from the major classes of IgG, IgM and IgA, and named H4L, N10K, T11F, showed a significant fungicidal activity at micromolar concentrations also against resistant strains (Polonelli et al. 2012). Furthermore, N10K displays immune-modulatory activity toward human monocytes *in vitro* (Gabrielli et al. 2012).

The *in vivo* potential antifungal activity of peptides generated by the less conserved Ab CDRs can be considered of relative importance due to the low amount of the fragments that may be delivered. However, the importance of CDRs as inspiration supplier of potentially bioactive sequences has been stressed in several studies. Indeed, it has been demonstrated that CDRs-derived peptides may display antimicrobial, antiviral and antitumor activities regardless of their specificity for a given Ag (Polonelli et al. 2008). Polonelli and colleagues (Polonelli et al. 2003) were the first to describe an Ab-derived microbicidal peptide, obtained by the substitution E→A in a very active synthetic CDR-related peptide, named “killer peptide” (KP). KP was active at micromolar concentrations against many pathogenic yeasts, even those resistant to conventional antifungal agents. KP also showed activity against pathogenic bacteria, protozoa, HIV-1 and influenza A viruses (Ciociola et al. 2014), as well as immunomodulatory effects. The different biological activities of KP have been related to its dimeric form derived by self-aggregation of β-sheet structures resulting in the formation of hydrogel-like aggregates (Pertinhez et al. 2009) able to provide protection against proteases. PEP3H originated by CDR H3 of RS-348 showed *in vitro* and *in vivo* antiviral activity against RSV (Burgeois et al. 1998), and synthetic peptides derived from CDRs of an anti-CD4 monoclonal

antibody were able to inhibit HIV-1 promoter activation (Monnet et al. 1999). Also a tyrosine sulfated peptide derived from an HIV-1-neutralizing Ab was demonstrated to be able to inhibit HIV-1 infections (Dorfam et al. 2006).

Among the different CDR peptides with antitumor activity, noteworthy is a 16-residue peptide (C7 H2) derived from CDR H2 of KAb mAbC7 able to inhibit both the germination of human umbilical vein endothelial cells and the lung colonization by melanoma cells in mice (Arruda et al. 2012). A screening on the antitumor activity of synthetic peptides derived from conserved CDR sequences of different immunoglobulins against human tumor cell lines and murine B16F10-Nex2 melanoma evidenced that rather frequent CDR sequences are endowed with specific antitumor properties and may be candidates to be developed as anti-cancer drugs (Figueiredo et al. 2014).

4. Hemoglobin cryptides

Hemoglobin (Hb), the milestone of quaternary cooperative protein, beside its fundamental role of oxygen transport and energy metabolism in vertebrates is a precious source of bioactive peptides originating from both α- and β-globin chains (Giardina et al. 1995). that constitute a tissue specific pool (Ivanov 1997). The latter concept originated from the recognition of different panels of peptide patterns in diverse tissues, suggesting the occurrence of tissue specific enzymatic cleavages on large circulating hemoglobin fragments (Ivanov et al. 1997).

An interesting paper (Zamyatnin 2009) compared the amino acid sequence of natural regulatory oligopeptides from the EROP-Moscow database with the primary structure of bovine hemoglobin in order to disclose, by a theoretical structure/function analysis, possible associations between specific sequences and selected functions. In addition to recognized bioactive hemoglobin peptides, many natural regulatory oligopeptides in the database resulted to contain sequence traits, at least of five amino acid residues, of bovine hemoglobin associated to antifreeze, antimicrobial, enzyme

inhibitor, hormone, neuropeptide, peptide potentiatory activities. More recently, the advances in the knowledge of endogenous peptides derived from hemoglobin chains have been interestingly reviewed also discussing about their biological activities and possible origins (Gomez et al. 2010).

The discovery of hemoglobin peptides begin in the 1980s with the identification of neokyotorphin, a five amino acid residues C-terminal peptide of the α-globin chain, containing the kyotorphin dipeptide and showing analgesic properties similar to Leu-enkephalin together with other biological roles (Gomes et al. 2010). Neokyotorphin and its des-Arg neokyotorphin showed cytolytic activity towards human erythroid leukemia and murine transformed fibroblast cell lines (Blishchenko et al. 1996). Additionally, hemoglobin fragments related to neokyotorphin were reported to have a proliferative effect on diverse cell cultures (Sazonova et al 2003).

Corresponding to specific sequences of the β-globin chain, the hemorphins peptides have been isolated from bovine blood treated with a mixture of gastrointestinal enzymes (Brantl et al. 1986) and successively isolated from extracts of cortex and subcortex bovine brain (Karelin et al. 1994). These tissue specific small peptides, all containing the tetrapeptide YPWT in the sequence, belong to the group of non-classical opioid peptides with affinity towards μ- and σ-opioid receptors, and were additionally reported to exert numerous other biological activities (Nyberg et al. 1997; Zhao et al. 1997; Gomez et al. 2010). Table 1 describes the diverse hemorphin's subfamilies based on their N-terminal sequence. Particularly, the LVV-hemorphin-7 and VV-hemorphin-7, head of the LVV- and VV- hemorphins' subfamilies, are 10 and 9 amino acids peptides, respectively, specific of the central nervous system (CNS) and corresponding to fragments 32-41 and 33-41 of β (or γ, δ and ε) globin chain (Nyberg et al, 1997). In addition to the opioid like activity, the LVV-hemorphin-7 was identified as the endogenous ligand of angiotensin IV receptor (Moeller et al. 1999) and showed inhibitory activity towards angiotensin converting enzyme (ACE) (Lantz et al. 1991) and insulin-regulated aminopeptidase (IRAP) (Lammerich et al. 2003) together with an important role of in

homeostasis (Barkhudaryan et al. 2010).

Hemorphins have been studied in relation to different physio-pathological conditions. LVV-hemorphin-7 seems to play a role in blood pressure regulation (Cejka et al. 2004), in connection to their ACE inhibitor capability, and in learning and memory by inhibition of IRAP (Albiston et al. 2004) and was identified in the bronchoalveolar lavage fluid of a non-small cell lung cancer patient (Duethman et al. 2000). Hemorphins have been also reported as potential drug candidate and putative biomarkers of breast cancer (Cohen et al. 2003; Song et al. 2012). Hemorphins showed citotoxicity towards tumor cell lines (Blishchenko et al. 2002a; Blishchenko et al. 2002b) and tumor growth inhibition capacity (Mikhailova et al. 1996; Blishchenko et al. 2005). A study on a rat sarcoma model demonstrated the capability of LVV-hemorphin-7 and hemorphin-7 to modulate calcineurin activity, DNA methylation and to form DNA complexes (Barkhudaryan et al. 2012, Hunanyan 2011). The top-down proteomic characterization of CSF in relation to posterior cranial fossa pediatric brain tumors identified the LVV- and VV-hemorphin-7 as candidate biomarkers for the prognosis of disease (Desiderio et al. 2012). Together with them, a panel of four peptides with molecular weight around 3 kDa and corresponding to specific sequences of both α- and β-globin chains has been also identified. These peptides, whose biological function is still not clear, have been also characterized in lipoaspirate fluid (Inserra et al. 2016) and craniopharyngioma adamantinomatous pediatric brain tumor intracystic fluid (Martelli et al. 2014). These peptides have been previously isolated in lysates of human erythrocytes (Karelin et al. 1995).

Together with its role in pain by modulation of enkephalin degradation, the LVV-hemorphin-4, also called spinorphin, was reported as possible anti-inflammatory endogenous regulator (Yamamoto et al. 2002). In addition to hemorphins, other bioactive fragments of hemoglobin, particularly of α-globin, have been identified under the name of hemopressins. Following the first identification of the α-globin hemopressin peptide (PVNFKFLSH), with CB1 cannabinoid receptor antagonist

activity, from rat brain extracts (Rioli et al. 2003), other hemopressins with N-terminal extensions, namely RVD- and VD-hemopressins, have been also identified in mouse, together with a similar VD-hemopressin peptide from the hemoglobin beta-chain. Differently from the original peptide, the N-terminal extended form of hemopressin showed agonist activity towards cannabinoid receptors (Gomes et al. 2010). Hemopressin additionally showed antinociceptive properties and capacity to reduce blood pressure (Gelman and Fricker 2010). Other bioactive peptides fragments from α-globin, corresponding to fragments 110-125 and 129-134, show bradykinin-potentiating action (Gelman and Fricker 2010). Another fragment of α-hemoglobin, isolated from human endometrial scraping samples, exhibited a potent antibacterial activity (Deng et al. 2010).

In conclusion hemoglobin still holds many mysteries to clarify. The diverse functions of its peptide fragments could represent a good recycling strategy of the cells to exert multiple actions in economy. Initially ascribed to the catabolism of blood hemoglobin at tissue level, the majority of its derived bioactive neuropeptides seems to origin directly from the hemoglobin expressed in brain tissues (Gelman et al. 2010). The process of synthesis and the mechanisms of action of the hemoglobin derived peptides need to be clarified yet making this issue an intriguing field of investigation.

5. Cryptides from saliva

In human saliva many putative latent peptides derive from proline-rich proteins (PRPs). Within whole human saliva PRPs account for more than 30% (w/w) of total protein content and for about 50-60% (w/w) of proteins secreted by parotid (Bennick 1982; Manconi et al. 2016). From this group of proteins three different families are distinguished, namely acidic PRPs (aPRPs), basic PRPs (bPRPs), and glycosylated (basic) PRPs (gPRPs) (Oppenheim et al. 2007). bPRPs are secreted only by parotid glands. On the contrary aPRPs are secreted by both parotid and submandibular/sublingual glands (in different percentages) and can be detected both as intact and

truncated proteoforms The intact proteoforms are called PRP-1, PRP-2, Pif-s, Db-s, and Pa, the first two codified by the locus *PRH2* and the last three codified by the locus *PRH1*. A convertase cleavage at Arg_{106}.(Arg_{127} for the Db-s proteoform) is responsible for the releasing from four truncated proteoforms (Pa is not cleaved for the substitution Arg_{106}→Cys) named PRP-3, PRP-4, Pif-f and Db-f and of a common C-terminal peptide of 44 amino acid residues, called P-C peptide. Differently from aPRPs, bPRPs encoded by *PRB1*, *PRB2* and *PRB4* genes are instead detectable in saliva only as proteins fragments from the pro-proteins, with a molecular weight ranging from 5 to 27 kDa. Today at least 18 bPRPs have been structurally characterized, namely II-2, P-E, IB-6, Ps-1, Ps-2, IB-1, P-J, IB-8a, P-F, P-H, P-D, II-1, protein glycosylated A, CD-IIg, and Gl1-4. Acidic and basic PRPs, after secretion, are further hydrolyzed in the mouth by endogenous and exogenous enzymes (Messana et al. 2008; Hemerhorst et al. 2008) in smaller putative cryptides 7-20 residue-long, mainly originating from cleavages at the XPQ↓G site (with X preferably K, to a lesser extent S or R (Helmerhorst et al. 2008), as reported in Table 2. From these observations Helmerhorst and colleagues were able to characterize a new glutamine endoprotease deriving from Rhodia bacteria (Helmerhorst et al. 2010, Zamakhchari et al. 2013).

The role of the parent proteins is different from that one of the fragment released. Indeed, aPRPs interact to hydroxyapatite with high affinity (Hay et al. 1987), can participate to the constitution of acquired enamel pellicle (Moreno et al. 1982) and are involved in oral calcium homeostasis inhibiting calcium phosphate precipitation (Bennick et al. 1983). bPRPs functional roles are not completely clarified. Even though their similar structure, the diverse proteoforms from the same pro-protein can exert proper biological activities. As an example, II-2 peptide and Ps-1 protein are involved in the PROP bitter taste responsiveness (Cabras et al. 2012; Melis et al. 2013) while P-E, IB-6, and Ps-2 together with IB-1, P-H (Lu and Bennick 1998; Cai et al. 2006) and P-D peptides (Charlton et al. 1996; Canon et al. 2013) can interact to and precipitate the harmful tannins. Furthermore, IB-6 promotes adhesion of *Candida albicans* on hydroxyapatite surface (O'Sullivan et

al. 1997).

The cryptides generated from the oral proteolysis have different properties of the parent proteins. For instance, six latent peptides from IB-8a ($Con1^{+}$/$Con1^{-}$), one from P-E, one from IB-1, and one from each PRB1-S, M, L proteins were identified in enamel pellicle (Vitorino et al. 2007). Other originated from PRB2-L (nine peptides) and from PRB1-S, M, L-related proteoforms (three peptides) were identified as enamel bounded peptides (Siqueira and Oppenheim 2009). A 20 residue proline-rich peptide (p1932: GPPPQGGNKPQGPPPPGKPQ), commonly present in human saliva and patented for its antiviral activity, was internalized within primary gingival fibroblast cell line and squamous cancer cell lines (Radicioni et al. 2015). The cytosolic localization was dependent on the cell type: p1932 peptide and its retro-inverso form were able to localize within nuclei of tumor cells, but not in the nuclei of gingival fibroblasts. It acts as an antagonist of the progesterone induced cytosolic Ca^{2+} mobilization in a tongue squamous carcinoma cell line and this dose-dependent activity is mainly confined in the C-terminal region characterized by a four proline repeat flanked by a lysine residue. The lack of activity of the retro-inverso form suggested the involvement of a specific molecular recognition mechanism at the basis of peptide antagonistic effect. The search for progesterone receptors in this oral cancer cell line, identified in the PRGMC1 the main expressed form, suggesting a modulation role of the peptide in the transduction signal pathway mediated by this receptor (Palmerini et al. 2016). The intrinsic propensity of p1932 to adopt a polyproline-II helix arrangement joined to PxxP motifs is suggestive for the interaction with the SH3 domain family. Surface plasmon resonance spectroscopy evidenced specific interactions only with Fyn, Hck and c-Src SH3 domains at nanomolar to micromolar values of dissociation constants. Interestingly, these interacting domains are all included in the Src kinases family, suggesting that p1932 can be involved in the signal transduction pathways modulated by these kinases (Righino et al. 2016). From Table 2 it is evident that p1932 is one of a large series of putative salivary cryptides waiting for a more detailed study of their functions.

Several peptides detected in human saliva derive from crevicular gingival fluid (CGF) arising from the gingival plexus that contains a diverse population of cells including bacteria from the adjacent plaque mass as well neutrophils, mononuclear cells, lymphocytes and migrating leukocytes and desquamated epithelial cells, which may release several secretory products, and microbial metabolites (Seguier et al. 2000). CGF is also distinctive for a very high concentration of thymosin β_4 ($T\beta_4$), a ubiquitous peptide with a pivotal role in the cytoskeletal system as G-actin sequestering peptide, activity probably related to its effects on the regulation and differentiation of T lymphocytes (Low et al. 1986). $T\beta_4$ can release a cryptide called seraspenide, i.e. the Ac-SPDK tetrapeptide corresponding to its N-terminal sequence (Grillon et al. 1990), which inhibits the entry of hematopoietic pluripotent stem cells into the S-phase *in vivo* blocking them in the G0-phase of the cell cycle (Lenfant et al. 1989). It was reported that seraspenide is a substrate of ACE (Rousseau et al. 1995) which in turn seems to be involved in its degradation in human plasma (Rieger et al. 1993). The protective effect of Tβ4 in acute myocardial infarction seems essentially due to the cardioprotective properties of seraspenide (Rossdeutsch et al. 2008).

6. Milk cryptides

More than 400 proteins are present in very different amounts in human breast milk (Roncada et al. 2012; 2013; D'Alessandro et al. 2010; Molinari et al. 2012), which, further than nutritive properties, exert relaxing, satiating, antimicrobial, immune-modulatory, metal-binding, anti-lipidaemic and anti-cancer activities (Nongonierma and Fitzgerald, 2015). During the digestion process these proteins are cleaved in smaller peptides by the action of endogenous (digestive enzymes) or exogenous proteinases (microbiota) that can modulate different biological pathways as reviewed (Korhonen and Pihlanto, 2003; Park and Nam, 2015; Capriotti et al. 2016; Théolier et al., 2014;). The major proteins present in human milk belong to the whey and casein families, while mucins represent a minor percentage of the total proteins (Lonnerdal 2004). Not all these proteins can

generate cryptides. Some of them may be resistant to digestion and act only in the intact form, others can generate bioactive peptides of different dimensions formed during digestion and some are completely digested and utilized as a source of amino acids (Lonnerdal 2014).

6.1 Whey proteins deriving cryptides

While mucins are confined in the milk fat globule membrane, the proteins detectable in high level in the whey fraction are lactoferrin, α-lactalbumin, lactoglobulin, IgS, lysozyme, and serum albumin (Lonnerdal 2004). Cryptides from serum albumin and immunoglobulins have been described previously in sections 1 and 2.

Lactoferrin (or lactotransferrin) is a protein responsible for the iron transport that can generate, after proteolysis, peptides with an antimicrobial and antiviral action which was partly attributed to its fragment called lactoferricin. Human lactoferricin (lactoferricin H) corresponds to the fragment 1-48 of the parent protein and consists of two subunits (namely fragment 1-11 and 12-48 connected by a disulfide bridge; Tab.3), while bovine lactoferricin (lactoferricin B; Tab. 3) corresponds to the fragment 17-41 of the parent protein (Wakabayashi et al. 2003). Lactoferricin binds to the bacterial surface and plays a relevant role in membrane-mediated activities of lactoferrin and reveals antiviral activity against papilloma infections (van der Kraan et al. 2004). Shestakov and colleagues (Shestakov et al. 2012) have shown that lactoferricin, but not lactoferrin, inhibits herpes simplex virus like 2 infection in mice. The N-terminal region of lactoferricin showed powerful antifungal activity against *Candida albicans* species (van der Kraan et al. 2004). A second antimicrobial peptide called lactoferrampin was detected in the N-1 domain of human and bovine lactoferrin (Tab. 3). Bovine lactoferrampin has high candidacidal activity and it is active against *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa* but not against *Actinomyces naeslundii*, *Porphyromonas gingivalis*, *Streptococcus mutans* and *Streptococcus sanguis* (van der Kraan et al. 2004). Bovine lactoferrampin, together with human lactoferricin can inhibit nuclear translocation of

HIV integrase (Wang 2016). Moreover, Eliassen and colleagues (Eliassen et al. 2006) have shown the cytotoxic effect of lactoferricin B against neuroblastoma cell *in vitro* by rapid destabilization of the cytoplasmic membrane and formation of membrane blebs. Depolarization of the mitochondria membranes and irreversible changes in the mitochondria morphology were also evident. Lactoferricin can in turn generate shorter peptides, which can be defined cryptides of second generation (Fig. 1), that are promising candidates with antibacterial activities suggesting the synthesis of peptidomimetics for the treatment of various infectious diseases (Haug et al. 2007). Some examples are represented by the fragment 1-11 deriving from the reduction of lactoferricin H (GRRRSVQWCAV) with antiviral and high antibacterial activity (Wang 2016, Bruni et al. 2016), which were confirmed in animal models and in a phase 1 study in human volunteers (Bruni et al. 2016), and a tetrameric peptide deriving from lactoferricin B ($(RRWQWR)_4$) exhibiting specific cytotoxic effects against oral squamous-cell carcinoma cell lines (Solarte et al. 2015). This antitumor activity was further confirmed by the PFR-peptide (Tab. 3) derived from lactoferricin H that induced necrotic cell death and G0/G1 cell cycle arrest in MEL and HL-60 leukemia cell-lines (Lu et al. 2016). In this study, researchers demostrated that PFR-peptide inhibited leukemia cell growth *in vivo* in immunocompromised mice transplanted with MEL cells.

Moreover, the fragment 18-40 of lactoferricin H, another cryptide of second generation called Lfpep (Tab 3), showed powerful fungicidal activity against *Candida* spp., including fluconazole- and amphotericin B-resistant clinical isolates. The killing activity of Lfpep is mediated by its permeabilizing activity on *Candida albicans* membranes (Viejo-Diaz et al. 2005). The candidacidal activity of Lfpep is higher than that observed for kaliocin-1, another cryptide corresponding to the fragment 171-201 of human lactoferrin (Tab. 3). The comparison among the structure of various antifungal cryptides deriving from lactotransferrin may be of interest in the design of new antifungal drugs.

An in vitro peptic digest of human lactoferrin generated three opiod antagonistic cryptides which were called lactoferroxins A-C (Tab 3) (Tani et al. 1990). While lactoferroxin A has antagonistic effect on mu-receptors, lactoferroxin B and C are more specific for kappa-receptors.

Several whey derived cryptides are termed lactokinins, peptides with inhibitory properties against angiotensin-1-converting enzyme (ACE). Among them α-lactorphin (human or bovine α-lactalbumin Fr. 50-53), β-lactorphin (β-lactoglobulin bovine Fr. 102-105) and β-lactotensin (β-lactoglobulin bovine Fr. 146-149) can be reported as relevant examples (Tab. 3), the first two with sequence similarity to enkephalins (YGGFM or YGGFL). However, the lactokinin with the highest inhibitory ACE activity *in vitro* is the fragment 142-148 of β-lactoglobulin bovine (ALPMHIR) (FitzGerald and Meisel 1999). A statistically significant hypotensive effect has been demonstrated in humans for a limited number of milk cryptides (FitzGerald et al. 2004). Alpha and beta-lactorphins increase the acetylcholine induced relaxation of mesenteric arterial preparations in spontaneously hypertensive rats, but the effect does not involve the prostanoid pathway, instead it seems related to the nitric oxide pathway (Sipola et al. 2002) Interestingly, despite the very similar structure, the beneficial effect of α-lactorphin is directed only towards endothelial function, while β-lactorphin enhances also endothelium-independent relaxation. Beta-lactotensin exhibits anxiolytic-like activity as an agonist for neurotensin NTS2 receptor via activation of dopamine D1 receptor (Hou et al. 2011) and rapidly reduces the levels of serum cholesterol (Yamauchi et al, 2003) in mice. The hypo-cholesterolemic activity was blocked by levocabastine showing the involvement of NTS2 receptor and was blocked by raclopride demonstrating an action of the peptide via dopamine D2 receptor too. Further experiments on mice showed that β-lactotensin increased memory consolidation in the step-through-type inhibitory avoidance test in mice. This effect is specifically mediated by the dopamine D2 receptor but not by the dopamine D1 receptor, because the effect is inhibited by raclopride, but not by SCH23390, an antagonist of D1 receptor (Ohinata et al.2007). Overall β-lactoglobulin derived peptides, although not having the potency of synthetic anxiolytic,

hypo-cholesterolemic or anti-hypertensive drugs, may represent potential natural, non-toxic food ingredients for prevention of stress, atherosclerosis and high blood pressure.

6.2 Caseins deriving cryptides

Caseins (CNs) are divided in different types according to their solubility. The best studied are bovine caseins which are named αS1, αS2, β and κ-casein (Nguyen et al. 2015). To date, the extensive study of β-casein allowed detecting at least 13 variants of this protein, including A1-4, B, C, D, E, F, H1 and H2, of which A1 and A2 are the most common variants (Kaminski et al. 2007). Human milk contains α-, β- and κ-caseins and they account for about 13% w/w of the total protein content, the lowest casein concentration in the breast milk of any studied mammals (Andreas et al. 2015). κ-Casein stabilizes the insoluble α- and β-caseins forming a colloidal suspension. During the digestion process CNs are differently degraded forming numerous fragments whose activity was only partly investigated. α- and β-CNs can generate phosphorylated peptides (caseinophosphopeptides, CPPs), which facilitate metal ion availability (calcium, iron, zinc). The most studied was the CPP β-CN(1-25)4P (4 phosphates) which exhibits a positive effects on iron availability (Bouhallab and Bouglé, 2004). By isothermal titration calorimetry carried out under experimental conditions mimicking those present in the ileum it was established that β-CN(1-25)4P binds Ca^{2+}, Mg^{2+} and Zn^{2+}, but not Cu^{2+} with 1:2 stoichiometry and low binding affinity constants, suggestive for the release of metal ions during intestinal absorption (Zidane et al. 2012). A study performed with three CPPs β-CN(1-25)4P, α(s1)-CN(64-74)4P and α(s2)-CN(1-19)4P bovine on Caco-2 cells established that they increase ferritin synthesis versus iron sulphate alone, β-CN(1-25)4P being the most effective (Garcia-Nebot et al. 2013). They also increase zinc uptake but in a way similar to the increase observed in the presence of zinc sulphate alone, suggesting that, for this ion, the peptide did not exert a specific function. A recent study showed that five CPPs named P1 to P5, characterized by means of LC-MS/MS, were able to selectively allow Mg^{2+} absorption towards

Ca^{2+} in Caco-2 cells growing in a medium enriched with Ca^{2+} and Mg^{2+} (Cao et al. 2017)]. The fragmentation of α- and β-CN generates several antimicrobial peptides. The first peptides detected after chymosin digestion were called casecidins and originated from the C-terminal sequence of β-CN bovine (Lahov and Regelson, 1996). Casecidins inhibited *in vitro* staphylococci, sarcina, *Bacillus Subtilis*, *Diplococcus Pneumoniae* and *Streptococcus pyrogenes*. However, the low activity of casecidins *in vitro*, compared with common antibiotics, induced to explore others CNs fragments and in particular a non-immunogenic product of chymosin digestion that was called isracidin and corresponded to the N-terminal fragment 1-23 of α(s1)-CN bovine, whom activity is comparable to that of commercial drugs. Casecidin 15, casecidin 17 and isracidin (Tab. 4) have been recently found naturally in bovine colostrums (Birkemo et al. 2009) where probably contribute to modulate microbiota in the early calf age. As we are aware these peptides until now have not been detected in human.

β-Casomorphins (BCMs) are a group of peptides with opioid-like activity and, among them, only casomorphin 7 (β-CN bovine 60-66; BCM7; Tab. 4) has been largely investigated (Nguyen et al. 2015). The release of BCM7 by hydrolysis of β-CN occurs in the presence of β-CN A1, B and C bovine, which differently from other β-CN variants such as β-CN A2 bovine, show a His residue instead of Pro at position 67 (Nguyen et al. 2015). Several epidemiological studies suggested a link between consumption of milk containing the A1 variant and increased risk of type 1 diabetes and heart diseases (Laugesen and Elliott, 2003). However, the European Food Safety Authority in 2009 concluded that the data were insufficient to establish a causal relationship between BCM7 ingestion and disease. A recent study showed that BCM7 has a protective effect against glucose-induced renal oxidative stress both *in vivo* (streptozotocin-induced diabetic rats) and *in vitro* (NRK-52E cells) (Zhang et al. 2013). Surely the availability of new analytical methods can in the next future help to elucidate the effective role of BMC7.

β-CN bovine can release casomokinin L (Tab. 4) a derivative of BCM with endothelium-dependent vasorelaxing activity probably mediated by NO. Although casomokinin L has only three residues in common with substance P, it binds to NK_1 receptors, relaxing the artery and exerting an antihypertensive effect (Fujita et al. 1996). Cleavage of β-CN bovine can release two short peptides acting as macrophage activators and with bradikinin-potentiating activity which were named casoparan, and casohypotensin (Tab. 4) and various antioxidant peptides (Lebrun et al. 2004).

During gastric digestion, κ-CN can release the caseinomacropeptide (CMP) a 7-kDa peptide exhibiting growth-promoting activity for lactobacilli and bifidobacteria. During the digestive process peptides derived from CMP can be detected in the intestinal lumen. Recent studies have shown that pepsin and trypsin treatments of CMP promoted the growth of probiotics and that the pepsin treatment was more effective (Robitaille and Champagne, 2014). Therefore, it appears that some casein derived cryptides could have a relevant role in the modulation of the intestinal microbiota, especially in the pediatric age. Kaye and Jollès (1978) evidenced structural similarity of the peptide fragments of bovine κ-CN (Fr. 106-116) and the γ-chain of human fibrinogen (Fr. 400-411). This finding stimulated the discovery of a class of κ-CN fragments called casoplatelins (Tab. 4) with antithrombotic activity, because they are inhibitors of both the aggregation of ADP-activated platelets and the binding of the human fibrinogen γ-chain to its receptor on the platelet surface (Fiat et al. 1993). Although the potential physiological effects of these antithrombotic peptides have not been determined, they have been detected in physiologically active concentrations in the plasma of newborn infants after ingestion of a cow's milk-based formula or human milk, respectively suggesting that these bioactive peptides are released from milk proteins during digestion (Gobetti et al. 2007).

The proteolysis of κ-CN by pepsin can generate various opioid antagonistic peptides called casoxins (Chiba et al. 1989). Among them, casoxin C (Tab. 4) evokes contraction of the ileum with a

contractile profile like that of the structurally homologous fragment 70-77 of human complement C3a. Indeed casoxin C had high affinity for C3a receptor and the induced ileum rapid contraction was mediated by histamine release, while the slow contraction was mediated by a prostaglandin E2-like substance (Takahashi et al, 1997). Finally, α-, β- and κ-caseins can release, as α-lactalbumin and β-lactoglobulin, more than twelve peptides ACE inhibitors, collectively termed casokinins having significant hypotensive effects (FitzGerald et al, 2004). On the whole this limited reported examples of milk cryptides indicated that an accurate study of their activities can be of great stimulus for the characterization of milk derived nontoxic nutraceuticals with the potential to significantly reduce global healthcare cost.

7. Miscellaneous cryptides

It is easily conceivable that many other sources of latent peptides exist: cell organelles, tissue protein matrices may provide a diversity of biologically active molecules. Mitochondria are in this view a rich source of cryptides (Raoof et al. 2010). As an example, cytochromes from porcine neutrophil mitochondria provide important biologically active cryptides. As reported in the introduction, the first discovered were named MCT-1 and MCT-2 (mitochondrial tricopeptide-1 and -2) (Mukai et al. 2009). The sequences of these two peptides coincided with the 23 C-terminal residues of porcine cytochrome c oxidase subunit VIII (LSFLIPAGWVLSHLDHYKRSSAA) and the 15 N-terminal residues of porcine mitochondrial cytochrome b (formyl-MTNIRKSHPLMKIIN), respectively. MCT-1 and MCT-2 are thought to play an important role in the innate immune system, contributing to enhance neutrophil responses to inflammation and traumas, thus to be considered as parts of the damage-associated molecular patterns (DAMPs) molecules produced in mitochondria (MTDs) (Hu et al. 2015). Interestingly, both these peptides present human counterparts (hMTC-1 and hMTC-2) showing identical biological activities (Mukai et al. 2009). Similarly, an octadecapeptide named mitocryptide-CYC (MCT-CYC), isolated from

porcine heart resulted to be identical to the 68-85 sequence of mitochondrial cytochrome C. It was shown to possess features comparable to those of MCT-1 and MCT-2 enhancing the β-hexosaminidase release from neutrophilic-differentiated HL-60 cells (Hokari et al. 2012). Bacterial and mitochondrial proteins are the only source of N-formyl peptides in nature being recognized by specific N-formyl-peptide receptors (FPRL-1, 2). FPRLs have probably evolved to mediate phagocyte migration to sites of bacterial invasion. Mitochondrial N-formyl peptides are damage-associated molecules generated from cytochrome c oxidase (CoxI, CoxII and CoxIII), NADH dehydrogenase subunits (ND1, 2, 3, 4, 4L, 5 and ND6) and ATP synthase sharing structural similarity to bacterial N-formylated peptides. Different novel mitochondrial formyl-peptides have been identified and characterized as agonists for human FPRL-1 and FPRL-2 suggesting that might play a role in inflammatory or degenerative processes upon their stimulation (Rabiet et al. 2005).

Collagen was seen to be another source of latent peptides possessing interesting features linked to wound healing, chemotaxis and antioxidant activities. In particular, a peptide named E1 (GETGPAGPAGPIGPVGARGPAGPQGPRGDKGETGEQ) was purified and structurally characterized after the treatment of collagen derived from bovine Achilles tendon with bacterial proteolytic enzymes. Although its sequence does not contain amino acid residues considered typical antioxidants such as tyrosine, a certain ability to scavenge radical species was observed (Banerjee et al. 2012). Further, the same peptide was shown to possess also wound healing properties (Banerjee et al. 2016). Collagen was seen to be a source of bioactive peptides helping in endothelial injury upon bacterial infection. When capillary-endothelial-derived extracellular matrices were treated with collagenase derived from *Clostridum histolyticum*, a series of peptides were identified by mass spectrometry which were demonstrated to possess wound healing properties (Demidova-Rice et al. 2010).

Autophagy is an important evolutionarily conserved cytoplasm-homeostatic complex of cellular

mechanisms aimed to eliminate damaged cellular components. During this function p62, a protein dedicated to this role also known as Sequestosome-1, sequesters cytosolic proteins (ribosomal protein rpS30 precursor FAU and ubiquitin) into autophagosomes where they are proteolytically converted into cryptides. In the case of *Mycobacterium tuberculosis* infection, p62 may capture bacteria in autophagosomes where they are challenged with the peptides originated as above mentioned that behave as antimicrobial peptides (Ponpuak et al. 2011).

Plasma proteins linked to coagulation were shown to be another rich source of cryptides. In particular, a wide range of peptides from C-terminal sequences of serine proteases, mainly from the coagulation and kallikrein systems, share characteristics common with classical antimicrobial peptides of innate immunity (Kasetty et al. 2011). A series of peptides were predicted by means of bioinformatics approaches identifying 68 S1 serine proteases deriving peptides with antimicrobial activity and sharing the X-[PFY]-X-[AFILV]-[AFY]-[AITV]-X-[ILV]-X(5)-W-[IL]-X sequence (Papareddy et al. 2010; Kasetty et al. 2011). In a similar fashion, antithrombin III (ATIII) beside its anticoagulant effect, exerts anti-inflammatory activity and preserves the microvascular leakage counteracting the bacterial growth in systemic infections. This last property was shown to be linked with the activity of the bacterial proteases which may release several peptides from the parent protein. One of these, named FFF21, deriving from the D helix in ATIII, was identified as an antimicrobial peptide effective against *E. coli* and *P. aeruginosa* through a membranolytic mechanism of action (Papareddy et al. 2014). Also thrombin releases various active peptides (Papareddy et al. 2010): it was indeed demonstrated that the thrombin derived host-defence peptide GKY25 (GKYGFYTHVFRLKKWIQKVIDQFGE) deriving from the C-terminus of the protein inhibits LPS-induced responses of monocytes, macrophages and neuthrophils *in vitro*, *ex vivo* and *in vivo* (Singh et al. 2013; Hansen et al. 2015; Lim et al. 2017). GKY25 have a broad and inhibitory effect on multiple sepsis pathologies. In a mouse model of *Pseudomonas aeruginosa* sepsis, while mediating a modest antimicrobial effect, GKY25 is able to significantly inhibit the pro-

inflammatory response, to decrease fibrin deposition in the lungs and to reduce mortality. It is therefore an attractive candidate for the treatment of invasive infections (Kalle et al. 2012).

8. Conclusions

It is important to remark that cryptides reported in this review are limited examples of a huge number of peptides described in a growing and vast literature impossible to cover in its totality. Apologizing for many and relevant omissions, the paradigms here described clearly indicate that "cryptides" research is only at the beginning and that they represent a wide biological event hitherto underestimated. Some of them were detected in bodily fluids (as cryptides from milk and saliva) that are in contact with the microbiota. These cryptides are at the most challenging to study, because they can be generated by the host proteinases and can exert retro-actions, modulating at-turn the colonization of the gastro-intestinal tracts. Therefore, their role has to be established in the complex interplay occurring in symbiosis. Other examples, i.e. cryptides either with opioid-like or with antimicrobial activity, suggests that their generation is strictly and finely connected to the life-span of the parent proteins, which, terminating its role, can release smaller peptides able to modulate functions in some way related to the function of the parent protein. The continuous discovery of new cryptides induces to postulate that bigger proteins are probably assembled during evolution from building blocks of smaller peptides with variegate function, often disconnected from those of the parent protein. Cryptides are therefore a strong suggestion for the presence of genomes with multiple information inside the exonic polynucleotide sequence. The investigation of the timing of cryptides production and of the enzymes responsible for their release is a challenge that enhances the complexity of proteomic studies. Nonetheless their knowledge adds new insights in the articulated interaction between the genome and the proteome.

The identification and characterization of many cryptides by *in vitro* experiments will require very accurate experimental plans in order to avoid false positive and negative determinations and to

exactly reproduce the specific proteolytic cascades occurring *in vivo*. Challenging will be the study of the cryptides role in the cellular pathways and of their interactions in the cellular machinery as well as the determination of the enzymatic cascade responsible for their generation. Also challenging will be the development of new –omic platforms for the fast and easy characterization of their function in complex mixtures. As mentioned, many cryptides are generated by a fine interplay between the host and his/her microbiota and the comprehension of these interactions will require the development of new throughput strategies to better understand reciprocal interactions. Proteomics, particularly with the use of top-down strategies, can contribute to large surveys for cryptides detection. However, demanding is the development of new high throughput platforms for the experimental identification of cryptides with specific functions. In this respect very interesting are theoretical predictions on the basis of common structural determinants found in cryptides with similar role. Several successful examples of the application of this strategy have been reported in the previous section for the theoretical detection of peptides with variegate activities from bovine hemoglobin (Ivanov et al. 1997) or for the detection of antimicrobial peptides from sequestesome-1 (Papareddy et al. 2010; Kasetty et al. 2011). Recently, Pane and colleagues (Pane et al. 2017) established that the antimicrobial potency of cationic antimicrobial peptides (CAMPs) linearly correlates to the product of C^mH^nL, where C is the net charge of the peptide, H is the measure of its hydrophobicity and L the length. The m and n exponents are related to the relative contribution of charge and hydrophobicity and, interestingly, are strain specific. Some strains are more sensitive to highly charged CAMPs, while others are more susceptible to the peptide hydrophobicity. This computational analysis represented a strategy to identify CAMPs included inside the structure of larger proteins and precursors. Among various cryptides, this strategy allowed to postulate the existence of a new CAMP from 11-hydroxysteroid dehydropgenase-1 β-like protein, called GVF27 that demonstrated to be not toxic towards human and murine cell lines and to trigger significant immune response by attenuating expression levels of pro-inflammatory interleukins and NO-release

in LPS induced macrophages (Bosso et al. 2017). This approach was designed for the screening of potential CAMPs in large pools of sequences and the development of further CAMPs surely will reinforce the predictive power of the therotical model. Indubitably similar platforms could allow in the future design creative classes of peptide-mimetics with powerful and specific antibacterial activity.

In conclusion, cryptide presence in biological systems affords new complexity to the comprehension of the phenotype expression and implicates superimposition in the information resident in the gene codifying the parent protein. Cryptide study could be hence of great help to decipher better the message included in polynucleotide sequences. Without any doubt a better definition of their presence and role in biological systems will offer to scientists new suggestions for the synthesis of bioactive peptides and peptido-mimetics characterized by low toxicity in numerous fields of application.

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References

Albiston AL, Fernando R, Ye S, Peck GR, Chai SY. 2004. Alzheimer's, angiotensin IV and anaminopeptidase. Biol Pharm Bull. 27(6):765-767.

Andreas NJ, Kampmann B, Le-Doare KM. 2015. Human breast milk: a review on its composition and bioactivity. Early hum dev. 91(11): 629-635.

Andrews SJ, Rothnagel JA. 2014. Emerging evidence for functional peptides encoded by short open reading frames. Nature Rev Gen. 15(3):193-204.

Arruda DC, Santos LC, Melo FM, Pereira FV, Figueiredo CR, Matsuo AL, Mortara RA, Juliano MA, Rodrigues EG, Dobroff AS, uPolonelli L, Travassos LR, et al. 2012. β-Actin-binding complementarity-determining region 2 of variable heavy chain from monoclonal antibody C7 induces apoptosis in several human tumor cells and is protective against metastatic melanoma. J Biol Chem. 287(18):14912-22.

Ascenzi P, di Masi A, Fanali G, Fasano M. 2015. Heme-based catalytic properties of human serum albumin. Cell Death Discov. 1:15025.

Autelitano DJ, Rajic A, Smith AI, Berndt MC, Ilag LL, Vadas M. 2006. The cryptome: a subset of the proteome, comprising cryptic peptides with distinct bioactivities. Drug Discov Today. 11(7-8):306-314.

Banerjee P, Shanthi C. 2016. Cryptic Peptides from Collagen: A Critical Review. Protein Pept Lett. 23(7): 664-672.

Banerjee P, Suseela G, Shanthi C. 2012. Isolation and identification of cryptic bioactive regions in bovine achille's tendon collagen. Protein J. 31(5):374-386.

Barkhudaryan N, Hunanyan OV, Sarukhanyan FP, Stepanyan HM, Zakaryan HH, Grigoryan IE, Dalyan EB. 2012. Study of molecular mechanisms of anti-tumor effect of hemorphin-7 in vivo, Med. Sci. Arm. LII:21-32.

Barkhudaryan N, Zakaryan H, Sarukhanyan F, Gabrielyan A, Dosch D, Kellermann J, Lottspeich F. 2010. Hemorphins act as homeostatic agents in response to endotoxin-induced stress. Neurochem Res. 35(6):925-933.

Bennick A. Salivary proline-rich proteins. 1982. Mol Cell Biochem. 45(2):83-99.

Bennick A, Chau G, Goodlin R, Abrams S, Tustian D, Madapallimattam G. 1983. The role of human salivary acidic proline-rich proteins in the formation of acquired dental pellicle in vivo and their fate after adsorption to the human enamel surface. Arch Oral Biol. 28(1) :19-27.

Birkemo GA, O'Sullivan O, Ross RP, Hill C. 2009. Antimicrobial activity of two peptides casecidins 15 and 17, found naturally in bovine colostrum. J Appl Microbiol. 106(1):233-240.

Blischenko EY, Mernenko OA, Yatskin ON, Ziganshin RH, Philippova MM, Karelin AA, Ivanov VT. 1996. Neokyotorphin and neokyotorphin (1-4): cytolytic activity and comparative levels in rat tissues. Biochem Biophys Res Commun. 224(3):721-727.

Blishchenko EY, Sazonova OV, Kalinina OA, Moiseeva EV, Vass AA, Karelin AA, Ivanov VT. 2005. Antitumor effect of valorphin in vitro and in vivo: combined action with cytostatic drugs. Cancer Biol Ther. 4(1):118-124.

Blishchenko EY, Sazonova OV, Kalinina OA, Yatskin ON, Philippova MM, Surovoy AY, Karelin AA, Ivanov VT. 2002a. Family of hemorphins: co-relations between amino acid sequences and effects in cell cultures. Peptides. 23(5):903-10.

Blishchenko EY, Sazonova O, Surovoy A, Khaidukov S, Sheikine Y, Sokolov D, Freidlin I, Philippova M, Vass A, Karelin A, et al. 2002b. Antiproliferative action of valorphin in cell cultures. J Pept Sci. 8(8):438-452.

Bosso A, Pirone L, Gaglione R, Pane K, Del Gatto A, Zaccaro L, Di Gaetano S, Diana D, Fattorusso R, Pedone E, et al. 2017. A new cryptic host defense peptide identified in human 11-hdroxysteroid dehydrogenase-1 β-like: from in silico identification to experimental evidence. Biochim Biophys Acta. 1861(19), 2342-2353.

Bouhallab S, Bouglé D. 2004. Biopeptides in milk: caseinophosphopeptides and mineral bioavailability. Reprod Nutr Dev. 44(5): 493-498.

Bourgeois C, Bour JB, Aho LS, Pothier P. 1998. Prophylactic administration of a complementarity-determining region derived from a neutralizing monoclonal antibody is effective against respiratory syncytial virus infection in BALB/c mice. J Virol. 72(1):807-810

Brantl V, Gramsch C, Lottspeich F, Mertz R, Jaeger KH, Herz A. 1986. Novel opioid peptides derived from hemoglobin: hemorphins. Eur J Pharmacol. 125(2):309-310.

Bruni N, Capucchio MT, Biasibetti E, Pessione E, Cirrincione S, Giraudo L, Corona A, Dosio F. 2016. Antimicrobial activity of lactoferrin-related peptides and applications in human and veterinary medicine. Molecules. 21(6):E752.

Cabras T, Melis M, Castagnola M, Padiglia A, Tepper BJ, Messana I, Tomassini Barbarossa I. 2012. Responsiveness to 6-n-propylthiouracil (PROP) is associated with salivary levels of two specific basic proline-rich proteins in humans. PLoS One. 7(2):e30962.

Cai K, Hagerman AE, Minto RE, Bennick A. 2006. Decreased polyphenol transport across cultured intestinal cells by a salivary proline-rich protein. Biochem Pharmacol. 71(11):1570-1580.

Canon F, Paté F, Cheynier V, Sarni-Manchado P, Giuliani A, Pérez J, Durand D, Li J, Cabane B. 2013 Aggregation of the salivary proline-rich protein IB5 in the presence of the tannin EgCG. Langmuir. 29(6):1926-1937.

Cao Y, Miao J, Liu G, Luo Z, Xia Z, Liu F, Yao M, Cao X, Sun S, Lin Y, et al. 2017. Bioactive Peptides Isolated from Casein Phosphopeptides Enhance Calcium and Magnesium Uptake in Caco-2 Cell Monolayers. J Agric Food Chem. 65(11):2307-2314.

Capriotti AL, Cavaliere C, Piovesana S, Samperi R, Laganà A. 2016. Recent trends in the analysis of bioactive peptides in milk and dairy products. Anal Bioanal Chem. 408(11); 2677-2685.

Cejka J, Zelezná B, Velek J, Zicha J, Kunes J. 2004. LVV-hemorphin-7 lowers blood pressure in spontaneously hypertensive rats: radiotelemetry study. Physiol Res. 53(6):603-607.

Charlton AJ, Baxter NJ, Lilley TH, Haslam E, McDonald CJ, Williamson MP. 1996. Tannin interactions with a full-length human salivary proline-rich protein display a stronger affinity than with single proline-rich repeats. FEBS Lett. 382(3):289-92.

Chiba H, Tani F, Yoshikawa M. Opioid antagonists peptides derived from κ-casein. 1989. J Dairy Res. 56(3): 363-366.

Ciociola T, Magliani W, Giovati L, Sperindé M, Santinoli C, Conti G, Conti S, Polonelli L. 2014. Antibodies as an unlimited source of anti-infective, anti-tumour and immunomodulatory peptides. Sci Prog. 97(Pt 3):215-233.

Cohen M, Fruitier-Arnaudin I, Sauvan R, Birnbaum D, Piot JM. 2003. Serum levels of Hemorphin-7 peptides in patients with breast cancer. Clin Chim Acta. 337(1-2):59-67.

D'Alessandro A, Scaloni A, Zolla L. 2010. Human milk proteins: an interactomics and updated functional overview. J Proteome Res. 9(7):3339-3373.

Demidova-Rice TN, Geevarghese A, Herman IM. 2011. Bioactive peptides derived from vascular endothelial cell extracellular matrices promote microvascular morphogenesis and wound healing in vitro. Wound Repair Regen. 19(1): 59-70.

Deng L, Pan X, Wang Y, Wang L, Zhou XE, Li M, Feng Y, Wu Q, Wang B, Huang N. 2009. Hemoglobin and its derived peptides may play a role in the antibacterial mechanism of the vagina. Hum Reprod. 24(1):211-218.

Desiderio C, D'Angelo L, Rossetti DV, Iavarone F, Giardina B, Castagnola M, Massimi L, Tamburrini G, Di Rocco C. 2012. Cerebrospinal fluid top-down proteomics evidenced the potential biomarker role of LVV- and VV-hemorphin-7 in posterior cranial fossa pediatric brain tumors. Proteomics. 12(13):2158-2166.

Dorfman T, Moore MJ, Guth AC, Choe H, Farzan M. 2006. A tyrosine-sulfated peptide derived from the heavy-chain CDR3 region of an HIV-1-neutralizing antibody binds gp120 and inhibits HIV-1 infection. J Biol Chem. 281(39):28529-28535.

Duethman D, Dewan N, Conlon JM. 2000. Isolation of the opioid peptide Leu-Val-Val-hemorphin-7 from bronchoalveolar lavage fluid of a patient with non-small cell lung cancer. Peptides. 21(1):137-142.

Eliassen LT, Berge G, Leknessund A, Wikman M, Lindin I, Løkke C, Ponthan F, Johnsen JI, Sveinbjørnsson B, Kogner P, et al. 2006. The antimicrobial peptide, lactoferricin B, is cytotoxic to neuroblastoma cells in vitro and inhibits xenograft growth in vivo. Int J Cancer. 119(3):493-500.

Fiat AM, Migliore-Samour D, Jollès P, Drouet L, Bal dit Sollier C, Caen J. 1993. Biologically active peptides from milk proteins with emphasis on two examples concerning antithrombotic and immunomodulating activities. J Dairy Sci; 76(1): 301-310.

Figueiredo CR, Matsuo AL, Massaoka MH, Polonelli L, Travassos LR. 2014. Anti-tumor activities of peptides corresponding to conserved complementary determining regions from different immunoglobulins. Peptides. 59:14-19.

FitzGerald RJ, Meisel H. 1999. Lactokinins: whey protein-derived ACE inhibitory peptides. Nahrung. 43(3):165-167.

FitzGerald RJ, Murray BA, Walsh DJ. 2004. Hypotensive peptides from milk proteins. J Nutr. 134(4): 980S-988S.

Fujita H, Suganuma H, Usui H, Kurahashi K, Nakagiri R, Sasaki R, Yoshikawa M. 1996. Vasorelaxation by casomokinin L, a derivative of β-casomorphin and casoxin D, is mediated by NK_1 receptor. Peptides. 17(4): 635-639.

Gabrielli E, Pericolini E, Cenci E, Monari C, Magliani W, Ciociola T, Conti S, Gatti R, Bistoni F, Polonelli L, et al. 2012. Antibody constant region peptides can display immunomodulatory activity through activation of the Dectin-1 signalling pathway. PLoS One. 7(8):e43972.

Gelman JS, Fricker LD. 2010. Hemopressin and other bioactive peptides from cytosolic proteins: are these non-classical neuropeptides? AAPS J. 12(3):279-289.

Gelman JS, Sironi J, Castro LM, Ferro ES, Fricker LD. 2010. Hemopressins and other hemoglobin-derived peptides in mouse brain: comparison between brain, blood, and heart peptidome and regulation in Cpefat/fat mice. J Neurochem. 113(4):871-880.

Giardina B, Messana I, Scatena R, Castagnola M. 1995. The multiple functions of hemoglobin. Crit Rev Biochem Mol Biol. 30(3):165-196

Garcia-Nebot MJ, Barberà R, Alegria A. 2013. Iron and zinc bioavailability in Caco-2 cells: influence of caseinophosphopeptides. Food Chem. 138(2-3): 1298-1303.

Gobbetti, M., Minervini, F., and Rizzello, C. G. 2007. Bioactive peptides in dairy products. In: Handbook of food products manufacturing. Y. H. Hui, (ed), John Wiley & Sons, Inc.pp. 489-517.

Gomes I, Dale CS, Casten K, Geigner MA, Gozzo FC, Ferro ES, Heimann AS, Devi LA. 2010. Hemoglobin-derived peptides as novel type of bioactive signaling molecules. AAPS J. 12(4):658-669.

Grillon C, Rieger K., Bakala J, Schott D, Morgat JL., Hannappel E, Voelter W, Lenfant M. 1990. Involvement of thymosin beta 4 and endoproteinase Asp-N in the biosynthesis of the tetrapeptide AcSerAspLysPro a regulator of the hematopoietic system. FEBS Lett. 274(1-2):30-34.

Hansen FC, Kalle-Brune M, van der Plas MJ, Strömdahl AC, Malmsten M, Mörgelin M, Schmidtchen A. 2015. The thrombin-derived host-defense peptide GKY25 inhibits endotoxin-induced responses through interactions with lipopolysaccharide and macrophages/monocytes. J Immunol. 194(11):5397-5406.

Hay DI, Carlson ER, Schluckebier SK, Moreno EC, Schlesinger DH. 1987. Inhibition of calcium phosphate precipitation by human salivary acidic proline-rich proteins: structure-activity relationships. Calcif Tissue Int. 40(3):126-132.

Helmerhorst EJ, Sun X, Salih E, Oppenheim FG. 2008. Identification of Lys-Pro-Gln as a novel cleavage site specificity of saliva-associated proteases. J Biol Chem. 283(29):19957-19966.

Helmerhorst EJ, Zamakhchari M, Schuppan D, Oppenheim FG. 2010. Discovery of a novel and rich source of gluten-degrading microbial enzymes in the oral cavity. PLoS One. 5(10):e13264.

Hokari Y, Seki T, Nakano H, Matsuo Y, Fukamizu A, Munekata E, Kiso Y, Mukai H. 2012. Isolation and identification of novel neutrophil-activating cryptides hidden in mitochondrial cytochrome C. Protein Pept Lett. 19(6):680-687.

Hosea Blewett HJ, Cicalo MC, Holland CD, Field CJ. 2008. The immunological components of human milk. Adv Food Nutr Res. 54:45-80.

Hou IC, Suzuki C, Kanewaga N, Oda A, Yamada A, Yoshikawa M, Yamada D, Sekiguchi M, Wada E, Wada K, et al. 2011. β-Lactotensin derived from bovine β-lactoglobulin exhibits anxiolytic-like activity as an agonist for neurotensin NTS(2) receptor via activation of dopamine D(1) receptor in mice. J Neurochem. 119(4):785-790.

Hu Q, Wood CR, Cimen S, Venkatachalam AB, Alwayn IP. 2015. Mitochondrial Damage-Associated Molecular Patterns (MTDs) Are Released during Hepatic Ischemia Reperfusion and Induce Inflammatory Responses. PLoS One. 10(10):e0140105.

Haug BE, Ström MB, Svendsen JS. 2007. The medicinal chemistry of short lactoferrin-based antibacterial peptides. Curr Med Chem. 14(1):1-18.

Hunanyan OV. 2011. Hemorphin-7 regulates interleukin-2 promoter activity by Ca2+/calmodulin/calcineurin/NFAT signalling pathway. Medical Sci. Arm. LI:43-50.

Inserra I, Martelli C, Cipollina M, Cicione C, Iavarone F, Taranto GD, Barba M, Castagnola M, Desiderio C, Lattanzi W. 2016. Lipoaspirate fluid proteome: A preliminary investigation by LC-MS top-down/bottom-up integrated platform of a high potential biofluid in regenerative medicine. Electrophoresis. 37(7-8):1015-1026.

Ivanov VT, Karelin AA, Philippova MM, Nazimov IV, Pletnev VZ. 1997. Hemoglobin as a source of endogenous bioactive peptides: the concept of tissue-specific peptide pool. Biopolymers. 43(2):171-88.

Kalle M, Papareddy P, Kasetty G, Mörgelin M, van der Plas MJ, Rydengärd V, Malmsten M, Albiger B, Schmidtchen A. 2012. Host defense peptides of thrombin modulate inflammation and coagulation in endotoxin-mediated shock and *Pseudomonas aeruginosa* sepsis. PLos One. 7(12), e51313

Kamiński S, Cieslińska A, Kostyra E. 2007. Polymorphism of bovine beta-casein and its potential effect on human health. J Appl Genet. 48(3):189-198.

Karelin AA, Philippova MM, Ivanov VT. 1995. Proteolytic degradation of hemoglobin in erythrocytes leads to biologically active peptides. Peptides. 16(4):693-697.

Karelin AA, Philippova MM, Karelina EV, Ivanov VT. 1994. Isolation of endogenous hemorphin-related hemoglobin fragments from bovine brain. Biochem Biophys Res Commun. 202(1):410-5.

Kasetty G, Papareddy P, Kalle M, Rydengård V, Walse B, Svensson B, Mörgelin M, Malmsten M, Schmidtchen A. 2011. The C-terminal sequence of several human serine proteases encodes host defense functions. J Innate Immun. 3(5):471-482.

Kaye NM, Jollès P. 1978. Characterization of the amino acids of bovine fibrinogen involved in the fibrinogen-thrombin interaction of the blood clotting process. Comnparison with the milk clotting process. Mol Cell Biochem. 20(3): 173-182.

Korhonen H, Pihlanto A. 2003. Food derived bioactive peptides: opportunities for designing future foods. Curr Pharm Des. 9(16):1297-1308.

Lahov E, Regelson W. 1996. Antibacterial and immunostimulating casein-derived substances from milk: casecidin, isracidin peptides. Food Chem Toxicol. 34(1): 131-145.

Lammerich HP, Busmann A, Kutzleb C, Wendland M, Seiler P, Berger C, Eickelmann P, Meyer M, Forssmann WG, Maronde E. 2003. Identification and functional characterization of hemorphins VV-H-7 and LVV-H-7 as low-affinity agonists for the orphan bombesin receptor subtype 3. Br J Pharmacol. 138(8):1431-1440.

Lantz I, Glämsta EL, Talbäck L, Nyberg F. 1991. Hemorphins derived from hemoglobin have an inhibitory action on angiotensin converting enzyme activity. FEBS Lett. 287(1-2):39-41.

Laugesen M, Elliott R. 2003. Ischaemic heart disease; type 1 diabetes and cow milk A1 β-casein. NZ Med J. 116(1168):U295.

Lebrun I, Cavallaro V, Juliano L, Juliano MA, de Sousa e Silva MC. 2004. Effects of 'casoparan', a peptide isolated from casein hydrolysates with mastoparan-like properties. Mediators Inflamm. 13(4):263-268.

Lenfant M, Wdzieczak-Bakala J, Guittet E, Prome JC, Sotty D. Frindel E. 1989. Inhibitor of hematopoietic pluripotent stem cell proliferation: purification and determination of its structure. Proc. Natl. Acad. Sci. USA, 86(3), 779-782.

Liepke C, Zucht HD, Forssmann WG, Ständker L. 2001. Purification of novel peptide antibiotics from human milk. J Chromatogr B Biomed Sci Appl. 752(2):369-377.

Lim CH, Puthia M, Butrym M, Tay HM, Lee MZY, Hou HW, Schmidtchen A. 2017. Thrombin-derived host defence peptide modulates neutrophil rolling and migration in vitro and functional response in vivo. Sci Rep. 7(1), 11201.

Lonnerdal B. Human milk proteins: key components for the biological activity of human milk. 2004. Adv Exp Med Biol. 554:11-25.

Lonnerdal B. 2014. Infant formula and infant nutrition: bioactive proteins of human milk and implications for the composition of infant formulas. Am J Clin Nutr. 99(3):712S-717S.

Low TL, Hu SK, Goldstain AL. 1981. Complete amino acid sequence of bovine thymosin beta 4: a thymic hormone that induces terminal deoxynucleotidyl transferase activity in thymocyte populations. Proc. Natl. Acad. Sci. USA 78(2):1162-1166.

Lu Y, Bennick A. 1998. Interaction of tannin with human salivary proline-rich proteins. Arch Oral Biol. 43(9):717-728.

Lu Y, Zhang TF, Shi Y, Zhou HW, Chen Q, Wei BY, Wang X, Yang TX, Chinn YE, Kang J, et al. 2016. PFR peptide, one of the antimicrobial peptides identified from the derivatives of lactoferrin, induces necrosis in leukemia cells. Sci Rep. 6:20823.

Manconi B, Castagnola M, Cabras T, Olianas A, Vitali A, Desiderio C, Sanna MT, Messana I. 2016. The intriguing heterogeneity of human salivary proline-rich proteins. J Proteomics. 134:47-56.

Martelli C, Iavarone F, Vincenzoni F, Rossetti DV, D'Angelo L, Tamburrini G, Caldarelli M, Di Rocco C, Messana I, Castagnola M, et al. 2014. Proteomic characterization of pediatric craniopharyngioma intracystic fluid by LC-MS top-down/bottom-up integrated approaches. Electrophoresis. 35(15):2172-2183.

Melis M, Aragoni MC, Arca M, Cabras T, Caltagirone C, Castagnola M, Crnjar R, Messana I, Tepper BJ, Tomassini Barbarossa I. 2013. Marked increase in PROP taste responsiveness following oral supplementation with selected salivary proteins or their related free amino acids. PLoS One. 8(3):e59810.

Messana I, Cabras T, Iavarone F, Vincenzoni F, Urbani A, Castagnola M. 2013. Unraveling the different proteomic platforms. J Sep Sci. 36(1):128-139.

Messana I, Cabras T, Pisano E, Sanna MT, Olianas A, Manconi B, Pellegrini M, Paludetti G,. Scarano E, Fiorita A, et al. 2008. Trafficking and post-secretory events responsible for the formation of secreted human salivary peptides. A proteomic approach. Mol Cell Proteomics. 7(5):911-926.

Mikhailova AA, Petrov RV, Fonina LA, Strelkov LA, Guriyanov SA, Gerassimova GK, Treshchalina EM. 1996. Hexapeptides possessing antitumor activity, patent WO 1996018652 A1.

Moeller I, Albiston AL, Lew RA, Mendelsohn FA, Chai SY. 1999. A globin fragment, LVV-hemorphin-7, induces [3H]thymidine incorporation in a neuronal cell line via the AT4 receptor. J Neurochem. 73(1):301-308.

Molinari CE, Casadio YS, Hartmann BT, Livk A, Bringans S, Arthur PG, Hartmann PE. 2012. Proteome mapping of human skim milk proteins in term and preterm milk. J Proteome Res. 11(3):1696-1714.

Monnet C, Laune D, Laroche-Traineau J, Biard-Piechaczyk M, Briant L, Bès C, Pugnière M, Mani JC, Pau B, Cerutti M, et al. 1999. Synthetic peptides derived from the variable regions of an anti-CD4 monoclonal antibody bind to CD4 and inhibit HIV-1 promoter activation in virus-infected cells. J Biol Chem. 274(6):3789-3796.

Moreno EC, Kresak M, Hay DI. 1982. Adsorption thermodynamics of acidic proline-rich human salivary proteins onto calcium apatites. J Biol Chem. 257(6):2981-2989.

Mukai H, Seki T, Nakano H, Hokari Y, Takao T, Kawanami M, Tsukagoshi H, Kimura H, Kiso Y, Shimonishi Y, et al. 2009. Mitocryptide-2: purification, identification, and characterization of a novel cryptide that activates neutrophils. J Immunol. 182(8):5072-5080.

Najjar VA, Nishioka K. 1970. “Tuftsin": a natural phagocytosis stimulating peptide. Nature. 228(5272):672-673.

Nakagomi K, Ebisu H, Sadakane Y, Fujii N, Akizawa T, Tanimura T. 2000. Properties and human origin of two angiotensin-I-converting enzyme inhibitory peptides isolated from a tryptic hydrolysate of human serum albumin. Biol Pharm Bull. 23(7): 879-883.

Nakagomi K, Fujimura A, Ebisu H, Sakai T, Sadakane Y, Fujii N, Tanimura T. 1998. Acein-1, a novel angiotensin-I-converting enzyme inhibitory peptide isolated from tryptic hydrolysate of human plasma. FEBS Lett. 438(3):255-257.

Nakagomi K, Takatsu K, Takagi S, Ebisu H, Sadakane Y, Fujii N, Akizawa T, Tanimura T, Hatanaka Y. 2002. Isolation of cathepsin B inhibitory peptides, cabin-A1 and -A2, from a tryptic and chimotryptic hydrolysate of human serum albumin. Peptides. 23(9): 1567-1571.

Navolotskaya EV. 2014. The second life of antibodies. Biochemistry (Mosc). 79(1):1-7.

Nguyen DD, Johnson SK, Busetti F, Solah VA. 2015. Formation and degradation of beta-casomorphins in dairy processing. Crit Rev Food Sci Nutr. 55(14): 1955-1967.

Nongonierma AB, FitzGerald RJ. 2015. The scientific evidence for the role of milk protein-derived bioactive peptides in humans: a review. J Funct Foods . 17:640–656.

Nyberg F, Sanderson K, Glämsta EL. 1997. The hemorphins: a new class of opioid peptides derived from the blood protein hemoglobin. Biopolymers. 43(2):147-156.

Oddy WH. 2001. Breastfeeding protects against illness and infection in infants and children: a review of the evidence. Breastfeed Rev. 9(2):11-18.

Ohinata K, Inui A, Asakawa A, Wada K, Wada E, Yoshikawa M. 2002. Albutensin A and complement C3a decrease food intake in mice. Peptides. 23(1):127-133.

Ohinata K, Sonoda S, Inoue N, Yamauchi B, Wada K, Yoshikawa M. 2007. Beta-lactotensin, a neurotensin agonist peptide derived from bovine beta-lactoglobulin, enhances memory consolidation in mice. Peptides. 28(7): 1470-1474.

Oppenheim FG, Salih E, Siqueira WL, Zhang W, Helmerhorst EJ. 2007. Salivary proteome and its genetic polymorphisms. Ann NY Acad Sci.1098:22-50.

O'Sullivan JM, Cannon RD, Sullivan PA, Jenkinson HF. 1997. Identification of salivary basic proline-rich proteins as receptors for Candida albicans adhesion. Microbiology. 143(Pt. 2):341-348.

Palmerini CA, Mazzoni M, Radicioni G, Marzano V, Granieri L, Iavarone F, Longhi R, Messana I, Cabras T, Sanna MT, et al. 2016. Antagonistic Effect of a Salivary Proline-Rich Peptide on the Cytosolic Ca2+ Mobilization Induced by Progesterone in Oral Squamous Cancer Cells. PLoS One. 11(1):e0147925.

Pane K, Durante L, Crescenzi O, Cafaro V, Pizzo E, Varcamonti M, Zanfardino A, Izzo V, Di Donato A, Notomista E. 2017. Antimicrobial potency of cationic of cationic antimicrobial peptides can be predicted from their amino acid composition: application to the detection of cryptic antimicrobial peptides. J Theor Biol. 419:254-265.

Papareddy P, Rydengård V, Pasupuleti M, Walse B, Mörgelin M, Chalupka A, Malmsten M, Schmidtchen A. 2010. Proteolysis of human thrombin generates novel host defense peptides. PLoS Pathog. 6(4):e1000857.

Papareddy P, Kalle M, Bhongir RK, Mörgelin M, Malmsten M, Schmidtchen A. 2014. Antimicrobial effects of helix D-derived peptides of human antithrombin III. J Biol Chem. 289(43): 29790-29800.

Park YW, Nam MS. 2015. Bioactive peptides in milk and dairy products: a review. Korean J Food Sci An. 35(6):831-840.

Pertinhez TA, Conti S, Ferrari E, Magliani W, Spisni A, Polonelli L. 2009. Reversible self-assembly: a key feature for a new class of autodelivering therapeutic peptides. Mol Pharm. 6(3):1036-39.

Polonelli L, Ciociola T, Magliani W, Zanello PP, D'Adda T, Galati S, De Bernardis F, Arancia S, Gabrielli E, Pericolini E, et al. 2012. Peptides of the constant region of antibodies display fungicidal activity. PLoS One. 7(3):e34105.

Polonelli L, Magliani W, Conti S, Bracci L, Lozzi L, Neri P, Adriani D, De Bernardis F, Cassone A. 2003. Therapeutic activity of an engineered synthetic killer antiidiotypic antibody fragment against experimental mucosal and systemic candidiasis. Infect Immun. 71(11):6205-6212.

Polonelli L, Pontón J, Elguezabal N, Moragues MD, Casoli C, Pilotti E, Ronzi P, Dobroff AS, Rodrigues EG, Juliano MA, et al. 2008. Antibody complementarity-determining regions (CDRs) can display differential antimicrobial, antiviral and antitumor activities PLoS One. 3(6):e2371.

Ponpuak M, Deretic V. 2011. Autophagy and p62/sequestosome 1 generate neo-antimicrobial peptides (cryptides) from cytosolic proteins. Autophagy. 7(3):336-337.

Rabiet MJ, Huet E, Boulay F. 2005. Human mitochondria-derived N-formylated peptides are novel agonists equally active on FPR and FPRL1, while Listeria monocytogenes-derived peptides preferentially activate FPR. Eur J Immunol. 35(8):2486–2495

Radicioni G, Stringaro A, Molinari A, Nocca G, Longhi R, Pirolli D, Scarano E, Iavarone F, Manconi B, Cabras T, et al. 2015. Characterization of the cell penetrating properties of a human salivary proline-rich peptide. Biochim Biophys Acta. 1848(11 Pt A):2868-2877.

Raoof M, Zhang Q, Itagaki K, Hauser CJ. 2010. Mitochondrial peptides are potent immune activators that activate human neutrophils via FPR-1. J Trauma. 68(6): 1328-1332

Rieger KJ, Saez-Servent N, Papet MP, Wdzieczak-Bakala J, Morgat JL, Thierry J, Voelter W, Lenfant M. 1993. Involvement of human plasma angiotensin I-converting enzyme in the degradation of the haemoregulatory peptide N-acetyl-seryl-aspartyl-lysyl-proline. Biochem J. 296(Pt 2):373-378.

Righino B, Pirolli D, Radicioni G, Marzano V, Longhi R, Arcovito A, Sanna MT, De Rosa MC, Paoluzi S, Cesareni G, et al. 2016. Structural studies and SH3 domain binding properties of a human antiviral salivary proline-rich peptide. Biopolymers. 106(5):714-725.

Rioli V, Gozzo FC, Heimann AS, Linardi A, Krieger JE, Shida CS, Almeida PC, Hyslop S, Eberlin MN, Ferro ES. 2003. Novel natural peptide substrates for endopeptidase 24.15, neurolysin, and angiotensin-converting enzyme. J Biol Chem. 278(10):8547-8555.

Robitaille G, Champagne CP. 2014. Growth promoting effects of pepsin- and trypsin-treated casienomacropeptide from bovine milk of probiotics. J Dairy Res. 81(3):319-324.

Roncada P, Piras C, Soggiu A, Turk R, Urbani A, Bonizzi L. 2012. Farm animal milk proteomic. J Proteomics. 75(14):4259–4274.

Roncada P, Stipetic LH, Bonizzi L, Burchmore RJ, Kennedy MW. 2013. Proteomics as a tool to explore human milk in health and disease. J Proteomics. 88:47-57.

Rossdeutsch A, Smart N, Riley PR. 2008.Thymosin beta4 and Ac-SDKP: tools to mend a broken heart. J Mol Med. 86(1):29-35.

Rousseau A, Michaud A, Chauvet MT, Lenfant M, Corvol P. 1995. The hemoregulatory peptide N-acetyl-Ser-Asp-Lys-Pro is a natural and specific substrate of the N-terminal active site of human angiotensin-converting enzyme. J Biol Chem. 270(8):3656-3661.

Samir P, Link AJ. 2011. Analyzing the cryptome: uncovering secret sequences. AAPS J. 13(2):152-158.

Sazonova OV, Blishchenko EY, Kalinina OA, Egorova NS, Surovoy AY, Philippova MM, Karelin AA, Ivanov VT. 2003. Proliferative activity of neokyotorphin-related hemoglobin fragments in cell cultures. Protein Pept Lett. 10(4):386-395.

Seguier S, Godeau G, Brousse N. 2000. Immunohistological and morphometric analysis of intra-epithelial lymphocytes and Langerhans cells in healthy and diseased human gingival tissues. Arch Oral Biol. 45(6):441-452.

Siemion IZ1, Kluczyk A. 1999. Tuftsin: on the 30-year anniversary of Victor Najjar's discovery. Peptides. 20(5):645-674.

Shestakov A, Jenssen H, Nordström I, Eriksson K. 2012. Lactoferricin but not lactoferrin inhibit herpes simplex virus type 2 infection in mice. Antiviral Res. 93(3):340-345.

Singh S, Kalle M. Papareddy P, Schmidtchen A, Malmsten M. 2013. Lipopolysaccaride interactions of C-terminal peptides from human thrombin. Biomacromolecules. 14(5):1482-1492

Sipola M, Finckenberg P, Vapaatalo H, Pihlanto-Leppälä A, Korhonen H, Korpela R, Nurminen ML. 2002. Alpha-lactorphin and beta-lactorphin improve arterial function in spontaneously hypertensive rats. Life Sci. 71(11):1245-1273.

Siqueira WL, Oppenheim FG. 2009. Small molecular weight proteins/peptides present in the in vivo formed human acquired enamel pellicle. Arch Oral Biol. 54(5):437-44.

Solarte VA, Rosas JE, Rivera ZJ, Arango-Rodriguez ML, Garcia JE, Vernot JP. 2015. A tetrameric peptide derived from bovine lactoferricin exhibits specific cytotoxic effects against oral squamous-cell carcinoma cell lines. Biomed Res. 2015:630179.

Song CZ, Wang QW, Song CC. 2012. Hemorphin as a prognostic biomarker and potential drug for breast cancer? Int J Cancer.131(4):1011-1012.

Takahashi M, Morigushi S, Minami T, Suganuma H, Shiota A, Takenaka Y, Tani F, Sasaki R, Yoshihawa M. 1998. Albutensin A, an ileum contracting peptide derived from serum albumin, acts through both receptors for complements C3a and C5a. Lett Pept Sci. 5(1):29-35.

Takahashi M, Morigushi S, Sugunuma H, Shiota A, Tani F, Usui H, Kurahashi K, Sasaki R, Yoshikawa M. 1997. Identification of casoxin C, an ileum-contracting peptide derived from bovine kappa-casen, as an agoniust fro C3a receptors. Peptides. 18(3):329-336.

Tani F, Iio K, Chiba H, Yoshikawa M. 1990. Isolation and characterization of opiod antagonist peptides derived from human lactoferrin. Agric Biol Chem. 54(7):1803-1810

Tavoulari S, Frillingos S, Karatza P, Messinis IE, Seferiadis K. 2004. The recombinant subdomain IIIB of human serum albumin displays activity of gonadothrophin surge-attenuating factor. Hum Reprod. 19(4):849-858.

Théolier J, Fliss I, Jean J, Hammami R. 2014. MilkAMP: a comprehensive database of antimicrobial peptides of dairy origin. Dairy Sci Technol. 94(2):181-193.

van der Kraan MI, Groenink J, Nazmi K, Veerrman EC, Bolscher JG, Nieuw Amerongen AV. 2004. Lactoferrampin: a novel antimicrobial peptide in the N-1 domain of bovine lactoferrin. Peptides. 25(2):177-83.

Viejo-Diaz M, Andrés MT, Fierro JF. 2005. Different anti-Candida activities of two human lactoferrin-derived peptides, Lfpep and kaliocin-1. Antimicrob Agents Chemother. 49(7):2583-2588.

Vento G, Tirone C, Lulli P, Capoluongo E, Ameglio F, Lozzi S, Cota F, Mosca F, Romagnoli C, Messana I, et al. 2009. Bronchoalveolar lavage fluid peptidomics suggests a possible matrix metalloproteinase-3 role in bronchopulmonary dysplasia. Intensive Care Med. 35(12):2115-2124.

Vitorino R, Calheiros-Lobo MJ, Williams J, Ferrer-Correia AJ, Tomer KB, Duarte JA, Domingues PM, Amado FM. 2007. Peptidomic analysis of human acquired enamel pellicle. Biomed Chromatogr. 21(11):1107-1117.

Wakabayashi H, Takase M, Tomita M. 2003. Lactoferricin derived from milk protein lactoferrin. Curr. Pharm. Des. 9(16):1277-1287.

Wang WY, Wong JH, Ip DT, Wan DC, Cheung RC, Ng TB. 2016. Bovine lactoferrampin, human lactoferricin and lactoferrin 1-11 inhibit nuclear translocation of HIV integrase. Appl Biochem Biotechnol. 179(7):1202-1212.

Yamamoto Y, Ono H, Ueda A, Shimamura M, Nishimura K, Hazato T. 2002. Spinorphin as an endogenous inhibitor of enkephalin-degrading enzymes: roles in pain and inflammation. Curr Protein Pept Sci. 3(6):587-599.

Yamauchi R, Ohinata K, Yoshikawa M. 2003. Beta-lactotensin and neurotensin rapidly reduce serum cholesterol via NT2 receptor. Peptides. 24(12):1955-1961.

Zamakhchari M, Wei G, Dewhirst F, Lee J, Schuppan D, Oppenheim FG, Helmerhorst EJ. 2011. Identification of Rothia bacteria as gluten-degrading natural colonizers of the upper gastro-intestinal tract. PLoS One. 6(9):e24455.

Zamyatnin AA. 2009. Hemoglobin as a potential source of natural regulatory oligopeptides. Biochemistry (Mosc). 74(2):201-208.

Zhang W, Miao J, Wang S, Zhang Y. 2013. The protective effects of beta-casomorphin-7 against glucose-induced renal oxidative stress in vivo and vitro. PLoS One. 8(5):e63472.

Zhao Q, Garreau I, Sannier F, Piot JM. 1997. Opioid peptides derived from hemoglobin: hemorphins. Biopolymers. 43(2):75-98.

Zidane F, Matèos A, Cakir-Klefer C, Miclo L, Rahuel-Clermont S, Girardet JM, Corbier C. 2012. Binding of divalent metal ions to 1-25 β-caseinophosphopeptide: an isothermal titration calorimetry study. Food Chem. 132(1):391-398.

Figure 1

Principal pathways for the generation of cryptides. In the present review the term cryptide refers to a bioactive peptide, encrypted inside a bigger functional polypeptide and released by a proteolytic event, with a function distinct or related, but not super-imposable, to that one of the parent polypeptide. The majority of cryptides are extracellular cryptides (right part of the Figure) generated by proteolytic processes occurring on functional polypeptides of any length after secretion (either *in vivo* or *ex vivo* or *in vitro*). According to this definition, the functional peptides generated by the cleavage (usually by convertases) of pre-pro-proteins before the secretion (see for instance the Section on saliva) are not discussed in this review, since they cannot be considered cryptides. Indeed, the cleavage occurring during the Golgi transit allows the maturation of the functional form of the polypeptide, which will exert its activity only after secretion. Anyhow, these functional peptides can hide inside their sequence functional cryptides. On the left part of the Figure the possible production of intracellular cryptides is briefly depicted. They are less studied than the extracellular ones. Their related proteolytic events can follow various pathways, such as lysosomal and proteasomal cleavages. Intracellular cryptides can exert their function either inside or outside the cell, for instance after apoptotic or necrotic events. If devoted to specific functions in tissues they could be generated before the maturation of the functional protein, however to verify this route remains a question mark. Recently, many evidences indicated that functional small peptides are encoded by short open reading frames (ORF) upstream from a downstream coding DNA sequence (CDS) (Andrews and Rothnagel 2014). These peptides are not considered cryptides in this review because they are not encrypted into a bigger functional polypeptide sequence. Any cryptide, in turn, can nest inside their sequence smaller cryptides (cryptides of second generation). The scheme does not include other particular routes for cryptides production, such as that of polypeptides encoded by the DNA of the mitochondrion.

Table 1. Hemorphins' subfamilies.

Hemorphins' subfamilies	amino acid sequence	name	
LVV-hemorphins	LVVYPWTQRF	LVV-hemorphin-7	
	LVVYPWTQR	LVV-hemorphin-6	
	LVVYPWTQ	LVV-hemorphin-5	
	LVVYPWT	LVV-hemorphin-4	(spinorphin)
VV-hemorphins	VVYPWTQRF	VV-hemorphin-7	
	VVYPWTQR	VV-hemorphin-6	
	VVYPWTQ	VV-hemorphin-5	(valorphin)
	VVYPWT	VV-hemorphin-4	
V-hemorphins	VYPWTQR	V-hemorphin-7	
	VYPWTQ	V-hemorphin-6	
	VYPWT	V-hemorphin-5	
	VYPW	V-hemorphin-4	
hemorphins	YPWTQRF	hemorphin-7	
	YPWTQR	hemorphin-6	
	YPWTQ	hemorphin-5	
	YPWT	hemorphin-4	

Table 2. Several fragments of human salivary proline-rich proteins recurrently detected in human saliva with potential protective and antibacterial activity.

amino acid sequence	Gene
SPPGKPQ	*PRB1-PRB2-PRB4*
PQGPPPQ	*PRB1-PRB2-PRB3-PRB4*
PEGPPPQ	*PRB3-PRB4*
GPPPPGKPQ	*PRB1-PRB4*
GPPPPPGKPQ	*PRB1*
GPPPQGGSKSR	*PRB2*
RPPPPPGKPQ	*PRB4*
RPPPPPGKPE	*PRB4*
GPPPQGGNKPQ	*PRB1-PRB2*
GPPPQGGNQPQ	*PRB1-PRB2*
GPPQQEGNNPQ	*PRB1-PRB2*
GPPPQGDKSRSP	*PRB1-PRB2*
SPPGKPQGPPPQGGNQPQ	*PRB1-PRB2*
GPPPQGGNKPQGPPPPGKP	*PRB1-PRB2*
GPPPPGGNPQQPQAPPAGKPQ	*PRB4-M,L*
GPPPQGGNQPQGPPPPPGKPQ	*PRB1-PRB2*

Table 3. Several cryptides detected in whey from breast milk

Parent polypeptide	Cryptide Sequence (one letter code)	Known function	Reference
Lactoferrin Human	Lactoferricin H * GRRRSVQWCA[10] VSQPEATKCF[20] QWQRNMRKVR[30] GPPVSCIKRD[40] SPIQCIQA	antimicrobial and antiviral activity	Haug et al. 2007
	Lfpep (from lactoferricin H) TKCF[20] QWQRNMRKVR[30] GPPVSCIKRD[40]	candidacidal activity	Viejo-Diaz et al. 2005
	PFR-peptide PFWRIRIRR-NH_2	antitumoral activity	Lu et al. 2016
	Kaliocin-1 FFSASCVPGA[10] DKGQFPNLCR[20] LCAGTGENKC[30] A	candidacidal activity	Viejo-Diaz et al. 2005
	Lactoferrampin human WNLLRQAQEK[10] FGKDKSPK	antimicrobial and antiviral activity	Bruni et al. 2016
	Lactoferroxin A YLGSGY Lactoferroxin B RYYGY Lactoferroxin C KYLGPQY	opioid antagonists	Tani et al. 1990
Lactoferrin Bovine	Lactoferricin B ** FKCRRWQWRM[10] KKLGAPSITC[20] VRRAF	antimicrobial and antiviral activity	Wakabayashi et al. 2003
	Lactoferrampin bovine DLIWKLLSKA[10] QEKFGKNKSR	antimicrobial and antiviral activity	Wang 2016
α-lactalbumin human or bovine	α-lactorphin YGLF	ACE-inhibitory activity	FitzGerald and Meisel 1999
β-lactoglobulin bovine	β-lactorphin YLLF	ACE-inhibitory activity	FitzGerald and Meisel 1999
	β-lactotensin HIRL	ACE-inhibitory activity	FitzGerald and Meisel 1999

* Lactoferricin H is cleaved between residues V_{11} and S_{12}. The fragments are connected by a disulfide bridge between C_9 and C_{19}.
**Disulfide bridge between C_3 and C_{20}.

Table 4. Several cryptides detected in casein from bovine breast milk

Parent polypeptide	Cryptide Sequence (one letter code)	Known function	Ref.
bovine β-casein	Casecidin 15 YQEPVLGPVR[10] GPFPI	antimicrobial activity	Birkemo et al. 2009
	Casecidin 17 YQEPVLGPVR[10] GPFPIIV	antimicrobial activity	Birkemo et al. 2009
	Casomorphin 7 (BMC7) YPFPGPI	opioid-like activity	Nguyen et al. 2015
	Casomokinin L YPFPPL	endothelium-dependent vasorelaxing activity	Fujita et al. 1996
	Casoparan INKKI	macrophage activator, bradikinin-potentiating activity	Lebrun et al. 2004
	Casohypotensin YPVEPFTE	bradikinin-potentiating activity	Lebrun et al. 2004
bovine α(S1)-casein	Isracidin RPKHPIKHQG[10] LPQEVLNENL[20] LRF	antimicrobial activity	Birkemo et al. 2009
bovine κ-casein	Casoplatelins MAIPPKKNQDK MAIPP MAIPPKK NQDK	antithrombotic activity	Fiat et al. 1993
	Casoxin C YIPIQYVLSR	opioid antagonistic	Takahashi et al, 1997

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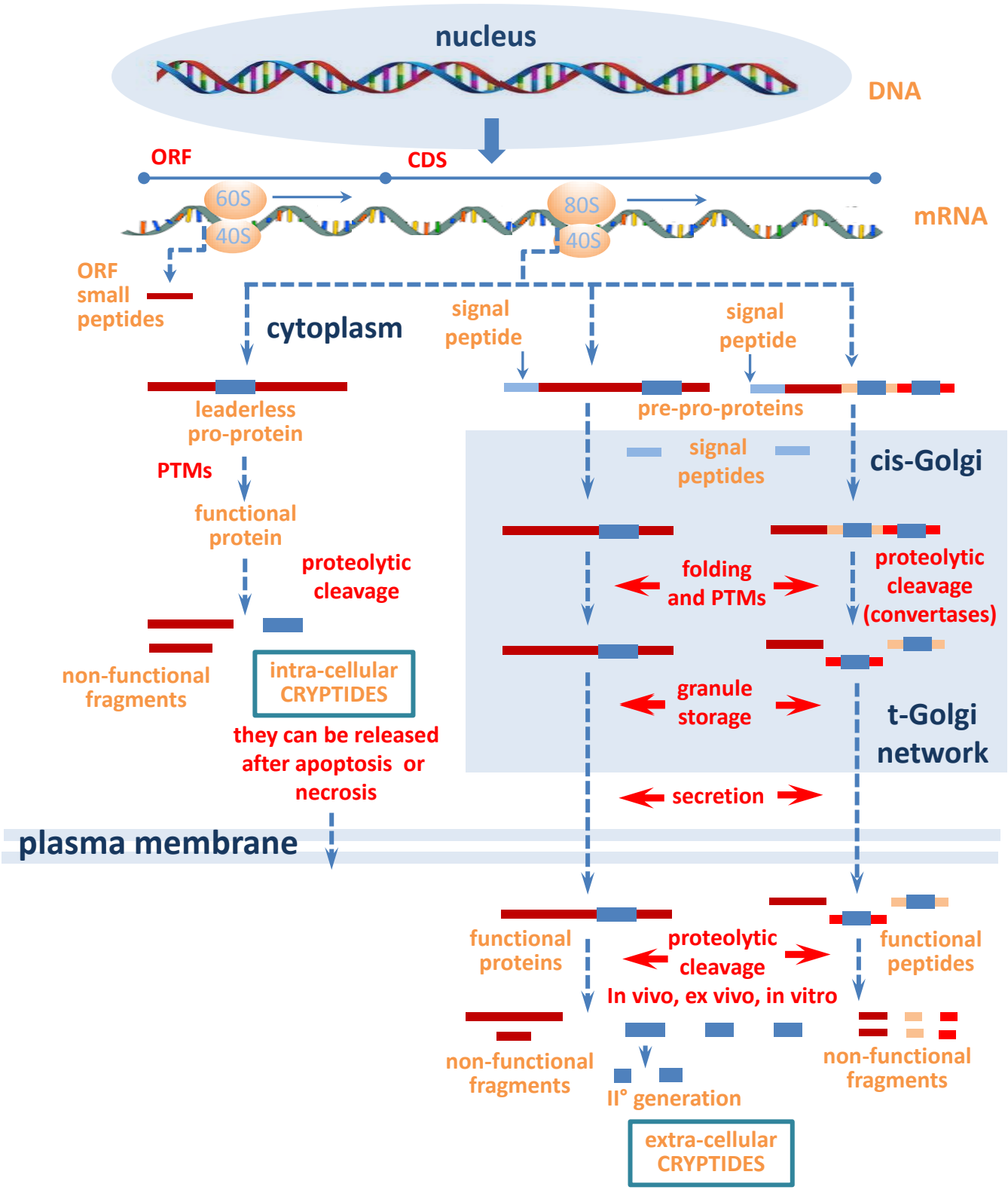
nucleus
DNA
ORF
CDS
60S
40S
80S
40S
mRNA
ORF
small
peptides
cytoplasm
signal
peptide
signal
peptide
leaderless
pro-protein
pre-pro-proteins
signal
peptides
cis-Golgi
PTMs
functional
protein
proteolytic
cleavage
folding
and PTMs
proteolytic
cleavage
(convertases)
non-functional
fragments
intra-cellular
CRYPTIDES
granule
storage
t-Golgi
network
they can be released
after apoptosis or
necrosis
secretion
plasma membrane
functional
proteins
proteolytic
cleavage
functional
peptides
In vivo, ex vivo, in vitro
non-functional
fragments
non-functional
fragments
II° generation
extra-cellular
CRYPTIDES