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Current and emerging approaches to define intestinal epithelium-specific transcriptional networks

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Submitted 6 September 2011; accepted in final form 13 November 2011

Olsen AK, Boyd M, Danielsen ET, Troelsen JT. Current and emerging approaches to define intestinal epithelium-specific transcriptional networks. Am J Physiol Gastrointest Liver Physiol 302: G277–G286, 2012. First published November 17, 2011; doi:10.1152/ajpgi.00362.2011.—Upon developmental or environmental cues, the composition of transcription factors in a transcriptional regulatory network is deeply implicated in controlling the signature of the gene expression and thereby specifies the cell or tissue type. Novel methods including ChIP-chip and ChIP-Seq have been applied to analyze known transcription factors and their interacting regulatory DNA elements in the intestine. The intestine is an example of a dynamic tissue where stem cells in the crypt proliferate and undergo a differentiation process toward the villus. During this differentiation process, specific regulatory networks of transcription factors are activated to target specific genes, which determine the intestinal cell fate. The expanding genomewide mapping of transcription factor binding sites and construction of transcriptional regulatory networks provide new insight into how intestinal differentiation occurs. This review summarizes the current overview of the transcriptional regulatory networks driving epithelial differentiation in adult intestine. The novel technologies that have been implied to study these networks are presented and their prospects for implications in future research are also addressed.

ChIP-chip; ChIP-Seq; differentiation; gene regulation

THE EXPLORATION ACROSS THE entire genome of the complex interactions between multiple transcription factors and gene targets are often termed “transcriptional regulatory networks.” Newly evolved technologies such as ChIP-chip (chromatin immunoprecipitation coupled to microarray hybridization) and ChIP-Seq (chromatin immunoprecipitation coupled to high-throughput sequencing) have enabled determination of genomewide distribution of transcription factor binding sites, histone modifications, RNA polymerase II occupancy, and DNA methylation (1). These two techniques have enabled researchers to gain massive new information of DNA-protein interactions distributed throughout the whole genome, contributing to a better understanding of the clusters of differently expressed genes in a given transcriptional regulatory network. In 2003, the US National Human Genome Research Institute established the ENCODE project. The main goal is to gather data identifying functional DNA elements in the entire human genome and release them into public databases; currently one of the most prominent assay used in ENCODE is ChIP-Seq. This very ambitious mission emphasizes the high relevance of investigations revealing transcriptional regulatory networks.

Chromatin immunoprecipitation (ChIP) is based on covalent linkage of DNA-binding protein to DNA by a cross-linking agent, e.g., formaldehyde (58). The chromatin is after cross-linking isolated and fragmented by sonication or enzymatic digestion into minor fragments between 200–1,000 bp. A specific antibody is used to perform immunoprecipitation of the appropriate DNA-protein complex. The cross-links are reversed and the DNA is purified and is ready for, e.g., cross-hybridization to microarrays or high-throughput sequencing. The sequences are finally aligned back to the genome to identify enriched DNA regions (Fig. 1) (77). ChIP-chip and ChIP-Seq analyses of intestinal expressed transcription factors have recently given us new insight in the transcriptional regulatory networks controlling the proliferation and differentiation of the adult intestine (18, 19, 102, 103).

The mammalian intestinal epithelium is a dynamic organ, which undergoes continuously renewal controlled by a coordinated regulation between proliferation and differentiation of epithelial stem cells and immature progenitor cells. This is the
Fig. 1. Workflow of chromatin immunoprecipitation (ChIP) coupled with hybridization genome tiling array (ChIP-chip) or with high-throughput sequencing (ChIP-Seq). Cells are treated with formaldehyde to create covalent cross-linkage between DNA and DNA-associated proteins such as transcription factors and histones (A). Transcription factor-bound DNA fragments are isolated by immunoprecipitation (B). After the transcription factor and DNA complexes have been de-cross-linked, the DNA fragments are prepared for either ChIP-chip (C) or ChIP-Seq (D). Highlighted screenshots are examples of HNF4α and CDX2 bindings on CDX2 gene analyzed by ChIP-chip (18) or ChIP-Seq (19, 103). HNF4α ChIP purified DNA resulted in high signal identities for probes (red circle) located in the CDX2 promoter (E). CDX2 ChIP purification led to enrichment of sequences (red circles) in CDX2 promoter (F). These results demonstrate binding of HNF4α and CDX2 to the CDX2 promoter and indicate a functional role of CDX2 regulation.

most rapidly renewed tissue in the human body (47). Several different epithelial cell types are organized into crypt-villus units along the vertical axis in the small intestine. These cells arise by asymmetric division of stem cells located in the crypts of Lieberkühn, where one stem cell is capable of self-renewal throughout a life span (Fig. 2) (7, 97). The progenitor cells, also known as transient amplifying cells, are highly proliferative and are responsible for the homeostasis of the tissue organization. Ultimately the progenitor cells in the small intestine differentiate into four principal lineages in the proliferative compartment in the lower part of the crypt: one lineage is the absorptive enterocyte and the remaining three are secretory lineages consisting of goblet, enteroendocrine, and Paneth cells (14, 67, 68). In contrast to Paneth cells, which migrate toward the crypt base, all secretory lineages and enterocytes migrate toward the lumen. Cells in the colonic epithelium also originate from crypt-localized stem cells, which differentiate into absorptive colonocytes and goblet cells (7).

There are several signaling pathways, which are involved in differentiation into either absorptive or secretory lineages. Notch signaling is involved in the determination whether a progenitor cell differentiates into the absorptive or a secretory lineage in the stem cell niche (51). Notch signaling is necessary to maintain a proliferative, undifferentiated cell population in the crypt as well as ensuring differentiation. For the most part these cells will differentiate into the absorptive enterocytic lineage (100). Cross talk between Wnt/β-catenin and Notch...
signaling is important for the coordination of proliferation and differentiation (72).

The Wnt/β-catenin pathway has a central role in regulation of intestinal stem cell proliferation mediated by gene activation by nuclear β-catenin associated with transcription factor 7-like 2 (TCF7L2), also known as TCF4 (9, 52, 79, 101). Mutations in Wnt-related genes, which involve activation of Wnt/β-catenin, lead to adenomatous polyp formation or colon cancer in mice and humans (52, 85). Furthermore, aberrant cell proliferation in the crypt of fetal mice can be a consequence of mutations in TCF4 (57). The activity of the Wnt/β-catenin pathway is highest in the crypt of colon or small intestine with a decreasing activity toward the lumen.

An opposite pattern is observed for transforming growth factor-β (TGF-β) superfamily signaling molecules, in which the highest activity is seen at the cells at the villus. TGF-β superfamily consists of secreted cytokines including the bone morphogenetic proteins (BMP), activins and TGF-β isoforms. TGF-β signaling can repress expression of c-Myc and cyclin D1, thus arresting the proliferation of intestinal epithelial cells (IECs) in the G1 phase (22, 53). Furthermore, the TGF-β/BMP signaling can activate transcription of genes, which are involved in repression of proliferation and promote differentiation (8, 29).

Under normal conditions, the fine-tuned equilibrium between proliferation and differentiation is critically important to maintain the appropriate size and function of the epithelium. Indeed, the coordination of Wnt/β-catenin, BMP, Notch, and other pathways located in the crypt regulates the fate of intestinal stem cells; this process is tightly controlled by transcription factors arranged in a regulatory network (Fig. 2) (65, 66). In this review, we have aimed to addresses which transcription factors and transcriptional regulatory networks are involved in proliferated and differentiated intestinal cells and how they interact with related signaling pathways in the adult intestinal epithelium.

Central Transcription Factors Involved in Proliferating and Differentiated IECs

Binding of transcription factors to specific DNA target sequences is the fundamental basis of gene regulatory activity. Basal transcription from a core promoter in the presence or absence of TATA box is generally very low, but it can be increased by the binding of site-specific factors to regulatory DNA elements, which stabilize the interaction of the general factors at the core promoter and/or recruit coactivators (92). Thus the regulation of these site-specific transcription factors, which number has been estimated to be 1,400 (34), is essential to adjust gene expression according to the needs of the cell. In many genes, an enhancer at some distance from the proximal promoter contains binding sites for transcription factors localized far from the transcriptional start site and repress transcription by blocking the general transcriptional machinery (60).

Controlling onset of intestinal differentiation is orchestrated by specific transcription factors or transcriptional regulatory networks; genes that are involved in differentiation are upregulated whereas those necessary for proliferation are downregulated (91). Furthermore, differentiation and polarization of epithelial cells is highly dependent on the relation between epithelial cells and extracellular matrix or stromal cells and reorganization of the cytoskeleton and correct vesicle trafficking (4, 42).

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The human colonic adenocarcinoma cell line Caco-2 is the only intestinal cell line that has been reported to spontaneously differentiate in vitro. Therefore, Caco-2 cells are a widely used in vitro model in the investigation of differentiation of IEC. Moreover, it resembles many of the migrating IECs in phenotypic changes and characteristics, including spontaneous contact inhibition-dependent cell cycle arrest and differentiation into the enterocyte-like cell lineage as a function of time in culture (35). One of the most investigated transcription factor involved in intestinal differentiation is CDX2, and a substantial number of those in vitro studies have been performed in Caco-2 cells.

**CDX2.** CDX2 is a member of the mammalian homeobox gene family related to the *Drosophila melanogaster caudal* gene. CDX2 is very important in differentiation of intestinal epithelium and is specifically expressed in epithelial cells lining the small and large intestine in human adults. CDX2 has the highest expression level at the distal small intestine and proximal colon. The CDX2 expression is increasing from the base of crypt toward the surface epithelium in colon (86).

CDX2 expression is regulated by an autoregulated loop via binding to the TATA box and another AT-rich motif in the promoter (106). It has recently been found that a distal enhancer region is contributing to the autoregulation with a higher impact than promoter binding site alone (Fig. 3) (19). Moreover, the transcriptional activity of CDX2 is negatively regulated by phosphorylation at Ser60 via the mitogen-activated protein kinase (MAPK) pathway in proliferating cells. Nonphosphorylated Ser60 is predominantly found in differentiated epithelial cells and exhibits an elevated transactivating capacity (81).

From earliest studies of CDX2, it has been suggested that CDX2 is a main player to modulate cellular cell proliferation and differentiation (90). Since then the role of CDX2 in regulating differentiation-specific genes has been elucidated in several studies (32, 48, 89, 94). As Cdx2-null embryos die before gastrulation (26) transgenic mice models use conditional intestine-specific inactivation of the Cdx2 gene to investigate its role in the development of the intestinal epithelium (27, 37, 103). These in vivo studies have shown that Cdx2 has a major impact on villus morphology and cytodifferentiation of the intestinal cells. Colonocytes differentiate into a gastric rather than into an intestinal phenotype by conditional knock-out of Cdx2 (38).

CDX2 acts in cooperative manner with other transcription factors and CDX2 regulates among other genes the presumptive morphogenetic regulator liver intestine-cadherin (LI-cadherin), a member of the MAPK superfamily, MOK, and ELMO3 (2, 25, 48, 96). It has been shown that CDX2 in interaction with HNF4α and GATA-4 regulate the claudin-2 (CLDN2), sucrase-isomaltase, and lactase-phlorizin hydrolase (LPH) expression (17, 69). Thus in the mature intestine CDX2 regulates several intestine-specific gene promoters and is responsible for expression of the digestive and absorptive proteins (32, 45, 89, 94).

From recent ChIP-Seq studies it has been shown that CDX2 binding on target genes are significantly elevated in differentiated compared with proliferating Caco-2 cells and interestingly, more than half of the CDX2 occupancy regions are located in enhancer regions (20, 105). Some of the CDX2 target genes that are upregulated during differentiation are cyclin-dependent kinase inhibitor 2D (CDKN2D) and lectin, galactosidase-binding soluble 8 (LGALS8), genes that are responsible for inhibition of cell cycle progression (20). The genes that are downregulated in differentiated Caco-2 cells mainly have functions in the Wnt/β-catenin signaling pathway (67).

HNF4α. HNF4α is another main transcription factor involved in intestinal differentiation. HNF4α is also expressed in liver, pancreas, and kidney. In the intestine, the highest expression is found in the jejunum (59). Along the crypt-villus axis, HNF4α is expressed in the upper two-thirds of the crypt cells and is equally expressed toward the top of villi (Fig. 2) (88). HNF4α has been found in nine isoforms generated by alternate promoter usage, alternate splicing, and differential use of polyadenylation sites (43).

Acetylation of HNF4α by the acetyltransferase activity of CREB binding protein (CBP) has been shown to be necessary for HNF4α-mediated transcriptional activity. Acetylated HNF4α enhances the formation of an activator-coactivator complex by stronger binding to CBP itself (87). On the contrary, phosphorylation by protein kinase A or AMP-activated protein kinase can suppress the HNF4α activity (49, 110).

The role of HNF4α in intestinal development and during intestinal epithelial differentiation in mature gut has also been investigated in several mice models. In embryonic mice, Hnf4α plays a major part during the visceral endoderm formation (54, 71). Disruption of the Hnf4α gene leads to a lethal phenotype or high mortality rate at the eighth week of life (23, 46). Using conditional intestine-specific inactivation of the Hnf4α gene to overcome lethality has demonstrated an increased proliferation of epithelial cells in crypts located in the mature small intestine (21). Crypt formation in colon is also disrupted and reduced goblet cell maturation is observed. However, different features of colon development occur normally, (39) and HNF4α is dispensable for cellular po-

![Fig. 3. Model of the transcriptional regulatory network in differentiated intestinal epithelial cells consisting of CDX2, HNF4α, and HNF1α.](http://ajpgi.physiology.org/)

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larization and microvilli formation after cytodifferentiation has occurred (5).

In the mature intestine, HNF4α has a central role in regulation of genes that are involved in lipid metabolism (88). Furthermore, HNF4α controls the expression of genes involved in the intestinal gluconeogenesis (40, 88). More specifically, selective markers of differentiated enterocytes in the small intestine such as LPH, apolipoprotein CIII, apolipoprotein IV, and intestinal alkaline phosphatase (IAP) are regulated by HNF4α (3, 61, 75, 88). ChIP-chip data have revealed that several important genes encoding digestive brush border enzymes like peptidases and disaccharidases, e.g., aminopeptidase N and trehalase also are bound by HNF4α (18). The number of expressed genes containing HNF4α-binding sites increases along the crypt-villus axis (88). In continuation, ~11% of the promoters of genes upregulated during differentiation are bound by HNF4α (18), which is in agreement with the observation that HNF4α expression is increased in differentiated Caco-2 cells compared with proliferating cells (105). Moreover, structural genes essential for a polarized cell type encoding tight junction proteins cingulin (CGN) and CLDN2 are potential HNF4α target genes (18). Finally, Hnf4α induces microvilli formation in mouse embryonic carcinoma cells (24), and it has been reported that HNF4α forms a link between cell-cell adhesion and genes that are involved in differentiation (78). Altogether this indicates that HNF4α is an important regulator of intestinal differentiation.

**HNF1 and GATA transcription factors.** Two other important transcription factor families for intestinal homeostasis are HNF1s and GATAs. Hnf1α and Hnf1β are essential to maintain IEC growth and cell lineage differentiation in adult mice (64). HNF1α and HNF1β can form homo- or heterodimers and the isoforms bind to same DNA sequences, indicating that these two transcription factors can complement each other (28). Hnf1α is expressed throughout the intestine in the adult mouse, and, whereas HNF1α protein is in abundance in villus enterocytes, a lower expression is observed in the crypt (84). Whereas conditional knockout of either Hnf1α or Hnf1β in the intestine of mice leads to minor changes of intestinal phenotypes, double-mutant mice die owing to a defect in water absorption (28).

GATA proteins are transcription factors that also are involved in regulation of proliferation, differentiation, and gene expression in multiple organs (70). In the adult mouse, Gata-4 protein is expressed primarily in the proximal regions of the gut, such as the jejunum and ileum, with very low expression in the colon. It is mainly expressed in villus intestinal cells (17). Gata-5 expression is confined to the small intestine, particularly the distal segments, and is not detected in the cecum or colon. The Gata-5 expression is thought to oppose the Gata-4 level in the longitudinal axis. Gata-6 is expressed throughout the intestine, including the colon, and it is located in both the crypt and villus (33). A conditional inactivation of Gata-4 or Gata-6 in mice reveals that these Gatas are responsible for maintenance of villus height and epithelial cell number in the small intestine (13). GATAs have furthermore been shown to interact with HNF1α to control expression of the intestinal differentiation marker LPH (101). Regulation of other differentiation markers such as IAP and intestinal fatty acid binding protein are dependent on cooperation between GATA-4 and the TGF-β pathway (10).

**TCF4.** TCF4 (TCF7L2) is the most prominent transcription factor expressed of the TCF family members in the intestine (6). TCF4 is the key transcription factor that mediates the effect of Wnt signaling by forming a complex with nuclear β-catenin. This complex regulates expression of several proliferation markers such as c-Myc (107, 108). Constitutive activation of the TCF4/β-catenin complex as a consequence of mutated APC causes initiation of polypl formation in colon (56). A cross talk between Wnt/β-catenin and JNK pathways is involved in intestinal homeostasis and tumorigenesis since c-Jun is able to regulate TCF4 expression and vice versa (82). Tcf4 knockout mice leads to depletion of the proliferative compartment in the crypt and Tcf4 

**Identification of Transcriptional Regulatory Networks**

**Differentiated IECs.** CDX2, HNF4α, HNF1α, GATAs, and TCF4 interact at different target genes and signaling pathways to tightly control intestinal epithelial differentiation. Interaction between CDX2, HNF1α, and GATA-4 is considered to be responsible for expression of intestinal differentiation markers and to drive enterocytic differentiation (12, 17, 69, 93). ChIP-Seq data have revealed additional insight of CDX2 interactions with other transcription factors, and CDX2 exhibits a dynamic mechanism throughout the proliferation and differentiation state (103). Near CDX2-occupied regions in differentiated Caco-2 cells HNF4α binds about double as many sites compared with CDX2 sites in proliferating cells. Binding sites are primarily highly conserved and far away from promoter regions (103). ChIP-chip data from Caco-2 and LS174t cells, which possess a similar TCF4 dependent transcriptional pattern as seen in the proliferative compartment of intestinal crypts (99), have revealed that, within 100 bp of CDX2 occupied regions, GATA and HNF4α are potential cofactors (102). This supports the above mentioned CDX2 cooccupancy thesis in differentiated Caco-2 cells.

ChIP-seq and ChIP-chip analyses have shown that CDX2 and HNF4α are part of a transcriptional regulatory network in the differentiated IECs (Fig. 3). Among these transcription factors, only CDX2 is strictly intestinally expressed and is thus a central node in the regulatory network. An important discovery revealed by ChIP-Seq analyses is that CDX2 regulates many of the other transcription factors involved in intestinal differentiation. HNF4α gene contains two 3’ end enhancer CDX2 binding sites and a CDX2 binding site in the promoter (Fig. 3) (38). In reporter gene assays, CDX2 regulates HNF4α transcription mainly through binding to the enhancer sites in Caco-2 cells (19). Likewise, the CDX2 ChIP-Seq data supported by data from Cdx2 gene ablation in mice reveal that
CDX2 can bind and regulate both HNF1α and β expressions (19, 38). Previously, it has been shown that CDX2 activation of HNF4α and HNF1α expression occurs in cocultured mesenchymal and IEC cells to initiate differentiation (63).

HNF4α is also an essential player in the intestinal transcriptional regulatory network. In vitro assays have shown that HNF4α activates HNF1α promoter (63). ChIP-chip analysis has further substantiated this finding providing in vivo data that HNF4α simultaneously activates HNF1α and CDX2 through binding to their promoter regions (Fig. 3) (18). Furthermore, an upstream cluster of regulatory binding sites by HNF4α, GATA-6, TCF4, and β-catenin located 8.5 kb from transcription start site of CDX2 is capable of activating CDX2 expression in nonintestinal HeLa cells when these factors are overexpressed (11). In addition, ChIP analyses and expression data on intestinal epithelium from mice have shown that Hnf1 regulates Cdx2 expression through binding to an enhancer region 10 kb upstream of the Cdx2 gene (Fig. 3) (28). ChIP-chip data from hepatic cells indicate that Hnf1α also regulates Hnf4α and furthermore controls its own expression in an autoregulatory loop (73, 74). Although this has not been investigated it is probably also the case in IECs.

In differentiated Caco-2 cells a downregulation of expression is most notably observed for genes with functions in the Wnt/β-catenin signaling pathway (65). There are indications that CDX2 and HNF4α are both directly and indirectly involved in this gene regulation. CDX2 and HNF4α are capable of decreasing Wnt/β-catenin driven transcriptional activity by interactions between CDX2 or HNF4α and TCF4 or β-catenin (19, 21, 41, 50), which is in line with significantly downregulated c-Myc expression in Caco-2 cells (41, 102). Furthermore, CDX2 binds and regulates AXIN2, which is a part of the multiprotein complex degrading cytoplasmic β-catenin by phosphorylation. The Wnt repressor HMG-box transcription factor 1 is also a CDX2-target gene (19).

In summary, ChIP-seq and ChIP-chip have gained insight of the transcriptional regulation network controlling developmental processes in the intestinal epithelium. These analyses have revealed that CDX2 is an important activator of HNF4α and HNF1α expression. Simultaneously, HNF4α activates HNF1α and CDX2 expressions and it is likely that HNF1α is able to regulate CDX2 and HNF4α. A regulatory network between CDX2, HNF4α, and HNF1α has been establishing that initiates and maintains the differentiation of the IECs (Fig. 3).

Proliferating IECs. ChIP-Seq analysis shows that CDX2 also binds target genes in proliferative Caco-2 cells (102, 103). Interestingly, many of the CDX2 target genes in the proliferative cells are not bound by CDX2 in differentiated cells and vice versa. There is an overrepresentation of GATA bindings sites located in close proximity to the CDX2-occupied regions in the proliferating cells (102). ChIP analysis has shown that many of these sites bound by GATA-6 in the proliferative cells (103). GATA-6 is mainly expressed in the proliferative cells and is downregulated during differentiation (103). GATA-6 is thus regarded to be a factor involved in cell division in the crypt of the small intestine. Hence it is reasonable that GATA-6 and CDX2 are colocated at target genes involved in proliferation in the crypt.

Moreover, colocalization between CDX2, HNF1, TCF4, and FOXA1 (HNF3β) on CDX2-target genes has been reported in proliferative Caco-2 cells (102). As described earlier, the interaction between CDX2 and HNF1 is well characterized, but it is new knowledge that TCF4 or FOXA3 interact with CDX2 to regulate intestinal gene expression during proliferation. Surprisingly, approximately one-third of all CDX2-interacting regions also contain TCF4 motifs in proliferating cells, suggesting that these transcription factors bind the same regulatory regions during proliferation (102). In some incidences, CDX2 is vital for TCF4 binding and cooccupancy of CDX2 and TCF4 is better associated with gene expression than binding by either factor alone (102). Interestingly, TCF4 exhibits autoregulation of its own expression, and the transcription factors involved in differentiation like HNF4α and HNF1α promoters contain a TCF4 motif (44). However, it is noteworthy that far from all TCF4 binding regions display direct regulatory activity on its target genes. Taking into account that CDX2 expression is enhanced by binding of β-catenin and TCF4 in a distal regulatory element (11), this indicates that TCF4 might have influence on the CDX2, HNF4α, and HNF1α transcriptional regulatory network.

In conclusion, a hypothesis based on the presented ChIP data explaining how proliferating IECs switch toward a differentiated phenotype could be that TCF4 enhances CDX2, HNF4α, and HNF1α expression in the proliferative cells resulting in initiation of the intestinal differentiation process. As a consequence of activation of the transcriptional regulatory network of CDX2, HNF4α, and HNF1α, the differentiated cells are maintained supported by a downregulated Wnt/β-catenin signaling activity mediated by CDX2 and HNF4α.

New Approaches

ChIP-Seq is an efficient technology to discover specific protein-DNA interactions. However, some challenges are associated with this technique. Regulation of gene expression can be controlled by regulatory DNA elements localized in chromatin loops or in interchromatin compartment (26). ChIP-Seq cannot immediately reveal such an interaction, e.g., as seen in regulation of intestinal lactase activity by an enhancer region (95). To investigate regulatory elements in chromatin loops chromosome conformation capture (3C) technology provides new insight how these DNA elements in native cellular state binds directly to distant target genes (30). Gene expression of the transcription factor c-Myc in the intestine has been explored by a comprehensive 3C-based study in which at least three novel distal enhancer elements or Wnt-responsive elements control the gene expression (107, 108). The 3C-carbon copied methods, 4C and 5C, are suitable in large-scale mapping of chromatin interactions compared with the original 3C, because of the inclusion of microarrays or sequencing (31).

The newly developed chromatin interaction analysis with paired-end tag sequencing (ChIA-PET), where high-throughput sequencing also is included, is able to reveal whether a given DNA-protein interaction localized in intra- or interchromosomal regions regulates gene expression (36, 62). This method can potentially reveal new unbiased genomewide chromatin interactions. To our knowledge there are only few or no studies describing the intestinal transcriptional regulatory network on the basis of 3C and ChIA-PET methods and therefore it is obvious that these techniques can
support and expand the knowledge of gene regulation within a regulatory network. However, all 3C-based technologies detect physical DNA-protein interactions. Additional experiments are required to demonstrate whether such interactions are actually functional.

Alternative splicing and alternative promoters are two of the most drastic ways cells can modify the function of genes resulting in different functional properties of the final protein. Therefore, just knowing that a certain gene is active is not enough; it is also required to know what form of the gene is present. Pre-mRNA splicing is the biochemical process by which introns are removed from the primary transcript to produce functional mRNA. Studies have shown that essentially all human genes (90–95%) generate several mRNAs as a result of alternative splicing (76, 104, 105) and skipping of exons can produce mRNAs lacking functional features (80). These alternative splice forms can be assessed by the genomewide RNA-Seq technique (104), which detects transcribed exons. Apart from splicing, the cell can produce gene isoforms by starting transcription at different places: alternative promoter usage. Recent studies using the novel cap analysis of gene expression (CAGE) technique have shown that most genes have two or more alternative promoters (20, 83, 98). Thus to get a complete understanding of the transcriptional network and of the functional outcome it is important to include the analysis of the actual isoforms produced in the IEC.

Conclusion

The current knowledge hints at a very complicated transcriptional network regulating the proliferation, migration, and differentiation of the IEC. The novel techniques described here are genomewide, thus enabling the research field to get the big overview. The result of these genomewide methods is generating amounts of data, and the interpretation of these not only relies on biologists but depends heavily on the use of skilled bioinformatics. The ultimate goal of the investigation of the transcriptional regulatory network controlling proliferation and differentiation in the IECs is to obtain the information required to perform targeted actions toward specific signaling pathways. Detailed information about the transcriptional regulatory network can contribute to identify potential prognostic markers for the disease cells. Furthermore, it can be analyzed to understand what makes the diseased cell dysfunctional: what parts of the network are missing in the disease-specific variant and what impact does it have to obtain a better treatment of intestinal disorders.

REFERENCES

Review

INTESTINAL TRANSCRIPTION NETWORKS


