# Appendix VII

## Locations of “anti-NBC3” immunoreactivity (see Section V)

The distribution of NBCn1 protein discussed in the review was disclosed using antibodies raised against an epitope at the Ct of rat NBCn1 (EDEPSKKYMDAETSL). Unusually, a rather different distribution of NBCn1 protein is indicated by the use of an antibody NBC3-C1—referred to here as “anti-NBC3”—raised against an epitope in human NBCn1 (21). The human epitope is two amino acid residues longer than the rat epitope and differs at the two (underlined) residues that are not conserved between rat and human NBCn1 (ISFEDEPRKKYVDAETSL). The reasons for the differences in their relativities remains obscure, and a direct comparison of the anti-NBCn1 and “anti-NBC3” antibodies is presented in ref. (6). In brief, anti-NBCn1 and “anti-NBC3” differ in three main respects.

[1] On western blots, anti-NBCn1 detects a ~180kDa protein that is reduced to ~140kDa by PNGaseF treatment (5), whereas anti-NBC3 detects a ~200kDa protein that is reduced to ~160kDa by PNGaseF treatment (12). Although these molecular masses are only estimates, the comparison presented in Figure 1 of ref. (6), makes it possible to discern that the anti-NBC3 reactive protein is indeed larger than the anti-NBCn1-reactive protein. The predicted molecular weight of non-glycosylated NBCn1 splice variants ranges between 123-141 kDa, favoring the results of the anti-NBCn1 antibody.

[2] Immunohistochemical studies reveal that anti-NBCn1 immunoreactivity is predominantly basolateral (3-6, 15, 18, 19, 24) whereas anti-NBC3 immunoreactivity is predominantly apical (6, 10-12, 14, 20, 21). An antibody raised against an epitope in the Nt of NBCn1 immunoreacts with a basolaterally located protein (5), favoring the results of the anti-NBCn1 antibody. Furthermore, NBCn1 is basolaterally located in the basolateral membrane of a rat parotid salivary gland cell-line (17).

[3] Anti-NBCn1 detects an abundance of NBCn1 immunoreactivity in mTAL epithelia (6, 8, 9, 11, 18, 23-25) and IMCD (15, 24) (25) but anti-NBC3 does not immunoreact with protein in the mTAL (12, 21) or the IM[ref]. The mTAL is a major site of NBCn1 expression, as determined by the detection in these tubules of a basolateral, stilbene-insensitive NCBT activity (2), NBCn1 transcripts (15), and anti–NBCn1-Nt immunoreactive protein (5). The IM is also a site of NBCn1 expression as NBCn1-derived phosphopeptides are detected by mass-spectroscopy of IMCD protein preparations (7) These observations favor the results of the anti-NBCn1 antibody.

Two observations indicate that the anti-NBC3 antibody might recognize a specific subset of NBCn1 variants. Anti-NBC3 detects NBCn1 overexpressed in HEK cells (12) and anti-NBC3 immunoreactivity is lost from the eyes of *Slc4a7* knockout mice (1). For all of the reasons mentioned above it is helpful to consider the anti-NBC3 data, that are presented below, in isolation.

*CENTRAL NERVOUS SYSTEM*

**Eye.** In the outer plexiform layer of mouse retinas, NBC3 protein is detected in the photoreceptor synaptic terminals (1, 22).

### *PERIPHERAL NERVOUS SYSTEM*

**Ear.** In the cochleas of mice, NBC3 protein is detected in spiral ligament fibrocytes-specifically in type I, type II, and type V fibrocytes (1). In the cochleas of rats, NBC3 protein is detected at the synaptic junctions between outer hair cells and efferent neurons, in hair cell stereocilia, and in the lateral membrane of the inner hair cell (22).

### *UPPER DIGESTIVE TRACT*

**Salivary gland.** NBC3 protein is detected in the submandibular glands of guinea pigs (13), humans (6), mice (14, 16), and rats (6, 16) and the parotid glands of rats and humans (6). In the SMG of mice and humans and in the parotid gland of humans, anti-NBC3 antibody labels the apical membranes of ducts and acini (6, 14). This contrasts with the findings of the anti-NBCn1 antibody that detect NBCn1 protein only in ducts and only at the basolateral membrane (6), but the NBC3 distribution does accord with a PCR analysis that detects NBCn1 transcripts in the acini and ducts (14).

### *LOWER DIGESTIVE TRACT*

**Liver and pancreas.** Anti-NBC3 antibody also recognizes protein in mouse liver and pancreas (16).

### *URINARY SYSTEM*

**Kidney.** In the kidneys of rats, anti-NBC3 immunoreactivity is stronger in the ISOM and OSOM than in the cortex and is absent from the IM (12). Apical and subapical anti-NBC3 immunoreactivity is detected in OMCD $\alpha$-intercalated cells of rabbits (21) and rats (11, 12), in CCD $\alpha$-intercalated cells of rabbits (21), rats (21), mice (10), and humans (6) and in non-$\alpha$, non-$\beta$ CNT intercalated cells of rats (10, 12). Basolateral anti-NBC3 immunoreactivity is detected in CCD $\beta$-intercalated cells of rats (12) and humans (6), in CCD non-$\alpha$-intercalated cells of mice (10), and in the CNT $\beta$-intercalated cells of rats (12).

### *REPRODUCTIVE SYSTEM*

**Male.** NBC3 protein with an apical distribution has also been detected along the epididymis of rats, most prominently in the clear cells of the caudus with an additional presence in the clear cells of the corpus and the narrow cells of the caput (20). Unpublished observations of NBC3 immunoreactivity in the apical membranes of vas deferens epithelia are cited in ref. (21).

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