Experimental Eye Research 89 (2009) 854–862

Contents lists available at ScienceDirect

Experimental Eye Research

journal homepage: www.elsevier.com/locate/yexer

Developmental regulation of the nuclear ferritoid–ferritin complex of avian corneal epithelial cells: Roles of systemic factors and thyroxine

Kelly E. Beazley [1], James P. Canner [1], Thomas F. Linsenmayer*

Department of Anatomy and Cellular Biology, Tufts University School of Medicine, 136 Harrison Avenue, Boston, MA 02111, USA

A R T I C L E I N F O

Article history:
Received 9 April 2009
Accepted in revised form 15 July 2009
Available online 21 July 2009

Keywords:
ferritin
ferritoid
thyroxine
CAM
corneal epithelium

A B S T R A C T

Previously we observed that avian corneal epithelial cells protect their DNA from oxidative damage by having the iron-sequestering molecule ferritin – normally cytoplasmic – in a nuclear location. This localization involves a developmentally-regulated ferritin-like protein – ferritoid – that initially serves as the nuclear transporter, and then as a component of a ferritoid–ferritin complex that is half the size of a typical ferritin and binds to DNA. We also observed that developmentally, the synthesis of ferritin and ferritoid are regulated coordinately – with ferritin being predominantly translational and ferritoid transcriptional. In the present study we examined whether the mechanism(s) involved in this regulation reside within the cornea itself, or alternatively involve a systemic factor(s). For this, we explanted embryonic corneas of one age to the chorioallantoic membrane (CAM) of host embryos of a different age – all prior to the initiation of ferritin synthesis. Consistent with systemic regulation, the explants initiated the synthesis of both ferritin and ferritoid in concert with that of the host. We then examined whether this systemic regulation might involve thyroxine – a hormone with broad developmental effects. Employing corneal organ cultures, we observed that thyroxine initiated the synthesis of both components in a manner similar to that which occurs in vivo (i.e. ferritin was translational and ferritoid transcriptional).

© 2009 Elsevier Ltd. All rights reserved.

1. Introduction

In the adult, reactive oxygen species (ROS) can be generated by UV light and molecular oxygen (O_2) (Shimmura et al., 1996); in the embryo ROS are present in amniotic fluid (Longini et al., 2007) – presumably resulting from metabolism. ROS can damage a wide variety of macromolecules ranging from DNA to proteins to lipids. The damage to DNA is potentially carcinogenic; in skin, for example, UV-induced damage to DNA is thought to be a major factor in the increasing incidence of epidermal cancers (Hart et al., 1977). Corneal epithelial (CE) cells, however, seem to be refractory to such damage. Primary cancers of these cells are extraordinarily rare, even though this tissue is transparent and constantly exposed to ROS-generating UV light and O_2 (Smolin and Thoft, 1987). In addition, within the central CE are multipotent progenitor cells that can repopulate the CE in normal tissue homeostasis, without activation of the pigment granule-containing limbal stem cells (Cotsarelis et al., 1989; Majo et al., 2008). This suggests that CE cells have evolved defense mechanisms – not involving pigmentation – that prevent damage to their DNA. Previous studies in our laboratory suggest that one mechanism for such protection involves having iron-sequestration molecule ferritin in a nuclear location rather than the cytoplasmic location it has in most other cell types. This nuclear ferritin seems to greatly diminish the effects of ROS on DNA, which are effected, at least in part, by the presence of free iron (Fe^{2+}) that can exacerbate oxidative damage through the Fenton reaction-mediated formation of hydroxyl radicals – the most energetic and damaging ROS (Henle et al., 1996; Luo et al., 1996; Stohs and Bagchi, 1995).

For the nuclear transport of ferritin in CE cells, we previously identified a novel protein that binds to ferritin and translocates it into the nucleus. This protein, that we have termed ferritoid for its similarities to the ferritin-H chain, consists of three domains. The largest domain is ferritin-like and is involved in the binding of ferritoid to ferritin; the second domain is NH2-terminal and contains an SV40-type nuclear localization signal that is involved in nuclear translocation (Millholland et al., 2003), and the third is a COOH-terminal that regulates the association of ferritoid and ferritin (Beazley et al., 2008).

Following nuclear transport, ferritoid remains associated with ferritin as a unique nuclear ferritoid–ferritin complex (Nurminskaya et al., 2009). This complex has properties that distinguish it from

* Corresponding author. Tel.: +1 617 636 6695; fax: +1 617 636 3676.
E-mail address: thomas.linsenmayer@tufts.edu (T.F. Linsenmayer).
[1] The first two authors contributed equally to this work.

0014-4835/$ – see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.exer.2009.07.007

a typical cytoplasmic ferritin molecule, including its size – which is approximately half that of a cytoplasmic ferritin – and its ability to bind to DNA – which could position it where iron-sequestration would be most effective in protecting DNA from ROS-mediated damage (Cai et al., 1998, 2008; Linsenmayer et al., 2005), and where it could afford additional protection through physical interaction with the DNA (Grant et al., 1998). In these characteristics, the nuclear ferritoid–ferritin complex(s) of CE cells is strikingly similar to a class of small, ferritin-like bacterial proteins (the DNA-binding Proteins of Starvation) that are synthesized under adverse environmental conditions and which protect DNA from oxidative damage (Grant et al., 1998).

As ferritoid and ferritin assemble as a complex, it is likely that their developmental synthesis is concomitant. Consistent with this hypothesis, we recently observed that the initiation of ferritoid synthesis occurs less than 6 h before that of ferritin (at E10.75 and E11, respectively) (Beazley et al., 2008).

Developmentally there are two ways in which the temporal initiation of the synthesis of ferritoid and ferritin could be regulated. One is that the mechanism(s) responsible reside within the developing cornea itself. The other is that their initiation is triggered by a systemic factor(s) produced elsewhere in the embryo. In the present study, to distinguish between these two possibilities, we employed heterochronic grafting of embryonic corneas to the chorioallantoic membrane (CAM) of host embryos. The CAM is an extraembryonic site that supports a full range of developmental processes in grafted tissues – including corneas, as we have shown previously (Zak and Linsenmayer, 1985a,b). As tissues explanted to the CAM are nourished by the host this should provide the explant with systemic factor(s) produced by the host embryo. For these experiments, corneas from various ages of embryos (all pre-ferritin/ferritoid) were grafted to the CAM of different ages of host embryos (also pre-ferritin/ferritoid). Then, at intervals following explantation, the appearance of ferritin and ferritoid in the CE of the explant and host were assayed by immunofluorescence. If systemic factors are responsible for the temporal initiation of synthesis of ferritin and ferritoid, these molecules should appear in the graft and host at the same time – irrespective of the age of the cornea used for explantation. As will be described in the results, the experiments show this to occur.

Also in the present study we examined whether thyroxine is one systemic factor responsible for the initiation of synthesis of ferritoid and ferritin. Thyroxine was chosen as a candidate for two reasons. One reason is that studies on another cell type, hepatocytes, have shown that: (1) thyroxine amplifies the synthesis of cytoplasmic ferritin (Deshpande and Nadkarni, 1992); (2) this effect of thyroxine involves translational regulation; and (3) the presence of low levels of iron is required for this effect (Leedman et al., 1996). Similarly, for CE cells our previous observations show that initiation of synthesis of ferritin and ferritoid are at least partly under translational control and that iron is required [(Beazley et al., 2008) and see Discussion]. The other reason we considered thyroxine to be a candidate is that in corneal development it is known to have a profound effect. The formation of the thyroid gland occurs around E10 (Thommes et al., 1992), shortly before the time we detect the appearance of CE nuclear ferritin and ferritoid (E11), and analyses employing RIA for thyroxine show an increase in thyroxine in the developing eye at that time (Prati et al., 1992). In addition, Conrad et al. (2006) have recently shown that thyroxine alters the expression of a number of corneal genes. In the present study we examined the effects of thyroxine on the initiation of synthesis of ferritoid and ferritin in cultured corneas. The results show that thyroxine does initiate the synthesis of both molecules in a dose-dependent manner that recapitulates their development in vivo.

2. Results and discussion

2.1. Heterochronic grafting of corneas to the CAM of a different age host

As described in the Introduction, the developmental initiation of the synthesis of ferritoid and ferritin could be regulated either by mechanism(s) that reside within the cornea itself, or involve systemic factor(s). To distinguish between these alternatives, corneas from different ages of pre-ferritin/ferritoid stage embryos were explanted to the chorioallantoic membrane (CAM) of host embryos that were also pre-ferritin/ferritoid, but of an age that differed from the cornea donor (i.e. heterochronic combinations). Then, at various times following explantation, the corresponding host and donor corneas were assayed for the presence of ferritin and ferritoid by immunofluorescence.

The heterochronic combinations we examined are schematically shown in Figs. 1A and 2A; also shown Figs. 1 and 2 are immunofluorescence micrographs for ferritoid and ferritin in the resulting explanted corneas and host corneas. The combinations consisted of explanted corneas from donors that, at the time of explantation, were either 3 days younger than the host [Fig. 1A, explant (E6); host (E9)], or 3 days older than the host [Fig. 2A, explant (E9); host (E6)]. Also shown in these schematic diagrams are the number of additional days, following explantation, that the hosts were allowed to develop before the corneas were analyzed (Fig. 1A: +1 d, and +3 d; Fig. 2A: +2 d, +4 d, and +6 d), and the day at which the host would normally initiate ferritoid (FTD) and ferritin (FTN) synthesis (E11) (Beazley et al., 2008). In all experiments, for each time point at least three explants and three host corneas were evaluated.

2.1.1. Younger corneas to older hosts (Fig. 1)

In the first series of experiments (Fig. 1), corneas from E6 embryos were explanted to the CAM of embryos that were three days older (E9), and then after one and three days of incubation both the host and explanted corneas were examined for the presence of ferritoid and ferritin (Fig. 1A, +1 d and +3 d). Consistent with our previous studies showing that ferritin and ferritoid are not present in the CE until ∼E11 (Beazley et al., 2008), the E9 host embryos, after one day of incubation had neither molecule [as they had progressed only to E10 (Fig. 1B, host E10)]. However, after three days of incubation (i.e. when the host was now E12) their CE now had both molecules in the nucleus (Fig. 1C, host E12), as expected.

For the explants, if the temporal synthesis of ferritin and ferritoid is inherently regulated within the cornea itself, after neither length of incubation should they have initiated synthesis of the molecules. After one day of incubation they chronologically would be E7 [Fig. 1C (E6 + 1 d)], and at 3 days of incubation they chronologically would be E9 [Fig. 1C (E6 + 3 d)], still two days before synthesis should be initiated.

At one day of incubation [Fig. 1B, explant (E6 + 1 d)], neither the explants nor the hosts showed ferritoid or ferritin. However, as neither the host nor the donor corneas had reached the time when the initiation of synthesis would be expected to occur (E11), no conclusions could be drawn. So these essentially served as negative controls.

However, after three days of incubation both the hosts (Fig. 1C, host E12) and explants [Fig, 1B, explant (E6 + 3 d)] had initiated the production of ferritoid and ferritin. Although chronologically the explants were only E9 (E6 + 3 d), with respect to the synthesis of ferritin and ferritoid they had differentiated past the point where synthesis would normally occur (i.e. E11). This suggests that developmentally they had progressed at least two days beyond

Download English Version:

https://daneshyari.com/en/article/4012000

Download Persian Version:

https://daneshyari.com/article/4012000

Daneshyari.com