The compass within the clock - Part 1: The hypothesis of magnetic fields as secondary zeitgebers to the circadian system—logical and scientific objections.

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ABSTRACT

For nearly fifty years, multiple experiments have repeatedly established the influence of the geomagnetic field (GMF) on the circadian system of various organisms, including humans. Recent work on the cryptochrome genes, core components of the circadian system, fundamentally involved with orchestrating biological adoptions to the day/night cycle, have also identified the gene product as a putative magnetoreceptor. Cryptochrome thus represents the idealised “candidate gene” for GMF-circadian interactions. Indeed, it has recently been demonstrated that this gene is responsible for the magnetosensitivity of the circadian system in Drosophila. Such investigations of circadian-GMF interactions have been primarily evaluated under the hypothesis that the GMF may be acting as a “secondary zeitgeber” (beyond the primary “time-giver” of the day/night cycle) for circadian systems. However, this paper demonstrates that there are clear logical and experimental objections to such a hypothesis. Most notably, no animal has ever been successfully entrained to the GMF in the absence of other stimuli, despite such experiments representing the most common control in circadian biology. Thus, it is argued that a new explanatory framework is required, the foundations of which are outlined in a second paper—“The Compass within the Clock - Part 2: Does cryptochrome radical-pair based signalling contribute to temperature-robustness of circadian systems?”

INTRODUCTION

Cryptochromes (CRYs) evolved from evolutionary ancestors—the photolyases—which are enzymes that perform the light-dependent repair of UV-damaged DNA. This is catalysed by the photolyase in a blue light-dependent, single enzyme reaction. Generation of a radical-pair upon light excitation is essential for the function of photolyases. In contrast to photolyases, cryptochromes function as signalling molecules that regulate diverse biological responses, not all of which require blue-light activation—such as roles within the circadian system, and the regulation of plant growth and development (reviewed in 1). The accepted definition of a cryptochrome is a protein with similarity to a photolyase that has lost or reduced DNA repair activity but that has gained a novel role in signalling.

The cryptochrome genes have been best characterised as core components of the circadian system, fundamentally involved in orchestrating biological adaptations to the daily cycle. The circadian system is classically described as a “transcription translation feedback loop” (TTFL), whereby gene products negatively regulate their own products, and thus oscillate endogenously with a period of approximately 24 hours. However, to precisely entrain the system to the solar day, the principle zeitgeber is light. Cryptochrome has variously been described in both these major roles of the circadian system (i.e. endogenous oscillations and light entrainment; see figure 1).

A widely held view is that cryptochrome functions as a circadian photoreceptor in Drosophila, but in mammals, evolutionary reconfiguration has led to a loss of its photoreceptive function, and it has become just another “cog” in the wheel of the molecular clock. Thus, within animals, it is revealed that cryptochromes can be generally divided into two categories: The “Drosophila-like” CRY type I molecule, which functions mainly as circadian photoreceptors, and the “vertebrate-like” CRY type II molecules, which are thought to function primarily as negative regulators of the clock’s transcriptional feedback loop (see figure 1). Only type II are present in vertebrates, where they are potent repressors of the clock proteins CLK/BMAL1 in a light-independent manner. In contrast, insects can have either type I or type II, or, in most instances, both type I and type II. However, such a schema necessarily over-simplifies the multiple roles of CRY.
MAGNETOSensitivity of the Circadian System

Experiments on various species have established the influence of the geomagnetic field (GMF) on the circadian system\(^3\)–\(^7\) (see (8) for detailed discussion), leading to the proposal that the GMF may be acting as a “secondary zeitgeber”, in addition to the primary temporal synchroniser of the day/night cycle. The notion of such a geomagnetic “time sense” can be traced back nearly half a century, with the observed influences of the GMF on the circadian system in mice\(^6\). Later isolation experiments with human subjects\(^5\) investigated various aspects of circadian behaviour. These experiments revealed that when humans are kept under constant lighting conditions their circadian rhythms become free-running and decoupled from the usual 24 hour cycle, adopting on average a 24.87 hour day. However, when subjects were placed in geomagnetically shielded conditions, the circadian period was demonstrated to be significantly (p < 0.01) longer, at 25.26 hours (see Table 1 (9)). It was concluded that the experiments provided significant proof that electromagnetic fields in the extremely low frequency (elf)-range influence human circadian rhythms, and therefore, human beings. Similar results have been obtained with other organisms, revealing the influence of shielding the geomagnetic field (or applied fields in the geomagnetic range) on a variety of rodents\(^13\)–\(^18\), *Musca* flies\(^3\), *Drosophila*\(^4\), and birds\(^7\). There is a notable paucity of negative results, although some studies revealed no effect in specific rodent strains\(^13\)–\(^18\), inconsistent results\(^19\), or no association with circadian responses in humans (with the authors discussing the shortcomings of the study replicating changes in the GMF)\(^20\). A novel application of the “candidate gene approach” for these GMF/circadian interactions would yield a rather specific list of candidate properties: a gene that was a molecular magnetosensor.

**CRYPTOCOrome as a geomagnetic Compass**

The biophysical mechanism for magnetodetection has been primarily investigated within the context of migrational navigation. The radical-pair mechanism (RPM) is one of the few molecular features that might plausibly be influenced by the GMF, with the yield of a biochemical reaction proceeding via a spin correlated radical pair (RP) based reaction being sensitive to the orientation of an external magnetic field. In the year 2000, it was proposed that cryptochrome was the prime candidate molecule for such a mechanism: it is the only known photoreceptor in vertebrates.
shown to be able to form a RP upon photoexcitation\(^2\). Over the intervening years, inferential evidence has provided increasing support for the role of cryptochrome in geomagnetic navigation in a variety of species, particularly birds (there are many good reviews). Direct evidence eventually involved transgenic experiments with *Drosophila* in a navigational paradigm, establishing that a variety of CRY genes—including those from *Drosophila*, humans, and monarch butterflies—are indeed functional magnetosensors\(^2\). Moreover, it has also been established using *Drosophila* transgenics that cryptochrome is indeed responsible for the circadian magnetosensitivity of this species\(^2\).

**THE PARADOX OF CRY: DISTINCT PLEIOTROPY UNDER STRONG PURIFYING SELECTION**

The RPM imposes a number of constraints—chemical, magnetic, kinetic, structural, and dynamic—that should be satisfied by a viable chemical magnetoreceptor\(^1\). For example, fine tuning of donor-acceptor distance is critical to permit weak enough inter-radical spin-spin interactions, with the terminal flavin-tryptophan radical pair in cryptochrome having the ideal separation\(^1\). Thus, it is difficult to conceive that the necessary biophysical features for RPM/magnetodetection could arise serendipitously. Instead, such molecules represent precisely-configured magnetoreceptors.

A cursory investigation of cryptochrome phylogenetics reveals several noteworthy features (see \(^2\) and ensembl.org). Of the cryptochromes tested, all have been established as functional magnetosensors, including CRYs on both major branches (i.e. types I and II) of the phylogenetic tree\(^2\). Moreover, concerted purifying selection (\(dN/dS\) ratios consistently < 0.1) (\(dN/dS\) ratios are a standard indicator of selective pressure acting on protein-coding genes, and can reveal whether genes are undergoing positive (i.e. directional) selection or purifying (i.e. stabilising) selection\(^2\)) suggest that RPM/magnetosensitivity may be a rather general feature of the cryptochrome family, as previously suggested\(^2\). This concerted purifying selection is observed in spite of the apparent pleiotropy of CRYs (i.e. a photopigment; a signalling molecule; a molecular compass).

Taking a particular example, the human and *Sylvia borin* CRY1 proteins have 93% sequence identity, with near-perfect homology in the photolyase-like domain, fundamentally responsible for forming radical pairs. The majority of differences are observed in the C-terminal tail (CTT), which is responsible for interactions with signalling partners. However, whilst the human CRYs are generally considered as light-independent core oscillators of the circadian system, the avian CRY1 is proposed as the prime candidate for light-dependent geomagnetic navigation\(^2\). Thus, despite >300 million years since the two species separated, and despite the radically different roles proposed for the gene, cryptochrome has not undergone duplication and separate evolution of function. Such observations are reaffirmed by experimental data—the human CRY2 has been revealed to be capable of functioning as a light-dependent RPM-magnetodetector within the *Drosophila* navigational paradigm\(^2\).

Finally, phylogenetic analysis of insect cryptochromes reveals the diverse ways in which these clocks can be constructed (e.g. type I systems, type II systems, or hybrid systems; see figure 5 in \(^2\)). Due to the distinct roles of cryptochrome (photic input versus circadian oscillator), it would therefore be expected that the insect circadian system could be configured to contain a CRY-less system. However, such systems are noticeably absent from the various insect configurations\(^2\). Thus, it appears that at least one CRY—either a type I or a type II—is an essential feature of any circadian clock.

**LIMITATIONS OF THE EXISTING PARADIGM**

The above observations lead naturally to the suggestion that RPM/magnetodetection plays a critical role within circadian systems. What might be the underlying biological rationale for such circadian/GMF interactions? Why would an apparently functional magnetosensor have been conserved within the circadian system throughout animal evolution? There is good reason to question the existing notion of a secondary zeitgeber:

1) A secondary GMF zeitgeber is of questionable biological utility—it is a variable proxy for a reliable primary indicator (i.e. day/night), as it would be rendered useless by frequent geomagnetic storms\(^2\) (approximately once every 10 days on average). Moreover, in marked contrast to other secondary zeitgebers (e.g. heat, food, and sociality), where the zeitgeber itself confers clear fitness benefits on organisms, the GMF has no obvious biological utility beyond navigation.

2) There is a notable absence of any experiment that has successfully entrained any animal to the natural GMF in the absence of other cues, even with particularly good timekeepers such as bees\(^3\). However, such experiments represent the most common control in circadian biology, where it is well known that rhythms become decoupled from the usual 24h cycle when animals are kept under constant conditions of light/temperature etc. In no experiment has an animal demonstrated the ability to resort to a secondary geomagnetic synchroniser (see \(3\)–\(5\) and numerous other examples).

3) Various experiments have successfully entrained animals to fields that are turned on/off every 12 hours\(^4\). However, such conditions do not in any way reflect the natural GMF (which is essentially constant, with only minor diurnal variation). In lieu of a positive entrainment to the natural GMF, such experiments can be considered nothing more than an experimental device.

4) It has further been argued that a functional secondary geomagnetic synchroniser may exist in a limited number of species where it has clear functional utility—e.g. polar extremes\(^4\) or hive animals\(^5\) where the light/dark cycle can be relatively constant. It is argued that the utility for such a geomagnetic secondary zeitgeber in a limited number of species could then lead to the vestiges of such a trait in other, related species (i.e. a magnetosensitive circadian system, but one that is no longer entrainable). However,
whilst bees do indeed reveal particularly good timekeeping in the absence of the usual cues, they reveal a drift in accuracy ± 15 min after 3 days. Moreover, the phylogenetics of cryptochrome are inconsistent with such proposals—they would predict periods of relaxed selection on cryptochrome, in contrast to the concerted purifying selection observed. 5) The most commonly discussed models involve the detection of Schumann Resonances by cryptochrome. However, these resonances are of picotesla size, far smaller than the modelled sensitivity of cryptochrome (nanotesla). Whilst there is repeated evidence of GMF/circadian interactions across a range of species, the existing explanatory framework of a secondary zeitgeber is logically incoherent and entirely unsupported by evidence. An alternative hypothesis is proposed in the following, accompanying paper—The Compass within the Clock - Part 2: Does cryptochrome radical-pair based signalling contribute to temperature-robustness of circadian systems? 4. H 4 CONFLICT OF INTEREST Author declares no conflicts of interest. 4. ABOUT THE AUTHOR James Close has previously held academic positions at King’s College London and the University of Oxford, where he applied his skills as a genome biologist to help unravel the biology of complex disorders in fields as diverse as haematology and psychiatry. He is now a freelance researcher and trader of financial markets.

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