Overview

Chronodisruption, cell cycle checkpoints and DNA repair

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Chronodisruption, a disturbance in “natural” daily light/dark regulation, is possibly linked to disturbances in cell cycle homeostasis. The association and the synchronization between circadian rhythms and mitosis are not yet clear. The circadian oscillator is involved in the major cellular pathways of cell division. A molecular link between the circadian clock and the mammalian DNA damage checkpoints has been outlined. Analyses suggest an association between light disruption and obstruction of the cell cycle homeostasis. Disruption in the homeostatic control of the cell cycle has been associated with cancer and acceleration of malignant growth, possibly as a result of the interruption of DNA damage check-points. Studies further indicate that light signal during the dark phase affects the transcription level of a substantial number of genes that are associated with cell cycle progression, cell proliferation and tumorigenesis. Indeed, the International Agency for Research in Cancer categorized “shift work that involves circadian disruption” as possibly carcinogenic. In this review the current finding on light pollution and its potential influence on cell cycle check-points and DNA repair is presented.

Keywords: Cell cycle homeostasis, Chronodisruption, Circadian oscillator, DNA damage

Living eukaryote cells present numerous periodic processes that oscillate in various paces. Two of the better characterized cell autonomous oscillators are circadian clock and cell division cycle. Each of these oscillatory systems seems to be self-directed and regulated by its own pacemaker. The period length of the circadian rhythm is about a day (~24 h) and the clock is compensated for various environmental fluctuations such as temperature. The period length of cell cycle is wide-ranging from minutes to days and the cycle is highly influenced by environmental variables like nutrient availability or temperature. Although there appears to be no link between these two oscillators, none the less, the circadian clock oscillators and the cell cycle oscillators are combined in several ways. The aim of this review is to provide an entrée into an exciting and promising new theme of chronobiology that links regulation of circadian oscillator to that of cell cycle homeostasis.

The circadian nature of cell division

A fundamental characteristic of circadian clocks in eukaryotes is that they are based on cell-autonomous interacting transcriptional/translational feedback loops that drive cyclical gene expression, which ultimately underlies many of the rhythms manifested by organisms. A number of the cell cycle oscillator core genes also show a circadian expression. Modification in expression levels of various canonical circadian clock genes results in changes in the level of several cell cycle genes, as well as in numerous cell cycle disorders.

Although period length of cell cycle is variable, a large variety of eukaryotic organisms exhibits a circadian mode of cell division. Hence, the nature of the association between the two oscillators, whether correlative or causative, is not yet clear. A support for a causal association between the circadian and the cell cycle oscillators is derived from several findings, suggesting that the daily oscillator is involved in gating the entry and exit for cell division.

The gating attribute of the circadian clock may be conserved from unicellular organisms to vertebrates. In cyanobacteria, Yang et al. showed a slowdown in the progress of cell cycle in a specific phase of the circadian interval. In yeast, DNA synthesis (S-phase) is timed to set apart of the oxidative phase, and in zebrafish, arrays of various cells are synchronized by the circadian oscillator to go into S-phase towards the end of the light phase. Along these lines, a daily mitotic cycle may be an evolutionary advantage, enabling cells to synchronize DNA synthesis to a specific time (dark phase for instance), whereby cells are able to escape deleterious and/or mutable effects such as UV radiation or oxidation.
Gating the cell cycle by the circadian oscillator can be accomplished by a molecular crosstalk between the core oscillator genes involved in the two rhythmic processes. The gene *period* (*per*) is one of the *bona fide* circadian clock canonical genes. The mammalian *period* paralogues *Perl* and *Per2* are molecularly linked to repression of G1-S transition, while the circadian transcription factors *Bmal1* and *Clock* are associated with G2-M transition. Additional putative role for BMAL1 protein is in suppressing cells from undergoing S-phase while reactive oxygen Species (ROS) levels are high.

Further support for a molecular crosstalk mode of gating operation was found recently by Kowalska *et al.*, who showed that the protein NONO, a multifunctional nuclear protein, operate together with the circadian protein PER, in controlling the circadian expression of the cell cycle checkpoint gene *p16-Ink4A*. The circadian control of *p16-Ink4A* via NONO is necessary for gating exit from cell cycle G1 phase.

### Cell cycle checkpoints and DNA damage control

In contrast to the continue cycling of the circadian pacemaker, the cell cycle oscillator is characterized by discrete checkpoints that monitor the integrity of DNA replication and repair. At least four major checkpoint pathways (S-phase, DNA damage, topoisomerase II – dependent and spindle assembly checkpoint) regulate phase transition during the cell cycle process.

The circadian oscillator is implicated in the checkpoint cellular pathways of the cell cycle. An involvement and the probable causative association between the core circadian oscillator genes and the cell cycle checkpoint processes are further manifested. A molecular association between the mammalian DNA damage checkpoints and the circadian clock genes has been proposed.

Modifications in expression levels of various circadian clock proteins alter checkpoint responses. PER1 protein is molecularly involved with DNA damage checkpoint protein ataxia telangiectasia mutated (ATM) and with checkpoint kinase 2 (Chk 2). Overexpression of PER1 induces apoptosis, while its inhibition reduces apoptosis. Mutation in *Per 2* gene eliminates cell response to gamma radiation and deregulates expression of several cell cycle checkpoint genes such as cyclin D1, cyclin A, mdm-2, gadd45 and c-myc.

Another implicating putative circadian clock gene is *timeless* (*tim*). The gene is a key element of the circadian oscillator in the fruit fly *Drosophila melanogaster*. In mammals, the probable descendant homologue TIM protein is not a true orthologue of *Drosophila* TIM, and its functional association with the circadian oscillator is under debate. A recent study suggested a probable role for TIM in the mammalian circadian clock speed and resetting. The mammalian TIM protein is involved in the cell cycle oscillator and is coordinated the intra-S checkpoint response to DNA damage induced by UV radiation.

Considering the circadian mode of cell proliferation and the causal association with the DNA damage checkpoint, an important question is whether altering circadian cycle can also affect the dynamics of cell division, with further comprehensible implications for tumor growth. Recent studies point out that interference in gene expression of the circadian oscillator can lead to cell cycle disorders and/or unrestrained cell proliferation. Indeed, in mammals, the association between malignancy and the interruption of the circadian cycle and interference in the regulation of cell cycle has been recognized for several years. However, most surveys are epidemiological studies, and there are no definitive investigations with a direct link between light-at-night, chronodisruption and cancer rate.

### Light at night, chronodisruption and malignancy

Light is the most prominent environmental signal that regulates daily and seasonal timing and as such, is an influential regulator of physiology and behaviour. An important adaptive feature of circadian clocks is that they can be synchronized (entrained) to local time. Synchronization of circadian oscillators is attained because light has acute effects on the levels of the clock’s components.

Modern life every so often comes across disturbances in "natural" light/dark cycles. Light phase is prolonged by light-at-night (LAT) and various professions involve night work or shift work. In addition, lights turned on during the dark phase generate light pulses, further triggering interferences. The resulting chronodisruption can cause complications for circadian clock organization. A substantial inference is whether such altering of circadian organization and entrainment also affects the dynamics of cell division. If so, the disruption of the circadian oscillator *per se* can further influence cancer growth and tumor progression.
There is increased evidence relating LAN to various malignancies in general and to breast cancer in particular. Nighttime satellite images and electricity consumption were monitored to estimate the correlation between LAN and various cancer incidents. The results indicate a significant association between rates of breast cancer and LAN. Wu et al. showed that to some extent, exposing transgenic rats to LAN accelerated tumor growth in vivo, through continuous activation of IGF-1R/PDK1 signaling. Nonetheless, the causative pathway of this association is not yet clear.

Night-shift work has long been associated with ill-health and the risk of cancer. Consequently, the International Agency for Research in Cancer (IARC) classified "shift work that involves circadian disruption" as possibly carcinogenic. In the past few years, there have been numerous supportive meta-analyses and evidence showing that night work does play a role in breast cancer. However, there are no clear indications for the exact pathway linking shift-work, chronodisruption and malignancy.

Nocturnal light pulses entrain and synchronize the circadian clock and are long known to affect physiology and behaviour. In mammals, evidence suggests that light pulses also operate as stressors that affect thermoregulation and impose a key threat to the physiological homeostasis of the organism. Ben-Shlomo and Kyriacou showed that light signal during the dark phase consistently activates transcription of numerous stress genes which are involved in the regulation of cell cycle progression, as well as levels of genes that are associated with the various cell cycle checkpoints. Thus, light pulse can operate as an environmental indicator and/or stressor that promptly affect transcription levels of genes that directly control cell cycle progression and play an indispensable role in cancer proliferation and survival.

Circadian disruption has been associated with interference to the homeostatic control of cell division, associated with malignancy, possibly as a result of interruption in the cell cycle DNA damage checkpoints. Chronodisruption by dim light at night or chronic jet lag accelerates tumor growth. Wood et al. suggested that the circadian clock gates tumor cell proliferation by coordinating clock-controlled proteins.

Ben-Shlomo and Kyriacou also showed that light pulse during the dark phase affected a considerable assembly of transcripts promoting cell proliferation, including tumor suppressors, oncogenes and genes that are involved in tumor growth and metastasis. The results suggest an association between light pulse, obstruction of the cell oscillator cycling and tumorigenesis. The molecular intracellular signaling pathway(s) by which light regulates and modifies the expression of genes related to cell cycle and tumorigenesis is not yet known. The increased risk for malignancy among shift-workers raised the debate of whether this is a causative phenomenon or a multifaceted association. Our results support the alternative of a causative link between light signal during the dark phase and the disruption of the cell cycle homeostasis.

The rapidity of tumor growth may also be related to the circadian oscillator and thus, to be affected by entrainment of the host clock. Consequently, chronodisruption may speed up abnormal proliferation. Hence, the circadian oscillator also drives daily cycles of detoxification drug metabolism, i.e. toxicity and anti-cancer xenobiotic efficacy is modified with the circadian time. A corresponding approach can consider chronotherapy, by means of using the circadian oscillator to down-regulate cell proliferation and malignant growth factors.

Concluding remarks
The accumulated data presented here suggests that artificial illumination via LAN, shift-work, and chronodisruption affects regulation and expression of genes that are associated with cell cycle homeostasis. Most of the available data to date merely reveals an association between the regulation of the circadian rhythms and the cell cycle progression. Nonetheless, the repeated found association and the suggested causality clearly has implications for the topic of whether there is an increased relative risk for cancer.

References
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