Alternately Spliced cDNAs Encode the Chicken Sarco/Endoplasmic Reticulum Slow Ca^{2+} ATPase

A. Malcolm Campbell, Paul D. Kessler, and D. M. Fambrough

Department of Biology, The Johns Hopkins University, Baltimore, Maryland 21218

Two similar forms of the cardiac/slow Ca^{2+} ATPase (SERCA2a and SERCA2b), differing in mobility on SDS-PAGE, are expressed in chicken heart and brain (Kaprielian et al., 1989). However, when the two subtypes of chicken SERCA2 were analyzed by two-dimensional peptide mapping, there was no discernible difference between Ca^{2+} ATPase isolated from brain or heart. In this study, cDNAs encoding each subtype were cloned and sequenced. Chicken SERCA2a is very similar to a variety of Ca^{2+} ATPases (Table I; MacLennan et al., 1985; Burk et al., 1989; Karin et al., 1989; Palmero and Sastre, 1989). The encoding sequence of SERCA2b is identi-

TABLE I
A Comparison of the Deduced Chicken SERCA2a Amino Acid Sequence against Four Other Ca^{2+} ATPases

	Rabbit SERCA2a	Chicken SERCA1a	Rat SERCA3	*Artemia*
Chicken SERCA2a	94%	88%	73%	72%

The numbers are the percentage of amino acid identity for each Ca^{2+} pump.

cal to SERCA2a except for the portion encoding the carboxyl terminus, where the final four amino acids of SERCA2a have been replaced with 49 amino acids. This extended carboxyl terminus of chicken SERCA2b is conserved in a variety of mammalian species (Table II; Gunteski-Hamblin et al., 1988; Lytton and MacLennan, 1988; Lytton et al., 1989). The extended terminus of SERCA2b accounts for the 5-kD shift in apparent M_r as seen on SDS-PAGE. A Kyte/Doolittle hydropathy plot analysis of the SERCA2b extended terminus suggests that SERCA2b may span the sarco/endoplasmic reticulum membrane an additional time.

Only SERCA2a mRNA is detectable in embryonic and adult heart, while SERCA2b and SERCA2a messages are expressed in adult and embryonic brain. Chicken SERCA2a- and SERCA2b-encoding cDNAs have been cloned into expression vector pRc/RSV (Invitrogen) and transfected into mouse L cells. Using a monoclonal antibody specific for chicken SERCA2 (Kaprielian and Fambrough, 1987), it appears by immunofluorescence microscopy that each Ca^{2+} ATPase subtype

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TABLE II
A Comparison of the Carboxyl-terminal 49 Amino Acids of Chicken SERCA2b against the Homologous Mammalian Termini

	Human	Rat	Rabbit
Chicken SERCA2b	38/49	37/50	35/49

The fraction is the number of identical amino acids out of the 49 or 50 residues of the extended terminus.

is expressed and correctly targeted to the endoplasmic reticulum (Fig. 1). Additional studies are in progress to characterize enzymatic differences between chicken SERCA2a and SERCA2b.

The mRNA encoding SERCA2b contains within its 3′ untranslated region the sequence encoding the carboxyl terminus of SERCA2a. Therefore, it is inferred from the nucleotide sequence of avian cDNAs that the two subtypes of chicken SERCA2 result from alternate splicing of a common primary transcript. Two different RNA splicing mechanisms (cassette type splicing or internal donor site splicing; Breitbart et al., 1987) could account for the production of mRNAs encoding both subtypes of

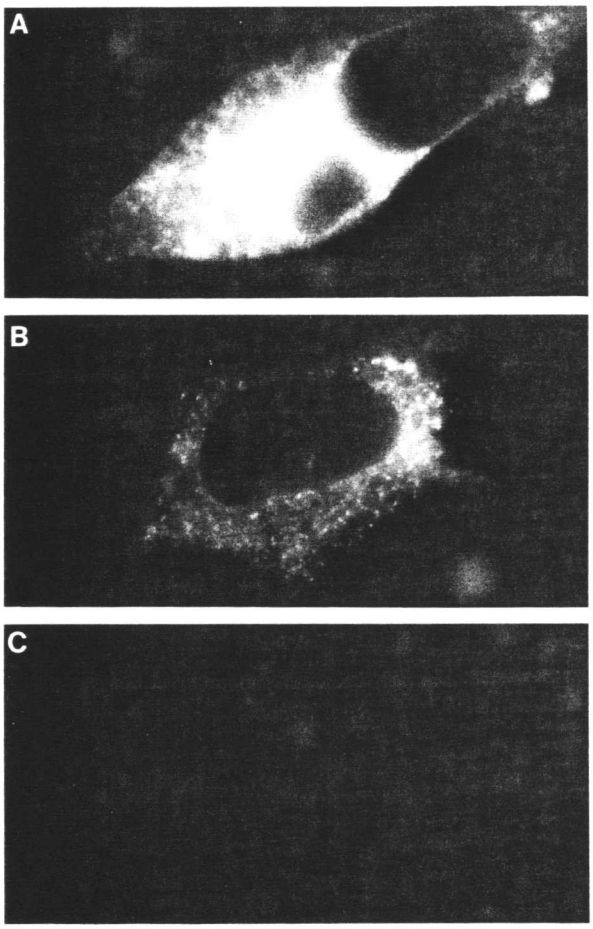

Figure 1. Mouse L cells were transfected with SERCA2a-(*A*) and SERCA2b-(*B*) encoding cDNAs cloned into the pRc/RSV (Invitrogen) expression vector or vector alone (*C*). The cells were fixed with paraformaldehyde, permeabilized with saponin, and stained with a monoclonal antibody specific for chicken SERCA2 and a rhodamine-conjugated secondary antibody.

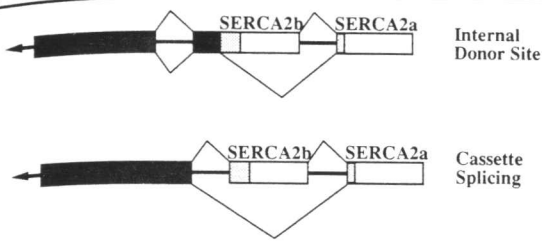

Figure 2. Diagrams depict the two possible alternate splicing mechanisms that might be utilized in chicken SERCA2 primary transcript processing. Boxes represent exons and lines are introns. The black boxes are the common coding portions of SERCA2 and the shaded boxes represent the subtype-specific carboxyl termini, while white boxes represent 3′ UT regions.

SERCA2, as shown in Fig. 2. Further analysis of chicken genomic sequence is underway to determine which of these two splicing mechanisms is utilized in chicken SERCA2 RNA processing.

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