Epithelial Cell Growth and Differentiation

IV. Controlled spatiotemporal expression of transgenes: new tools to study normal and pathological states

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Robine, Sylvie, Frédéric Jaisser, and Daniel Louvard. Epithelial Cell Growth and Differentiation. IV. Controlled spatiotemporal expression of transgenes: new tools to study normal and pathological states. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G759–G762, 1997.—The gut epithelium represents a dynamic, well-organized developmental system for examining self-renewal, differentiation, repair, and tumorigenesis. The apical pole of the enterocytes, the brush border, is composed of an array of well-organized actin microfilaments that support the plasma membrane. Villin, one actin-binding protein that contributes to the assembly and dynamics of the microvillus bundle, exhibits special features such as restricted tissue specificity and early expression in the immature crypt cells. The regulatory elements of the villin gene are suitable to control the expression of transgenes in intestinal cells. Engineering genetically modified animals by classic transgenesis using the villin promoter or by gene targeting in the villin locus will allow the establishment of animal models that may recapitulate human intestinal disorders.

signal transduction; epithelium; brush-border membrane; villin gene

The formation of a selective barrier between two compartments is a feature common to all epithelia and requires the generation and maintenance of specialized domains of the plasma membrane. The cortical actin cytoskeleton, membrane trafficking, and signal transduction are all involved in the establishment of cell polarity. A major interest in the field of cell polarity is to understand how these processes are integrated at the cell and tissue levels and consequently how they contribute to both normal epithelial cell function and epithelial cell disease states. Recent developments in mouse genetics have opened new possibilities to bridge molecular cell biology with physiology and so work toward a molecular understanding of pathophysiological disorders.

The gut epithelium represents a dynamic, geographically well-organized developmental system for examining self-renewal, differentiation, repair, and tumorigenesis of the intestinal mucosa. This epithelium is composed of a layer of cuboidal cells that are able to establish regulatory interactions with the mesodermal layer through the basement membrane. Its crypt-villus unit provides a unique model for studying many processes that occur during tissue morphogenesis, such as control of proliferative status, specification of cell fate, regulation of differentiation, and induction of cell death. Several genes implicated in those functions have been widely investigated in recent years (10, 17, 19).

In contact with the intestinal lumen, enterocytes have an apical pole composed of a highly specialized domain made of numerous microvilli, the brush border. This domain is often considered to be a polarized epithelium-specific "organelle" specialized for luminal absorption. However, the molecular mechanisms involved in the induction of brush-border assembly remain poorly understood.

Over the last two decades, research on brush-border biogenesis has been driven by the characterization of gene products that could play a specific and important role in its morphogenesis. This has led several laboratories to focus their interest on the microfilaments supporting the microvillus plasma membrane (3, 15). It is now well established that four major actin-binding proteins contribute to the assembly and the dynamics of the microvillus bundles of actin (ezrin, fimbrin, brush-border myosin 1, and villin) (1, 2, 6, 7). Among these well-characterized proteins, villin exhibits special features that suggest the villin gene has a suitable locus to control the expression of transgenes in carefully designed animal models that may recapitulate human intestinal disorders.

The villin gene has a restricted profile of tissue expression. In embryonic and in adult normal or neoplastic tissues, villin is only produced by differentiated and immature epithelial cells of the intestinal mucosa and the kidney proximal tubules (16). A single villin gene in the mouse or human genome encodes for a unique protein highly conserved throughout evolution (17). Biochemical studies have shown that villin is a member of a subfamily of actin-bundling proteins (2). It is worthwhile to stress that the ability of villin to organize actin microfilaments and to stimulate micro-
villi assembly is strictly dependent on a unique amino acid sequence not found in other proteins of the same subfamily (8).

These structural and functional characteristics have been at the origin of our effort to disrupt the villin gene in mice. Contrary to our expectations derived from previous work with in vitro models (5, 8), villin null mice could be developed and raised for months in the standard environment of a research animal house. Moreover, histological and ultrastructural studies of the intestinal mucosa revealed a conspicuously well-organized brush border. These observations suggest an ability to compensate for the lack of villin during the assembly of the brush border, potentially either via redundancy and/or substitution by analogous proteins (E. Ferrary, unpublished observations). However, there may be other essential functions of the villin gene in vivo waiting to be discovered. For instance, the effects of harsh nutritional conditions or the result of infections by pathogenic microorganisms on the physiology of animals unable to produce villin remain to be investigated.

Laboratories throughout the world are generating an increasing number of knockout mice and, with increasing frequency, facing difficulties in assessing the function(s) of genes using this experimental approach. The challenge for investigators is to discover experimental conditions that reveal the function(s) of the gene under investigation.

Our observations with the villin knockout mice, which did not develop obvious intestinal and kidney disorders, demonstrated that the disruption of a single villin allele should be harmless. Indeed, we have been able to show that villin heterozygote mice [vil(+/−)] produce almost normal levels of villin. Because only a single functional villin allele was required to sustain normal function, we were able to initiate new strategies aimed at driving the expression of genes in the intestinal mucosa by taking advantage of the endogenous villin promoter.

A few genes and their regulatory sequences primarily or exclusively expressed in the intestinal mucosa have been characterized (10, 19). The pioneering work of Dr. J. Gordon and his colleagues on the Fabp gene has unequivocally demonstrated the power of transgenic animal approaches to unravel numerous features of the development, differentiation, and transduction occurring in the gastrointestinal tract (18).

As observed for other genes, the regulatory sequences of the villin mouse gene have proven to be very dispersed and distant from the 5’ initiation site (D. Pinto, unpublished results). Hence, the use of this tissue-specific promoter to target the expression of exogenous genes using standard transgenic methods...
Homologous recombination provides a major advantage over a random insertion in the genome of the transgene of interest. The complete endogenous set of regulatory cues controlling the expression of the chosen gene can be exploited. In addition, this strategy prevents ectopic expression of the transgene. If the intact allele of the targeted gene can sustain its natural function, no additional effect should be expected. This is in sharp contrast to the uncontrolled random insertion at an unidentified locus. The major drawback of the homologous recombination approach is its low efficiency and the laborious work involved to generate mouse strains expressing the transgene in the germinal lineage. To overcome these difficulties, we have designed a novel gene-targeting strategy applied to mouse embryonic stem cells (ES cells). This method is based on the induction of a double-strand break (DSB) in the gene to be mutated (12). To induce the DSB, an I-Sce I yeast meganuclease restriction site is first introduced by gene targeting to the villin gene (Fig. 1) (4, 11). This strategy has been successfully used to first target a reporter gene, i.e., nls-LacZ in the murine villin locus. Thus, in these mice, the expression of nls-LacZ is now under the control of the complete regulatory elements of the natural endogenous villin promoter. Analysis of chimeric animals clearly shows that LacZ expression reproduces the full villin expression pattern (Fig. 2). The combination of the I-Sce I strategy associated with reconstitution of a functional resistance gene has been used and resulted in 100% gene replacement fidelity (M. Cohen-Tannoudji, unpublished results). This strategy should allow us to achieve a locus-targeted introduction (knock-in) of mutated genes of interest or heterologous genes, leading to gain or loss of function in the intestinal mucosa. Indeed, this protocol should also be applicable to other tissue-specific genes, providing that their mouse 5′ and 3′ sequences are available. Furthermore, temporal and tissue-specific expression of the gene can be achieved by combining the above-described gene-targeting approach with the tetracycline-dependent regulatory systems (tet systems) developed in Dr. H. Bujard’s laboratory. These expression systems permit investigators to stringently control gene expression over a wide range in cell culture as well as in transgenic organisms (9, 13). A hallmark of the tet systems is the tightness of control as well as the potential to regulate a gene activity in a tissue-specific manner when combined with tissue-specific promoter sequences. Moreover, the use of the Cre recombinase system in vivo for conditional knock out has been successfully applied together with the inducible tet system, leading to spatiotemporal control of the transgene (14).

Using the villin gene or other tissue-specific promoter sequences and this combined replacement strategy, a variety of gene products might be delivered with high efficiency as well as spatiotemporal control in the intestinal epithelium of transgenic mice. Different projects can be undertaken, such as epithelial cell immortalization, colorectal carcinogenesis models, or analysis of wild-type or modified protein expression. Because the villin gene is expressed in the stem cells of the crypt epithelium, the targeting of an immortalizing gene might result in the isolation of a cell line that
should recapitulate the complete profile of intestinal differentiation. The targeting of the various oncogenes implicated in colorectal carcinogenesis should promote tumorigenesis when expressed under the villin control in the crypt cell-proliferating region. This approach may also be a powerful tool to target a wild-type, mutated, or truncated gene in the intestine to evaluate the role of such a protein. For instance, several laboratories have attempted to develop an animal model for cystic fibrosis by inactivation of the cystic fibrosis transmembrane conductance regulator (cfr) gene. However, these animals died early after birth due to intestinal obstruction. One way to overcome this problem is to target the expression of wild-type CFTR in the intestine of cfr null mice. This approach can be very useful for delivering a variety of gene products in the villin-expressing cells of transgenic animals, allowing complementary molecular studies using both in vivo models and in vitro cell culture models.

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