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Epigenetic basis of neuronal plasticity: Association with R/G-band boundaries on human chromosomes

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ABSTRACT

Epigenetic mechanisms have been suggested to have roles in neuroplasticity, in particular with regard to learning and memory formation, and in a range of neural diseases. In addition to epigenetic marks, the human genome also contains large-scale compartmentalized structures that might also influence neuroplasticity and neural disease. These structures result from variations in the amounts of GC% and in the timing of DNA replication and give rise to longitudinal differentiation (light and dark bands) along chromosomes after the appropriate staining. Here we describe our current understanding of the biological importance of the boundaries between these light and dark bands (the so-called R/G boundaries). We propose that the R/G-band boundaries on human chromosomes can be altered by epigenetic mechanisms, and that these changes may affect neuroplasticity, which is important to memory and learning, and may also have a role in the development of neural diseases associated with genomic instability.

1. Introduction: DNA sequences and chromosome bands

Considerable research effort is currently being expended on elucidating the genetic and epigenetic bases of neuroplasticity associated with learning and memory formation and with neural diseases. One aspect of this research is investigation of the relationship between large-scale chromatin structures and the epigenetic mechanisms that influence normal and abnormal mental development in humans. This review discusses the potential medical significance of one particular type of large-scale chromatin structure for conditions related to neuronal plasticity and to neural diseases associated with genomic instability.

Eukaryotic genomes contain regions with differing amounts of guanidine and cytosine residues (GC%); in mammals, these different regions have been shown to vary consistently in their replication timing patterns (Bernardi et al., 1985; Ikemura, 1985; Aota and Ikemura, 1986; Holmquist, 1989; Gardiner et al., 1990; Saccone et al., 1999; Pilia et al., 1993). Bernardi suggested the term "isochores" for regions (>300 kb) with homogenous GC contents (Bernardi et al., 1985). As isochores can show differences in staining intensity with the appropriate cytological dyes, such as Giemsa, chromosomes appear to have bands of light and dark staining regions. Giemsa dark (G) bands replicate late in S-phase and are mainly composed of AT-rich sequences (Table 1). Some Giemsa pale (R) bands are GC rich and replicate very early; these are termed T bands. The remaining R bands replicate early but have variable GC contents (Ikemura and Aota, 1988; Bernardi, 1989; Ikemura et al., 1990; Ikemura and Wada, 1991; Bernardi, 1993; Craig and Bickmore, 1993; Saccone et al., 1993). Although chromosome banding is a cytological phenomenon, the variations in sequence contents and replication timing of the bands have been used to define boundaries at the genomic level.

Epigenetic modifications, such as methylation, acetylation, or phosphorylation of histone proteins or methylation of the DNA, can cause changes to chromatin structure. These changes in chromatin structure may alter the binding of transcription factors or enhancer element binding proteins to promoter sites, thereby modifying gene transcription patterns (Gibbs et al., 2010). Consequently, regulation of changes to chromatin structure is important in the control of mRNA production (Bagot and Meaney, 2010). The epigenetic changes to chromatin structure are not permanent but can be reversed; this characteristic makes them suited to a role in memory formation. For example, the addition or removal of modifying functional groups on histones causes folding or unfolding changes in chromatin structure. In mature neurons, the addition of such functional groups to histones is transient and can be reversed in response to environmental stimuli (Bagot and Meaney, 2010).

The methylation of DNA is another epigenetic modification with a potential role in long-term memory formation. DNA methyltransferase (DNMT) enzymes, such as DNMT1, DNMT3A and DNMT3B, are highly expressed in the adult central nervous system (Fatemi et al., 2002; Feng et al., 2005; Feng et al., 2010); the activity of these enzymes, and the level of DNA methylation, is influenced by environmental cues (Feng et al., 2005). *In vitro* experiments using cortical neuronal cultures have

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Table 1
Characterization of chromosome bands.

Chromosome band	G/Q band	R band	T band (R subgroup)
Replication timing	Late	Early	Very early
GC%	AT-rich	Medium	GC-rich
Gene density	Low	High	Very high
Chromatin appearance	Compact	Loose	Loose

shown that the DNMT inhibitor 5-azadeoxycytidine can diminish DNA methylation levels and reduce expression of the memory-related gene, *REELIN* (Feng et al., 2005). DNMT mRNA (and its protein) has been shown to be present and active in cells of the central nervous system, indicating that changes in DNA methylation levels are involved in the normal functioning of the adult central nervous system. DNA methylation marks act either through steric inhibition of transcription factor binding or by recruiting reader proteins containing methyl-binding domains. Thus, these methylation marks may either reduce transcriptional efficiency or affect chromatin structure by recruiting histone deacetylases (Jones et al., 1998).

The *GRIK1*, *GRIA2* and *GRIA4* loci contain sequence motifs or similar sequences for the binding of the protein SATB1 (special AT-rich sequence binding protein 1) and these may be involved in controlling changes in replication timing in and around transition regions (Oda et al., 2012). Replication profiles, which correspond with chromosomal bands, are cell-type specific, and changes to these profiles identify chromosomal segments that are reorganized during differentiation (Hiratani et al., 2008). Moreover, cell pluripotency is associated with smaller replication domains and a higher density of timing transition regions that interrupt isochore replication timing (Hiratani et al., 2008).

2. Characteristic chromatin structures in chromosome band boundaries

The differences in GC contents between early and late replicating chromosomal regions are mirrored by differences in chromosomal structure at interphase and at metaphase of mitosis. In particular, early replicating regions have a looser chromatin structure than late replicating regions (Holmquist et al., 1982; Holmquist, 1989; Bernardi, 1989; Craig and Bickmore, 1993). Thus, chromosomal regions that show a transition in replication timing also show a transition in chromatin compaction. In terminally differentiated cells, such as neurons, these patterns of chromatin compaction are generally stable (Fig. 1). If a transition in chromatin compaction occurs within a gene, it might increase genomic instability and susceptibility to agents that influence gene expression. Consequently, chromatin instability and likelihood of DNA damage (including DNA rearrangements) may be greater in regions with replication transition than elsewhere in the genome (Fig. 1) (Bierne and Michel, 1994; Verbovaia and Razin, 1997; Rothstein et al., 2000; Watanabe et al., 2002, 2004, 2008, 2009; Puliti et al., 2010; Poretti et al., 2011; De and Michor, 2011).

Replication timing is often considered in terms of the interaction of transcriptional activity and chromatin structure (Hiratani et al., 2009). There is strong evidence that cellular differentiation is accompanied by coordinated changes in replication timing and transcription. These changes take place at the level of megabase-sized domains (R, G-chromosome bands) and are greater than localized alterations in chromatin structure or transcription (Hiratani et al., 2009). On the basis of these results, we propose that a key transition stage occurs during the middle of S-phase (R/G-band boundaries of human chromosomes) and that changes in replication timing that traverse this period are associated with changes in the activity of groups of promoters.

Interestingly, regions with an early to late switch in replication timing, so-called R/G-chromosomal boundaries, have been found to have a greater incidence of DNA sequences that can form non-B-DNA structures compared to other areas in the genome (Watanabe and Maekawa, 2010a, 2010b, 2013). Non-B-DNA structures are more likely to impede replication fork movement than normal chromatin. Therefore, R/G-chromosomal boundaries are genomic regions with a higher likelihood of stalled replication. Failure to complete replication has been suggested to increase the risk of genetic instability and to cause genetic mutations that have been found to be associated with many human diseases (Watanabe and Maekawa, 2010a, 2010b, 2013). It has been hypothesized that R/G-chromosomal boundaries are also more susceptible to epimutations than other parts of the genome (Watanabe and Maekawa, 2010a, 2010b, 2013).

3. Relationships between neuronal plasticity and/or neural disease and chromosomal R/G-band boundaries

Human chromosome 21 is not only responsible for Down syndrome (trisomy 21), but also carries genes associated with the development of neural diseases such as Alzheimer's disease and amyotrophic lateral sclerosis. In order to investigate the molecular pathology of neural disease, DNA replication timing on human chromosome 21q was analyzed (Watanabe et al., 2002). Three large genes, amyloid beta precursor protein (*APP*), glutamate ionotropic receptor kainite type subunit 1 (*GRIK1*) and Down syndrome cell adhesion molecule (*DSCAM*), were found to be located in regions where DNA replication timing switched from early to late S phase (Watanabe et al., 2002, 2014). Interestingly, *GRIK1* was located in a replication transition region in neural precursor cells (NPCs) but not in embryonic stem cells (ESCs); in the latter, it was located in a later replication timing region (Watanabe et al., 2014). Other large glutamate receptor genes are also located in or near transition zones in NPCs but not in ESCs where they often locate in later replication timing zones or in late replication zones. Early replicating regions tend to have “looser” chromatin structures compared to late replicating zones (Holmquist et al., 1982; Holmquist, 1989; Bernardi, 1989; Craig and Bickmore, 1993; Watanabe and Maekawa, 2010a, 2010b, 2013). Therefore, a change in relative chromatin compaction is likely to occur within a transition region of replication timing. This effect is illustrated by the four genes that encode the transmembrane AMPA glutamate receptor that mediates fast synaptic transmission in the central nervous system. Analysis of their replication timings indicated that they were clearly located in transition zones in NPCs but not in ESCs (Watanabe et al., 2014). We propose that this transition is universal in patients with neurological diseases, and that many neural genes and/or neural disease genes are located in the early/late-switch regions that correspond to the R/G-band boundaries on human chromosomes.

DNA methylation changes in three glutamate receptor genes, before and after replication transition, have been analyzed in NPCs and ESCs. A lower rate of methylation was found in the 5′-upstream genomic regions of *GRIK1*, *GRIA2* and *GRIA4* in NPCs compared to ESCs; these genes were clearly located in early replication transition zones in NPCs but not in ESCs (Watanabe et al., 2014). Thus, there was a clear relationship between replication timing and DNA methylation: earlier replication timing regions were relatively hypomethylated and later replication timing regions were hypermethylated.

One possible explanation for the different replication timing of large glutamate receptor genes and *APP* in different cell lines is that it is altered by epigenetic mechanisms (Watanabe et al., 2014). Recent studies have demonstrated that environmental factors can alter epigenetic marks in the genome that control gene expression. Thus, transition zones of DNA replication timing might be altered by epigenetic mechanisms in response to environmental factors. Such changes might be associated with the epigenetic basis of neuroplastic changes, such as the synaptic plasticity involved in learning and memory formation, and neural diseases associated with mutation of