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Roles of cell-intrinsic and microenvironmental factors in photoreceptor cell differentiation

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Abstract

Photoreceptor differentiation requires the coordinated expression of numerous genes. It is unknown whether those genes share common regulatory mechanisms or are independently regulated by distinct mechanisms. To distinguish between these scenarios, we have used in situ hybridization, RT-PCR, and real-time PCR to analyze the expression of visual pigments and other photoreceptor-specific genes during chick embryo retinal development in ovo, as well as in retinal cell cultures treated with molecules that regulate the expression of particular visual pigments. In ovo, onset of gene expression was asynchronous, becoming detectable at the time of photoreceptor generation (ED 5–8) for some photoreceptor genes, but only around the time of outer segment formation (ED 14–16) for others. Treatment of retinal cell cultures with activin, staurosporine, or CNTF selectively induced or down-regulated specific visual pigment genes, but many cognate rod- or cone-specific genes were not affected by the treatments. These results indicate that many photoreceptor genes are independently regulated during development, are consistent with the existence of at least two distinct stages of gene expression during photoreceptor differentiation, suggest that intrinsic, coordinated regulation of a cascade of gene expression triggered by a commitment to the photoreceptor fate is not a general mechanism of photoreceptor differentiation, and imply that using a single photoreceptor-specific "marker" as a proxy to identify photoreceptor cell fate is problematic.

Keywords: Photoreceptor; Differentiation; Chick embryo; Visual pigments; Cell culture; Growth factors

Introduction

Retinal cell differentiation is still incompletely understood, despite recent progress in the identification of transcription factors and extracellular signaling molecules involved in its regulation (Jean et al., 1998; Cepko, 1999; Seigel, 1999; Adler, 2000; Lupo et al., 2000; Galli-Resta, 2001; Vetter and Brown, 2001; Marquardt and Gruss, 2002; Zhang et al., 2002; Hatakeyama and Kageyama, 2004; Malicki, 2004). It has been proposed that proliferating retinal progenitor cells are multipotential, becoming restricted to a specific lineage only after undergoing terminal mitosis (Adler and Hatlee, 1989; Belecky-Adams et al., 1996; Cepko, 1999). Analysis of this hypothesis has been predominantly phenomenological, through experiments testing whether populations of retinal progenitor cells change their developmental fate under different microenvironmental conditions. These studies have led to the categorization of progenitor cells as "multipotent", "restricted", or "committed", but molecular descriptions of these hypothetical cell states and/or their underlying inductive event(s) are still lacking. Experiments addressing these issues are frequently confounded by the use, as markers of progenitor cell commitment, of molecules expressed during the terminal differentiation of the cells. Such use would only be justified in cases in which the entire process of cell differentiation is

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controlled cell-autonomously by intrinsic mechanisms set in motion at the time of progenitor cell commitment to a particular lineage, but would not be warranted if many separate inductive events are necessary before a committed progenitor can reach terminal differentiation (Adler, in press).

Several unique properties of retinal photoreceptor cells make them particularly suitable for the investigation of these questions. Photoreceptor differentiation, for example, can be evaluated by assessing the development of characteristic structural properties (e.g., an elongated, compartmentalized cell body, an outer segment, complex ribbon-containing synaptic terminals, and structural and molecular polarity), and the expression of many photoreceptor-specific genes (e.g., those encoding visual pigments and other members of the phototransduction cascade, outer segment structural proteins, and transporters and binding proteins involved in retinoid metabolism; Nathans, 1987; Adler, 2000; Hargrave, 2001; Gonzalez-Fernandez, 2002; Parsons and Sterling, 2003; Palczewski et al., 2004). Photoreceptor subtypes (rods and several types of cones) can be distinguished based on further structural and molecular criteria.

There is some evidence suggestive of both cell-autonomous and non-cell-autonomous mechanisms in photoreceptor differentiation. We have hypothesized that many of its aspects are controlled by a cell-intrinsic "master plan", which retinal progenitors acquire shortly after terminal mitosis and express autonomously (Adler, 2000). Supportive evidence was derived from isolation experiments, in which chick embryo retinal progenitor cells were dissociated before the onset of overt differentiation, and cultured at low densities that minimized contact-mediated intercellular interactions. Under these conditions, some progenitor cells developed as non-photoreceptor (predominantly amacrine) neurons, while others differentiated as photoreceptors (Adler et al., 1984; Belecky-Adams et al., 1996). The relative frequency of photoreceptors and non-photoreceptor neurons was developmental stage-dependent, with photoreceptors being more abundant when cells were isolated from younger embryos (Adler and Hatlee, 1989; Belecky-Adams et al., 1996). Photoreceptor progenitors underwent a series of complex morphogenetic changes even in the absence of contacts with other cells, including elongation, development of structural and molecular polarity, and formation of a small outer segment process and a short axon (Madreperla and Adler, 1989; Madreperla et al., 1989; Saga et al., 1996). Structural differentiation was accompanied by molecular differentiation, e.g., expression of visinin and of the red cone pigment by 100% and 50% of the photoreceptors, respectively (Adler et al., 2001), and by functional differentiation, e.g., photomechanical responses to light (Stenkamp and Adler, 1993; Stenkamp et al., 1994).

A role for cell-intrinsic mechanisms in the regulation of early aspects of photoreceptor differentiation was also suggested by the patterns of photoreceptor gene expression observed in vivo during retinal development in the ferret (Johnson et al., 2001) as well as in other species (Cook and Desplan, 2001). However, inductive signals from the microenvironment appeared necessary for later events, including the expression of genes which became detectable around the time of outer segment formation (see Discussion). The possible existence of independent regulatory mechanisms for different photoreceptor genes, however, has not been experimentally tested. Towards this goal, we have now used in situ hybridization, RT-PCR, and real-time PCR to investigate the expression of visual pigments and several other photoreceptor-specific genes, both during normal chick embryo development in vivo and in retinal cells cultured under different experiment conditions. These results indicate that many photoreceptor genes are independently regulated during development, suggesting that expression of a visual pigment gene cannot be considered by itself an indication that photoreceptors have already acquired the complex molecular machinery necessary for their visual function. The results are also consistent with the existence of at least two distinct stages of gene expression during photoreceptor differentiation, and raise some concerns about the use of molecules expressed during terminal differentiation (a late event) as indicators of the commitment of retinal progenitor cells to the photoreceptor lineage (a much earlier event).

Methods

Materials

Reagents were purchased from the following suppliers: Roche Molecular Biochemicals (previously known as Boehringer Mannheim; Indianapolis, IN): T1 RNAse, alkaline phosphatase (AP)-labeled anti-digoxigenin antibody, horseradish peroxidase (HRP)-labeled anti-digoxigenin antibody, HRP-labeled anti-fluorescein antibody, blocking reagents, 5-bromo-4-chloro-3-indolyl-phosphate (BCIP), DNAse I, 4-nitroblue tetrazolium chloride (NBT), T7 and T3 polymerases, nylon membranes, digoxigenin-labeled nucleotide (NTP) mix, LightCycler FastStart DNA Master^{Plus}SYBR Green 1 kit, Dig-labeled Control RNA, bovine serum albumin. Gibco BRL (Bethesda, MD): formamide, proteinase K, yeast tRNA. Sigma (St. Louis, MO): staurosporine, DMSO, sucrose, heparin, 3-[(3-cholamidopropyl) dimethylammonio]-1-propanesulfonate (CHAPS), EDTA, saponin, ahydride, maleic acid, paraformaldehyde, Triton X-100, Tween 20, triethanolamine HCl, polyornithine, linoleic acid, phenol–chloroform, ethidium bromide. VectorLabs (Burlingame, CA): DAPI-containing Vectashield. Invitrogen (Carlsbad, CA): Trizol, RNase A, DNAse, Superscript II, Recombinant Taq Polymerase, SP6 polymerase, oligo(dT), random primers, 10 mM dNTP, PCR enhancer. Perkin Elmer Life Sciences (Shelton, CT): cyanine-3 fluorophore tyra-