Available online at www.sciencedirect.com
ScienceDirect
Developmental Biology 303 (2007) 591–600
DEVELOPMENTAL BIOLOGY
www.elsevier.com/locate/ydbio

SOCS3 is required to temporally fine-tune photoreceptor cell differentiation

Yoko Ozawa [a,b], Keiko Nakao [b,c,d], Takuya Shimazaki [b,c,d], Shigeto Shimmura [a], Toshihide Kurihara [a,b], Susumu Ishida [a], Akihiko Yoshimura [e], Kazuo Tsubota [a], Hideyuki Okano [b,c,d,*]

[a] *Department of Ophthalmology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[b] *Department of Physiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan*
[c] *Core Research for Evolutional Science and Technology (CREST), Japan Science and Technology Corporation (JST), 4-1-8, Honcho, Kawaguchi, Saitaima 332-0012, Japan*
[d] *Solution Oriented Research for Science and Technology (SORST), Japan Science and Technology Corporation (JST), 4-1-8, Honcho, Kawaguchi, Saitaima 332-0012, Japan*
[e] *Division of Molecular and Cellular Immunology, Medical Institute of Bioregulation, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan*

Received for publication 16 May 2006; revised 28 October 2006; accepted 22 November 2006
Available online 1 December 2006

Abstract

Suppressor of cytokine signaling 3 (SOCS3) is an intracellular, ligand-induced negative feedback modulator of STAT3 activation that acts during inflammation. Here, we demonstrate that SOCS3 expression is important for normal retinal development in the perinatal period. STAT3 is highly activated in the late-embryonic retina, then downregulated at postnatal day 0 (P0), presumably by the depletion of upstream ligands. We found that SOCS3 was required after P0 to shut down the residual STAT3 activation; this loss of activated STAT3 leads to Rhodopsin expression and rod photoreceptor cell differentiation. *SOCS3* deficiency failed to terminate STAT3 activation, thereby delaying expression of Rhodopsin and its upstream transcription factor, *crx*. Development subsequently continued, but its course was temporally erratic, probably because of faulty compensation. Interestingly, SOCS3 protein expression was first detected postnatally, after STAT3 activation was mostly downregulated. It initially appeared in some of the presumptive photoreceptor cells and gradually spread. *SOCS3* mRNA level was constant from the late-embryonic to early-postnatal period. Post-transcriptional inhibition of SOCS3 protein expression maintains a high STAT3 activation during late embryogenesis, and after P0, releasing from the inhibition promptly terminates STAT3 activation. Thus, SOCS3 can act as a temporal fine-tuner of STAT3 activation during photoreceptor cell differentiation.

Keywords: SOCS3; STAT3 activation; Retina; Post-transcriptional; Fine-tune; Differentiation; Photoreceptor

Introduction

During development, differentiation is tightly regulated in a temporal and cell type-specific manner via the orchestration of many different mechanisms. The vertebrate retina provides an excellent model system for investigating such mechanisms. In the developing mammalian retina, seven types of neural cells differentiate from common retinal progenitor cells, under the influence of extrinsic and intrinsic factors that act at specific times (Altshuler and Cepko, 1992; Altshuler et al., 1993; Cepko et al., 1996; Ezzeddine et al., 1997; Hicks and Courtois, 1992; Kelley et al., 1994; Levine et al., 2000; Lillien, 1995; Livesey and Cepko, 2001; Morrow et al., 1998a,b; Watanabe and Raff, 1990; Yourey et al., 2000). In this system, postmitotic presumptive photoreceptor cells generated during the late-embryonic period are kept in an undifferentiated state by strong STAT3 activation, which is induced by extrinsic diffusible factors, such as ciliary neurotrophic factor (CNTF) (Caffe et al., 2001; Neophytou et al., 1997; Rhee et al., 2004; Zhang et al., 2004), at least until postnatal day 0 (P0). Since CNTF

* Corresponding author. Department of Physiology, Keio University School of Medicine, 35 Shinanomachi, Shinjuku-ku, Tokyo 160-8582, Japan. Fax: +81 3 3357 5445.
E-mail address: hidokano@sc.itc.keio.ac.jp (H. Okano).

0012-1606/$ - see front matter © 2006 Elsevier Inc. All rights reserved.
doi:10.1016/j.ydbio.2006.11.032

expression is high in the embryonic retina but declines postnatally (Kirsch and Hofmann, 1994; Schulz-Key et al., 2002), it is thought to be responsible for the downregulation of STAT3 activation at P0, which leads to the onset of Rhodopsin expression and rod photoreceptor cell differentiation (Ozawa et al., 2004). However, one remaining puzzle is that, although STAT3 activation in most of the presumptive photoreceptor cells has already been substantially lowered at P0, compared with E18 (4.5-fold decreased) (Ozawa et al., 2004), the onset of Rhodopsin expression does not start simultaneously in all postmitotic cells (Morrow et al., 1998a,b). Therefore, there may be other regulatory systems that reduce the residual STAT3 activation in each postmitotic presumptive photoreceptor cell to a level low enough for the initiation of Rhodopsin expression.

In this study, we focused on suppressor of cytokine signaling 3 (SOCS3), one of the intracellular negative modulators of STAT3 activation, since SOCS3 sharply terminates STAT3 activation through the direct inhibition of Janus kinase (JAK) (Yasukawa et al., 2001). SOCS3 is in the cytokine inducible SH2-protein (CIS)/SOCS family, whose members act in a cell type-specific and receptor-specific manner (Hanada et al., 2003; Kubo et al., 2003; Yasukawa et al., 2003). SOCS3 mRNA and protein are rapidly induced by several cytokine signals, including interleukin-6/gp130 signals, through STAT3 activation (Auernhammer et al., 1999; Hanada et al., 2003; Suzuki et al., 2001; Yoshimura et al., 2003), and the STAT-binding site in the promoter of the SOCS3 gene. SOCS3 protein binds to Tyr759 of the gp130 receptor and inhibits the JAK dependent activation of STAT3, even when extracellular ligands bind to the gp130 receptor (Nicholson et al., 2000). Thus, SOCS3 is categorized as a negative feedback regulator of STAT3 activation.

Here, we first show that SOCS3 protein is expressed in a unique pattern in the developing retina, which is restricted to the photoreceptor cell layer, and that its expression becomes temporally and spatially upregulated after the major downregulation of STAT3 activation at P0, just prior to the onset of Rhodopsin expression. Interestingly, SOCS3 protein is not observed when STAT3 is highly activated during the late-embryonic period, and it gradually increases in the postnatal period, when STAT3 activation is low. *SOCS3* mRNA is expressed at a constant level from the late-embryonic to the early-postnatal period, indicating the involvement of a temporally controlled, post-transcriptional inhibitory mechanism for SOCS3 expression. We also demonstrate here that *SOCS3* deficiency prolongs STAT3 activation postnatally and delays the expression of Rhodopsin and its upstream transcription factor, *crx* (Chen et al., 1997; Furukawa et al., 1997, 1999, 2002). In retina-specific *SOCS3*-deficient mice (α-Cre *SOCS3*$^{flox/flox}$), the delayed differentiation was compensated by adulthood; however, the timing of this process was erratic, and the catch-up speed varied from mouse to mouse. Therefore, SOCS3 is required for the temporal fine-tuning of STAT3 activation in normal rod photoreceptor cell differentiation, where it acts cell-autonomously under post-transcriptional regulation.

Materials and methods

Animals

ICR and C57B/6 mice were purchased from Clea Japan, INC. and the Charles River Laboratory. SOCS3 floxed mice were generated in Dr. Yoshimura's Laboratory (Mori et al., 2004), and α-Cre transgenic mice were generously provided by Dr. P. Gruss (Marquardt et al., 2001). The mice were genotyped in conjunction with their use in experiments. CAG-CAT-EGFP transgenic mice, which were used to show the expression of Cre-recombinase, were kindly provided by Dr. J. Miyazaki (Niwa et al., 1991). All the procedures conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research, and the procedures were approved by the Institutional Safety Committee on Recombinant DNA Experiments and the Animal Research Committee of Keio University.

Neural retinal explant culture

Retinal explant culture was performed using P0 mouse neural retina as described previously (Ozawa et al., 2004), using a modification of the protocol described by Tomita et al. (1996). Briefly, eyes were enucleated and the neural retinas were isolated and placed on a Millicell chamber filter (Millipore: pore size; 0.4 μm) with the ganglion cell layer facing up. The chamber was then placed on a 6-well culture plate, containing 50% MEM (GIBCO), 25% HBSS (GIBCO), 25% horse serum (Thermo Trace), 200 mM L-glutamine, and 6.75 mg/ml D-glucose. The explants were incubated at 34 °C in 5% CO_2, and the medium was changed every 1–2 days. CNTF was used at a concentration of 50 ng/ml in this system, which is just enough to completely inhibit the Rhodopsin expression in the outer nuclear layer (ONL) of the P0 retinal explants without causing any obvious morphological changes, as described previously (Ozawa et al., 2004).

Electroporation

Electroporation was performed as described previously (Ozawa et al., 2004). Briefly, a retinal explant on the membrane was placed onto the DNA solution (5 μg/μl in PBS) set on the agalose gel, and electric pulses (20 V 50 ms, 6 times) were applied with an electroporator (CUY21 NEPPA GENE), without contacting the tissue. An expression vector carrying the cDNA for human SOCS3, a dominant-negative form of SOCS3, F59D-JAB (Hanada et al., 2001; Suzuki et al., 2001), or Cre-recombinase under control of the CAG promoter (Niwa et al., 1991), was co-transfected with pCAG-EGFP (an expression vector containing the enhanced green fluorescent protein (EGFP)) (10:1). F59D-JAB destabilizes the endogenous SOCS3, thus overcoming the inhibitory effect of SOCS3 on JAK (Hanada et al., 2001; Suzuki et al., 2001). An empty transfection vector, pCAG, with pCAG-EGFP was electroporated as a control.

Immunohistochemistry

Cryosections of retinal explants (12–16 μm) were fixed with 4% paraformaldehyde and prepared as described elsewhere (Ozawa et al., 2004). The sections were first incubated with 0.1% Triton and 10% goat serum in PBS and then at 4 °C with primary antibodies to mouse anti-Rhodopsin (1:100 Abcam), rabbit anti-Rhodopsin (1:2000, LSL), mouse anti-myc (1:100, Cell Signaling), or mouse anti-GFP (1:300, Molecular Probe) antibodies diluted in 0.1% Triton and 2% goat serum. The sections were then incubated with the secondary antibody Alexa 568- or 488-conjugated goat anti-mouse or anti-rabbit IgG (1:500, Molecular Probes), respectively. For immunostaining with rabbit anti-phospho-STAT3 (1:50, Cell Signaling) and rabbit anti-SOCS3 (1:100, anti-SOCS3 C-terminal, generated in Dr. Yoshimura's Laboratory (Mori et al., 2004; Sasaki et al., 2003)), the sections were pre-incubated at 100 °C for 5 min in tissue retrieval solution (TRS, Sigma #1699) and then incubated at 4 °C overnight with primary antibody diluted in blocking agent with 0.3% or 0.1% Triton, respectively. Finally, the immunoreactions were detected with a tyramide signal amplification (TSA) fluorescein system (Perkin Elmer Life Science), as described previously (Ozawa et al., 2004). The TRS treatment was performed before staining with rabbit anti-Cre recombinase (1:100, Invitrogen), which was