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REVIEW ARTICLE

DNA replication, development and cancer: a homeotic connection?

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Abstract

The homeotic proteins are transcription factors, highly conserved in metazoan organisms, exerting a pivotal role in development and differentiation. They individually display a loose specificity for the DNA sequence they can bind, but operate mainly in multi-molecular associations that assure their target and function specificity. Homeotic proteins are known to play a role in the positive or negative regulation of cell proliferation. Furthermore, many homeotic proteins are actually proto-oncogenes, since different translocations involving their genes cause tumors, particularly in the hematopoietic system. A one-hybrid screen to detect proteins with affinity for the lamin B2 replication origin identified three homeotic proteins, namely HoxA13, HoxC10 and HoxC13. Recent data demonstrate that the HoxC13 oncoprotein specifically associates with replication foci and binds *in vitro* and *in vivo* to several human DNA replication origins. Moreover, Hox proteins interact with geminin, a regulator of cell cycle progression, and control the interaction of this protein with the DNA replication licensing factor Ctd1. Thus, the homeotic proteins, by participating directly in the function of DNA replication origins, may provide a direct link between the accurate regulation of DNA replication required by the morphogenetic program and the deregulation of this process typical of cancer.

Keywords: *DNA replication origins; DNA replication regulation; Hoxc13; Hoxc10; geminin; lamin B2 origin*

Introduction

The process of development and differentiation of metazoans, from the fertilized egg to the adult organism, requires an accurate regulation of faithful genome duplication, capable to cope, at one extreme, with the need of segmentation embryos to duplicate their several Mbp-long DNA in less than 20 minutes (as in Xenopus), and, at the other extreme, with the total abolition of the replication capacity of terminally differentiated cells. Conversely, the uncontrolled proliferation of cancer cells is characterized by a loss of DNA replication regulation, which provides the basis for the continuation of cell duplication. Thus, it can be expected, a priori, that the mechanisms of DNA replication regulation must involve factors that are at the basis of the "dialectic" link between these two processes.

Among the actors in the development process of vertebrates, the homeodomain-containing Hox proteins play a central role; in mammals 39 such proteins, orthologous to the *Drosophila* ones, are present (Krumlauf, 1994) and several other "diverged" homeobox-containing proteins are also coded in the genome. All these proteins demonstrably exert positive or negative modulation of the transcription of specific target genes, via their interaction with the promoter sequences, generally in combination with other associated Hox or non-Hox proteins. A role of Hox or Hox-related proteins in the regulation of cell proliferation has been proposed for a long time (see the next paragraph); in parallel, an involvement of Hox proteins in oncogenesis has become apparent, particularly (but not exclusively) in leukemogenesis. Finally, recent data demonstrate a direct involvement of Hox proteins with

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human DNA replication origins, pointing to a specific role in origin function.

In the present review we shall analyze the combination of observations just summarized and propose that Hox proteins may provide a link between development and cancer through their interaction with the replication origins, i.e. the DNA elements essential for activating or deactivating the process of genome duplication.

Hox proteins and cell proliferation

The proteins encoded by the *Hox* genes are transcription factors, evolutionarily highly conserved, that determine the morphogenesis of metazoa along the antero-posterior axis (McGinnis and Krumlauf, 1992; Krumlauf, 1994). All these proteins, belonging to 13 paralogous groups, are characterized by a 60-aa homeodomain, through which they interact with their DNA target. The 13 paralogs are organized into four clusters, A to D, located respectively on human chromosomes 7, 17, 12, and 2; for their homology with the *Drosophila* homeotic genes, groups 1 to 8 are considered analogous to the *antennapedia* genes (*Antp)* and groups 9 to 13 to the *abdominal-B* (*AbdB*) ones (Figure 1). In addition, "diverged" homeobox genes exist that are dispersed throughout the genome. The homeodomain contains three α-helices interacting with the major and minor groove as well as with the DNA phosphate chain (Gehring *et al.*, 1994). The sequence-specificity of binding of the different Hox proteins is rather loose: the bound elements are characterized by a preponderance of A and T and they often (but by no means invariably) contain an ATTA motif. In spite of this loose specificity, the Hox proteins can affect the transcription of a large variety of genes in a very precise way, because they invariably interact with the promoters as members of multi-protein complexes (Nolte *et al.*, 2006; Paris *et al.*, 2006; Svingen and Tonissen, 2006; Gong *et al.*, 2007) (see also the next section).

The first indication of a relationship between Hox proteins and cell proliferation offered also one of the first indications of a connection of these proteins with cancer: in rat cells cellular transformation and proliferation are induced by the products of the *Hoxb3* and *Hoxb4* genes, in cooperation with the product of the *Pbx1* gene (Krosl *et al.*, 1998). More evidence for a connection of *Hox* gene expression with cell proliferation came from two different systems, namely *Xenopus* embryos and retinoic acid-induced differentiation of pluripotent P19 mouse cells (Fisher and Méchali, 2003). The completion of the first cell cycles following the *Xenopus* mid-blastula transition is necessary for the expression of *HoxB* genes, whereas later cycles are necessary for assuring the correct pattern of expression of those genes. Similarly, in P19 cells *HoxB* expression requires proliferation and is concomitant with the S phase. Furthermore, in this system, the activation of *HoxB* transcription causes the silencing of the replication origins present in the 100 kb of the HoxB locus (Grégoire *et al.*, 2006). In analogy with these observations, in a system of murine CNS-derived cells, the homeodomain transcription factor Phox2a coordinates exit from the cell cycle and the start of neuronal differentiation (Paris *et al.*, 2006).

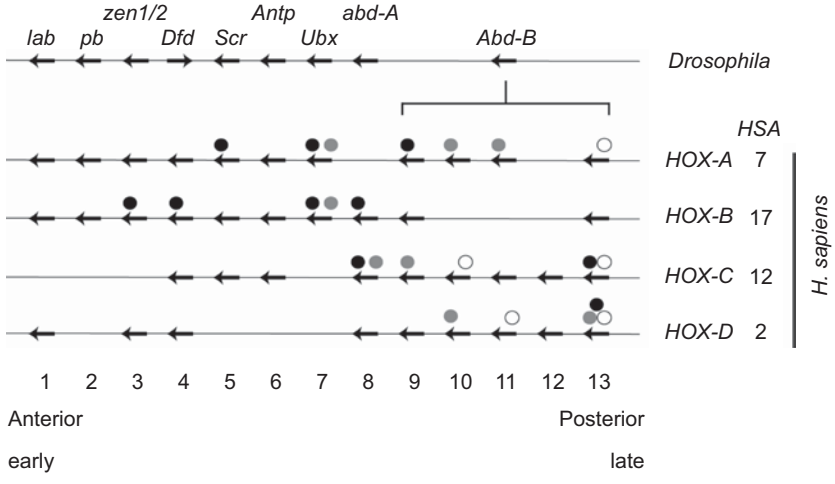

Figure 1. Schematic representation of the *Hox* gene clusters in the human genome. The chromosome (HSA: *Homo sapiens*) of each cluster is indicated on the right. Genes are represented by arrows that indicate the direction of transcription. Paralogous group numbers are shown on the bottom of the figure alongside the spatial and temporal expression profile in the developing embryos. The organization of the single *Hox* gene cluster in *Drosophila* is shown in the upper part of the figure to highlight the functional conservation of the paralogous genes during evolution. *Hox* genes involved in cancer are marked by a black circle; gray circles indicate proteins that interact with geminin, while white circles identify proteins shown to bind the lamin B2 origin.

Finally, and again related to the role of Hox genes in cancer development, which will be the subject of the next section, several Hox proteins play essential roles in hematopoiesis, by stimulating the proliferation of different blood cell progenitors (Sitwala *et al.*, 2008). In particular, *HoxA7*, a gene overexpressed in acute myeloid leukemia, is downregulated when HL-60 cells switch from proliferation to monocytic differentiation (Leroy *et al.*, 2004).

These and other similar studies clearly established a pivotal role of Hox proteins in modulating cell proliferation (Del Bene and Wittbrodt, 2005). This combination of observations is, at first sight, not univocal, insofar as the Hox proteins appear to play opposite roles relevant to their morphogenetic activities, in different systems, sometimes inhibiting proliferation to channel the cell into the differentiation pathway, sometimes stimulating the proliferation of appropriate precursors of mature differentiated cells. This is not in itself surprising, as often DNA-binding factors can play opposite roles in gene expression according to the cellular environment and availability of other cofactors, including post-translationally modifying enzymes. In this perspective, it comes as no surprise that Hox proteins are deeply involved in oncogenic transformations.

Hox proteins and oncogenesis

The earliest indications of a role of Hox proteins in oncogenesis came from hematopoietic tumors: *Hox 2.4* is the retrovirally activated gene of several myeloid tumors and is capable of transforming 3T3 cells (Blatt *et al.*, 1988; Aberdam *et al.*, 1991). Translocations involving a diverged homeobox gene, *Pbx1*, cause pre-B acute lymphatic leukemias and can transform 3T3 cells (Kamps *et al.*, 1990; 1991). Translocations activating the transcription factor *Hox11* cause T-cell leukemias (Dear *et al.*, 1993).

It was soon evident that the oncogenic potential of Hox proteins is not confined to the hematopoietic system: HoxB7, HoxB8, HoxC8 and HoxA9 are dominant oncoproteins in fibroblasts (Maulbecker and Gruss, 1993).

As mentioned in the previous section, the interaction of the Hox proteins with their target occurs probably in association with other proteins. In particular, the homeobox Pbx1 protein, that, as we have just seen, is an oncoprotein, performs its transforming function by binding cooperatively to a target ATCAATCAA motif together with either HoxA5, HoxB7, HoxB8 or HoxC8 (Lu *et al.*, 1995). The need for cooperative association with other Hox proteins to achieve the interaction of Pbx1 with its target was confirmed also for the HoxA1 and HoxD4 proteins (Phelan *et al.*, 1995), for the HoxA7 protein (Nakamura *et al.*, 1996b), for the HoxB1 protein (Di Rocco *et al.*, 1997) and for the HoxB3 and HoxB4 proteins (Krosl *et al.*, 1998).

The functional association of Pbx proteins with Hox proteins does not extend to all members of the Hox family; the association concerns groups 1–8, but does not occur with the other three paralogous groups, 9–13, corresponding to the *Abdominal B* family of *Drosophila* homeotic genes (Krumlauf, 1994). But these Hox proteins need cooperation with another cofactor to bind their target; this appears to be, for the proteins HoxA9, HoxA10, HoxA11, HoxD12 and HoxB13, a protein coded by another homeobox gene, *Meis1*, a common oncogene in murine myeloid leukemias (Shen *et al.*, 1997).

Several demonstrations of the proto-oncogenic character of many *Hox* genes are particularly concentrated, as indicated above, in the hematopoietic system. Fusion of *HoxD13* with the gene for a nucleoporin, *Nup98*, causes acute myeloid leukemia (Taniguchi *et al.*, 1995; Raza-Egilmez *et al.*, 1998); a similar effect is caused by fusions of *Nup98* with *HoxA9* (Borrow *et al.*, 1996; Nakamura *et al.*, 1996a). Subsequently, also the *HoxC13* gene was demonstrated to participate in the induction of acute myeloid leukemias when fused to *Nup98* (La Starza *et al.*, 2003; Slape and Aplan, 2004). The induction of tumors by *HoxD13* overexpression is not limited to the hematopoietic system: a recent review reports the involvement of this gene in breast cancer, melanoma, cervical cancer and astrocytomas (Cantile *et al.*, 2009). *HoxC8* expression correlates with prostate, cervical and esophageal cancer (Lei *et al.*, 2006).

Among the diverged homeobox proteins, a particular role is played by the so-called TALE (three amino acid loop extension) proteins, of which the most studied are the above-mentioned proteins Pbx and Meis (Owens and Hawley, 2002). They are commonly co-factors for the interaction of "canonical" Hox proteins with their targets; Pbx proteins, as we have just seen, cooperate with *Antp* paralogs, whereas Meis proteins associate mainly with *AbdB* paralogs (but paralogs 9 and 10 can interact with both). For instance, the interaction of the HoxA13 and HoxD13 proteins with MeisA1 and Meis1B appears essential for function (Williams *et al.*, 2005). Through these interactions the Hox proteins contribute to different stages of hematopoiesis and their overexpression is often the trigger of a number of leukemias, an aspect for which the interaction of Meis1 with the HoxA9 protein is prominent (Sitwala *et al.*, 2008). Meis can also cause myeloid leukemias in association with another divergent homeobox gene, *Cdx4* (Bansal *et al.*, 2006).

The importance of the Pbx/Hox interaction for determining the cancerous phenotype is highlighted by the initial promising results with small molecules that disrupt this interaction: a cell-permeable peptide designed to interfere with the bimolecular association inhibits melanoma growth (Morgan *et al.*, 2007); another designed peptide shows inhibition of several cancer cells (Aulisa *et al.*, 2009).

Other diverged homeobox genes with a demonstrated role in cancerogenesis are: *Six1*, which enhances metastasis in rhabdomyosarcomas (Yu *et al.*, 2004) and is overexpressed in breast cancer (Reichenberger *et al.*, 2005); *Tlx3* and *Nkx2-5*, which cause T-cell acute lymphoblastic leukemias (Nagel *et al.*, 2007); and *Hox11/Tlx1*, also the trigger of T-cell acute lymphoblastic leukemias (Riz and Hawley, 2005).

In summary, the oncogenic role of Hox proteins is demonstrated by multiple lines of evidence (in Figure 1 a black circle indicates the Hox paralogs for which a connection with oncogenesis has been demonstrated at this moment). These observations are not surprising considering the delicate game that these morphogenetic agents play in carefully regulating, *inter alia*, the proliferation of the cells that will eventually produce a well-functioning organ and organism. As for the basis for this regulation, the dogmatic conviction that these molecules are essentially transcription factors has always oriented the search on the specific promoters target of the regulation, whereas the start of DNA replication was considered secondary to the Hox-induced expression of the genes for replicative complex assembly and function. Conversely, in recent years, evidence for a direct involvement of Hox proteins in these processes has begun to emerge.

Hox proteins and DNA replication origins

Regulation of DNA replication in eukaryotes relies on the programmed activation of a number of replication origins, spaced approximately every 100 kb in the human genome, at precise moments of the S phase of the cell cycle (for a review on the subject, see the appropriate chapters in DePamphilis (2006)). Each origin is "licensed" to initiate replication only once in each cell cycle, thanks to the build-up of a multi-protein replication complex, assembled in G1 on the origin (pre-replicative complex); the composition of this complex was first described in yeast, but the essential components are conserved also in metazoa. A six-protein origin recognition complex (Orc1 to Orc6) is later joined by the Cdc6 and Cdt1 proteins; the latter enables the loading of a helicase complex (composed of the Mcm2 to Mcm7 hetero-hexamer plus the Cdc45 protein and the GINS hetero-tetramer) that constitutes (together with other proteins) the pre-initiation complex. This highly controlled assembly of the pre-initiation complex, at the appropriate moment of the cell cycle, allows the start from the origin of two diverging semi-conservative replicative forks that progress until they converge with the replicative forks coming from the adjacent origins. After origin firing, the rearrangement of the replicative complex into a different structure (missing Orc1 in the case of the human cells) and the inactivation or destruction of Cdc6 and Cdt1 inhibit origin firing again before the next cell cycle. The reformation of the pre-replicative complex in G2 and M is inhibited by the action of cyclin-dependent kinases.

In vertebrates, a further mechanism acts to prevent re-replication, namely a 33-kD protein called geminin that interacts with Cdt1 inhibiting the assembly of the helicase complex on the origin (Wohlschlegel *et al.*, 2000; Tada *et al.*, 2001; Kerns *et al.*, 2007). Geminin offered the first indication of a link between Hox proteins and the activation of replication origins. After its discovery, it became apparent that geminin plays a role in embryonic development by interacting with the Polycomb complex that represses *Hox* genes and also by interacting with the HoxB7 protein (thus inhibiting the transcription of its target gene (Luo and Kessel, 2004; Luo *et al.*, 2004) and with the Six3 protein, disturbing retinal development (Del Bene *et al.*, 2004; Pitulescu *et al.*, 2005; Seo and Kroll, 2006). In Figure 1 the gray circles indicate the Hox paralogs demonstrated so far to interact with geminin.

Hox and Six (homeobox divergent) proteins, in their association with geminin, compete with Cdt1 (Kroll, 2007) and thus geminin is critical in the balance between a differentiation and a proliferation pathway, particularly as concerns neural development. Thus, in mice, geminin and its binding partner Cdt1 are expressed abundantly in neural progenitor cells in early neurogenesis, but disappear during terminal differentiation (Spella *et al.*, 2007). More generally, the levels of Cdt1 and geminin follow a parallel modulation, being down-regulated when cells exit the cycle and overexpressed in cancer cells (Xouri *et al.*, 2004).

It has thus been proposed that geminin organizes a sort of "molecular platform" balancing cellular proliferation and differentiation (Yoshida, 2007), by exploiting its property, on the one hand, to bind Hox proteins and thus inhibit the expression of the genes important for morphogenesis, and, on the other hand, to bind Cdt1 and thus repress DNA replication and cell proliferation. The interaction with geminin prevents Hox proteins from binding to DNA, inhibits Hox-dependent transcriptional activation of reporter and endogenous downstream target genes, and displaces Cdt1 from its complex with geminin (Luo *et al.*, 2004).

Thus, geminin links Hox protein function with DNA replication origin function, but still would not seem to require a direct contact of those proteins with the origins, considering also that geminin itself has no affinity for specific DNA and is brought to the origin by Cdt1, which, in turn, is positioned there by the Orc/Cdc6 complex. More recent studies demonstrate instead that Hox proteins interact directly with replication origins and proteins of the replicative complexes.

Contrary to the situation in unicellular eukaryotes, metazoan Orc does not display sequence specificity

in binding DNA (Vashee *et al.*, 2003). This is certainly related to the lack of a sequence consensus for metazoan origins, a condition that has hampered for many years the identification of bona fide origins in human and other metazoan genomes (DePamphilis, 2006). The origin located near the human *LaminB2* gene is one of the best characterized human origins (Biamonti *et al.*, 1992; Giacca *et al.*, 1994). A pre-replication complex covering 110 bp assembles on it in G1 and is transformed in S into a post-replicative complex that covers only 70 bp (Dimitrova *et al.*, 1996; Abdurashidova *et al.*, 1998). On this origin, the precise positions of binding of the Orc1, Orc2 and Cdc6 proteins have been determined, as well as their modifications during the cell cycle (Abdurashidova *et al.*, 2003); furthermore, the start sites of leading strand synthesis have been mapped at two specific nucleotides overlapping by four bp (Abdurashidova *et al.*, 2000) and the precise position and dynamics of interaction within the replicative complex of the DNA topoisomerases I and II have been described (Abdurashidova *et al.*, 2007; Falaschi *et al.*, 2007).

A one-hybrid screen in yeast identified only three proteins specifically binding to a 74 bp sequence within the replicative complex area, namely the HoxA13, HoxC10 and HoxC13 proteins (de Stanchina *et al.*, 2000). The ability of the HoxC10 and HoxC13 proteins to bind the origin was confirmed *in vivo* by CAT assay and *in vitro* by band-shift analysis; an *in vitro* footprinting analysis with the HoxC13 protein showed a weak but significant protection within the area of the replicative complex. The HoxC10 protein was shown to be degraded early in mitosis by the ubiquitin-dependent pathway (Gabellini *et al.*, 2003), while its permanence (mediated by the mutation of two destruction boxes) causes the accumulation of cells in metaphase.

These observations have prompted a more in-depth study of the possible role of these proteins in origin function. Two lines of experimental evidence suggest the involvement of Hox proteins in origin definition: fluorescence microscopy analysis with a HoxC13 fluorescent construct demonstrated that this protein co-localizes with early S replication foci (when also the lamin B2 origin fires); chromatin immuno-precipitation assays with anti HoxC13 antibody demonstrated a clear enrichment of the precipitated DNA in the lamin B2 origin sequence in cycling cells, whereas no enrichment was found in non-cycling G0 cells (Comelli *et al.*, 2009).

The fluorescence microscopy data hint at a possible general involvement of HoxC13 with replication initiation (i.e. not specific for the lamin B origin). This indication was corroborated by the data of chromatin immunoprecipitation at two other human replication origins, close to the *Top1* and *Mcm4* genes. Also in these cases the HoxC13 protein was clearly bound to the origins, but only in proliferating cells.

A further confirmation of a possible general role of homeotic proteins in origin function came from recent data demonstrating that another *abdominal-B* ortholog protein, HoxD13, interacts *in vivo* with the lamin B2 origin and with the origins located close to the *TOP1*, *MCM4*, *c-MYC* and *FMR1* loci (Salsi *et al.*, 2009); this property was also confirmed for the HoxA13 protein and extended to the HoxD11 one; HoxD13 interacts with Cdc6, appears to stimulate pre-replication complex assembly and its interaction with the origins is competed by the binding to geminin.

Thus, binding of Hox proteins to DNA replication origins is now well established (In Figure 1 the white circles indicate the Hox proteins for which the binding to replication origins has been demonstrated at this moment); however, it remains unclear whether this interaction is relevant for the activity of this chromosomal element. Interestingly, unpublished data from the authors' labs (manuscript in preparation) show that the HoxC13 protein binds well within the replicative complex; furthermore, also the HoxC10 protein is bound at the same origins and interacts with HoxC13, as shown by tandem affinity purification.

Thus, some Hox proteins may be bona fide members of the replicative complexes; as appears to be the rule for trancription regulation by Hox proteins, they probably bind the origins in association with other proteins. Since cells can survive without HoxC13 (knock-out mice have defects in hair morphogenesis (Godwin and Capecchi, 1998)), it is conceivable that the role of this protein in origin function may be compensated by other similar molecules, given the redundance of Hox proteins and their tendency to functionally associate in multi-molecular complexes.

The observations summarized here invite a more thorough analysis of the precise sites and dynamics of the interactions and of the precise function of Hox proteins within the pre-replication, pre-initiation, initiation and post-replication stages, as well as of their interactions with other replication complex members. In Figure 2 we propose a tentative scheme of the chain of events leading to origin activation and involving Hox proteins (a detailed chronological and spatial analysis of the interaction of HoxC13 with the lamin B2 origin is at present under study in the authors' labs). In this context, it may be worth to consider the possibility that the (admittedly rather loose) sequence recognition specificity of these proteins may contribute, in a cooperative fashion with other factors, to the selection of the replication origin, a problem that remains puzzling at this moment in metazoans. Moreover, it is unclear whether most origins or only early firing ones are recognized and bound by Hox protein members. Finally, it is unclear whether Hox proteins have a role in activation or silencing of origins, the latter possibility being relevant for the inhibition of DNA replication during cell differentiation.

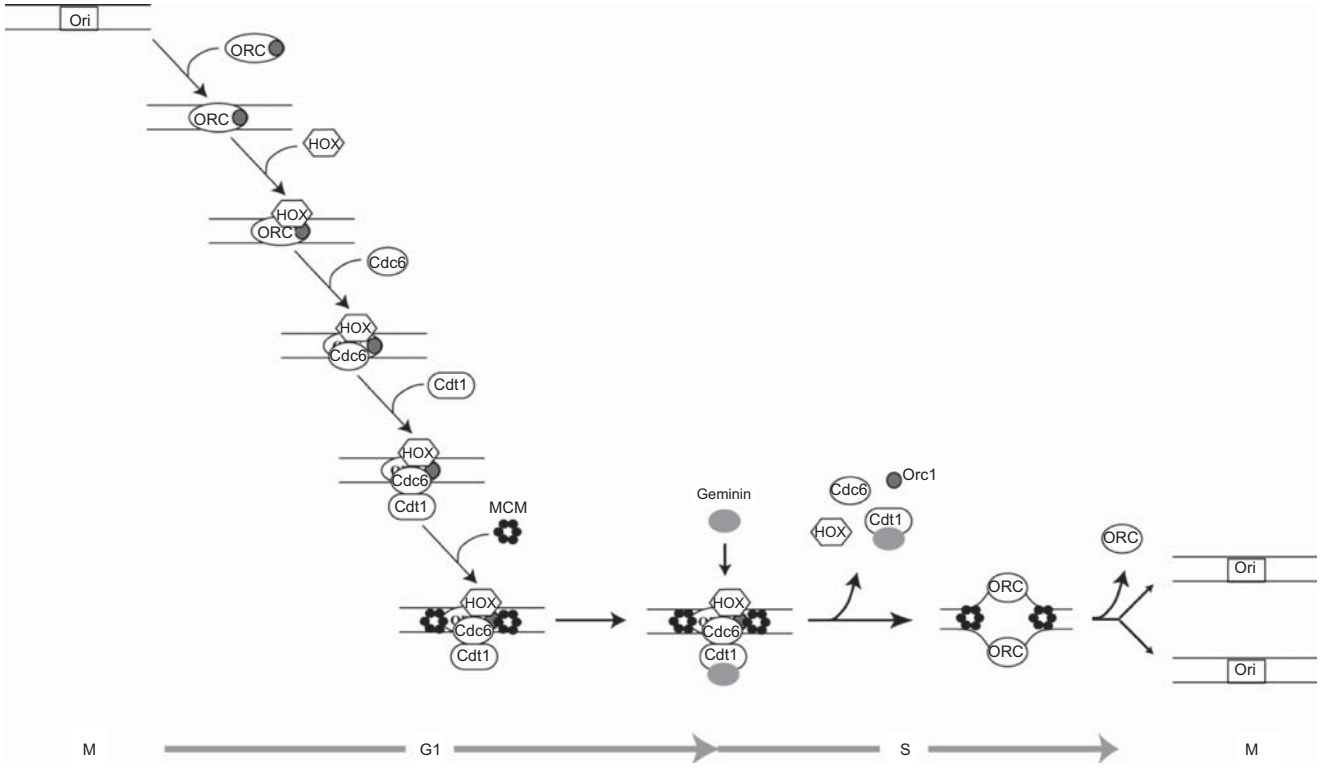

Figure 2. Tentative representation of Hox protein involvement in the process of activation and deactivation of replicative origins. It is proposed that the protein intervenes during the assembly of the pre-replicative complex before the successive loading of Cdc6, Cdt1 and the MCM heteroexamer; origin firing is accompanied by the removal of Cdc6, Orc1 and Cdt1 (sequestered by geminin that thus impedes the re-firing of the origin). It is proposed that also Hox may leave the origin at this moment, while the Orc reassembled in the post-replicative complex leaves the origin before the M phase.

The demonstrated direct interaction of Hox proteins with replication origins opens the possibility of new roles in development, in addition to their roles as transcription factors. Moreover, considering the data reviewed in the previous section, an important role can be envisaged in oncogenesis, which may involve regulation of the replication program besides gene expression.

Oncoproteins and DNA replication complexes

As indicated above, translocations of the *HoxD13* or of the *HoxC13* gene to the *Nup98* gene cause acute myeloid leukemias (Taniguchi *et al.*, 1995; Raza-Egilmez *et al.*, 1998; La Starza *et al.*, 2003; Slape and Aplan, 2004). This oncogenic behavior appears a common theme for proteins belonging to pre-replicative complexes or bound to DNA replication origins. Two other members of the lamin B2 replication complex DNA topoisomerases I and II, when fused with Nup98, also cause acute myeloid leukemias (Iwase *et al.*, 2003; Nebral *et al.*, 2005).

These four are not the only oncogenes that functionally interact with replication origins; c-Myc has been demonstrated to functionally interact with the lamin B2 (and other) origin(s) (Dominguez-Sola *et al.*, 2007); in *Drosophila* the Myb protein is essential for origin function (Beall *et al.*, 2002); in the same organism, an RB analog is essential to limit replication origin activity (Bosco *et al.*, 2001); in human cells the RB protein localizes on origins following DNA damage (Avni *et al.*, 2003). Finally, a recent high-throughput approach to identify human replication origins has produced 283 candidate sequences in which a significant abundance of sites for binding c-fos and c-jun is observed (Cadoret *et al.*, 2008). Each of these proteins also plays roles in developmental processes and, again, finding them implicated in the function of replication origins appears a very reasonable situation.

The description of the composition, supra-molecular structure and dynamics of human replication complex is still at an early stage. Recent work has uncovered new members that are all involved in oncogenesis; the replicative complexes have not yet been the object of search for specific "bullets" addressed to their members, their interactions and their function. It is to be expected that the newly identified Hox members, which are very likely to operate as multi-protein complexes, may offer, through description of their sites of interaction

with other replicative complex members, novel targets for cancer therapy, in analogy with the first promising attempts reported above that disrupt Pbx/Hox interaction in some cancers (Morgan *et al.*, 2007; Aulisa *et al.*, 2009).

Conclusions

The demonstration of a direct interaction of Hox proteins with replication origins and of their immediate participation in the process of DNA replication regulation sheds new light on the mode of action of these extraordinary molecules. The long envisaged connection between the highly ordered differentiation program and the deregulated proliferation typical of cancer now has a potential physical link, namely certain homeotic proteins. In this context, it is worth recalling that four members of the replicative complexes (DNA topoisomerases I and II, HoxC13 and Hoxd13) are oncogenic when fused to a protein of the nucleopore, which regulates nucleo-cytoplasmic traffic. Since these proteins have been demonstrated to reach the replication sites at precise times in the cell cycle, it is conceivable that such fusions disturb the accurate regulation of the traffic and this could trigger de-regulated replication. In view of the results reported above, in order to gain a greater comprehension and possibility of control of the connected seminal biological processes of development and cancer induction, it appears essential to achieve a detailed description of the structures and dynamic processes involved in origin activation and deactivation.

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