The Tandem: Telocytes - Stem Cells

Lawrence M. Popescu

***Abstract* -** We have recently described a novel type of interstitial (stromal) cells – **Telocytes** (**TC**) – in several cavitary and non-cavitary organs from humans and other mammalians. **TC** have a small cell body, but specific (unique) prolongations that we named **Telopodes** (**Tp**). Therefore, the simplest definition for **TC** is: cells with **Tp**. **Tp** are characterized by: **a)** *number* (1-5/cell, frequently 2 or 3); **b)** *length* (tens up to hundreds of μm); **c)** *moniliform aspect* – an alternation of thin segments, *podomeres* (with caliber under 200 nm, below the resolving power of light microscopy) and dilated segments, *podoms*, which accommodate mitochondria, (rough) endoplasmic-reticulum and caveolae, the so-called "*Ca^{2+} uptake/release units*"; **d)** *dichotomous branching* pattern forming a 3D network, a labyrinthine system with complex homo- and heterocellular junctions, revealed only by *electron tomography*. Significantly, **TC** (especially **Tp**) release shed vesicles and exosomes, sending macromolecular signals to neighbor cells, thus modifying their transcriptional activity, eventually. The lenght and ramifications of **Tp** together with the intercellular junctions and the releasing of shed vesicles or exosomes suggest an essential role of **TC** in *intercellular signaling* and coordination. Noteworthy, at least in some organs (e.g. heart and lungs) **TC** and stem cells **(SC)** are located in *tandem* within the so called stem cell niches, where **Tp** surround stem cells (**SC**). **TC** heterocellular contacts, as well as the impulsion of shed vesicles assure **TC - SC** *sinergy*. Presumably, **TC** "nurse" **SC** in stem cell niches.

***Keywords* -** Telocytes, Telopodes, Stem cells, Stem-cell niches, Shed vesicles / Exosomes, Electron tomography

I. RATIONALE FOR THE TERM "TELOCYTE"

During the last 5 years we described a new type of cell which became known as "Intersitial Cajal-Like Cells" (acronym - ICLC). We named these cells ICLC because they (apparently) seemed similar, at first glance, with the canonical gastrointestinal interstitial cells of Cajal (ICC). However, little by little, it became clear that the ultrastructure of ICLC was (completely) different from that of ICC, and that the difference between these cells was not only semantic, as they have different ultrastructure and immunophenotype, and therefore should be functionally distinct [1]. Hence, we coined the terms Telocyte (**TC**) - for these cells, and Telopodes (**Tp**) [1] for their extremely long but thin prolongations [1-7] in order to prevent further confusion with other interstitial (stromal) cells (*e.g.*, fibroblast, fibroblast-like cells, myofibroblast, mesenchymal cells) (see Figs. 1-8). The concept of **TC** was promptly addopted by other Laboratories [8-14].

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II. TELOCYTES AND/OR FIBROBLASTS ?

The interstitium (stroma) is in most of the cases seen as a connecting "device" for the specific structures of an organ. Usually, people are perceiving interstitial cells as being mainly (or even, only) fibroblasts. However, fibroblasts have the function of generating connective tissue matrix, specifically, collagen. The distinction between **TC** and fibroblasts is obvious since they have different ultrastructure and phenotype. Therefore, their functions should be mostly different: **TC** - intercellular signalling (connections), but fibroblasts - collagen synthesis. In other words, **TC** are "more" functionally oriented, and fibroblasts are "more" structuraly oriented, responsible for fibrosis.

There are some clear ultrastructural features that differentiate **TC** from fibroblasts. For instance, the general aspect of **TC** is of a small oval(piriform/spindle /triangular/stellate)-shaped cellular body, containing a nucleus surrounded by a small amount of cytoplasm. Anyway, the shape of the cell body depends on the number of **Tp**. Fibrobast cell body is pleiomorphic (phenotype heterogeneity ?). **TC** cellular body average dimensions are, as measured on EM images, 9.3 μm ± 3.2 μm (min. 6.3 μm; max. 16.4 μm). Fibroblast nucleus is tipycally euchromatic, but **TC** nucleus is mostly heterochromatic. Mitochondria represent only 2% of cell body volume and the Golgi complex is small in **TC**. Fibroblasts Golgi complex is prominent and the rough endoplasmic reticulum is very well devloped (usually 5-12%) of cell volume.

Since we are thinking that telopodes are distinctive for telocytes, we would like to emphasize at least the following characteristics:

(1) *Number*: 1–5, frequently only 2–3 telopodes are observed on a single section, depending on site and angle of section, since their 3D convolutions prevent them to be observed at their full length in a 2D very thin section;

(2) *Length*: tens – up to hundreds of μm, as measured on EM images (*e.g.* Figs. 2-10). However, under favourable conditions in cell cultures, their entire length can be captured in several successive images (Fig. 1);

(3) *Thickness*: uneven calibre, mostly below 0.2 μm (below the resolving power of light microscopy), visible under electron microscopy; moniliform aspect: *podoms* and *podomeres*; average caliber of podomeres: 0.1 μm ± 0.05μm, min. = 0.003 μm; max. = 0.24 μm;

(4) *Podoms* accommodate: mitochondria, (rough) endoplasmic reticulum, caveolae, a trio called '*Ca^{2+}-uptake/release units*'.

(5) *Branching*, with a *dichotomous pattern*;

(6) Organization in a labyrinthine system forming a *3D network* anchored by *hetero-* and *homocellular junctions*.

III. SUMMARY

We have presented here visual evidence (electron microscopy, electron tomography, phase-contrast microscopy) for the ex-

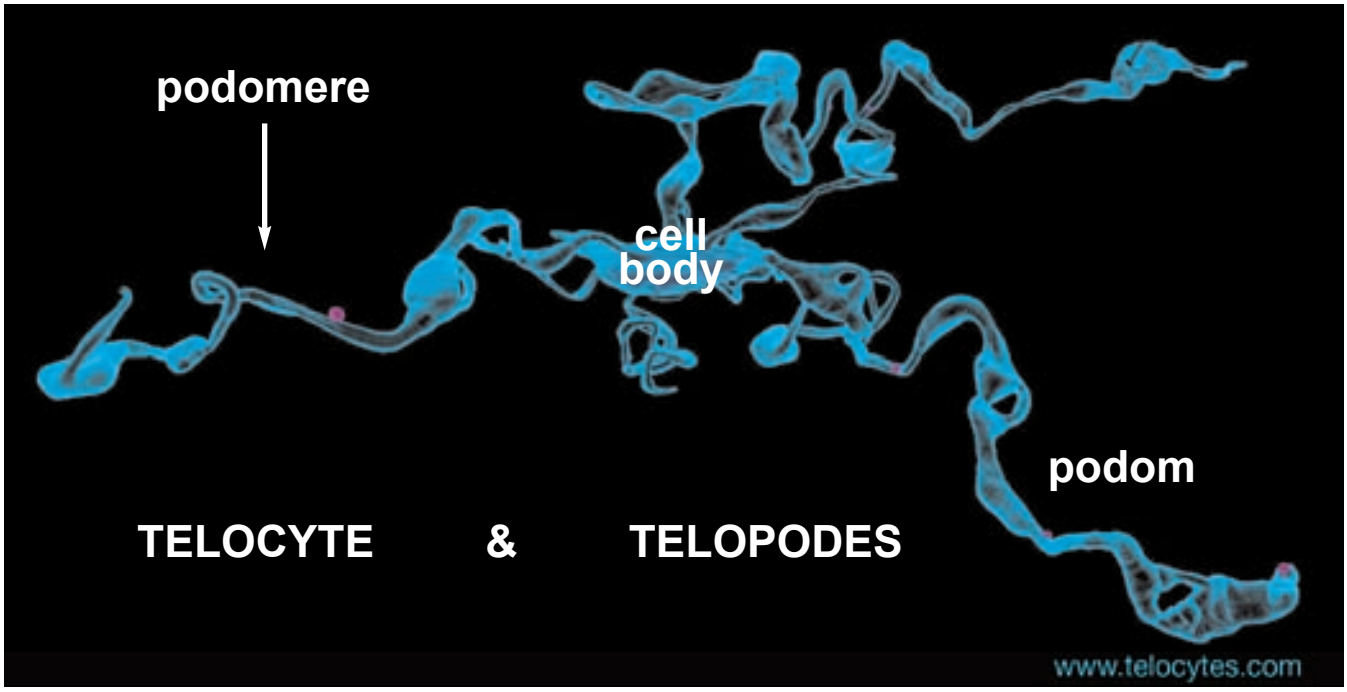


Fig. 1 Computer simulation of a 3D representation of a TC outside the tissular environment. Note a small cel body and 5 very long and convoluted Tp. Tp consist an alternation of thin segments - podomeres - and thick segments - podoms. Also note the shed vesicles (violet colored) emerging from the surface of Tp.

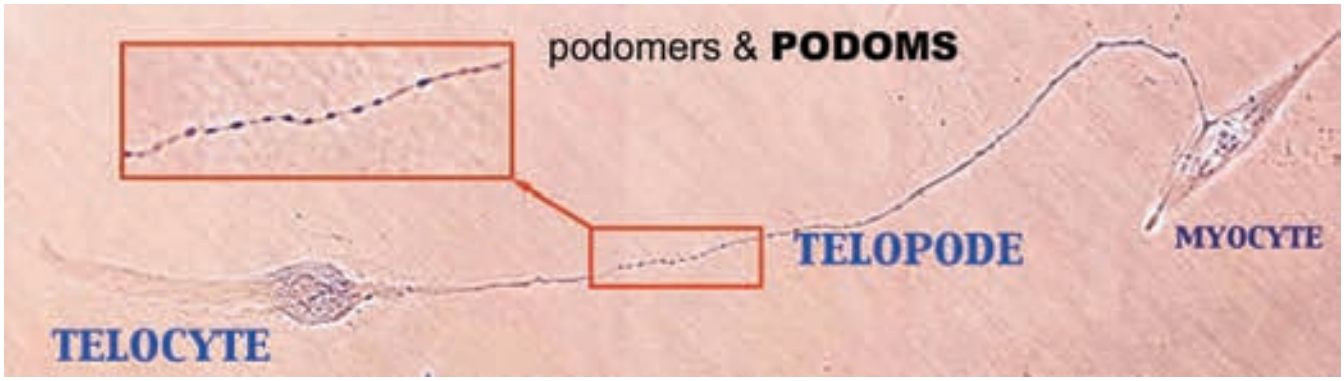
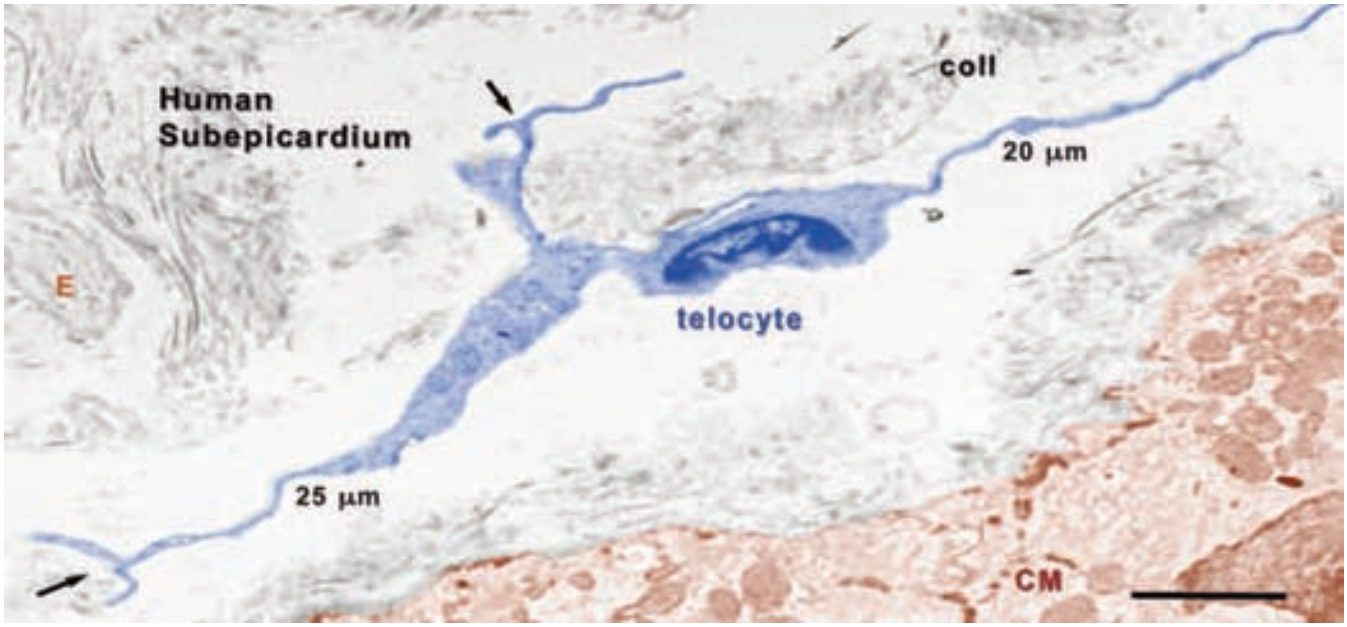


Fig. 2 Human non-pregnant myometrium in cell culture; day 3; the first passage. Giemsa staining. One **TC** establishing contacts with a myocyte by a **Tp** of about 65 μm long. Photographic composition of 4 serial phase contrast images; original magnification 40x. In red rectangles, a higher magnification clearly shows the moniliform aspect; at least 40 specific dilations (podoms) interconnected by thin segments (podomeres) are visible in a 'bead-like' fashion. Reproduced with permission from [1].



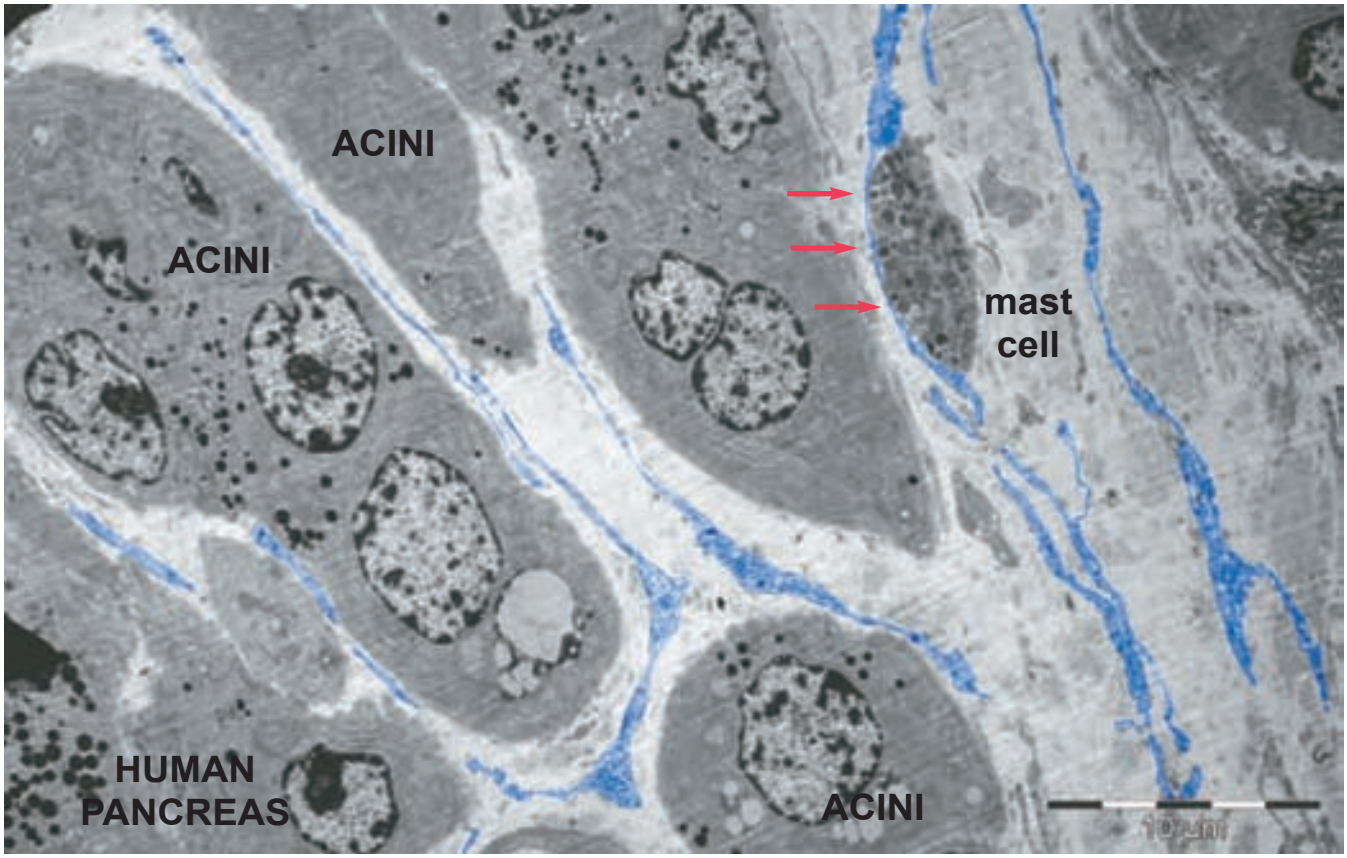


Fig. 4 Human exocrine pancreas. **TC** (blue) form with their typical **Tp** a network around acini. Note the stromal synapse (red arrows) between a mast cell and the **Tp** of a **TC**.

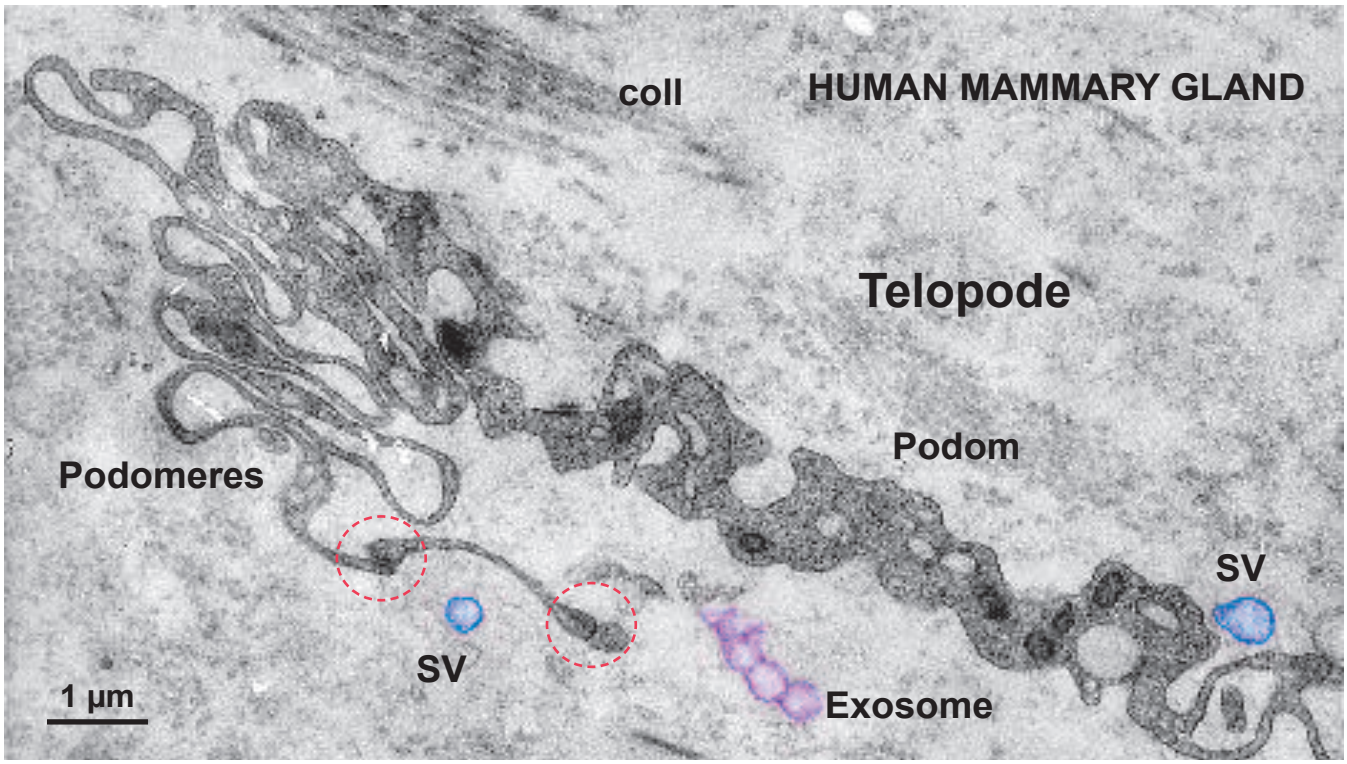


Fig. 5 Human resting mammary gland stroma. One **TC** hallmark, namely **Tp**, appears very long and convoluted. Note homocellular junctions marked by red circles, as well as shed vesicles (blue) and an exosome (violet). Reproduced with permission from [15].

Fig. 3 Digitally coloured TEM image shows **TC** (blue) in human subepicardium, bordering the peripheral cardiomyocytes (CM, highlighted in brown). The **TC** has three telopodes, illustrating: a) the distinctive dichotomous pattern of branching (arrows); b) **Tp** are very thin at the emergence of the cell body; c) alternating *podoms* and *podomeres*. Note that *some portions of podomeres have the same thickness as collagen fibrills*, which make them impossible to be observed under light microscopy. E - elastin Scale bar - 2 µm

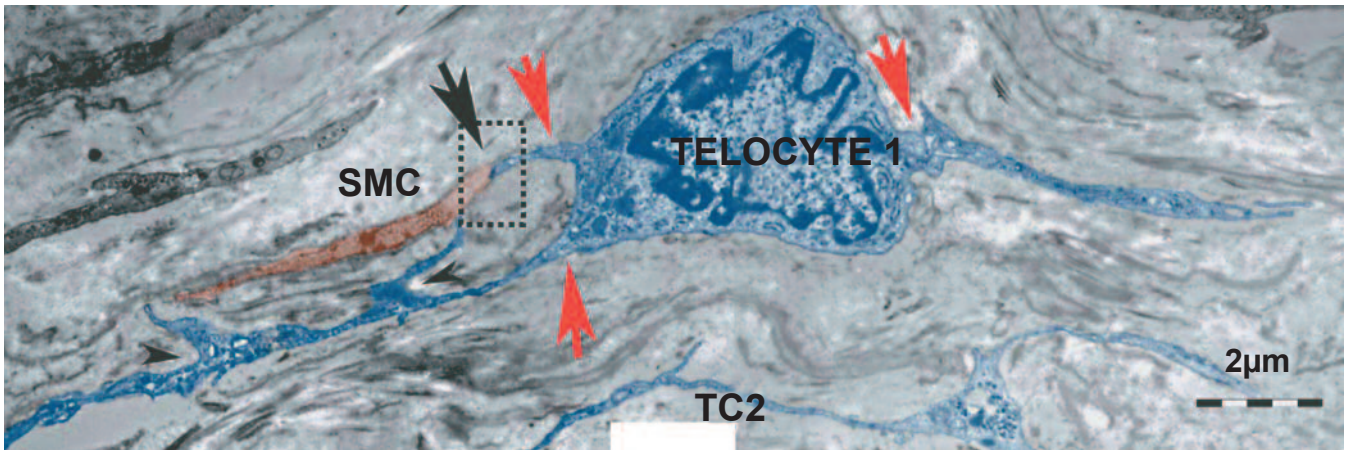


Fig. 6 Human term placenta. The **TC** (blue) has few organelles in the perinuclear area and 3 emerging **Tp** (red arrows); black arrowheads mark the dichotomic branching points. Note the *podoms* and *podomeres*. Black arrow points the junction between a **Tp** and a smooth muscle cell (SMC, colored in brown).

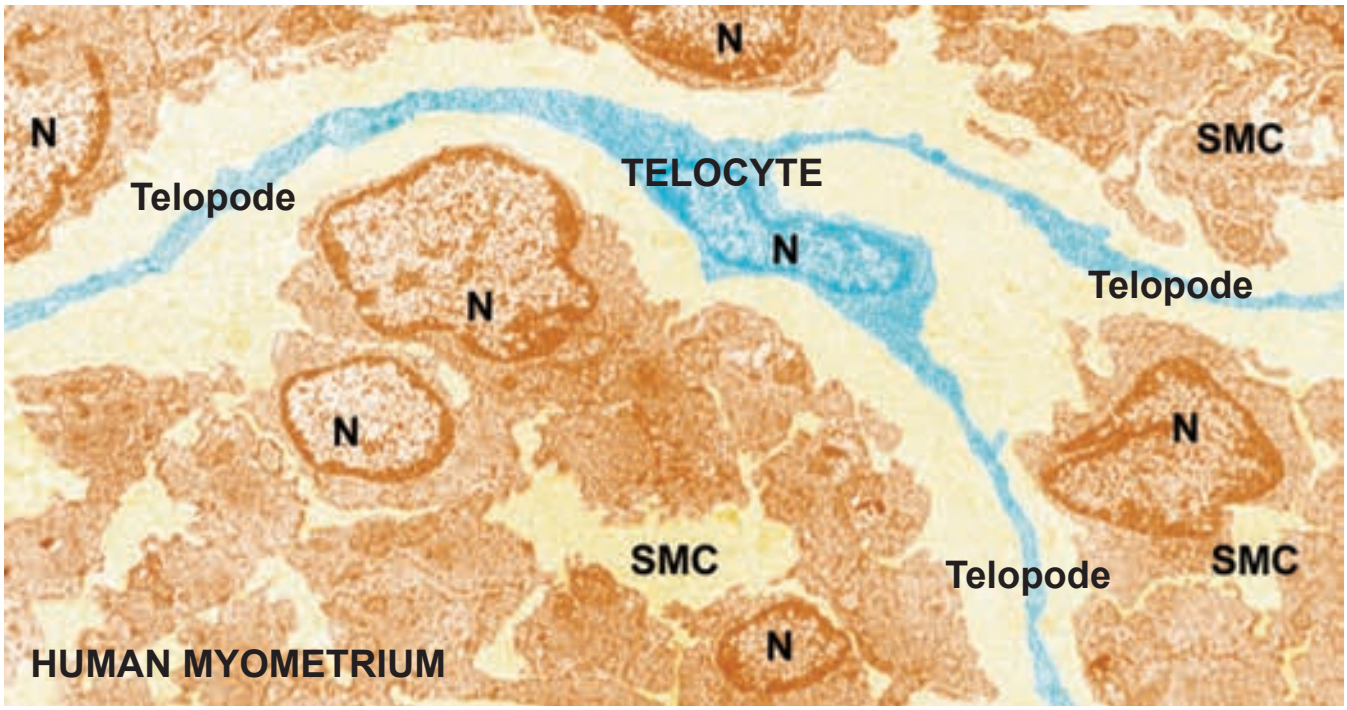


Fig. 7..Non-pregnant myometrium. Digitally colored **TC** (blue) with 3 **Tp** that encircle bundles of cross-cut smooth muscle cells (SMC, Sienna brown); N - nuclei. Reproduced with permission from [1].

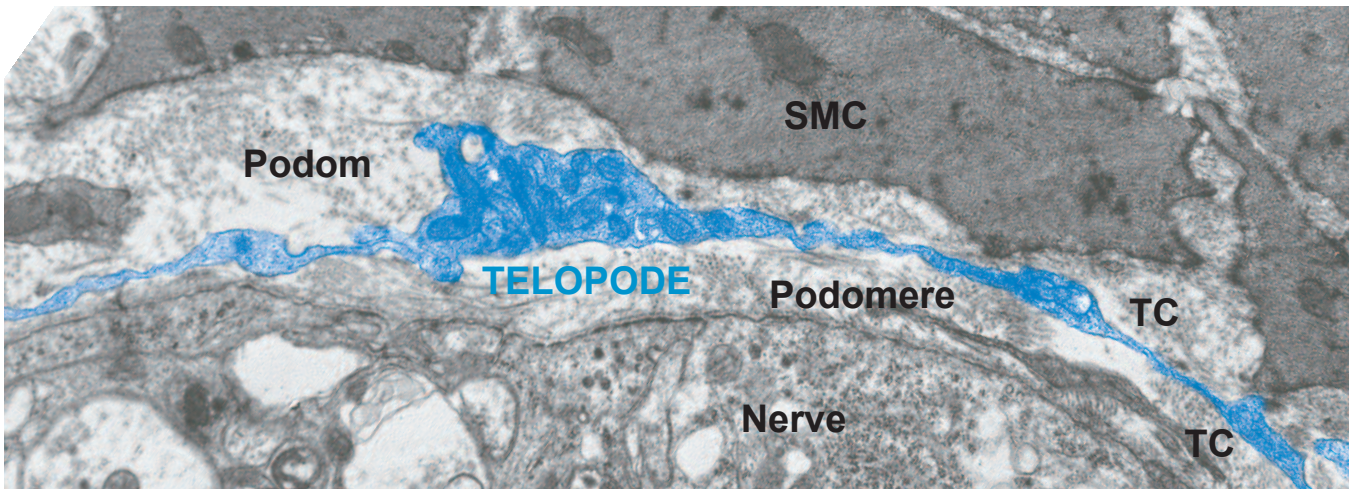


Fig. 8 Rat jejunum. A typical **Tp** (blue) located between smooth muscle cells (SMC) and nerve endings. Note a large *podom* and the corresponding *podomeres*. **TC** body is not captured in the image.

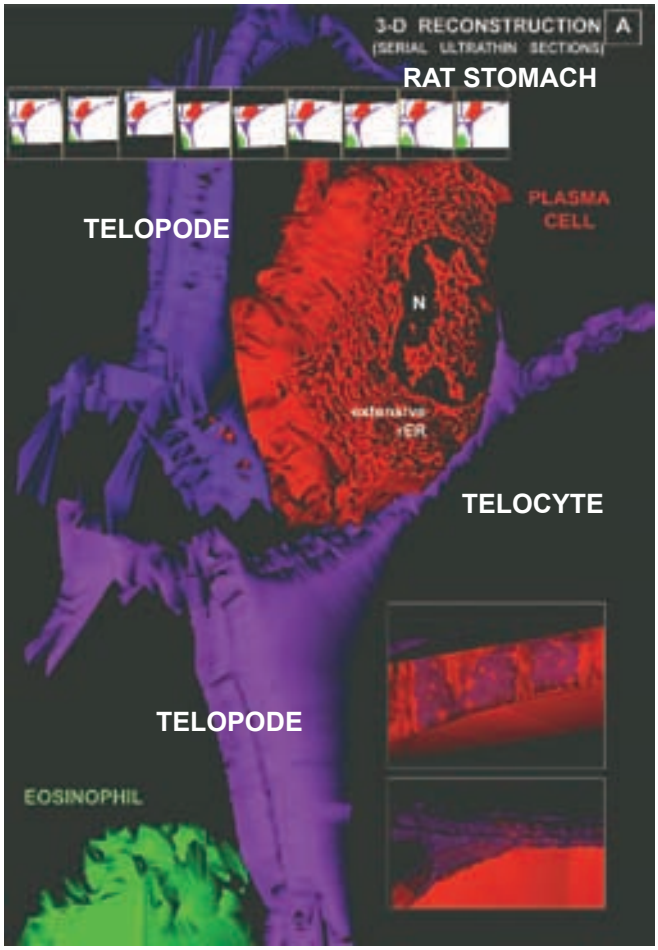


Fig. 9 Rat stomach, multicontact *stromal synapses* between two **TC**, a plasma cell and an eosinophil, respectively. 3-D image computer-aided reconstruction from 9 serial ultrathin sections; original magnification 1,500x. The upper inset shows contact points where the distance between both cell membranes (**Tp** membrane and plasma-cell membrane) is 15 nm or less (in violet), seen from the plasma cell cytoplasm. In the lower inset **Tp** were rendered transperent in order to depict the same synapse. Reproduced with permission from [16].

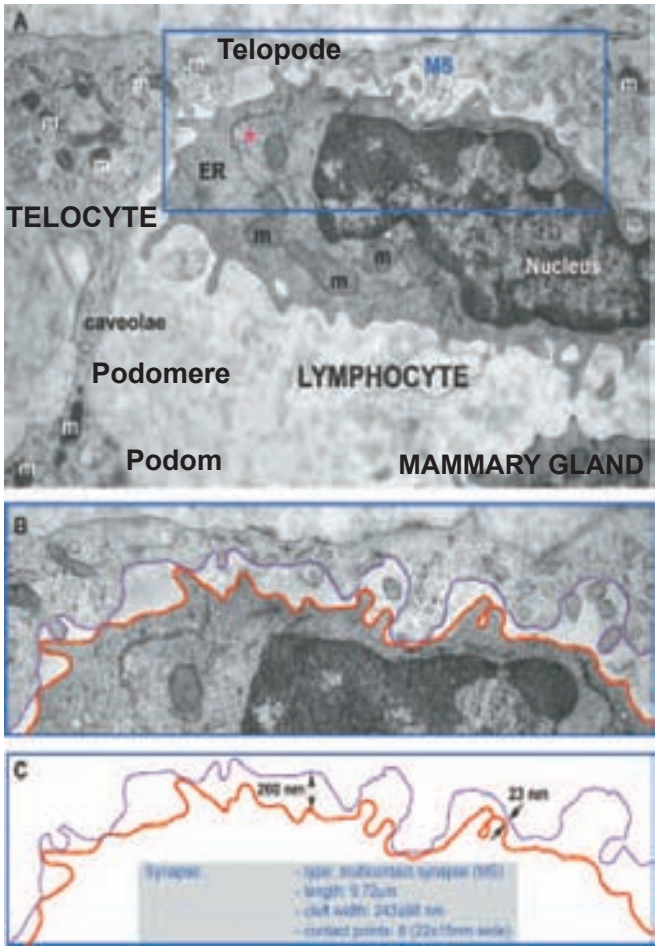


Fig. 10 Human mammary gland stroma: TEM; original magnification 9,100x. **A**: Lymphocyte establishing a multicontact synapse (MS) with a **TC**. The blue rectangle shows the synaptic 'kiss and run' region. The synaptic membranes appear traced in **B** (violet - **TC**, orange - lymphocyte). The distances between membranes are shown in **C**. Note (asterisk) a peculiar conformation of ER connecting mitochondria with the cell surface, suggestive for a possible role in synaptic Ca^{2+} homeostasis. Reproduced with permission from [16].

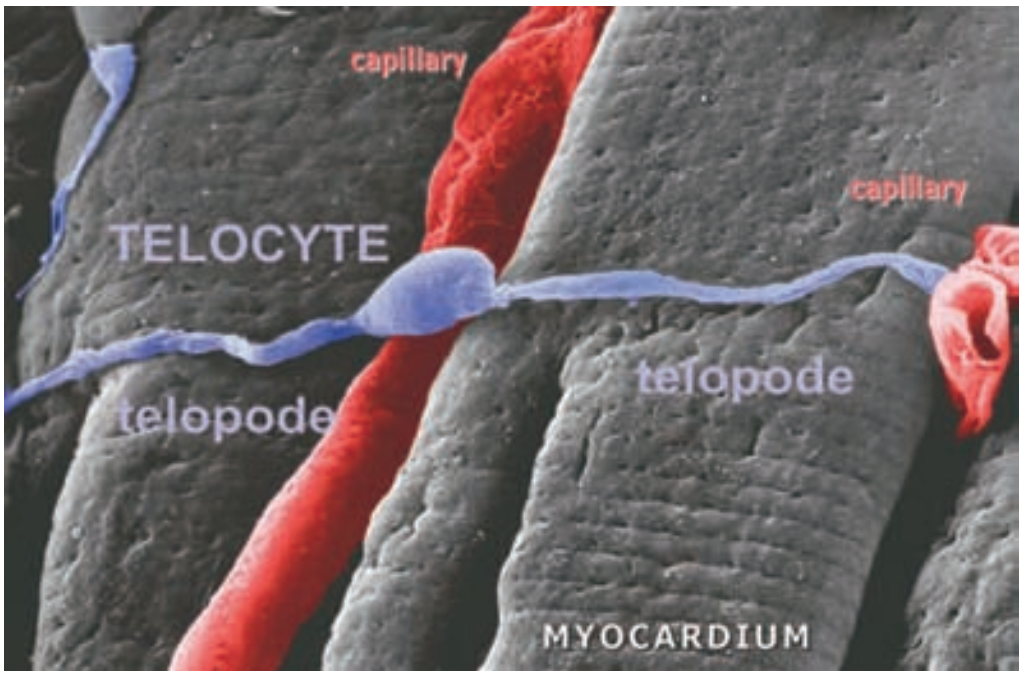


Fig. 11 Scanning electron micrograph of monkey left ventricular myocardium. A typical **TC** is located across the cardiomyocytes, in close contacts with blood capillaries. Note, the cardiomyocytes striations and the openings of T tubules. Reproduced with permission from [1].

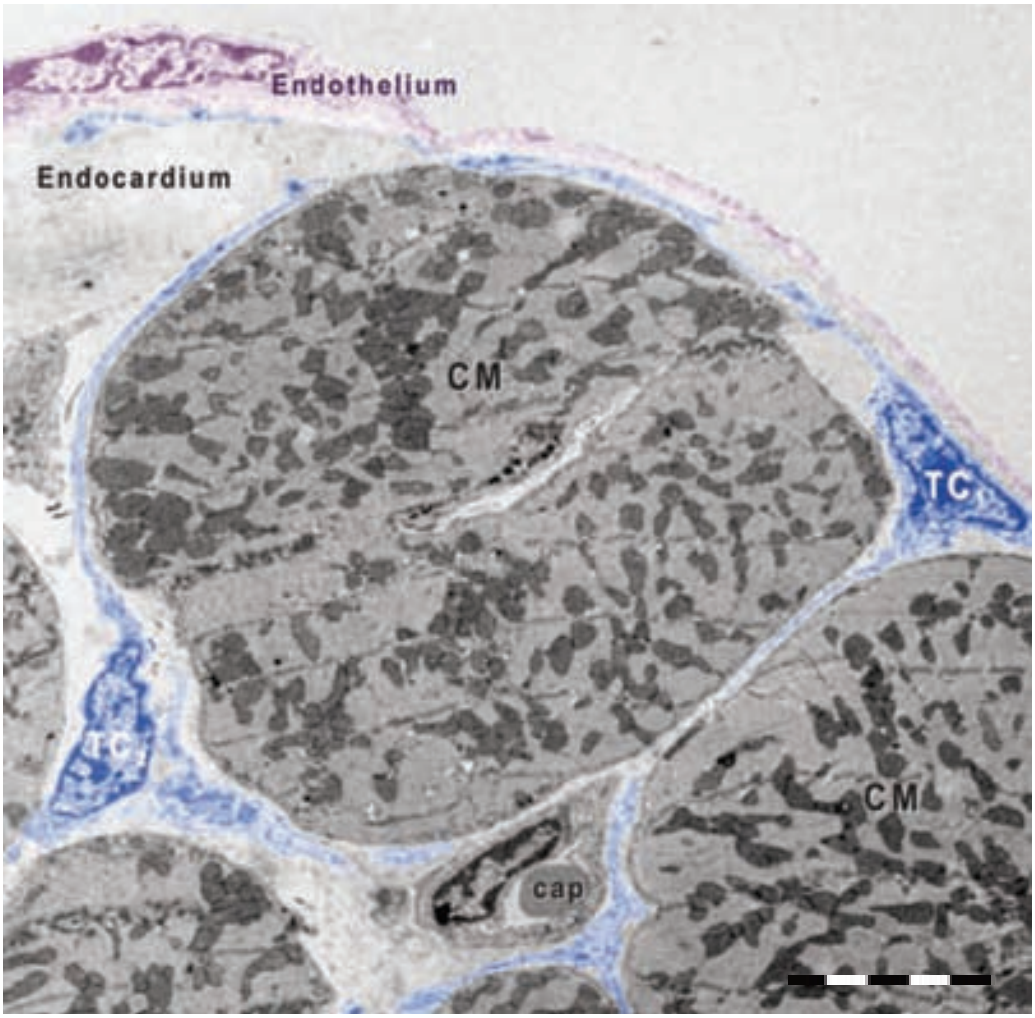


Fig. 12 Digitally coloured electron micrograph of mouse ventricular endocardium (burgundy). **TC** (blue) make an interstitial network in the heart. Subendocardial telocytes ($\mathbf{TC_1}$) sends **Tp** between cardiomyocytes (CM) and communicate with $\mathbf{TC_2}$. Cap, blood capillary. Scale bar 5µm. Reproduced with permission from [4].

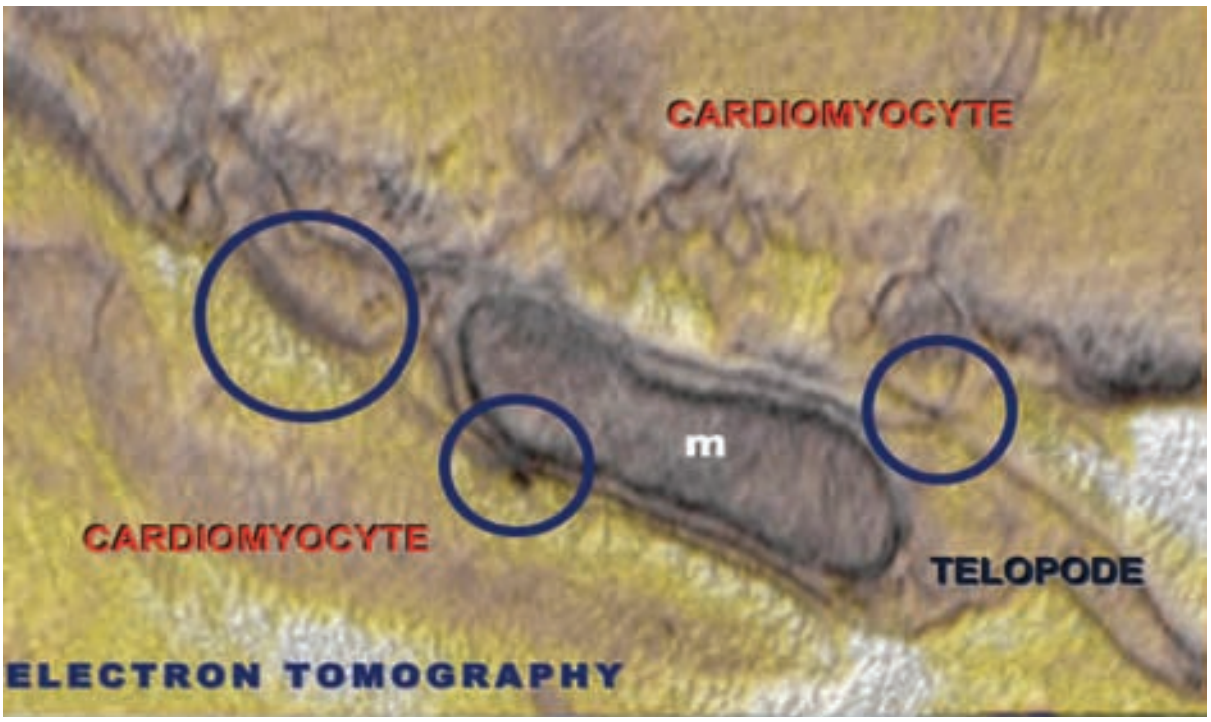


Fig. 13 This electron tomography (thick section of about 300 nm) shows nanostructures connecting the **TC** and cardiomyocytes in adult mouse heart. The bridging structures (encircled) have 10-15 nm and suggest a molecular interaction between the **Tp** of one **TC** and the two adjacent cardiomyocytes. The dilated segment of **Tp** involved in the heterocellular connection (podom) - contains a mitochondrion (m).

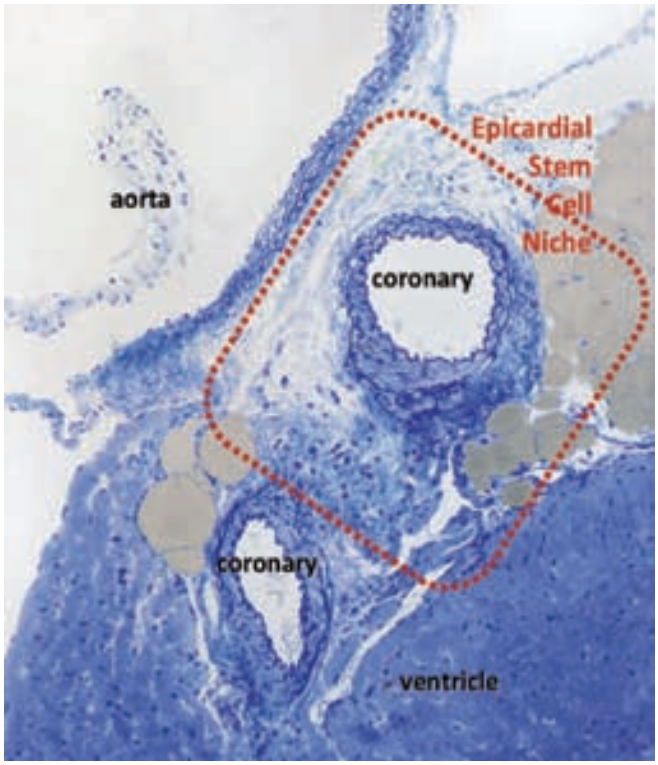


Fig. 14 High resolution light microscopy on toluidine blue stained semithin section (~1 μm thick ultramicrotome section) of Epon-embedded mouse heart (6 month old) shows the limited space where cardiomyocyte progenitors have been found by electron microscopy. Cardiac stem cell niche is located in the subepicardial area surrounding coronary artery next to the emergence from aorta (red rectangle). EM revealed a dozen of cell types, including **TC** as well as cardiomyocyte progenitors and precursors. Reproduced with permission from [6].

istence of **Telocytes** (**TC**) in many organs from human and rodents. **TC** and **Tp** and also *podoms* and *podomeres* were found in:

- **cavitary organs**:
  - heart (endo-, myo-, and pericardium);
  - stomach and intestine, with mesentery;
  - gallblader [15];
  - uterus and Fallopian tube [16];
- **non-cavitary organs**:
  - lungs and pleura;
  - pancreas (exocrine);
  - mammary gland;
  - placenta;

Anyway, recent evidence shows the involvement of **TC** in pathology [19].

TC are strategically located in between blood vessels (capillaries), nerve endings and the specific resident cell population(s) of a given organ. **TC** establish via **Tp** homo- and heterocellular junctions and release shed vesicles and exosomes. Thus, **TC** could be "Le Violin d'Ingres" that locally orchestrate the intercellular "concerto".

4 PERSPECTIVES: REGENERATIVE MEDICINE

TC and **SC** make a *tandem* (due to specific intercellular junctions) within the so called **SC** *niches*, at least in heart [20] and lungs. Hence, **TC** could be key-players in regenerating and repair of some organs. The **tandem TC-SC** could be a better option fot therapy rather than **SC** alone.

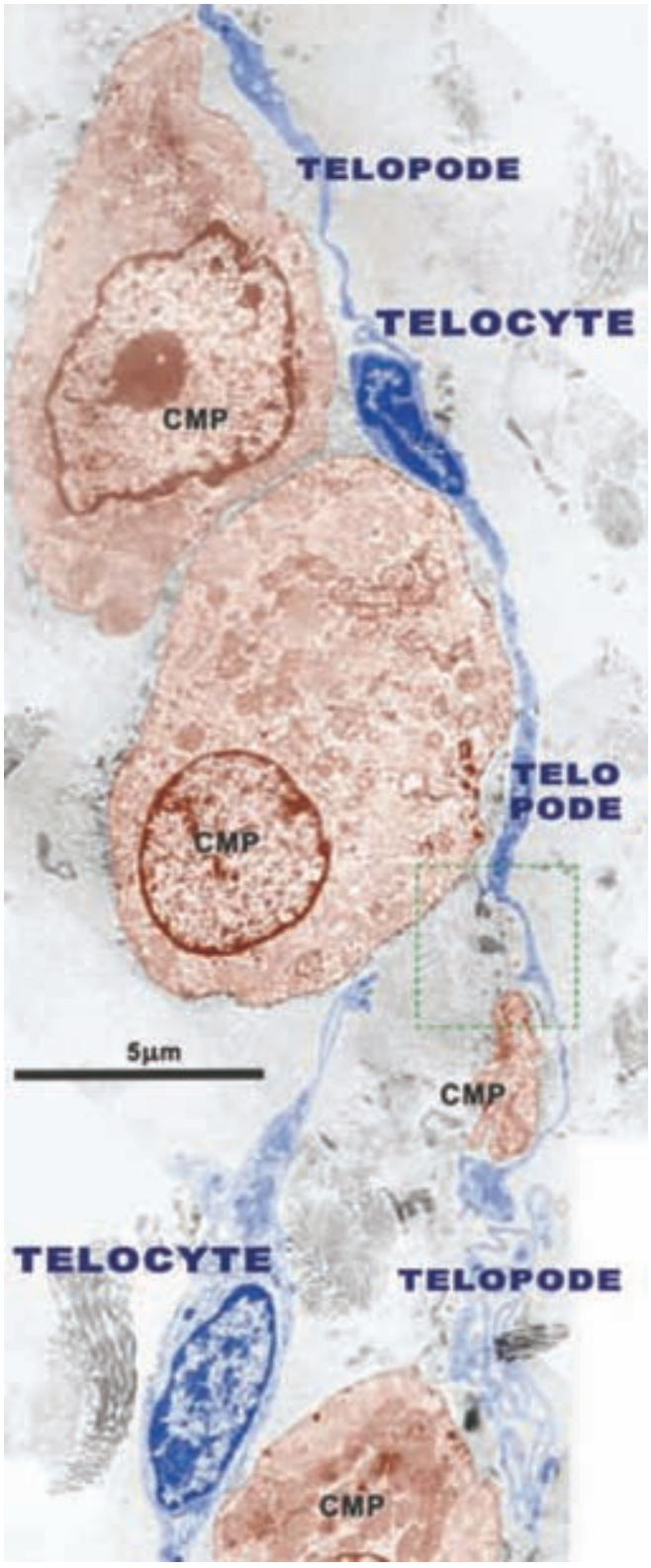


Fig. 15 Electron micrographs illustrates the relationships of **TC** (blue) with cardiomyocyte progenitors (CMP, brown). The **Tp** run parallel with the main axis of the CMP and seem to establish their direction of development. Reproduced with permission from [4].

Epilogue:

Men are only as good as their technical development allows them to be

George Orwell (1903-1950)

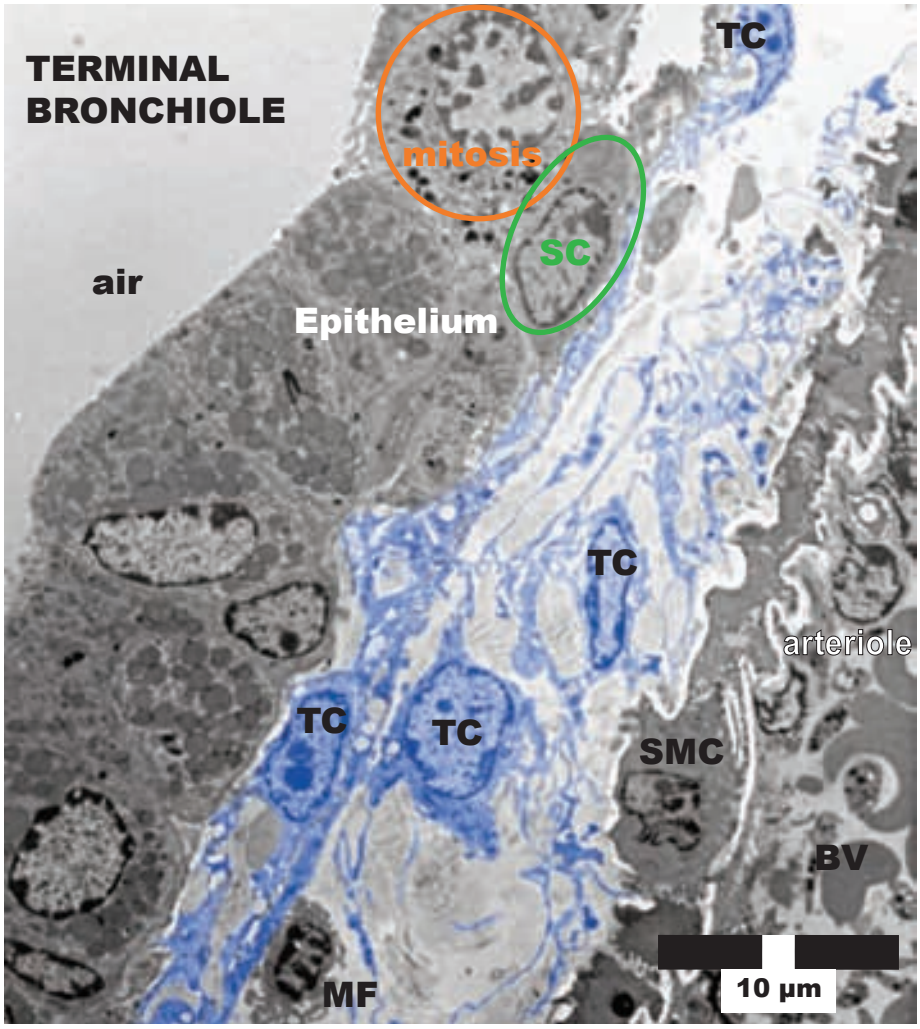


Fig. 16 Mice lung. Terminal bronchiole. At least 4 **TC** with their extensive **Tp** are visible between the epithelium and an arteriole (SMC - smooth muscle cells). Note, the striking labyrinthine network formed by **Tp**. In the upper part a mitosis (prophase) is obvious (orange circle). In addition, a putative stem cell (**SC**, green oval) is in close contacts with telocytes prolongations, establishing a heterocellular junctions, visible at higher magnification only). The tandem **TC-SC** forms, presumably, a **TC-SC** *niche*. In the lower part, a macrophage (MF) makes a *stromal synapse* with **Tp**.

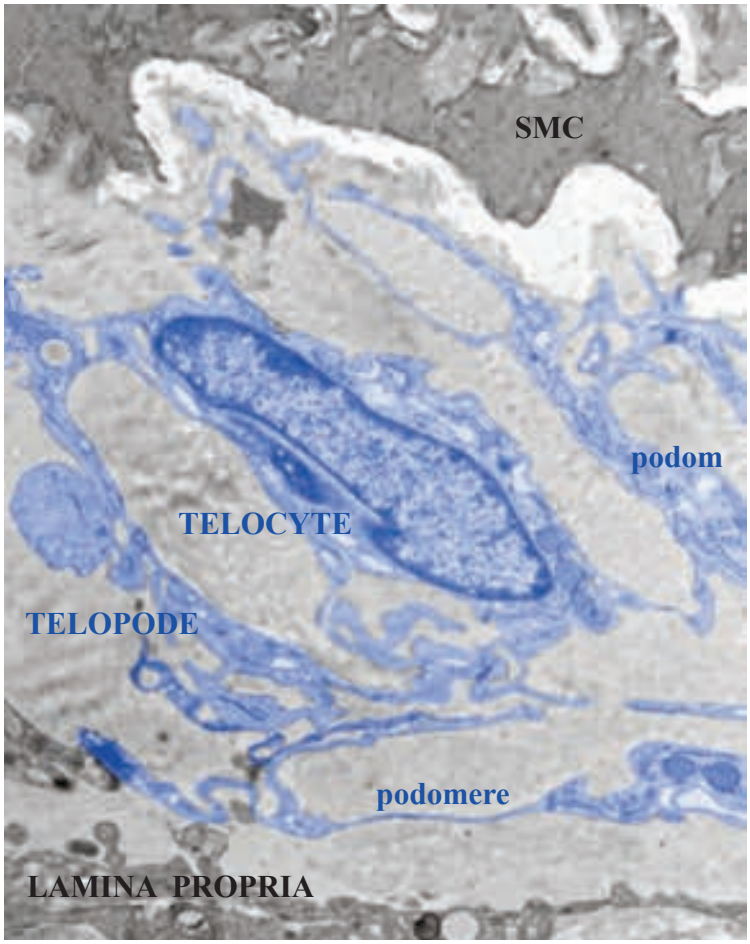


Fig. 17 Mice lung, terminal bronchiole. Electron microscopy shows one **TC** with its highly convoluted **Tp**, creating a labirinthyne system in between smooth muscle cell (SMC) and lamina propria of brochiole. Note the appearance of Tp as a alternation of podomeres and podoms. Podoms are accommodating mitochondirae.

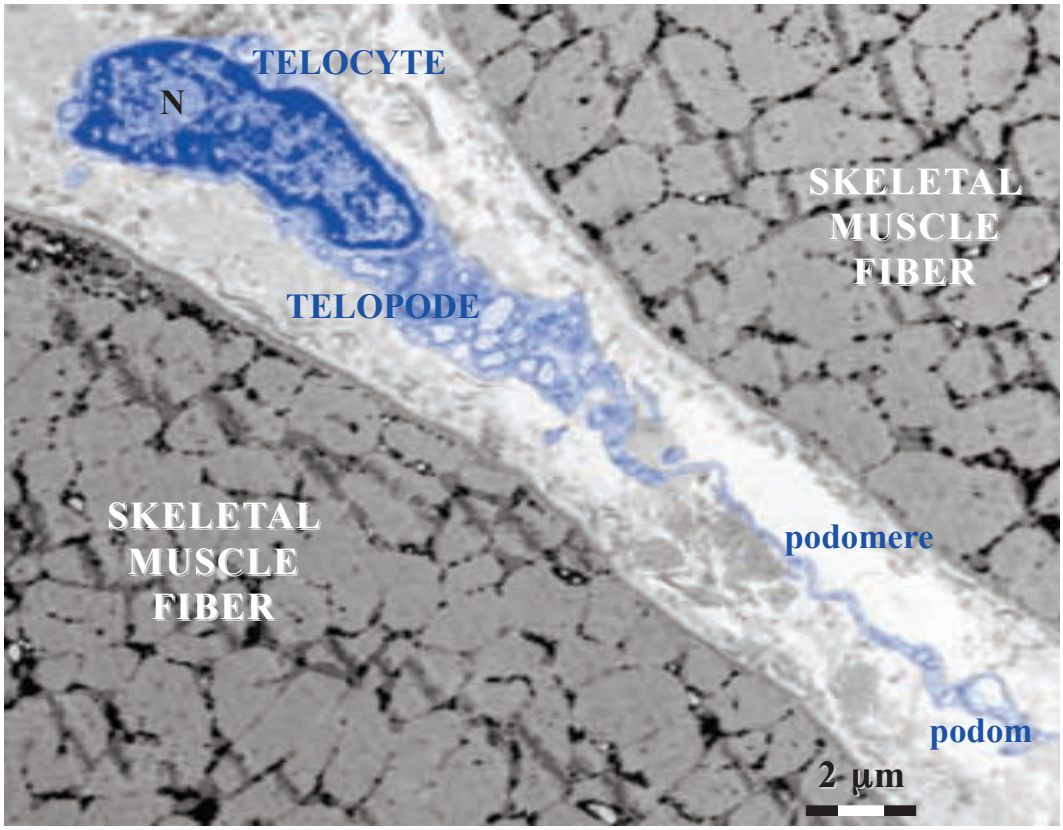


Fig. 18. Rat striated skeletal muscle. A typical **TC** in between two skeletal muscle fibers with one convoluted **Tp** is shown, by transmission electron microscopy. Note,the typical uneven caliber of the Tp, the thin podomere and dilated segments - podoms - harboring mitochondriae.

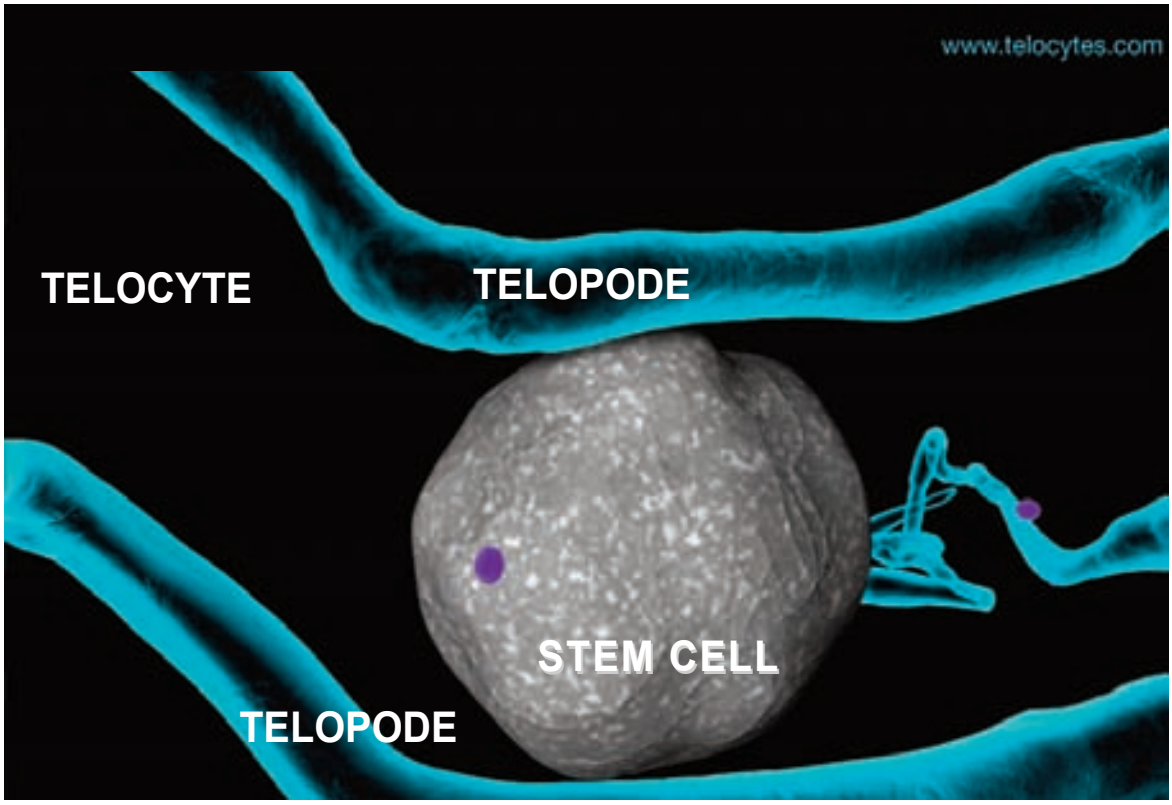


Fig. 19 Computer simulation of a 3D representation of the tandem TC - stem cells outside the tissular environment. Only Tp (of uneven caliber) are observable surounding the stem cell. Shed vesicles (violet colored) are emerging from the surface of Tp targetting the stem cells, being involved in intercellular communication.

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