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Intracellular peptides: From discovery to function

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ABSTRACT

Peptidomics techniques have identified hundreds of peptides that are derived from proteins present mainly in the cytosol, mitochondria, and/or nucleus; these are termed intracellular peptides to distinguish them from secretory pathway peptides that function primarily outside of the cell. The proteasome and thimet oligopeptidase participate in the production and metabolism of intracellular peptides. Many of the intracellular peptides are common among mouse tissues and human cell lines analyzed and likely to perform a variety of functions within cells. Demonstrated functions include the modulation of signal transduction, mitochondrial stress, and development; additional functions will likely be found for intracellular peptides.

1. Historical perspectives

Ever since the discovery of the proteasome, a multisubunit complex that converts proteins into peptides, it was recognized that peptides would transiently exist inside of cells (Fig. 1). The formation and degradation of intracellular peptides is as complex as the production and degradation of microRNA (Fig. 1). Intracellular peptides typically range in size from 2 to 21 amino acids, with the average size of the proteasome degradation products 10–12 amino acids in length [1]. The conventional view is that the proteasome-produced peptides are rapidly broken down by cytosolic aminopeptidases with a half life of several seconds [2,3]. Some of the peptides produced by the proteasome are protected from cytosolic aminopeptidases by transport into the endoplasmic reticulum (ER) by a peptide transporter. Once in the ER, if the peptide is the correct size and contains the appropriate amino acids in key positions, the peptide binds to major histocompatibility complex I (MHC-I) and is transported to the cell surface where it serves in antigen presentation [2]. If the peptide is too long to bind to MHC-I, an ER resident aminopeptidase trims the peptide until it is either the correct length and able to bind to MHC-I, or if unable to bind to MHC1, the aminopeptidase continues until the peptide is degraded. Ultimately, only a very small fraction of intracellular peptides (*e.g.* one peptide of 9–11

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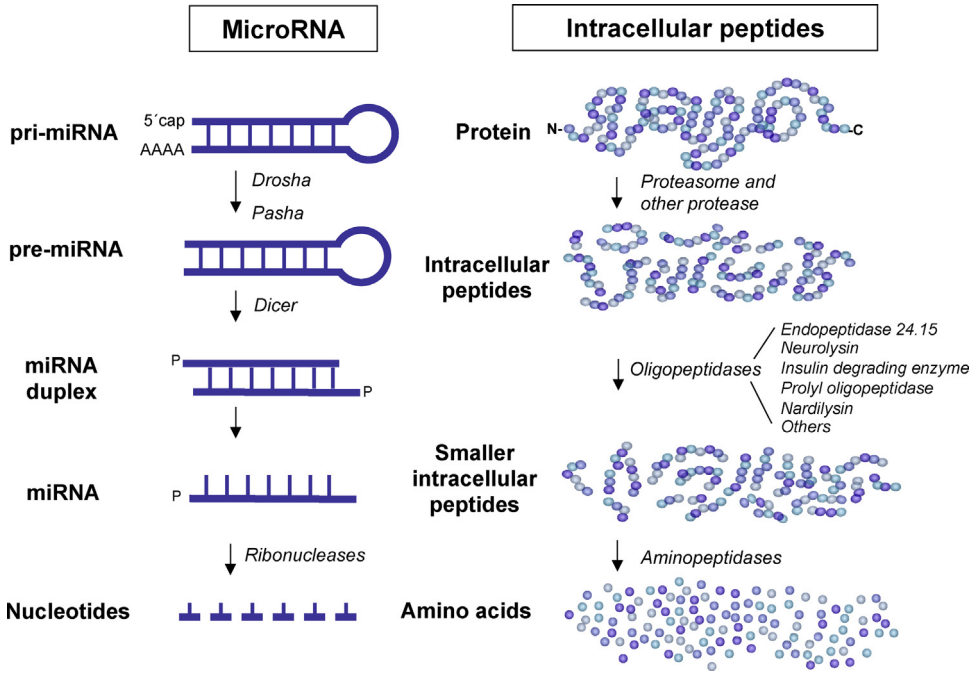

Fig. 1 – Comparison of the key steps in the formation and degradation of microRNA and intracellular peptides. Micro RNA (miRNA) is produced from primary transcripts (pri-mRNA) by a series of nucleases: Drosha, Pasha, and Dicer. Once the miRNA duplexes dissociate to form the miRNA, it is able to form an RNA-induced silencing complex that contains many associated proteins including members of the argonaute protein family. Degradation of miRNA–RNA complex is mediated by ribonucleases. Intracellular peptides are primarily produced by the proteasome although other proteases such as calpains, caspases, and mitochondrial enzymes are also known to generate intracellular peptides. There are several intracellular peptidases that cleave moderately sized peptides, which are not able to directly cleave proteins. These oligopeptidases include endopeptidase 24.15 (also known as thimet oligopeptidase), neurolysin (also known as endopeptidase 24.16), insulin-degrading enzyme, prolyl oligopeptidase, and nardilysin. These enzymes have different substrate specificities such that they each produce distinct patterns of intracellular peptides from the proteasome-produced peptides. There is evidence that some of the intracellular peptides are biologically active. Final degradation of the intracellular peptides is mediated by aminopeptidases, including tripeptidyl peptidase II, leucine aminopeptides, puromycin-sensitive aminopeptidase, and bleomycin hydrolase. In addition to the formation of intracellular peptides from proteins, it is also possible that intracellular peptides are produced directly from RNA with short open reading frames [74,75] or from defective ribosome products [76].

amino acids for each protein) end up on cell surface, and until recent peptidomics analyses detected a large number of intracellular peptides (described below), only a small number of intracellular peptides were known.

One of the best-studied intracellular peptides is the yeast *Saccharomyces cerevisiae* a-factor, which functions as a mating pheromone [4]. Yeast has two peptide mating factors, named alpha-factor and a-factor. Alpha-factor is produced within the secretory pathway, much like mammalian peptide hormones (although with different processing enzymes), stored in vesicles, and secreted when the vesicles fuse with the cellular membrane. In contrast, a-factor is produced within the cytosol by a series of steps involving lipid attachment (prenylation), N-terminal proteolytic cleavages by Ste24p and Axl1p, and transport from the cytosol to the extracellular space by Ste6p [4]. The secreted a-factor binds to a specific receptor (Ste3p) and stimulates mating.

Despite the precedence for cytosolic peptides functioning in plasma membrane receptor-mediated cell-cell signaling that was provided from studies on yeast a-factor, there has been resistance to the idea that mammalian cells signal each other using peptides produced from cytosolic proteins. Some peptides were isolated from mammalian brains based on bioactive properties, but when these molecules were found to represent fragments of intracellular proteins, there was little enthusiasm amongst the scientific community. Examples of bioactive peptides from cytosolic proteins include diazepam binding inhibitor (DBI) from acyl-coA binding protein and hippocampal cholinergic neurostimulating peptide (HCNP) from phosphatidylethanolamine-binding protein [5–7]. During the 1980s and 1990s while the above studies were on-going, a number of cellular endopeptidases were discovered and characterized (Fig. 1). Some of these enzymes, such as endopeptidase 24.15 (also named endo-oligopeptidase A and thimet oligopeptidase), are able to convert neuropeptides into smaller fragments; in some cases this conversion causes the neuropeptide to be inactive, while in other cases the product is also biologically active but with differing receptor specificity [8–11]. In the latter cases, the endopeptidase was thought to play a modulatory role, rather than simply activating or inactivating the peptide. While the extracellular processing of neuropeptides may represent a bona fide