Targeting of Sodium Pumps to Basolateral Membranes in Polarized Cells
- Analysis of β-subunit chimeras -

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Summary

Two sets of chimeric β-subunits were constructed from subunits of *Torpedo californica* Na^+/K^+-ATPase and pig gastric H^+/K^+-ATPase. Five unique restriction sites (*Sna*BI, *Eco*RV, *Mun*I, *Sph*I and *Eco*T22I) were created at equivalent positions of the respective cDNAs and were used as joining points for the constuction. One set of chimeras (HxN series) was made by exchanging the 5' portion of Na^+/K^+-ATPase β-subunit cDNA with the corresponding portion of the H^+/K^+-ATPase β-subunit cDNA at the respective joining point. Complementary constructs were also prepared (NxH series).

In the HxN series, the chimera joined at the *Sna*BI site formed a stable trypsin resistant complex with the Na^+/K^+-ATPase α-subunit, which was functional with respect to ATP hydrolysis and pump current generation, although the activities were less than those of the complex with the Na^+/K^+-ATPase β-subunit. Trypsin resistance decreased for the complex of the chimera joined at the *Eco*RV site. In the NxH series, the chimeras joined at the *Sna*BI site and the *Eco*RV site formed rather trypsin-resistant complexes, but the expressions of the α-subunits were below 50% of the control. The chimera joined at the *Eco*T22I site formed a complex susceptible to tryptic digestion. None of the chimeras in the NxH series were functional.

These results suggest that at least two regions of the Na^+/K^+-ATPase β-subunit (*Sna*BI site to *Eco*RV site and *Eco*T22I site to C-terminus) are involved in stable assembly with the Na^+/K^+-ATPase α-subunit and that the cytoplasmic domain (N-terminus to *Sna*BI site) is functionally replaceable with the corresponding domain of the H^+/K^+-ATPase β-subunit.