Acting Locally and Globally: Myc’s Ever-Expanding Roles on Chromatin

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Abstract

Myc regulates key cellular processes including cell cycle, differentiation, and apoptosis. It has long been thought to direct these functions by acting solely as a classic transcription factor regulating expression of a small number of key target genes through discrete chromatin events in their promoters. A recent wave of genomics studies together directly challenge the narrowness of this model. For example, Myc binds to tens of thousands of sites in the human genome. It also regulates histone acetylation at and transcription of a remarkable number of genes, far beyond that expected of a classical transcription factor. The influence of Myc on chromatin also surprisingly extends to both genic and expansive intergenic regions. These studies support an evolving model in which Myc activity on chromatin is far more complex than previously imagined. The ability of Myc to act both locally and globally on chromatin may be responsible for its wide-ranging effects on the biology of stem and tumor cells. [Cancer Res 2009;69(19):7487–90]

A Long-Standing Puzzle for Myc: What’s a Protein Like You Doing in a Place Like This?

The family of myc proto-oncogenes encodes proteins belonging to the basic helix-loop-helix leucine zipper (bHLHZ) group of classical transcription factors. Myc proteins regulate a wide variety of cellular processes including cell growth, proliferation, differentiation, cell cycle progression, and apoptosis (reviewed in refs. 1, 2). Myc overexpression is strongly associated with tumorigenesis, and knockout of either c- or N-myc profoundly disrupts embryogenesis indicating that it is essential for all proliferating cells, stem and progenitor alike, to maintain myc levels within a narrow range. One potential link between Myc-driven tumorigenesis and the requirement for Myc during development is an emerging role for Myc in regulating stem cell biology. Indeed, several recent studies have indicated that Myc regulates the establishment [induced pluripotent stem cells (iPSC)] and maintenance of pluripotency (3–5).

Despite its central role in coordinating many cellular processes and its homology to other bHLH proteins, the molecular mechanisms of Myc’s biological functions in both stem and tumor cells have remained surprisingly difficult to define. Myc has been generally modeled to function only as a classic transcription factor that binds to a set of specific target genes and regulates transcription though recruitment of chromatin-modifying complexes to very discrete regions mostly in promoters. More then a thousand putative target genes of Myc have been identified in mammalian cells including genes involved in cell cycle, ribosome biogenesis, protein synthesis, and mitochondrial function (3, 4). The sheer number of Myc target genes sets it apart from most transcription factors, but other recent work has also indicated that Myc is a very atypical bHLH protein. For example, in neural stem and precursor cells, loss of Myc leads in some cases to nuclear condensation and widespread loss of euchromatin, which is totally at odds with what would be expected of classical transcription factors including bHLHZ proteins (5–7). Also puzzling for a supposed classical transcription factor, a host of expression microarray and genomics studies indicate that Myc binds to and likely regulates widespread chromatin (8, 9), as well as expression of a huge number of genes, including collectively perhaps as much as 15% of all genes (reviewed in ref. 10). Although unexpectedly widespread, the magnitude of transcriptional regulation of most target genes by Myc, with few important exceptions including nucleolar genes, is nonetheless relatively modest compared with other bHLHZ family members and transcription factors more generally, producing yet another puzzle (4, 10). Although the mostly modest transcriptional regulation by Myc is likely still quite important for the regulation of various cellular processes, it is very uncharacteristic of classical transcription factors, which more often regulate fewer target genes, but in a much more pronounced manner.

Why would a bHLHZ protein such as Myc influence widespread euchromatin, bind potentially tens of thousands of sites throughout the genome, and most often only relatively weakly regulate transcription, but at large numbers of target genes (3, 7, 10)? Myc’s potent influence on cell biology coupled with its relatively modest transcriptional regulation suggests the notion that even changes in gene expression that are viewed as relatively weak can collectively be extremely important control mechanisms for cell behavior, but making meaning out of the complexity of the Myc-regulated program remains a challenge. A relatively recent collection of genomics studies have gone a long way toward addressing this challenge and have together transformed how we think about Myc. They may also have lain to rest once and for all the model that Myc strictly is a classical transcription factor, suggesting a new model in which Myc acts both locally and globally.

Beyond Binding: Functional Genomics Studies of Myc Chromatin Activity

Myc has been previously linked to widespread active histone modifications in tumors and stem cells (8, 11), and genomics studies on Myc binding suggest a potential direct mechanism involving Myc bound regions, but there have been few genomics studies that specifically address Myc chromatin function in addition to binding. Amati and Guccione’s key studies in this area examined Myc and associated chromatin in the human genome...
Interestingly, this work suggested Myc not only regulates euchromatin but also requires a preexisting active chromatin state. They found evidence of widespread Myc regulation of acetylation of histones H3 and H4 at a surprisingly large number of lysine residues, whereas there was no clear pattern linking Myc levels to triMeK4. In the most comprehensive study of Myc regulation of histone modifications to date, they found many additional marks associated with Myc including intriguingly diMeH3K79 (9). Myc also induced deposition of H2A.Z. Together the Myc-induced chromatin program strongly correlated with transcriptional activation as well. In our ChIP-chip study of neuroblastoma cells with a Tet-regulatable N-Myc transgene (TET21N; ref. 12), we found upon Tet treatment that overall most AcK9 and triMeK4 genomic peaks were greatly diminished directly in parallel to decreased N-Myc levels and, both spatially and temporally, to loss of N-Myc genomic binding (13). These findings indicate that Myc does not just bind to the thousands of genomic sites, but also initiates or maintains an expansive euchromatic program. Because changes in global histone modifications have been reported to be associated with tumorigenesis (14, 15), this expansive chromatin function for Myc may manifest both in normal and neoplastic cells. For example, both c- and N-Myc as well as a host of genes involved in histone methylation have been linked to medulloblastoma formation (16). The fact that N-Myc also regulates histone methylation in cerebellar granule neural progenitors, a possible cell of origin of medulloblastoma, as well as in neuroblastoma also supports the notion of a convergence on histone methylation in primitive neuroectodermal tumors (11, 13).

### Direct and Indirect

In our ChIP-chip studies, we also found that a subset of the N-Myc dependent AcK9 and triMeK4 peaks are localized in regions not detectably bound by N-Myc. This observation might reflect an indirect mechanism of chromatin regulation by Myc. There are several potential explanations for the apparent indirect regulation of global chromatin by Myc. Myc might directly regulate the expression of target genes that encode histone-modifying enzymes, which, in turn, would modify histones in the genomic regions not bound by Myc (Fig. 1). Indeed, it has been previously reported that the histone acetyltransferase gene GCN5 is transcriptionally upregulated by Myc. GCN5, in its turn, can strongly increase

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(8, 9). Although these studies vary on the specific euchromatic marks globally regulated by Myc, perhaps because of differences in cells used (fibroblasts and/or lymphocytes versus neuroblastoma), they consistently point to a very widespread role for Myc proteins in regulating euchromatin. This expansive chromatin function for Myc may manifest both in normal and neoplastic cells. For example, both c- and N-Myc as well as a host of genes involved in histone methylation have been linked to medulloblastoma formation (16). The fact that N-Myc also regulates histone methylation in cerebellar granule neural progenitors, a possible cell of origin of medulloblastoma, as well as in neuroblastoma also supports the notion of a convergence on histone methylation in primitive neuroectodermal tumors (11, 13).
acetylation in Myc null cells indicating that GCN5 also has widespread effects on chromatin independent of Myc (11). Alternatively, Myc binding in one chromatin location might influence another chromatin location owing to a multidimensional organization of cellular chromatin via an enhancer-like phenomenon. In fact, several multigene clusters including β-globin locus are regulated by a remote enhancer via a chromatin loop formation (reviewed in ref. 17). It is also conceivable that Myc controls focal chromatin domains, which establish boundaries for heterochromatin spreading. In this scenario local changes in the chromatin associated with the Myc loss would disrupt boundaries of heterochromatin allowing it to spread over large regions. Finally, Myc may depend upon complex formation with other DNA binding factors and in such contexts its own DNA binding may become dispensable. Consistent with this idea, we found that non-transcriptional start sites (TSS) seem to be enriched for binding sites for other transcription factors that may recruit Myc. The binding data we obtained also indicated that whereas TSS regions bound by N-Myc were enriched with Myc canonical E-box motifs, surprisingly non-TSS regions bound by N-Myc were not significantly enriched in CACGTG E-boxes, suggesting that regulation of non-TSS regions might involve a mechanism of N-Myc recruitment to the DNA other than its recruitment to canonical consensus sequences such as through other transcription factors. Thus, there seems to be a great deal more to Myc activity on chromatin than simple direct E-box binding and promoter activation. An extreme maneuver is in order for how we think about Myc in the form of a new model that includes but goes far beyond direct transcriptional regulation of protein-coding genes to encompass the new chromatin activities described above.

Functional genomics studies on Myc binding have been remarkably consistent in arguing for very expansive, direct genomic DNA binding by Myc proteins. For example, the ChIP-chip assays in neuroblastoma revealed very widespread N-Myc genomic binding with an estimated 25,000 to 40,000 total binding sites out of which almost 40% were binding sites lying a distance of >10 kb from the TSS domains. These findings are similar to the predictions for c-Myc genomic binding (18, 19). Interestingly, in HeLa cells, the figure of 17,000 to 35,000 Myc binding sites from Farnham’s laboratory (19) almost perfectly matches the estimate of 30,000 Myc protein molecules in HeLa cells by Evan’s group, suggesting most Myc molecules are bound to DNA (20). A ChIP-PET approach found fewer c-Myc binding sites (~4,000), but still suggests very widespread binding (21).

Myc, Chromatin, and Pluripotency

The evolving model of Myc activities on chromatin has wide implications in stem cell as well as IPSC biology (reviewed in ref. 22). Recent studies have uncovered a specific global euchromatic state in embryonic stem (ES) cells encompassing both genic and intergenic regions, which are essential to the maintenance of ES cell state (7). The work by H. Chang’s group has delineated a “stem cell” expression signature, a core transcription program characteristic of ES cells, which includes genes involved in regulation of transcription and cell cycle, as well as nucleolar, mitochondrial, and ribosomal genes (6). Interestingly, Myc is able to activate the ES cell-like program in both normal and cancer cells (6). Moreover, Myc has been linked to the generation of IPSC and to signal transducers and activators of transcription (STAT) signaling in murine ES cells indicating its important role in regulating stem cell self-renewal and pluripotency (23, 24). The molecular mechanisms of Myc’s normal function in ES cells and IPSC, however, remain unknown. One notion is that Myc contributes to the control of stem-cell pluripotency and self-renewal through global chromatin reprogramming involving widespread histone modifications that might facilitate the action of other stem cell-related transcription factors on chromatin. However, Myc regulation of expression of specific stem cell transcription factors could also play a key role.

Myc’s function in IPSC is likely to be similar to its function in ES cells. Indeed, recent findings indicate that during the reprogramming process, Myc promotes the repression of fibroblast-specific genes, which is an important early step required for the induction of the ES-like expression program (25). Reprogramming fibroblasts to IPSC, therefore, might involve Myc-dependent reversal of the pre-existing differentiated state of fibroblast genomic chromatin to a globally “active” state bearing an overall signature pattern with much in common with ES cells. Such a process, unfortunately, when deregulated, could readily lead to oncogenic transformation so there may be a fine line between pluripotent and oncogenic transformation. The mechanism by which Myc influences pluripotency, particularly in IPSC, remains somewhat unclear. The extent to which Myc may cooperate with other stem-related transcription factors, an intriguing possibility, also remains unclear as various genomic studies have suggested differing degrees of overlap in binding sites between Myc and core pluripotency transcription factors (25, 26).

Where Does Myc Go from Here?

The recent studies on Myc and chromatin raise many important, open questions. Is widespread chromatin activity unique to Myc or are there other as yet to be discovered transcription factors and oncogenes that regulate global chromatin structure? In other words, is Myc unique in this multifaceted influence on the genome or simply the first factor discerned to behave in this manner? Because Myc deregulation is strongly associated with many different types of tumors, could Myc-dependent changes in widespread chromatin structure contribute to tumorigenesis, and if so, how? If there are other oncogenic factors assumed to be transcription factors that behave in similar fashion, could deregulation of global chromatin be a new general mechanism for oncogenesis? Does the Mdx/Mnt family of Myc antagonists have global repressive effects and promote widespread heterochromatin? Does Myc regulate widespread euchromatin in normal stem cells, suggested by studies in neural stem cells (11), in the same fashion as it does in neuroblastoma? What about Myc chromatin activity in other kinds of tumors and their normal cognate stem or progenitors as well? Many of these questions are likely to be answered in the not-so-distant future by the continuing application of genomics studies to Myc-Max superfamily function and to mapping of chromatin events in tumors more generally.

Although the emerging role of Myc as a general chromatin regulator provides important insights into the molecular mechanisms of Myc function, the importance of its role as a gene-specific transcription factor should not be underestimated. Both genomic and functional analyses of Myc targets suggest that Myc globally regulates groups of genes involved in cell cycle, protein synthesis, and metabolism (27–29). Recent studies have shown a number of genes involved in ribosome biogenesis to be direct targets of Myc and linked them and Myc in the control of cell size (30–32). Moreover, in mice, ribosomal proteins have been reported to be required for Myc-mediated tumorigenesis (33). Myc regulation of stem and tumor cell...
biology is also suggested by recent work demonstrating Myc transcriptional activation of plurioty-related genes in neuroblastoma (34). Thus, key aspects of Myc function can be attributed to its ability to directly influence the expression of individual genes.

MicroRNAs (miRNA), small RNA molecules that can control their target gene expression post-transcriptionally and via their effects on epigenetic machinery, are also specific Myc-regulated genes (35–37). They are likely to be extremely important for Myc regulation of N-Myc activation of the miR-17/92 polycistron has been implicated in medulloblastoma genesis and neural progenitor cell function as well (38). In addition c- and N-Myc regulation of lin28 and lin28β expression in tumors, with miRNAs may play a role in Myc's enhancement of its ability to directly influence the expression of individual genes.

Understanding the chromatin-based mechanisms by which Myc regulates the balance between normal stem cell functions, such as self-renewal and pluripotency, and oncogenic transformation is certain to provide important insights essential for the development of cancer therapies as well as perhaps ES cell- and IPSC-based regenerative medicine therapies. The clearest path toward this objective is through a concerted multifunctional laboratory genomics-based effort to in parallel globally map not only Myc protein family genomic binding, but also, importantly, its regulation of specific key histone modifications, so we can further our understanding of its role on chromatin. This endeavor should be conducted in both normal stem cells and a variety of tumor types associated with Myc deregulation so that tumor-specific chromatin functions can be defined. The global, unbiased mapping of N-Myc-regulated histone modifications in neuroblastoma is just another stepping stone forward toward the goal of therapeutically targeting Myc's chromatin program, but there is likely a long road ahead as Myc's known chromatin-related functions are ever expanding.

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