Masking of Signal Sequences in CREC Proteins by cDNA Subcloning in Epitope Vectors

Tsukumo and colleagues (1) have recently reported that a proline residue at position 2 from the signal peptide cleavage site determines the export of nucleobindin 1 from the endoplasmic reticulum (ER) and subsequent transport to the Golgi. They also studied the localization of three CREC proteins, reticulocalbin, calumenin, and Cab45 that contain a proline at position 2 from the N-terminal signal sequence. The cDNAs for each of the CREC proteins were subcloned into the pcDNA3.1/V5-His-TOPO vector (Invitrogen) used to transfect eukaryotic cells. Each of the CREC proteins, synthesized with a V5-His epitope at the C terminus, are localized in the Golgi (1).

The Golgi localization of the recombinant V5-His containing CREC proteins is in striking contrast to the heterogeneous localization of endogenous CREC proteins (2). Indeed, Cab45 is the only CREC protein localized strictly within the Golgi (3). Reticulocalbin is present in the ER due to a C-terminal ER retention signal, HDEL (4), and may, by an unknown mechanism, be found on the surface of bone endothelial cells and prostate cancer cells (5). Calumenin is distributed throughout the secretory pathway with a significant proportion localized within the endoplasmic or sarcoplasmic reticulum. Here calumenin interacts with several proteins and, probably due to an inefficient C-terminal ER retention signal, HDEF, is also secreted (2). Thus, the addition of a V5-His epitope to the C termini of the CREC proteins may have masked the C-terminal signals, which appear to be more important for the localization in vivo than the proline at position 2.

Bent Honoré
Department of Medical Biochemistry, Aarhus University, Denmark


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1E-mail: bh@biokemi.au.dk
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Bent Honoré

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