4 Animal Models of Experimental Myopia: Limitations and Synergies with Studies on Human Myopia

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While interest in human myopia has a long history [1], research into experimental myopia in animal models is much more recent. After some early attempts at experiments in animals [2, 3], the field took off after the publication of two papers – the first a paper on induced myopia in primates in 1977 by Wiesel and Raviola [4], which was an offshoot of the research on visual pathways which later won Hubel and Wiesel the Nobel Prize. This was rapidly followed by a paper by Wallman and colleagues on experimental myopia in chickens [5]. Since then, experimental myopia has been expanded to a much wider range of species, including common laboratory animals such as mice [6, 7] and guinea pigs [8, 9], as well as more exotic species such as tree shrews [10].

While experimental myopia is a biologically interesting problem in its own right [11], we will deal primarily with what experimental myopia can tell us about human myopia. This perspective means that the ideal animal model should reproduce the developmental features of human myopia in the time-course of change in the ocular determinants of refraction and use methods of inducing experimental myopia which mimic those important in human myopia – although departures from this ideal do not doom a model to irrelevance.

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4.1 Refractive Development and Incident Myopia in Children

Given these criteria, a brief overview of refractive development in children is necessary. Children are born with a normal (Gaussian) distribution of spherical equivalent refraction (SER), with a mean hyperopic refraction [12]. Rapid changes over the first year or two after birth result in a narrower distribution of SER, often described as leptokurtotic, due to a reduction in both myopic and highly hyperopic refractive errors [13–15]. These changes involve loss of corneal power, loss of lens power and axial elongation. While the mean SER moves towards emmetropia, at the end of this developmental period the mean SER remains distinctly hyperopic. From then on, the cornea stabilises, and up to the age of 5–6, these characteristic features of the distribution of SER (hyperopic mean SER and narrow distribution) are seen in all populations that have been studied, even those that subsequently become highly myopic [16]. By the age of 5–6, the distribution of the ratio of AL to CR is also narrow, suggesting that an important part of the changes up to this age involves matching the axial length of the eye to the corneal power, but the underlying distributions of AL and CR remain normal [17]. In general, the prevalence of myopia is low over this period.

After the cornea stabilises, axial elongation can continue for as much as 20 years, at rates which seem to be influenced by the environments in which the children are growing up [18, 19]. This period of development appears to create the marked differences in the prevalence of myopia currently seen around the world [16]. Up to 10–12 years, there are rapid decreases in lens thickness and power [18, 20], which minimise increases in myopia associated with axial elongation. The rate of loss of lens power decreases after the age of 10–12, and the lens starts to thicken. Mutti and colleagues [21] have reported that, close to the onset of myopia, loss of lens power ceases abruptly, but this phenomenon has not been reported in all studies [22]. After this age, with a slower rate of loss of lens power, axial elongation is translated almost completely into myopic shifts in refraction.

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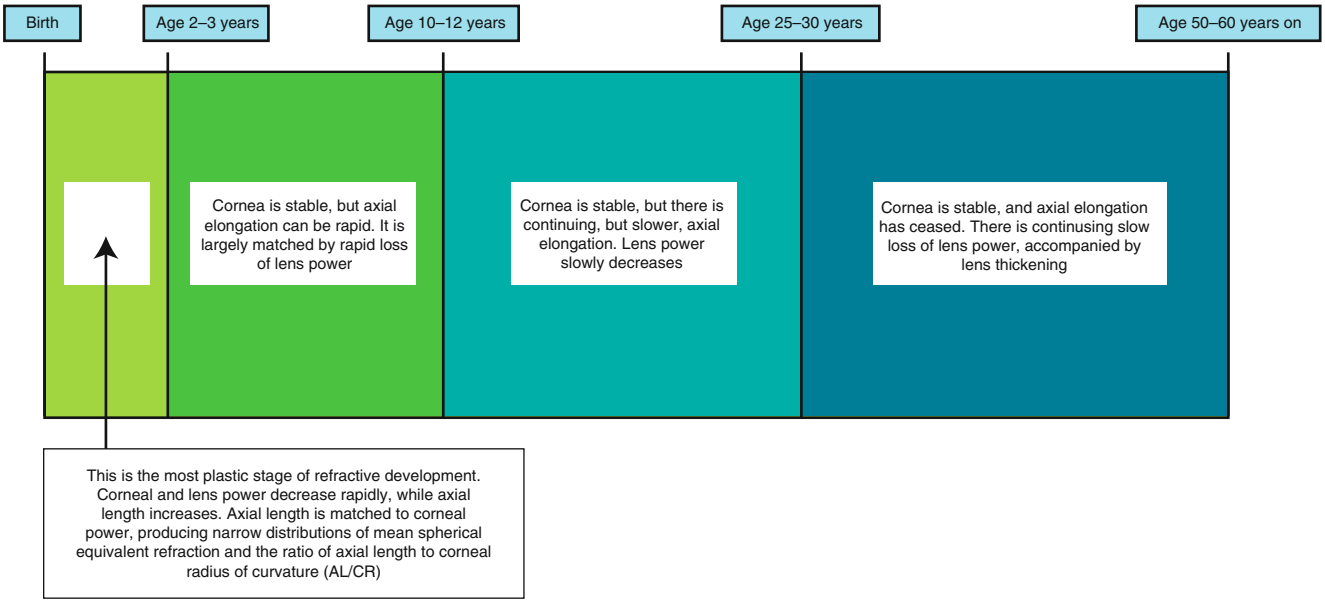

Fig. 4.1 Phases of refractive development in humans. Four phases can be distinguished. A highly plastic neonatal stage lasts 2–3 years, after which the cornea stabilises. Following this, axial elongation can continue for at least 20 years. For some of this time, loss of lens power tends to minimise the myopic refractive shift associated with axial elongation, but around the age of 10–12, the rate of loss of lens power decreases but is then maintained at a slow rate for several decades. This results in hyperopic shifts for much of adult life, except where marked increases in lens power associated with cataract lead to marked myopic shifts in the elderly

It is important to note that most human myopia appears after the age of 5–6 across a range of ethnic groups [16]. In children of European origin, the prevalence is generally less than 1 % up to the age of 5–6 [23]. In children of African-American and Hispanic origin in the United States [23, 24], it has been reported that the prevalence of myopia is higher up to this age (5–10 %). In particular the prevalence of myopia is higher in neonates and drops during development. Particularly high myopia prevalence rates, as high as 20 %, have been reported in children of Chinese origin in this age group in Singapore [25]. At present, it is not clear whether these differences represent genuine ethnic differences, whether they are specific features of the sites examined or whether they are due to problems with cycloplegia in children with dark irises, but it should be noted that little myopia is detected in children of Chinese origin when more rigorous cycloplegia is used [26].

An important feature of human myopia is that, although the term emmetropisation is widely used [27], the end point of refractive development is not, in fact, emmetropia [16]. Rather the "normal" refractive state is mild hyperopia (anywhere in the range of +0.5 D to +2.00 D) – a level at which normal visual acuity can be achieved through accommodation by most people up to the age of about 40 [28]. In populations where the prevalence of myopia is low, this refractive state persists into at least the early adult years, provided that cycloplegia is used [29, 30]. In contrast, in populations which later develop significant myopia, the refractive distribution shifts towards myopia, but emmetropia rarely becomes the dominant refractive category, because it appears that, as some children enter the emmetropic category, others pass from emmetropia to myopia [16].

Thus, in human refractive development, we need to consider several developmental phases defined by changes in refraction and in the biometric components of refraction. At least four phases can be distinguished (Fig. 4.1). The boundaries between these stages are not tightly defined but provide an important point of reference for studies on experimental myopia.

4.2 Experimental Myopia

4.2.1 The Basic Paradigms in Experimental Myopia

The basic methods for research in experimental myopia, and the results obtained, have been extensively reviewed [11]. Wiesel and Raviola [4] carried out their pioneering studies on monkeys with sutured eyelids, an approach with analogies to the myopia associated with infantile ptosis [31]. In contrast, Wallman and colleagues placed translucent diffusers over the eyes of chickens to induce myopia [5]. Roughly 10 years later, a different technique for inducing experimental myopia was introduced, in which negative lenses were placed over the eyes, and compensatory changes in eye growth were observed [32].

These studies have led to general use of two paradigms for inducing experimental myopia.

4.2.1.1 Form-Deprivation Myopia (FDM) or Deprivation Myopia (DM)

This paradigm uses translucent diffusers, fitted over the experimental eye, which allow considerable light through, typically with a reduction of light intensity of less than one log unit. These diffusers, however, markedly reduce spatial contrast, and, in moving animals, the reduced spatial contrast translates automatically into reduced temporal contrast. This kind of manipulation results in rapid development of myopia – for example, in chickens as much as 20 D of myopia is achieved in less than 2 weeks, although the development of myopia is somewhat slower in other animals. As in humans, the development of myopia in this paradigm primarily depends on axial elongation and particularly elongation of the vitreous chamber.

4.2.1.2 Lens-Induced Myopia (LIM)

When negative lenses are fitted over the developing eye, the eye responds rapidly with compensatory increased growth, which continues until the imposed defocus has been neutralised [32, 33]. Thus the experimental eyes move towards emmetropia with the lens in place and develop an intrinsic refractive error which, after the lens is removed, corresponds to the power of the lens fitted. This occurs largely through modulation of vitreous chamber depth. The compensation of imposed defocus achieved in LIM appears to be quite precise.

4.2.1.3 How Different Are FDM and LIM?

These paradigms differ in several ways. Eyes fitted with diffusers have no way of overcoming the reduced spatial and temporal contrast to which they are exposed, for this is not affected by axial elongation. Thus, continued growth does not reduce the level of the stimulation towards growth, and the eyes continue to grow until natural reductions in body growth terminate the process. Accommodation does not seem to be important for the development of FDM [34, 35]. This is therefore an open loop process, in which there is no feedback to limit growth.

In contrast, in LIM, the eyes are generally fitted with lenses of powers that are within the accommodative capacity of the eyes. It is therefore expected that, for at least part of the time, the animals use accommodation to neutralise the imposed defocus to produce focused images. This accommodative response does not appear to be a crucial factor, since eyes with impaired accommodation seem to develop lens-induced myopia [36, 37]. It is thus generally assumed that the growth responses are stimulated by the magnitude or the sign of defocus, which is detected in some way by the retina, although the mechanisms involved are not understood. Since axial elongation leads to compensation for the imposed hyperopic defocus, as the eye grows there is a constant reduction in the stimulus to growth, and the process terminates when growth has compensated for the imposed refractive error. In other words, this is a closed loop system.

Despite the different properties of the paradigms at the level of visual input and feedback, the responses in FDM and LIM are very similar at the cellular and molecular levels [38, 39], suggesting that many of the pathways leading to axial elongation and myopia are shared between the two systems. But, this is currently a controversial area, and the many similarities do not mean that they are identical.

4.2.1.4 Recovery from Experimental Myopia (REC)

After the introduction of the FDM paradigm, it was discovered that if the diffusers were removed, provided that the animals were still young, the eyes responded by slowing the rate of axial elongation [40]. As a result, the refractive state could return to, or at least towards, emmetropia, due to continued development of the anterior segment of the eye. This process does not appear to be driven by the different shape of the myopic eye but is driven by the defocus, because optical correction of the myopic defocus prevents the changes in eye growth [41, 42]. Unlike the FDM paradigm used to induce the myopia initially, this is therefore also a closed loop paradigm.

4.2.1.5 Lens-Induced Hyperopia (LIH)

In the same set of experiments that introduced LIM [32, 33], the effects of fitting positive lenses were examined. This should impose myopic defocus on the eye, which cannot be corrected by accommodation, and in this case, the rate of axial elongation slows. Since the anterior segment of the eye continues to develop, associated loss of corneal and lens power can lead to hyperopic shifts in refraction. As with LIM, this is a closed loop paradigm, and compensation for the imposed lens appears to be quite precise.

4.2.1.6 How Similar Are the REC and LIH Paradigms?

These two paradigms enable investigation of the processes leading to reduced rates of eye growth. It is generally assumed that the paradigms involve reductions in or cessation of the rate of axial elongation, but recently strong evidence that a proportion of eyes can actually become shorter through active remodelling of the sclera has been published [43]. These paradigms which involve reduced axial elongation. The REC paradigm involves an eye and a retina which have already been supporting an excessive rate of eye growth,

and thus they are in a different state to that of a normal eye, at both the retinal and scleral levels. In contrast, the eye and retina in the LIH paradigm are effectively in their control state. Much less work has been done on the cellular and molecular basis of these paradigms, but there are some indications that they may be different.

4.2.2 What Is the Best Model in Terms of Stimulus Relevance to Human Myopia?

The unfortunate answer to this question is "probably none". With the exception of the very low percentage of myopia associated with congenitally blurred vision, such as with ptosis, congenital cataract or corneal scarring, children do not grow up with the equivalent of translucent plastic goggles over their eyes, and thus the FDM model is not regarded as a good model of human myopia. In contrast, it is generally believed that LIM provides a better model, because the imposed hyperopic defocus generated by fitting negative lenses over the eye can be regarded as analogous to the demands placed on children's eyes by too much near work. But the evidence that near-work demands and myopia are linked has become much weaker, as more quantitative studies have been performed [44].

Initially, it was believed that the increased accommodation associated with high levels of near work might be the important factor. But studies on animals have shown that experimental myopia can be induced in species without accommodative capacity [34], or with experimental interruptions to accommodation [36], and that atropine can block eye growth in a species (chicken) in which it does not block accommodation [35]. Collectively, this is strong evidence that active accommodation is not a crucial factor in the development of myopia.

In parallel with these developments, while there is a consistent correlation between schooling and educational outcomes and myopia [45], attempts to quantify the association using precise measurement of near-work exposures have produced less than stunning results, and some have concluded that near work may have little role [44]. Emphasis then shifted to the idea that, rather than accommodation itself, it was accommodative lag in periods of near work which was important. However, while accommodation is less accurate in children with myopia than in those with emmetropic refractions [46], there is conflicting evidence on whether this difference precedes or follows the development of myopia [47–50]. Overall, it is far from clear that the mechanisms involved in LIM are really similar to those involved in human myopia. More recently, attention has shifted to the interplay in space and time between hyperopic and myopic defocus on the retina, where myopic defocus appears to be a stronger stimulus [51–53]. But it is not clear how this would work in detail in the human context.

Quite recently, it has been shown that chickens raised on light-dark cycles in which the light phase consists of dim light (50 lx), slowly become myopic [54, 55]. There has been some interest in this as a model for human myopia, but children becoming myopic are not generally exposed to conditions of this kind, even where there is an epidemic of myopia. It is, in fact, clear that human myopia involves a response to environmental exposures, which needs to be part of a good animal model.

Overall, none of the animal models fits well with what we know about human myopia. A simple but powerful illustration of this point is that in both FDM and LIM [56–58], brief removal of the optical devices prevents the development of myopia. In contrast, it seems almost certain that children are not constantly exposed to risk factors such as near work or low light intensities, and periods without these conditions do not seem to block the development of myopia. Equally, given the strong effect of imposed myopic defocus, there is a paradox, because the ability of myopic defocus to slow axial elongation and the recovery observed in the REC paradigm, if simply applied to human myopia, would suggest that human myopia should be a self-limiting condition, which it clearly is not.

Another important difference is that, while compensation appears to be quite precise in the LIM and LIH paradigms, the same precision is not obvious in human emmetropisation, given that characteristically in 3–5-year-olds, the mean SER is distinctly hyperopic. Thus some of the principles which appear to apply to experimental myopia do not seem to apply to human myopia.

Clearly, none of the existing paradigms provides an adequate model of human myopia, which means that human epidemiology will continue to play a critical role as the point of reference. But this does not mean that these models are useless. Irrespective of the mechanism by which myopia is induced, these models can be used to study the nature of the changes in ocular components and the corresponding details of changes in gene expression and biochemistry, at a level which is simply impossible in humans.

4.2.3 Which Is the Best Species to Study for Relevance to Human Myopia?

At one level, the answer to the question about the most relevant species is self-evident – non-human primates or monkeys. Detailed studies of the development of the refractive components of the eye in rhesus monkeys have shown that humans and monkeys share common processes of early loss of corneal power, followed by stabilisation, and more prolonged loss of lens power and thickness, followed by relative stabilisation, as well as a mean SER which is in the mildly hyperopic rather than emmetropic range [59]. They have also shown that development of myopia in humans and monkeys

Table 4.1 Temporal characteristics of refractive development in humans and monkeys (Time to reach the midpoint between the measure at birth and the measure at the developmental plateau, assuming non-linear regression) [59]

	Human (days)	Monkey (days)
Ocular component		
Refraction	276	213
Corneal power	251	75
Axial length	584	196
Anterior chamber depth	384	133
Vitreous chamber depth	815	258

Data were taken from a study of refractive development in rhesus monkeys. Asymptotic regression models were used to define the half-time to a developmental plateau. Data on humans was taken from a range of studies on humans. Original references are given in the paper on rhesus monkeys. The time to half-plateau is higher in humans than in monkeys. Studies on humans may overestimate some of these parameters because of continuing axial elongation and development of myopia, which does not normally occur in monkeys

predominantly depends on changes in axial length [60]. But while the pattern of change is similar, the absolute timing is different (Table 4.1). In humans, corneal power (radius of curvature) stabilises at about 700 days, while in monkeys it stabilises at around 200 days. This difference is not surprising given the relative differences in maturation and life span and is quite consistent with the data on time of half-change. It is particularly important to note that most studies on monkeys have been carried out in this early developmental period, typically from 21 days up to around 140 days, corresponding to a developmental period which does not correspond to that in which most myopia develops in humans.

This difference in developmental age also applies to the other species that are studied. As a general rule, studies on experimental myopia have overwhelmingly been carried out during developmental periods that correspond most closely to the neonatal period of development in humans, for the simple reason that large and rapid changes can be observed in this period. In humans, this is a period in which neonatal myopia is naturally reduced or eliminated, significant hyperopia is substantially reduced, and there is loss of corneal power, major loss of lens power and substantial matching of the axial length of the eye to the corneal and lens powers, to produce a tight distribution of refraction. In contrast, during the period in which myopia typically develops in humans, corneal power is stable, lens power loss decreases and after the age of 10–12 slows even further. It would therefore not be surprising if there were substantial differences in the regulatory processes in operation. For example, a simple resolution of the paradox that myopic defocus does not prevent the development of myopia in humans, although recovery from both FDM and LIM is effective in experimental myopia, may be that the signals generated by myopic defocus are weaker or nonexistent at later developmental stages. There is very limited experimental support for this idea [61], but there is also evidence that myopic defocus can still exert effects later in human development [22].

In the other species that are commonly studied as models of experimental myopia, the developmental events deviate more markedly from the human pattern, and their use is consequently more contestable. Guinea pigs [8, 9, 62] and mice [7, 63–65] show thickening of the crystalline lens, in contrast to humans, although lens power decreases in all three. However, tree shrews show the human pattern of combination of loss of lens power and thinning of the crystalline lens but otherwise have a complicated pattern of development, including a period after eye opening where experimental myopia develops very slowly [66, 67].

Again, this does not mean that the use of models other than non-human primates is irrelevant. Experimentation on monkeys is limited by justified ethical concerns, as well as by logistic and other considerations, and other species have their advantages. Chickens are easy and cheap to obtain, and induction of experimental myopia is extremely rapid. Mice are easy to obtain, but induction and monitoring of myopia is more difficult. The great advantage of mice lies in the existing detailed knowledge of the mouse genome and of their cellular and molecular biochemistry. Guinea pigs provide a diurnal mammalian model, although they probably differ most in terms of the changes in ocular components from the human model. Tree shrews provide a diurnal mammalian model and are close to the primate line, but again their developmental profile does not correspond closely to the human model, and they require specialised breeding facilities. However, provided that allowance is made for the different responses of the ocular components of refraction, these models can be used to usefully address many basic questions about changes at the cellular and molecular levels which lead to myopia.

One important limitation on the use of animal models is that most vertebrates, including birds, have a sclera which consists of two components – a fibrous layer and a cartilaginous layer [68, 69]. In lower vertebrates, the dominant response of the sclera involves expansion of the cartilaginous layer, whereas the fibrous layer appears to become thinner. Mammals, including humans, appear to have lost the cartilaginous layer, and thus the scleral response consists only of thinning and weakening of the fibrous layer. Thus, chickens are not a good model in which to study changes in the sclera.

4.3 Important Features of Experimental Myopia

4.3.1 Local Control and Spatial Localisation

One of the most striking discoveries from experimental myopia is that the control of eye growth primarily occurs within the eye, presumably involving interactions between the retina and sclera, with little impact from central

pathways. In both FDM and LIM, there is minimal impact of sectioning of the optic nerve, cutting off the eye from centrifugal input [36, 70]. Equally, lesions to the ciliary nerve have little effect [36].

This point is further emphasised by the evidence that use of partial diffusers and lenses produces differential growth changes. For example, half diffusers tend to produce excessive growth in roughly half the eye, the half experiencing form deprivation [71], and the same is true of partial lenses [72]. These findings place some important limitations on mechanisms, since global processes such as accommodation would not be expected to operate in this way. But, at the same time, it is not clear how precise this spatial localisation is, since most experiments have demonstrated differential control over quite large areas, and it should not be assumed that the spatial localisation is as precise as point-to-point neural pathways can be. Rather, it is probably best to think in terms of circles of influence for any pathway being considered, just as blur in the image turns a point focus into a blur circle. For example, if the release of dopamine from dopaminergic neurons in the retina is important, as the evidence strongly implies [39], then this could be controlled by spatially precise modulation of activity within defined pathways linking photoreceptors to ON-bipolar cells to dopaminergic cells. However, once the transmitter has been released, then diffusion of the transmitter, including lateral spread of its effects within the retina and choroid, is likely to produce a circle of influence. How large this circle of influence is will depend on the speed with which the transmitter or messenger diffuses, but this principle is likely to apply at any stage in the growth control pathway where the message is transmitted by soluble, diffusible messengers.

4.3.2 Choroidal Changes

Another important observation from animal experimentation is that, particularly in the chicken, there are major changes in the thickness of the choroid, which swells in response to myopic defocus and thins in response to hyperopic defocus [73, 74]. In chickens, the choroid can expand by some hundreds of microns in response to myopic defocus, although thinning is of lesser magnitude. In other species, including non-human primates [75], changes in choroidal thickness are much less marked. This is also true for humans [76, 77].

In chickens, it has been suggested that the swelling of the choroid in response to myopic defocus may act to reduce the level of myopic defocus on a time-scale intermediate between that of accommodation and changes in axial length, by bringing the retina towards the myopic focal plane within minutes to hours of the imposition of myopic defocus, although the accuracy of the compensation is still to be determined. Given the smaller magnitude of the responses to hyperopic defocus, less effective compensation would be achieved.

Whatever, the reasons for these changes, they may be involved in the transmission of growth control signals from the retina to the sclera, since there is some evidence that choroidal changes are linked to slowing of axial elongation in response to myopic defocus [78–81]. The role of the choroid has been extensively reviewed recently [82].

4.3.3 Summary

Despite the many limitations and cautions on the use of animal models of experimental myopia, studies on animal models can investigate issues that cannot be addressed in humans – in particular animal models of experimental myopia can be used to elucidate details of the molecular and cellular processes involved, which may open up opportunities for pharmacological intervention.

Of the various limitations of studies on experimental myopia, probably the most fundamental is that experimental myopia is generally induced during a different developmental phase to that in which human myopia appears. In addition, LIM involves a level of precision that does not appear to apply to human myopia. Specifically, the compensation process in LIM (and LIH) appears to be very precise, but, in humans, refractive development appears to rather imprecise, with refractions in the range +0.5 to +2.0 D appearing after the first 2–5 years of life. These are maintained into adult life in populations in which the prevalence of myopia is low.

The distinction concerning developmental period may be crucial. Pooled data from four leading laboratories in the field of experimental myopia has shown that in the REC and LIH paradigms, there is evidence that the eyes can actually shrink in chickens, monkeys (both macaques and marmosets) and tree shrews [43], suggesting a more active remodelling process than just the slowing of growth normally assumed. Not all eyes shrink however, and shrinking was more common in tree shrews than in the monkeys, which the authors attributed to the earlier developmental age of the tree shrews. All the studies were carried out in the rapid developmental period, and, given the evidence in the paper that this active remodelling becomes less active with age, it is questionable whether anything like this would occur in human myopia, even if methods are developed for preventing myopic progression, given the differences in developmental stage.

4.4 Synergies Between Research on Human Myopia and Experimental Myopia

We suggest that greater integration of the results from these two streams of research, human myopia and experimental myopia, will increase understanding of the aetiology of myopia and assist in achieving the ultimate goal of controlling

human myopia. The interaction is two way – sometimes starting with discoveries in human epidemiology and sometimes with discoveries in human myopia. We will discuss some case studies which illustrate the synergies that have already occurred and suggest some areas that can be more systematically explored in the future. Later, we propose and discuss a heuristic model of the control of refractive development (Fig. 4.2), which can be used for orientation at this stage.

4.4.1 Genes and Environment

One of the most immediate conclusions that could have been drawn from the animal models of myopia is that refractive development could be profoundly altered by changes to visual input – stressing the potential for environmental influences. Unfortunately, there has often been little interchange between the two approaches to myopia research, and conclusions about the tight genetic determination of myopia derived from twin studies [83] were not seriously contrasted with the evidence that refractive development was extremely responsive to environmental manipulation. It is, of course, equally true that sensitivity to environmental manipulation in experimental myopia does not prove that environmental variation makes a major contribution to phenotypic variation in humans, and the two conflicting conclusions simply coexisted. In fact, it took the emergence of an epidemic of myopia in developed parts of East and Southeast Asia to bring the issue to the fore [45]. The realisation of the implications of the rapid increase in the prevalence of myopia in East and Southeast Asia and the development of research in experimental myopia covered much the same period, and, in combination, they played a major role in the reassessment of the balance between genes and environment in the aetiology of myopia that has taken place over the past decade [84].

So far, genetic studies on apparently genetic (generally early onset, severe and highly familial) forms of human myopia have only identified a limited set of genes, and there has been a major difficulty in replicating many reported associations. These account for only a low percentage of myopia in most populations. This topic has been extensively reviewed [85–87]. Nevertheless, two clusters of mutations, one associated with scleral constituents and another associated with visual processing in the photoreceptor to ON-bipolar cell pathway and in particular with various forms of stationary night blindness, have been identified from candidate gene studies. Similarly, in GWAS studies, after some years with little return, two recent studies based on large cohorts have identified a limited core group of around 30 genes that show significant associations with myopia, but which collectively explain less than 5 % of phenotypic variation [88, 89]. There is some overlap between discoveries using GWAS in human myopia, results on the genetic basis of human syndromic myopias, and changes observed using microarray technology in experimental myopia in animals – in particular the identification of the important role of changes in the outer retina and the sclera.

Further examination of changes in expression of at least some of these genes in animal models could be very illuminating. For example, one of the genes implicated in the development of myopia in several studies is RASGRF1 [90]. It codes for a nuclear exchange factor that promotes the exchange of GTP for GDP on Ras family GTPases, and as such it is likely to be involved in a range of functions in a variety of tissues. Studies on gene expression in human eye tissue show strong expression in RPE, photoreceptors and choroid, leaving the site of action uncertain. Knockout mutants show defects in photoreception, which could be associated with myopia by analogy with other mutations, but equally these knockout animals show other changes such as an enlarged lens, which could equally impact on refractive status. RASGRF1 contains a phosphorylation site, where stimulation of muscarinic receptors leads to increased phosphorylation and increases exchange activity [91].

GJD2 [92] provides a different example. It encodes connexin36, a gap junction protein, which is expressed in both the outer and inner plexiform layers of the retina and appears to play a role in coupling and uncoupling of rods and cones, horizontal cells, amacrine cells and ganglion cells. Its function is regulated by phosphorylation, which is in turn controlled by dopamine, which promotes uncoupling of cells to allow for higher-resolution vision under photopic conditions [39, 93]. D1-dopamine receptors are involved in this regulation, whereas most dopaminergic effects in experimental myopia involve D2-dopamine receptors.

It is clear that to unravel the complexities of the retinal pathways involved in just these two examples requires the use of animal models. In both cases, the modulation of function involves second messenger systems and protein phosphorylation, which might not involve changes in mRNA expression, making it difficult to detect changes in microarray experiments. The links of these two candidate genes to muscarinic and dopaminergic mechanisms are of considerable interest, since these mechanisms have been implicated in pathways controlling eye growth (see below), and these should certainly be explored.

Studies of changes in gene expression in experimental myopia can help to define the site of action of mutant genes identified for human myopia, since they can define, at least in some cases, where relevant changes in the expression of genes and gene products take place. The link between sites of mutations which affect myopia in humans and where changes in gene expression take place in experimental myopia is not likely to be absolute, but substantial overlap would be anticipated. Where genes identified in human myopia studies correspond to those in which changes in expression are reported in experimental myopia, the case will be particularly strong. There are many more opportunities for synergies, since, as of March 2013, the OMIM database listed

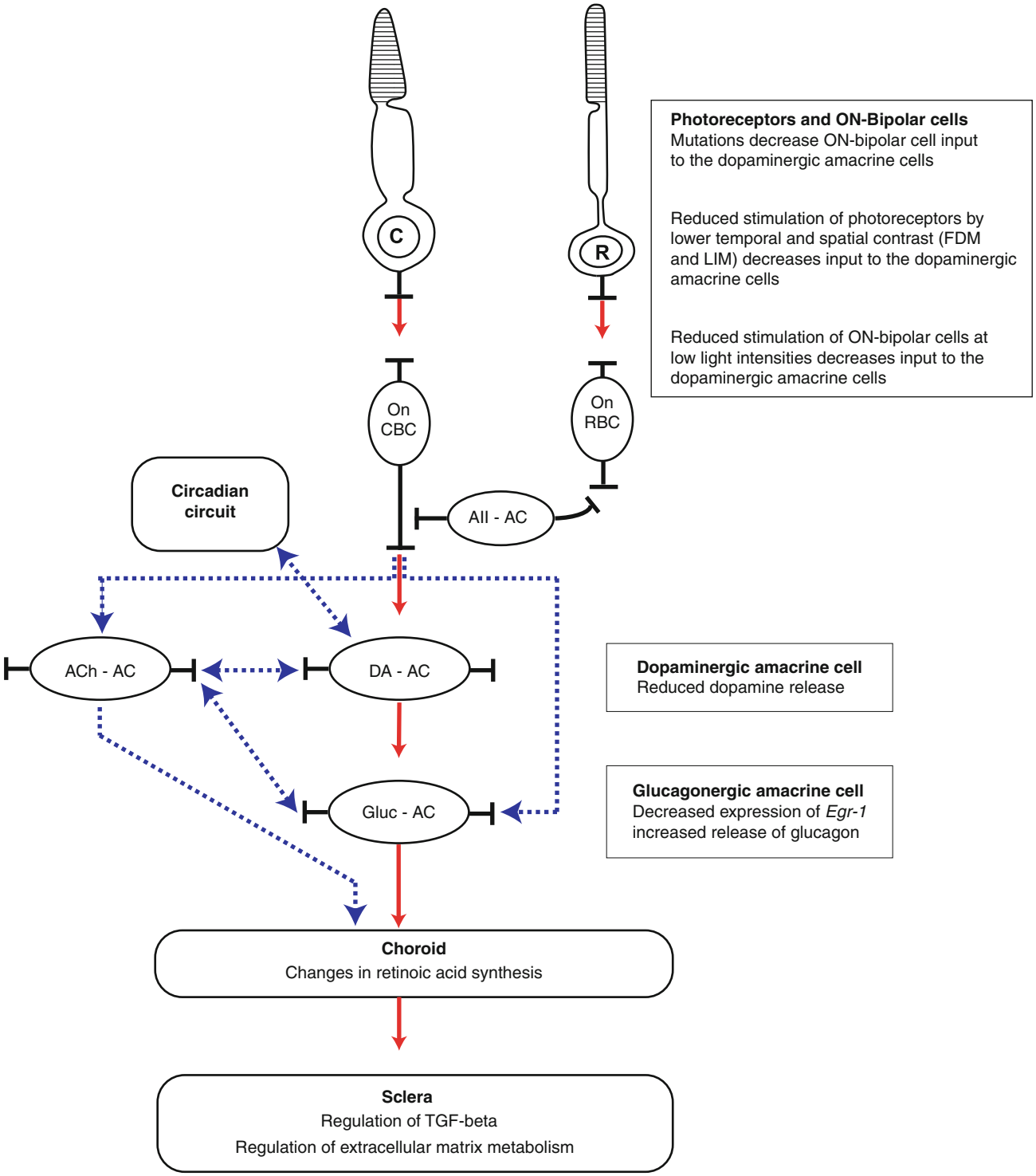

Fig. 4.2 A schematic diagram of a general pathway that may be important in control of eye growth. A key element is the link from photoreceptors though ON-bipolar cells and dopaminergic amacrine cells into the inner retina, where the first stages of growth signal cascades, which ultimately control scleral metabolism and growth, are generated. Animal studies strongly support a role for dopaminergic amacrine cells in growth control, and recent studies on the protective effect of time outdoors in children also suggest that dopamine may be involved in human myopia. *Key*: *C* Cone photoreceptor, *R* Rod photoreceptor, *On CBC* On-cone bipolar cell, *On RBC* On-rod bipolar cell, *All-AC* All amacrine cell, *Ach-AC* Acetylcholine amacrine cell, *DA-AC* Dopaminergic amacrine cell, *Gluc-AC* Glucagonergic amacrine cell

300 inherited conditions in which myopia is a symptom, although not necessarily a defining symptom.

The reverse flow is also possible. Studies on animal models have identified a list of candidate genes on the basis of changes in gene expression [87, 94, 95]. These need to be used as candidate genes in studies on human myopia, since the chances of the gene playing a significant role seem likely to be higher if there are large changes in gene expression during the development of myopia. However, so far the list of changes in mRNA expression is relatively short, and they are small in magnitude. But parallel studies are bound to become more systematic in the future.

An interesting approach which is impossible in humans, but highly feasible in experimental animals, is selective breeding. After only two cycles of selective breeding of chickens which show large or small responses to form deprivation, the strains showed marked differences in their responses to FDM, indicating a strong genetic component to the differences [96–98]. Whether this is relevant to human myopia is not clear, since, for these characteristics to segregate in human populations would require selective mating on the basis of sensitivity to develop FDM, which seems unlikely. Nevertheless, selectively bred strains could enable elegant dissection of the pathways involved.

Now that there is a common list of genes associated with myopia from the CREAM [89] and 23andMe studies [88], one of the important next steps is to examine whether there are any genetic differences in susceptibility between ethnic groups. So far, limited analysis suggests that there are no major differences between ethnic groups, consistent with the evidence on similar myopia prevalence values in the different ethnic groups in Singapore, with Chinese, Malays and Indians all showing very high rates of myopia. Another important next step is to look for gene-environment interactions involving these identified SNPs and identified environmental factors such as education, near work and time outdoors.

4.4.2 Site of Action of Atropine

Atropine was introduced to control the progression of myopia, based on the idea that myopia was due to excessive accommodation, and the initial successes seemed to give strong support to the excessive accommodation theory [99]. It is still the best validated technique for preventing myopic progression [99–101], and it has been extensively used, particularly in Taiwan [102].

When muscarinic agents were first used in experimental myopia, their ability to block axial elongation was taken as strong evidence for an effect on accommodation. However, this assumption was critically explored by McBrien and colleagues, who showed that experimental myopia could be induced in animals with little accommodative capacity, such as grey squirrels [34]. Other studies suggested that experimental myopia developed normally in animals in which accommodation had been experimentally disrupted [36, 37]. McBrien [35] also pointed out that atropine was effective in chickens, where accommodation was controlled by nicotinic rather than muscarinic acetylcholine receptors. Collectively, this evidence decisively ruled out a role for excessive accommodation per se in the development of experimental myopia, which has important implications for human epidemiology.

This shifted attention to alternative sites of action. Studies on chicken chondrocytes and scleral tissue in culture showed that many muscarinic antagonists were able to exert direct effects on these tissues [103]. The other obvious site was the retina itself, given that it has an extensive cholinergic system, with both muscarinic and nicotinic elements. However, evidence on whether retinal sites are involved is ambiguous. Fischer and colleagues [104] used a cholinergic toxin, which had been shown to destroy most cholinergic neurons in the chicken retina [105], to show that eyes in which the cholinergic system had been extensively disrupted could still develop FDM and LIM, which could be blocked with atropine. This evidence tended to favour a non-retinal (scleral?) site of action.

However, other evidence tends to support a retinal site. Specifically, one of the earliest responses detected in response to myopigenic optical devices, which can be detected within 30 min, is decreased expression of the immediate early gene Egr-1 at both the mRNA and protein levels in the glucagon-immunoreactive amacrine cells of the chicken retina [106]. It should be noted that this part of the pathway may be specific to chickens and may not be applicable to the human retina. Atropine reverses this downregulation within 1 h of the fitting of a diffuser or negative lens [38, 107]. It is hard to explain the rapidity of this effect in terms of a primary action of atropine on the sclera, with feedback to the retina. The ultimate test of site of action should come from a full pharmacological analysis of the three processes affected by muscarinic antagonists – block of axial elongation by muscarinic antagonists, block of scleral glycosaminoglycan synthesis and reversal of downregulation of Erg-1 in the retina. Whichever of the latter two replicates the pharmacology of the block of axial elongation is likely to be the site of action, although the subtleties of muscarinic cholinergic pharmacology may make discrimination difficult.

Irrespective of the outcome of this three-way comparison, more detailed analysis of the receptors involved in blocking the development of myopia has been pursued. McBrien and colleagues have shown that the M4 antagonist himbacine blocks experimental myopia [108], and use of snake toxins which have a somewhat greater differential affinity for receptor subtypes has given further support to the idea that M4 receptors are involved [109]. In the chicken, which appears

to lack M1 receptors [110], predominantly M4 receptors may be involved. However, in mammals, it appears that both M1 and M4 receptors are involved [109].

This pharmacological characterisation is important because the use of atropine to control myopic progression has been limited because of the associated pupil dilation and block of accommodation which underlie its use as a cycloplegic agent. One approach to this problem is to use lower doses of atropine, which avoid some of the side effects [100, 111–115]. The other is to more precisely define the receptors involved, so that agents with more specific actions can be developed.

4.4.3 Interplay of Defocus Signals

In part due to the demolition of the excessive accommodation hypothesis and in part due to the evidence that growth control mechanisms are intrinsic to the eye and do not require central input, attention in experimental myopia shifted to an emphasis on the ability of the retina to detect sign of defocus, with hyperopic defocus stimulating GO (or GROW) signals and myopic defocus stimulating STOP signals, even if the nature of the signals is poorly defined. This area has been extensively reviewed [11, 51].

The kinetics of these signals and their spatial and temporal interactions have been extensively studied. Interruption of the signals, which is possible experimentally, showed that both were less effective if exposure to the stimuli was not constant. However even short periods of stimulation which generated STOP signals were effective, whereas effective GO signals required essentially constant stimulation. Interruptions to FDM of as little as 15 min significantly reduced the development of myopia [56, 57], and the effectiveness of this reduction was markedly increased if the light intensity was increased over this period [116] and decreased if the animals were kept in the dark [117]. In addition, the D2-dopamine antagonist spiperone blocked the inhibitory effects of diffuser removal in the light, and, thus, the inhibitory effect of removal of the diffusers seems to involve light-stimulated release of dopamine. This may be relevant to the protection from myopia that children who spend more time outdoors receive (see below).

Experiments involving temporal interactions between these signals have also shown that relatively brief periods of exposure to myopic defocus are able to block the effects of otherwise continuous exposure to hyperopic defocus [51–53, 118]. This is also true when spatial interactions were examined. When only 25 % of the field was myopically defocused, the amount of myopia was substantially reduced, and with one third of the field myopically defocused, hyperopic refractions were achieved [119, 120].

The major impact of the discovery of the highly non-linear interactions between different sorts of defocus has been on thinking about the kinds of defocus exposure that humans could receive in different environments. This issue has been extensively discussed [11, 121]. How this would work in human environments is unclear. Consider, for example, reading a book. With considerable accommodation exerted to bring the pages of the book into focus, more distant peripheral objects would be myopically defocused, which would be expected to prevent the development of myopia. However, accommodative lag might lead to hyperopic defocus centrally. Also consider the situation outdoors – with focus on the horizon, all closer objects would be hyperopically defocused, which would be expected to promote axial elongation, the reverse of the normal assumption that relaxed accommodation would prevent myopia. By contrast, a focus on closer objects outdoors would leave most other objects myopically defocused, which would be expected to prevent myopia. How these would add up to a final overall response is unclear, given the non-linearity of the interactions in both time and space. Given the current interest in the protective effects of time outdoors in children, Flitcroft [121] has suggested that an important factor is that the differences in outdoor accommodative demands are much smaller than in indoor environments, producing a more uniform dioptric space, but whether this difference is involved in the protective effects of time outdoors is, at the moment, purely speculative. At a more immediately practical level, spectacles incorporating bands of alternating bands of focus and myopic defocus are currently the subject of clinical trials.

4.4.4 Peripheral Defocus

A closely related area of interest is the role of peripheral defocus in the development and control of myopia. This idea originated in observations on eye shape in Dutch trainee pilots [122], which suggested that more prolate eyes at baseline (eyes with axial diameter greater than equatorial diameter) were more likely to become myopic, which led to the hypothesis that peripheral hyperopic defocus in such eyes might promote the development of myopia and/or myopic progression.

There was initial scepticism about this idea, but a series of seminal papers by Smith and colleagues showed that lesions to the central retina of monkeys did not prevent the normal process of emmetropisation or prevent the development of FDM and LIM [123–127]. This showed clearly that the peripheral retina was able to control central axial elongation in the absence of central signals, but further experiments were unable to definitively show that peripheral signals could

override central signals. This area has now been extensively reviewed [128, 129].

This area has been pursued in two ways. Firstly, the idea that incident myopia was dependent upon hyperopic eye shape has been extensively pursued. Since myopic eyes tend to be more prolate than emmetropic eyes, the critical question is whether eyes that became myopic were more prolate prior to the onset of myopia. This idea has not fared well, and it appears that the appearance of a prolate eye shape is a consequence of, rather than a cause of, myopia [130–132]. It has even been suggested, given the vagueness in the original paper on Dutch pilots, that the original results may have been misinterpreted [133].

However, even if a role for peripheral defocus in the appearance of incident myopia has not stood up, a possible role for peripheral defocus as a continuing drive to myopic progression could still be valid, as is the idea that peripheral myopic defocus might inhibit myopia. These questions have been addressed through the design of spectacles or contact lenses which reduce the level of peripheral hyperopia or which impose peripheral myopia [134, 135]. These have produced some benefits in terms of slowed progression, although the results are not yet consistent. Zeiss now sells a myopia control lens of this design, which in clinical trials over 6 months showed no significant protection in the whole sample but a significant effect in the subsample with myopic parents. Clearly more comprehensive observations including longer-term follow-up are required before this design can be regarded as validated.

4.4.5 Protective Effects of Time Outdoors

One of the observations that has excited considerable recent interest is that children who spend more time outdoors are less likely to be, or become, myopic [136]. After considering a range of possibilities, we [137, 138] suggested that the most plausible explanation of this effect was that bright light outdoors stimulated the release of dopamine from the retina, which then acted as an inhibitor of axial elongation. This suggestion was based on considerable prior research on experimental myopia, both FDM and LIM, which suggested that one of the early steps in the development of experimental myopia was the suppression of dopamine release [139–141]. This area has recently been reviewed in detail [39].

This hypothesis was immediately translated into experimental situations, and it was shown that raising animals in lights brighter than those normally used in animal houses, from 15,000 to 30,000 lx as compared to normal experimental conditions of 100–500 lx, could substantially inhibit the development of FDM in chickens [116], primates [142] and tree shrews, and slow the development of LIM in chickens [143] and tree shrews, and more marginally in primates. It was also shown that the ability of bright light to block FDM was itself blocked by a D2-dopamine receptor antagonist, spiperone [143]. However, subsequent experimentation has suggested that the pharmacological properties of the block of LIM by light might be different, since the results suggest that the effect is not blocked by either D1 or D2 antagonists [144]. This clearly requires further exploration, given that dopamine agonists block LIM as well as FDM [39].

These very promising results need to be put into perspective in two ways. Firstly, the ranges of light intensity involved in the protective effects are commonly encountered in human environments. Thus indoor light intensities are generally in the range from 200 up to 1,000 lx, with light intensities in animal houses at the lower end. Outdoor light intensities can range during the day and even in the shade on cloudy days, from several thousand Lux up to 150,000 to 200,000 lx on bright sunny days at lower latitudes. These, of course, can vary quite significantly by latitude and season, both in intensity and duration.

Secondly, the protective effects seem to be quite substantial in the experimental studies discussed above and in epidemiological studies. For example, longitudinal data from the CLEERE study have shown that the risk of developing myopia is around three times lower for children spending more than 15 h per week outdoors as compared to those spending less than five [145], and this risk reduction applies irrespective of whether the parents are myopic or not. Similarly, longitudinal data from the Sydney Myopia Study suggest that children from the top tertile of time outdoors are substantially less likely to become myopic than those in the bottom tertile by a similar factor [146]. Comparisons of those who combine low near work with high time outdoors (low risk), compared to those who combine high near work with low time outdoors (high risk) are even more stark. The much higher prevalence of myopia in Orthodox Jewish boys [147, 148] compared to that in boys studying in general schools, as well as that in girls irrespective of the school attended, which is generally attributed to intensive study habits, also provides evidence of the power of environmental effects, although in this case a link to time outdoors is not established but is plausible.

The evidence for the involvement of dopaminergic pathways from human epidemiology and from experimental myopia is very detailed, albeit not entirely consistent, but there is no direct evidence for involvement of dopaminergic pathways in studies on human genetics. However dopaminergic pathways have been implicated indirectly. Mutations which affect outer retinal processing, and in particular photoreceptor and ON-bipolar cell pathways, could exert their effects on the development of myopia by altering the release of dopamine, since the ON-bipolar cells provide a major input to the dopaminergic cells [149–152]. The glutamate

agonist, 2-amino-4-phosphonobutyric acid (2APB), which hyperpolarises the ON-bipolar cells and presumably reduces dopamine release, leads to the development of myopia in kittens [153]. Mutant mice with a mutation of nyctalopin similar to that which causes congenital stationary night blindness in humans [154] have lower pools of dopamine and are more sensitive to the development of form-deprivation myopia [155]. But there is a clear gap in the evidence which needs to be followed up by more detailed analyses of dopaminergic function in human syndromic myopia and in human genetic studies with candidate gene approaches.

Secondly, the involvement of the GJD2 gene, which codes for connexin36, also implicates dopamine, since the permeability of gap junctions involving connexin36 is regulated by dopamine [156–160], although in this case via D1-dopamine receptors. If dopamine release is reduced either by genetic defects in the photoreceptor to ON-bipolar cell pathway, which appears to control dopamine release [147–150], or by lack of stimulation of the pathway by environmental light, this might be expected to lead to reduced light adaptation, more diffuse signalling through rod and cone pathways and a reduction in the narrowing of receptive fields that normally occurs in light adaptation. In many ways this could be analogous to the lowered spatial and temporal stimulation that occurs in the FDM paradigm.

How this can be further investigated in human myopia is problematic. Changes in the electroretinogram (ERG) may provide a relatively non-invasive approach to measuring the functions of retinal circuits [158–160]. In fact, studies on ERG responses in myopic human eyes have tended to implicate changes in the inner retina, with normal a-waves and reduction in b-waves. There are also more complex changes in the oscillatory potentials of the ERG which are believed to involve dopaminergic circuits, and changes in adaptation, which could be related to changes in dopaminergic function. This evidence lends some support to the hypothesis that dopaminergic function is depressed in the inner retina of humans with myopia but falls short of proving that this has taken place.

Given the magnitude of the potential effects of outdoor exposure, these developments have been rapidly translated into clinical trials. Two small trials have reported positive results [161, 162], and the interim results of a larger trial, in which schools have increased the amount of time that children spend outdoors, have reported small but statistically significant protection from myopic shifts in refraction and incident myopia.

4.4.6 Changes in Scleral Metabolism

This area has been extensively reviewed [68, 69, 163]. The sclera is the endpoint tissue for both human and experimental myopia, because it is the structure and metabolism of the sclera which ultimately determines the axial length of the eye. It is also the site of the development of staphyloma, one of the most destructive pathological features of high myopia. Studies on human myopia have shown that the sclera from myopic eyes is thinner than normal and that marked reductions in the content and structure of collagens, as well as scleral glycosaminoglycans have taken place. Studies on human sclera are obviously limited to single-point determinations, except when culture systems, such as human scleral fibroblasts (HSF), can be developed. The HSF culture system has been used to document regulation of synthesis of brain morphogenetic proteins (BMPs) by retinoic acid [164], both of which have been implicated in the development of experimental myopia [165–167], and identified as candidates in human genetic studies [88, 89]. More systematic use of this approach to examining changes in scleral metabolism looks promising.

Several animal experiments have documented changes in scleral collagens and glycosaminoglycans, which parallel those seen in human myopic sclera. There are reductions in the synthesis of collagens and glycosaminoglycans and in addition increased catabolism. In fact, one of the early events in the development of myopia appears to be up-regulation of matrix metalloproteinase (MMP) activity [168], which has also been implicated in genetic studies [169–171].

Further work has implicated myofibroblasts in controlling the properties of the sclera [172, 173]. Myofibroblasts differentiate from fibroblasts and are highly contractile cells which express the smooth muscle protein alpha-smooth muscle actin. The differentiation of these cells can occur in response to local stresses, a process involving regulation of the synthesis of extracellular matrix consitutents. They could therefore play a role in enabling the sclera to adjust for fluctuating intraocular pressure and other stresses. Cell adhesion molecules such as integrins play a key role in mediating cell-matrix interactions, and again McBrien and colleagues have shown rapid downregulation of the expression of alpha1 and alpha2 integrin subunits [174]. The expression of these two subunits seems to be differentially regulated during the development of myopia.

McBrien has proposed that transforming growth factor-beta (TGF-beta) is a key regulator [163]. The three mammalian isoforms of TGF-beta change rapidly in response to stimuli that induce experimental myopia and regulate collagen and glycosaminoglycan production, as well as differentiation of scleral fibroblasts to myofibroblasts. TGF-beta is found in retina, choroid and sclera, but it is only in the sclera that regulation occurs in relation to myopigenic stimuli.

It is important to note that mutations in many genes involved in this complex integrated response have been identified in human genetic studies as candidate genes, along with a number of other scleral constituents. This suggests that the sclera in myopia can be both the direct site of action

of mutations which affect extracellular matrix metabolism and cause a weaker sclera, and the site of modulation of scleral metabolism in response to upstream mutations which may affect retinal dopamine release, or in response to changes in dopamine release caused by environmental exposures. It is not clear how changes in dopamine release in the retina are propagated to the sclera, and at present there are only a few signposts along the way. It seems unlikely that dopamine acts directly on the sclera, since no effects of dopamine agonists on the sclera were detected in experiments in vitro (unpublished results). But the model developed by McBrien implies that regulation of TGF-beta expression could be a key event worth further study and is a site for potential pharmacological intervention.

4.4.7 Circadian Rhythms and Myopia

There has also been considerable interest in the potential role of circadian rhythms in the development of myopia and in particular interruptions to normal dark periods [95, 175]. This was, in part, stimulated by a report which suggested that children who slept with night lights, and even more so with room lights, were more likely to be myopic [176]. The effects reported were very substantial, but most subsequent studies have failed to replicate this effect. The few that reported positive effects showed changes that were much smaller. Other evidence from human epidemiology has given, at best, very limited support to these ideas, since there are only small albeit significant effects of season of birth [177] or latitude of birth [178] which could implicate circadian phenomena but which could also have other explanations.

Studies of experimental myopia have also given some support to this idea. Eye growth shows clear circadian rhythms, which are perturbed under conditions which change the rate of eye growth, and it has been argued that key timing events around the transitions between light and dark phases might be important for correct regulation of eye growth [175, 179, 180]. Stone [95] has argued that many of the changes in mRNA expression in microarray analysis of animal experiments involve changes in clock genes, but it needs to be remembered that dopamine rhythms are perturbed in experimental myopia and probably in human myopia, and these rhythms, substantially light-driven in the case of dopamine [140], are quite closely linked to circadian rhythms [181]. It is therefore not clear whether these changes represent an independent response to myopigenic conditions or whether they reflect a fundamental effect on dopamine metabolism. While human epidemiology does not suggest substantial effects of circadian rhythms, with the possible exception of the epidemic of myopia that appeared in some Eskimo populations under conditions of quite mild urbanisation and engagement in schooling [182, 183], this area deserves further study.

4.4.8 A Role for Retinoic Acid

In a seminal paper in this area, Mertz and Wallman [167] showed that, in the chicken retina, the choroid synthesised retinoic acid at a much greater rate than any other ocular tissue. The rate of choroidal retinoic acid synthesis was markedly decreased under conditions that increased the rate of eye growth (both FDM and LIM) and markedly increased under conditions that decreased the rate of eye growth. They also provided evidence that retinoic acid was released from the choroid and accumulated in a nuclear fraction from the sclera, where retinoic acid decreased the rate of scleral glycosaminoglycan synthesis. In contrast, they found that changes in the retina were much smaller in magnitude and reversed in direction, consistent with previous evidence [184, 185]. They therefore suggested that regulation of the rate of synthesis of retinoic acid in the choroid could be a crucial element in regulation of the rate of eye growth, at least in the chicken. These changes were subsequently confirmed in the chicken [186].

In one of the early studies, it was reported that retinoic acid stimulated the proliferation of sclera chondrocytes but inhibited the proliferation of scleral fibroblasts [184]. Given the different composition of the sclera in chickens (predominantly chondrocytes) compared to mammals (predominantly fibroblasts), it is not clear whether these results obtained on chickens can be generalised to mammals, and indeed several studies suggest that in experimental myopia in mammals, increased retinoic acid synthesis and levels, possibly in both the retina and the choroid, are involved [187–189]. Human fibroblasts in culture express a variety of retinoic acid receptors [190], and retinoic acid has been shown to inhibit the synthesis of brain morphogenetic proteins [165], which have been implicated in both human and experimental myopia, as well as another extracellular matrix constituent fibulin [191].

While the results are not entirely consistent, the idea that retinoic acid may be a mediator of changes in retinal or choroidal visual processing to the sclera is worth pursuing and intersects with evidence for a role of retinoic acid receptors and synthetic enzymes in experimental myopia [186, 192]. The recent CREAM and 23andMe studies [88, 89] have identified mutations in retinol dehydrogenase 5 as associated with myopia, but whether this primarily affects retinal recycling in the outer retina, or retinoic acid as a messenger in eye growth control, is currently unclear. Studies on experimental myopia suggest that retinaldehyde dehydrogenase 2, which converts retinal to retinoic acid, is more likely to be involved [186].

4.4.9 Fibroblast Growth Factor

Based on research on the general regulation of extracellular matrix by FGF-beta and TGF-beta, Rohrer and Stell [193]

tested the hypothesis that these growth factors might act as regulators of scleral growth in chickens. They showed that exogenous FGF-beta reduced the development of FDM and that TGF-beta blocked the effect of FGF-beta. This work was followed up in a subsequent paper [194] in which it was demonstrated that FDM reduced the rate of retinal dopamine synthesis, which was reversed by strobe lighting, which in turn was associated with increased expression of cfos in the dopaminergic amacrine cells. They also showed that FGF-beta did not affect cfos expression, tyrosine hydroxylase levels or dopamine synthesis, suggesting that its effect was exerted downstream of the dopaminergic amacrine cells. In complete contrast to these results, Seko and colleagues found that FGF-beta stimulated proliferation of scleral chondrocytes and fibroblasts [195]. Again in contrast, in tree shrews, Gentle and McBrien found that FGF-beta did not change in FDM but that FGFR-1 did [196].

Subsequent experimentation has not clarified further the pathways that might be involved but has suggested that in addition to links with TGF-beta, interactions with IGF may also be involved [197]. It is not clear if these interactions are related to the effects of insulin and glucagon on experimental myopia [198, 199]. How important these interactions are is far from clear, but both FGF [200, 201] and IGF [202–204] have been inconsistently implicated in high myopia, and there is other supporting evidence for a role of IGF in experimental myopia [205, 206].

4.4.10 Summary

These case studies illustrate the way in which evidence from human studies, both of epidemiology and genetics can cross-fertilise with studies on experimental myopia. In some cases the flow is from human to experimental studies, in some cases the other way around. We have chosen examples which illustrate the potential for successful integration, where we believe that continuing research could deliver major returns. This list is in no way comprehensive. For example, major effects of GABA agonists in preventing myopia have been reported in animal studies [207, 208] which seem to be linked to changes in the dopaminergic and cholinergic systems [209]. The magnitude of the effects suggests significant potential for pharmacological intervention, but at the same time GABA is such a widespread transmitter, both within the body and within the eye and retina than any approach to prevention based on manipulation of GABAergic pathways would have to be exceptionally cautious. Similar caution has been exercised in making use of dopaminergic agents, despite the strong evidence base in animal studies. But the ability to use natural modulation of dopamine release with light, of the kind that appears to be involved in the protective effects of light, offers a way of avoiding some of the problems.

4.5 A Heuristic Model of Growth Control

Based on the evidence we have reviewed in this chapter, we propose a model which we believe will be useful for orienting future studies on both human and experimental myopia (Fig. 4.2). A key player is the dopaminergic amacrine cell, which may be involved in the appearance of myopia in a number of human diseases in which myopia is a feature – most notably in congenital stationary night blindness of various forms. These mutations, which primarily affect the functioning of a photoreceptor to ON-bipolar cells pathway, may mediate a reduction in the normal release of dopamine by increasing light intensities. The nob mutant mouse provides a relevant animal model. Dopamine release also appears to be regulated by environmental stimulation of the dopaminergic amacrine cells, and increased release of dopamine by bright light may mediate the protective effects of increased time spent outdoors by children. Some of the large number of mutations in the OMIM database which result in myopia may also affect this pathway – particularly those with the potential to affect visual processing in the outer retina. Within the inner retina, experimental myopia has shown that dopaminergic function is regulated, at least in part, by GABAergic and cholinergic amacrine cells, and the effects of the three transmitters – dopamine, acetylcholine and GABA – may converge, at least in the chicken retina, on the glucagon-immunoreactive amacrine cells in which decreased expression of the immediate early gene *Egr-1* appears to correlate with an increased rate of eye growth. Glucagon may be an important messenger released at this point in the pathway, but it should be noted that evidence for the involvement of glucagon-immunoreactive amacrine cells in humans is limited, and another amacrine cell may play a critical role in humans. It is important to note that, so far, none of the mutations detected in human myopia appear to involve these mechanisms documented in the inner retina but primarily involve the outer retina and sclera.

After that, the potential pathway is poorly defined, although changes in the retinal pigment epithelium and choroid may be required to transmit growth control signals to the sclera, and retinoic acid seems to be the best candidate for future studies. McBrien has proposed that the key regulatory event in the sclera involves changes in transforming growth factor-beta, which in turn regulates, directly or indirectly, collagen and glycosaminoglycan synthesis, catabolism mediated by matrix metalloproteinases, levels of integrins and conversion of fibroblasts to myofibroblasts. Other mutations associated with myopia appear to directly affect scleral constituents and may produce myopia by generally weakening the sclera, just as reduced function in this pathway results in changes in the sclera which promote sclera thinning and weakness. This model does not cover all of the

multitude of observations that have been made on human and experimental myopia, but we believe that it covers a quite extensive range and is capable of explaining much of the current evidence on both genetic and environmental control of myopia.

Conclusions

A range of paradigms in which changes in eye growth can be induced experimentally have been developed. None of the paradigms precisely matches the features of human myopia, with particular issues in relation to the methods used to induce myopia and the developmental stage at which myopia is induced. Monkeys provide the model which most closely follows the human pattern in terms of the pattern of change in ocular biometry and refraction, but any of the models can be used, with appropriate caution, to investigate the molecular details of the changes in ways which are not possible in humans.

There has already been considerable synergy between human and animals studies, with flow in both directions and critical testing of hypotheses. In his classical book, published nearly 30 years ago, Curtin commented that "…many theories of myopia genesis were the product of pure speculation. It would appear at one point towards the close of the 19th century that any ophthalmologist who experienced a night of insomnia arose in the morning with a new, and usually more bizarre, theory".

We have now moved well beyond that point and have the ability to critically scrutinise new theories through both human studies in epidemiology and genetics and through animal studies. Hopefully, some of the new ideas that have emerged, such as the importance of peripheral defocus and the amount of time that children spend outdoors, are close to delivering preventive strategies to control both incident myopia and myopic progression. But only future research will tell.

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