Variant AE2 Anion Exchanger Transcripts Accumulate in Multiple Cell Types in the Chicken Gastric Epithelium*

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Molecular analyses have resulted in the isolation of two chicken stomach AE2 anion exchanger cDNAs, AE2-1 and AE2-2. The ~4.3-kilobase (kb) AE2-1 cDNA contains an open reading frame that encodes a predicted polypeptide of ~135 kDa that is homologous to AE2 anion exchangers from other species. The partial ~1.7-kb AE2-2 cDNA, which differs from the AE2-1 cDNA in two regions, would be predicted to encode an AE2 polypeptide with an alternative N-terminal cytoplasmic tail. Examination of the distribution of these variant transcripts has revealed that AE2 transcripts ranging in size from ~4.4 to ~7.3 kb accumulate in various adult tissues. However, in the stomach, the unique sequence at the 5'-end of AE2-1 is preferentially associated with transcripts that range in size from ~4.5 to ~4.9 kb, while the unique sequence at the 5'-end of AE2-2 is preferentially associated with the ~7.3-kb AE2 RNA species. In situ hybridization analyses have further revealed that AE2 transcripts accumulate to very high levels within the acid-secreting epithelial cells of the profound gland in the stomach and, to a lesser extent, within the mucus-secreting cells of the superficial gland that line the stomach lumen. This result suggests that AE2 anion exchangers are involved in the regulation of intracellular pH in each of these gastric epithelial cell types.

Electroneutral anion exchange activities have also been characterized in a variety of other cell types, including gastric parietal cells (12), cardiac Purkinje fibers (13), and renal mesangial cells (14, 15). The genes that encode most of these anion exchange activities are not known. However, immunolocalization studies using AE2 anion exchanger-specific peptide antibodies have suggested that the AE2 anion exchanger mediates the basolateral chloride/bicarbonate exchange activity of the acid-secreting parietal cells of the mammalian stomach (16). The studies described here have revealed that variant chicken AE2 anion exchanger transcripts are generated by a complex pattern of alternative transcriptional initiation and differential RNA splicing. These transcripts are expressed in a wide variety of tissues including the proventriculus, the equivalent of the mammalian stomach, where they accumulate to very high levels. Examination of the cell type-specific pattern of expression of AE2 transcripts in the proventriculus has revealed that they primarily accumulate within the epithelial cells of the profound gland and, to a lesser extent, within the mucus-secreting cells that line the stomach lumen. Previous studies have suggested that the epithelial cells of the profound gland are functionally equivalent to the parietal cells of the mammalian stomach, secreting acid into the stomach lumen (17). The high level of AE2 expression observed in this acid-secreting cell type suggests that the AE2 gene encodes the basolateral anion exchanger of these cells. The detection of AE2 transcripts within the mucus-secreting cells of the stomach further suggests that AE2 anion exchangers mediate the apical bicarbonate-secreting activity that has been characterized in this epithelial cell type in other species (18).

MATERIALS AND METHODS

Isolation and Characterization of Chicken AE2 Anion Exchanger cDNAs—A λgt10 cDNA library was constructed from poly(A)⁺ RNA isolated from the proventriculus of an 18-day chicken embryo. First strand cDNA for this library was primed using oligo(dT) and a specific oligonucleotide (nucleotides 1744–1763 in Fig. 1) complementary to a sequence near the 5'-end of a previously characterized chicken kidney AE2 cDNA (8). This cDNA, which was ~3.2 kb in size, corresponded to the 3'-end of the chicken kidney AE2 transcript. Following cDNA synthesis, cDNA was size-fractionated on a low melting point agarose gel, and cDNAs greater than 1.5 kb in size were ligated into a λgt10 vector. This library was screened using the nick-translated kidney AE2 cDNA as a probe. cDNA inserts from two positive clones, AE2-1 and AE2-2, were subcloned into a pGEM-3 vector (Promega) and sequenced by the dideoxy chain termination method using specific oligonucleotides as primers.

RNA Blotting Analysis—RNA was isolated from the following adult chicken tissues by the guanidinium/CsCl method (8): perfused heart, perfused kidney, perfused liver, perfused proventriculus, perfused gizzard, perfused intestine, skeletal muscle, and brain. Following purification of poly(A)⁺ RNA by oligo(dT)-cellulose chromatography, 2 μg of poly(A)⁺ RNA from each tissue were electrophoresed on a formalde-
hyde-agarose gel and transferred to nitrocellulose. A $^{32}$P-labeled antisense RNA probe complementary to nucleotides 2783–2979 of the AE2-1 cDNA was synthesized using SP6 RNA polymerase. Alternatively, $^{32}$P-labeled antisense RNA probes complementary to nucleotides 14–278 of the AE2-1 cDNA or nucleotides 1–194 of the AE2-2 cDNA were synthesized using SP6 RNA polymerase. These probes were incubated with RNA blots as described previously (8). Hybridizing species were detected by autoradiography using an intensifying screen.

Analysis of the Structure of the Variant AE2 Transcripts by Reverse Transcription-PCR—The structure at the 5′-end of the variant AE2 transcripts was examined by the reverse transcription-polymerase chain reaction (PCR) technique. Poly(A)$^+$ RNA isolated from the embryonic chicken proventriculus of an 18-day-old chick embryo was reverse-transcribed using an oligonucleotide complementary to nucleotides 986–1006 of the AE2-1 cDNA. This first strand cDNA was PCR-amplified using sense primers corresponding to nucleotides 3–23 of the AE2-1 cDNA or nucleotides 113–133 of the AE2-2 cDNA and an antisense primer complementary to nucleotides 707–727 of the AE2-1 cDNA (nucleotides 693–713 of AE2-2). Each primer contained the sequence for a restriction endonuclease site at its 5′-end. The resulting amplification products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide, and transferred to nitrocellulose. This blot was probed with a $^{32}$P-end-labeled oligonucleotide corresponding to nucleotides 449–469 of the AE2-1 cDNA (nucleotides 378–398 of AE2-2). Alternatively, an identical blot was hybridized with a $^{32}$P-end-labeled oligonucleotide complementary to nucleotides 650–670 of the AE2-2 cDNA. This oligonucleotide corresponds to a sequence in the 57-nucleotide insert of AE2-2 that is absent in the AE2-1 cDNA (see Fig. 2B). Following washing of the filters, hybridizing species were visualized by autoradiography using an intensifying screen. The hybridizing species were digested with the appropriate restriction endonuclease and isolated on a low melting point agarose gel. The isolated DNA fragments were subcloned into a pGEM-3 vector and sequenced by the dideoxy chain termination method using specific oligonucleotides as primers.

In Situ Hybridization—The cell type-specific pattern of expression of the variant chicken AE2 transcripts was determined by a modification of the in situ hybridization protocol of Cox et al. (19). The proventriculus from a 21-day-old chicken embryo was isolated and fixed by incubation for 30 min in 4% paraformaldehyde in 136 mM NaCl, 3 mM KCl, 2 mM KH$_2$PO$_4$, and 10 mM Na$_2$HPO$_4$, pH 7.4 (PBS), at 4°C. After fixation, the tissue was rinsed in PBS and incubated overnight at 4°C in 0.5 μg sucrose in PBS. The tissue was then frozen in embedding medium, and 4-μm tissue sections were cut. The tissue sections were post-fixed in 4% paraformaldehyde in PBS for 5 min; rinsed three times in PBS for 5 min each; dehydrated by incubation through a series of 30, 60, 80, 95, and 100% ethanol; and dried under vacuum for 2 h. Following prehybridization, the sections were incubated overnight at 50°C with 1 × 10$^6$ cpm of a $^{32}$P-labeled antisense or sense RNA probe corresponding to nucleotides 14–278 of the AE2-1 cDNA, nucleotides 3671–4295 of the AE2-2 cDNA, or nucleotides 1–194 of the AE2-2 cDNA. The tissue sections were then treated with RNase for 30 min at 37°C. After RNase digestion, the sections were washed in 1 × SSC for 30 min at room temperature and in 0.1 × SSC for 1 h at 57°C and dried under vacuum. Finally, the processed slides were dipped in Kodak NTB-2 Liquid Track emulsion and exposed for 7–90 days. After developing, the pattern of AE2 expression was visualized by dark-field microscopy using a Zeiss Axioshot microscope equipped with a Dark-lite illuminator.

Hybridization signals using different RNA probes were quantitated by counting silver grains over at least 30 randomly chosen areas of the profound gland, the superficial gland, and the mucosal tissue separating these glands, as well as background grains in the emulsion. Dark-field images resulting from the hybridization with each probe were collected with a Kodak DCS 420 digital camera. These images were imported into Adobe Photoshop, and silver grains were counted. The grain densities over specific regions of the stomach represent the number of grains over a fixed area of tissue minus the number of grains over the same area of emulsion alone. Essentially identical results were obtained in two separate experiments.

RESULTS

Isolation and Characterization of Chicken AE2 cDNAs—Upon stimulation, the parietal cells of the mammalian gastric epithelium secrete H$^+$ into the lumen of the stomach (12). Intracellular pH is regulated in this acid-secreting cell type by a basolateral anion transporter, which mediates the electro-neutral exchange of internal bicarbonate for external chloride (12). Immunolocalization analyses have suggested that the AE2 anion exchanger mediates this basolateral anion exchange activity (16). To further investigate the role of the AE2 anion exchanger in regulating intracellular pH within the cells of the chicken stomach, a size-fractionated cDNA library was constructed from poly(A)$^+$ RNA isolated from the proventriculus of an 18-day-old chicken embryo. This library was screened using a previously characterized partial kidney AE2 cDNA (8) as a probe. Two positive cDNA clones were isolated, and characterization of the largest cDNA, AE2-1, revealed that it was 4339 base pairs in size. The 3′-end of AE2-1 was identical to the previously characterized kidney AE2 cDNA, and AE2-1 contained an open reading frame of 3657 nucleotides that initiated at nucleotide 233 and extended to nucleotide 3889 (Fig. 1). This open reading frame encoded a predicted polypeptide of 135,288 Da.

The second cDNA, AE2-2, was 1749 nucleotides in size, and sequence analysis revealed that the 3′-end of this partial cDNA corresponded to nucleotide 1763 of the AE2-1 cDNA. The sequence of AE2-2 was identical to AE2-1 from nucleotide 222 to its 3′-end with the exception of an insert of 57 nucleotides that initiated at nucleotide 621 of AE2-2 (Fig. 2B). This insert encodes 19 amino acids (amino acids 186–204 in AE2-2) that are absent in AE2-1 (Fig. 3). In addition, the 221 nucleotides at the 5′-end of AE2-2 were different than the 292 nucleotides at the 5′-end of AE2-1 (Fig. 2A). These results suggest that these cDNAs were derived from variant chicken AE2 transcripts that had been generated by alternative transcriptional initiation and differential RNA splicing.

Both of the variant AE2 cDNAs would be predicted to initiate translation from an AUG codon that is present in their unique 5′-sequences (Fig. 2A). The AE2-1 variant contains 20 unique amino acids at its N terminus, while the AE2-2 variant contains a larger unique sequence of 52 amino acids at its N terminus (Fig. 3). The putative translation initiation site of the AE2-1 variant is preceded immediately upstream by an in-frame stop codon (Fig. 2A).

The variant chicken AE2 anion exchangers share significant homology with AE2 polypeptides from other species. This homology is most striking in the C-terminal transmembrane domain, where the chicken AE2–1 polypeptide is ~90% identical to AE2 anion exchangers from human (20, 21), mouse (22), rat (23), and rabbit (24). This region of the predicted polypeptide possesses 10 hydrophobic stretches that may span the membrane 12–14 times (data not shown). These membrane-spanning regions are virtually identical to the homologous regions of previously characterized AE2 anion exchangers. Those substitutions that occur are primarily conservative in nature. The only region of the transmembrane domain that has significantly diverged from other AE2 anion exchangers is a putative extracellular loop that lies between the fourth and fifth hydrophobic regions (data not shown). This region also exhibits extensive variability among AE2 anion exchangers from other species (20–24). In contrast to the transmembrane domain, the N-terminal cytoplasmic domain is only ~70% identical to AE2 anion exchangers from other species. This sequence divergence is most striking at the N termini of the chicken AE2 variants, which exhibit little homology to previously characterized AE2 polypeptides (Fig. 3).

Tissue Distribution of Variant Chicken AE2 Transcripts—RNA blotting analyses have examined the tissue distribution of the variant chicken AE2 transcripts. Poly(A)$^+$ RNA isolated from adult chicken heart, kidney, liver, skeletal muscle, brain, proventriculus, gizzard, and intestine was electrophoresed on a formaldehyde-agarose gel; transferred to nitrocellulose; and probed with a $^{32}$P-labeled antisense RNA probe complementary to nucleotides 2783–2979 of the AE2-1 cDNA. At the criterion used for this analysis, this transmembrane domain probe does
not hybridize with either the chicken AE1 or AE3 anion exchanger transcripts (data not shown). This experiment revealed that multiple AE2 transcripts accumulate in the various chicken tissues. The most abundant species is a transcript of 4.4 kb, which can be most clearly seen in Fig. 4B. This 4.4-kb transcript is detected in the proventriculus (Fig. 4B), which is the equivalent of the mammalian stomach, and, to a lesser extent, in the liver (Fig. 4A). Additional transcripts, ranging in size from 4.5 to 4.9 kb, were observed in all of the tissues (Fig. 4A). Finally, a transcript of 7.3 kb was observed in the proventriculus (Fig. 4A). Longer exposure of the autoradiogram in Fig. 4 indicated that the 7.3-kb transcript accumulates in each tissue (data not shown).

To determine if the variant AE2-1 and AE2-2 cDNAs correspond to specific RNA species detected in the blotting analysis, 32P-labeled antisense RNA probes were generated that were complementary to the unique sequences at the 5'-ends of the variant cDNAs. Blotting analyses with these variant-specific probes revealed that the probe specific for AE2-1 recognizes a similar array of transcripts (Fig. 5A) as those recognized by the transmembrane domain probe. However, the AE2-1-specific probe did not recognize the major 4.4-kb proventricular transcript detected by the transmembrane domain probe (compare Figs. 4B and 5B). In contrast, the AE2-2-specific probe primarily recognized the 7.3-kb transcript present in the proventriculus (Fig. 5C). This probe also weakly hybridized to the 4.5-4.9-kb AE2 transcripts from each tissue. Longer exposure of these autoradiograms indicated that both probes recognize a 7.3-kb transcript in each tissue (data not shown).

These data indicate that the unique sequences at the 5'-ends of the AE2-1 and AE2-2 cDNAs are associated with multiple variant AE2 transcripts. However, the transcripts containing the unique sequence at the 5'-end of AE2-1 are much more abundant in each tissue we have examined than transcripts containing the unique sequence at the 5'-end of AE2-2 (Fig. 5, FIG. 1.

**Fig. 1. Nucleotide and predicted amino acid sequences of the chicken AE2-1 anion exchanger.** The nucleotide and amino acid sequences of the chicken AE2-1 anion exchanger are illustrated. This AE2 variant contains an open reading frame that initiates at nucleotide 233 and extends to nucleotide 3889.

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compare A and C). Furthermore, the alternative 5'-ends of the variant AE2 cDNAs are differentially spliced onto the different size AE2 transcripts. This is best illustrated in the proventriculus, where the unique sequence at the 5'-end of AE2-1 is preferentially spliced onto the −4.5-4.9-kb class of AE2 transcripts (Fig. 5A), while the unique sequence at the 5'-end of AE2-2 is preferentially spliced onto transcripts of −7.3 kb (Fig. 5C). In addition, AE2 transcripts in the proventriculus must possess 5'-ends in addition to those of the AE2-1 and AE2-2 cDNAs since neither the AE2-1 nor the AE2-2-specific probe recognizes the major −4.4-kb proventricular transcript.

The relative abundance of the variant AE2 transcripts varied dramatically in the different tissues. This highly regulated pattern of expression can be seen when comparing the AE2 variant composition in the different compartments of the gastrointestinal tract. The −4.4-kb AE2 transcript is very abundant in the proventriculus (Fig. 4A), while it is undetectable in the gizzard (Fig. 4A), the portion of the chicken stomach that is primarily involved in mechanical aspects of digestion, and in the intestine (Fig. 4A). In addition, the −7.3-kb transcript is the most prevalent transcript recognized by the AE2-2-specific probe in the proventriculus, while the −4.5-4.9-kb species are the most prevalent transcripts recognized by this probe in the other tissues (Fig. 5C).

Structure of the Alternatively Spliced Chicken AE2 Transcripts—The blotting analyses have shown that the unique 5'-ends of each of the variant chicken AE2 cDNAs can associate with multiple size transcripts. Reverse transcription-PCR analyses have investigated whether AE2 transcripts that initiate with each of these alternative sequences contain the 57-nucleotide insert that is present in AE2-2 and absent in AE2-1 (Fig. 2B). Poly(A)^+ RNA isolated from the proventriculus of a 1-day-old chicken was reverse-transcribed using an oligonucleotide complementary to nucleotides 986-1006 of the AE2-1.

Fig. 2. Comparison of the nucleotide sequences at the 5'-ends of the AE2-1 and AE2-2 anion exchanger cDNAs. The nucleotide sequences at the 5'-ends of the AE2-1 and AE2-2 cDNAs are illustrated in A. The putative translation initiation codons of the variant cDNAs are in boldface. The in-frame stop codon immediately preceding the translation initiation site of the AE2-1 cDNA is underlined. The sequence of the 57-nucleotide insert that is present in AE2-2 and absent in AE2-1 is illustrated in B.

Structure of the Alternatively Spliced Chicken AE2 Transcripts—The blotting analyses have shown that the unique 5'-ends of each of the variant chicken AE2 cDNAs can associate with multiple size transcripts. Reverse transcription-PCR analyses have investigated whether AE2 transcripts that initiate with each of these alternative sequences contain the 57-nucleotide insert that is present in AE2-2 and absent in AE2-1 (Fig. 2B). Poly(A)^+ RNA isolated from the proventriculus of a 1-day-old chicken was reverse-transcribed using an oligonucleotide complementary to nucleotides 986-1006 of the AE2-1.
There was no detectable hybridization of probe 2 with the unique sequence at the 5'-end of the transcribed mRNA. The 57-nucleotide insert present in the AE2 cDNA migrates species, either contain (slower migrating species) or lack (faster migrating species) the 57-nucleotide insert. The 57-nucleotide insert corresponds to a sequence that is shared by the chicken AE2-1 and AE2-2 cDNAs. Analysis of the PCR products revealed a single species when using the AE2-1-specific antisense RNA probe (lane 1) and two species when using the AE2-2-specific sense primer (lane 3). Blotting analysis of the amplification products revealed that each species hybridized with a 32P-labeled oligonucleotide that corresponds to a sequence that is shared by the AE2-1 and AE2-2 cDNAs. In addition, the larger species in the AE2-2-specific amplification hybridized with a 32P-labeled oligonucleotide that corresponds to a sequence in the unique 5'-ends of the AE2-1 cDNA (Fig. 6, lane 1) and AE2-2 cDNA (lane 3). This indicates that transcripts initiate with the unique sequence at the 5'-end of the AE2-2 cDNA (Fig. 6, lane 3) either contain (slower migrating species) or lack (faster migrating species) the 57-nucleotide insert present in the AE2-2 cDNA. In contrast, this alternatively spliced 57-nucleotide insert does not associate with transcripts initiating with the unique sequence at the 5'-end of AE2-1. Sequence analysis of the PCR amplification products supports this conclusion. The fact that the amplification products contain only those sequences that were present in the AE2-1 and AE2-2 cDNAs suggests that extensive variability must reside in other regions of the AE2 transcripts to account for the wide range in sizes (−4.4–7.3 kb) observed.

Cell Type-specific Pattern of Expression of the Chicken Stomach AE2 Transcripts—The expression of all size classes of chicken AE2 transcripts is highest in the proventriculus, the region of the chicken stomach that is primarily involved in enzymatic digestion. Unlike the mammalian stomach, which possesses secretory glands that are composed of a simple branched tubular epithelium, the proventriculus contains superficial glands, which line the lumen of the stomach and are composed of a simple branched tubular epithelium, and profound glands, which are located in the mucosal tissue beneath the superficial glands and are composed of a compound tubular epithelium. The superficial glands are primarily involved in the secretion of mucus, while the profound glands secrete both H+ and pepsinogen into the stomach lumen (17). Furthermore, in contrast to the mammalian stomach, in which acid secretion is mediated exclusively by the parietal cells of the gastric epithelium, all of the epithelial cells of the profound gland are thought to secrete both H+ and pepsinogen (17).

In situ hybridization studies have determined the cell types that are AE2 transcripts accumulate. Initial analyses employed an antisense RNA probe that was complementary to a portion of the C-terminal transmembrane domain and the 3'-untranslated region of the variant AE2-1 cDNA (nucleotides 3671–4295). Blotting analyses have indicated this antisense probe does not recognize the chicken AE1 or AE3 anion exchanger transcripts at the criterion used for the in situ studies (data not shown). 4-μm frozen tissue sections from the proventriculus of a 21-day chicken embryo were hybridized with this 32P-labeled antisense RNA, and following processing of the tissue sections, the results were visualized by dark-field microscopy (Fig. 7, A and D–F). This analysis revealed that AE2 transcripts accumulate to very high levels within the epithelial cells of the profound gland (Fig. 7, A...
and F) as well as within the epithelial cells that line the duct connecting the profound gland with the stomach lumen (Fig. 7, D–F). AE2 transcripts also accumulate at much lower levels in the mucus-secreting epithelial cells of the superficial gland (Fig. 7A). There was no detectable accumulation of AE2 transcripts in the mucosal tissue that separates these two glands, which includes the lamina propria mucosa and the inner muscularis mucosa, or in the outer muscle layer that separates the profound glands from the serosa, the outer wall of the proventriculus (Fig. 7A).

The hybridization signal obtained with this antisense RNA probe was compared with the signal obtained with a sense probe corresponding to the same region of the AE2-1 cDNA. This analysis revealed that the antisense probe exhibited grain densities >10-fold higher in the epithelial cells of the profound gland and 2.5-fold higher in the epithelial cells of the superficial gland than that observed with the control sense probe. The high level of AE2 expression in the epithelial cells of the profound gland suggests that like the mammalian AE2 anion exchanger (16), chicken AE2 anion exchangers mediate a basolateral anion exchange activity in the acid-secreting cells of the chicken stomach. In addition, the accumulation of AE2 transcripts in the mucus-secreting cells of the superficial gland suggests that AE2 anion exchangers may also mediate the apical bicarbonate-secreting activity that has been characterized in this epithelial cell type in other species (18).

Similar in situ analyses using antisense RNA probes complementary to the unique sequences at the 5'-ends of the AE2-1 and AE2-2 cDNAs have investigated the cell type-specific pattern of expression of transcripts containing these sequences. These studies have revealed that the AE2-1-specific antisense probe (Fig. 7B) exhibited a hybridization signal 3-fold higher than that observed with a control sense probe in the epithelial cells of the profound gland. However, the signal with this AE2-1-specific antisense probe in the epithelial cells of the superficial gland was identical to that observed with the control sense probe. In addition, the AE2-2-specific antisense probe (Fig. 7C) exhibited levels of hybridization similar to those of the control sense probe in the cells of the profound and superficial glands. The inability to detect transcripts containing the unique sequences of the AE2-1 and AE2-2 cDNAs in the cells of the superficial gland suggests that AE2 variants containing these sequences do not accumulate within the mucus-secreting cells of this gland. However, it is possible that the in
situ hybridization technique is not of sufficient sensitivity to detect the AE2-1 and AE2-2 variants that may be present in this cell type at low abundance. The hybridization signal observed with the variant-specific probes (Fig. 7, B and C) is much lower than that observed with the probe corresponding to a portion of the transmembrane domain and the 3'-untranslated region of the AE2-1 cDNA (Fig. 7A). This is consistent with the blotting analysis that indicated that the major prov- entricular AE2 transcript of ~4.4 kb does not hybridize with either of the variant-specific probes (Figs. 4 and 5). Similar in situ studies using probes specific for the chicken AE1 and AE3 anion exchangers have revealed that only transcripts derived from the chicken AE2 anion exchanger gene accumulate to detectable levels in the cells of the superficial and profound glands (data not shown).

**DISCUSSION**

Molecular analyses have indicated that electroneutral anion transporters are encoded by a multigene family, which includes the AE1, AE2, and AE3 anion exchangers. The widespread distribution of transcripts derived from these genes (23) suggests that these anion exchangers are important in regulating intracellular pH in a variety of cell types. Recent analyses have suggested that the electroneutral anion exchange activity that has been characterized in the basolateral membrane of mammalian gastric parietal cells is mediated by the AE2 anion exchanger (16). Physiological studies have demonstrated that upon stimulation, parietal cells secrete H⁺ across their apical membrane, thereby acidifying the lumen of the stomach. To prevent intracellular alkalization during acid secretion, the anion exchange activity localized in the basolateral membrane of parietal cells mediates the exchange of intracellular bicarbonate for extracellular chloride (12). The demonstration that polypeptides recognized by AE2-specific peptide antibodies accumulate in the basolateral membrane of gastric parietal cells in both rabbit and rat (16) strongly suggests that the AE2 anion exchanger mediates the basolateral anion exchange activity of this acid-secreting cell type. The studies presented here indicate that variant AE2 transcripts accumulate to very high levels within the epithelial cells of the chicken profound gland and, to a lesser extent, within the mucus-secreting cells of the superficial gland. These results suggest that these chicken AE2 gene products may not only mediate the basolateral anion exchange activity of the acid-secreting cells in the profound gland, but also may mediate the apical bicarbonate-secreting activity that has been characterized in the gastric mucus-secreting cells of other species (18).

In situ hybridization studies have shown that variant AE2 anion exchanger transcripts accumulate to very high levels within the acid-secreting epithelial cells of the profound gland in the chicken stomach. Higher power magnification of regions within the profound gland indicates that all of the epithelial cells lining the profound gland accumulate AE2 transcripts (data not shown). This observation suggests that each of the epithelial cells of the gland mediates acid secretion, as had been previously proposed (17). The high level of AE2 expression in the cells lining the ducts that connect the profound glands with the lumen was somewhat surprising since these epithelial cells are morphologically distinct from the epithelial cells of the profound gland (17). Furthermore, histochemical staining has shown that the epithelial cells lining these ducts secrete mucopolysaccharides similar to those secreted by the epithelial cells of the superficial glands (17). Although it is not known whether the cells of these ducts mediate acid secretion, the in situ results taken together with previous analyses indicate that they exhibit properties of both the acid-secreting and mucus-secreting cells of the stomach.

Previous immunolocalization studies failed to detect AE2 anion exchangers in the mucus-secreting cells of rabbit and rat gastric epithelia, suggesting that the apical bicarbonate-secreting activity of this cell type is not encoded by the AE2 gene in these species (16). However, the in situ data presented here have shown that AE2 transcripts accumulate in the mucus-secreting cells of the chicken superficial gland, albeit at much lower levels than detected in the profound gland. This result suggests that one or more of the variant AE2 anion exchangers mediate the apical bicarbonate-secreting activity of these cells, which has been proposed to serve a protective function by buffering the epithelial lining of the stomach against the acidic environment of the lumen. Physiological studies have shown that luminal bicarbonate transport in the stomach ranges from 2 to 20% of luminal proton transport (18). It is interesting to note that this large difference in the extent of luminal bicarbonate and proton transport is reflected by the differing levels of AE2 expression in the mucus-secreting and acid-secreting cells.

All of the chicken tissues examined accumulate multiple AE2 transcripts that are generated by a complex pattern of alternative transcriptional initiation and differential RNA splicing. Both the abundance and the repertoire of these transcripts vary dramatically between tissues. Blotting analyses have revealed similar diversity among the transcripts derived from the AE2 anion exchanger gene in rat (23) and human (20), which range in size from ~3.9 to ~4.4 kb. The studies in rat also demonstrated that individual AE2 transcripts exhibit differences in tissue-specific expression (23). However, the data presented here are the first to define the molecular basis for some of this observed heterogeneity.

The ~7.3-kb AE2 transcripts we have detected in chickens have not been observed in other species. Although a transcript of ~8 kb hybridized with a probe derived from the cytoplasmic domain of the human AE2 anion exchanger (20), this RNA species did not hybridize with a transmembrane domain probe, suggesting that it was not derived from the AE2 gene. Immunoblotting analyses have shown that polypeptides of ~145 and ~165 kDa are the primary AE2 species in the rat stomach. These species correspond to differentially glycosylated forms of the polypeptide derived from transcripts homologous to the smaller size class of chicken AE2 transcripts (~4.4–4.9 kb). Less abundant AE2 polypeptides much greater than 200 kDa in size were also detected in the rat stomach (16). Whether these higher molecular mass AE2 species result, at least in part, from translation of transcripts homologous to the ~7.3-kb chicken AE2 transcripts awaits further analysis.

The variant AE2 anion exchanger transcripts encode polypeptides with alternative N-terminal cytoplasmic tails. Similar N-terminal cytoplasmic diversity has been observed among AE1 (4–9) and AE3 (23, 26–28) anion exchangers. Recent studies have indicated that the alternative sequences at the N termini of the variant chicken erythropoietin AE1 anion exchangers are involved in targeting these variant transporters to different membrane compartments within transfected human erythrocytes (9). Additional analyses will be required to ascertain whether specific AE2 variants mediate the basolateral and apical anion exchange activities, respectively, of the acid-secreting cells of the profound gland and the bicarbonate-secreting cells of the superficial gland. However, it is tempting to speculate that the alternative N-terminal cytoplasmic sequences of the variant AE2 anion exchangers may be involved in targeting these electroneutral transporters to specific membrane domains within other epithelial cell types.

The mechanisms involved in directing plasma membrane transporters to specific membrane domains within cells are not...
well understood. One mechanism that has been proposed for restricting the distribution of membrane proteins is their association with elements of the membrane cytoskeleton (29). Consistent with this hypothesis, immunolocalization studies have shown that the rat AE1 anion exchanger colocalizes with ankyrin in the basolateral membrane of A-intercalated cells of the kidney collecting duct (10). Given the potential role of ankyrin in restricting the distribution of AE1 anion exchangers in the epithelial cells of the kidney, it is of interest that sequences that have been implicated in mediating the association of the human AE1 anion exchanger with ankyrin (30, 31) are highly homologous to regions that are conserved among all characterized AE2 anion exchangers (amino acids 398–449 and 477–485 of AE2-1). Whether these sequences are involved in mediating the association of AE2 anion exchangers with any of the ankyrin isoforms that have been characterized (32–34) is not known.

Recent data have suggested that AE2 anion exchangers do not associate with the membrane cytoskeleton via interaction with ankyrin. Immunocytochemical studies have shown that the membrane cytoskeletal elements ankyrin and fodrin do not colocalize with the AE2 anion exchanger in the epithelial cells of the choroid plexus (35). Furthermore, the murine AE2 anion exchanger, unlike the murine AE1 and AE3 anion exchangers, could not be communoprecipitated with the repeat domain of human erythroid ankyrin (ANK1) from human embryonic kidney cells cotransfected with these polypeptides (36). These results, however, do not exclude the possibility that one or more of the variant chicken AE2 anion exchangers associate with the peripheral membrane cytoskeleton through interaction with one of the multiple ankyrin isoforms that are encoded by the three ankyrin genes (32–34). Future studies will further investigate the potential role of the membrane cytoskeleton in restricting the distribution of the variant AE2 anion exchangers in the epithelial cells of the stomach.

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