

Diurnal expression of NGF1-A mRNA in retinal degeneration slow (*rds*) mutant mouse retina

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Abstract

The *retinal degeneration slow (rds)* mutant mouse is a model for studying the retinal dystrophy for human disease, retinitis pigmentosa (RP). To continue our effort towards a possible mechanism of photoreceptor cell death in retinal dystrophies, we have studied the impact of the *rds* mutation on diurnal expression of a 'zinc-finger' DNA-binding protein, NGF1-A mRNA in the isolated retinas of *rds* mutant mice compared to those of BALB/c mice. Background levels of NGF1-A mRNA were maintained during the subjective light period. Higher levels of NGF1-A mRNA were observed immediately after the light offset and peaked two hours into the light offset for both the BALB/c and the *rds* mutant retinas and remained higher for several hours in the dark. If the animals were left continuously in light during the subjective dark period, NGF1-A mRNA levels were not induced and remained lower. On the other hand NGF1-A mRNA levels were transiently induced during the transition of the dark-to-light phase. These data suggest that NGF1-A mRNA is differentially regulated by light and dark stimuli in the retina and an absence of rod outer segments in the *rds* mutant retina does not alter the normal diurnal cycle of NGF1-A mRNA expression.

Key words: Retinal dystrophy; *rds* mutation; NGF1-A mRNA; Retina; Photoreceptor cell death

1. Introduction

The events leading to photoreceptor cell death in *rds* mutation, might be a result of a series of events which are precipitated by the lack of outer segments. The genetic defect underlying the *rds* mutation was shown to be in the *rds*/peripherin protein, responsible for disk morphogenesis in the photoreceptor differentiation [1–3]. The *rds* mutant mouse has been considered a model for human retinitis pigmentosa and has become even more important since several point mutations of the peripherin gene have been reported in humans with autosomal dominant retinitis pigmentosa (ADRP) [4–5] and in macular dystrophy [6]. We showed the expression and synthesis of opsin and interstitial retinal binding protein (IRBP) was normal in the *rds* mutant retinas [7–8]. We also studied the arrestin mRNA levels and biosynthesis through the diurnal cycle and showed that the mRNA as well as the levels of this soluble protein in the *rds* mutant retinas remained higher during the 24 h cycle as compared to the BALB/c controls. In these controls, the arrestin mRNA expression was under diurnal control, with high levels of expression in the light period and lower levels in the darkness [9]. The results of the defective diurnal expression of arrestin mRNA led us to be-

lieve that there may be a generalized defect in the diurnal cycle of the *rds* mutant mouse retinas.

NGF1-A encodes a 'zinc finger' DNA binding protein which has been shown to be a transcriptional regulator in response to growth factors and other stimuli such as hormones [10–16]. In the present study we show that its mRNA responds to light and dark changes as well. Since the failure to form the rod outer segments (ROS) and the missense mutations of peripherin gene are not a self-evident cause of photoreceptor cell death, we have studied the expression of a transcription regulatory factor NGF1-A in the *rds* mutant retinas in response to light and dark. These studies further test the hypothesis, whether there is a generalized loss of the diurnal cycles in the *rds* mutant retinas as evidenced by a loss of diurnal expression of arrestin mRNA and its gene product [9] and if the expression of one of the early response genes NGF1-A, is normal in the *rds* mutant retina in response to light and dark.

2. Materials and methods

2.1. Animals

The BALB/c and mutant 020/A *rds/rds* mice were maintained under a 12 h light/dark (L/D) cycle at low light levels (3–5 foot-candles) as previously described [7]. The age of the animals used in this study was 1–2 months for both the BALB/c and *rds* mutant mice. The light came on at 8 a.m. and went off at 8 p.m. and was monitored daily with an automatic timer and a recorder. The animals were sacrificed with an

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overdose of CO₂ exposure and were in accordance with the ARVO and AAALAC guidelines.

2.2 Northern blot analysis

Total RNA from BALB/c and *rd*s mutant mice retinas frozen in liquid N₂ and stored at –70°C, was isolated by extraction in guanidinium isothiocyanate and centrifugation through a CsCl gradient as previously described [17]. Purified total retinal RNA was separated by electrophoresis in 1.1% agarose–formaldehyde denaturing gels as described in [18] and blotted to a Nytran nylon membrane (S & S, Inc.) for a period of 24 h in 20×SSC. Following the blotting, the nylon membrane was baked at 80°C for a period of 2 h under vacuum. The blot was then subjected to hybridization with ³²P-labeled NGF1-A cDNA (kindly given by Dr. Deborah H. Damon of UTHSC at San Antonio, TX) or 18S ribosomal RNA probes using the procedure described in [7]. After hybridization, the blot was subjected to washing. For NGF1-A and 18S RNA transcript, the blot was first washed twice with 2×SSC with 0.1% SDS at room temperature for a period of 20 min. The blot was further washed in 0.2×SSC with 0.1% SDS at 42°C for a period of 15 min with shaking. Afterwards, the blots were subjected to fluorography using Kodak X-Omat X-ray film at –70°C with an intensifying screen and developed using an automatic developer (Konica or Kodak).

3. Results

3.1. NGF1-A expression is diurnal in BALB/c mice retina

We studied the expression of NGF1-A at various times during the 12 h L/D cycle for BALB/c retinas. The lights came on at 8 a.m. in the morning and went off at 8 p.m. in the evening. Fig. 1 shows a typical Northern blot for isolated BALB/c retina total RNA, probed with ³²P-labeled NGF1-A cDNA and an 18S ribosomal RNA probe. A peak of NGF1-A mRNA induction was ob-

served within 2 h of the light off-set and high levels of NGF1-A mRNA persisted for several hours (lanes 11–12). Thereafter, the NGF1-A mRNA declined to lower levels (lanes 1–4). Low-to-background levels of NGF1-A mRNA were observed throughout the subjective light period (lanes 8–9). On the other hand NGF1-A mRNA levels were transiently induced during the transition of the dark-to-light phase (lanes 5,6) early in the morning.

3.2. Effect of light and dark interruptions during the night and day period on the expression of NGF1-A

As expected, low-to-undetectable levels of NGF1-A mRNA were observed during the light period (Fig. 2, lanes 3 and 5). A group of BALB/c mice were kept in the light for 6 and 8 h and then they were transferred to the dark for 4 and 2 h during their subjective light periods, respectively. This treatment of light and dark combination resulted in an induction of NGF1-A mRNA for both groups of animals. Higher levels of NGF1-A mRNA were reported for the animals which were kept in the light for 8 h as compared to the animals left in light for 6 h before transferring to dark for 4 h (lanes 1 and 2). These results suggest that it is essential for the animals to be in the light period before they can show an induction of NGF1-A mRNA due to darkness. The group of animals which was forced to stay continuously in the dark during its subjective light period failed to show induced levels of NGF1-A mRNA (Fig. 2, lanes 4 and

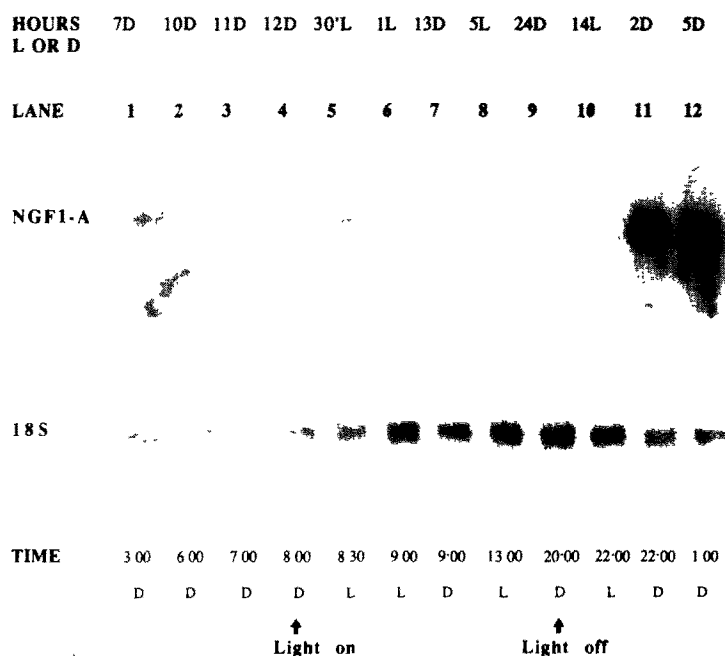


Fig. 1. Northern blot analysis of NGF1-A mRNA of the BALB/c mouse retinas at various times of light and dark. The mice were maintained on a 12 h light/12 h dark cycle with light on at 08.00 h and off at 20.00 h. The total RNA (5 g) isolated from the BALB/c retinas was run on the gel, blotted to nylon membranes and probed with either a [³²P]NGF1-A cDNA or the same blot was probed with a [³²P]18S ribosomal probe. The 18S ribosomal RNA was used as a control for loading of total RNA in each lane. D and L denote dark and light, respectively. The animals in lanes 1–4 were in dark for 7 h, 10 h, 11 h, and 12 h, respectively; lanes 5, 6, 8, and 10 for 30 min, 1 h, 5 h and 14 h in light, respectively; lanes 7, and 9 for 13 h and 24 h in dark, respectively; and lanes 11 and 12 for 2 h and 5 h in dark, respectively.

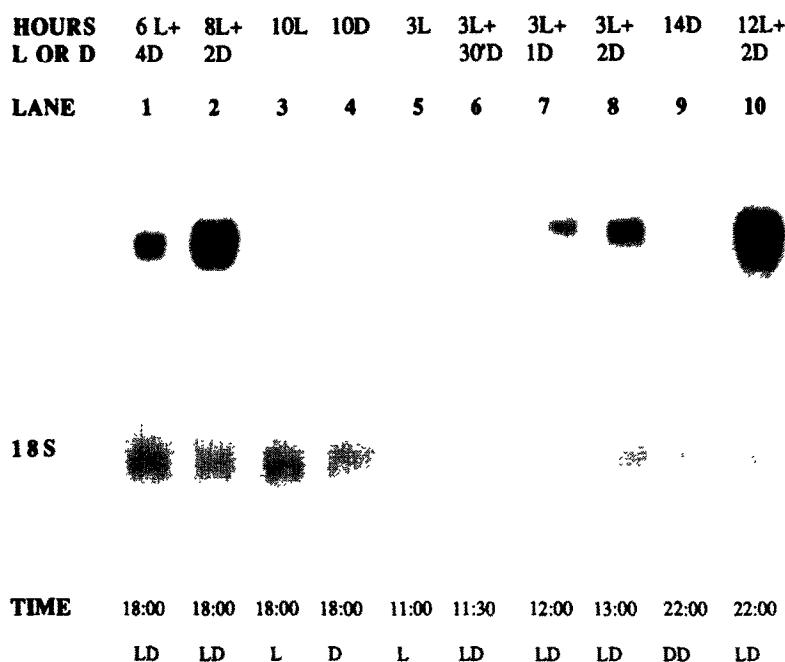


Fig. 2. Effect of light and dark interruptions on the expression of NGF1-A mRNA in BALB/c mice retinas. The mice were sacrificed at various times in their light and dark cycle and the total RNA from the isolated retinas was subjected to Northern analysis with NGF1-A cDNA probe as detailed in Section 2. D and L denote dark and light, respectively. The animals in lanes 1 and 2 were sacrificed at 18:00 h and were 6 and 8 h in light and 4 and 2 h in dark in their subjective light period, respectively, before being sacrificed. The animals in lanes 3, 4 and 9 were sacrificed after 10 h of light at 18:00 h, 10 h of dark at 18:00 h or 14 h of dark at 22:00 h in their subjective light periods, respectively. The animals in lanes 5, 6, 7 and 8 were sacrificed after 3 h in light at 11:00 h, 3 h of light and 30 min of dark at 11:30 h, 3 h of light and 1 h of dark at 12:00 h, or 3 h of light and 2 h of dark at 13:00 h in their subjective light periods, respectively. The animals in lane 10 were sacrificed after 12 h of light and 2 h of dark at 22:00 h.

9, respectively). The NGF1-A mRNA as expected was not induced in animals sacrificed after 3 h of light at 11:00 a.m. (lane 5). On the other hand it was induced in the animals sacrificed after 3 h of light and 30 min of dark at 11:30 a.m.; 1 h of dark at 12:00 p.m. and 2 h of dark at 1:00 p.m. in their subjective light period respectively (lanes 6–8). These results also suggest that there may not be a free running cycle for NGF1-A mRNA as it is necessary for the animals to be kept in light before NGF1-A mRNA could be induced by the dark. Normal induction of NGF1-A mRNA was observed when the animals were kept in the normal light cycle and were sacrificed after 2 h in the dark cycle (lane 10).

3.3. The *rds* mutant animals have a normal L/D cycle for NGF1-A mRNA

As shown in Fig. 3 (lanes 1–2) normal induction of NGF1-A mRNA were observed also for *rds* mutant mouse retinas. The *rds* mutant mice were sacrificed at 5 h into the light period and 2 h into the dark period after 12 h of subjective light period and the retinas were isolated to study the induction of NGF1-A mRNA. As seen in the control BALB/c retinas, there was a major induction of NGF1-A mRNA during the dark phase as compared to the light levels in the *rds* mutant mouse retinas. Since normal induction of NGF1-A mRNA was observed in *rds* mutant mouse retinas, subsequent light and

dark interruptions were not done using the *rds* mutant mouse retinas and the results are shown for BALB/c retinas only.

4. Discussion

We have shown the presence of diurnal cycle of NGF1-A mRNA in both the BALB/c and *rds* mutant mouse retinas. These results suggest that the absence of rod outer segments in the photoreceptors of the *rds* mutant mouse retinas does not affect the diurnal expression of the NGF1-A mRNA in the mutant animals. This observation is of interest in view of our earlier report in which we showed a distinct lack of the diurnal expression of arrestin mRNA in the *rds* mutant mouse retinas such that the arrestin mRNA as well as its gene product was expressed at a high level as compared to control BALB/c retinas throughout the L/D cycle as if they were locked in a permanent light phase of the cycle [9]. The results of arrestin diurnal expression suggested that there may be a generalized loss of the diurnal cycle of the *rds* mutant mouse retinas, however in light of the NGF1-A mRNA results this does not seem to be the case. Normally, the arrestin mRNA in the BALB/c retinas is induced by light and suppressed by dark exposure [9,19,20].

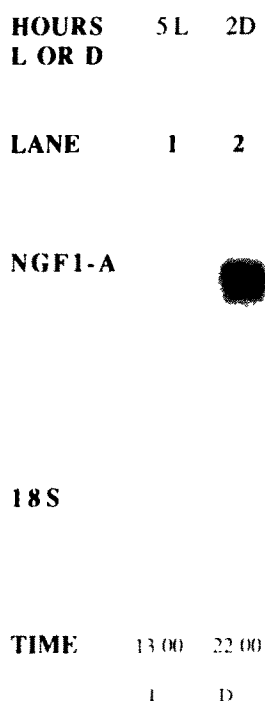


Fig. 3. Northern blot analysis of NGF1-A mRNA of the *rds* mutant mouse retinas at various times of light and dark. The *rds* mutant mice were sacrificed at various times in their light and dark cycle and the total RNA from the isolated retinas was subjected to Northern analysis with NGF1-A cDNA probe as detailed in Section 2. D and L denote dark and light, respectively. The animals in lane 1 were sacrificed after 5 h into the light in their subjective light period at 13:00 h. The animals in lane 2 were sacrificed after 2 h into the dark in their subjective dark period at 22:00 h. The NGF1-A mRNA is induced by dark as in the case of BALB/c retinas.

Another early response gene, *c-fos* mRNA was also shown to be regulated diurnally and was induced early in the subjective dark period of the L/D cycle in BALB/c retinas [21]. This indicates that several early response genes might be induced by dark exposure in the retina and together they may exert their effects in concert on retinal function. Also the presence of light activated short lived NGF1-A mRNA expression along with a long lasting dark induced NGF1-A mRNA in the retinas may suggest that NGF1-A mRNA might be expressed in two different cell types and is regulated differentially by light and dark. The light-evoked induction of NGF1-A mRNA may be associated with the light-activated functions of the retina such as disk shedding and renewal. A number of other metabolic processes in the retina are under diurnal control, such as retinomotor movement, and the expression of visual genes arrestin, opsin, and transducin [9,19,22–24]. In view of these studies, NGF1-A, being a transcriptional regulator, might therefore be responsible for the regulation of the genes under its own control. It will be of interest to localize the expression of NGF1-A mRNA in the retinas by in situ hybridization. These studies will show us which cell

type(s) express the NGF1-A mRNA. It has been shown earlier in *Xenopus* retinas that a separate circadian clock exists in the photoreceptor cell layer [25]. Therefore based on the present results in *rds* mutant retinas, it is reasonable to assume that the outer segments might not play a role in maintenance of the clock since the *rds* mutant retinas lack ROS. Circulating hormones such as corticosterone and ACTH have been shown to be correlated with diurnal expression of *c-fos* in the dentate gyrus [26] and in the renal cortex [27]. NGF1-A expression in retina might also be under the control of these hormonal changes. NGF1-A contains three tandemly repeated 'zinc finger' domains characteristic of many DNA-binding proteins. One of the initial nuclear responses to stimuli such as growth factors and light, is the rapid transcriptional activation of genes that encode putative transcriptional regulatory factors. It is though that the expression of these factors results in a cascade of gene activation that ultimately results in the differentiated phenotype. It is not clear in retinas why there should be such a long lived induction in NGF1-A mRNA by the dark cycle. It has been shown earlier for *c-jun* which remained induced for several hours in human monocyte cell line U937 treated with phorbol ester [28]. In conclusion, these studies have shown that the NGF1-A mRNA is regulated diurnally with lower levels in light and high levels in dark. Also absence of an outer segment does not alter the diurnal expression of NGF1-A mRNA unlike arrestin mRNA [9] in *rds* mutant retinas.

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