Targeting of PKA in Mammary Epithelial Cells
Mechanisms and functional consequences

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Abstract: Targeting of protein kinases, promoting association with specific partner-molecules and localisation to particular sites within the cell, has come to be recognised as a key mechanism for attributing specificity to these enzymes. In mammary epithelial cells, the repertoire of acute regulatory roles played by cyclic AMP-dependent protein kinase (PKA) differs from that in other lipogenic cell-types. Furthermore, PKA is implicated in the regulation of mammary-specific function, mediating a tonic stimulation of the flux of newly-synthesised casein through its basal secretory pathway. Both these observations imply mammary-specific properties of either PKA targeting systems or of PKA itself. Evidence for the latter is currently lacking. Pulse-chase labelling experiments in the presence and absence of selective effectors of PKA have enabled the site(s) of action of this protein kinase on casein secretion to be localised to the early stages of the secretory pathway. Possible mechanisms are considered for the physical targeting of PKA to the membrane-enclosed components of the secretory pathway and evidence for their occurrence in mammary epithelial cells is presented.

1. INTRODUCTION

Targeting of protein kinases is a mechanism of imposing functional selectivity on their action, additional to that which their own sequence-recognition specificity gives them. Furthermore, the characteristics of an individual targeting mechanism may be such as to enable a single enzyme to
manifest diverse specificities both between cells and, within a single cell, at different points in time or during a programme of growth and differentiation.

1.1 PKA isozyme diversity

Cyclic AMP-dependent protein kinase (PKA) is one of a relatively small number of generic protein kinases acting pleiotropically as primary transducers of regulatory signals within cells. It is catalytically activated in proportion to the prevailing concentration of cAMP in its immediate environment. In its inactive state, PKA exists as a heterotetramer consisting of two catalytic (C- ) and two regulatory (R- ) subunits. Multiple isozymes of both R- and C- subunit are encoded by different genes (RIα,β; RIIα,β; Cα,β,γ). Splice variants of C-subunit also exist, most strikingly in C. elegans but also in mammalian species. The precise function of all this diversity is not known with certainty either in C. elegans or in mammals, but evidence is beginning to emerge to support the suggestions that determinants of targeting and/or stability and turnover of the C-subunit protein molecule may reside in these alternative terminal sequences.

1.2 Basal activity and activation of PKA

The presence of R-subunit sequences occluding the substrate cleft of C-subunit causes the latter to remain catalytically inactive in the holoenzyme. The cAMP signal is transduced via its high-affinity binding to two sites on each R-subunit, causing them to dissociate as an R-subunit dimer, concomitantly liberating two catalytically active C-subunits. Phosphorylation of proteins by C-subunit can lead to a variety of biological effects depending on the identity of the phospho-acceptor substrate protein. Examples of these include: enzymes catalysing biosynthesis, degradation and metabolic interconversion; transcriptional regulators; ion channels; transmembrane receptors and other signalling mediator proteins. The PKA activation cycle is reversed by the action of cAMP phosphodiesterases. When making experimental measurements of PKA activity, a “snapshot” of the dissociation status at the time of sampling can be made by assaying in the absence of added cAMP to determine the “basal” or “expressed” activity. The “total” activity (measured in the presence of a saturating excess of cAMP) measures the maximum potential of the tissue for C-subunit catalysed protein kinase activity in the event of complete dissociation of all the cellular holoenzyme. In real situations within living cells, activation of PKA is not an “all-or-none” phenomenon but a proportional response to the prevailing cAMP level which may itself vary from place to place within the cell. Thus the tonic or basal level of PKA activation is an important