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# Fluorescence Analysis of the SERCA:Sarcoplipin Regulatory Complex

Sarcoplasmic reticulum (SR) is an intracellular membrane system that functions to uptake, store, and release calcium during the muscle contraction/relaxation cycle. Muscle relaxation is stimulated by the sarcoplasmic reticulum calcium ATPase (SERCA), a 994-amino acid protein that transports calcium ions from the cytosol into the SR lumen using energy from ATP hydrolysis. Sarcoplipin (SLN) is a 33 residue protein that inhibits SERCA by decreasing the calcium affinity of SERCA through direct protein:protein interactions.

The structure of the SERCA:SLN regulatory complex was examined using fluorescence resonance electron transfer (FRET), a biophysical tool that measures intermolecular distance and protein complex formation. SERCA and SLN were tagged with cyan and yellow derivatives of green fluorescent protein using recombinant DNA technology. The fluorescent fusion proteins were then expressed in *Spodoptera frugiperda* (*Sf21*) insect cells using the baculovirus system. FRET spectroscopy (*in vitro*) and microscopy (*in vivo*) were used to monitor protein-protein interactions between CFP-SERCA:YFP-SLN (regulatory complex formation) and CFP-SLN:YFP-SLN (self-association).

FRET microscopy between CFP-SERCA and YFP-SLN yielded an average of 25% FRET efficiency between proteins, revealing that SERCA and SLN form a 1:1 binary heterocomplex. FRET microscopy between CFP-SLN and YFP-SLN yielded an average of 50% FRET efficiency between proteins, and data analysis indicates that SLN oligomerizes with a distance of 40 angstroms between monomer subunits. However, mutations of two SLN transmembrane residues (<sup>14</sup>Valine, <sup>17</sup>Isoleucine) decreases SLN:SLN FRET by nearly 60%, indicating that these are critical residues that stabilize the SLN oligomer. Therefore, the data suggest that SLN dissociates into monomers in order to inhibit SERCA. Further experiments are in progress to test if there is a competing equilibrium between SLN oligomerization and SERCA:SLN regulatory complex formation.

These are the first experiments to directly measure SLN oligomerization and SLN binding to SERCA *in vivo*. Results from these experiments will help determine the mode of functional interaction between SLN and SERCA. In addition, the experiments may provide new insights into research pertaining to the manipulation of SERCA: To increase SR calcium uptake and thereby increase contractility in human muscle diseases.

