SER and Spine Apparatus Identification

The purpose of this protocol is to assist with the identification of SER and spine apparatus in serial section electron microscopy.

Smooth Endoplasmic Reticulum (SER)

The primary criteria for identifying SER:

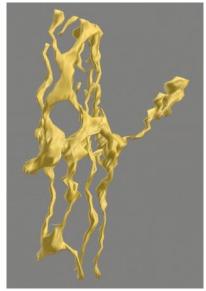
- **SER is continuous** (anastomosing throughout the dendrite) -- although the continuity can sometimes be very tenuous and unclear.
- SER is variable in shape and appearance (SER can look like a grey smudge, a tiny black dot, a snake-like black line, or large vesicle-like lakes -- from snake-like to lake-like).

Here is a snake-like tubule of SER entering a spine:



Spacek & Harris, 1997. Fig. 2c.

Here is a 3D reconstruction of SER demonstrating its continuity:



Spacek, Atlas of Ultrastructural Neurocytology, 1.1.2.14.

The best way to trace SER is to pick one thread and follow it as far as you can; then go back to the starting section and follow another thread as far as you can. If you simply trace all of section 1, then move to trace all of section 2, etc, then you will miss the tenuous connections and you might trace other objects that look like SER but are not connected to other SER.

SER can easily be confused with various endosomes, so read <u>2002 Cooney et al.</u> to familiarize yourself with the shapes of various endosomes. The clearest way to distinguish SER from endosomes:

- Endosomes are individual, discrete, having a beginning and an end.
- Endosomes are more consistent in their shape and appearance and are unlikely to vary from snake-like to lake-like.

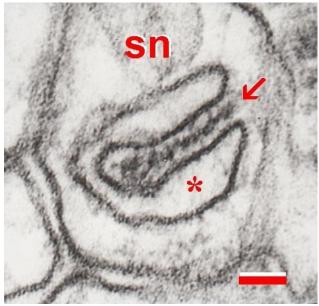
For example, an endosomal tubule will usually have a consistent width, a cigar-like shape, and will have a clear beginning and end, being a single object. SER, in contrast, will have variable width and will connect with other SER.

When a single thread of SER is cut cross-sectioned, it can look like a grey smudge, a ribosome-like black dot, a glycogen granule-like black circle, or it can seemingly disappear for a couple sections. The thread of SER entering spines can appear extremely tenuous and almost non-existent.

Spine Apparatus (SA)

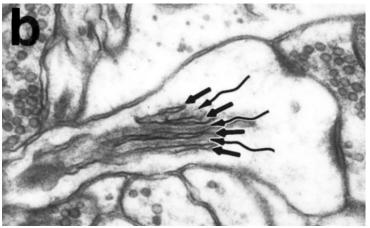
The spine apparatus is a complex derivative of the SER, commonly seen in large mushroom spines with a perforated synapse. Despite the name, the spine apparatus can also appear in the dendritic shaft (aka the "shapparatus" or "shaftaratus" in the Harris Lab Lexicon).

The most basic appearance that can still be considered a spine apparatus is 2 flat cisternae with a single dense plate in between. Even if this only appears on one section, it can be stamped as a spine apparatus. Here is an example of the most basic unit of a spine apparatus:



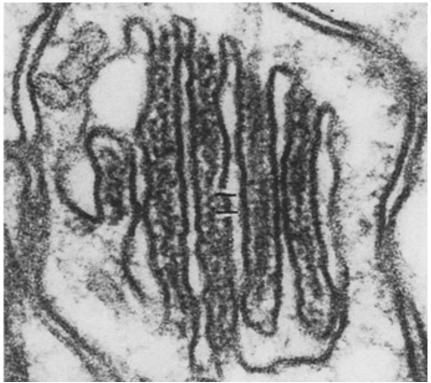
Spacek, Atlas of Ultrastructural Neurocytology, 1.4.1.47. The simplest form of spine apparatus in a dendritic spine neck (sn). Inner dense plate (arrow) is interposed between two U-shaped cisterns of endoplasmic reticulum (asterisk).

The clearest and most unmistakable appearance of the spine apparatus shows multiple flat cisternae with dark dense plates in between them:



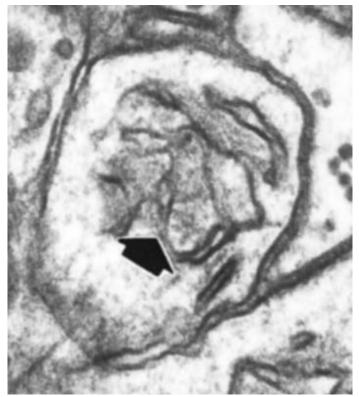
Spacek & Harris, 1997. Fig. 3b.

Here is another clear example of the flat cisternae with dark dense plates:



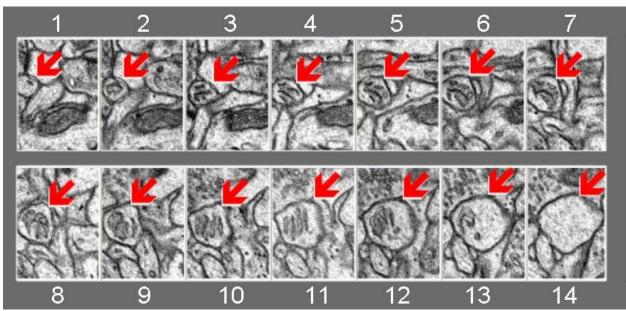
Spacek, 1985. Fig. 10.

However, sometimes, it is not so easy to identify:



Spacek & Harris, 1997. Fig. 3c.

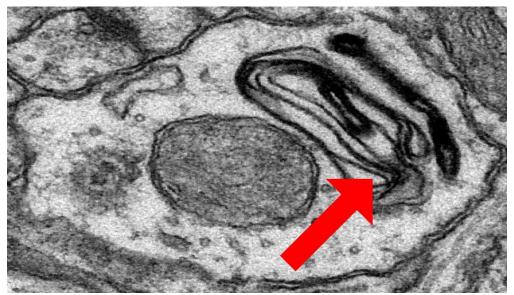
Here is the spine apparatus in serial section as it emerges from a single strand of SER:



Spacek, Atlas of Ultrastructural Neurocytology, 1.4.1.36.

The spine apparatus can also appear as a black smudge (presumably when the dark dense plate is viewed *en face*). When the spine apparatus is difficult to identify, determine whether it connects with SER. An amorphous vesicular clump could look somewhat similar, but that has its own appearance and it would not connect with SER.

Note that a whorl could possibly confuse someone -- be familiar with what whorls look like (see <u>Kulik et al. 2019</u>). Whorls are typically huge objects in the dendritic shaft, with an invagination point. They are much larger than a shaft apparatus ever would be. Here's an example of a whorl:



Whorl (red arrow) in a dendritic shaft, to the right above the mitochondrion (XRZCT_TEM D17).

References:

Smooth Endoplasmic Reticulum in Dendrites tutorial. SynapseWeb.

Spacek J. Spine Apparatus tutorial. SynapseWeb.

Spacek J. <u>Endoplasmic Reticulum</u>, <u>1.1.2.11</u>, <u>1.1.2.14</u>, etc. Atlas of Ultrastructural Neurocytology, SynapseWeb.

Spacek J. Spine Apparatus, <u>1.4.1.03</u>, <u>1.4.1.18</u>, <u>1.4.1.21</u>, <u>1.4.1.26</u>, <u>1.4.1.27</u>, <u>1.4.1.36</u>, <u>1.4.1.47</u>, etc. Atlas of Ultrastructural Neurocytology, SynapseWeb.

Spacek J, Harris KM (1997) Three-dimensional organization of smooth endoplasmic reticulum in hippocampal CA1 dendrites and dendritic spines of the immature and mature rat. J. Neurosci. 17:190-203. (PDF)

Spacek J (1985) Three-dimensional analysis of dendritic spines. II. Spine apparatus and other cytoplasmic components. Anat. Embryol. 171:235-243. (PDF)
