Colinearity Loops Out

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Abstract
Modulation of chromatin structure has long been proposed to underlie the colinear regulation of Hox genes during animal development. In a recent paper, Chambeyron and Bickmore explore this possibility in retinoic acid-induced ES cells. They show that, while chromatin remodeling confers transcriptional competence to the gene cluster, subsequent sequential extrusion of genes from their chromosome territory may determine their coordinated expression in time.

Reference


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and hence may mark genes for activation by Pdx during pancreas development. Further, if Pbx and Meis proteins act as markers that enable transcriptional activation by sequence-specific factors, this may have relevance to the role of Pbx and Meis in cancer. For instance, fusion of Pbx1 to the activation domain of E2A, as a result of chromosomal translocations, leads to pre-B-cell leukemia. Since E2A binds histone acetyl transferases (HATs), the E2APbx1 fusion protein might be particularly detrimental because it can penetrate silent chromatin, recruit HATs, and activate transcription without a requirement for other sequence-specific transcription factors.

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Selected Reading

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Modulation of chromatin structure has long been proposed to underlie the colinear regulation of Hox genes during animal development. In a recent paper, Chambery and Bickmore explore this possibility in retinoic acid-induced ES cells. They show that, while chromatin remodeling confers transcriptional competence to the gene cluster, subsequent sequential extrusion of genes from their chromosome territory may determine their coordinated expression in time.
the expression specificity of clustered genes depends upon their genomic topography. Genes located at one extremity of the cluster are activated earlier and in more anterior structures than genes lying progressively toward the opposite end (Gaunt et al., 1988; Krumlauf, 1994; Kmita and Duboule, 2003).

The molecular mechanisms governing this process are still unclear. In particular, the regulation of the progressive temporal activation of these genes during vertebrate embryogenesis has remained elusive. Based on genetic evidence, it was hypothesized that the transcription of neighboring genes in the developing trunk was progressively activated as a result of concomitant modifications in the structure (accessibility) of chromatin (reviewed in Kmita and Duboule, 2003). In a recent paper, Chambeyron and Bickmore (2004) take this question to a biochemical and subcellular level and provide support to this proposal, yet with some unexpected observations.

To overcome the difficulty of obtaining pure embryonic cellular populations representing different extents of Hox genes activation, these authors used ES cells treated in vitro with retinoids. In this system, Hoxb genes somehow recapitulate their temporal sequence of activation (Simeone et al., 1990). Because ES cell cultures, both undifferentiated and after induced differentiation, tend to be nonhomogeneous, a selection of either undifferentiated or differentiated cells was applied, such that two fairly pure cell populations could be isolated either before or at various time points after treatment. Transcription of Hoxb1 was found to be induced by 2 to 4 days of retinoic acid (RA) treatment, whereas Hoxb9 required 10 days of RA exposure, at which time Hoxb1 was no longer expressed.

With this material in hand, the authors looked at histone modifications at these two loci, at three time points (0, 4, and 10 days of RA treatment) using ChIP and anti-H3 antibodies. They observed an increase in both K9 acetylation and K4 methylation at the Hoxb1 locus after 4 days of treatment. These modifications disappeared at day 10, when the gene was silenced again. Surprisingly, however, the same modifications were detected at the Hoxb9 locus after 4 days, i.e., 6 days before the gene was detectably transcribed. In this latter case, the modifications persisted after 10 days of treatment. Methylation at K9 was not scored under any circumstances.

To investigate a potential “opening,” or decondensation of the chromatin structure, the authors applied FISH to ES cell nuclei, before and after RA induction. Hoxb1 and Hoxb9 are separated by 90 kb, a distance that does not allow to separate their signals in undifferentiated, nonexpressing cells. After a few days of RA treatment, however, the loci appeared significantly separated, suggesting chromatin decondensation. Control experiments showed this was due neither to the ES cell differentiation protocol nor to the increase of histone acetylation per se, since it did not occur upon treatment of ES cells with trichostatin A (TSA). The authors conclude that RA induces a rather specific and transient decondensation of chromatin at the Hox loci.

As Hoxb9 was not expressed at the time chromatin modifications and decondensation were observed, nuclear reorganization does not seem to solely account for transcriptional activation. Previous work (Mahy et al., 2002, and references in Chambeyron and Bickmore, 2004) suggests that physical exclusion of a gene from the chromosome territory (CT) is selectively associated with transcriptional activity. The authors then combined FISH for Hoxb1 and Hoxb9 with a paint for chromosome 11 and calculated the distance between the two signals and the nearest CT edge. They conclude that Hoxb1 and Hoxb9 sequentially loop out from their CT at the time they are selectively expressed. Furthermore, Hoxb1 is located closer to the CT periphery than Hoxb9 even before RA induction, which may reflect a greater availability of 3’-located Hox genes for transcription. In addition, looping out of Hoxb genes at the time of their expression appeared directional, toward the center of the nucleus, suggesting that it does not merely result from chromatin decondensation.

Based on these results, Chambeyron and Bickmore propose a two-step model whereby RA induction initially leads to alterations in histone modification and chromatin decondensation that confer a transcriptional “awareness” to the locus. Subsequently, ordered looping out from the CT allows the genes to be sequentially transcribed.

This model nicely refines previously proposed mechanisms, and the underlying experiments are the first to provide direct evidence for chromatin remodeling and chromosome dynamics accompanying the modulation of Hox gene expression. It is nevertheless important to consider that collinear regulation occurs several times and in different contexts in the course of embryonic development. While the onset of Hox gene transcription during gastrulation occurs sequentially, subsequent gene regulatory events leading to the establishment of fetal Hox expression domains also depend upon gene order (Forlani et al., 2003). The time scale and modalities of these multiple manifestations of colinearity vary extensively, and the question remains as to whether the same molecular mechanisms are at work (see Kmita and Duboule, 2003). The challenge now is to apply the technologies used by Chambeyron and Bickmore to mouse embryos and fetuses.

While genetic evidence for a direct role of RA in controlling general colinear gene expression during early embryogenesis is still lacking, the progressive refinement of Hox gene transcription in the spinal cord is RA dependent (Oosterveen et al., 2003). Consequently, while the present results may shed light on a particular case of concerted Hox gene regulation, their general relevance for the various colinear processes is as yet unclear.

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The presence of a nuclear envelope necessitates the movement of molecules between the nucleus and the cytoplasm. Nuclear export of RNAs and proteins is achieved through their association with soluble transport receptors that recognize substrates directly or through the use of adaptor proteins. Transport receptors escort their cargo across the nuclear membrane by making specific contacts with proteins of the nuclear pore. Increasing evidence demonstrates that mRNA export is coupled to other cotranscriptional processes such as splicing, surveillance, and ribonucleoprotein (mRNP) packaging, indicating that export plays an important role in determining the fate of mRNA transcripts (for review see Reed, 2003; Stutz and Izaurralde, 2003). As the nuclear envelope poses as a physical barrier between transcription and translation, the control over export exerted by nuclear transport, adaptor, and nuclear pore proteins provides a powerful means of gene regulation.

Typically, proteins and mRNAs exit the nucleus by different export pathways (for review, see Lei and Silver, 2002). Proteins containing nuclear export signals are exported via an interaction with the Leptomycin B (LMB)-sensitive export receptor, CRM1. Nuclear export of most mRNA transcripts is believed to occur primarily through the nuclear export factor NXF-1. However, some RNAs may rely upon an export pathway that is sensitive to LMB (reviewed in Cullen, 2003). Presently, it is unclear how the choice of mRNA nuclear export pathway is determined. Adaptor proteins such as those in the RNA export factor (REF) family, which are deposited on pre-mRNA, bind to both RNA and mRNA export factors and are believed to facilitate mRNP export (reviewed in Stutz and Izaurralde, 2003). Yet, whether adaptor or other proteins confer selection of export pathway remains to be determined. Moreover, it is not understood how the route of export influences the cytoplasmic fate of mRNAs.

In a study of the C. elegans sex-determining gene tra-2, Kuersten et al. (2004) demonstrate that the pathway of nuclear export can affect the translational control of a specific mRNA. In particular, the association of adaptor proteins and nuclear export factors with an element in the 3’ untranslated region (UTR) of tra-2 directs the pathway of nuclear export and affects the translation of tra-2. These findings significantly impact our understanding of both sex determination in C. elegans and the role of nuclear export in gene regulation.

A pathway of sex-determining genes specifies sexual cell fate in C. elegans (for review see Kuwabara and Perry, 2001). tra-1 and tra-2 are required for female development (Hodgkin and Brenner, 1977) (Figure 1A). Specification of male cell fate, which occurs in males and in hermaphrodites during spermatogenesis, requires repression of the tra genes (Figure 1A). Repression of the zinc finger transcription factor TRA-1 is achieved via its export to the cytoplasm. Translational repression of the tra-2 mRNA, which encodes a transmembrane protein, is mediated in part through elements in its 3’ UTR (Goodwin et al., 1993).

The importance of the tra-2 3’ UTR in promoting male development is further evinced by findings that it regulates tra-2 mRNA and TRA-1 nuclear export (Graves et al., 1999; Segal et al., 2001). Previous data demonstrated that, in the absence of TRA-1, tra-2 is retained in the nucleus via a 3’ UTR tra-2 retention element (TRE) (Graves et al., 1999). Notably, the binding of TRA-1 to tra-2 mRNA in a region of its 3’ UTR that overlaps the TRE overcomes nuclear retention of tra-2 (Graves et al., 1999; Segal et al., 2001). Nuclear export of TRA-1 and tra-2 mRNA, which occurs through an LMB-sensitive pathway, relies upon the formation of a TRA-1/tra-2 mRNA complex (Segal et al., 2001). Export of the TRA-1/tra-2 mRNA complex effectively reduces nuclear levels of TRA-1, thereby promoting male development (Segal et al., 2001). Now, Kuersten et al. show that the route of tra-2 mRNA export depends upon the TRE. Removal of the TRE results in nuclear export of tra-2 that requires NXF-1 but is no longer sensitive to LMB (Kuersten et al., 2004).

The presence of a nuclear retention element in tra-2 suggested the existence of TRE-interacting factors. Kuersten et al. reasoned that removal of these factors would have the same effect as loss of the TRE. RNAi experiments performed to reduce the activity of select nuclear export factors and adaptor proteins demonstrated that the loss of either NXF-2 (a second NXF family member), REF-1, or REF-2 leads to tra-2 export that utilizes NXF-1 but is no longer sensitive to LMB.

Selected Reading


REF-ereeeing the Cytoplasmic Fate of mRNA via Nuclear Export

The C. elegans sex-determining gene tra-2 is subject to multiple forms of regulation. A report in the June 4 issue of Molecular Cell now shows that proteins associated with the tra-2 mRNA determine its pathway of nuclear export and influence its cytoplasmic fate. These findings demonstrate an additional level of control and link nuclear export to the regulation of sexual development.

The presence of a nuclear envelope necessitates the movement of molecules between the nucleus and the cytoplasm. Nuclear export of RNAs and proteins is achieved through their association with soluble transport receptors that recognize substrates directly or through the use of adaptor proteins. Transport receptors escort their cargo across the nuclear membrane by making specific contacts with proteins of the nuclear pore. Increasing evidence demonstrates that mRNA export is coupled to other cotranscriptional processes such as splicing, surveillance, and ribonucleoprotein (mRNP) packaging, indicating that export plays an important role in determining the fate of mRNA transcripts (for review see Reed, 2003; Stutz and Izaurralde, 2003). As the nuclear envelope poses as a physical barrier between transcription and translation, the control over export exerted by nuclear transport, adaptor, and nuclear pore proteins provides a powerful means of gene regulation.

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