5294 (1979)]. Total cellular RNA (10 to 15 μg) was fractionated in 0.7% agarose-formaldehyde gels, transferred to nylon membranes (Nytran, Schleicher and Schuell), and prehybridization and hybridization were performed as previously described [(*8*); H. Lehrach, D. Diamond, J. M. Wozney, H. Boedtker, *Biochemistry* **16**, 4743 (1978)]. The murine c-*kit* cDNA labeled with [^{32}P]phosphate, prepared by the random primer method, was used as a probe for hybridization [A. P. Feinberg and B. Vogelstein, *Ann. Biochem.* **132**, 6 (1983)].

22. Mast cells were labeled with [^{35}S]methionine for 5 hours and Triton X-100 lysates were prepared. Equal amounts of trichloroacetic acid precipitable counts were immunoprecipitated with rabbit antisera to c-*kit* conjugated to protein A–Sepharose and analyzed by SDS-PAGE (10%), and autoradiography as previously described (*8*). Immune complex kinase reactions were performed essentially as described (*3, 8*); equal numbers of cells were lysed in Triton X-100 and immunoprecipitated as above. Kinase reactions were carried out in 30 μl of 20 m*M* 1,4-piperazinediethanesulfonic acid (Pipes) (*p*H 7.2), 10 m*M* $MnCl_2$, 10 μ*M* ATP, 20 μ*M* sodium vanadate, and 20 μCi of [γ-^{32}P]ATP for 10 min at 30°C.

23. Flow cytometry was performed by labeling mast cells with kit antisera specific for extracellular determinants of c-*kit*. The antiserum to c-*kit* was obtained by immunization of rabbits with a recombinant vaccinia virus expressing the c-*kit* protein product (a detailed description of this reagent will be published elsewhere). Mast cells were labeled with preimmune and immune sera in phosphate-buffered saline (PBS) containing 5% bovine serum at 4°C for 30 min, washed, and labeled with fluorescein isothiocyanate (FITC)–conjugated goat antiserum to rabbit immunoglobulin G (Becton Dickinson). Cells were then washed and fixed in 1% paraformaldehyde in PBS and analyzed with a flow cytometer (FACSCAN, Becton Dickinson). Dead cells were gated out on the basis of forward and 90° light scatter; results are expressed as cell number (linear scale) versus fluorescence (log scale).

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Localization of an Acetylcholine Receptor Intron to the Nuclear Membrane

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The first intron of the RNA for the acetylcholine receptor (AChR) α subunit shows a ringlike distribution around nuclei in multinucleated myotubes by in situ hybridization. This pattern is not observed for an actin intron or U1 RNA. Quantitation of the intron sequences reveals large variations in the amount of both the AChR and actin introns between nuclei within the same myotube, although all nuclei express equivalent amounts of U1 RNA. This differential RNA expression indicates that nuclei can individually control expression of messenger RNAs. The restricted distribution of the AChR intron RNA suggests a previously unknown step in RNA processing.

IN SITU HYBRIDIZATION WITH DNA probes can reveal the distribution of mRNA in cultured cells. When embryonic chick myoblasts are cultured, they fuse to produce multinucleated myotubes, which spontaneously contract. Studies on the distribution of mRNA for the α subunit of the AChR, an essential component of the neuromuscular junction, have shown that nuclei in the same myotube differ in their expression of this transcript (*1–3*). We have used a probe for the first intron of the AChR α subunit mRNA (*4*) in such an in situ hybridization experiment with skeletal muscle cells from 11-day-old chick embryos. We hybridized the myotubes 4 days after plating with a highly radioactive, single-stranded DNA probe. After autoradiography, we stained the preparation with bisbenzamide and visualized the nuclei and the silver grains simultaneously by fluorescence and dark-field microscopy (*5*). A ringlike distribution of grains, confined to the periphery of the nuclei, was observed (Fig. 1, A to C). The peripheral distribution of grains was more apparent in dark-field images (Fig. 1B), with the centers of the nuclei being almost completely devoid of silver grains.

To confirm that this peripheral distribution of grains was not due to a permeability barrier, we hybridized the cells with a probe of comparable length for U1 small nuclear RNA (*6*), a member of a class of small RNAs that are present in the nucleus complexed with certain proteins to form small nuclear ribonucleoproteins (snRNPs) (7). In situ hybridization studies have shown that the U1 RNA is present in the nucleus with no preferential intranuclear localization (*8*). The U1 probe labeled all nuclei in the myotubes with a homogeneous distribution of grains (Fig. 1G).

As a further control, we hybridized the cells with a probe of similar length corresponding to the fifth intron (intron E) of chicken cardiac α actin, one of the most abundant actin species expressed during embryonic myotube development (*9*). The actin intron probe produced a pattern of silver grains confined diffusely to the nuclei (Fig. 1, D to F). Exon probes for both the AChR α subunit (Fig. 1H) and actin (Fig. 1I) mRNAs produced a homogeneous distribution of grains that was confined within the cytoplasm of the muscle cells. Because the labeling and exposure times were the same for all the probes, there would appear to be comparable amounts of actin and AChR α subunit mRNAs and introns.

A quantitative analysis of silver grain distribution revealed heterogeneity in the degree of nuclear labeling. An examination of 463 nuclei, identified with bisbenzamide, from three different cultures that were hybridized with the AChR α subunit intron probe showed that 21% of the nuclei had more than ten grains per nucleus, whereas 20% of nuclei were either devoid of grains or had less than two grains (Fig. 2A). Cells hybridized with the actin intron probe also showed variability among nuclei; however, the grain distribution was very broad and 90% of nuclei had more than ten grains per nucleus (Fig. 2B). In contrast, cells hybridized with the U1 RNA probe showed a uniform grain distribution among nuclei, with 99% of nuclei having greater than ten grains and none possessing less than two grains (Fig. 2C). The background counts in each of these experiments were equivalent to 0.2 to 0.3 grains per nucleus (*10*).

The localization of the AChR α subunit intron (or the entire pre-mRNA) to the periphery of the nucleus represents a regionalization of transcripts of a type not seen before. The U1 probe labels the nucleoplasm of all nuclei without noticeable intranuclear regionalization. This shows that the nuclei are penetrable by the probes and that all nuclei are functional. The general nuclear localization of the actin intron further shows that the ring localization is an unusual feature of the AChR α subunit intron. The pattern seen with the AChR α subunit intron resembles the peripheral labeling described by Hutchison and Weintraub (*11*) after their in situ nick translation of the exposed chromatin in mouse L cells; they hypothesized that an exposed peripheral region of nuclei was the site of transcription. However, the rings in our experiments are unlikely to be a direct indication of transcription of the AChR α subunit gene because only two copies of the gene exist in

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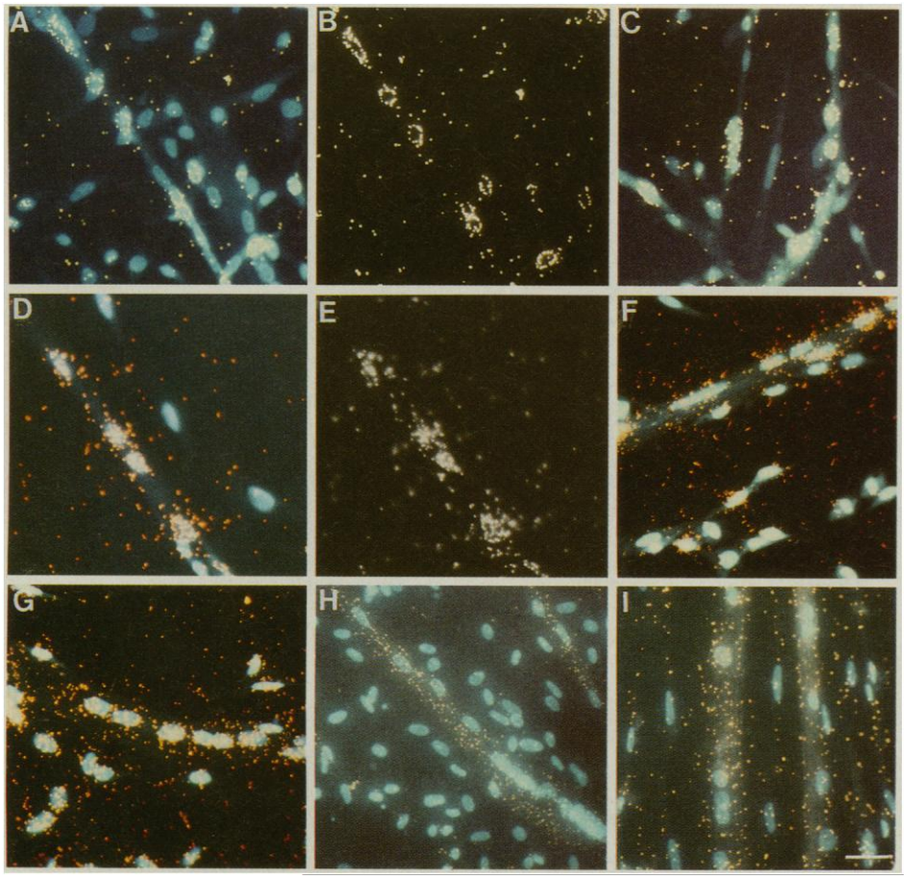

Fig. 1. In situ hybridization of myotubes with ^{35}S-labeled probes to an AChR α subunit intron (**A** to **C**), an actin intron (**D** to **F**), U1 RNA (**G**), an AChR α subunit exon (**H**), and an actin exon (**I**). The nuclei, stained with bisbenzamide, are blue, and the silver grains appear orange or white in dark-field images. All images are double-label exposures except (B) and (E), which are corresponding dark-field images of (A) and (D), respectively. The outlines of myotubes are readily seen when focusing with the microscope. The neighboring nuclei that are most predominant in (H) are either in myoblasts or fibroblasts. Scale bar, 50 μm.

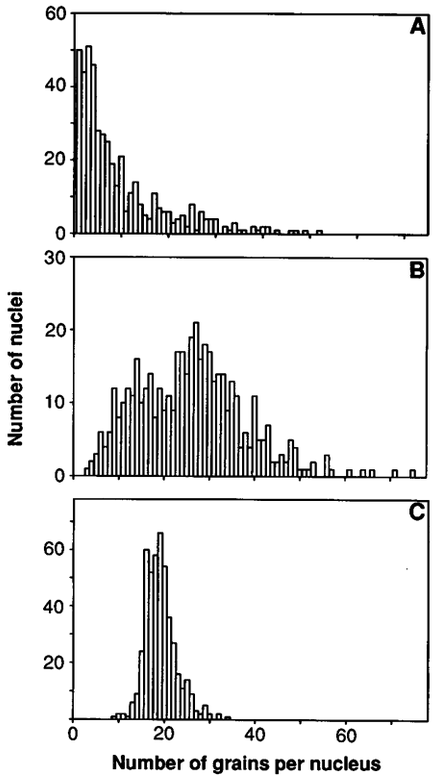

Fig. 2. Histograms showing heterogenous distribution of RNA among nuclei of chick myotubes. Muscle cells were hybridized with probes to the AChR α subunit intron (**A**), the actin intron (**B**), or U1 RNA (**C**) (*4*). Cells were viewed with fluorescent optics to visualize nuclei and a dark-field condenser to allow the simultaneous observation of the position of silver grains with respect to the nuclei. All counts were done with a ×60 objective and a microscope containing a drawing tube. Grains over the nuclei were counted on a digitizing tablet connected to an Apple IIe computer (*19*). By focusing up and down it was possible to quantitate all the grains with a Bioquant morphometry program. The results of one of two separate experiments is shown. In each experiment three cover slips bearing myotubes containing 463 nuclei were counted.

each nucleus of these diploid cells; if the mRNA were localized as it is transcribed, one would expect two foci of grains (*12*). Thus the intron RNA must be either freely diffusing in some compartment beneath the outer nuclear membrane or bound in some way in this compartment. This perinuclear accumulation of the AChR α subunit intron may reflect an aspect of RNA processing or efflux.

Blobel (*13*) has suggested that the nuclear pores interact specifically with the active genomic regions to gate transcripts to specific pores. This type of mechanism also may allow for selective transport of exons out of the nucleus, whereas the introns are retained within. Other nuclear-associated matrix proteins, such as lamins, may interact with certain intron sequences, allowing for their accumulation at the periphery of the nucleus. Our results support the concept of such differential interactions between various RNA species and structures in the vicinity of the nuclear membrane. We may be observing the result of quantitative differences in the proportion of RNA available to specific perinuclear binding sites such as occurs in the case of transfer RNA ligase, in which a ringlike pattern is observed at low levels of production (allowing all the ligase to be bound to binding sites in the nuclear membrane) and a diffuse nucleoplasmic pattern is observed at high levels of ligase production (*14*). Alternatively, there may be perinuclear binding sites for the first intron of the AChR α subunit RNA, but none for the fifth intron of α actin.

Our results may be a reflection of the differences between the proteins encoded by AChR α subunit and actin mRNAs. Actin is a cytoplasmic protein and its mRNA presumably could exit immediately into the cytoplasm to associate with free ribosomes. The AChR α subunit is a membrane-bound protein that must be directed to the endoplasmic reticulum (*15*); perhaps, in this case, the final splicing of certain introns occurs at binding or anchoring sites close to the nuclear envelope, thus allowing the mRNA to be directed to specialized pores.

Our comparison of three nuclear markers, an AChR α subunit intron, an α actin intron, and U1 RNA, demonstrates that nuclei within the muscle cell can vary in their production of different RNA species. Apparent differences in AChR production have been detected in sectioned skeletal muscle and cultured myotubes (*1, 2*), as has differential responsiveness to putative innervation factors (*3*) or actual innervation (*16*). A capacity of nuclei to vary individually would facilitate a regional specialization and response within the cell, such as the localization of AChR and other proteins to certain domains near one or more specific nuclei, and could explain the association between AChR clusters and myotube nuclei (*17*), as well as other evidence of nuclear domains (*18*).

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5. Pectoral muscle cells were obtained from 11-day-old chick embryos and plated on collagen-coated cover slips [S. Bursztajn, *J. Neurocytol.* **13**, 501 (1984)]. Cells were maintained in Eagle's minimum essential medium made with Earle's balanced salt solution and supplemented with 10% (v/v) horse serum and embryo extracts. Culture medium was changed every other day, and cells were fixed in 4% paraformaldehyde 4 days after plating. We used a modified hybridization protocol of J. B. Lawrence and R. H. Singer [*Nucleic Acids Res.* **13**, 1777 (1985)]. Cells were washed at room temperature with phosphate-buffered saline (PBS) or stored in 70% ethanol; in the latter case cells were rehydrated for 10 min in PBS containing 5 m*M* $MgCl_2$, followed by a solution containing 0.1*M* glycine and 0.2*M* tris-HCl (*p*H 7.4) for 10 min. Cells were hybridized for 10 min with 50% formamide containing 2× SSC (standard saline citrate) at 65°C (*1*). For each cover slip, 4×10^5 cpm of probe was lyophilized with 40 μg of *Escherichia coli* transfer RNA and 40 μg of sheared salmon sperm DNA, and the mixture was then resuspended in 10 μl of deionized formamide and combined with 10 μl of 4× SSC, 0.4% bovine serum albumin (BSA), 20 m*M* vanadyl ribonucleoside inhibitor, 20% (w/v) dextran sulfate, heparin (1 μg/μl), and 20 m*M* dithiothreitol (DTT). Cells were incubated with the hybridization mixture for 4 hours at 37°C. Cells were then washed at 37°C for 30 min each with 2× SSC containing 50% (v/v) formamide and then 1× SSC containing 50% formamide and finally at room temperature with 1× SSC until almost no radioactivity was detected by a minimonitor. Cover slips were dehydrated through 70%, 95%, and 100% ethanol, attached to slides with Permount, and allowed to dry. Cells were stained with bisbenzimide (Hoechst 33258) and were coated with emulsion (Kodak NTB-3). After 12 days of storage, slides were developed in Kodak D19, and cells were mounted under a cover slip with PBS and glycerol. Cells were observed with a Zeiss microscope equipped with fluorescence optics and a dark-field condenser.
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10. Background counts were determined by counting the number of grains present in areas on cover slips devoid of muscle cells. The average area occupied by 100 nuclei in muscle cells was also determined. The number of background grains per square micrometer of nonmyotube area was determined with the Bioquant morphometry program. This number was then multiplied by the average area of a nucleus to give 0.25 ± 0.10 (mean ± SD) grains per nucleus.
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Perceptual Deficits and the Activity of the Color-Opponent and Broad-Band Pathways at Isoluminance

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The deficits in texture, motion, and depth perception incurred in monkeys at isoluminance were compared with the responses of neurons of the color-opponent and broad-band systems in the lateral geniculate nucleus. Texture perception, assumed to be carried by the color-opponent system, and motion and depth perception, ascribed to the broad-band pathway, were all found to be compromised but not abolished at isoluminance. Correspondingly, both the color-opponent and the broad-band systems were affected at isoluminance, but the activity of the neurons in neither system was abolished. These results suggest that impairment of visual capacities at isoluminance cannot be uniquely attributed to either of these systems and that isoluminant stimuli are inappropriate for the psychophysical isolation of these pathways.

INFORMATION IN THE VISUAL SYSTEM, as in sensory systems in general, is processed along several parallel channels. The geniculostriate system of the primate consists of two major pathways with distinct physiological properties, the color-opponent and the broad-band. These pathways remain segregated through several cortical stages and are believed to subserve different visual capacities (*1*). Because cells in the broad-band system do not respond selectively to different wavelengths of light (*2*), it has been thought that this "color-blind" system becomes inactive at isoluminance, leaving only the color-opponent system functional (*3*). On the basis of these ideas, Livingstone and Hubel proposed that those aspects of visual perception compromised at isoluminance (*4*) are mediated by the broad-band system and that such experiments, therefore, could reveal what visual functions are carried by each of these two channels (*5, 6*). To test this hypothesis directly, we set out to compare the perceptual deficits incurred in monkeys at isoluminance with the responses of single cells of the color-opponent and broad-band systems.

We trained rhesus monkeys to detect or discriminate motion, stereoscopic depth, and texture differences in red-green stimuli of various luminance and color contrasts (Fig. 1). Choice of these three visual tasks was based on the assumption that the color-

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