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Subcellular Localization of an Intermediate Filament Protein and Its mRNA in Glial Cells

P. VIJAY SARTHY,* MARLENE FU, AND JING HUANG

Department of Ophthalmology, Physiology and Biophysics, University of Washington, Seattle, Washington 98195

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Eucaryotic mRNAs are generally localized in the cell body, where most protein synthesis occurs. We have found that mRNAs encoding the glial intermediate filament protein are spatially distributed in the glial cell cytoplasm close to the location of the glial filaments. Whereas the glial filament protein mRNA was located predominantly in the distal process, actin mRNA was found almost exclusively in the apical portion of the glial cell. This pattern of mRNA localization might provide a mechanism for synthesis of proteins in specific subcellular compartments by mRNA translation locally.

In eucaryotic cells, mRNA localization studies have shown that mRNAs encoding cellular and secreted proteins are usually present in the cellular perikarya, where most protein synthesis occurs. In the nervous system, where newly synthesized proteins often have to be transported from the cell body to the synaptic terminals over long distances, mRNA compartmentalization might provide an alternative mechanism for synthesis of proteins near their sites of function (9). During a study of the expression of the glial intermediate filament protein (GFAP) gene in the mouse retina by in situ hybridization, we observed that GFAP mRNAs were distributed in the cytoplasm of retinal glial cells, where GFAP is also localized. These data suggest that mRNAs may be spatially distributed in close proximity to the products they encode, thus enabling maintenance of protein levels by local protein synthesis.

Figure 1 presents the localization of GFAP and GFAP mRNA in sections of mouse retina. Immunocytochemistry and in situ hybridizations were carried out according to published protocols (3, 8, 17). When retinas were stained with antibodies to GFAP, a radial staining pattern was noted (Fig. 1B). The distribution of immunostaining corresponded to the disposition of Muller cells in the retina (Fig. 1A). In sections processed for in situ hybridization with a GFAP cDNA probe (11), we found that in addition to labeled cell bodies located in the inner nuclear layer, there was widespread distribution of silver grains across the retina. Silver grains were distributed in the inner nuclear layer, inner plexiform layer, and ganglion cell layer and extended all the way to the internal limiting membrane (Fig. 1C to F). This labeling pattern was found in both light-damaged (Fig. 1C and D) and dystrophic (Fig. 1E and F) retinas. Pretreatment of retinal sections with RNase before mRNA localization eliminated labeling of the tissue. Similar results were obtained with GFAP riboprobes. Whereas the GFAP antisense-strand transcript gave tissue labeling, the sense-strand transcript did not show labeling above the tissue background (data not shown). The riboprobes were not used because they gave too high a background to be suitable for work with single cells. Northern (RNA) blot analysis of retinas showed that there was little GFAP mRNA degradation (15); hence, the mRNAs found in the Muller cell processes were full-length transcripts rather than RNA fragments.

* Corresponding author.

To determine whether the radial labeling pattern that we observed was specific to GFAP mRNA or was a common feature of all mRNAs in Muller cells, we hybridized retinal sections to a ^{35}S-labeled β-actin probe. We chose the actin probe as a control because (i) both actin and GFAP are cytoskeletal proteins and (ii) mRNAs for the two proteins are equally abundant in the retina (15). Our results showed that actin mRNA was present at high levels in the retina (Fig. 1G and H). Furthermore, labeling was primarily confined to the inner nuclear layer and the ganglion cell layer, whereas the inner plexiform layer had relatively little labeling. A similar pattern of cell body labeling has been reported for actin mRNA in the chick retina (1, 14). Hence, unlike GFAP mRNA, actin mRNA appears to be mainly confined to the cell bodies in the retina. A comparison of labeling intensity in the different layers suggested that ganglion cells had the highest actin mRNA content, followed by inner nuclear layer somata; photoreceptor cell bodies showed the lowest actin mRNA levels.

The cytoplasmic distribution of GFAP mRNA was also examined in single Muller cells dissociated from the retina (Fig. 2A to C). These studies were carried out to ensure that the silver grains in the inner plexiform layer were in fact present in Muller cell processes. We found that some cells had mRNA in both the cell processes and the somata (Fig. 2A and B), whereas other cells showed mRNA only in the processes and end feet (Fig. 2C). From an examination of retinal sections with different degrees of light damage, it is our impression that initially GFAP mRNA is found primarily in the cell bodies. Subsequently, the RNA migrates to other areas of the Muller cells, particularly to the end-foot area (Fig. 2B and C). On the other hand, such highly restricted GFAP mRNA localization could also result from preferential loss of mRNA from other regions of the cell. The intensity of labeling of cellular structures was higher than that of the background on the slides or on neighboring cells (Fig. 2A and C).

For comparison, we also examined actin mRNA localization in dissociated retinal cells (Fig. 2D and E). We found that every Muller cell examined had a high concentration of silver grains in the cell body. In addition, silver grains were also found along the apical processes of most of these cells, although labeling in this region was less intense (Fig. 2D and E). This distribution of actin mRNA is in agreement with actin localization in isolated Muller cells (18). Most interestingly, we did not find actin mRNA in the distal portion of the

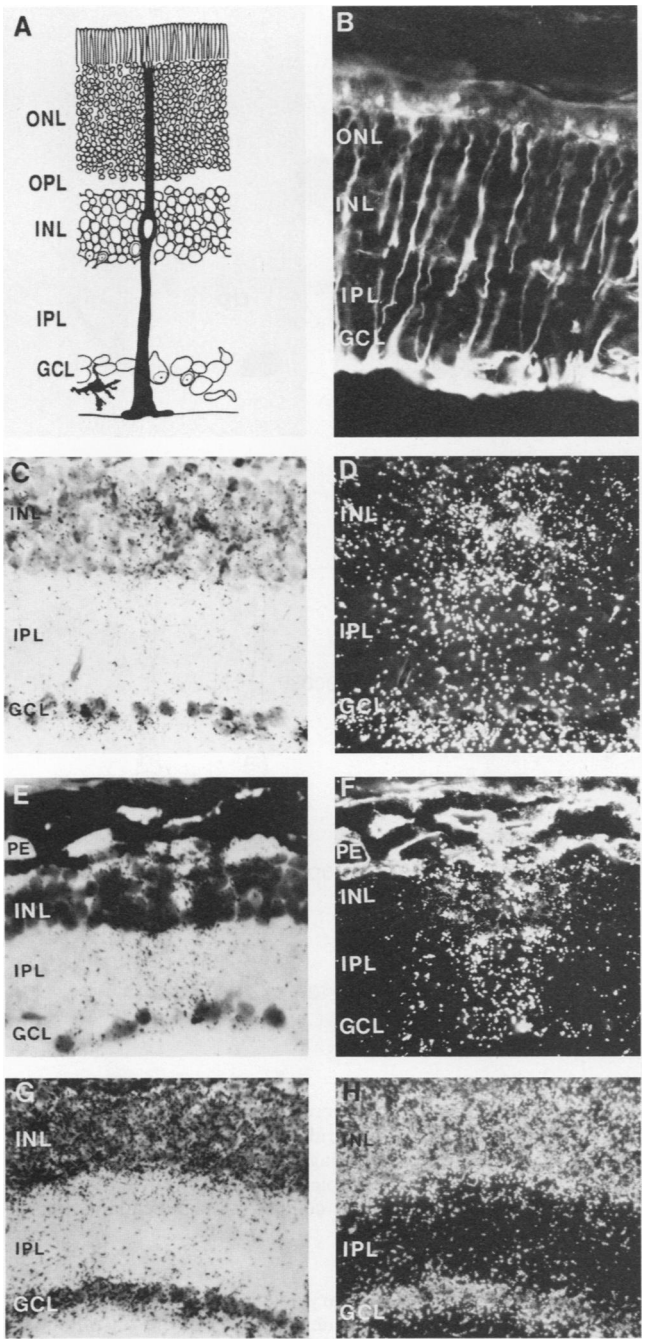

FIG. 1. Localization of GFAP, GFAP mRNA, and actin mRNA in the mouse retina. Eyes from retinal dystrophic (*rd/rd*) mice or BALB/c mice with retinal damage from constant light exposure for 2 weeks were fixed in 4% paraformaldehyde for 6 h. After immersion in 10% sucrose overnight, eyes were embedded and cut on a cryostat into 8-μm sections. The sections were processed for GFAP immunocytochemistry or in situ hybridization with ^{35}S-labeled DNA probes derived from GFAP (11, 15) and β-actin (pRβA-1) cDNA clones, using published protocols (3, 8, 17). (A) Diagram of the retina showing the different layers and the radial distribution of Muller cells. An astrocyte is shown in the ganglion cell layer (GCL). ONL, Outer nuclear layer; OPL, outer plexiform layer; INL, inner nuclear layer; IPL, inner plexiform layer. (B) Section immunostained with anti-GFAP. Note the staining of processes running radially. (C and D) Light-field (C) and dark-field (D) photographs of light-damaged retina labeled with the GFAP probe. (E and F) Light-field (E) and dark-field (F) photographs of *rd/rd* retina labeled with the GFAP probe. (G and H) Light-field (G) and dark-field (H) micrographs of light-damaged retina labeled with actin probe. Note the heavy distribution of silver grains in the INL, IPL, and GCL in panels C to F and labeling of the INL and GCL in panels G and H. The bright structure above silver grains in panels E and F is the pigment epithelium (PE). Magnification, ×640.

cell, particularly in the end foot (Fig. 2D and E). Hence, it appears that GFAP mRNA and actin mRNA are distributed differently in the Muller cell; whereas GFAP mRNA is located predominantly in the distal portion of the cell, actin mRNA is almost exclusively present in the apical region (Fig. 2H).

We also examined astrocytes in these cell suspensions to determine whether the distribution of GFAP mRNA was restricted to the cell body or, as with Muller cells, the mRNA was located in the cytoplasmic processes as well. We found that the mRNA was present in some of the cellular processes and the cell bodies (Fig. 2F and G). In the case of astrocytes, however, cell body labeling was stronger than labeling of the processes (Fig. 2F and G). Astrocytes with different morphologies showed the same labeling pattern.

The results presented in this study show that GFAP mRNA is not only present in the cell body but is also found in the processes of retinal glial cells. Since smooth endoplasmic reticulum is observed throughout Muller cell cytoplasm (19), it is possible that GFAP synthesis occurs in regions outside the perikaryon. In hippocampal neurons in culture, RNA has been shown to be transported from the cell bodies to the dendrites by an energy-dependent process (2). High levels of microtubule-associated protein 2 mRNA have been reported in dendrites in postnatal rat brain (5). In muscle fibers, Northern blot analysis and in situ hybridizations show that acetylcholine receptor subunit mRNAs are present at a higher concentration close to synaptic areas where acetylcholine receptors are present (4, 13). In situ hybridization studies with chicken myoblasts demonstrate that the actin mRNA level is about 16-fold higher at the lamellopodia than in the nucleus (9). The level of vimentin mRNA, on the other hand, is higher near the nucleus (9). In the study presented here, we found that the highest levels of actin mRNA were in the cell body region in both tissues and single cells. It is possible that cells grown in vitro show an mRNA distribution different from that found in vivo.

In the eggs and embryos of *Xenopus laevis*, *Drosophila melanogaster*, and ascidians, spatial compartmentalization of certain mRNAs has also been reported (6, 7, 12, 20). Interestingly, in *X. laevis* and *D. melanogaster*, the sequences involved in the asymmetric distribution appear to be present in the RNA itself (20) and, in the case of the bicoid mRNA, in its 3′ untranslated region (12). Hence, sequence-specific receptors are likely to be involved in mRNA anchoring (12). In the case of cytoskeletal proteins, it would be interesting to investigate whether a single receptor or a family of receptors is involved in mRNA trapping in different subcellular compartments. Alternatively, these mRNAs may be transported and anchored by the cytoskeletal proteins themselves.

Taken together, our results and those of other investigators show that in eggs, embryos, and fully developed organisms, certain mRNAs may be distributed in close proximity to the products they encode. Although the functional significance of the colocalization of a protein and its mRNA is not understood, it has been speculated that this mechanism may enable regulation of protein synthesis locally (9). Such a

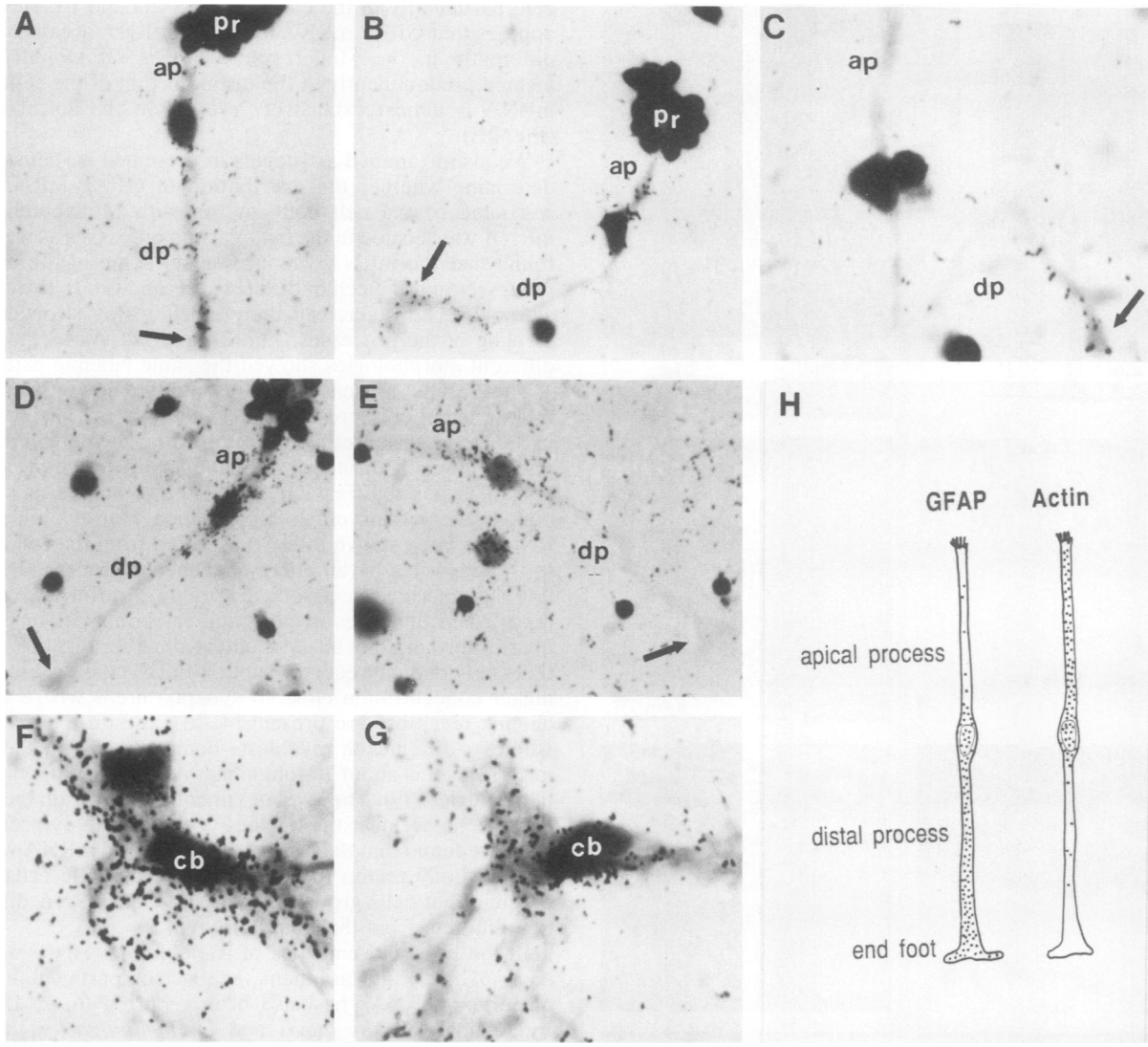

FIG. 2. Localization of GFAP and actin mRNAs in dissociated Muller cells. Single-cell suspensions were obtained from BALB/c retinas with 2 weeks of light damage according to previously published protocols (16). (A to C) Muller cells hybridized to the GFAP probe; D and E, Muller cells labeled with the actin probe; (F and G) astrocytes hybridized to the GFAP probe; (H) diagram showing the distribution of GFAP and actin mRNA in single Muller cells. pr, Clump of photoreceptor cell bodies attached to the apical process (ap) of a Muller cell. Arrows show the end-foot region of Muller cells located at the tip of the distal process (dp). cb, Astrocyte cell body. Magnifications, ×1,260 for panels A to E and ×2,000 for panels F and G.

mechanism may be particularly useful in the case of cytoskeletal systems, such as intermediate filaments, that turn over slowly (10).

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