Unusual degradation of α-β complexes in *Xenopus* oocytes by β-subunits of *Xenopus* gastric H-K-ATPase

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Unusual degradation of α-β complexes in *Xenopus* oocytes by β-subunits of *Xenopus* gastric H-K-ATPase

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Chen, Pei-Xian, Paul M. Mathews, Peter J. Good, Bernard C. Rossier, and Käthi Geering. Unusual degradation of α-β complexes in *Xenopus* oocytes by β-subunits of *Xenopus* gastric H-K-ATPase. *Am. J. Physiol.* 275 (*Cell Physiol.* 44): C139–C145, 1998.—The catalytic α-subunit of oligomeric P-type ATPases such as Na-K-ATPase and H-K-ATPase requires association with a β-subunit after synthesis in the endoplasmic reticulum (ER) to become stably expressed and functionally active. In this study, we have expressed the β-subunit of *Xenopus* gastric H-K-ATPase (βHK) in *Xenopus* oocytes together with α-subunits of H-K-ATPase (αHK) or Na-K-ATPase (αNK) and have followed the biosynthesis, assembly, and cell surface expression of functional pumps. Immunoprecipitations of *Xenopus* βHK from metabolically labeled oocytes show that it is well expressed and, when synthesized without α-subunits, can leave the ER and become fully glycosylated. *Xenopus* βHK can associate with both coexpressed αHK and αNK, but the α-β complexes formed are degraded rapidly in or close to the ER and do not produce functional pumps at the cell surface as assessed by ^{86}Rb uptake. A possible explanation of these results is that *Xenopus* βHK may contain a tissue-specific signal that is important in the formation or correct targeting of functional α-β complexes in the stomach but that cannot be recognized in *Xenopus* oocytes and in consequence leads to cellular degradation of the α-β complexes in this experimental system.

intracellular transport; oligomerization; pre-Golgi degradation; *Xenopus* oocyte expression

CELLS CONTROL THE FIDELITY of secretory protein biosynthesis before transport through the Golgi complex, assuring that proteins in distal compartments of the secretory pathway are conformationally intact and targeted to the correct cellular compartment. To a major extent, cells achieve this by synthesizing proteins that form multimeric complexes and by permitting only fully assembled multimers to exit the endoplasmic reticulum (ER). In many cases, unassembled and ER-retained subunits are degraded rapidly by a pre-Golgi degradation system (for review see Ref. 6).

Our laboratory has studied the subunit assembly and transport from the ER to the plasma membrane of the heterodimeric α-β cation-transporting ATPases expressed in *Xenopus laevis* oocytes (7, 11, 14). The multi-membrane-spanning α-subunit (relative mol mass ~100 kDa) is the catalytic subunit that hydrolyzes ATP and undergoes the E_1-to-E_2 conformational transition during ion translocation. The glycosylated β-subunit (relative mol mass ~45–80 kDa) is required for the plasma membrane expression of functional pumps (for review see Ref. 6), and it may influence the apparent K affinity (12, 14). Well characterized in vertebrates are the Na-K-ATPase, a ubiquitous component of the plasma membrane, and the gastric H-K-ATPase, expressed only in the parietal cell of the stomach mucosa. The α-subunits share ~65% amino acid sequence identity (17), whereas the β-subunits are less well conserved (24).

In the parietal cell of the stomach, the H-K-ATPase resides in the apical membrane and subapical tubulovesicles (20), whereas the Na-K-ATPase is basolateral, as it is in most polarized epithelia (18). Gottardi and Caplan (9) have identified apical targeting domains in the α- and β-subunits of the gastric H-K-ATPase (αHK and βHK, respectively), suggesting a mechanism for the correct plasma membrane localization of the molecule. Arrival in the apical or basolateral membrane, however, must be preceded by appropriate subunit assembly in the ER. The available data indicate that assembly can be promiscuous, with Na-K-ATPase α-subunits (αNK) and gastric βHK forming heterodimers and functional pumps in the plasma membrane (5, 10, 14, 16). Clearly, however, the parietal cell must extend greater control over the expression of mixed pumps.

To better understand these control mechanisms, we expressed gastric αHK or βHK in combination with αNK or Na-K-ATPase β-subunits (βNK) in *Xenopus* oocytes and analyzed their ability to assemble and to support the expression of functional H-K or Na-K pumps at the cell surface. With this approach, we wanted to test whether *Xenopus* oocytes lack the parietal cell-specific control factors for correct assembly and transport of cation pumps. Our previous work has involved the functional expression of rabbit gastric βHK in oocytes along with *Xenopus* αNK (10) or *Xenopus* gastric αHK (17). To have a strictly homologous *Xenopus* system, we isolated a cDNA encoding *Xenopus* βHK and generated an antibody against this protein. Using these tools and the *Xenopus* oocyte expression system, we show that, like rabbit gastric βHK, the *Xenopus* βHK assembles with *Xenopus* αHK as well as αNK in the ER. However, α-β heterodimers formed with *Xenopus* βHK were not expressed at the cell surface due to early degradation. This may suggest a mechanism by which the parietal cell can assure that only appropriate α-β heterodimers are routed to the

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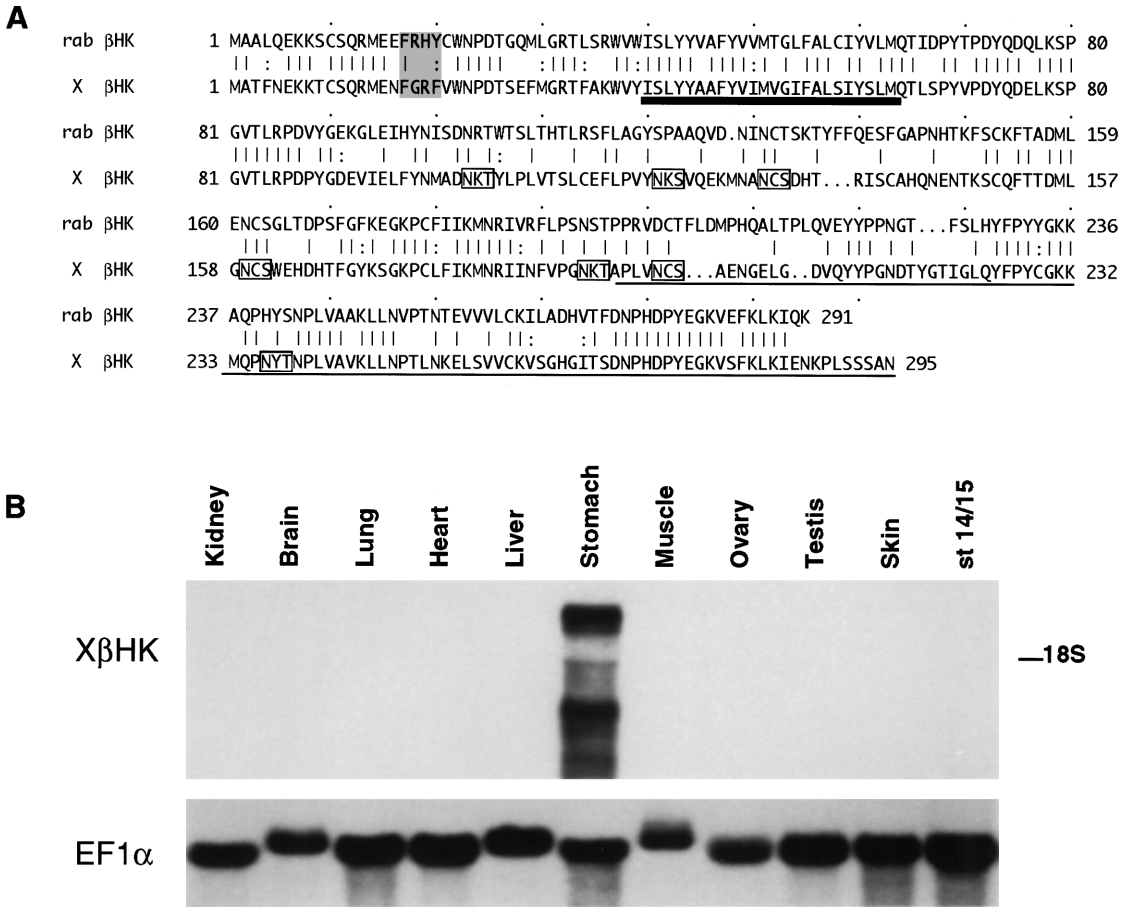

Fig. 1. *A*: primary structure and tissue distribution of *Xenopus* gastric H-K-ATPase β-subunit (βHK). Isolated cDNA coding for *Xenopus* βHK is ~1.1 kb long and contains a 295-codon open reading frame with start codon beginning at nucleotide 22. Shown is an alignment of amino acid sequences of *Xenopus laevis* (X) and rabbit (rab) gastric βHK. Identical amino acids are indicated by dashes and conservative substitutions by dots. Single transmembrane domain is underlined in bold. The 7 putative glycosylation sites are boxed. Two additional, less conservative consensus glycosylation sequences NDT and NPT are also present. A glutathione *S*-transferase fusion protein that was used to generate antibodies against βHK contained the carboxy-terminal 100 amino acids (underlined). *B*: Northern blot analysis of tissue distribution of *Xenopus* βHK. Total RNA (7.5 μg) from different tissues of adult *X. laevis* was hybridized with cDNA probes. *Top*: RNA hybridized with a *Xenopus* gastric β-subunit probe. *Bottom*: same filter reprobed with a *Xenopus* elongation factor 1α (EF1α) probe. mRNA encoding EF1α is a major transcript at midblastula transition in *Xenopus* (15). Lanes are labeled with source of RNA; st 14/15 RNA is from stage 14–15 embryos. Migration of 18S RNA is indicated at *right*.

trans-Golgi network for trafficking to either the apical or basolateral membranes. Additionally, this presents a novel paradigm for the ER exit of a multisubunit protein. Unassembled βHK is capable of rapidly leaving the ER, whereas α-assembled βHK is not transported to the cell surface.

METHODS

Cloning of a cDNA encoding the Xenopus laevis gastric βHK. A cDNA fragment of *Xenopus* βHK was generated by PCR, using as template cDNA prepared from *Xenopus* stomach mRNA with oligo(dT) priming. The degenerate oligonucleotide primers used in the PCR were a sense primer encoding the amino acid sequence Pro-Asp-Tyr-Gln-Asp-Gln with an added *Eco*R I site at the 5′ end (CGGAATTCCNGAYTAYCARGAYCA) and an antisense primer encoding the amino acid sequence His-Tyr-Phe-Pro-Tyr-Tyr with an added *Bam*H I site at the 5′ end (CCGGATCCRTARTANGGRAARTARTG). A 480-bp fragment was isolated, subcloned, sequenced, and found to encode the appropriate region of *Xenopus* βHK.

A *Xenopus* stomach mucosal cDNA library was constructed in the vector pBluescript (Stratagene) from size-fractionated poly(A)$^+$ RNA enriched for *Xenopus* βHK message (see Ref. 17 for details). Approximately 40,000 independent colonies were screened in pools of 2,000 colonies by PCR using 2 oligonucleotides (GGTGTGACATTGAGACC and TGAACAGTTCACAAGAGG), the sequences of which were based on the *Xenopus* βHK cDNA fragment described above. Sequential pool size reduction and PCR screening yielded a clone containing a full-length *Xenopus* βHK cDNA, which was sequenced on both strands. The full-length cDNA was subcloned into the expression vector pSD3 (8) for cRNA synthesis (19).

Xenopus βHK and αHK antibodies. A glutathione *S*-transferase (GST) fusion protein containing the carboxy-terminal 100 amino acids of *Xenopus* βHK (Fig. 1) was constructed in the vector pGEX-2T (Pharmacia). A *Bam*H I site, in frame with the GST open reading frame, was intro-

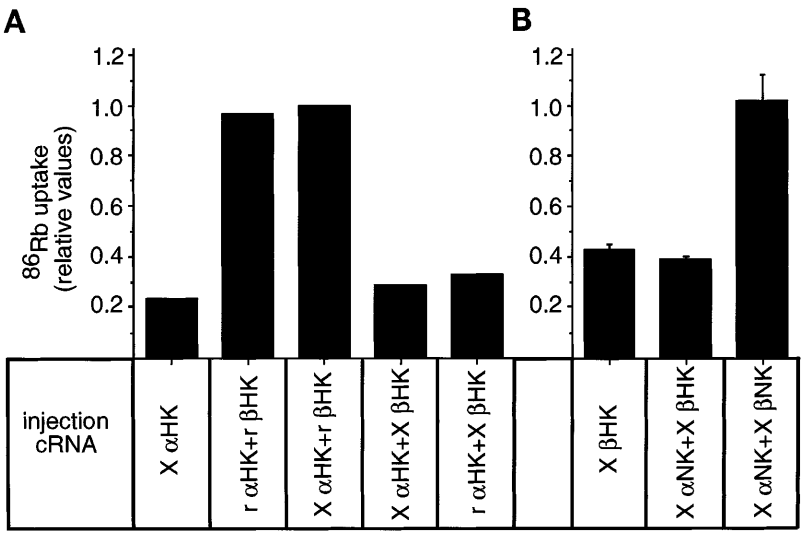

Fig. 2. Expression of *Xenopus* gastric βHK in *Xenopus* oocytes does not produce functional H-K or Na-K pumps at cell surface. Oocytes were injected with indicated combinations of cRNAs for different α-subunits of N-K-ATPase (αNK; 11 ng) or H-K-ATPase (αHK; 11 ng) and βHK or β-subunit of N-K-ATPase (βNK) [2 ng rabbit (r) βHK, 0.5 ng *Xenopus* βHK, or *Xenopus* βNK]. Three days after injection, ^{86}Rb uptake into oocytes was measured as described in METHODS. *A*: ^{86}Rb uptake mediated by expressed H-K pumps. Shown is mean of 2 experiments performed on 2 different batches of oocytes. In each experiment, 16–20 oocytes were measured for each condition. Values obtained in oocytes expressing rabbit αHK-rabbit βHK complexes were set to 1 and amounted to 14.3 ± 0.5 and 12.8 ± 0.4 pmol·min^{-1}·oocyte^{-1}, respectively, in 2 experiments. *B*: ^{86}Rb uptake mediated by expressed Na-K pumps. Values obtained in oocytes expressing *Xenopus* αNK-*Xenopus* βNK complexes were set to 1 and amounted to 129.2 ± 15.5 pmol·min^{-1}·oocyte^{-1}. Data are means ± SE (n = 20).

duced by PCR into the *Xenopus* βHK cDNA before the codon for Pro-195. Bacterially produced fusion proteins were affinity-purified using glutathione-Sepharose 4B (Pharmacia), recovered by elution with glutathione, and used to immunize rabbits. The specificity of the antiserum was tested by immunoprecipitation of digitonin extracts of metabolically labeled *Xenopus* βHK following cRNA injection into oocytes (see below). Preimmune serum and immune serum preabsorbed on the GST-*Xenopus* βHK fusion protein used as antigen did not immunoprecipitate any labeled proteins (data not shown). On the other hand, both immune serum preabsorbed on GST and immune serum without treatment immunoprecipitated a core-glycosylated ER form (~50 kDa) and a fully glycosylated post-Golgi form (60–75 kDa) of the *Xenopus* βHK.

A synthetic peptide corresponding to a 15-amino acid sequence near the amino terminus of the *Xenopus* αHK (Ser-Val-Glu-Met-Glu-Arg-Glu-Gly-Asp-Gly-Ala-Met-Val-Lys) linked to a lysine core (multiple antigenic peptide; Ref. 21) was used to immunize rabbits to generate the αHK antiserum. This antiserum recognizes in vitro-translated αHK and does not recognize αNK either from in vitro translation or expressed in oocytes (data not shown). The anti-rabbit βHK monoclonal antibody was a gift of P. Mangeat (20). Antibodies against αNK and βNK have been described (1).

Protein expression in Xenopus oocytes and immunoprecipitations. Oocytes were obtained from *X. laevis* as previously described (7). Oocytes were injected with the indicated amounts of β-subunit cRNA alone or in combination with α-subunit cRNA, metabolically labeled in modified Barth's solution (MBS) containing 0.6 μCi/ml [^{35}S]methionine, and chased in MBS containing 10 mM unlabeled methionine. Digitonin extracts were prepared, and immunoprecipitations were performed under denaturing or nondenaturing conditions as described (7). In some instances, immunoprecipitated β-subunits were digested with endoglycosidase H (Calbiochem-Novabiochem, La Jolla, CA) (13). SDS-PAGE, fluorography, and laser densitometry were performed as previously described (7).

^{86}Rb uptake. Three days after cRNA injection of oocytes, ^{86}Rb uptake was measured as previously described (13). The assay solution used throughout was (in mM) 90 NaCl, 1 $MgCl_2$, 0.33 $Ca(NO_3)_2$, 0.41 $CaCl_2$, 5 $BaCl_2$, and 10 HEPES (pH 7.4). For H-K-ATPase transport measurements, oocytes were preincubated for 15 min in an assay solution containing 10 μM ouabain, which completely inhibits the endogenous oocyte Na-K-ATPase but has no effect on H-K-ATPase activity (17). After preincubation, oocytes were incubated for 12 min in an assay solution containing 5 μCi/ml ^{86}RbCl (Amersham) and 0.5 mM KCl for H-K-ATPase transport measurements or 5 mM KCl for Na-K-ATPase transport measurements. Oocytes were washed, and the ^{86}Rb uptake in single oocytes was determined by scintillation counting.

RESULTS

Primary structure and tissue distribution of the Xenopus gastric βHK. We characterized recently a *Xenopus* gastric αHK after coexpression with rabbit gastric βHK in *Xenopus* oocytes (17). To test whether functional H-K pumps could be formed in a strictly homologous system different from parietal cells, we isolated a cDNA encoding *Xenopus* gastric βHK (GenBank accession no. BankIt165014 AF042812). *Xenopus* βHK shares 55% amino acid sequence identity with rabbit βHK, with two striking differences (Fig. 1*A*). One area of difference is at the carboxy terminus, where the *Xenopus* βHK extends eight amino acid beyond that of the rabbit βHK. A second difference is in the cytoplasmic amino terminus, where a tyrosine-based endocytosis motif has been identified in mammalian βHK (4). In *Xenopus* and chicken βHK, however, phenylalanine replaces this tyrosine.

Northern blot analysis of *Xenopus* βHK performed in different tissues (Fig. 1*B*) revealed its exclusive expression in the stomach, confirming that the isolated cDNA encoded the gastric βHK.

Xenopus βHK does not support the functional expression of αHK or αNK. To test the ability of *Xenopus* βHK to produce functional H-K-ATPase α-β complexes in *Xenopus* oocytes, we expressed *Xenopus* βHK or rabbit βHK together with *Xenopus* or rabbit αHK and compared the expression of functional H-K pumps at the cell surface by ^{86}Rb uptake measurements. As previously shown, coexpression of rabbit βHK with rabbit (10) or *Xenopus* (17) αHK led to a significant, approximately fivefold increase in ^{86}Rb uptake compared with that measured in oocytes expressing αHK alone (Fig.

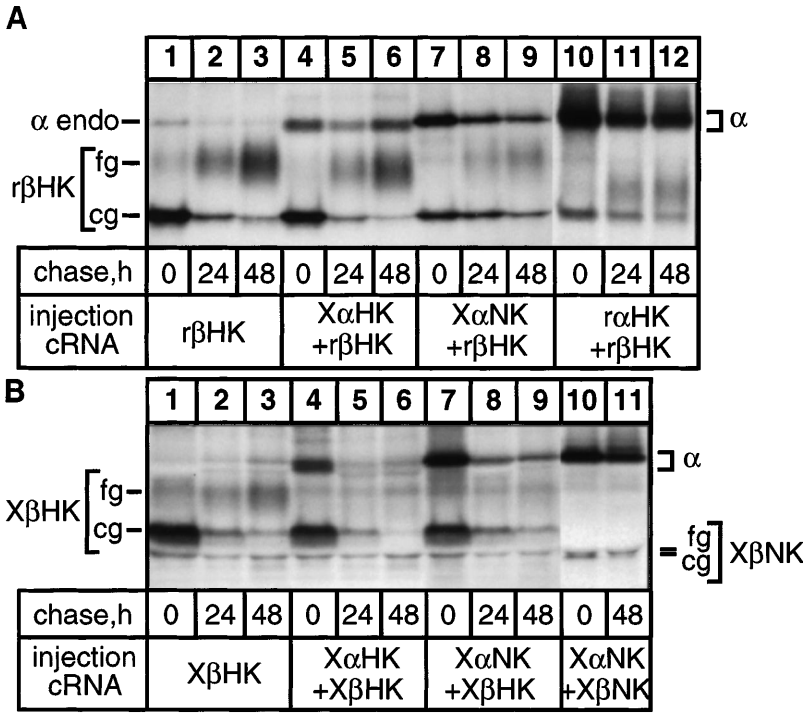

Fig. 3. *Xenopus* βHK assembles with but does not stabilize αHK and αNK. Oocytes were injected with βHK (0.25 ng *Xenopus* βHK, 2.0 ng rabbit βHK) cRNA alone or in combination with αHK (8 ng) or αNK (7 ng) as indicated. After metabolic labeling for 16 h and chase periods of 0, 24, or 48 h, digitonin extracts were prepared and subjected to immunoprecipitation under nondenaturing conditions with an anti-rabbit βHK monoclonal antibody (*A*) or a *Xenopus* βHK antiserum (*B*). Core-glycosylated (cg) and fully glycosylated (fg) βHK and βNK, coprecipitated exogenous α-subunit (α), and endogenous α-subunit (α endo) are indicated.

2*A*). Surprisingly, coexpression of *Xenopus* βHK with *Xenopus* or rabbit αHK did not result in a significant change in the H-K pump activity compared with that in αHK-expressing oocytes. Similarly, *Xenopus* βHK coexpressed with *Xenopus* αNK did not increase Na-K pump activity at the cell surface compared with that found in oocytes expressing *Xenopus* βHK alone (Fig. 2*B*).

Xenopus βHK can associate with but not stabilize αHK or αNK. The lack of an increased pump expression after coexpression of *Xenopus* βHK with either αHK or αNK could be an indication that *Xenopus* oocytes lack mechanisms for the sorting of H-K-ATPases vs. Na-K-ATPases that parietal cells need to express H-K pumps in the correct cellular compartment. However, it is also possible that the results obtained were only due to inefficient translation of the injected cRNA or to lack of assembly of the newly synthesized *Xenopus* βHK with the α-subunits. To test the two latter possibilities, we compared the biosynthesis and assembly of metabolically labeled *Xenopus* or rabbit βHK expressed alone or together with αHK or αNK in oocytes. After cRNA injection, oocytes were subjected to a 16-h pulse with [^{35}S]methionine and various chase periods, digitonin extracts were prepared, and the expressed proteins were immunoprecipitated under nondenaturing conditions that preserve subunit interaction. As previously observed (10), rabbit βHK was well expressed in oocytes and when expressed alone was immunoprecipitated mainly in its core glycosylated form after a pulse period (Fig. 3*A*, *lane 1*) and in its fully glycosylated form after various chase periods (*lanes 2* and *3*). Although rabbit βHK could associate with endogenous oocyte αNK, as reflected by coimmunoprecipitation with a βHK antibody (*lanes 1–3*), it was synthesized in large excess over the endogenous α-subunit. Therefore our data confirm that rabbit βHK is able to be transported to the plasma membrane without association with α-subunits. Rabbit βHK associated efficiently with coexpressed rabbit (*lane 10*) and *Xenopus* (*lane 4*) αHK or αNK (*lane 7*) and typically stabilized the αHK (*lanes 5*, *6*, *11*, and *12*) and to a somewhat lesser extent the αNK (*lanes 8* and *9*), which in an unassembled form are degraded completely during the chase period (1). This result reflects the higher specificity of βHK for αHK than for αNK and explains the previously observed difference in the cell surface expression of functional αHK-βHK and αNK-βHK complexes (14).

Inefficient synthesis of *Xenopus* βHK is not responsible for the lack of formation of functional H-K pumps. *Xenopus* βHK also was well expressed in oocytes when expressed either alone (Fig. 3*B*, *lane 1*) or together with αHK (*lanes 4* and *7*). When expressed alone, *Xenopus* βHK was found mainly in its core glycosylated form after a pulse period (*lane 1*) and in its fully glycosylated form after chase periods (*lanes 2* and *3*), indicating that, like rabbit βHK, *Xenopus* βHK can leave the ER without α-association.

The association efficiency of *Xenopus* βHK with α-subunits was tested by following the coimmunoprecipitation of coexpressed *Xenopus* αHK or αNK. The results revealed that *Xenopus* βHK was indeed able to associate with both α-subunits during a pulse period (*lanes 4* and *7*) but that the association was lost during the chase periods (*lanes 5*, *6*, *8*, and *9*). The results indeed suggest that association of *Xenopus* βHK with *Xenopus* αHK or with αNK, which is stabilized completely by association with βNK (*lanes 10* and *11*), provokes the degradation not only of the associated α-subunit but also of the *Xenopus* βHK itself rapidly after synthesis.

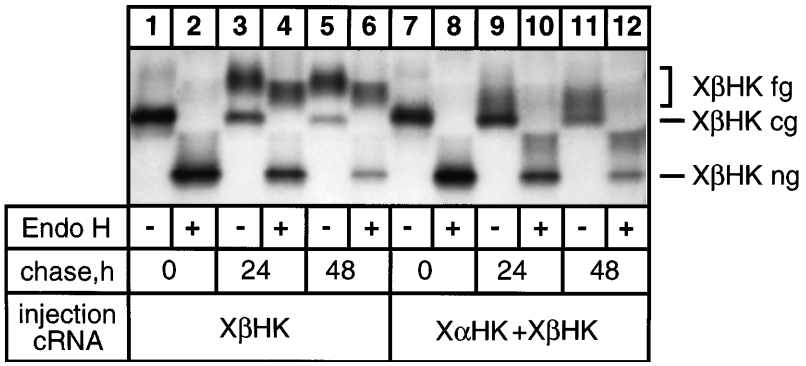

Fig. 4. Carbohydrate processing of *Xenopus* βHK expressed alone or with αHK. Oocytes were injected with *Xenopus* βHK cRNA (0.25 ng) alone or in combination with αHK cRNA (8 ng), metabolically labeled for 16 h, and chased for indicated times. Digitonin extracts were denatured and immunoprecipitated with *Xenopus* βHK antiserum. One-half of eluted proteins were digested with endoglycosidase H (Endo H) before SDS-PAGE. Nonglycosylated (ng), core-glycosylated, and fully glycosylated forms of *Xenopus* βHK are indicated.

This event is responsible for the lack of formation of functional H-K or Na-K pumps at the cell surface.

To further document this finding, we expressed *Xenopus* βHK alone or together with *Xenopus* αHK in oocytes and followed the degradation and the glycosylation processing of the *Xenopus* βHK after a pulse and various chase periods. The glycosylation processing was followed via the sensitivity to endoglycosidase H digestion, which characteristically cleaves only high-mannose core sugars acquired during synthesis and not complex type sugars added to the protein in the trans-Golgi compartment after mannose trimming. Typically, after a 12-h pulse, the total population of *Xenopus* βHK synthesized in *Xenopus* oocytes in the absence (Fig. 4, *lanes 1* and *2*) or presence (*lanes 7* and *8*) of *Xenopus* αHK was endoglycosidase H sensitive, indicating that the protein resides at the level of the ER. In the absence of coexpressed αHK, *Xenopus* βHK became progressively fully glycosylated and thus was transported to the plasma membrane, as reflected by the decrease in endoglycosidase H-sensitive species and a parallel increase in higher molecular mass species that were partially but not completely endoglycosidase H resistant (*lanes 3–6*). Analysis of the N-linked sugars in βHK revealed recently that oligomannose structures persist on some of the seven glycosylation sites even in fully glycosylated βHK (23), which could explain the partial endoglycosidase H sensitivity of the high-molecular-mass species. Compared with individual *Xenopus* βHK, α-assembled *Xenopus* βHK are not processed to the same high-molecular-mass species during the chase periods but are slowly degraded, mainly in their core-glycosylated, endoglycosidase H-sensitive form (*lanes 7–9*) and to a lesser extent in an intermediate, poorly defined, endoglycosidase H-resistant form. This result suggests that association of *Xenopus* βHK with α-subunits induces retention of the α-β complex in or close to the ER compartment and its concomitant degradation.

Xenopus oocytes contain an endogenous αNK pool that is not associated with β-subunits and that is in a highly trypsin-sensitive form (13). Expression of exogenous βNK is able to recruit this immature αNK pool and permits the formation of functional Na-K pumps at the cell surface. In contrast to exogenous α-subunits, which are degraded when expressed alone in oocytes, the endogenous, unassembled α-subunits are stable for unknown reasons. To further characterize the degradation event induced by *Xenopus* βHK assembly, we tested in a final series of experiments whether association of *Xenopus* βHK with the endogenous, stable α-subunit would promote its degradation or rather permit expression of functional Na-K pumps at the cell surface. Figure 5*A* (*lanes 1* and *2*) shows the metabolically labeled, endogenous oocyte α-subunit pool, which was stable during a 48-h chase period. Expression of either exogenous rabbit (*lanes 5* and *6*) or *Xenopus* (*lanes 3* and *4*) βHK did not destabilize the endogenous αNK pool but indeed led to a small but significant increase in the number of functional Na-K pumps at

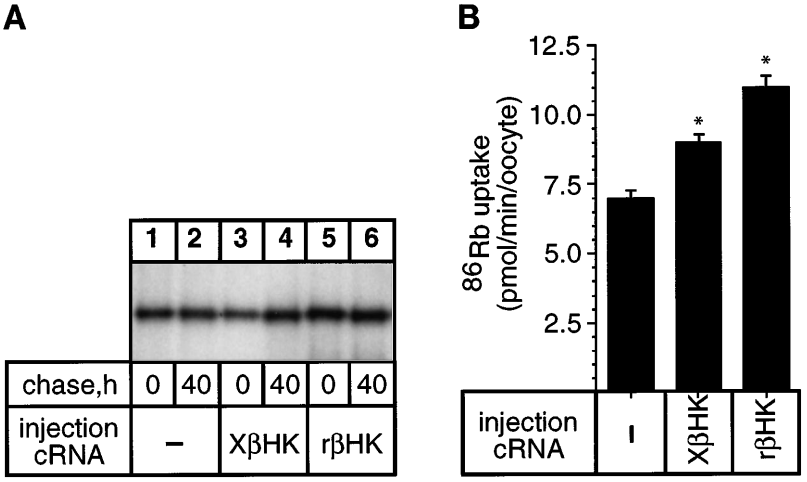

Fig. 5. Assembly and functional maturation of endogenous α-subunit with *Xenopus* βHK. Oocytes were injected or not with *Xenopus* βHK (0.25 ng) or rabbit βHK (2 ng). *A*: immunoprecipitation of endogenous α-subunits. Oocytes were labeled for 16 h and chased for 40 h. Extracts were denatured and immunoprecipitated with αNK antiserum. *B*: ^{86}Rb uptake. After cRNA injection, oocytes were incubated for 3 days before ^{86}Rb uptake measurements. Data are means ± SE (n = 20). *$P < 0.5$ vs. noninjected control oocytes.

the cell surface, as measured by ^{86}Rb uptake (Fig. 5*B*). These data indicate that *Xenopus* βHK can indeed associate with both αNK and αHK and induce their functional maturation but only if the α-subunit is in a stable form, possibly due to association with an unknown factor and/or a particular cellular localization that protects it from pre-Golgi degradation.

DISCUSSION

In this study, we document an unusual characteristic of *Xenopus* βHK expressed in *Xenopus* oocytes that may be indicative of specific mechanisms governing the selective formation or targeting of H-K-ATPase and Na-K-ATPase α-β complexes in the stomach.

Our data show that *Xenopus* βHK expressed in *Xenopus* oocytes can indeed associate with rabbit or *Xenopus* αHK and αNK but does not permit the formation of functional pumps at the cell surface due to rapid degradation of the α-β complexes. The *Xenopus* βHK behaves in this respect differently from all other β-subunits so far tested in the *Xenopus* oocyte system, including *Xenopus*, rat, and human βNK and *Bufo* nongastric βHK and rabbit gastric βHK. Although heterologous assembly between αNK and βHK or αHK and βNK was found to be less efficient than homologous assembly, β-assembly was always accompanied by a complete or at least partial stabilization of the coexpressed α-subunit and a corresponding increase in the number of functional pumps at the cell surface (for review see Ref. 6).

A possible explanation for the observation that rabbit as well as *Xenopus* αHK expressed in *Xenopus* oocytes can be stabilized and form functional H-K pumps with rabbit βHK but not with *Xenopus* βHK could involve the presence of a molecular signal in the *Xenopus* βHK, which might be important for a tissue-specific control of the stable formation or targeting of H-K-ATPase α-β complexes in the *Xenopus* stomach but which cannot be interpreted correctly in the *Xenopus* oocyte expression system. At first sight, two domains in the *Xenopus* βHK could be of interest in this respect. Analysis of the carboxy termini of βNK and βHK has revealed that the last 10 amino acids may form an amphipathic β-strand that exposes on one side a hydrophilic domain and on the other side a continuous hydrophobic domain that is important for subunit assembly (2). *Xenopus* βHK differs from all other known βHK as well as from βNK by an extension of eight amino acids, which could be necessary for a tissue-specific control of subunit assembly.

A second domain in the *Xenopus* βHK that could be involved in the particular behavior of this protein when expressed in *Xenopus* oocytes, is the cytoplasmic amino terminus. All βHK so far identified, with the exception of chicken and *Xenopus* βHK, contain a tyrosine-containing sequence in the amino terminus that is a reversed version of the motif that is responsible for transferrin receptor internalization (3). Recently, it was shown in transgenic mice that the Phe-Arg-His-Tyr motif in the mammalian βHK is necessary for endocytosis of H-K pumps and termination of acid secretion in the stomach (4). In addition, tyrosine-based motifs have been shown to be responsible for targeting to various endosomal compartments or lysosomes (for review see Ref. 22).

It is not known whether the signals that are involved in these processes are similar in amphibia or birds and in mammals, but it could be that the corresponding Phe-Arg-Arg-Phe or the Phe-Gly-Arg-Phe sequences in chicken or *Xenopus* βHK have functions similar to those of the tyrosine-based motifs in mammals. If this is the case, the signal present in *Xenopus* gastric βHK might specifically mediate targeting to the tubulovesicular structure in the *Xenopus* stomach cells. Due to the lack of these structures in *Xenopus* oocytes, the newly synthesized *Xenopus* H-K-ATPase α-β complexes might be sorted to lysosomes or another degradation compartment.

Finally, it is interesting to note that there is degradation only of assembled α-β complexes but not of *Xenopus* βHK expressed alone in *Xenopus* oocytes. This result indicates that a putative targeting signal present on *Xenopus* βHK is exposed only after the β-subunit associates with the α-subunit, due either to a conformational change or to targeting to a particular ER subcompartment that is involved in the recognition of the signal.

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