

Synapse formation in retinoblastoma tumours

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Although several papers have recently been published on the ultrastructure of retinoblastoma tumours (Ts'o, Fine, and Zimmerman, 1969a, 1970; Ts'o, Fine, Zimmerman, and Vogel, 1969b; Choux, Tripier, Bérard, Hassoun, and Toga, 1972; Sevel, Röhm, and Sealy, 1974; Radnót, 1975) the discovery of synapse formation in this

type of tumour pointed to new information on the histogenesis of retinoblasts.

Materials and methods

A portion of a retinoblastoma tumour which had been fixed in 10 per cent neutral buffered formalin was prepared for transmission electron microscopy. After postfixation in osmium tetroxide and dehydration in graded alcohols and propylene oxide the tissue was embedded in Durcupan, and sectioned and examined in a Philips 300 electron microscope.

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Results

Previous light microscopical examination of the globe had confirmed the presence of a retinoblas-

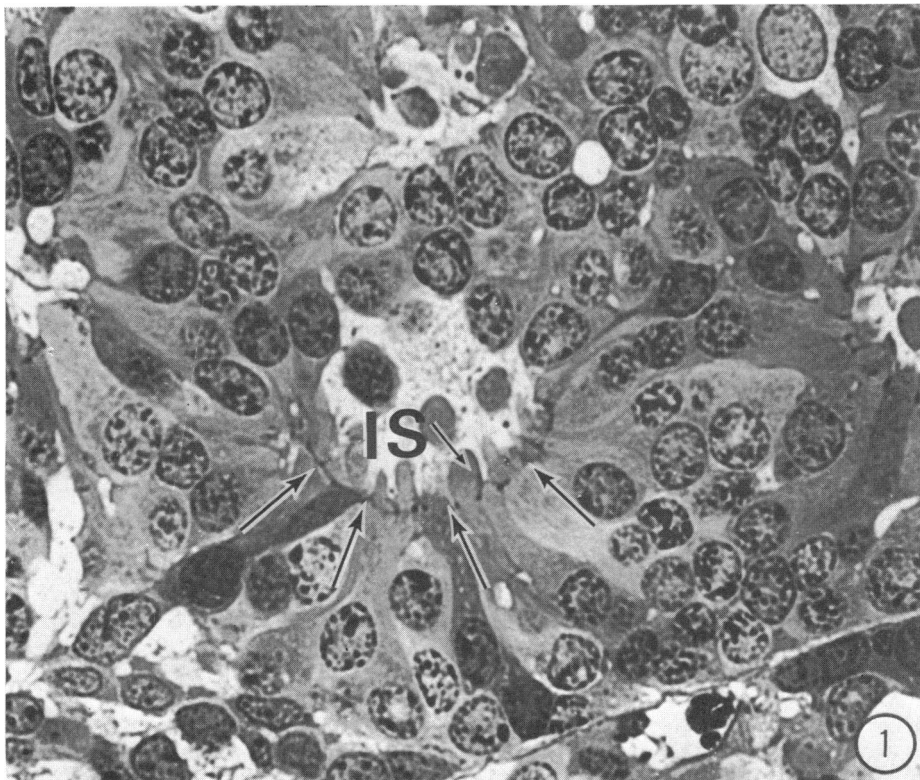


FIG. 1 *Light micrograph of 0.5 µm plastic section of retinoblastoma fleurette. Some cells show features of photoreceptor differentiation which include elongated inner segment regions (IS) that project beyond fenestrated membrane (arrows) into lumen of fleurette. × 2000*

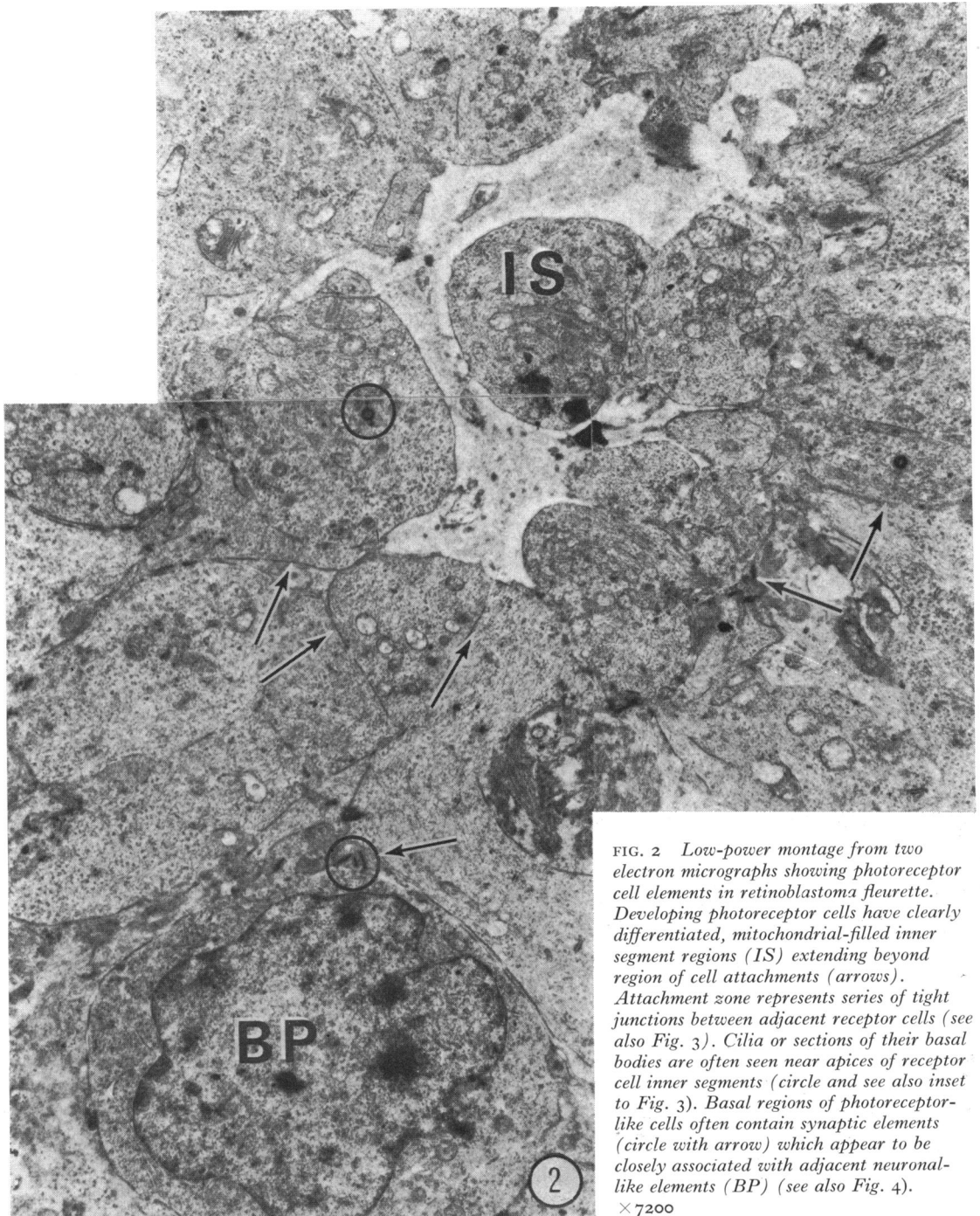


FIG. 2 *Low-power montage from two electron micrographs showing photoreceptor cell elements in retinoblastoma fleurette. Developing photoreceptor cells have clearly differentiated, mitochondrial-filled inner segment regions (IS) extending beyond region of cell attachments (arrows). Attachment zone represents series of tight junctions between adjacent receptor cells (see also Fig. 3). Cilia or sections of their basal bodies are often seen near apices of receptor cell inner segments (circle and see also inset to Fig. 3). Basal regions of photoreceptor-like cells often contain synaptic elements (circle with arrow) which appear to be closely associated with adjacent neuronal-like elements (BP) (see also Fig. 4).*
 ×7200

toma with tumour-cell appearances ranging from undifferentiated neuroblastic cells to incomplete rosettes and to well-formed Flexner-Wintersteiner rosettes and fleurettes (Dickson, Ramsey, Tonus, and Goldberg, 1976).

The fleurettes were in the form of asymmetric tumour-cell clusters with some of the cells forming elongated photoreceptor-like elements (Figs 1 and 2). These cells had long apical cytoplasmic processes joined by a fenestrated membrane which the

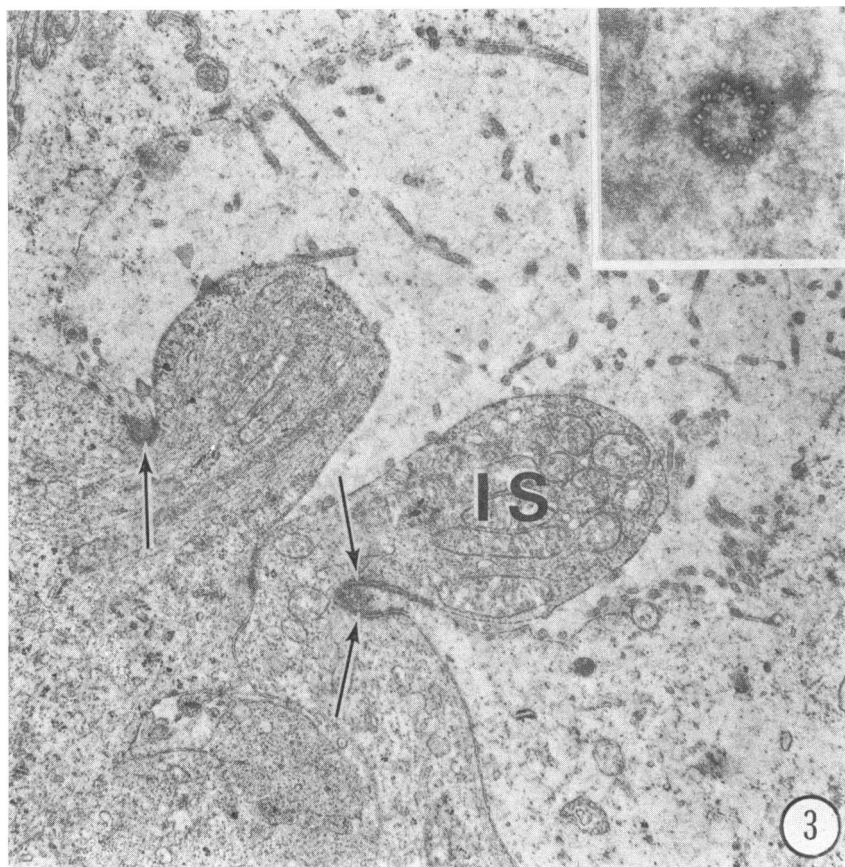


FIG. 3 *Electron micrograph of mitochondrial-filled inner segment regions of two developing photoreceptor elements from retinoblastoma fleurette. Mitochondria and microtubular structures seen within bulbous inner segment regions (IS) and apical tight-junctional complexes between adjacent cells (arrows). Inset: high resolution micrograph of basal body region of cilium in Fig. 2. $\times 9500$. Inset $\times 53\,300$*

electron microscope showed to be composed of a series of tight junctions between adjacent receptor-like cells (Figs 2 and 3). The distal cytoplasmic processes of these developing receptor-like elements were expanded to form bulbous mitochondrial-filled endings. The apices of these cells contained a cilium and an abundance of microtubular structures typical of the inner segment regions of normal human photoreceptors. In addition, their basal regions contained synaptic elements which included elongated synaptic ribbons each with an arciform density and surrounded by clear synaptic vesicles measuring about 45 nm in diameter (Figs 2, 4, and 5). These terminals appeared to be making functional invaginated synapses with adjacent neuronal-like elements. The postsynaptic cells located near the basal regions of the receptor elements presented spherical profiles and were more electron dense than the presynaptic receptor elements.

The synaptic arrangements presented here are identical in appearance to the photoreceptor-bipolar cell synapses in the normal human retina.

Discussion

The electron microscopical appearance of retinoblastoma fleurettes was described first by Ts'o and others (1969b) and was reported to represent a much more advanced stage of tumour cell differentiation than the Flexner-Wintersteiner rosettes described by Ts'o and others (1969b). Our data would clearly substantiate the present evidence on the ultrastructural characteristics of photoreceptor-like elements in retinoblastoma fleurettes. These include apical tight-junctional complexes between adjacent cells; apical mitochondrial-filled expansions (presumably developing inner segment ellipsoids), and apically positioned cilia. In addition, we have found further evidence of the advanced state of differentiation of this tumour in the form of receptor-cell synapses with adjacent neuronal elements.

The synaptic elements reported here are composed of synaptic ribbons (lamellae) and arciform densities within the basal region of the developing receptor-like cells. These ribbons were found to be surrounded by clear, 45 nm vesicles. Further,

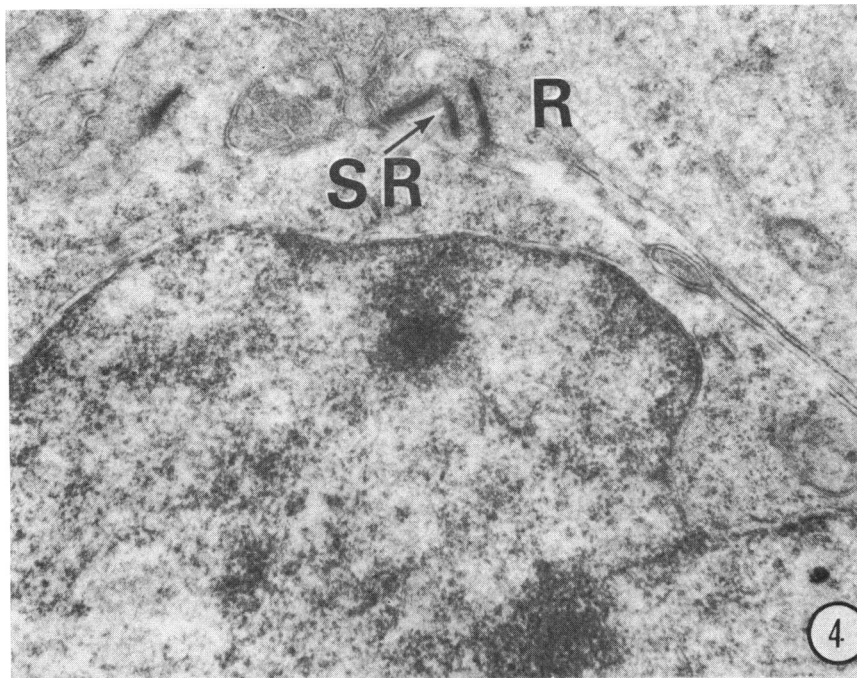


FIG. 4 *Electron micrograph of synaptic area indicated by circle with arrow in Fig. 2. Synaptic elements visible include synaptic ribbons (SR) and associated synaptic vesicles. R= receptor cell. BP (Fig. 2) = postsynaptic neuronal element. $\times 28\ 500$*

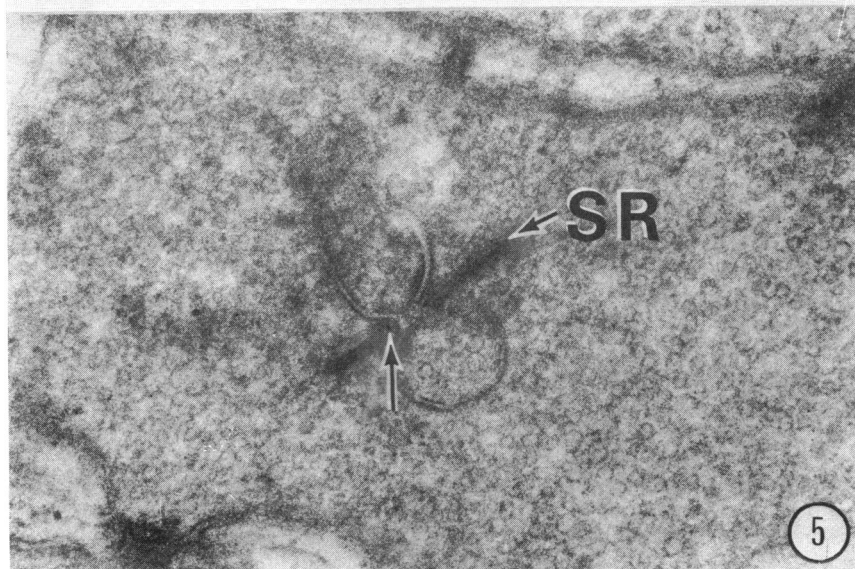


FIG. 5 *High resolution electron micrograph of receptor cell terminal (R) with two synaptic ribbons (SR), one with arciform density (arrow) and both surrounded by clecr, 45-nm synaptic vesicles. Invaginated synapse occurring with adjacent neuronal element (BP (Fig. 2)). $\times 57\ 300$*

these receptor-like cells made close invaginated contact with basally located cells, which we suggest represent tumour cell differentiation along the lines of bipolar neurons. This whole complex appears in all respects to be analogous to the outer plexiform layer synaptic associations between normal human photoreceptor cells and bipolar neurons.

Synaptic vesicles were first reported in retino-

blastoma Flexner-Wintersteiner rosettes by Choux and others (1972), but they did not identify synaptic ribbons associated with the vesicles. Radnót (1975) has identified isolated examples of synaptic lamellae in retinoblastoma tumour cells. She has not, however, reported these structures within developing receptor cells.

According to Spira and Hollenberg (1973)

functional synapses are seen in developing human retinas before the formation of outer segment disc membranes. The presence of a synaptic apparatus but absence of outer segment discs from the developing receptor cells in the fleurettes of this tumour suggests further proof of a similarity in development between normal human retinal receptors and the receptor-like cells of retinoblastoma tumours.

Further, our study would suggest that retinoblastoma rosettes and fleurettes represent sequential or progressive stages of tumour differentiation along photoreceptor lines. From the work of Choux and others (1972) and our findings (Dickson and others, 1976) initial synapse formation appears to begin at the rosette stage with a bulbous vesicle-filled sac near the base of the developing receptor

cell. Later, in the fleurette stage, a synaptic ribbon forms and invaginated postsynaptic contact is established with other developing neuronal elements to form a functional synapse.

Summary

Developing photoreceptor-like cells in retinoblastoma tumour fleurettes were found to contain apical tight junctions, mitochondrial-filled inner segment regions with cilia, and basally located synaptic ribbons and vesicles. Synaptic contact appeared to be established with adjacent neuronal-like elements. We suggest that photoreceptor differentiation in retinoblastoma tumours follows a sequence similar to normal human photoreceptor embryogenesis.

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