Isolation of the Mouse Nyctalopin Gene Nyx and Expression Studies in Mouse and Rat Retina

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PURPOSE. It has been shown recently that mutations in NYX (nyctalopin on chromosome X), encoding a novel protein associated with the leucine-rich repeat (LRR) protein superfamily, are responsible for the complete form of X-linked congenital stationary night blindness (CSNB1). This study describes the isolation and molecular characterization of the mouse orthologue Nyx and its expression pattern in the retina.

METHODS. Nyx was isolated by conventional DNA library screening and polymerase chain reaction (PCR)-based approaches. Gene expression in different mouse tissues was studied by RT-PCR. Subsequently, the expression pattern of Nyx and its gene product in mouse and rat retinas was investigated by RNA in situ hybridization and immunohistochemistry with Nyx-specific antibodies.

RESULTS. The Nyx gene encodes a protein of 476 amino acids that contain 11 consecutive LRR motifs flanked by aminoterminal and carboxylterminal cysteine-rich LRRs. At the amino acid level, Nyx is highly homologous to its human orthologue (86% identity). The gene is expressed in the eye but also, at lower levels, in brain, lung, spleen, and testis. Nyx expression was found during all stages of postnatal retinal development and was confined to cells of the inner nuclear layer and the ganglion cell layer in adult mouse and rat retinas.

CONCLUSIONS. These data suggest an important function of the Nyx protein in the inner retina and provide evidence that CSNB1 is based on a defect in the inner retinal circuitry. (Invest Ophthalmol Vis Sci. 2003;44:2260–2266) DOI:10.1167/iovs.02-0115

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Isolation of Genomic Nyx Sequences

High-density filters of a genomic P1-derived artificial chromosome (PAC) library from mouse strain 129 (Library 711; Resource Centre/Primary Database [RZPD], Berlin, Germany) were hybridized with a 32PdCTP-labeled human NYX probe (800 bp of exon 5). DNA from positive clones was isolated with a kit (Plasmid Midi Kit; Qiagen, Hilden, Germany); digested with EcoRI, HindIII, HindII, and PstI; blotted on a nylon membrane; and rehybridized with the human NYX probe. A single positive 7-kb EcoRI fragment was preparatively isolated on an agarose gel, purified with a gel extraction kit (Qiagen; Qiagen), and cloned into a vector (pBluescript II SK; Stratagene, La Jolla, CA). The 7-kb EcoRI insert was then digested with PstI, and the fragments were further subcloned in the same vector. Sequence analysis of subclones was performed using standard M13 forward (5'-GTTTTCCAGTCCAGGACG-G') and reverse primers (5'-CAGGAAA-CGCTATGACC-3'). Analysis of sequence data revealed that one of the subclones contained part of exon 3 of the orthologous mouse gene (Nyx). This nucleotide sequence information was used to design mouse-specific primers for RT-PCR and rapid amplification of cDNA ends (RACE)-PCR.

Synthesis of cDNA and RACE-PCR

cDNA was synthesized from total mouse eye RNA by using a commercial technology (SMART; Clontech, Palo Alto, CA). 5'-RACE experiments were performed with a forward primer derived from the mouse cDNA.
genomic sequence (5'-GCTAAGGGCCAGTTTCTCCTC-3'). The 2kb
RACE product was cloned into a vector (pCR2.1-TOPO; Invitrogen,
Groningen, Germany) and sequenced with M13 and a gene-specific
primer (5'-GCATCTGGCTTTAATCGT-3'). 5'-RACE experiments were
only successful with the implementation of the polymerase
provided by the manufacturer (Advantage-GC 2 PCR Polymerase; Clon-
tech) for PCR amplification and the application of a nested PCR
strategy with reverse primers in the 5' untranslated region (UTR)
(5'-CATGAGTTATGCTGACGCCGAC-3') and the coding sequence
of exon 3 (5'-GGCCATTCCGCTCAGATGATG-3').

RNA Isolation and RT-PCR
Total RNA was isolated from various mouse tissues (RNeasy Midi and
Mini Kit; Qiagen). Tissues were homogenized (PT3100 Polytron; Brick-
man Instruments, Westbury, NY) in lysis buffer. The RNA preparation
was treated with Dnase I and tested for the absence of DNA contam-
ination by control PCR amplification with two mNd gene intron
primers in combination with two neo primers. Reverse transcription of
total RNA was performed by random hexanucleotide priming with
reverse transcriptase (Omniscript; Qiagen). cDNA amplification
from total RNA was performed by random hexanucleotide priming
with reverse transcriptase (Omniscript; Qiagen). cDNA amplifi-
cations were examined by microscope (AX70; Olympus, Tokyo,
Japan) with Normarski optics. Images were adjusted for brightness and
contrast on computer (Photoshop; Adobe Systems, San Jose, CA).

Animals and Tissue Preparation
for Histological Studies
All experiments performed in this study were in accordance with the
ARVO Statement for the Use of Animals in Ophthalmic and Vision
Research. To determine cell specificity of Nyx mRNA expression in the
rodent retina, in situ hybridization was performed on mouse and rat
retina tissue. For the mouse experiments retinal tissue from adult
animals at postnatal day (P)76 and from the developmental stages P3,
P5, P10, P15, P20, and P30 were used. The rat retina was mature at
P44, and the developmental stages were equal to the mouse stages.
The day of birth was designated as P0. Nyx protein was localized
immunohistochemically in adult rat retina.

C57BL/6 mice and Brown Norway rats were killed by a short CO2
incubation and decapitation. The eyes were removed and dissected
along the ora serrata, and the posterior eyecups were fixed in 2% (in
situ hybridization) or 4% (immunohistochemistry) paraformaldehyde
in phosphate buffer (PB; 0.1 M, pH 7.4) for 13 to 30 minutes at 4°C.
After washing in PB, tissues were cryoprotected by immersion in 30%
(wt/vol) sucrose in PB overnight at 4°C. Sections were cut on a
cryostat. For in situ hybridization, sections were mounted in tissue
adhesive (Vetabond; Vector Laboratories, Burlington, Canada) for
immunohistochemistry on silane-coated glass slides and dried at 60°C for
2 to 3 hours. Slides were stored at −80°C until further use.

Samples processed for either in situ hybridization or immunohisto-
chemistry were examined by microscope (AX70; Olympus, Tokyo,
Japan) with Normarski optics. Images were adjusted for brightness and
contrast on computer (Photoshop; Adobe Systems, San Jose, CA).

In Situ Hybridization
As the probe template, a 520-bp PCR fragment of the human NYX
(nucleotide positions 1887-2406, GenBank accession number
AJ278865; http://www.ncbi.nlm.nih.gov/Genbank; provided in the
public domain by the National Center for Biotechnology Information,
Bethesda, MD) was subcloned into an Smal-linearized vector (pBlue-
script II SK+; Stratagene) by blunt-end ligation, and a 551-bp fragment
of the murine Nyx (corresponding to nucleotide positions 557-1107 in
the human sequence) was subcloned into a PstI-linearized vector
(pBluescript II SK+; Stratagene). The plasmid pRO4 containing the rat
rhodopsin cDNA was kindly provided by Armin Huber, Institut für
Zoologie, Universität Karlsruhe, Germany.

One microgram of plasmid DNA was linearized by restriction en-
zyme digestion and purified by phenol-chloroform extraction. Digoxi-
genin-UTP-labeled sense and antisense riboprobes were generated by in
vitro transcription of linearized plasmids with a kit (DIG RNA
Labeling Kit; Roche Molecular Biochemicals, Mannheim, Germany).

Before the in situ hybridization, sections were treated with proteinase
K buffer (0.1 M Tris-HCl [pH 8] and 0.05 M EDTA) for 5 minutes at
37°C and digested with 0.3 μg/ml protease K (Sigma, Deisenhofen,
Germany) for 8 minutes at 37°C. Slides were then washed two times for
3 minutes each in diethyl pyrocarbonate (DEPC)-treated water,
postfixed for 15 minutes in paraformaldehyde (PFA; 4% PFA in 0.2 M
PB), washed again three times with DEPC-treated water, and air dried.

Sixty microliters of hybridization solution (50% deionized formi-
amide [Sigma], 5% SSC, 5% Denhardt's solution, 0.5 mg/ml tRNA
[Fluka, Buchs, Switzerland]) and 0.2 mg/μl of the digoxigenin-labeled
riboprobe were denatured for 5 minutes at 80°C and applied to the
slides. The slides were then incubated in a humidified chamber for
16 hours at 64°C. Posthybridization washing steps were performed
for 30 minutes each in 0.1X SSC at 64°C. After a 10-minute
wash in Tris-buffered saline (TBS; 0.15 M NaCl and 0.1 M Tris-HCl [pH
7.5]) at room temperature (RT), the slides were incubated for 30
minutes with blocking solution (10% blocking reagent in 0.1 M maleic
acid, 0.15 M NaCl [pH 7.5]; Roche Molecular Biochemicals). Drained
slides were incubated with alkaline-phosphatase–conjugated anti-
digoxigenin antibody (1:500, in 10% blocking solution, 0.15% Triton
X-100 in TBS; Roche Molecular Biochemicals) for 45 minutes at
37°C. Sections were then briefly rinsed two times for 15 minutes each in
TBS and preincubated for 10 minutes in substrate buffer (0.1 M Tris-HCl
[pH 9.5], 1 mM MgCl2, 10% tetramisole-hydrochloride [Fluka]). Four
microliters nitroblue tetrazolium salt (NBT; 30 mg/ml; Bio-Rad Laborato-
ries, Munich, Germany) and 4 μl 5-bromo-4-chloro-3-indolyl phos-
phate (BCIP; 15 mg/ml; Bio-Rad Laboratories) were mixed with 1 mL
of substrate buffer, and each section was incubated in 200 μL of this
solution, in a humidified chamber in the dark for 24 to 72 hours at RT.
Color reaction was stopped with stop buffer (0.1 M Tris-HCl [pH 7.5]
and 0.01 M EDTA), and covered with sorbitol (Merck, Darmstadt,
Germany).

Immunohistochemistry
To raise antibodies against NYX, two peptides comprising the amino
acid sequences LTTSPGPSPEPAATT and ASLSDSSLRSGVG were pre-
pared by solid-phase peptide synthesis, using the Fmoc-But-strategy.12

The peptides were purified to a homogeneity of more than 95% by
HPLC, and their identity was confirmed by electrospray mass chroma-
tography. The peptides were coupled to keyhole limpet hemocyanin
(KLH) by the glutaraldehyde method13 and used as antigens to raise
domestic antibodies in New Zealand White rabbits, according to
standard immunization protocols (Charles River Service Laboratory,
Sulzfeld, Germany). The resultant antisera was purified by affinity
chromatography (Protein A Sepharose; Amersham Biosciences,
Freiburg, Germany) and subsequently by immunoaffinity chromatog-
raphy (applying the peptides used for immunization) and finally concen-
trated by ultrafiltration on a 20kDa cut-off membrane.

In sections used for immunohistochemistry endogenous peroxi-
dase was blocked with 3% H2O2 in 40% methanol. To reduce back-
ground staining, slides were preincubated for 1 hour in 10% normal
goat serum (NGS; Sigma, Deisenhofen, Germany) and applied to the
sections. The primary antibody was diluted 1:1000 in PBST containing
0.1% NGS and incubated for 3 hours at RT or overnight at 4°C. After a wash
with phosphate-buffered saline (PBS), the samples were incubated for
1 hour with a biotin-conjugated secondary antibody (dilution 1:200,
VectorStain Elite Kit; Vector Laboratories) in PBST with 10% NGS and
0.3% Triton X-100 in TBS. Sections were washed in TBS, washed
three times with 0.1% Tween in TBS, and incubated in 100 μL of a
digoxigenin antibody (1:500, in 10% blocking solution, 0.15% Triton
X-100 in TBS; Roche Molecular Biochemicals) for 45 minutes at
37°C. After washing in Tris-buffered saline (TBS; 0.15 M NaCl and 0.1 M
Tris-HCl [pH 7.5]) at room temperature (RT), the slides were incubated for
30 minutes with blocking solution (10% blocking reagent in 0.1 M maleic
acid, 0.15 M NaCl [pH 7.5]; Roche Molecular Biochemicals). Drained
slides were incubated with alkaline-phosphatase–conjugated anti-
digoxigenin antibody (1:500, in 10% blocking solution, 0.15% Triton
X-100 in TBS; Roche Molecular Biochemicals) for 45 minutes at
37°C. Sections were then briefly rinsed two times for 15 minutes each in
TBS and preincubated for 10 minutes in substrate buffer (0.1 M Tris-HCl
[pH 9.5], 1 mM MgCl2, 10% tetramisole-hydrochloride [Fluka]). Four
microliters nitroblue tetrazolium salt (NBT; 30 mg/ml; Bio-Rad Laborato-
ries, Munich, Germany) and 4 μl 5-bromo-4-chloro-3-indolyl phos-
phate (BCIP; 15 mg/ml; Bio-Rad Laboratories) were mixed with 1 mL
of substrate buffer, and each section was incubated in 200 μL of this
solution, in a humidified chamber in the dark for 24 to 72 hours at RT.
Color reaction was stopped with stop buffer (0.1 M Tris-HCl [pH 7.5]
and 0.01 M EDTA), and covered with sorbitol (Merck, Darmstadt,
Germany).

The Murine Orthologue of NYX 2261

NYX

NYX

NYX
minobenzidine-nickel solution as the chromogen (1 mg/mL diaminobenzidine, 0.2% glucose 0.004% NH₄Cl, 0.09% (NH₄)₂Ni(SO₄)₂, and 1 μL/mL glucose oxidase in PB). After terminating the reaction in PB the slides were coverslipped with glycerol/PBS (9:1). To determine the specificity of the antigen-antibody reaction, negative control experiments were performed, either by omitting the primary antibody or preabsorbing it with the appropriate peptide.

**Results**

**Cloning of the Mouse Orthologue of NYX**

As a prerequisite for gene expression studies, we isolated the mouse orthologue of NYX. A genomic PAC library from mouse strain 129 was screened with a human probe corresponding to part of exon 3 (codons 242-418 and 3'-UTR). In this way, three PAC clones were identified and characterized in more detail. Analysis of 881-bp sequences revealed a 1410-bp segment with 85% identity to exon 3 of the human NYX gene. Based on this partial genomic mouse sequence, primers were designed and used for the isolation of the full-length *NYX* cDNA from reverse-transcribed total mouse eye RNA by RACE-PCR. Sequence analysis of amplification products and their comparison with the human nucleotide sequence revealed 85% identity in the ORF. In addition, the splice site within the ORF (between amino acid residues 12 and 13 in the human sequence) is conserved in the mouse, as shown by the alignment of genomic and cDNA sequence data. The cDNA sequence of *NYX* has been deposited in the GenBank database (Accession-No. AY114303). The mouse gene encode 476 amino acid residues, whereas a 481-amino-acid protein is predicted from the human sequence (Fig. 1). Computational protein sequence analysis and motif predictions of NYX identified a characteristic domain structure: The core sequence consists of 11 LRRs that are flanked by two cysteine-rich LRRs. This core segment is preceded by a putative signal sequence and followed by a GPI anchor at the very C terminus. Amino acid sequence identity between human and mouse is much higher in the LRR core (>90%) than in the signal sequence and GPI anchor (62% and 52%, respectively). However, virtually all previously identified mutations affect conserved amino acid residues (Fig. 1).

Alignments of the *NYX* cDNA with our PAC sequences and the draft sequence of the mouse genome indicate that the murine gene is split into 4 exons. The ORF is confined to the final two exons, analogous to the human gene. In silico analysis with the draft mouse genome assembly placed *NYX* close to the centromere of the murine X chromosome in the vicinity of *cask*. The proximity of these two genes was also demonstrated by positive hybridization of human CASK sequences to the PAC clones harboring *NYX*. Thus, *NYX* localizes to a region on the murine X chromosome that is syntenic to the p11.4 segment of the human X chromosome.

Gene expression in different mouse tissues was explored by RT-PCR with forward and reverse primers in exons 2 and 3, respectively (Fig. 2). Transcripts were detected in the eye, brain (cerebrum and cerebellum), lung, spleen, and testis, but not in the kidney, heart, and liver.

**Expression of NYX in Mouse and Rat Retinas**

To examine cell-specific mRNA expression in retinal neurons, in situ hybridization was performed on retinal sections from rat and mouse, using human and mouse *NYX* antisense riboprobes.

Hybridization with a rat rhodopsin cDNA antisense riboprobe was performed to verify the quality and reliability of our in situ hybridization protocol and the specificity of the staining in the different retinal layers (compare also Bech-Hansen et al.). The reaction time of the rhodopsin probe was the same as for the NYX probe in Figures 3 and 4. Rhodopsin signals were observed solely in the photoreceptor layer (Fig. 3B)—that is, in the cell bodies and the myoid regions of the photoreceptor cells.

A clear and pronounced expression of *NYX* was found in the inner nuclear layer (INL) and the ganglion cell layer (GCL) in fully differentiated retinas (rat: P4/4, mouse: P76; Figs. 3A, 3C). Staining of the INL was mostly confined to the inner row of cells, which largely corresponds to the localization of amacrine cells. In addition, some stained cells were occasionally localized superior to this innermost row. However, the most intense *NYX* staining was observed in the GCL (Figs. 3A, 3C). In rodents, the GCL consists of only slightly more than 50% of ganglion cells, whereas the remaining represent displaced amacrine cells. Using a size criterion (soma diameter larger than 15 μm), at least a subset of the stained cells can be considered ganglion cells. Those cells showed the most intense staining, suggesting a particularly high level of *NYX* expression. Our hybridization protocol was adjusted in such a way that a background-free and unquestionable signal could be obtained with the *NYX* probe that can be clearly allocated to single cells. This was nicely achieved in the inner retina where the signal filled the entire cell body (Figs. 3A, 3B). Compared with this staining, the signal in the outer retina was extremely faint and not assigned to cell bodies in the same way as in the inner retina.

A weakly stained band was found at approximately the level of the outer limiting membrane, along the border of the outer nuclear layer (ONL) with the myoid region of the photoreceptors (Figs. 3, PhR), and where the photoreceptors terminate, at the border to the outer plexiform layer (OPL; Figs. 3A, 3C, 4). At early postnatal stages (<P5), the retina is not fully developed, and only the GCL and the adjacent inner plexiform layer (IPL) have already separated from the neuroblast layer (NBL). Strong expression was observed in the GCL and the INL at P10, when the INL had separated from the photoreceptors. Staining was clearly confined to the GCL and INL. With eye opening, which occurred around P15 in both species, the expression pattern of *NYX* was essentially the same as in the retina of adult animals, with the exception of a few labeled cells in the more proximal part of the INL. Later developmental stages reflected the murine *Nyx* expression pattern of the mature rat retina.

To examine cell-specific protein localization we performed immunohistochemistry on retinal sections of adult rats, with polyclonal antibodies against the NYX carboxyl terminus. Immunoreaction to the antibodies was found in the GCL, IPL, INL, and OPL. The photoreceptor region—the ONL and the inner and outer segments—were devoid of immunoreactivity, except for weak staining at the level of the ONL-myoid border, similar to the situation found with the mRNA probe (Fig. 3E).

The immunoreaction in the GCL and in the inner row of the INL resembled the pattern of mRNA expression. Cells of different size and more than 50% of the somata were labeled in the GCL (Fig. 3E, large arrows), and individual cells were distinguished along the INL-IPL border (Fig. 3E, arrowheads). Besides an overall punctuate labeling of the entire IPL two bands of enhanced immunoreaction were present in sublayers 1 and 3, within which horizontally running processes were observable (Fig. 3E, asterisks). Neurons along the outer margin of the INL were also labeled (Fig. 3E, small double arrows) and, in addition, the OPL showed immunoreactivity with more
pronounced staining of fibers running through the proximal part of the OPL above the INL somata (Fig. 3E, small arrow). No labeling was observed when the primary antibody was either omitted (Fig. 3F) or preabsorbed with the peptide used to raise the antibody.

**DISCUSSION**

Cloning of the mouse orthologue of NYX and sequence comparison to its human counterpart revealed a high degree of conservation at the sequence level and a similar structural composition of the protein in both species. All hitherto identified mutations in patients with CSNB1 affect conserved amino acids. The deduced amino acid sequence of the mouse gene is five residues shorter at the N terminus. The putative start codon in the mouse gene coincides with a second in-frame ATG codon in the human sequence. Thus, this second ATG codon may represent the functional translation initiation in the human gene, too.
In the adult mouse, Nyx is expressed in several tissues (brain, lung, spleen, and testis) with highest expression levels observed in the eye (Fig. 2). Similarly, expression of NYX in neural and several non-neural tissues was found in humans. Expression in kidney, consistently shown in humans, is absent or largely reduced in the mouse (Fig. 2).

On RNA in situ hybridization, we observed Nyx expression in the cells of the GCL and the inner part of the INL in the mouse and rat retinas. This pattern is consistent with expression of Nyx in amacrine and ganglion cells in the rodent retina. Localization of the Nyx protein by immunohistochemical analysis showed predominant staining in the inner retina, from the GCL up to the terminals of the photoreceptors. However, the photoreceptor layer itself was free of anti-Nyx immunoreactivity, except for a very faintly stained band along the border of the ONL toward the inner segments. In addition to the localization of Nyx mRNA in the cells of the GCL and the inner INL, numerous cells in the outer INL along the INL–OPL border were immunoreactive. These Nyx protein-positive cells outnumbered the sporadically detectable mRNA-expressing cells in the outer region of the INL considerably, thus supporting the idea that Nyx may also be vertically transported to cells that are not able to produce it themselves.

Immunostaining in the outer part of the INL raises the question of the identity of these cells. There is evidence from ERG recordings that the function of depolarizing bipolar cells is impaired in CSNB1.13–15 However, location, size, and shape of these immunoreactive neurons make it likely that they are horizontal rather than bipolar cells, an assumption further supported by single horizontally oriented fibers running in the OPL close to these cells. Even though the identity of these cells still has to be determined, it is obvious from the in situ hybridization data that most of them do not express Nyx and therefore depend on Nyx produced in the proximal retina. Our results clearly exclude a pronounced expression of Nyx in photoreceptors in the rodent retina. Even though there was a very faint signal in both in situ hybridization and immunohistochemistry approximately at the level of the external limiting membrane, we never observed (in any developmental stage examined) staining in the cell bodies of the ONL or the myoid regions of the photoreceptors comparable to the lucid Nyx expression in the INL and GCL. In the human retina, NYX expression of similar intensity has been reported in all nuclear layers, including the ONL and the inner segments.5  This discrepancy may reflect species differences in Nyx expression. A similar difference was found in the kidney, where Nyx expression was consistently shown in humans, but was absent, or at least largely reduced, in the mouse (Fig. 2).

It has been argued that, analogous to the Drosophila LRR proteins chaoptin and capricious, NYX may be involved in the formation of synaptic connections between neurons during development and maturation of the retina. Our in situ results at various developmental stages showed early expression of Nyx (at least as early as P3) and no gross differences in the temporal and spatial expression pattern during postnatal retinal development. The early expression in the developing retina may indicate that NYX at this stage plays a role in synaptogenesis and neuronal circuit formation. Continuous expression in the
adult retina suggests a functional relevance throughout life—for example, in the maintenance of the extracellular matrix or cell interaction processes.

CSNB1 was initially thought to be caused by a defect in signal transmission from rods to rod bipolar cells, but more recent studies have shown that there is a general impairment of the retinal ON-pathway that involves both rod and cone signaling and is apparently due to a functional defect postsynaptic to the photoreceptors. 18

In the mammalian retinal circuitry, the main (sensitive) signaling pathway of rods involves a single type of depolarizing bipolar cell (rod ON-bipolar), which in turn contact all amacrine cells through a sign-preserving glutamate synapse. Signals from the AII amacrine cells then infiltrate the cone signaling pathways by exciting the cone ON bipolar cells through gap junction electrical contacts and inhibiting OFF cone bipolar cells through glycinergic synapses. 20 The available electrophysiological data suggest that function of rod bipolar cells and also the cone ON pathway through the depolarizing bipolar cells are compromised in CSNB1. Our histologic analyses in the rodent retina localize Nyx expression to cells of the inner half of the INL and the GCL, which probably represents amacrine cells and ganglion cells, respectively. This expression pattern contrasts with the principal electrophysiological findings in CSNB1, which propose a main defect in the depolarizing bipolar cells. 15–18

However, there is also evidence for impairment of more distal neuroretinal function in patients with CSNB1. Miyake et al. 28 possibly by depolarizing amacrine cells or near the NBL–IPL in P3 retinas. The same pattern was observed at P5. At P10, when the INL was completely separated from the photoreceptors, staining was confined to the GCL and INL. In P15 the expression pattern of Nyx was essentially the same as in the retina of adult mouse retinas, with the exception of a few labeled cells in the more proximal part of the INL. Later developmental stages (P20, P30) reflect the Nyx expression pattern of the mature rat retina. Scale bar, 50 μm.

Because there is no evidence that amacrine or ganglion cells contribute to the rod b-wave or the cone ON response, its loss in patients with CSNB1 cannot be readily explained if the function of NYX is restricted to the inner retina. However, it might be assumed that the absence of a functional defect of nyctalopin in amacrine and ganglion cells will impair the formation of regular synaptic contacts with their input bipolar cells and thus indirectly have also an adverse effect on the functional differentiation of the bipolar cells themselves. Further studies on the neuroretinal circuitry in patients with CSNB1 are necessary to solve this question.

Of note, a naturally occurring mouse model nob (no b-wave) has been described that resembles CSNB1 in humans: stationary course, preserved a-wave, and absent b-wave and oscillatory potentials in ERG recordings. Moreover the nob trait displays X-linked recessive inheritance, and recent linkage analysis excludes the gene involved in the incomplete form of CSNB (CSNB2) but maps the nob locus to a region syntenic to the human CSNB1 locus. 31

Our in silico mapping results localize Nyx to the centromeric region of the murine X chromosome within the nob-critical interval. This localization further supports the idea that a mutation in Nyx gives rise to the nob phenotype. Thus, further studies of the nob mouse model may provide more insight into the complex pathophysiology of CSNB1 in humans and may help to elucidate the relationship between the principal electrophysiological findings and the restricted expression pattern of NYX in the retina.

Note Added in Proof

After final submission of this paper, Gregg and coworkers published an article in which they showed that nob mice do indeed have a mutation in the Nyx gene (Gregg RG, Mukhopadhyay S, Candille SI, et al. Identification of the gene and the mutation responsible for the mouse nob phenotype. Invest Ophthalmol Vis Sci. 2003;44:378–384).
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