Ultrastructural Demonstration of ICC

9

ICC are characterized by such ultrastructural features as the presence of numerous mitochondria, abundant intermediate filaments, moderately developed Golgi apparatus, granular and smooth endoplasmic reticulum, close contacts with nerve varicosities and formation of gap junctions with each other and with smooth muscle cells. However, ICC show a certain range of morphological heterogeneity ranging from features similar to fibroblasts to those specific to smooth muscles cells such as caveolae, a basal lamina and subsurface cisterns, depending on their anatomical location and species. Indeed an early ultrastructural study described ICC-DMP of the dog small intestine as "hybrid cells" [40].

ICC are classified into three types. On one hand, Type 1 ICC are the least like muscle cells and the most like fibroblasts, while on the other hand, Type 3 are the most similar to smooth muscle cells. Type 2 ICC have an intermediate character (Table 9.1).

Table 9.1 Three types of ICC classified by their ultrastructural features. All types of ICC are positive for c-Kit immunoreactivity. (Modified from Komuro [37])

ICC type	Basal lamina	Caveolae	Gap junctions	Intermediate filaments	Mitochondria	Nerve contacts
Type 1 ICC (least like smooth muscle cells)	–	+–	++	++	++	++
Type 2 ICC (intermediate type)	+–	++	++	++	++	++
Type 3 ICC (most like smooth muscle cells)	++	++	++	++	++	++

Abundance: (++) present or numerous; (+/-) fuzzy or few; (-) absent.

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9.1 Ultrastructural Characteristics of ICC

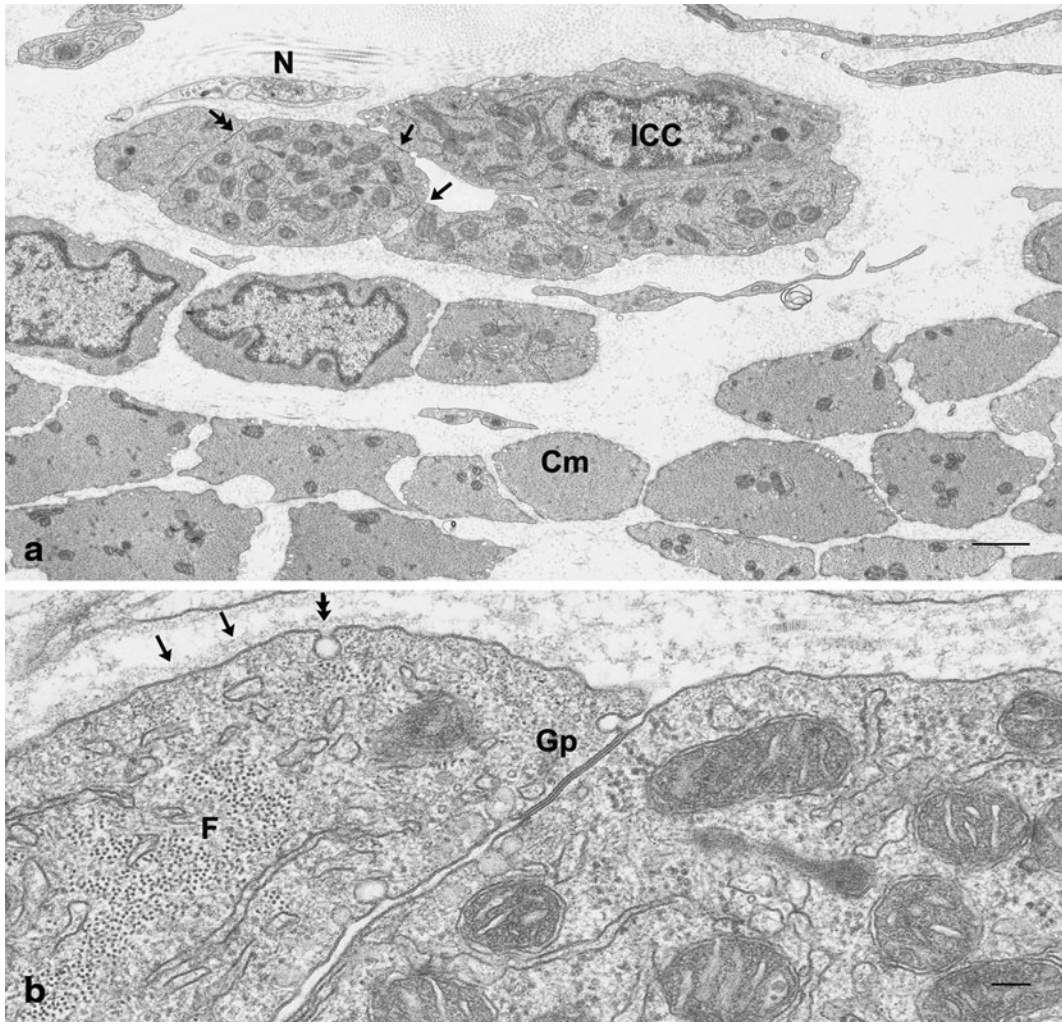

Fig. 9.1 **a** Cytoplasm and the cell membrane (I). **a** ICC-SM (*ICC*) located at the submucosal border of the circular muscle layer (*Cm*) of the guinea-pig gastric antrum. This type of cell has typical ultrastructural features of Type 3 ICC and is characterized by the presence of many mitochondria and large gap junctions (*arrow* and *double-headed arrow*). Cisterns of glandular endoplasmic reticulum are also observed in the perinuclear cytoplasm. Nerve fibres (*N*) are found in their close vicinity. ×9,600. *Bar* 1 μm. (Reproduced from Komuro [54] with permission of the publisher). **b** Higher magnification of a part of **a** (surrounding area of *double-headed arrow*) showing caveolae (*double-headed arrow*), basal lamina (*arrows*), cross section of the intermediate filaments (*F*) and a gap junction (*Gp*). ×62,000. *Bar* 0.1 μm.

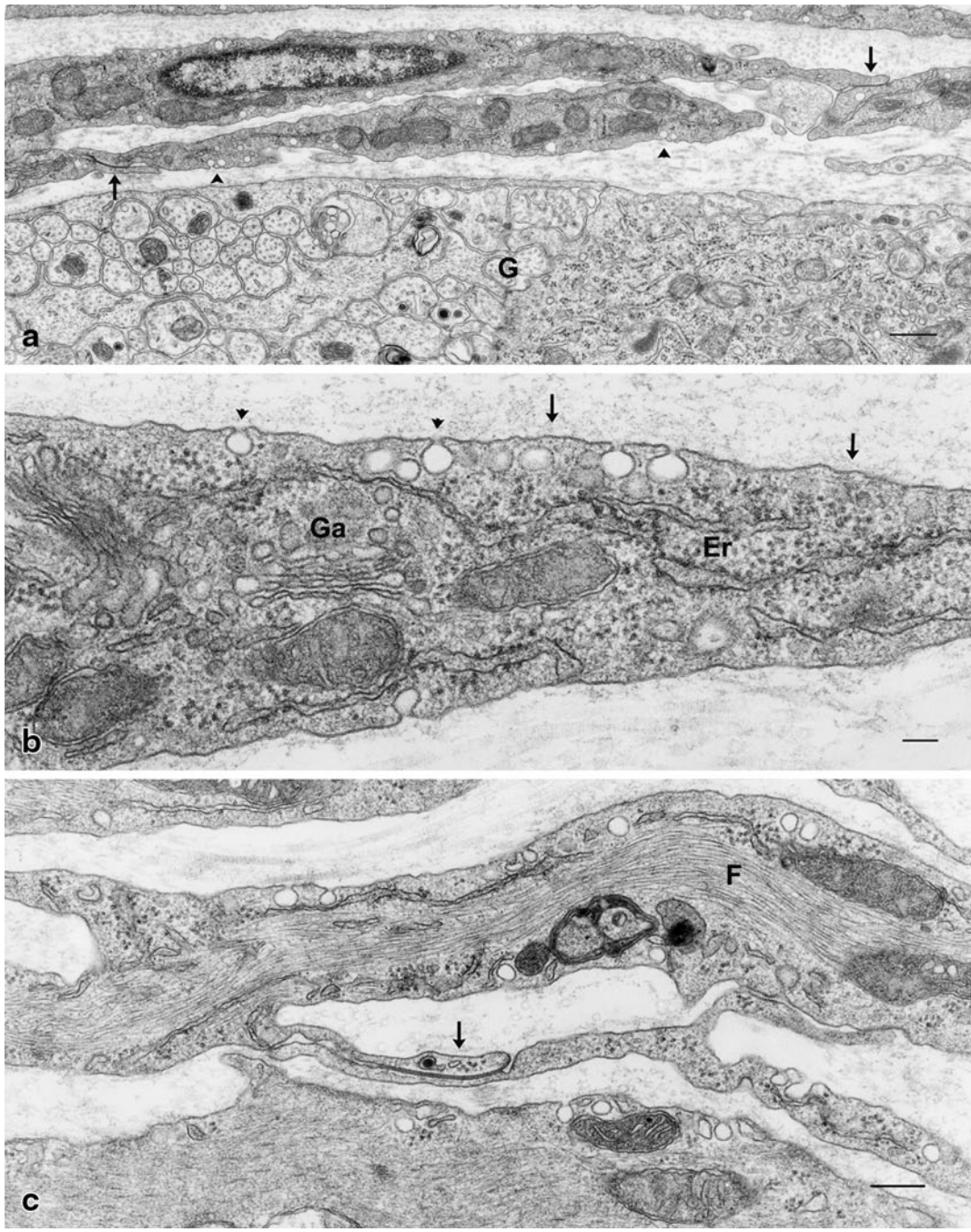

Fig. 9.2 Cytoplasm and the cell membrane (II). **a** ICC-MP located over the myenteric ganglion (*G*) of the mouse pylorus which form gap junctions (*arrows*) with the processes of the same type of cells even though the cytoplasm of those cells shows a close similarity to fibroblasts. Many caveolae are also observed along the cell membrane (*arrow heads*). ×8,600. *Bar* 1 μm (Figure 9.2a, c: Reproduced from Komuro et al. [74] with permission of the publisher). **b** A cell process of ICC-MP of the mouse stomach. The cytoplasm contains granular endoplasmic reticulum (*Er*) and Golgi apparatus (*Ga*), but also has caveolae (*arrow heads*) and basal lamina (*arrows*) along the cell membrane. Cisterns of the granular endoplasmic reticulum are not dilated unlike those often seen in the fibroblasts. ×63,000. *Bar* 0.1 μm (Reproduced from Komuro [54] with permission of the publisher). **c** A bundle of intermediate filaments (*F*) found in the cell process of ICC-MP in the mouse stomach. A gap junction is formed between thin processes (*arrow*). ×56,000. *Bar* 0.2 μm.

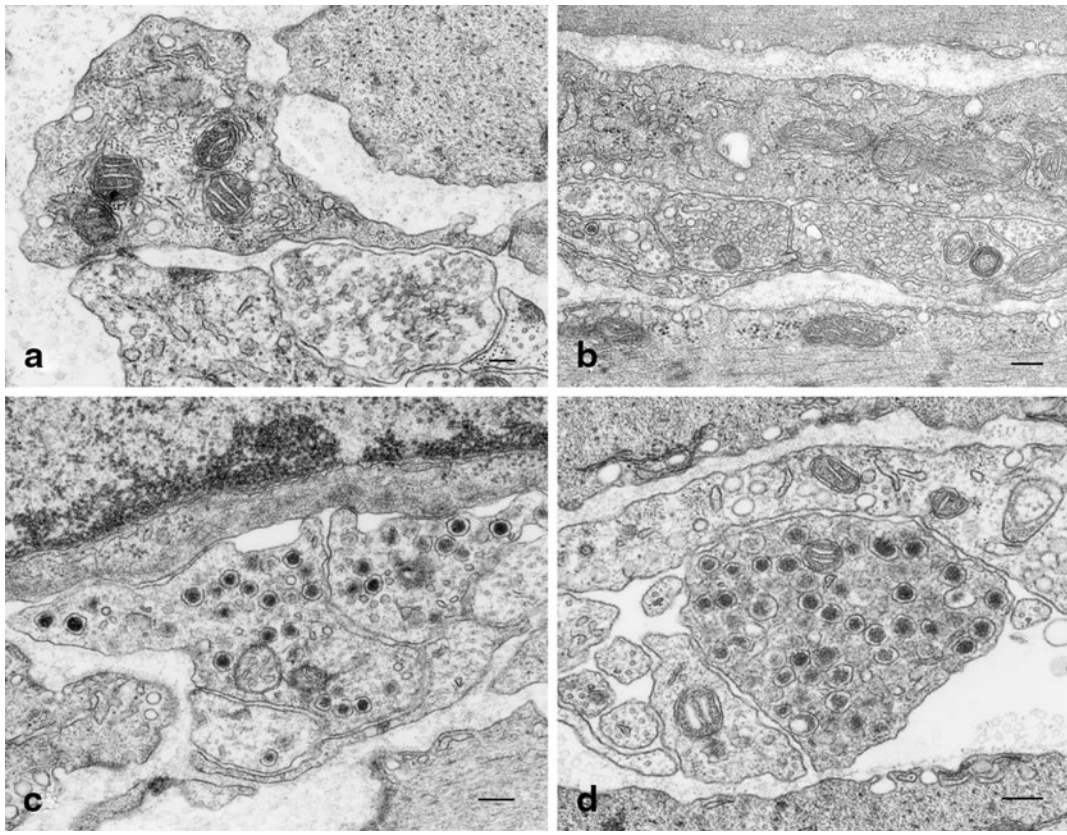

Fig. 9.3 Close contacts with nerve varicosities. **a** An axon varicosity containing clear flat vesicles closely adjacent to the process of ICC-SM in the rat stomach. ×40,000. *Bar* 1 μm (Reproduced from Mitsui and Komuro [68] with permission of the publisher). **b** An axon varicosity containing clear round vesicles closely adjacent to ICC-DMP in the rat small intestine. ×26,000. *Bar* 0.2 μm (Reproduced from Komuro and Seki [82] with permission of the publisher). **c** Close contact between an axon varicosity containing large cored vesicles and the cell body of ICC-MP in the mouse stomach. ×29,000. *Bar* 0.2 μm (Reproduced from Komuro et al. [74] with permission of the publisher). **d** Close contact between an axon varicosity containing large cored vesicles and ICC-DMP of the mouse small intestine. ×35,000. *Bar* 0.2 μm (Courtesy of Dr. Horiguchi, Fukui University).

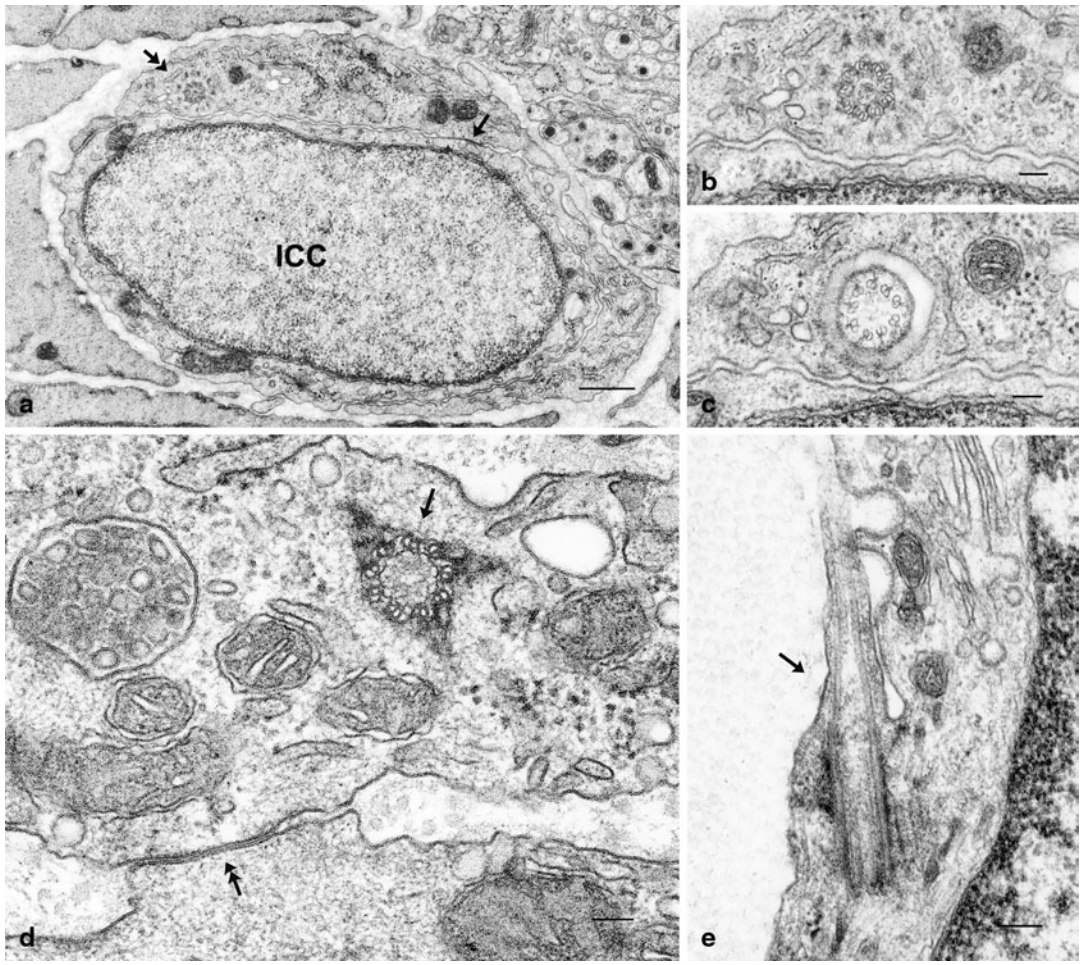

Fig. 9.4 Cilia and basal bodies. **a** ICC-CM (*ICC*) and a process of the same cell type in the guinea-pig colon are connected with each other with a gap junction (*arrow*). The process contains a basal body of cilium (*double-headed arrow*). Interestingly, ICC located in the connective tissue space often have cilia and the basal bodies. However, the frequent observations of cilia throughout every subtype of ICC suggest that cilia are one of the characteristic features of ICC. ×18,000. *Bar* 0.5 μm. **b** Higher magnification of the basal body indicated by double-headed arrow in **a**. ×50,000. *Bar* 0.1 μm. **c** A neighbouring section of **b** showing a cross section of the cilium. ×50,000. *Bar* 0.1 μm (Fig. 9.4a–c: Courtesy of Dr. Ishikawa, Waseda University). **d** A part of the cytoplasm of ICC-DMP in the rat small intestine containing a basal body (*arrow*). A gap junction is also observed between this cell and the neighboring cell (*double-headed arrow*). ×70,000. *Bar* 0.1 μm. **e** A longitudinal section of the cilium found in ICC-SS in the guinea-pig colon (*arrow*). ×75,000. *Bar* 0.1 μm (Courtesy of Dr. Tamada, Waseda University).

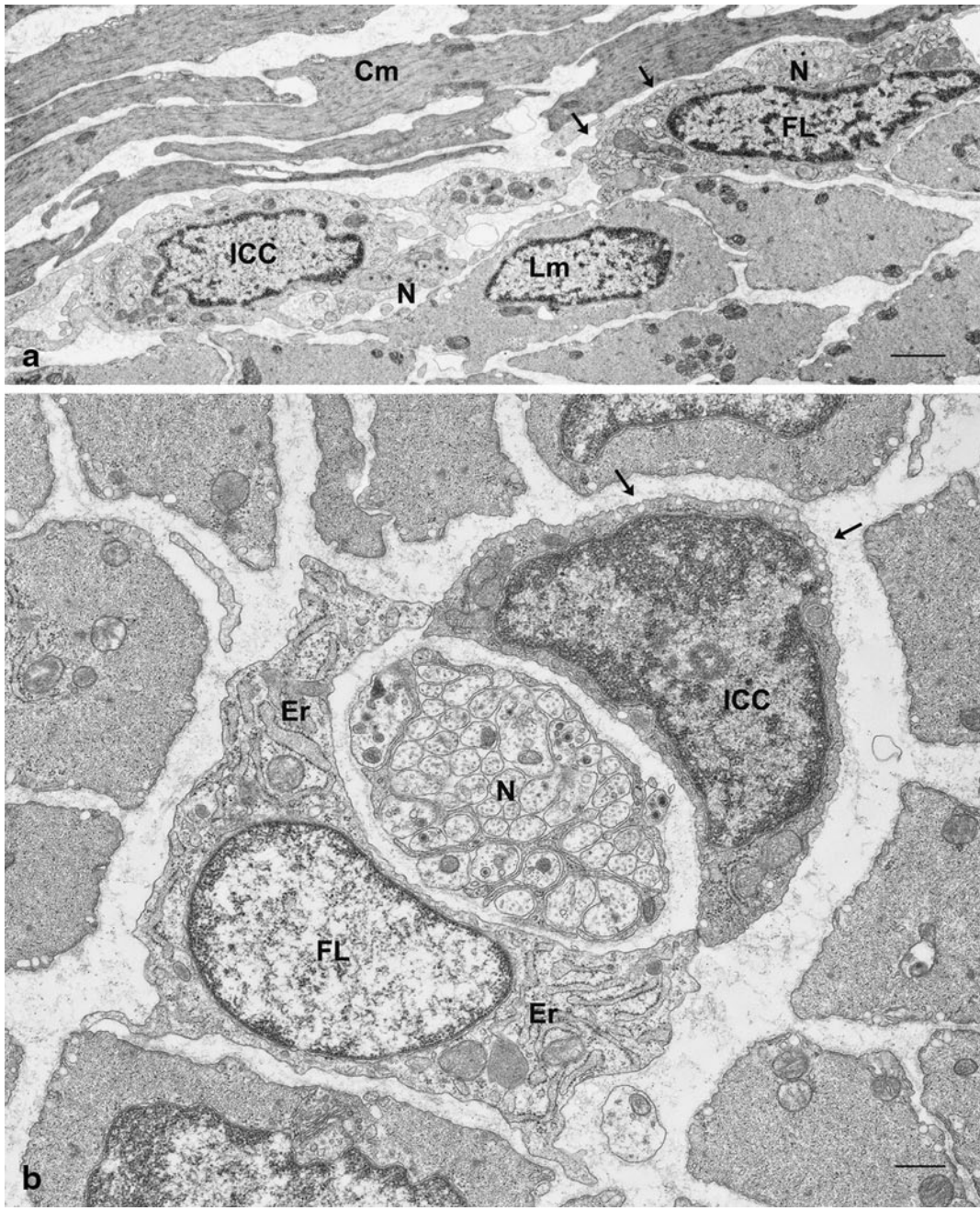

Fig. 9.5 Distinguishing ICC from other cell types (I). **a** ICC-MP (*ICC*) and fibroblast-like cell (*FL*) found in the region between the circular (*Cm*) and longitudinal (*Lm*) muscle layers of the rat small intestine. ICC is characterized by the presence of many mitochondria, while the fibroblast-like cell contains well-developed granular endoplasmic reticulum with dilated cisterns (*arrows*). Nerve fibers (*N*) are found in close vicinity to both types of cells. ×9,800. *Bar* 1 μm (Reproduced from Komuro et al. [15] with permission of the publisher). **b** ICC-CM (*CM*) and fibroblast-like cell (*FL*) associated with a nerve bundle in the circular muscle layer of the rat colon. The ICC is characterized by electron-dense cytoplasm and caveolae along the cell membrane (*arrows*), while the fibroblast-like cell shows well-developed granular endoplasmic reticulum (*Er*) in the cytoplasm and no caveolae along the cell membrane. ×18,000. *Bar* 0.5 μm (Reproduced from Komuro [30] with permission of the publisher).

The electron density of the cytoplasm is often discussed in relation to the identification of ICC, but it seems to be an unreliable criterion, as can be seen from the two types of cells in a and b.

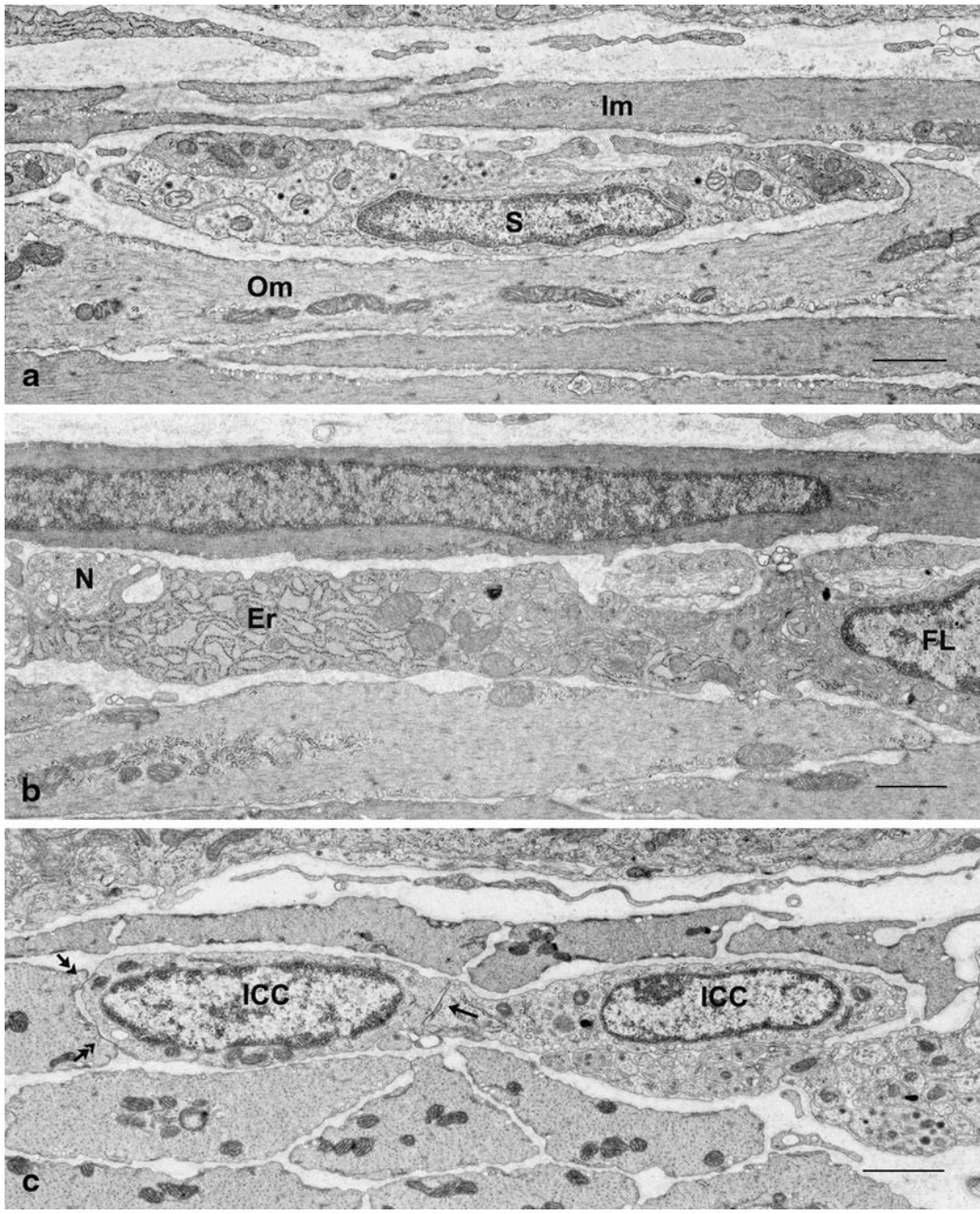

Fig. 9.6 Distinguishing ICC from other cell types (II). **a** Schwann cell (*S*) located in the region of the deep muscular plexus between the inner (*Im*) and outer (*Om*) sublayers of the circular muscle in the rat small intestine. It closely holds many nerve fibres by the thin processes. (Note, the poor cytoplasm in the perinuclear region). ×13,000. *Bar* 1 μm (Reproduced from Komuro and Seki [82] with permission of the publisher). **b** Fibroblast-like cell (*FL*) found in the region of the deep muscular plexus of the rat small intestine. Dilated cisterns of granular endoplasmic reticulum (*Er*) contain moderate electron dense materials. An axon varicosity containing many synaptic vesicles (*N*) is lodged in a surface indentation of the cell. ×13,000. *Bar* 1 μm (Reproduced from Komuro and Seki [82] with permission of the publisher). **c** ICC-DMP (*ICC*) of the mouse small intestine. These cells are connected by a gap junction with each other (*arrow*) and with the smooth muscle cell (*double-headed arrow*). Large gap junctions are the only reliable criteria in this profile of the cell that otherwise has few organelles. ×13,000. *Bar* 1 μm. (Reproduced from Komuro [54] with permission of the publisher).

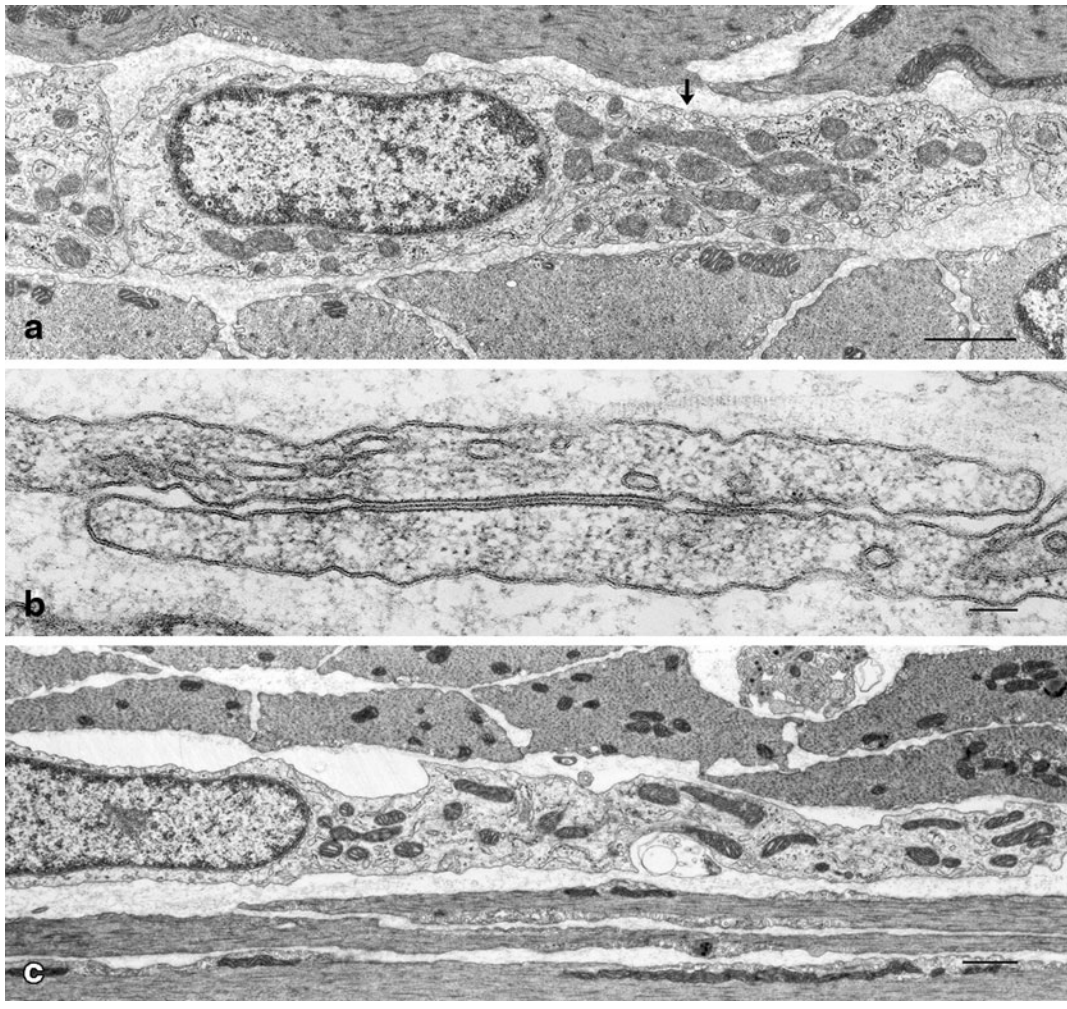

Fig. 9.7 ICC-MP of the small intestine. **a** ICC-MP of the rat small intestine belong to Type 1 ICC and are characterized by abundant mitochondria embedded in the electron-lucent cytoplasm. Caveolae are occasionally observed (*arrow*). ×16,000. *Bar* 1 μm (Reproduced from Komuro [50] with permission of the publisher). **b** A typical large gap junction between the slender processes of ICC-MP of the rat small intestine. ×1,65,000. *Bar* 0.05 μm (Reproduced from Horiguchi and Komuro [70] with permission of the publisher). **c** ICC-MP of the mouse small intestine which show very similar features to the rat small intestine described above. ×8,800. *Bar* 1 μm (Reproduced from Komuro et al. [74] with permission of the publisher).

9.2 Ultrastructural Features of ICC-MP

ICC-MP show some variations depending on the organ and the species in which they are found, and thus a specific set of ultrastructural features cannot be generalized. Important criteria of ICC distinguishing these cells from fibroblasts, such as the basal lamina and caveolae, are not always found in ICC-MP, though abundant mitochondria in the cytoplasm and large gap junctions are common. For example, ICC-MP of the mouse stomach described above (Fig. 9.2a–c) show Type 3 ultrastructural features (most muscle-like features), while ICC-MP of the mouse small intestine have the least muscle-like features described below (Fig. 9.7c). In this respect, ICC-MP require careful observation to correctly identify and interpret them.

9.3 Ultrastructural Features of ICC-CM and ICC-LM

ICC-CM and ICC-LM are prominent in the stomach and colon but not in the small intestine in the laboratory rodents including mice, rats and guinea-pigs, as already shown by immunohistochemical preparations. ICC-CM and ICC-LM of the stomach and colon have similar ultrastructural features, or Type 2 intermediate characteristics.

These cells connect with neighbouring smooth muscle cells via many large gap junctions and often show close contacts with nerve terminals containing many synaptic vesicles.

9.4 Ultrastructural Features of ICC-DMP

Ultrastructural features of ICC-DMP do not show major differences among the species and these cells belong to Type 3 most muscle-like ICC. A basal lamina and numerous caveolae are observed along the cell membrane. Subsurface cisterns of smooth endoplasmic reticulum can be found immediately beneath the cell membranes. Intermediate filaments are abundant in the cytoplasmic processes.

The most conspicuous feature of ICC-DMP is the frequent occurrence of large gap junctions that interconnect these cells with each other and also with smooth muscle cells. Their gap junctions with muscle cells are mainly formed with those of the outer subdivision, but some gap junctions with the muscle cells of inner sublayer are also observed. ICC-DMP have close contacts with nerve varicosities containing accumulations of synaptic vesicles. This means that ICC-DMP are intercalated between nerves and smooth muscle cells. Therefore, it is quite possible that ICC-DMP can act as an accessory route for neuromuscular transmission, as originally suggested by Cajal.

Two types of ICC-DMP have been described in the rat and guinea-pig below.

The functional significance of gap junctions in ICC-DMP can be evaluated from evidence that the percentage of the total cell area occupied by gap junctions is 1.3% in rats [42] and 4% in guinea-pigs [43]. These values are about 6 and 20 times greater respectively than the corresponding percentage area (0.2%) occupied by gap junctions on smooth muscle cells of the guinea-pig intestine [44]. The presence of these highly developed gap junctions also seem to be consistent with the notion that the well organized network of ICC-DMP acts as an impulse-conducting system analogous to that in the heart.

9.5 Ultrastructural Features of ICC-SMP

ICC-SMP of the colon are observed at the interface between the submucosa and the circular muscle layer. They have similar features to ICC-DMP and belong to Type 3 most muscle-like ICC. They are characterized by the presence of a basal lamina, caveolae and many mitochondria. They have gap junctions that interconnect with the same type of cells and connect with smooth muscle cells. Intermediate filaments are particularly abundant in the small processes. They often have close contacts with nerve varicosities containing many synaptic vesicles.

A specialized pacemaker function has been proposed in the colon, with ICC-SMP primarily responsible for generating the slow waves, and ICC-MP acting as secondary pacemaker cells described above. However, it remains to be elucidated as to the reason why ICC-SMP are the primary pacemaker cells in the colon.

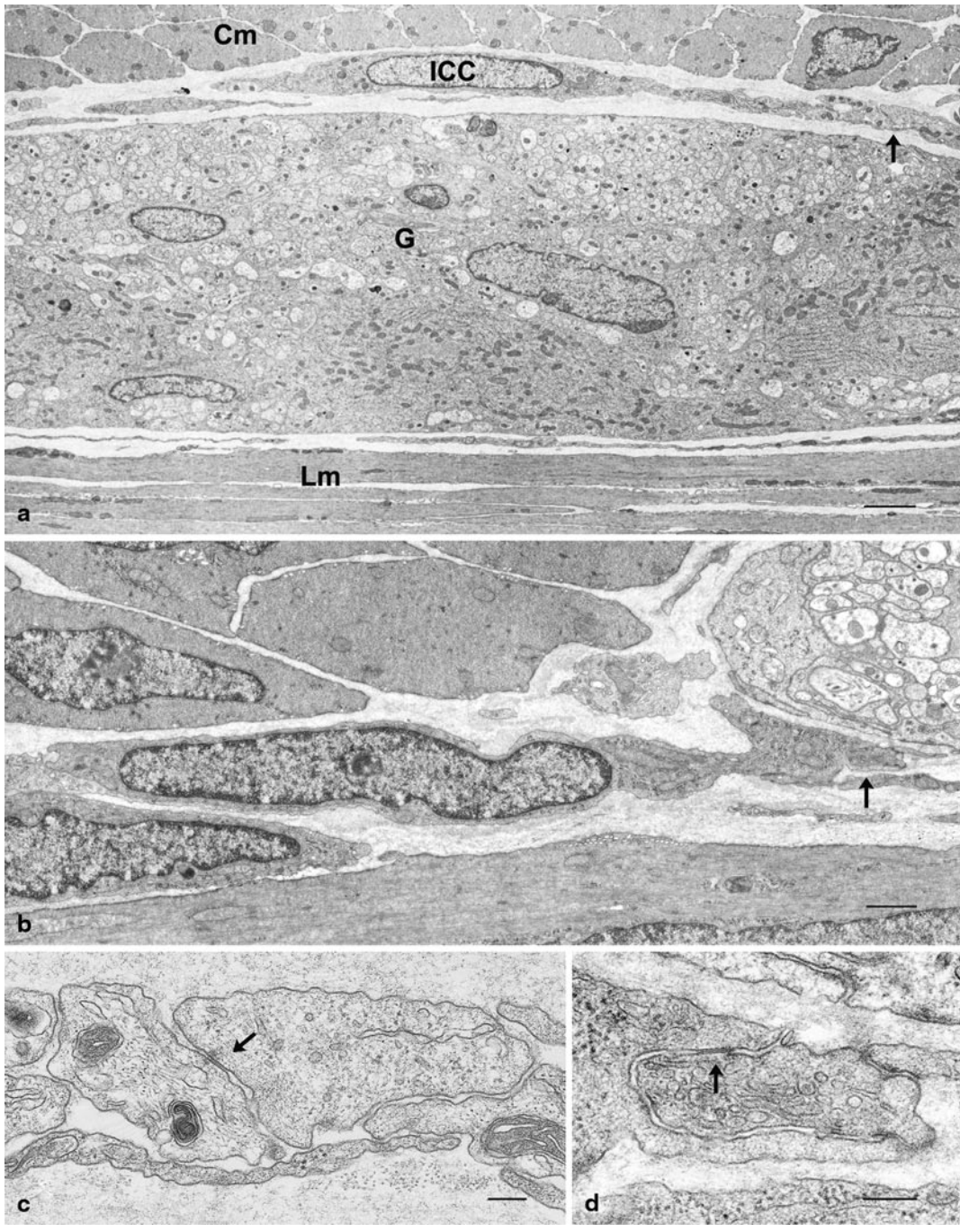

Fig. 9.8 ICC-MP of the small intestine and colon. **a** ICC-MP (*ICC*) of the guinea-pig small intestine located along the myenteric ganglion (*G*) between the inner circular (*Cm*) and outer longitudinal (*Lm*) muscle layers. ICC-MP of the guinea-pig small intestine belong to the least muscle-like cell type of ICC (Type 1) and at first glance resemble fibroblasts. But they contains bundle of intermediate filaments and form a gap junction at their tips (*arrow* and **c**). ×4,800. *Bar* 2 μm. **b** A ICC-MP of the rat colon that also shows similar cytoplasmic features to fibroblasts, but it forms a gap junction with the same type of cell (*arrow* and **d**). ×9,000. *Bar* 1 μm (Courtesy of Dr. Ishikawa, Waseda University). **c** Higher magnification of the region with arrow in **a**, showing the gap junction (*arrow*) observed between fine processes of ICC-MP. ×35,000. *Bar* 0.2 μm (Reproduced from Komuro et al. [15] with permission of the publisher). **d** Higher magnification of the gap junction (*arrow*) indicated by arrow in **b**. ×50,000. *Bar* 0.2 μm.

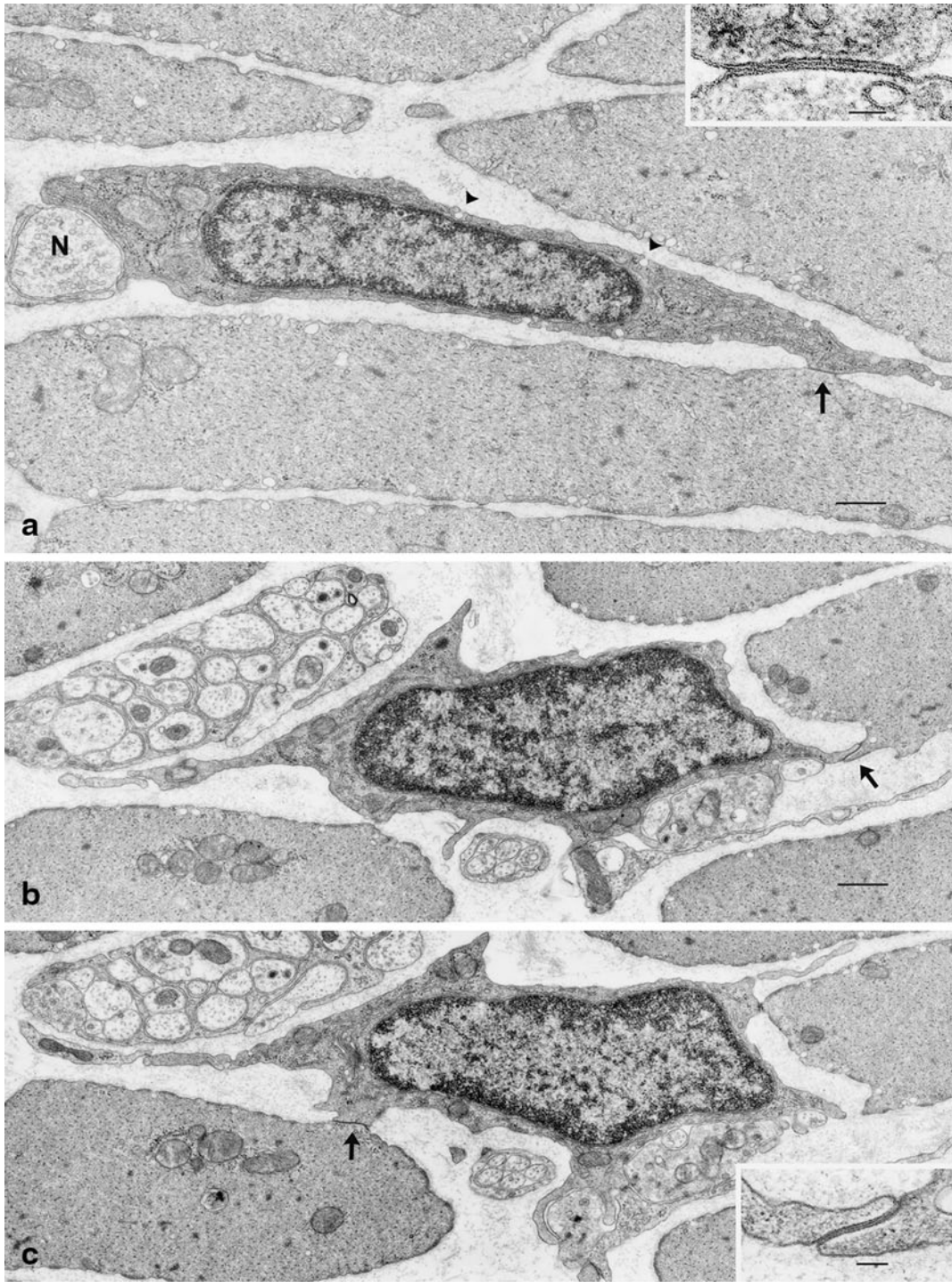

Fig. 9.9 ICC-CM intercalated between the nerve and muscles. **a** ICC-CM of the rat stomach, which show a close contact with a nerve terminal (*N*) on one hand and form a gap junction (*arrow*) with a smooth muscle cell on the other. Caveolae are indicated by *arrow-heads*. ×18,000. *Bar* 0.5 μm. *Inset* Higher magnification of the gap junction indicated by an *arrow*. ×66,000. *Bar* 0.1 μm (Reproduced from Ishikawa et al. [83] with permission of the publisher). **b**, **c** Serial sections of ICC-CM in the rat stomach showing frequent connections by gap junctions with neighboring smooth muscle cells (*arrows*). ×18,000. *Bar* 0.5 μm. *Inset* The gap junction indicated by the *arrow* in **b**. ×56,000. *Bar* 0.1 μm (Reproduced from Komuro [30] with permission of the publisher)

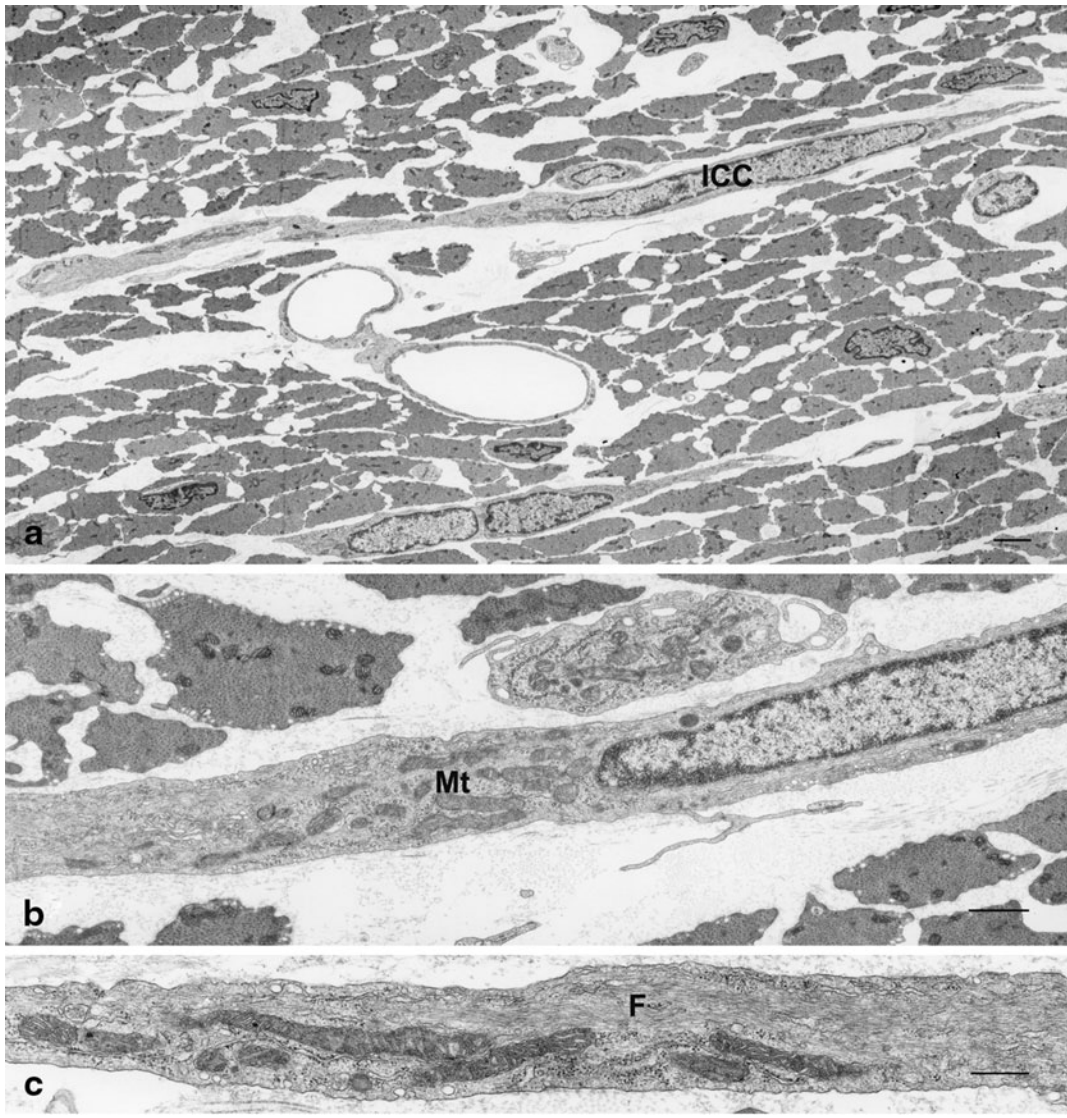

Fig. 9.10 ICC-CM in the rat stomach. **a** ICC found in the connective tissue septum in the circular muscle layer of the rat stomach. ×3,200. *Bar* 2 μm.

These cells are specially designated as ICC-SEP and are claimed to have different physiological significance, to transfer pacemaker depolarizations from ICC-MP to distant bundles of the circular muscle in the dog stomach [41]. **b** Higher magnification of the perinuclear cytoplasm of the same cell as **a** in a neighboring section characterized by many mitochondria (*Mt*). ×11,000. *Bar* 1 μm. **c** The further distal portion of the same cell as **a**, which is characterized by abundant intermediate filaments (*F*). ×19,000. *Bar* 0.5 μm (Reproduced from Mitsui and Komuro [68] with permission of the publisher).

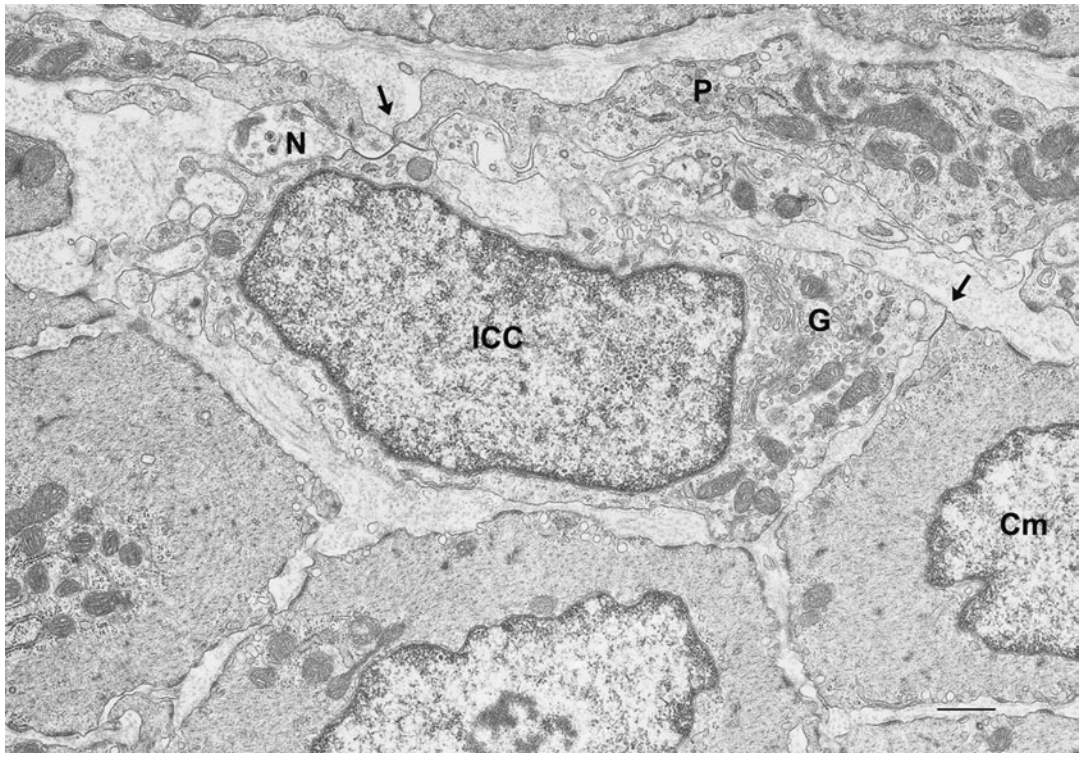

Fig. 9.11 Cross section of ICC-DMP of the rat small intestine. ICC-DMP (*ICC*) are characterized by electron-lucent cytoplasm and gap junctions (*arrows*) with processes of the same type of cell (*P*) containing many mitochondria and other gap junctions with the muscle cell of the circular layer (*Cm*). A nerve varicosity (*N*) is closely adjacent to the cell. Golgi apparatus (*G*) is located in the perinuclear region. ×18,000. *Bar* 0.5 μm (Reproduced from Komuro and Seki [82] with permission of the publisher).

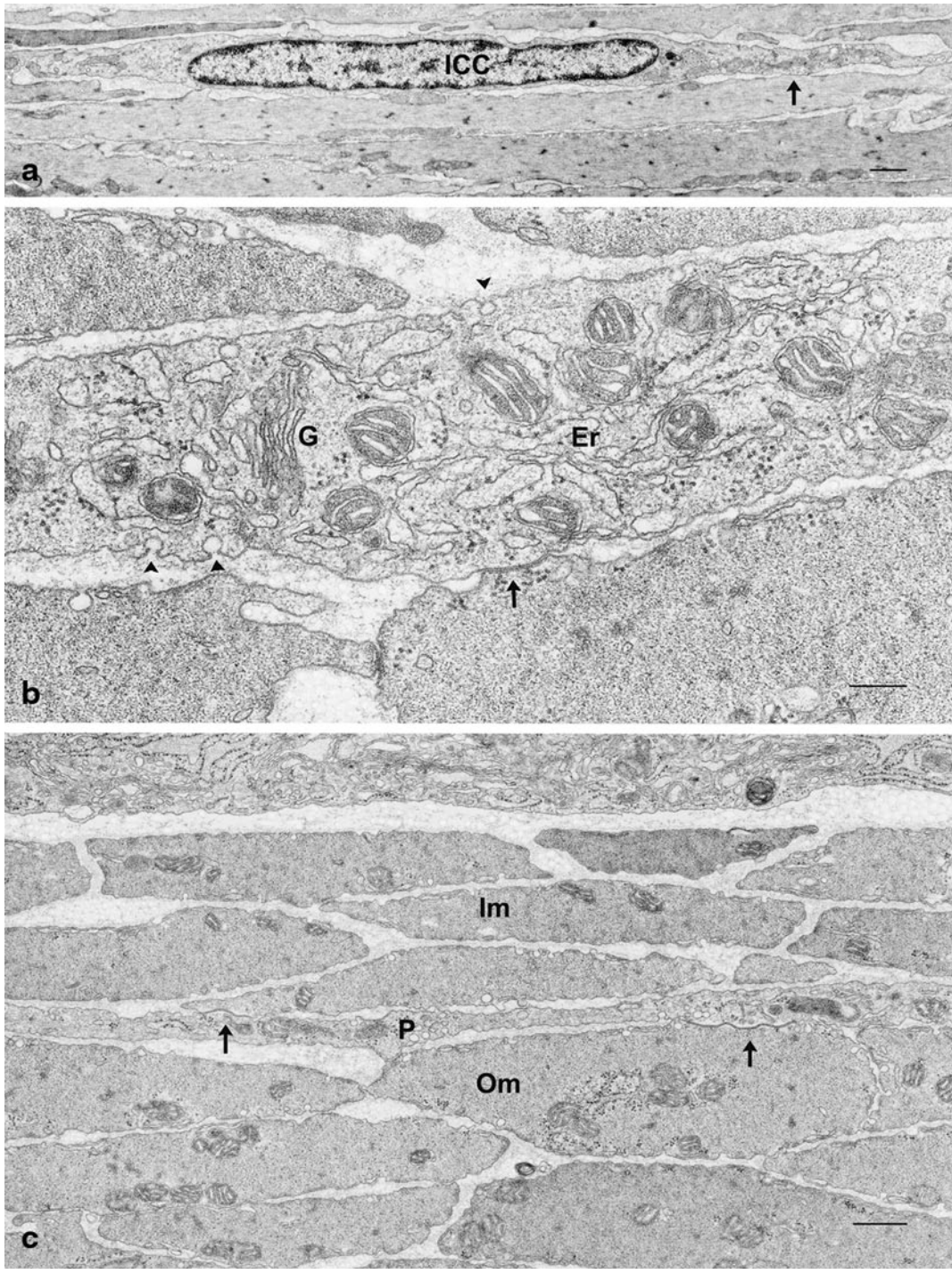

Fig. 9.12 Longitudinal section of ICC-DMP of the rat small intestine. **a** A slender cell body of an ICC-DMP (*ICC*) is located in the narrow space between the inner thin and outer thick circular muscle layers. It forms a gap junction with the adjacent muscle cell (*arrow*). ×7,000. *Bar* 1 μm. **b** The cytoplasm of an ICC-DMP of the rat small intestine, which is characterized by electron-lucent cytoplasm, caveolae (*arrowheads*), many mitochondria and formation of gap junction with the muscle cell (*arrow*). Golgi apparatus (*G*) and granular endoplasmic reticulum (*Er*) are also observed. ×46,000. *Bar* 0.2 μm. **c** A slender cell process (*P*) of an ICC-DMP forming gap junctions (*arrows*) with the muscle cells of inner (*Im*) and outer (*Om*) sublayers. ×19,000. *Bar* 0.5 μm (Reproduced from Komuro and Seki [82] with permission of the publisher).

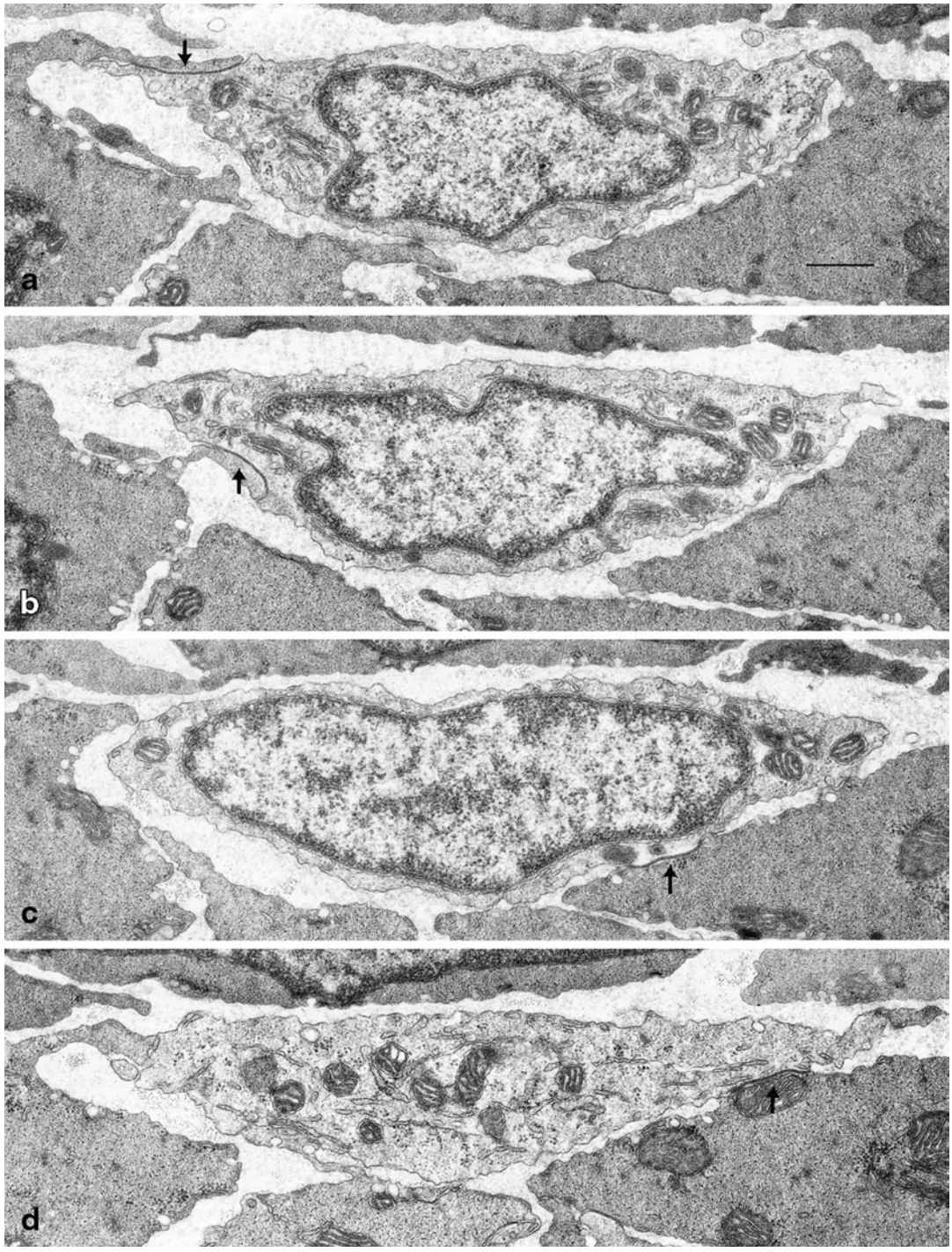

Fig. 9.13 Serial sections of ICC-DMP of the rat small intestine (**a–d**) showing how often a single cell of ICC-DMP forms gap junctions (*arrows*) with the adjacent smooth muscle cells. ×23,000. *Bar* 0.5 μm (Reproduced from Seki and Komuro [42] with permission of the publisher).

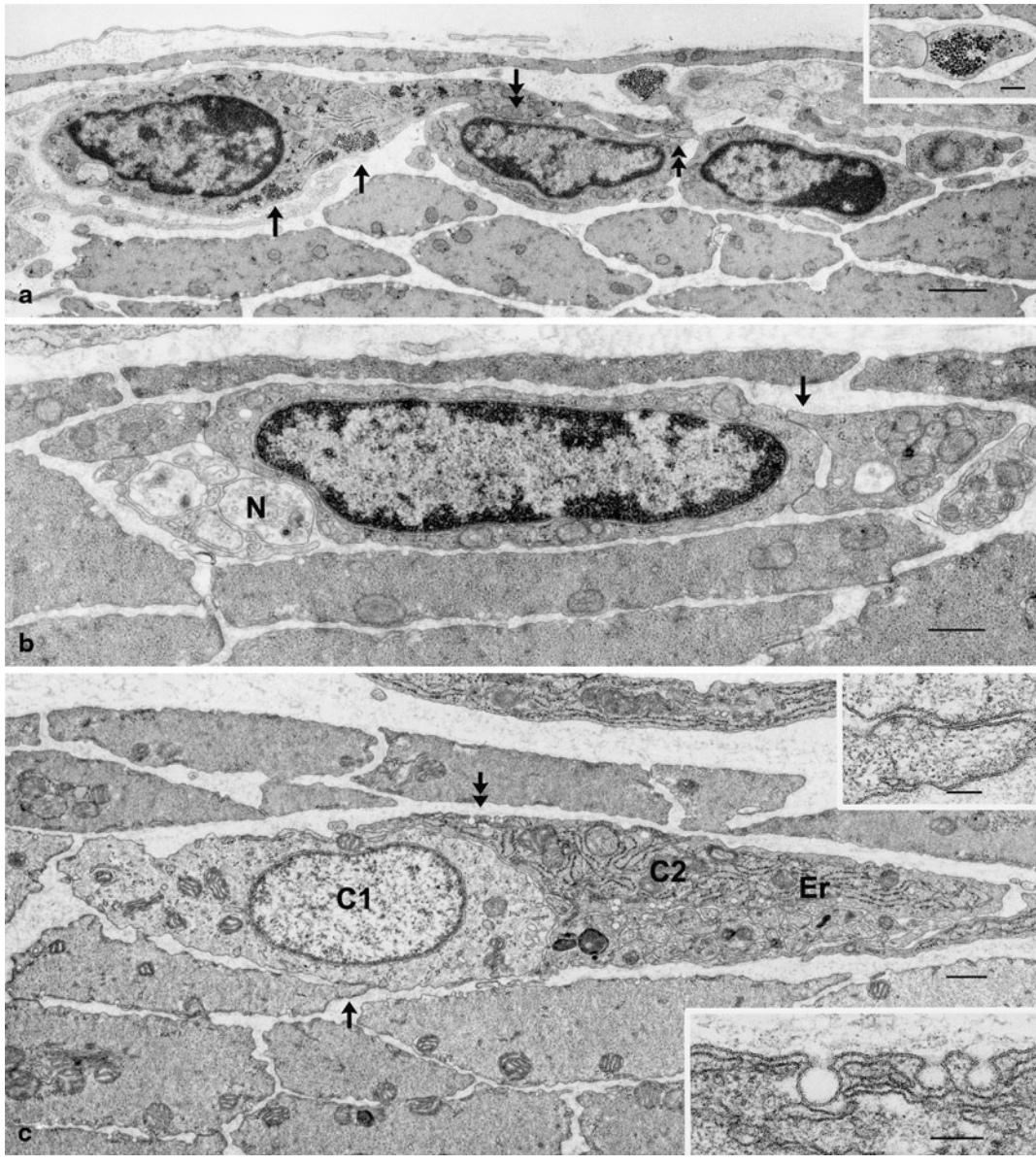

Fig. 9.14 Evidence potentially showing two subtypes of ICC-DMP. **a** A possible subtype of ICC-DMP in the guinea-pig small intestine, which is characterized by clusters of glycogen granules (*arrows*) and by forming gap junctions with the same type of cells (*double-headed arrow*). ×10,000. *Bar* 1 μm (Reproduced from Komuro et al. [15] with permission of the publisher). *Inset* Higher magnification of the small process containing glycogen granules and form a gap junction with the same type of the cell. *Bar* 0.5 μm. **b** Another subtype of ICC-DMP of the guinea-pig small intestine showing a gap junction with a process of the same type of cell containing many mitochondria (*arrow*) and close contact with a nerve varicosity containing many synaptic vesicles (*N*). ×18,000. *Bar* 0.5 μm (Reproduced from Komuro [30] with permission of the publisher). *These two types of cells are densely distributed in an alternate fashion in the space between the inner and outer sublayers of the circular muscle [43, 45].* **c** Possible two types of ICC-DMP of the rat small intestine. One (*C1*) is characterized by electron-lucent cytoplasm and the other (*C2*) is characterized by electron-dense cytoplasm containing many cisterns of granular endoplasmic reticulum (*Er*). The arrow indicates a gap junction of the former cell and double-headed arrow indicate caveolae and basal lamina of the latter. ×15,000. *Bar* 0.5 μm. *Top inset* The gap junction indicated by the *arrow* ×60,000, *Bar* 0.1 μm; *Bottom inset* Caveolae and the basal lamina indicated by the double-headed arrow. ×90,000, *Bar* 0.1 μm (Reproduced from Seki and Komuro [48] with permission of the publisher).

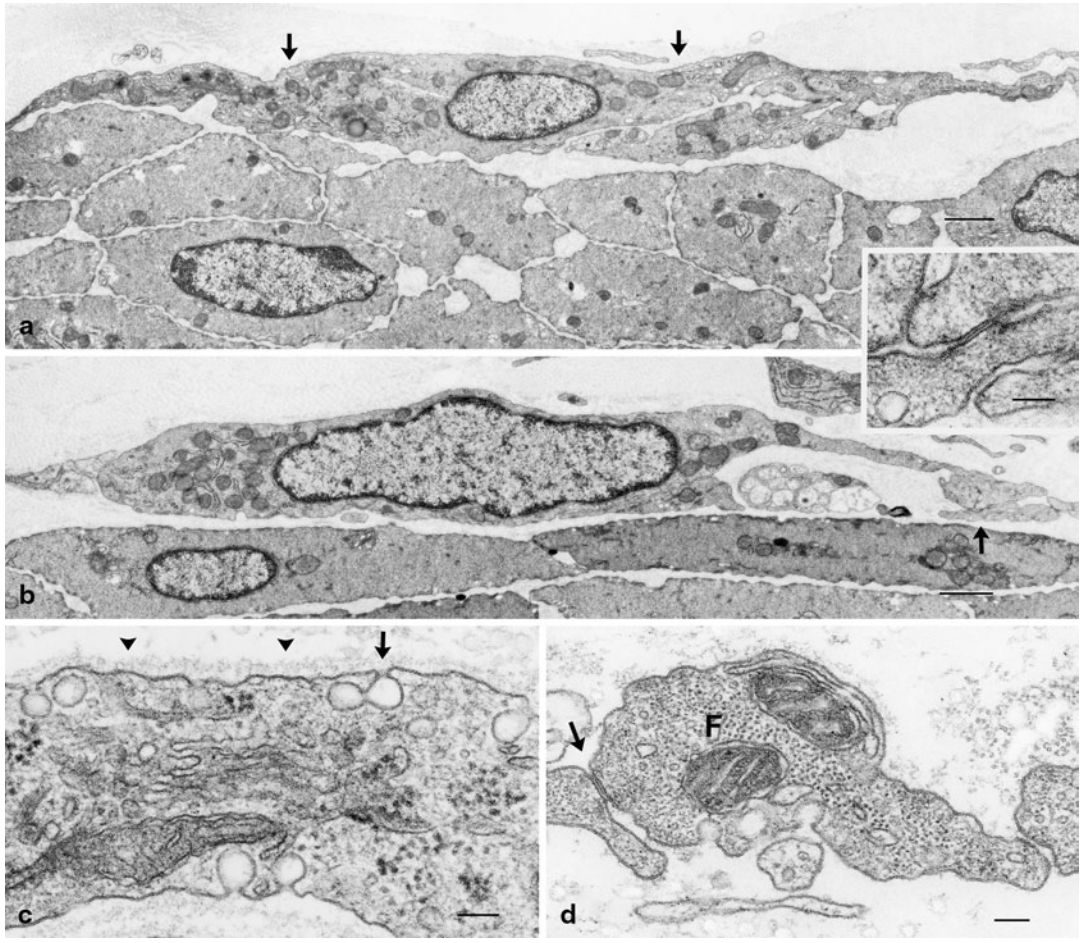

Fig. 9.15 ICC-SMP of the colon. **a** ICC-SMP at the submucosal border of the circular muscle layer of the guinea-pig colon. Many mitochondria and caveolae (*arrows*) are observed. ×8,200. *Bar* 1 μm (Reproduced from Komuro [30] with permission of the publisher). **b** ICC-SMP of the rat colon. This cell also contains many mitochondria and form gap junctions with the processes of the same type of cell (*arrow*) (Courtesy of Dr. Ishikawa, Waseda University). ×9,000. *Bar* 1 μm. *Inset* Higher magnification of the gap junction indicated by an *arrow* in **b**. ×70,000. *Bar* 0.1 μm. **c** Higher magnification of the process of the rat ICC-SMP showing the continuous basal lamina (*arrow heads*) and caveolae (*arrow*). ×68,000. *Bar* 0.1 μm. **d** Process of ICC-SMP of the guinea-pig colon showing abundant intermediate filaments (*F*) and gap junction (*arrow*). ×57,000. *Bar* 0.1 μm (Reproduced from Ishikawa and Komuro [73] with permission of the publisher).

9.6 Ultrastructural Features of ICC-SP

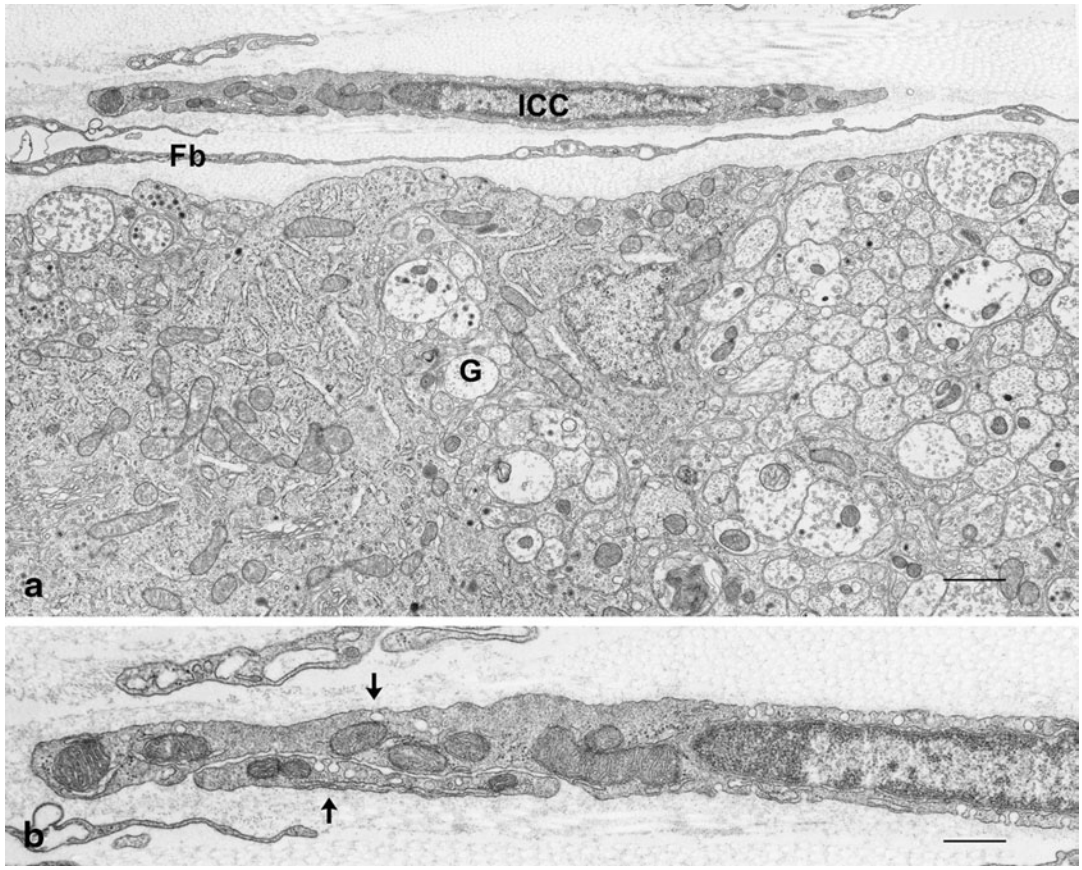

Fig. 9.16 ICC-SP in the guinea-pig proximal colon. **a** ICC-SP (*ICC*) located over the submucosal ganglion (*G*) in the guinea-pig proximal colon. Slender processes of the fibroblasts (*Fb*) are intervened between them. ×10,000. *Bar* 1 μm. **b** Higher magnification of the left half of the cell body of ICC-SP in **a**, showing caveolae (*arrows*) and many mitochondria in the cytoplasm. ×20,000. *Bar* 0.5 μm (Reproduced from Tamada and Komuro [84] with permission of the publisher).

Ultrastructural features of ICC-SP have only been observed in the guinea-pig colon so far, but ICC-SP, at least, in this material seems to have similar features to ICC found in/on the external muscle coat. They can be categorized as Type 3 cells or most muscle-like ICC.