# Notch and Wnt signals cooperatively control cell proliferation and tumorigenesis in the intestine

Silvia Fre[a], S. K. Pallavi[b], Mathilde Huyghe[a], Marick Laé[c], Klaus-Peter Janssen[d], Sylvie Robine[a], Spyros Artavanis-Tsakonas[b,e,1], and Daniel Louvard[a]

[a]Morphogenesis and Intracellular Signaling, Institut Curie–Unité Mixte de Recherche 144 Centre National de la Recherche Scientifique, 75248 Paris Cedex 05, France; [b]Department of Cell Biology, Harvard Medical School, Boston, MA 02115; [c]Department of Pathology and [e]Collège de France and Department of Developmental Biology and Genetics, Institut Curie, 75248 Paris Cedex 05, France; and [d]Department of Surgery, Technische Universitaet Muenchen, 81675 Muenchen, Germany

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**Notch and Wnt signals play essential roles in intestinal development and homeostasis, yet how they integrate their action to affect intestinal morphogenesis is not understood. We examined the interplay between these two signaling pathways in vivo, by modulating Notch activity in mice carrying either a loss- or a gain-of-function mutation of Wnt signaling. We find that the dramatic proliferative effect that Notch signals have on early intestinal precursors requires normal Wnt signaling, whereas its influence on intestinal differentiation appears independent of Wnt. Analogous experiments in *Drosophila* demonstrate that the synergistic effects of Notch and Wnt are valid across species. We also demonstrate a striking synergy between Notch and Wnt signals that results in inducing the formation of intestinal adenomas, particularly in the colon, a region rarely affected in available mouse tumor models, but the primary target organ in human patients. These studies thus reveal a previously unknown oncogenic potential of Notch signaling in colorectal tumorigenesis that, significantly, is supported by the analysis of human tumors. Importantly, our experimental evidence raises the possibility that Notch activation might be an essential initial event triggering colorectal cancer.**

colorectal cancer | Hes1

The intestinal epithelium defines a paradigm of rapid tissue renewal from a source of multipotent stem cells and is composed of fields of proliferating and differentiating cell populations that are spatially separated. Early precursors are generated continuously in the crypts of Lieberkühn, from where they begin an apical migration without ceasing to divide. Upon reaching the crypt–villus border, they exit the cell cycle and differentiate, giving rise to the enterocyte, goblet, enteroendocrine, or Paneth cell population (1). The morphogenetic process along the crypt–villus axis and the acquisition of particular cell fates are controlled by the interplay between distinct signaling pathways. How cell signals coordinate their action to organize the crypt–villus architecture and control the patterning and renewal of the gut epithelium remains unclear.

The Wnt and Notch signaling pathways have been shown to play a major role in intestinal morphogenesis and homeostasis (2–6). Blocking Wnt signaling abolishes cell proliferation in the prospective crypt regions of the fetal small intestine (7–9) and influences differentiation events. Modulation of Notch signals has also profound effects on intestinal development. Inhibition of Notch signals results in the arrest of crypt cell proliferation and guides all crypt cells into a goblet cell fate (5). Conversely, constitutive activation of Notch signals in the developing intestine leads to an increased number of dividing cells and, concomitantly, to a dramatic impairment of differentiation of all intestinal cell types (6). Accurate coordination of Notch and Wnt signals is essential in normal development, and, consequently, it may play an important role in intestinal tumorigenesis (10). We explore the integration of Wnt and Notch signals in the mouse intestine and uncover synergistic effects that have profound consequences in controlling proliferation of intestinal precursors, as well as in promoting tumorigenesis in this organ. Using *Drosophila*, we demonstrate that the synergistic effects uncovered in the mouse intestine are conserved across species. Importantly, the analysis of human tumor samples shows that our findings are relevant to human intestinal tumorigenesis.

## Results

**Notch Activation in the Developing Intestine Cannot Restore Proliferation in Tcf4 Knockout Mice.** To assess whether and how the documented proliferative action of Notch in the intestine (5, 6) is affected by Wnt signal modulation, we expressed a constitutively active form of the mouse Notch1 receptor in the intestinal epithelium (vilCre/Nic) (6) in a Tcf4-null genetic background (2). Lack of Tcf4 function, the major effector of Wnt signals in the mouse intestine, results in perinatal lethality, and Tcf4 knockout mice display a complete absence of proliferative cells in the prospective crypt regions of the small intestine (2). On the contrary, activation of the Notch pathway through the villin promoter (vilCre/Nic mice) dramatically increases the number of dividing cells and impairs their differentiation, leading also to lethality at birth (6). However, when we activate Notch in an intestine where Wnt signaling is blocked, as is the case in the compound mutant mice vilCre/Nic;Tcf4$^{-/-}$, expansion of the proliferative compartment is not observed. These animals die at birth, lacking any dividing cells in the prospective crypt regions, a phenotype undistinguishable from that reported for the Tcf4 knockout mice (2) (Fig. 1 *A–D*). Confirming that Notch signaling is activated in the compound vilCre/Nic;Tcf4$^{-/-}$ mice, we find the canonical Notch target Hes1 to be ectopically expressed in the entire intestinal epithelium (Fig. 1*H*). We conclude that the intestinal proliferation phenotype induced by activation of the Notch receptor requires Tcf4, indicating that only cells in which the Wnt cascade is intact are "competent" to respond to the Notch-dependent mitogenic stimulus.

**Notch Controls Cell Differentiation in the Developing Intestine Independently of Tcf4.** We established that Notch pathway activation results in a complete absence of goblet cells (6), whereas intestines lacking Tcf4 are known to retain a normal goblet cell number (2). To establish the impact of Notch and Wnt signal integration on the differentiation of intestinal cell types, we examined the intestine of compound transgenic animals (vilCre/Nic;Tcf4$^{-/-}$) and found it to be completely depleted of goblet cells. Significantly, this phenotype is indistinguishable from that associated with vilCre/Nic mice (Fig.

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[1]To whom correspondence should be addressed at: Department of Cell Biology, Harvard Medical School, 240 Longwood Avenue, Boston, MA 02115. E-mail: artavanis@hms.harvard.edu.

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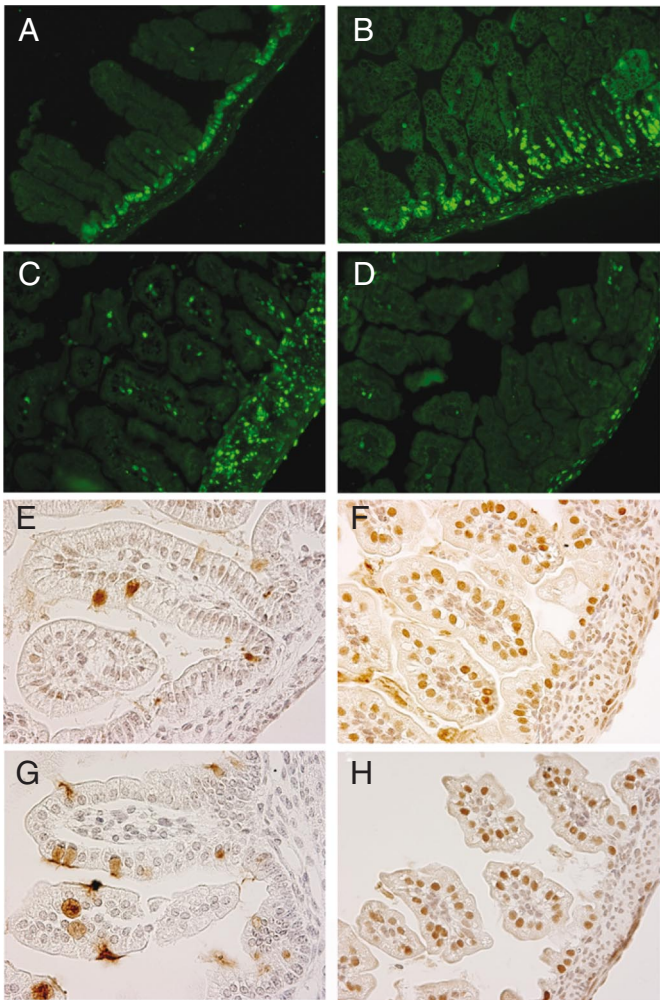


**Fig. 1.** Notch activation is not sufficient to restore proliferation in Tcf4$^{-/-}$ mice. Paraffin longitudinal sections of neonatal small intestine from wild-type (*A* and *E*), vilCre/Nic (*B* and *F*), Tcf4$^{-/-}$ (*C* and *G*) and vilCre/Nic;Tcf4$^{-/-}$ (*D* and *H*) mice stained with the proliferation marker Ki67 in *A–D* and with an antibody anti-Hes1 in *E–H*. The increase in cell proliferation induced by Notch activation (green cells in *B*) is not visible in the absence of Tcf4 (*D*). The Notch target Hes1 is strongly induced in vilCre/Nic mice and in vilCre/Nic;Tcf4$^{-/-}$ mice (brown nuclei in *F* and *H*), indicating that Notch signaling is activated in these transgenic mice. Goblet cells show nonspecific staining with this polyclonal antibody (*E* and *G*). [Scale bar, 80 $\mu$m (*A–D*) and 50 $\mu$m (*E–H*).]

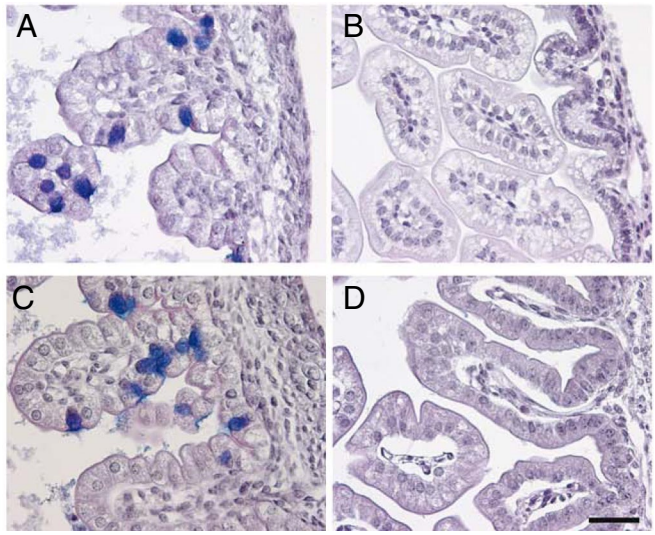


**Fig. 2.** Notch impairs goblet cell differentiation independently of Tcf4. Paraffin longitudinal sections of neonatal small intestine from wild-type (*A*), vilCre/Nic (*B*), Tcf4$^{-/-}$ (*C*), and vilCre/Nic;Tcf4$^{-/-}$ (*D*) mice stained with Alcian blue to reveal differentiated goblet cells are shown. The light blue cells evident in *A* and *C* are absent in the presence of the activated Notch transgene (*B*), even in a Tcf4$^{-/-}$ background (*D*). (Scale bar, 50 $\mu$m.)

2 *A–D*), indicating that, in the double transgenic mice, Notch activation exerts its inhibitory action on goblet cell differentiation independently of Wnt signaling.

These results indicate that the effect of Notch activation on goblet cell differentiation is independent of Wnt signals, whereas the proliferative state of the crypt cells depends on the integrated action of the Notch and the Wnt/Tcf4 cascades.

**Notch and Wnt Signals Cooperate to Trigger Intestinal Tumorigenesis.** Given the uncovered cooperation between Notch and Wnt signals in controlling intestinal cell proliferation, we were interested in examining the oncogenic potential of Notch and Wnt in the adult intestine, especially in view of the well-documented involvement of both pathways in tumorigenic events (7, 11–17).

We thus crossed the Nic transgenic mice (18) with animals expressing a tamoxifen-inducible Cre recombinase expressed under the control of the villin promoter (vilCreERT2) (19). Inducible expression of Nic in the adult intestine (vilCreERT2/Nic mice) leads to a mosaic expression of the transgene, resulting in a nonlethal, less severe phenotype than the one we obtain through constitutive expression of Nic during embryonic development (6). Notwithstanding the lower penetrance of recombination, we clearly find that Notch activation results in both blocking secretory cell differentiation [goblet cells are shown in supporting information (SI) Fig. S1 *A* and *B*] and augments crypt cell proliferation (Fig. S1*C*).

The great majority of human intestinal tumors, whether associated with hereditary syndromes or sporadic colorectal cancers, display loss of the tumor suppressor *Apc* (*adenomatous polyposis coli*) (20–22), a key negative regulator of $\beta$-catenin/Wnt signals. Consistent with this notion, mice heterozygous for a loss-of-function allele of *Apc* (23) (*Apc*$^{+/1638N}$, henceforth referred to as Apc$^{+/-}$ mice) spontaneously develop intestinal adenomas, initially detectable ≈6 months after birth, upon somatic loss of the wild-type *Apc* allele (loss of heterozygosity or LOH) (23). Activation of Notch in these mice (vilCreERT2/Nic; *Apc*$^{+/1638N}$, hereafter referred to as Notch/Apc mice), results in a remarkable increase in the number of adenomas developed, leading to lethality within 4 months after birth ($n$ = 118 Notch/Apc mice) (Fig. 3 *A–E*). In Notch/Apc mice, tumors appear much earlier than in Apc$^{+/-}$ littermates, indicating a decrease in the latency period required for the development of the adenomas. At 3 months of age, Notch/Apc mice already present a high number of intestinal tumors (>30 per intestine), which further increases to an average of ≈115 polyps per intestine in 4-month-old animals, in contrast to no lesions found in Apc$^{+/-}$ control mice at the same age. Histopathological analysis reveals that all polyps found in Notch/Apc mice are low-grade adenomas, closely resembling nascent polyps found in early stages of intestinal tumorigenesis. The majority of adenoma cells in Notch/Apc mice express the activated Notch transgene (Fig. 3*K*), actively proliferate (Fig. 3 *F* and *G*), and present nuclear localization of $\beta$-catenin, a hallmark of active Wnt signaling (Fig. 3 *H* and *I*).

Notably, Notch activation in the *Apc* mutant background correlates with the presence of a remarkably large number of dysplastic lesions in the colon (Fig. 3 *C* and *D*), a region where tumors are rarely found in Apc$^{+/-}$ control mice. This feature is of particular importance considering the pathogenesis of human gastrointestinal tumors, the vast majority of which occurs in the distal colon and rectum and not in the small intestine (24).

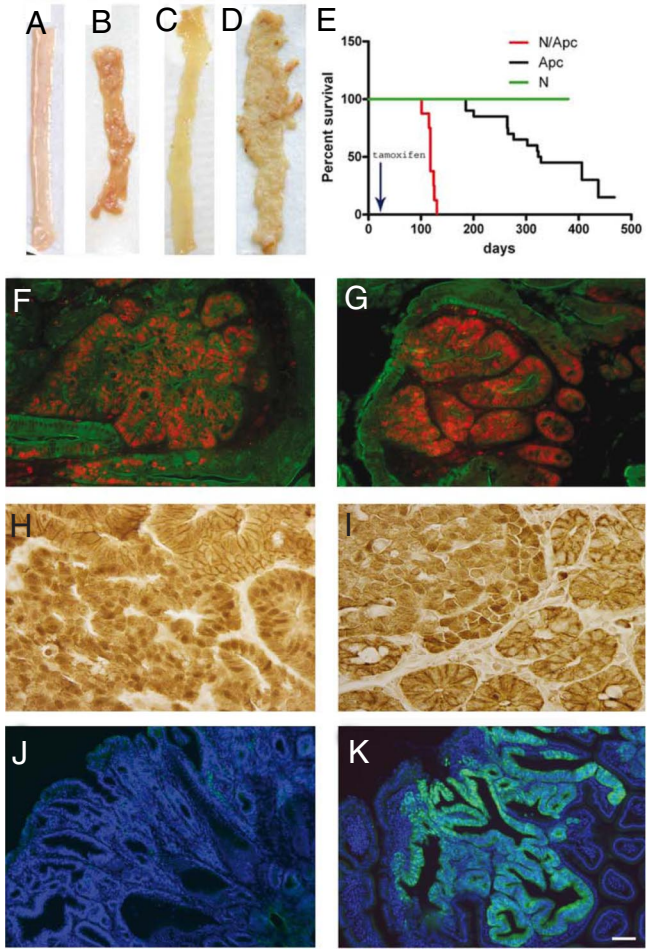


**Fig. 3.** Notch and Wnt signals show a synergistic impact on intestinal tumorigenesis. (*A–D*) Small intestine (*A* and *B*) and colon (*C* and *D*) of N/Apc mice (*B* and *D*) present a high number of macroscopically visible lesions compared with control tissues (*A* and *C*). (*E*) Kaplan–Meier survival of N/Apc mice and their single transgenic littermates over a period of 437 days. The N/Apc compound mice (in red) are all dead by 4 months, whereas the Apc single-mutant mice (in black) start dying at ≈6 months of age, and the N mice (in green) present a normal lifespan. (*F–K*) Thin sections of intestinal adenomas from $Apc^{+/-}$ (*F*, *H* and *J*) and N/Apc (*G*, *I*, and *K*) mice. (*F* and *G*) Tissues are stained with the proliferation marker Ki67 (in red) and an antibody recognizing the villin protein (in green). (*H* and *I*) Antibody identifying β-catenin reveals cytoplasmic and nuclear localization of β-catenin in both tumors, indicative of active Wnt signaling. (*J* and *K*) Anti-GFP staining reveals the presence of the Notch-ires-GFP transgene in N/Apc tumors (*K*). [Scale bar, 1 cm (*A–D*), 50 μm (*F*, *G*, *J*, and *K*), and 25 μm (*H* and *I*).]

**Notch/Wnt Cross-Talk Is Conserved Across Species.** To investigate further the Notch/Wnt–Wingless relationship uncovered in the mouse intestine and examine its possible generality, we explored the activities of these genes in the developing *Drosophila* eye. Previous studies have shown that modulation of both Notch and Wingless signals can affect proliferation in the *Drosophila* eye imaginal disc (25, 26). Ectopic expression of an activated form of the Notch receptor (Nic), a form analogous to the Nic transgene used in the mouse studies, under the early-acting *eyeless* promoter, results in a large eye phenotype in the adult fly (UAS-Nic;eyGAL4, Fig. 4*B*). Consistent with the adult phenotype, BrdU incorporation in the eye discs of UAS-Nic;eyGAL4 third-instar larvae reveals an increase in proliferation (Fig. 4*B*'), as evidenced by an obviously expanded morphogenetic furrow (compare arrowheads between Fig. 4*A*" and 4*B*") and ectopic pockets of dividing cells (indicated by an arrow in Fig. 4*B*'). Consistent with our observations in the mouse intestine, when Nic is expressed in a genetic background where Wnt signaling is inhibited through the expression of a dominant-negative form of *pangolin*, the *Drosophila* homolog of Tcf4 (27), the adult large-eye phenotype is suppressed (Fig. 4*C*), and the underlying BrdU-expression pattern in the larval disc is essentially indistinguishable from the wild type (Fig. 4*C*'). In contrast, the expression of Nic in a background-inducing constitutive Wnt activation by using two independent gain-of-function Wnt mutants (d08266, a Gal4-dependent UAS insertion in *wingless* (28, 29) (Fig. 4 *D–D*") and UAS-arm S10, an activated form of *armadillo*, the *Drosophila* homolog of β-catenin (30), displays a dramatic enhancement of the adult eye phenotype, reflected by a wrinkled and heavily distorted larval eye disc, as shown in Fig. 4 *D*' and *D*".

Importantly, these observations are confirmed when we analyze the connection between Notch and the Wnt negative regulator Apc (20). When both Wnt and Notch are activated by combining a loss-of-function allele of *Apc* (c00746, a P-element insertion in the *Apc* locus) (28, 29) and UAS-Nic;eyGAL4 (Fig. 4 *E–E*"), we observe a strong synergy, giving rise to a phenotype analogous to that resulting from coexpression of Nic and gain-of-function Wnt mutations (Fig. 4 *D–D*").

We note that staining of the discs with the marker of neuronal differentiation *elav* does not reveal obvious abnormalities in the differentiation pattern (Fig. 4 *B–E*). Even in the cases where the disc is heavily distorted, *elav*-positive cells are exclusively found posterior to the morphogenetic furrow, as is the case in a wild-type disc.

These observations are consistent with our results in the mouse intestine and support the mechanistic notion that the proliferative activity of Notch is exerted through Wnt/Wingless signals, both in the *Drosophila* eye disc and in the mouse intestine. We conclude that the synergistic interaction between Notch and Wnt signals is conserved across species barriers, suggesting that the coordinated control of Notch and Wnt on proliferation reflects a fundamental mechanism.

**Notch Signaling Is Active in Mouse and Human Intestinal Adenomas.** The conserved nature of the Notch/Wnt cross-talk that we uncovered and their synergy in the development of intestinal adenomas in Notch/Apc mice raise the possibility that this interaction may be relevant to tumor formation in humans. In considering the underlying mechanistic circuitry of this synergy, several observations warrant comment. Although, as expected, the Notch target Hes1 is up-regulated in all cells of Notch/Apc mouse adenomas (Fig. 5*D*), we also detect a strong expression of Hes1 in tumors originated in $Apc^{+/-}$ control mice (Fig. 5*C*). Quantification of the expression levels of Hes1 mRNA by real time qPCR in $Apc^{+/-}$ and N/Apc mice confirms that Hes1 is significantly up-regulated in $Apc^{+/-}$ adenomas, compared with normal intestine (Fig. 6*A*).

The analysis of Hes1 expression in colon cancer specimens from human patients shows that 12 of 15 polyps of both sporadic and hereditary [familial adenomatous polyposis or FAP (31)] low-grade adenomas present strong nuclear expression of the Notch target Hes1 ($n = 15$) (Fig. 5 *F* and *I*), whereas Hes1 is either not detectable or expressed at very low levels in human adenocarcinomas ($n = 14$) (Fig. 5 *G* and *J*). Consistently, real-time quantitative PCR (qPCR) on human adenomas and adenocarcinomas reveals that the Notch targets Hes1 (Fig. 6*B*), HeyL (Fig. 6*C*), and Hey1 are expressed at higher levels in adenomas than in carcinomas. In addition, we find a significant up-regulation of the Notch ligands Jagged1 and Jagged2 (Fig. 6*E*) in human adenomas. The expression levels of the Notch1 receptor (Fig. 6*D*) are also slightly increased in adenomas, albeit at a lesser extent than the other genes tested. These observations suggest that elevated Notch signaling in benign adenomas may contribute to the initiation of colorectal cancer.

## Discussion

How signaling pathways integrate their action to affect cell fates is a fundamental issue in developmental biology (32). Our analysis reveals a hitherto unknown synergy between Notch and

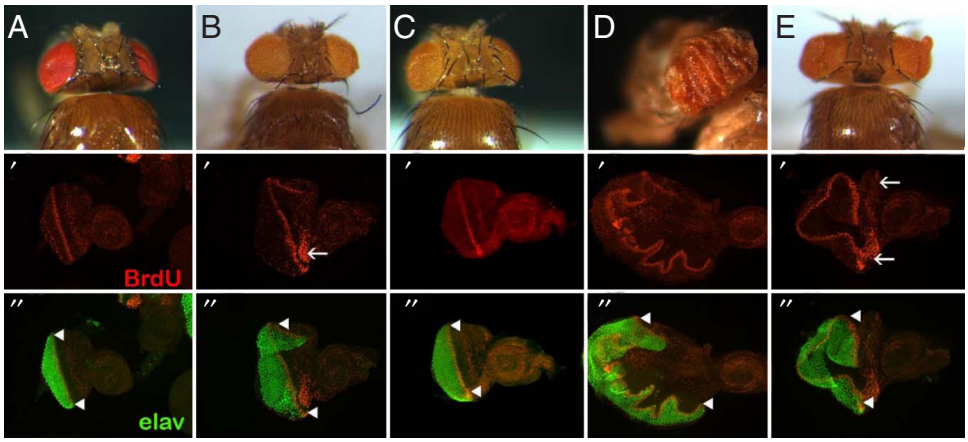


**Fig. 4.** Synergy of Notch and Wingless in *Drosophila*. Adult eyes (*Top*) and corresponding larval eye imaginal discs of *Drosophila* in different genetic backgrounds (see *Results* for detailed genotypes), stained for BrdU (in red, *Middle*) to indicate cells in S phase and elav (in green, *Bottom*) a marker of neuronal differentiation. (*B′* and *E′*) White arrows in indicate ectopic proliferation in the eye imaginal discs. (*Bottom*) White arrowheads indicate the positions of the morphogenetic furrow. (*A*–*A″*) Wild-type animals. (*B*–*B″*) Nic-expressing flies show a large-eye phenotype induced by Notch activation. Abnormal, ectopic BrdU incorporation is indicated by the arrow in *B′*, whereas neuronal differentiation is unaffected in *B″*. This phenotype is suppressed by expression of dominant-negative Pangolin, the *Drosophila* homolog of Tcf4. Flies expressing Nic and dominant-negative Pangolin present adult eye morphology, BrdU incorporation, and elav pattern of expression undistinguishable from wild-type flies (compare *A*–*A″* with *C*–*C″*). In contrast, the expression of a gain-of-function *wingless* allele in these flies dramatically enhances the Nic phenotype (compare *B*–*B″* with *D*–*D″*). (*E*–*E″*) A similar synergy is also observed in flies expressing Nic and harboring a loss-of-function mutation of the Wnt antagonist Apc.

Wnt in controlling proliferation events in the mouse intestine, which may have important implications in the initiation of intestinal tumorigenesis.

The observed synergy between Notch and Wnt could be explained by a model where the outcome depends on whether Notch activation occurs before, after, or concurrently with LOH of the *Apc* allele (Fig. 6*F*). Activation of Notch before the loss of the wt *Apc* allele (model 1), as is the case for the N/Apc mice used in this work, could lead to a localized expansion of early progenitor cells, thus increasing the chances of additional intervening mutations that would trigger tumor formation, such as LOH at the *Apc* locus. Alternatively, Notch activation may influence the proliferative potential of $Apc^{-/-}$ cells, accelerating their proliferation and the formation of adenomas (model 2). A combination of these scenarios, such as the simultaneous activation of both pathways in a given cell (model 3), would also be possible and still be consistent with our experimental results. We favor, however, the first possibility, because the number of tumors that develop in Notch/Apc mice is at least 20-fold increased compared with $Apc^{+/-}$ mice, suggesting that $Apc^{-/-}$ cells in the absence of Notch signals do not have the same potential of inducing tumor formation. Indeed, in humans we observe Notch signal activation in the majority of adenomas, suggesting that Notch signaling enhances Apc-driven tumor initiation.

In this context, it is worth mentioning that when we induce Notch activation with a lower dose of tamoxifen, therefore considerably decreasing the number of Nic-expressing cells, Notch/Apc mice can survive longer (up to 9 months of age). These mice still present a significantly higher number of adenomas than control $Apc^{+/-}$ littermates, whereas the number of intestinal adenocarcinomas is

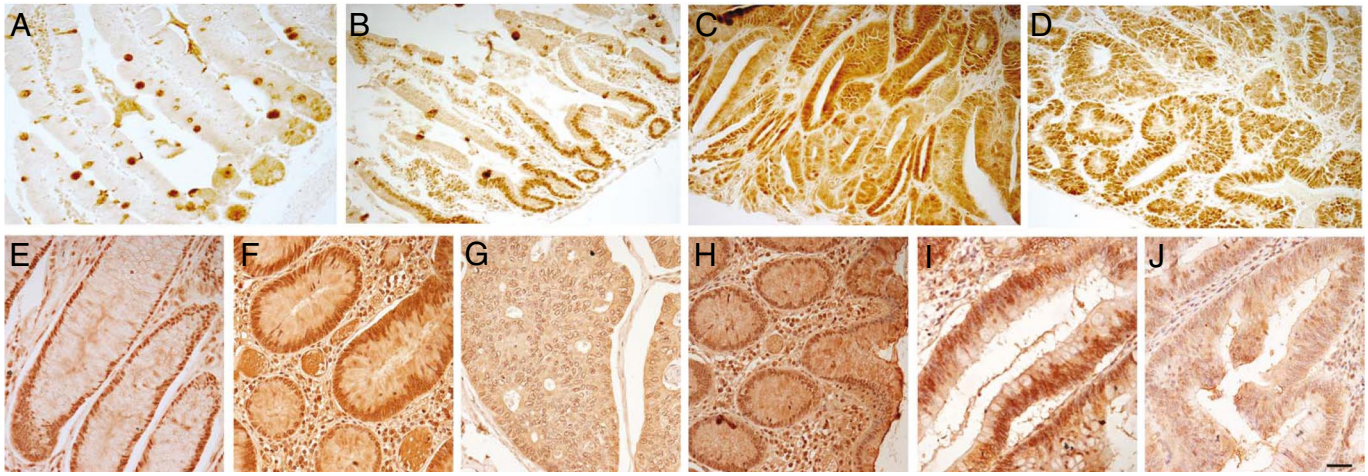


**Fig. 5.** The Notch target Hes1 is highly expressed in mouse and human adenomas but not in carcinomas. (*A*–*D*) Paraffin longitudinal sections of normal small intestine (*A* and *B*) and tumor tissues (*C* and *D*) from $Apc^{+/-}$ (*A* and *C*) and N/Apc (*B* and *D*) mice, stained with a rabbit antibody recognizing the Hes1 protein. Hes1 is found exclusively in the crypts of adult normal intestine (*A*), whereas in N/Apc mice its expression correlates with the mosaic expression of the Nic transgene (*B*). In mouse adenomas, Hes1 is strongly expressed both in N/Apc mice (*D*) and in $Apc^{+/-}$ control mice (*C*). Goblet cells present nonspecific staining with this antibody in *A*. (*E*–*J*) Paraffin representative sections of human specimens of normal colon (*E* and *H*), sporadic and FAP low-grade colon adenomas (*F* and *I*, respectively), and sporadic and FAP adenocarcinomas (*G* and *J*, respectively) stained with a rat anti-Hes1 antibody. Hes1 is readily detectable in the nuclei of both normal and dysplastic human colonic crypts (*E*, *F*, *H*, and *I*), but it is not expressed in human colon adenocarcinomas (*G* and *J*). [Scale bar, 100 $\mu$m (*A* and *B*) and 50 $\mu$m (*C*–*J*).]

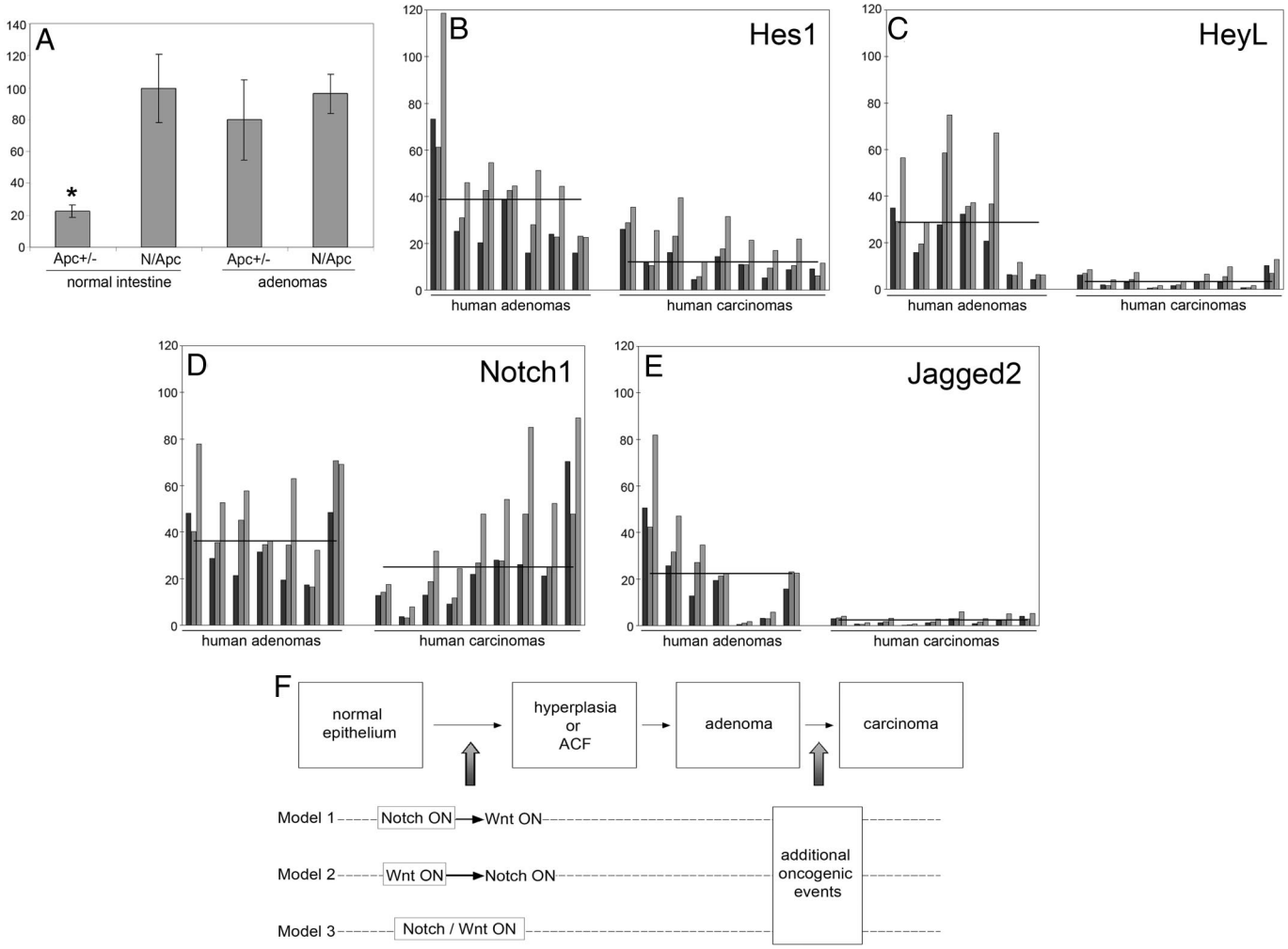


**Fig. 6.** Notch signaling is active in mouse and human tumors. (*A*) Real-time qPCR was used to quantify the expression levels of Hes1 mRNA in mouse samples. Hes1 expression is elevated in adenomas derived from the small intestine of $Apc^{+/-}$ mice to levels comparable with those found in N/Apc mice (compare the bar of $Apc^{+/-}$ adenomas with the bars corresponding to N/Apc normal intestine and adenomas). Expression of Hes1 in the normal intestine of $Apc^{+/-}$ mice is restricted to the crypt proliferative compartment, as illustrated in Fig. 5*A*. The standard deviation represents the variability between at least 5 different mice. Each sample was normalized to $\beta_2$-microglobulin. (*B–E*) Real-time qPCR reveals that the Notch targets Hes1 (*B*) and HeyL (*C*) and the Notch ligand Jagged2 (*E*) are significantly up-regulated in human adenomas ($n = 7$) compared with human adenocarcinomas ($n = 9$). The levels of Notch1 mRNA (*D*) are also elevated in adenomas versus carcinomas, albeit to a lesser extent. The black horizontal lines represent the median value for each group of individual tumors. Each sample was normalized to $\beta$-actin (black bars), hypoxyanthine-guanine phosphoribosyltransferase (dark gray bars), and glyceraldehyde-3-phosphate dehydrogenase (light gray bars). (*F*) Schematic diagram illustrating the synergy of Notch and Wnt signals during intestinal tumor initiation. Activation of Notch signaling may occur before (Model 1), after (Model 2), or concomitantly (Model 3) with the occurrence of *Apc* mutations leading to constitutive activation of the Wnt cascade. Our results, however, favor Model 1 because in mice where Notch activation precedes LOH for *Apc* (N/Apc mice) we observe ≈20-fold more adenomas than in $Apc^{+/-}$ mice.

the same as in age-matched control $Apc^{+/-}$ mice, leading to an adenoma/carcinoma ratio of 24.1 in N/Apc and 2.7 in $Apc^{+/-}$ mice ($n = 12$ N/Apc mice and 24 $Apc^{+/-}$ mice). These observations suggest that the observed lack of adenocarcinomas in Notch/Apc mice is not simply because of the short survival of these animals. Therefore, both the mouse model and the human data we have gathered favor the notion that Notch activation has a strong impact on the initial phases of tumor development, although it may not have a role in the progression to malignant carcinomas.

This work supports the notion that it is the synergy between Notch and other cellular signals that can provide a developmental context that is favorable for the accumulation of oncogenic mutations (32, 33). We propose a model in which aberrant Notch activation results in hyperplastic conditions representing a preneoplastic state, in which the chances of secondary mutagenic events that can drive full-fledged malignancy are increased. Notwithstanding the fact that oncogenic Notch mutations exist (13, 16, 34), our hypothesis does not demand mutant Notch pathway elements. Notch signal activation could be achieved through several distinct mechanisms, such as epigenetic control of positive or negative regulators of the Notch pathway, eventually leading to elevated signaling levels. This possibility is reinforced by the observation that in advanced stages of human colorectal cancer, Notch signaling is not aberrantly activated, strongly suggesting the existence of reversible modulations of Notch signals that would not be achievable in the case of acquired somatic mutations in the tumor cells. We thus consider it essential to define further the genetic circuitries capable of synergizing with Notch signals to affect cell proliferation.

## Materials and Methods

**Transgenic Mice and Tamoxifen Administration.** All mice used in this work have been described: Rosa-N1ic mice (18), vilCre and vilCreERT2 mice (19), vilCre/Nic mice (6), $Tcf4^{-/-}$ mice (2), and $Apc^{+/1638N}$ mice (23). These strains were crossed for >10 generations to reach an isogenic C57BL/6 background and were used to generate the compound mice vilCre/Nic;$Tcf4^{-/-}$, VilCreERT2/Nic, and vilCreERT2/

Nic;$Apc^{+/1638N}$, the last being referred to as Notch/Apc mice in this article. For the experiments on vilCreERT2/Nic;$Apc^{+/1638N}$ mice, each triple transgenic mouse was analyzed at the same time as a littermate control of either the genotype Nic;$Apc^{+/1638N}$ (without the CreERT2 transgene) or the genotype vilCreERT2;$Apc^{+/1638N}$ (without the Nic transgene). The VilCreERT2 transgenic mice express the CreERT2 transgene under the control of the villin promoter (35–37). Four-week-old mice, including all control mice, were injected i.p. with tamoxifen (ICN) (50 $\mu$g/g of animal body weight) for 2 consecutive days/week for a total of 4 weeks.

***Drosophila* Lines.** The *Drosophila* strains used in this study are eyGAL4 (a gift from Gerald Rubin, Janelia Farm Research Campus, Ashburn, VA), UAS-Nic (38), UAS-dominant-negative Pangolin (27). The Apc (c00746) and *wingless* (d08266) alleles were derived from the Exelixis collection (http://drosophila.med.harvard.edu/) (28). All crosses were carried out at 25 °C.

**Human Samples of Colorectal Cancer.** Twelve sporadic adenomas of low-grade and 13 sporadic adenocarcinomas were obtained from the Gastroenterology and Pathology Unit of the Curie Hospital in Paris. Four samples from FAP patients, consisting of 3 adenomas and 1 carcinoma, were provided by the Pathology Service of St. Antoine Hospital in Paris. In addition, 7 sporadic adenomas and 9 sporadic adenocarcinomas were obtained from patients admitted to the Department of Surgery, Klinikum rechts der Isar in Munich, Germany. The tissue samples were obtained with informed, written consent of the patients and approval of the local ethics committee. Tumor grading and TNM staging were according to International Union Against Cancer (UICC) criteria. Stained sections were evaluated by two researchers and one pathologist independently. In cases of disagreement, a third examination under a multiheaded microscope was carried out.

**Histology and Immunohistochemistry.** Freshly dissected intestines were fixed in 4% neutral-buffered paraformaldehyde, paraffin, or OCT-embedded and sectioned at 5 $\mu$m. Paraffin sections were stained with H&E or subjected to immunohistochemistry, as described in *SI Materials and Methods*. For staining of *Drosophila* imaginal discs, eye-antennal discs were dissected from third-instar larvae, and BrdU labeling was performed as described in ref. 39. Primary antibodies used were anti-BrdU (1:50, BD Biosciences), and anti-elav (1:50, Developmental Studies Hybridoma Bank). Alexa Fluor-coupled secondary antibodies (Molecular Probes) were used at 1:1,000 dilution. All images were taken with a Zeiss Axiovision LE and assembled with Adobe Photoshop.

**RNA Isolation and qPCR.** Total RNA isolation was performed by using TRIzol reagent (Invitrogen) or an RNeasy kit (Qiagen), according to the manufacturers' recommendations. First-strand cDNA was synthesized by using SuperScript III reverse transcriptase (Invitrogen). After ribonuclease H treatment (Invitrogen), PCR was performed. Control reactions omitting reverse transcriptase were performed in each experiment. Oligonucleotide sequences are described in Table S1. For qPCR, reactions were run on a real-time PCR system (ABI Prism 7900; Applied Biosystems). Gene expression was detected with SYBR Green (Applied Biosystems), and relative gene expression was determined by normalizing to reference genes using the comparative $C_T$ method (40). For mouse cDNAs, $\beta_2$-microglobulin and TATA box-binding protein TFIID were used as reference genes, whereas for human cDNAs, each sample was normalized to $\beta$-actin, hypoxyanthine-guanine phosphoribosyltransferase, and GAPDH. A list of the oligonucleotide sequences used for the qPCR can be found in Table S1.

**Statistical Analysis.** To determine statistical significance, the nonparametric Wilcoxon matched-pairs test was performed. Statistical significance was taken at $P$ values $\leq 0.05$. The $P$ value corresponds to $P \leq 0.004801$ in Fig. S1C.

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