Introduction 1

1.1 What are ICC?

1.1.1 First Description of ICC by Cajal

Santiago Ramon y Cajal, who contributed to the establishment of the neuron theory and was awarded the Nobel Prize for Medicine and Physiology in 1906, regarded the demonstration of the histological basis of autonomic innervation and how nerves transmit signals to effector tissues as a major challenge. He described a fine cellular network that he designated as "*cellules interstitielles*" or "*neurones sympathiques interstitiels*" in association with the terminal arborization of the autonomic nerves of intestines, glands, and blood vessels stained with methylene blue or the Golgi method [1, 2]. Cajal considered these cells as primitive nerve cells that mediate nerve impulses from the terminal portions of the sympathetic nerves to the smooth muscle cells, because at that time these staining methods were believed to be specific to the nerves. Since then, interstitial cells of Cajal (ICC), as referred to by later microscopists, have been a subject of a historical debate in respect of their cytological nature. ICC were regarded by different microscopists as neurons or Schwann cells or connective tissue cells or smooth muscle cells [3–5]. With the development of electron microscopy, the ultrastructural identification of ICC was attempted by several investigators [4, 6, 7], but the cytological definition and the developmental origin of ICC remained unsettled.

1.1.2 Pacemaker Hypothesis

A breakthrough in ICC research was triggered by the novel hypothesis proposed by Thuneberg [8], suggesting that ICC act as pacemaker cells and that they conduct impulses in the gut musculature in an analogous fashion to that in the heart. This hypothesis greatly stimulated both morphological and physiological studies of ICC. Subsequently, various cells in different regions of the digestive tract from a variety of species were described by many authors by a variety of methods [9–11]. However, again, it was uncertain whether different cytological features of these cells represented different profiles of the same cell type, morphological variations of the same cell type, or a mixture of different cell types. Part of the reason for the confusion was the lack of a truly specific staining method for ICC, and the difficulty of correlating ultrastructural observations with the traditional histological descriptions by silver-impregnations and methylene blue staining. Therefore, to establish an unambiguous set of cytological criteria, it was essential that the whole shape of a given cell type and its relation to nerve and muscle cells should correspond closely to that originally described by Cajal.

1.1.3 Finding of c-Kit in ICC

The discovery of a significant role of c-*kit* in the maturation of ICC and the finding that ICC cor-

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respond to the cells expressing c-Kit receptor tyrosine kinase was a major advance. Abnormal development of ICC was demonstrated in studies using experimental blockade of c-Kit [12, 13]. Then immunohistochemical staining for c-Kit became accepted as a useful marker of ICC at the light microscopic level.

Studies using combinations of c-Kit immunostaining, the zinc iodide-osmium tetroxide (ZIO) method, which shares many staining properties with methylene blue staining and the Golgi method, and ultrastructural observations, contributed to bridging the gap between the old histological descriptions and more recent findings on ICC [14, 15].

1.1.4 Definition and Developmental Origin

As described above, c-Kit-immunostaining proved to depict exactly the same features of the cells as demonstrated by methylene-blue or Golgi methods originally used for the detection of ICC. Therefore, ICC are defined here as c-Kit immunoreactive cells showing bipolar or multipolar shape within the gastrointestinal tract. Meanwhile, studies using chick-quail chimeras [16] and studies using transplants of intestinal segments of the mouse embryo [17] demonstrated that all classes of ICC share a common embryological origin from mesenchymal cells. Developmental studies also showed that ICC in the myenteric plexus originate from the same mesenchymal progenitor cells expressing c-Kit as smooth muscle cells of the longitudinal muscle layer [18, 19].

1.1.5 Distribution of ICC

The presence of ICC has been reported in a wide variety of species, including the frog, lizard, turkey, and many mammals including opossum, bat, rabbit, hedgehog, pig, horse, and conventional experimental animals such as the mouse, rat, guinea-pig and dog, and also in the monkey and human. In the human, ICC have been found throughout the digestive tract from the esophagus to the inner sphincter region of the anus.

1.1.6 Functional Role

Soon after the proposal of the pacemaker hypothesis [8], physiological studies started to provide evidence for a pacemaker function of ICC associated with the myenteric plexus (ICC-MP) and for their generation of slow waves [20, 21]. After the discovery that the c-Kit receptor is essential for the normal development of ICC [12, 13], further strong evidence for such a pacemaker function came from the demonstration of the loss of pacemaker activity of mice with a genetic defect in c-Kit [22, 23]. Eventually, direct recording of the pacemaker activity and slow waves were made from ICC-MP in the stomach of guinea-pig [24].

A pacemaker function was also reported for the ICC associated with the submuscular plexus (ICC-SMP) of the colon in the dog [25–27], human [28], and rat [29].

On the other hand, an intermediary role in the neuromuscular transmission was suggested by morphological studies that certain types of ICC were closely apposed to nerve terminals and formed numerous gap junctions with neighboring smooth muscle cells at different levels of the gastrointestinal tract in many species [30]. Indeed, cytochemical and physiological studies [31, 32] supported that idea that ICC of the circular (ICC-CM) and the deep muscular plexus in the small intestine (ICC-DMP) had a functional significance in both inhibitory and excitatory neurotransmission in the smooth muscles in the GI tract.

In addition, mechanosensitive functions has also been suggested for ICC. Arrays of intramuscular vagal nerves innervating smooth muscles and ICC-CM are believed to be intramuscular mechanoreceptors [33, 34]. A role as stretch receptors was also proposed for ICC located in the subserosal layer (ICC-SS) in the guinea-pig colon to detect the circumferential expansion and swelling of the colon caused by active absorption of water and electrolytes [35]. However, in general, further convincing evidence is required to demonstrate mechanosensitive functions of ICC.

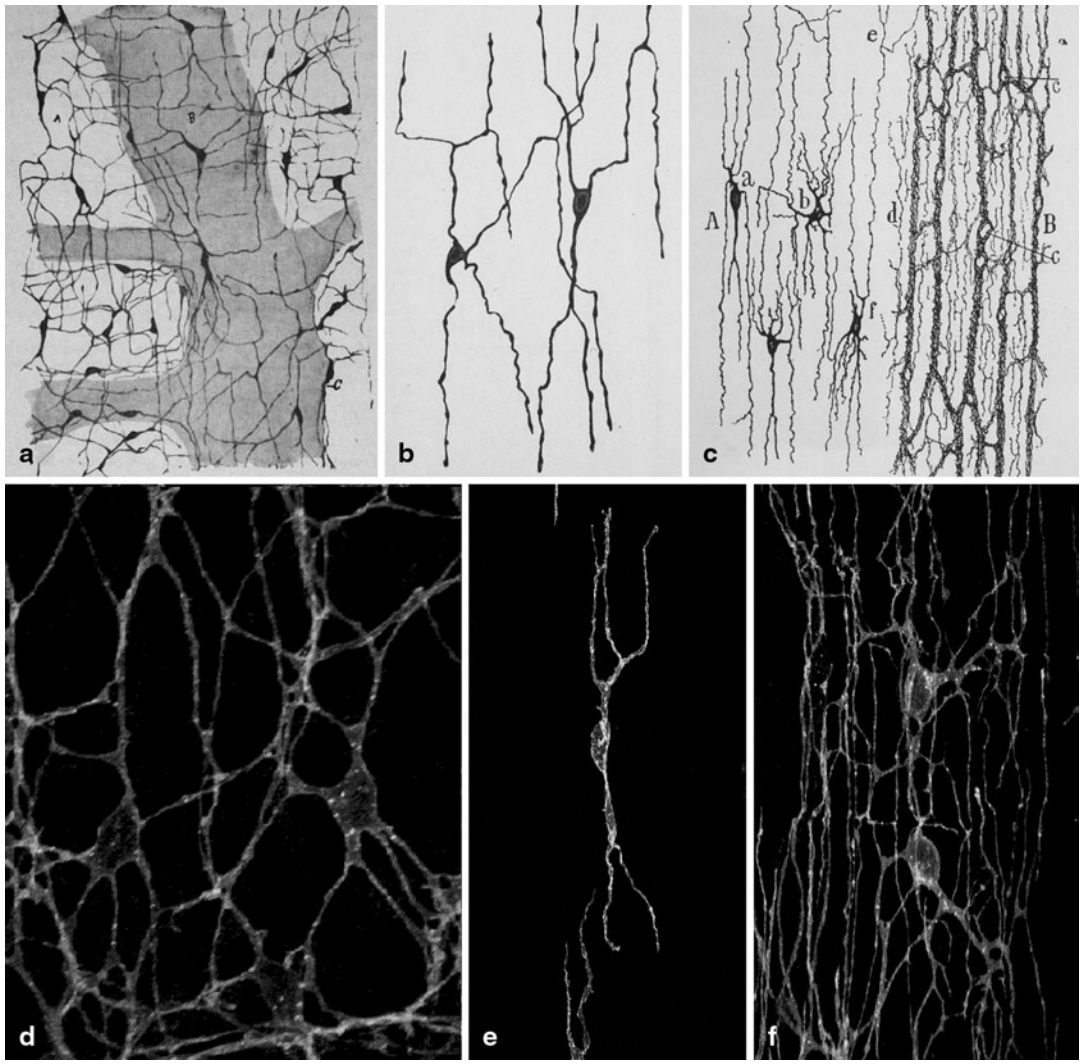

Fig. 1.1 Drawings by Cajal and ICC depicted by modern method. **a** A drawing by Cajal of ICC-MP in the rabbit intestine stained with methylene blue. (Reproduced from Cajal [2], Fig. 572). **b** A drawing by Cajal of ICC-CM in the rabbit stained with methylene blue. (Reproduced from Cajal [2], Fig. 573). **c** A drawing by Cajal of ICC-DMP in the guinea-pig small intestine stained with Golgi method. (Reproduced from Cajal [2], Fig. 575). **d** ICC-MP of the guinea-pig small intestine stained with c-Kit immunohistochemistry. **e** ICC-CM of the guinea-pig small intestine stained with c-Kit immunohistochemistry. **f** ICC-DMP of the guinea-pig small intestine stained with c-Kit-immunohistochemistry. (Note, close similarity of the whole shape and branching patterns of their processes between the pairs of **a** and **d**, **b** and **e**, and **c** and **f**, respectively).

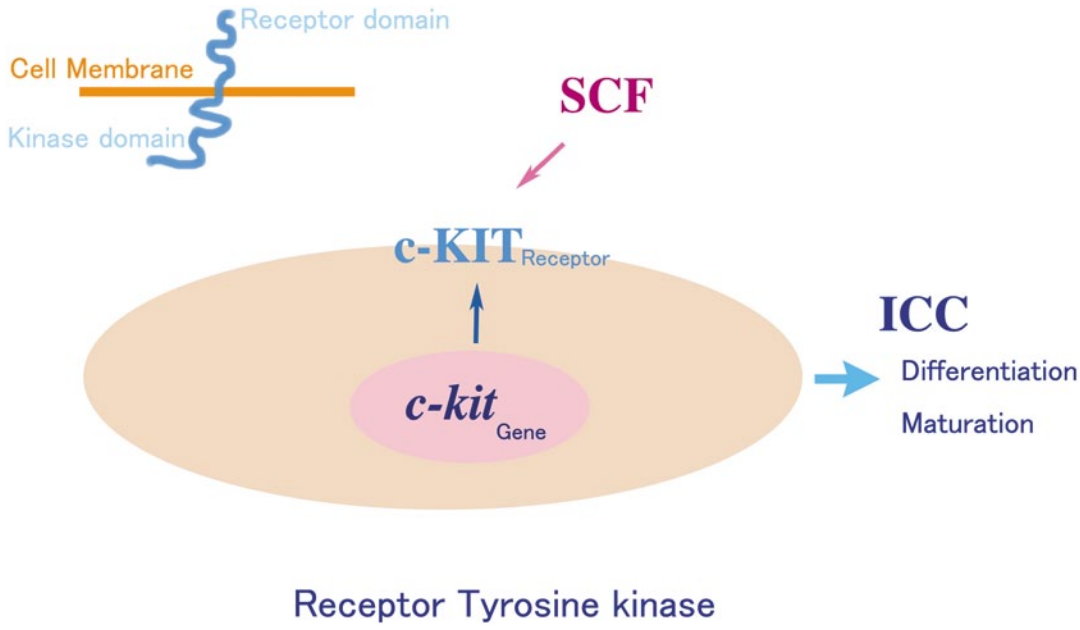

Fig. 1.2 Schematic illustration of c-Kit. c-Kit is a receptor tyrosine kinase that is encoded by the proto-oncogene (*c-kit*) and its ligand is stem cell factor (*SCF*). c-Kit consists of an extracellular domain, a transmembrane domain and an intracellular tyrosine kinase domain. Antibodies against a part of the extracelluar domain can be used immunohistochemistry for labelling ICC. Accumulated evidence indicates that ICC depend on SCF signaling via c-Kit for their development, proliferation and maintenance of function.

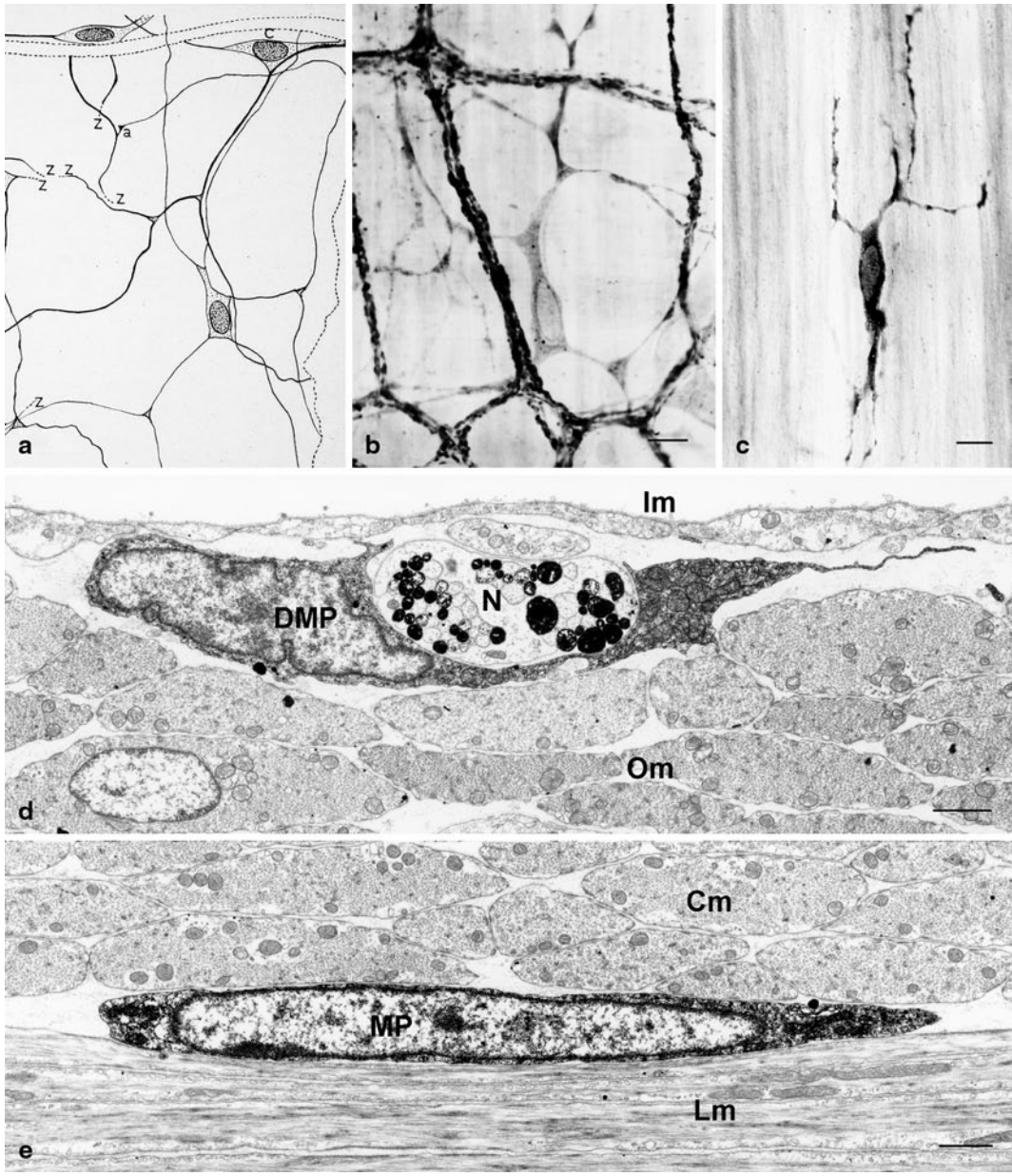

Fig. 1.3 ICC depicted by traditional staining methods. **a** Drawing of ICC-MP by Taxi in the guinea-pig intestine stained with the Bielschowsky-Gross method. Note close similarity of the cell shape with those of Fig. 1.1a, d. (Reproduced with permission from Taxi [4], Fig. 43). **b** ICC-MP of the guinea-pig small intestine stained by the zinc-iodide tetroxide (ZIO) method, which shows a very similar appearance to the cell in a. ×650. *Bar* 10 μm. (Reproduced from Komuro and Zhou [14] with permission of the publisher). **c** ICC-CM of the guinea-pig small intestine stained by ZIO method, which shows close similarity to those of Fig. 1.1b, e. ×600. *Bar* 10 μm. (Reproduced from Komuro et al. [15] with permission of the publisher). **d** Electron micrograph showing ICC-DMP (*DMP*) densely stained by ZIO method located between the inner (*Im*) and outer (*Om*) circular muscle layers of the guinea-pig small intestine. Many mitochondria can be noticed in the cytoplasm. Nerve bundles (*N*) containing ZIO-positive fibres are observed in the cytoplasmic indentation of the ICC-DMP. ×10,000. *Bar* 1 μm. **e** Electron micrograph showing ICC-MP (*MP*) densely stained by ZIO method located between the inner circular (*Cm*) and outer longitudinal (*Lm*) muscle layers of the guinea-pig small intestine. ×9000. *Bar* 1 μm.

Here, it is worth noting that ICC and neurons share staining affinity in several histological methods including the Bielschowsky-Gross method and ZIO method as well as the supravital methylene blue staining and Golgi method used for their original demonstration of ICC. These features have been one of the main reasons for difficulty in understanding true nature of ICC. The reason why ICC and neurons share similar staining affinity has not been fully clarified.

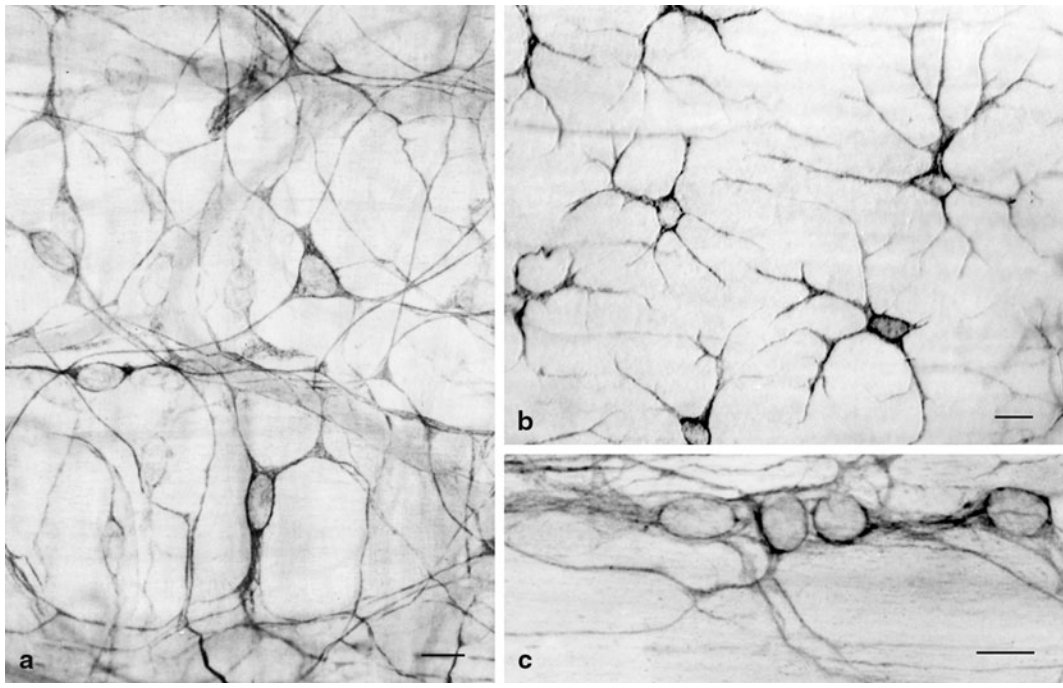

Fig. 1.4 ICC demonstrated by immunohistochemistry for vimentin. **a** ICC-MP of the guinea-pig small intestine. The ICC in his picture also show close similarity in shape to Fig. 1.1a, d and Fig. 1.2a, b. ×650. *Bar* 10 μm. (Modified from Komuro et al. [74]). **b** ICC-SS of the guinea-pig colon. ×600 *Bar* 10 μm. (See section on Colon). **c** ICC-DMP of the guinea-pig small intestine. ×900. *Bar* 10 μm. (See section on small intestine).

Vimentin filaments are commonly found in ICC and seem to function as cytoskeletal elements for supporting well-developed long slender processes, in a similar way in which neurotubules and neurofilaments support long processes of the neurons. Thus vimentin staining can be used as a substitute for c-Kit staining in a similar manner to using neurotubule staining to depict neurons.

1.2 Structure of the Gastrointestinal Tract

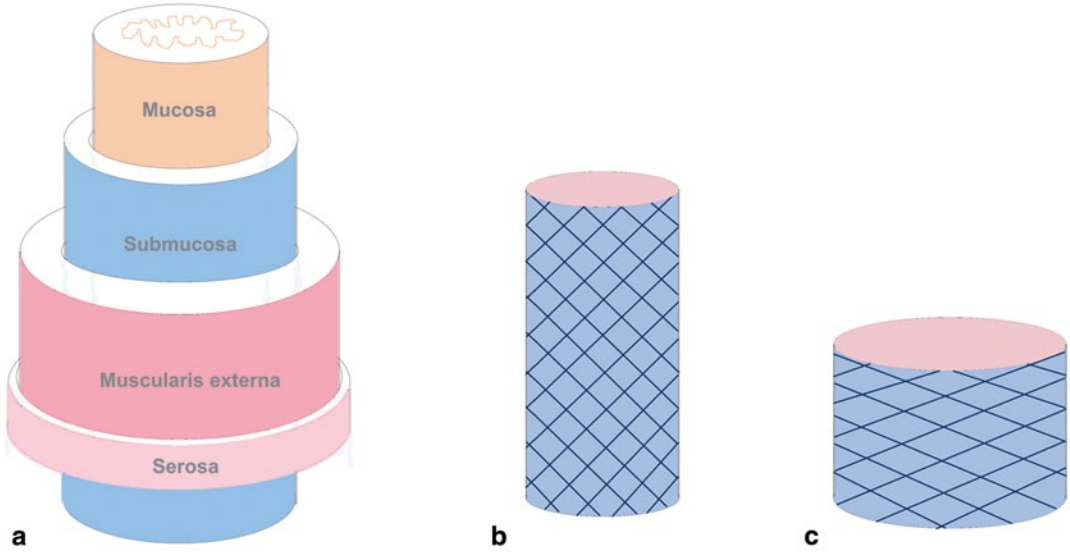

Fig. 1.5 General organization of the gastrointestinal wall. The wall of the alimentary tract is made up of four layers from the inside to outer surfaces: the mucosa, the submucosa, the tunica muscularis and the serosa (**a**). The innermost of these layers, the mucosa is responsible for the main role peculiar to each segment of the GI tract, such as secretion or digestion or absorption. In most parts of the GI tract, the tunica muscularis is composed of two layers of smooth muscle cells—a circumferentially oriented inner layer and a longitudinally oriented outer layer. The myenteric plexus located between two muscle layers coordinates their peristaltic contractions to move the luminal contents along the GI tract. Between the mucosa and muscle layers, the submucosa acts as a skeleton of the hollow organ to connect the mucosa with tunica muscularis and transmit the contractile power of the muscle to the mucosa. This results in the movement of the whole organ, reminiscent of the relationship between skeletal bones and body muscles. The diagonal arrangement of collagen bundles in the submucosa (**b c**, see below) is essential for the flexibility of the gut skeleton in allowing the deformation of the gastrointestinal tract during peristaltic movements.

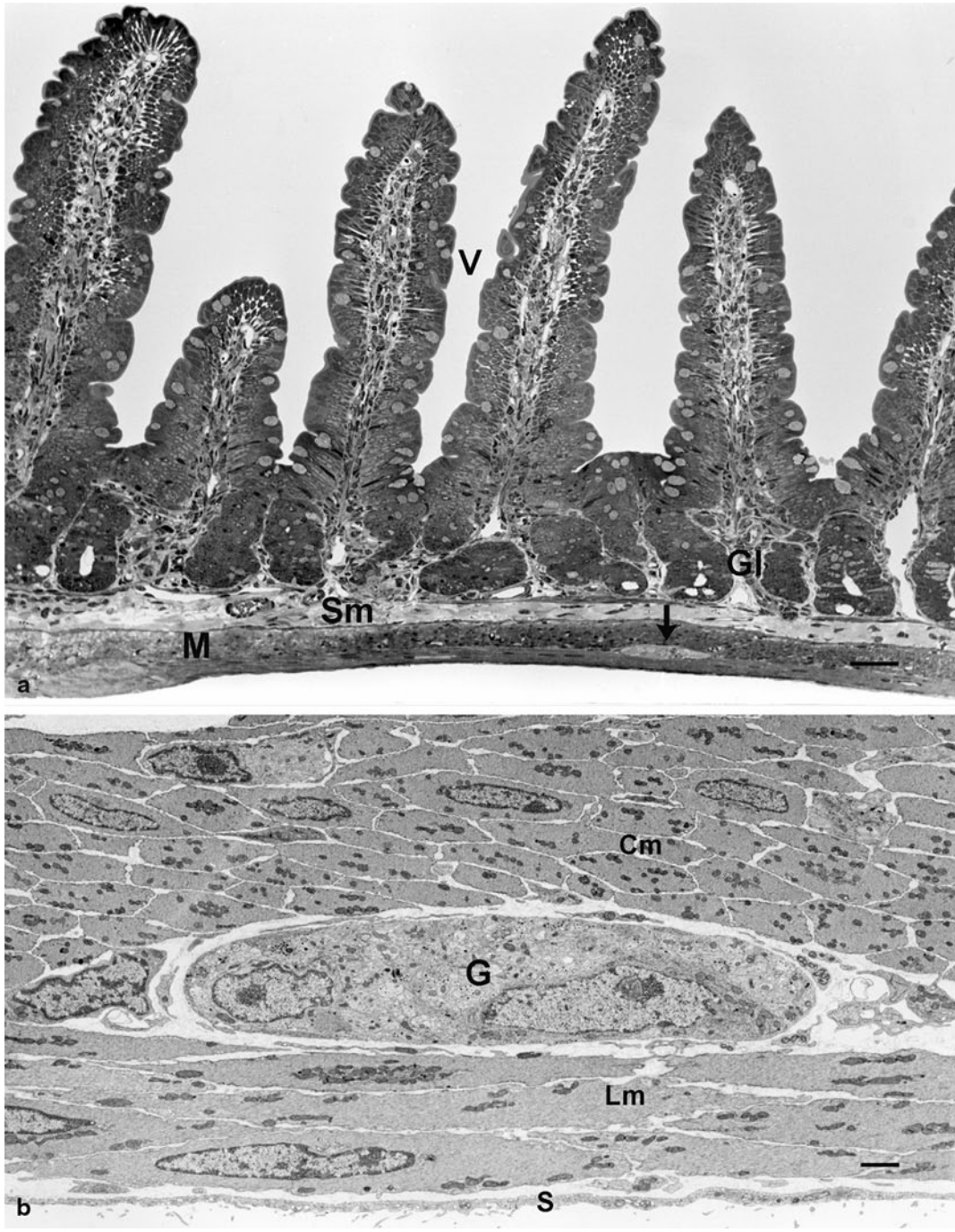

Fig. 1.6 A sectioned profile of the gut wall. **a** A longitudinal section of the rat small intestine stained with toluidine blue. The mucosa including villi (*V*) and glands (*Gl*) are separated by the pale-staining layer of the submucosa (*Sm*) from the tunica muscularis or external muscle layer (*M*). Myenteric ganglia (*arrow*) are located between two muscle layers. ×180. *Bar* 50 μm. (Reproduced from Komuro and Hashimoto [77] with permission of the publisher). **b** An electron micrograph showing the tunica muscularis consisting of the inner circular (*Cm*) and the outer longitudinal (*Lm*) muscle layers of the mouse intestine. A myenteric ganglion (*G*) is observed between two muscle layers. Mesothelium of the serosa (*S*) is observed at the outermost layer of the intestinal wall. ×7000. *Bar* 1 μm. (Reproduced from Hanani et al. [36] with permission of the publisher).

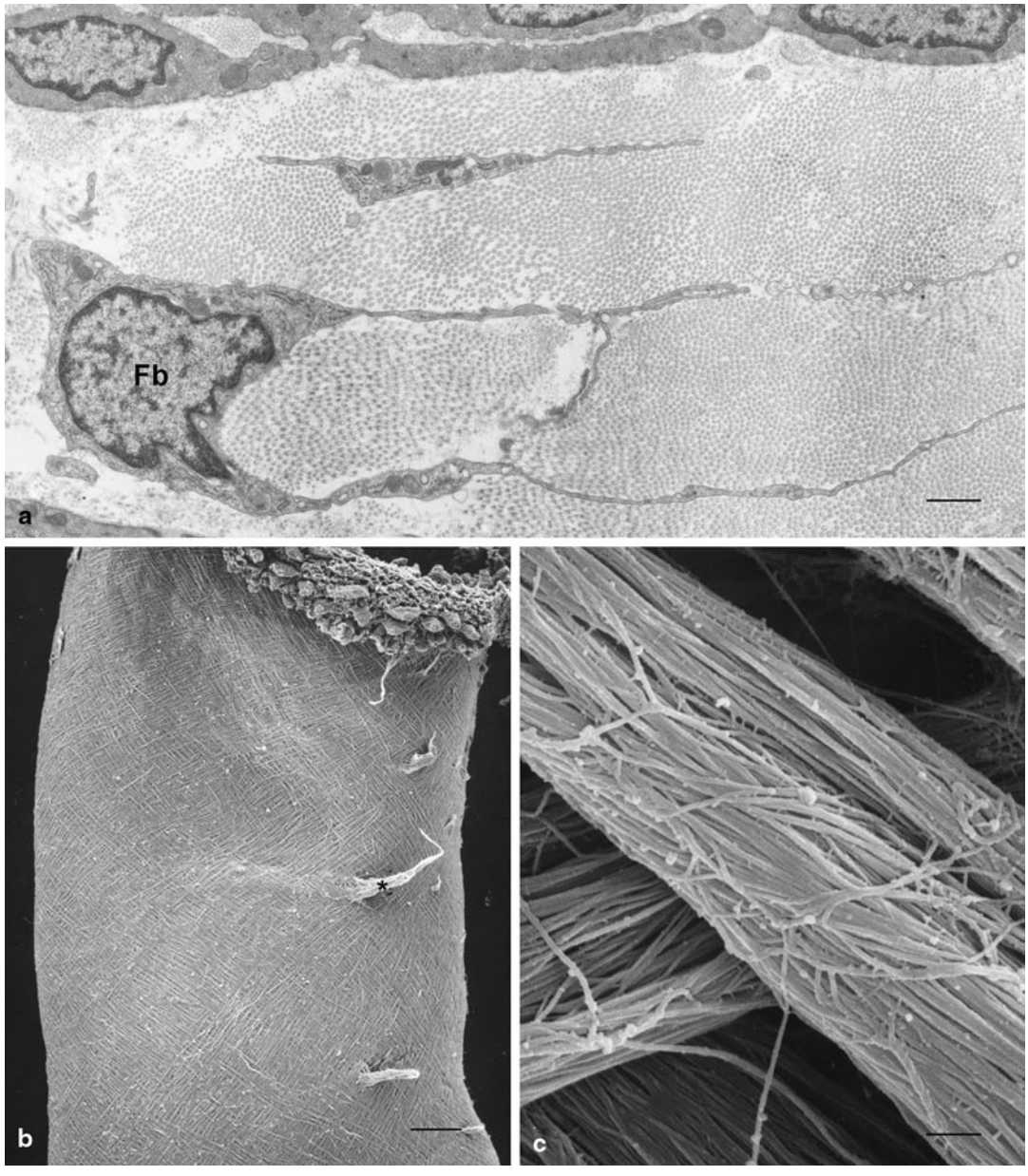

Fig. 1.7 Ultrastructural demonstration of the framework of the submucosa. **a** Transmission electron micrograph showing the submucosa of the rat intestine composed of layered, abundant collagen fibrils. The thin processes of a fibroblast (*Fb*) are located between layered collagen fibrils. ×9000. *Bar* 1 µm. (Reproduced from Komuro and Hashimoto [77] with permission of the publisher). **b** Scanning electron micrograph showing collagenous framework of the submucosa of the rat small intestine. The collagen fibres are arranged in two sets of interweaving helices. Cord-like structures are traces of blood vessels (*). ×45. *Bar* 200 µm. (Figure 1.3b, c: Reproduced from Komuro [78] with permission of the publisher). **c** Higher magnification of the collagen fibers composed of densely packed collagen fibrils. ×9200. *Bar* 1 µm.

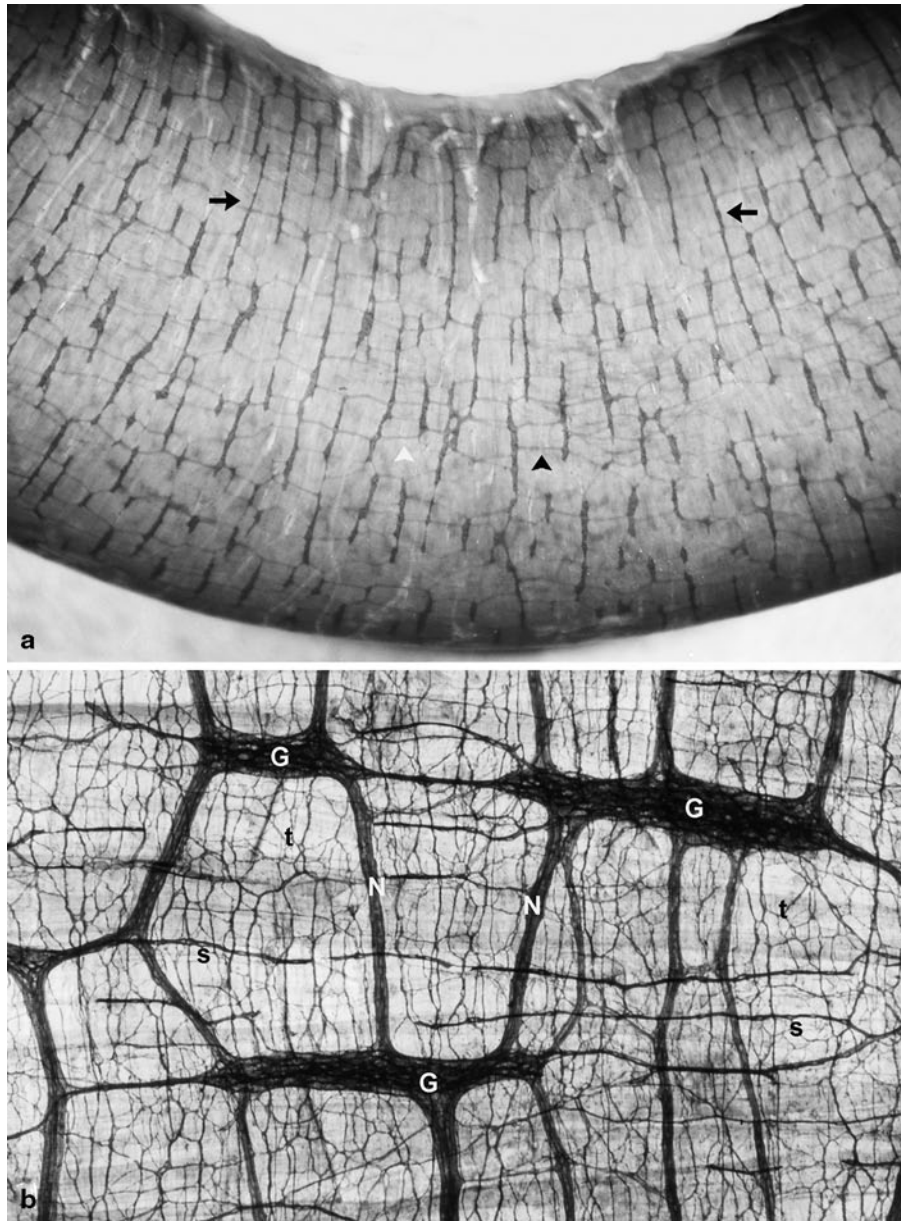

Fig. 1.8 Myenteric plexus. **a** Surface view of the whole tube of the guinea-pig small intestine stained by NADH histochemistry. A rectangular pattern of the myenteric plexus can be seen through the longitudinal muscle layer and the serous membrane. The plexus consists of elongated ganglia (*arrows*) mainly orientated along the axis of the circular muscle connected by nerve strands (*arrow heads*) running perpendicularly. The pattern of the myenteric plexus is different depending on the level of the digestive tract and shows features specific for each region. ×15. *Bar* 400 μm. **b** Whole-mount stretch preparation of the guinea-pig small intestine stained with ZIO method. The primary network of the myenteric plexus consists of ganglion strands (*G*) and connecting nerve strands (*N*). Fine nerve bundles of the secondary (*s*) and tertiary network (*t*) are clearly observed among the primary framework. ×150. *Bar* 40 μm.

The myenteric plexus is distributed throughout the GI tract and plays a central role in regulating the motor activity of the gastrointestinal tract. Accumulated evidence shows that ICC-MP associated with the myenteric plexus act as the primary pacemaker cells both in the stomach and small intestine and as secondary pacemaker cells in the colon. Thus, the specific features of the myenteric plexus in each organ are key in the movement of the external muscle layer.

Table 1.1 Abbreviations of subtypes of ICC in the GI tract

ICC	Location	Synonym
ICC-MP	Associated with myenteric plexus	ICC-AP Thuneberg [9] IC-MY Sanders [52]
ICC-DMP	Associated with deep muscular plexus of the small intestine	IC-DMP Sanders [52]
ICC-SMP	Associated with submuscular plexus of the colon	IC-SM Sanders [52]
ICC-SM	Located at submucosal border of the circular muscle in the antrum	IC-SM Sanders [52]
ICC-CM	Located within the circular muscle layer	IC-IM Sanders [52]
ICC-LM	Located within the longitudinal muscle layer	IC-IM Sanders [52]
ICC-IM	General term for ICC within the muscle layer	IC-IM Sanders [52]
ICC-SS	Located in the subserous connective tissue space	
ICC-SP	Associated with submucosal plexus	

1.3 Nomenclature of ICC

The terminology adopted in this Atlas is a minor modification of that used in Hanani et al. [36] and Komuro [37]. It is based on the Thuneberg's invention [8] of classifying ICC depending on the tissue layer with which they are associated, and follows the modern practice of avoiding attribution of the individual name of discoverer of cells and tissues, and the addition of new subtypes.

Three principles are set in this terminology. First, ICC is used as an abbreviation for Interstitial Cells of Cajal to make clear their nature, since the abbreviation IC has also been used in the literature but represents interstitial cells in general and only has a neutral meaning regarding connective tissue cells. Second, where a nerve plexus is associated with the ICC, the initial letters of the plexus are added to ICC with a hyphen, for example, DMP for the deep muscular plexus, or MP for the myenteric plexus. Third, where the nerve plexus has no particular name, hyphenated abbreviations of the tissue layer are added to ICC, e.g. ICC-CM in the circular muscle layer and ICC-SM in the submucosal layer. ICC-IM is also adopted as a general term for ICC within a muscle coat (Table 1.1) (Fig. 1.9).

ICC-SM and ICC-SMP are found at the submucosal border of the circular muscle layer of the gastric pylorus and the colon, respectively. ICC-DMP are observed in association with the deep muscular plexus located between the inner thin and outer main layers of the circular muscles in the small intestine. ICC-MP are seen in association with the myenteric plexus located between the circular and longitudinal muscle layers throughout the gastrointestinal tract except the proximal part of the stomach. ICC-CM and ICC-LM are distributed within the circular and longitudinal muscle layers, respectively. ICC-SS are found in the subserosal connective tissue space.

ICC-SP associated with the submucosal plexus are not included in this illustration. To date, ICC-SP have been only found in the gastric corpus, proximal colon and caecum in the guinea-pig.

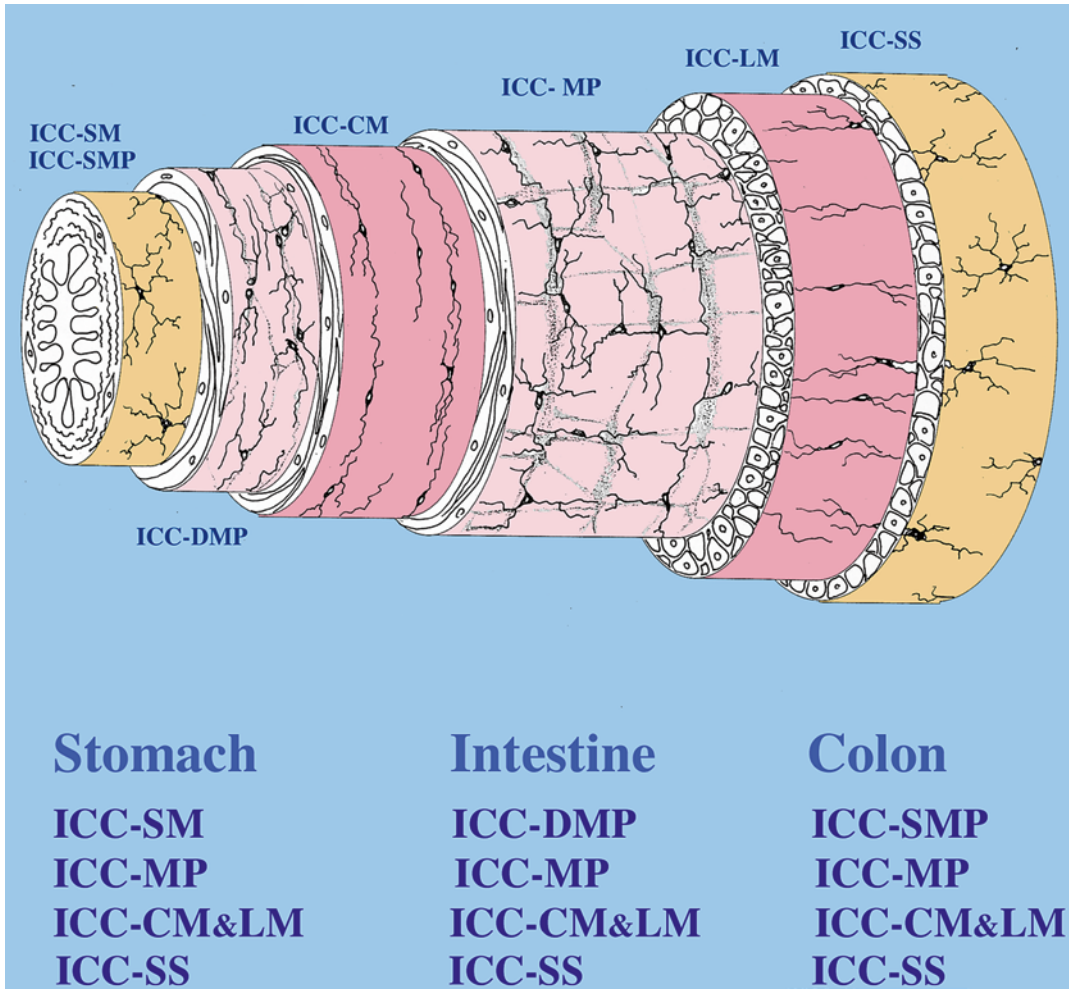

Fig. 1.9 Schematic demonstration of ICC found in the different tissue layers in different regions of the GI tract. (Modified from Hanani et al. [36]).

1.4 Shape and Size of ICC

The cell shape of ICC appears to be determined by several factors, including the presence or absence of a nerve plexus, their relationships to those plexuses and the frequency of connections between ICC themselves.

ICC-IM located within the muscle layers are mainly bipolar cells oriented parallel with the axis of surrounding smooth muscle cells. The secondary and tertiary processes are generally not well developed.

On the other hand, some of ICC-IM show a multipolar shape with three to five primary processes while they maintaining their longer cell axis among the muscle cells. These cells are found in the circular muscle layer of the guinea-pig small intestine and in the colon. ICC-DMP of the small intestine, which are regarded as a special type of ICC-CM, can take a variety of forms depending on the sites of their associated nerve bundles. At straight portions of the nerves, they show slim spindle shapes with long bipolar processes, while at the intersections the cells project three to five processes along the nerve bundles.

In contrast, ICC-MP located in the meshes of the primary network of the myenteric plexus do not show a clear cell axis but project several processes in multiple directions. Similar multipolar cells with no obvious cell axes are found in the subserosal layer of the guinea-pig proximal colon.

Footnote Morphological features of ICC illustrated in this Atlas were mainly demonstrated by immunohistochemistry using whole-mount stretch preparation. Specimens were pre-incubated in 4% Block Ace solution for 20 min and then incubated with a rat monoclonal antibody against mouse CD117 (c-Kit) to label ICC and with a rabbit antibody against human protein gene product (PGP) 9.5 to label nerve components. Then specimens were incubated with a fluorescein isothiocyanate (FITC)-conjugated secondary antibody and a Cy^{TM}3-conjugated secondary antibody. Specimens were observed with a confocal laser scanning microscope (Leica TCS SP2; Leica Microsystems, Wetzlar, Germany).

Specimens (jejunum) from the guinea-pigs (weighing 300 ~ 400 g) fixed slightly distended condition measure about 2 cm in the circumference. In these intestines, an approximate estimation indicate, that at least 40 smooth muscle cells are needed to encircle the whole tube if they contact each other tip to tip. Similar estimates indicate that more than 100 ICC-CM and about 80 ICC-DMP are needed to cover the whole circumference of the intestine. Such estimates suggest that role of ICC as intermediate cells in the neural transmission to the smooth muscles may be effective in the lateral direction but not in the axial direction of the circular muscle.

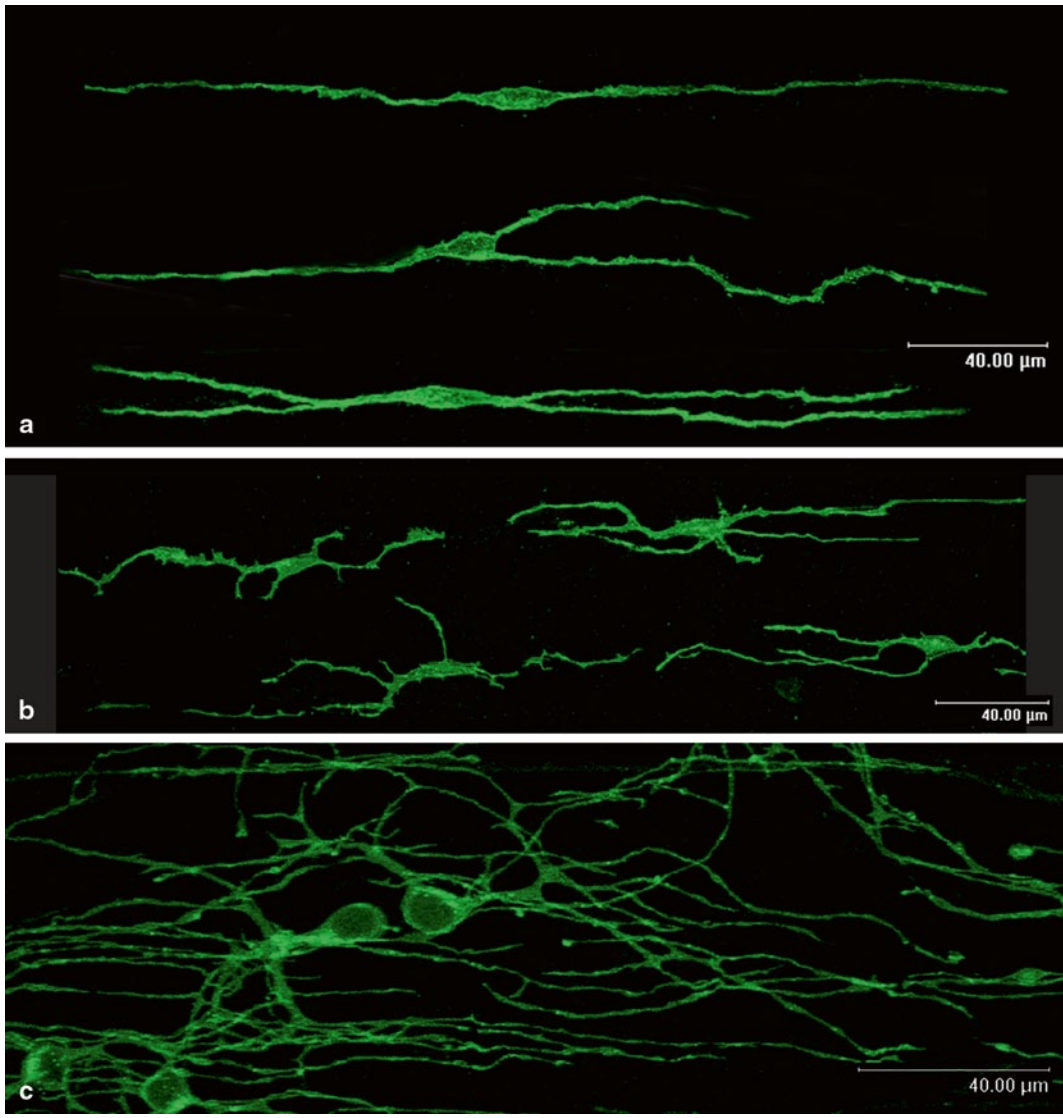

Fig. 1.10 ICC within the muscle layer. **a** Bipolar cells found within the circular muscle layer (ICC-CM) in the guinea-pig stomach. Simple bipolar cell with only primary processes projecting to the opposite directions (*top of the figure*) are frequently observed in the fundus, and cells with secondary branches in both ends (*bottom of the figure*) are numerous in the antrum. These types of cells are observed in almost every muscle layer in the GI tract, but the cell size differs depending on the organ or region. For example, their cell lengths measure around 200 µm in the guinea-pig gastric fundus and more than 300 µm in the guinea-pig caecum. *Bar* 40 µm. **b** Multipolar cells found in the circular muscle layer (ICC-CM) in the guinea-pig small intestine. They project 3~5 primary processes while they maintain their longer cell axis aligned with that of the muscle layer (*horizontal direction*). They are slightly shorter than the bipolar cells and their length measures around 180 µm in the small intestine and around 120 µm in the colon, though the small intestine contains long bipolar cells around 350 µm long. *Bar* 40 µm. **c** Multipolar cells associated with the deep muscular plexus (ICC-DMP) of the guinea-pig small intestine. Their secondary and tertiary processes are well developed and are mainly arranged in parallel with the axis of the muscle layer (*horizontal direction*). The processes of ICC-DMP often span lengths of 300 µm and they are aligned with the axis of the circular muscle cell. *Bar* 40 µm.

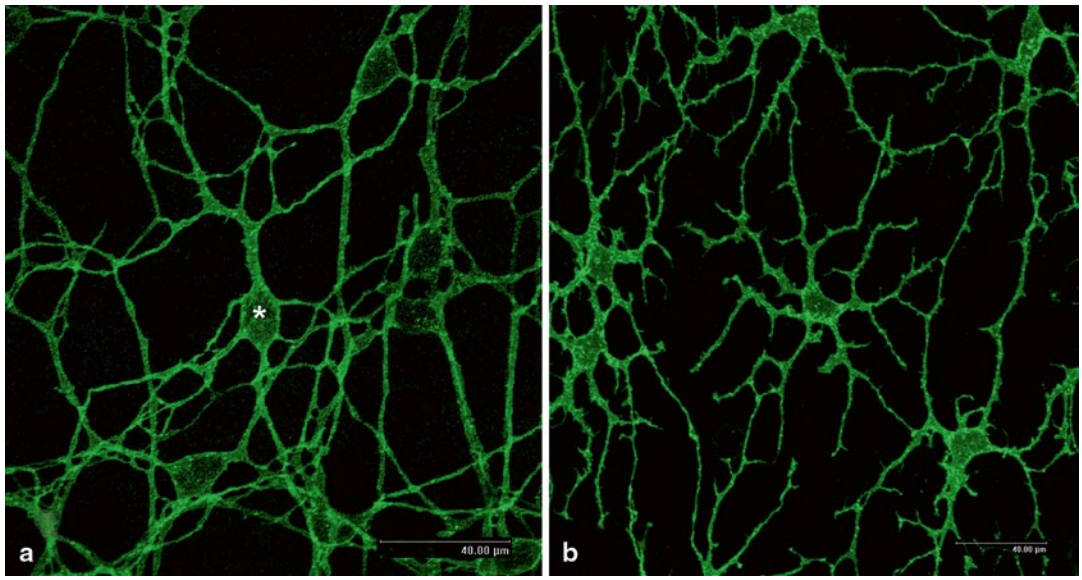

Fig. 1.11 ICC forming their own network. **a** Multipolar cells associated with the myenteric plexus (ICC-MP) of the guinea-pig small intestine. These cells form a net on the framework of the primary nerve plexus located between two muscle layers and thus ICC-MP (*) do not show a clear cell axis. They usually have three to five primary processes that branch off repeatedly to form secondary, tertiary and further branches. The cell processes of these cells measure around 100 µm in one direction from the base of the cell body to the tip. *Bar* 40 µm. (Modified from Hanani et al. [36]). **b** Multipolar cells found in the subserosal layer (ICC-SS) of the guinea-pig proximal colon. These cells are distributed in the connective tissue space beneath the serosal mesothelium without firm connection with any structures and thus do not show clear cell axis. Spiny processes along the long processes are one of the different features from ICC-MP described above. The cell processes also measure around 100 µm in one direction from the base to the tip. (Reproduced from Aranishi et al. [35] with permission of the publisher). *Bar* 40 µm.

Here, it is worth noting that ICC-MP and ICC-SS send their processes into the different tissue layers and form cellular networks in three-dimensions. These features will be described in a later chapter with stereo-micrographs (see section on colon).

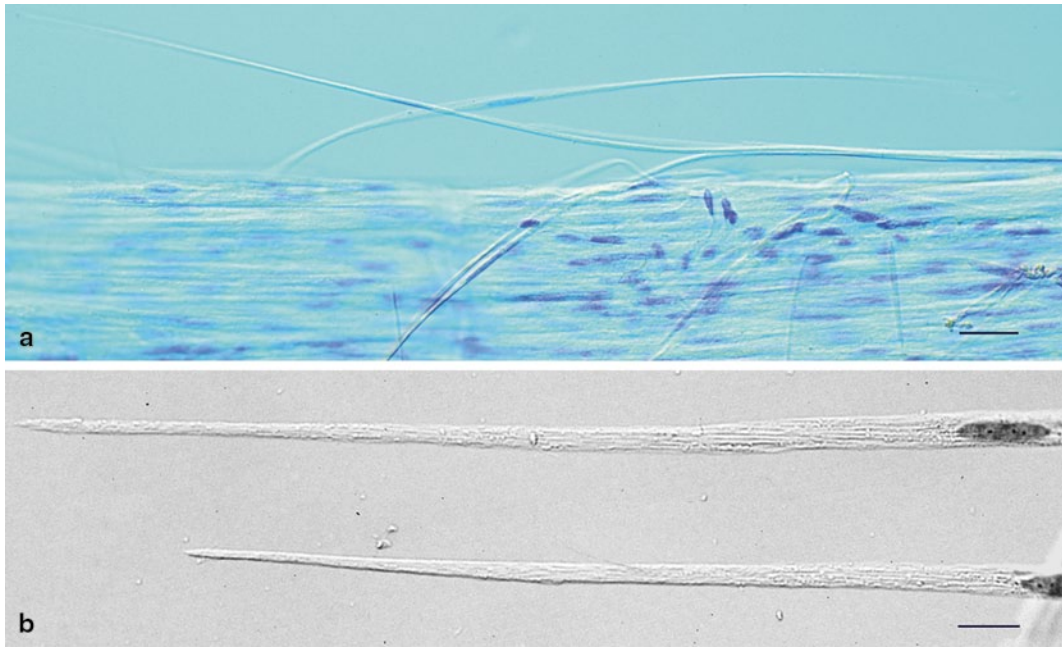

Fig. 1.12 Smooth muscle cells. **a** Images with Nomarski optics of the smooth muscle cells of the guinea-pig small intestine which are isolated under a dissection microscope and stained with toluidine blue. *Bar* 50 µm. **b** Higher magnification of the smooth muscle cells in the same preparation as a. These cells measure about 500 ~ 600 µm in length. *Bar* 20 µm.