Notch pathway: **Making sense of Suppressor of Hairless**

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Suppressor of Hairless (Su(H)) is a DNA-binding protein component of the Notch signalling pathway, thought to be required, with a fragment of the Notch receptor, for target gene activation. Recent studies show that this is only one side of the story: target gene enhancers may be regulated by Su(H) in a variety of different ways.

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Signalling via the cell-surface protein Notch has been implicated in many developmental processes, but is best known for its role in lateral inhibition. In this situation, a cell differentiating along one pathway produces a signal, detected via Notch, which prevents neighbouring cells from differentiating along the same pathway. This system, which limits the number of cells that adopt the Notch-repressed cell fate and also spaces them out within a development field, was initially shown to work during neurogenesis in *Drosophila*, where *Notch* is one of a small set of 'neurogenic genes', so-called because loss-of-function of such a gene leads to the production of excess neurons. Analysis of these genes has helped define the pathway for signalling inside the cell from Notch at the cell surface. A key downstream component of this pathway is a DNA-binding protein known as Suppressor of Hairless (Su(H)), the mammalian homologue of which goes by various names but is mostly commonly referred to as CBF-1.

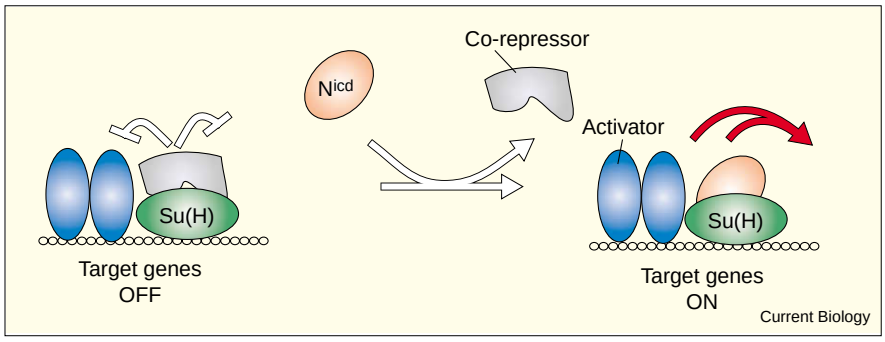
The first link between Su(H) and Notch came from genetic screens in *Drosophila* to identify mutations that modified the phenotypes produced by an activated form of Notch [1]. It subsequently became clear that Su(H) is pivotal in the regulation of the *Enhancer of split* (*E(spl)*) class of target genes, which code for basic helix–loop–helix proteins involved in many cell-fate decisions and have Su(H)-binding sites that are needed for transcriptional activation [2–6]. More recently a number of new targets for Su(H) have come to light, including a Su(H) autoregulatory element that is active in the sense organ [7] and some Notch-responsive genes that were originally thought not to require Su(H) [8,9]. Analyses of these and other more familiar target genes have shown that there are different modes of Su(H)-mediated regulation.

Activation of Notch receptors by their ligands is accompanied by proteolytic processing that releases an intracellular fragment, N^{icd}, from the membrane (reviewed in [10]). This fragment can enter the nucleus and can also interact directly with Su(H)/CBF-1. The presence of N^{icd} inside a cell stimulates transcription from enhancers containing Su(H)/CBF-1-binding sites [3]. The model that first emerged from these observations was that N^{icd} confers on Su(H) the capacity to activate transcription, either by supplying an activation domain itself and/or by helping to recruit a co-activator complex [11–13].

Studies of the mammalian homologue of Su(H), CBF-1, at first seemed at odds with this proposed role, as they indicated that CBF-1 is a repressor of transcription [14]. But in cell culture transcription assays, addition of N^{icd} converted CBF-1 into an activator, thus leading to the elegant model that activation of Notch switches Su(H)/CBF-1 from a transcriptional repressor to a transcriptional activator (Figure 1) [15]. Two different co-repressor complexes that interact with CBF-1 in mammalian cells have now been identified [16,17], as well as an adaptor protein, SKIP, that may be important in

Figure 1

The switch model for Notch target gene regulation by Su(H) [15,16]. In the absence of Notch, DNA-bound Su(H) (green) prevents activators (blue), from promoting transcription. This is likely to be an indirect effect mediated by co-repressors (grey) that are recruited by Su(H), and may act by local modification of chromatin. N^{icd} (orange) is able to alleviate the repression, and Su(H)–N^{icd} cooperate with trans-activators, probably via the recruitment of additional cofactors, to promote transcription.



facilitating the switch between the repression and activation functions [18].

Although attractive, it has been difficult to apply this 'switch' model to the data obtained *in vivo* during development. The tally of genes that have now been shown to be direct targets of Notch and Su(H) include *E(spl)bHLH*, *E(spl)m4*, *single-minded*, *vestigial* (*vg*), *pax2/sparkling* and *Su(H)* itself [3–7,9,19–22]. These genes all contain binding-sites for Su(H) that are essential for activation, but there was little evidence for any involvement of Su(H)-mediated repression in their regulation. We shall discuss recent analyses in *Drosophila* which have started to uncover repressive effects that consolidate the switch model, and have implications for different types of Notch-dependent regulation of enhancers.

Suppressor of Hairless as a repressor

The regulation of the mesectodermal gene *single-minded* (*sim*) at first appeared to be independent of Su(H) [4]: *sim* expression in the mid-line of the *Drosophila* embryo was absent in *Notch* mutant embryos, but not in *Su(H)* mutants. Subsequent analysis, however, showed that the *sim* gene has ten binding sites for Su(H) [9]. When these were mutated, the *sim* enhancer directed expression in a broader domain of the *Drosophila* embryo, but the levels of expression were reduced. This can be explained if the Su(H) sites are required both to repress the *sim* enhancer, in a Notch-independent manner, and then to activate the enhancer in the presence of Notch. Furthermore, re-examination of the effect of eliminating *Su(H)* became possible using a newly generated allele that completely deleted the locus [9]: in embryos devoid of Su(H), *sim* was found to be expressed in a broader domain than in wild-type, providing the first *in vivo* evidence for Su(H)-mediated repression.

Another example of a Notch function that seemed not to require Su(H) was the initiation of *atonal* (*ato*) expression at the morphogenetic furrow in the developing eye [23]. Little or no *ato* expression was detected in the absence of Notch function, whereas its expression appeared normal in *Su(H)* mutant cells. In reassessing which components of the Notch pathway might be involved in mediating this effect, Li and Baker [8] tested the new null allele of *Su(H)* and observed a different result. In clones of cells that lacked *Su(H)*, *ato* expression was initiated prematurely and to higher levels than in the surrounding wild-type cells. These results are most compatible with the view that *ato* has an enhancer that requires Su(H) for repression, but not for activation. The role of Notch in this case would be to alleviate the repression mediated by Su(H). Subsequently, neither Su(H) nor N^{icd} would be required, as removal of Su(H) does not compromise activity, so other activators must be responsible for promoting *ato* transcription.

To directly test for Su(H)-mediated repression, binding-sites for Su(H) were placed adjacent to sites for binding by a widely expressed activator, Grainyhead, and activity of the reporter gene assayed in transgenic flies [24]. This model Notch response element conferred clear Su(H)-mediated repression in tissues where Grainyhead is present but Notch is inactive, and high levels of expression where Notch activity overlapped with that of Grainyhead. As expected, the inhibition by Su(H) could be overcome by supplying N^{icd}. These results, along with those on *ato* and *sim*, provide strong *in vivo* support for the 'switch' model, and highlight the fact that this leads to differences in the phenotypes produced by mutations in *Notch* and *Su(H)*.

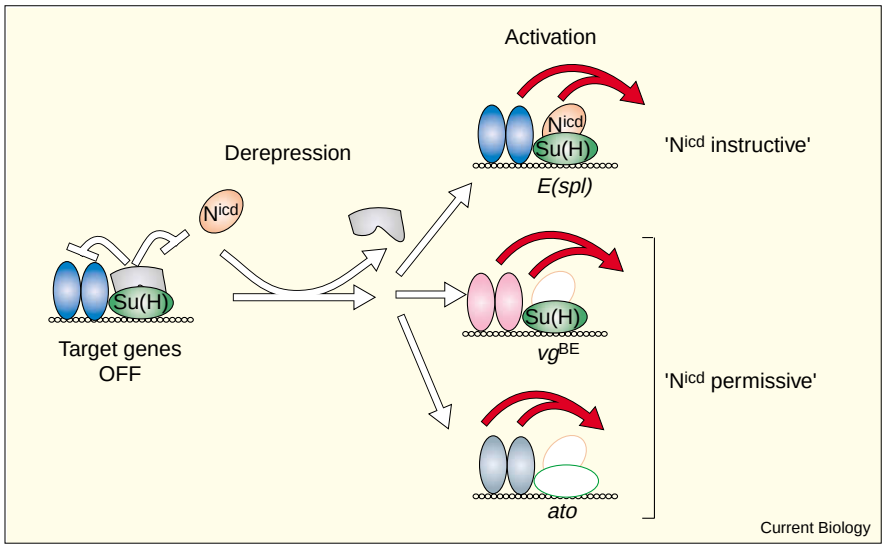
Why has the repressive aspect of Su(H) function been difficult to uncover in previous genetic studies? There are two contributory factors that probably account for this. One is technical: until recently there were no alleles of Su(H) available that completely eliminate its function, so in most *Drosophila* experiments a low level of protein probably persisted. The second is the involvement of Su(H) in both activation and repression, which may lead to target genes having no net activity when Su(H) is eliminated and thus mask the repressor action of Su(H).

N^{icd}-instructive versus N^{icd}-permissive enhancers

The switch model invokes two functions for N^{icd}: the first to displace the co-repressors, so alleviating repression of target enhancers; and the second to supply or recruit co-activators to promote transcription. A number of genes, such as *sim* and the *E(spl)/HES* genes, seem to need N^{icd} at both of these steps (Figure 2). Others, such as *ato* [8] (Figure 2), appear only to require N^{icd} for the first step, to alleviate repression; their subsequent activation can occur independently of N^{icd}, presumably because of the presence of other DNA-bound transactivators. Another example of an enhancer which appears to fall into the 'N^{icd} permissive' category is vg^{BE}, even though it loses its activity in cells that are mutant for either *Notch* or *Su(H)* [21].

The difference between vg^{BE} and *E(spl)* was revealed in experiments where Su(H) was expressed ectopically in *Drosophila* [25,26]. *E(spl)* enhancers are repressed by ectopic Su(H), presumably because the excess Su(H) is able to titrate the available N^{icd}, as well as any corepressors. In contrast, vg^{BE} was found to be activated strongly by ectopic Su(H), suggesting that it can be activated without N^{icd} when there is excess Su(H) present to titrate the co-repressors. In agreement with this, ectopic Su(H) could promote expression from vg^{BE} even in cells that lacked Notch [25]. Under normal circumstances, vg^{BE} requires N^{icd} for its expression, but these data indicate that it is needed only to alleviate repression and not for co-activation (Figure 2). On the other hand, *E(spl)* enhancers appear to be 'N^{icd} instructive', needing N^{icd} for co-activation as well as to alleviate repression (Figure 2). The

Figure 2



Nicd-instructive and Nicd-permissive enhancers. Depending on the other trans-activators present (vertical ovals), enhancers have different requirements for Su(H) and Nicd during the activation step. Solid, shaded shapes indicate a requirement for the protein; unshaded shapes indicate that a protein is not essential for activation. Note that *ato* has not yet been shown to be a direct target of Su(H), so this is speculative. Symbols as in Figure 1.

difference between instructive and permissive enhancers is likely to reside in the other trans-activators that bind to the individual enhancers, and their capacity to activate transcription autonomously.

A third example of a Nicd permissive enhancer is found in the *Su(H)* gene itself. The Su(H) protein is found at high levels in the future socket cell of the developing sense organs. Barolo *et al.* [7] have now shown that this is due to an autoregulatory enhancer (*ASE*) within *Su(H)*, which contains eight binding-sites for Su(H). Once activated, the *ASE* enhancer continues to be positively autoregulated by Su(H), even in the absence of Notch. The initial activation of the *ASE*, however, does require Notch function, suggesting that Nicd is needed initially, to alleviate Su(H)-mediated repression. This interpretation is supported by the observation that the *ASE* becomes active in other cells in the lineage when Su(H) is removed. Nicd is thus required to switch between repression and activation at the *ASE*, but is not subsequently necessary to maintain activity.

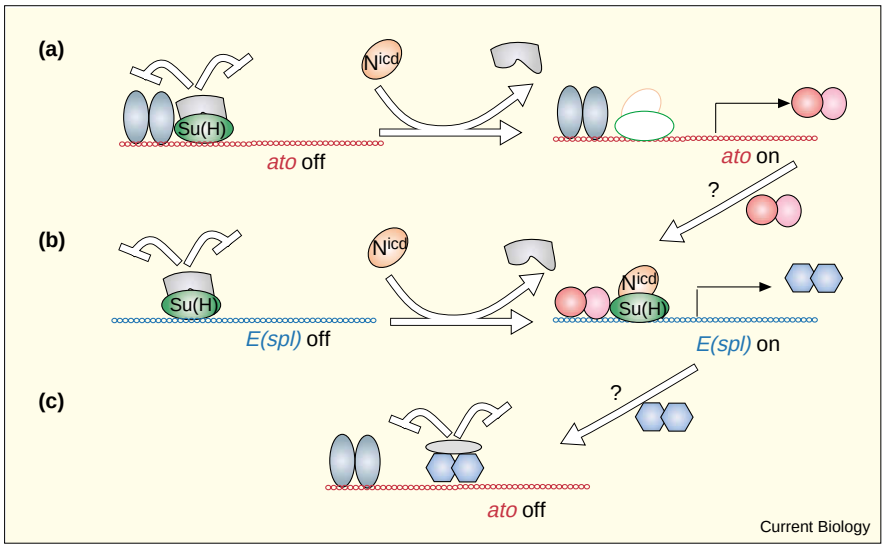
Autoregulation of *Su(H)* is important for differentiation of the socket cell in *Drosophila*, as the electrophysiological properties of this sense organ are abnormal if it is perturbed. This raises the intriguing question of whether the Su(H) that accumulates in the socket cell may be regulating specific genes independently of Notch. The observation that high levels of Su(H) can result in derepression of vg^{BE} [25,26] suggests a possible model. If autoregulation of *Su(H)* in the socket cell leads to Su(H) being present in excess over the components of the co-repressor complex, it might bind to target genes but not repress them. This would obviate any requirement for Nicd to displace the co-repressors. But although *Su(H)* autoregulation continues in the absence of Notch, we cannot tell yet whether the targets of Su(H) in the socket cells can be activated independently of Nicd. If they can be, manipulations that disrupt Notch function in the adult socket cell should not perturb the electrophysiology of the sense organ.

Variations on a theme

The switch model can thus accommodate a variety of different mechanisms for Su(H)-mediated regulation. Nicd-instructive enhancers, such as those at the *E(spl)* and *sim* loci, require Nicd both to displace the co-repressor complex from Su(H) and to recruit a coactivator complex. Nicd-permissive enhancers can be subdivided into at least two types. One class, illustrated by vg^{BE} and the *Su(H) ASE*, require Nicd to alleviate repression, but Su(H) can maintain activity of the enhancer in the absence of Nicd. A second class is illustrated by *ato*, which appears to be repressed by Su(H) and requires Notch to alleviate this repression, but has no further requirement for Su(H) in its activation. A final possibility is that the socket cell differentiation may involve enhancers that require Su(H) but are totally independent of Nicd.

Within this general framework there are considerable variations in both the number and organisation of Su(H)-binding-sites. Some enhancers, such as those mediating regulation of *sim*, *Su(H)* and *Pax2/sparkling*, contain many Su(H)-binding sites [7,9,22], whereas others, such as vg^{BE} or the *E(spl)* enhancers, contain few such binding sites [4,6,19–21]. The *E(spl)* enhancers also have a conserved organisation of paired Su(H)-binding sites [6]. Do these different arrangements of binding sites confer significant

Figure 3



A speculative scheme to explain two modes of Notch-dependent regulation of *ato* during *Drosophila* eye development. **(a)** At the furrow, Notch activation would lead to derepression of *ato* (red, circles indicate Ato protein, other symbols as in Figure 1). **(b)** *E(spl)* (blue) expression is promoted by a combination of N^{icd} and other activators [20], one of which could be Ato. This system would have a built-in delay with *E(spl)* being activated after *ato* (blue hexagons indicate E(spl) protein). Unlike *ato*, which would no longer be affected by N^{icd} after the initial activation, *E(spl)* expression appears to be directly contingent upon N^{icd} activity. **(c)** Levels of E(spl) proteins would continue to escalate, and could in turn repress *ato* expression.

properties on their regulation, for example altering the threshold of the response?

Four Su(H)-binding sites are more effective than two at mediating repression in the context of a model Notch response element, indicating a correlation between the number of sites and the strength of repression [24]. The behaviour of the *pax2/sparkling* enhancer also suggests that the number of Su(H)-binding sites might influence responsiveness [22]. Activation of *pax2/sparkling* in cone cells is mediated through a combination of Ras and Notch activation, yet this enhancer is not normally active in the R7 photoreceptor, where both signals are also present [22,27]. However, *pax2/sparkling* can be activated in R7 if extra N^{icd} is supplied [22]. This suggests that N^{icd} levels are not normally sufficient in R7 to activate the *pax2/sparkling* enhancer, which contains twelve binding-sites for Su(H). If the number of Su(H) sites is important in determining the response threshold, mutation of some but not all of the *pax2/sparkling* sites should lead to derepression in R7.

A final twist in the tale comes from the recent analysis of *ato* regulation in the eye [8]. Initially, Notch is required to derepress the *ato* enhancer, permitting *ato* expression and promoting neural fates. Subsequently, Notch activity is required for lateral inhibition to repress *ato* in all but the presumptive R8 cell. How can the same pathway lead to two opposing effects? This can be explained if the initial activation is a direct effect of N^{icd}, which results in the enhancer becoming derepressed and no longer dependent on Su(H), and the second repressive effect is indirect, mediated by DNA-binding proteins encoded by the *E(spl)* locus (see Figure 3).

Within the paradigm set by the switch model, there is evidently considerable room for different deployment of N^{icd} and Su(H). Unravelling these differences has helped to explain several examples of Notch-dependent gene regulation that were previously thought to be independent of Su(H). The schemes outlined here do not, however, account for all the observed Su(H)-independent activities of Notch so there may yet be other mechanisms of transduction (for example, see [28]). As more Notch-dependent target genes are analysed, we shall be able to evaluate the extent to which they fall into the different categories of Su(H)-mediated regulation. Defining the full set of target genes may, however, be quite difficult, as one final point that has emerged from the recent analyses is that Notch-dependent regulation may contribute only a small part of the overall expression pattern of a gene (for example, see [7]).

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