Review Letter

Posttranslational attenuation of peptide gene expression

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Studies of the cell-specific processing of neuroendocrine peptides have shown that neuroendocrine cells occasionally fail to mature the biosynthetic precursors to bioactive peptides, or that they do so to a negligible extent only. Instead, inactive precursors and processing intermediates accumulate in the cells. Thus, the expression of genes encoding hormonal peptides is in certain cells and under certain conditions attenuated at the posttranslational level. The exact molecular mechanisms of posttranslational atenuation are still largely unknown. The review emphasizes that postttranslational attenuation may play a significant role during normal cell differentiation and in the carcinogenic transformation of cells. The existence of posttranslational attenuation has significant biological and clinical implications

Cholecystokinin; Gastrin; Gene expression; Neuroendocrine peptide; Posttranslational processing

1. INTRODUCTION

It is a well-known fact that gene expression comprises multiple steps from initiation of the transcription to the appearance of the encoded protein or peptide in its active, functional form. It is also common knowledge that although transcriptional control is of paramount significance, expression may in addition be controlled at steps following transcription. Thus, attenuation mechanisms have been described, which suppress the expression of procaryotic and eucaryotic genes both at transcriptional and translational levels (for reviews, see [1,2]).

Some proproteins and many neuroendocrine propeptides undergo complex and highly elaborate posttranslational processing [3,4]. Consequently, there are ample opportunities for control of the expression of neuropeptides and peptide hormones at several steps along the posttranslational pathways. Attenuation mechanisms might therefore operate also in the posttranslational phase of expression in addition to the earlier described transcriptional and translational attenuations [1,2]. So far, however, the possibility of posttranslational attenuation has received little attention, if any.

During studies of the cell-specific processing of precursors for the neuroendocrine peptides, cholecystokinin (CCK) and gastrin, we have during recent years observed a striking accumulation of precursors in certain cells almost devoid of smaller precursor fragments, whether bioactive or not [5,6]. These observations suggest that attenuation of gene expression occurs indeed also at the posttranslational level. The following review describes examples of posttranslational attenuation in normal adult cells. Moreover, the significance of posttranslational attenuation of peptide genes during ontogenetic development and carcinogenic transformation will also be discussed. Since the CCK and gastrin systems of peptides will be used for illustration, a description of the normal, unattenuated processing of proCCK and progastrin will first be presented.

2. UNATTENUATED proCCK AND PROGASTRIN PROCESSING

In adult mammals, by far most gastrin is synthesized in the antral G-cells, and by far most CCK in intestinal I-cells and cerebrocortical neurons. After cotranslational removal of the N-terminal signal peptides, proCCK and progastrin are subjected to extensive covalent trimming before the mature bioactive peptides are ready for release from the cells. The posttranslational modifications comprise proteolytic cleavage at several di- and monobasic processing sites, *O*-sulphations at several tyrosyl residues, serine phosphorylations, glutamic acid cyclizations and phenylalanine α-carboxyamidation (for review, see [7–9]).

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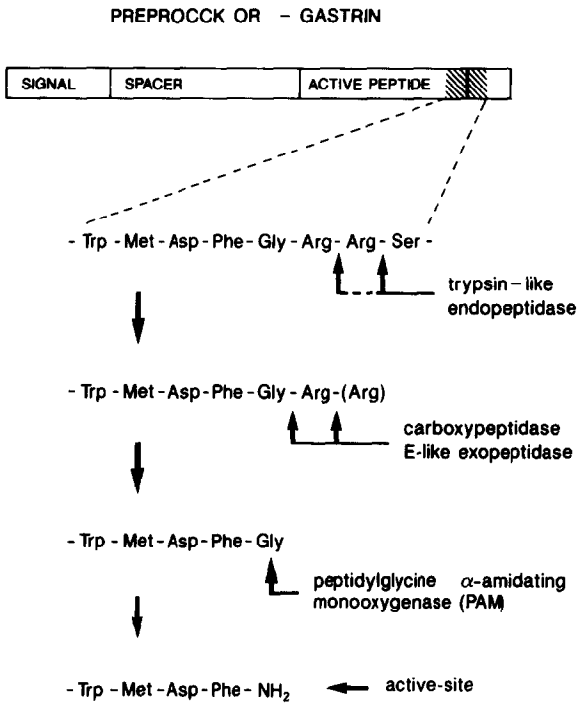

Fig. 1. Posttranslational maturation of proCCK and progastrin. The identical active-site of the propeptides is -Trp-Met-Asp-Phe-NH_2. Only fragments with this α-amidated C-terminus have biological activity. The maturation process exposing the active-site, i.e. the enzymes and cofactors required, is now well-known (see also [4,9]).

The CCK and gastrin systems are particularly useful models for examination of the posttranslational phase of gene expression for three reasons. First, the trimming of the precursors to bioactive mature peptides comprises as mentioned a large number of common processing steps. Second, the final bioactive products have an unusually well-defined active-site, i.e. the C-terminal Trp-Met-Asp-Phe-NH_2 sequence. Finally, the processing steps required for synthesis of peptides having this carboxyamidated tetrapeptide sequence are well-known (Fig. 1). In other words, expression of the CCK and gastrin genes is phenotypically defined with great precision, i.e. by synthesis and release of peptides having the Trp-Met-Asp-Phe-NH_2 C-terminus.

In the above-mentioned 'main factories', the gastrointestinal endocrine cells and the cerebral CCK-neurons, proCCK and progastrin are processed almost completely ($\geq$98%) to bioactive carboxyamidated peptides [4,10]. This is truly unattenuated processing.

3. ATTENUATED proCCK AND PROGASTRIN PROCESSING IN ADULT CELLS

Attenuated posttranslational processing occurs in cells that translate a given mRNA, but subsequently fail to mature the translation product to a functionally useful protein or peptide. Such condition might also be called silent gene expression.

Our first example of attenuated posttranslational processing was discovered in normal adult porcine corticotrophs, which – as indicated by the name – produce ACTH and other fragments of proopiomelanocortin (POMC) in large quantities. The corticotrophs, however, also express significant amounts of progastrin and proCCK [5], but process only a negligible fraction to peptides having the carboxyamidated active-site [5,11]. The corticotrophs therefore release neither bioactive CCK nor gastrin peptides. Nevertheless corticotrophs are abundantly equipped with enzymes for cleavage at dibasic processing sites and for α-carboxyamidation [3]. In addition the pituitary and

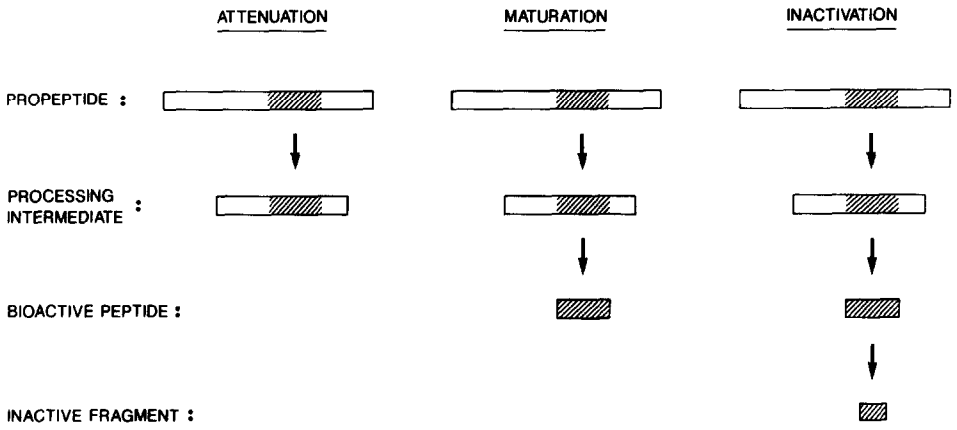

Fig. 2. Three different courses of the posttranslational phase of gene expression: by *attenuation* there is either no processing of the propeptide or a partial processing to biosynthetic intermediates without significant maturation of proper bioactive peptides. Examples of posttranslational attenuation are the corticotrophic expression of progastrin [5] and the cardiac expression of proenkephalin A [25]. By *maturation* the propeptide is fully processed to release the bioactive peptide. Maturation, cell-specific or not, is the classical and well-elucidated posttranslational course of the expression of peptide genes. By *inactivation* the matured bioactive peptide loses its biological activity either by proteolytic fragmentation or by derivatization of the active-site like the pituitary *N*-acetylation of β-endorphin [26–28]. It is possible that some inactivation occurs in most peptide-producing cells.

gastrointestinal amidation enzymes appear to be almost identical structurally as well as functionally [12]. It is therefore surprising that corticotrophs fail to mature both progastrin and proCCK [5].

We do not yet know the explanation. Perhaps the substrate specificity of processing enzymes is so high that those used to process POMC have only little effect on proCCK and progastrin. Perhaps the processing sites on pituitary progastrin and proCCK are modified in a way that interferes with the maturation process. Perhaps the proCCK and progastrin concentrations in the corticotrophs are too low for the corticotrophic processing enzymes, although the progastrin concentrations in the pituitary and antral cells are the same. The pituitary gastrin mRNA is apparently identical with that of the antral mucosa [13]. Therefore, alternative splicing cannot explain the lack of posttranslational processing. So far there are no reasons to believe that unprocessed or partly processed pituitary progastrin and proCCK per se have any biological activity. Also ontogenetic studies have so far failed to provide evidence of a more comprehensive foetal or neonatal pituitary synthesis and processing of which the progastrin and proCCK in the adult might be a reflection (unpublished studies).

In contrast with the pituitary, the low level of progastrin in the adult mammalian pancreas [6] reflects, however, an extensive foetal and neonatal synthesis of bioactive, carboxyamidated gastrins [6,14,15]. The foetal synthesis in the pancreas seems to serve local growth control [16]. As discussed later, the fact that the normal adult pancreas expresses progastrin – although at a low level – shows that the gastrin producing carcinomas in the pancreas are not ectopic.

Other examples of low-level synthesis of progastrin and proCCK with attenuated posttranslational processing have been found in bronchial endocrine cells [17] and cerebellar neurons (unpublished results). The different posttranslational pathway during attenuation is schematized in Fig. 2.

4. ATTENUATED PROCESSING IN FOETAL AND NEONATAL CELLS

Posttranslational attenuation may occur in two opposite directions during the ontogenic development. In the first situation, considerable amounts of propeptides may accumulate in foetoneonatal cerebral neurons and peripheral endocrine cells in which the enzymatic machinery necessary for the processing to bioactive peptides is not yet available [18,19]. Then at a certain stage of development, for instance in rats, immediately before or at weaning, the processing machinery matures [18,19].

The other situation concerns bioactive peptides that act also as growth factors, which are required to regulate foetal growth. In the adult the local requirements for growth factors have apparently diminished to such a degree that the posttranslational maturation of peptide growth factors ceases, so that the peptide-producing cells are left with moderate amounts of propeptides [5,6]. Thus, the developmental expression of bioactive peptide seems to be regulated not only at the transcriptional but also at the posttranslational level.

5. ATTENUATED PROCESSING IN TUMOUR CELLS

The phenomenon of posttranslational attenuation may turn out to have specific implications for the understanding of carcinogenetic mechanisms and for improvement of cancer diagnosis. First, closer analysis of tissues from which the so-called peptide-producing ectopic tumours are developed indicates that genes encoding the peptides produced in the tumours are expressed also in the corresponding normal cells. A modest level of expression and subsequent posttranslational attenuation, however, has silenced the phenotypic expression so that only inactive precursors are present in the normal cells (for review, see [20]). Consequently, several peptide-producing tumours cannot be considered ectopic. They are eutopic. But the eutopic synthesis is camouflaged by posttranslational attenuation. Second, the size of the synthesis and the efficiency of the posttranslational processing in peptide-producing transformed tumours display gross individual variations. Many carcinomas therefore synthesize substantial quantities of propeptides but fail to mature the propeptides to any significant extent. In clinical terms these tumours are therefore silent and less easily diagnosed. By so-called processing-independent immunoanalysis [21] it seems possible now to diagnose peptide-producing carcinomas irrespective of the degree of posttranslational attenuation [22]. Third, since many neuroendocrine peptides also are growth factors, as previously mentioned, it is likely that the peptides synthesized in carcinomas may stimulate the growth of the tumour by local autocrine secretion [23]. Thus, even low-level expression with attenuation may by local secretion play a significant role in tumour growth. It is therefore important to detect expression of peptide genes in tumour cells, also when the expression is attenuated posttranslationally.

6. ATTENUATED PROCESSING OF PROPEPTIDES OTHER THAN proCCK AND PROGASTRIN

So far only few laboratories have described findings that correspond to posttranslational attenuation as defined in this review. The paucity is probably due to analytical shortcomings, because only few laboratories have developed the complex precursors assay pro-

cedures of sufficient sensitivity and specificity. Precursor analysis generally requires libraries of monospecific radioimmunoassays used in combination with appropriate enzymography and chromatography [21,24].

One valid example has been reported by Howells et al. [25], who in the rat heart in addition to high concentrations of proenkephalin A mRNA found significant amounts of a precursor protein corresponding to proenkephalin A, but only traces of mature enkephalin peptides. The precursor-product ratio >30 [25] corresponds to those of proCCK-CCK and progastrin-gastrin found in our laboratory [5,6,19].

Posttranslational attenuation as defined in this review (Fig. 2) should not be confused with inactivation or degradation of the matured peptide products. *N*-Acetylation of pituitary and cerebral β-endorphin is an example of the latter [26–28]. Although peptide inactivation or degradation may lead to the same result as the posttranslational attenuation, i.e. absence of physiologically active peptides, the mechanisms nevertheless differ substantially. In order to understand how gene expression is controlled in normal as well as sick and especially in transformed cells a clear conception of posttranslational attenuation is necessary.

7. CONCLUDING REMARKS

Our studies of cell-specific processing of neuroendocrine peptides has revealed the so far overlooked phenomenon of posttranslational attenuation of gene expression. Such attenuation occurs in relation to the ontogenetic development of neuroendocrine cells, which in their normal adult state are characterized by their high degree of differentiation. Thus, it seems expedient for highly differentiated cells to have mechanisms for fine-tuning the expression of peptide genes also at the posttranslational level. Recognition of attenuation phenomena in gene expression are also important for the understanding of the role of peptides as signals and growth factors in development of cancers. Thus, peptides seem to play an ever increasing role both in the genesis and growth of cancers and as tumour markers. In this context, it has been striking to observe that peptide-producing tumours always seem to originate from cells, which also in their normal state express the same peptide genes, but at low-level and incomplete processing (for review, see [20]). The existence of posttranslational processing in normal cells therefore questions the concept of ectopic tumour synthesis of peptides.

The molecular mechanisms of posttranslational attenuation are still unknown. They probably vary with each peptide system and each cell type. Elucidation of the mechanisms involved in the attenuation of gastrin and CCK peptides is in progress in our laboratory. The extent to which posttranslational attenuation plays a role for the expression of eucaryotic genes in general remains to be seen. It is possible that the phenomenon mainly occurs in the biogenesis of proteins and peptides have complex, multistep posttranslational processing pathways.

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