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CORRESPONDENCE

Transcriptional interpretation of Shh morphogen signaling: computational modeling validates empirically established models

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In an article published in Development titled 'A theoretical framework for the regulation of Shh morphogen-controlled gene expression', the Briscoe laboratory presented a mathematical model of the transcriptional interpretation of graded Shh signaling and bifunctional Gli transcription factors (TFs) in neural patterning (Cohen et al., 2014). This model makes the following major predictions: (1) gene expression boundaries will be shifted as a function of the affinity properties of Gli-binding sites (GBSs) in *cis*-regulatory modules (CRMs); (2) target gene expression is determined by the combinatorial input of transcriptional effectors which are integrated in individual target gene CRMs. In addition to Gli TFs, such input includes (3) uniformly expressed TFs and (4) morphogen-regulated target genes that are dynamically regulated downstream of the morphogen and which comprise repressor or activator functions.

While overall we do not dispute the theoretical model itself, we are troubled that the study largely overlooks previous experimental work on Shh-regulated CRMs by the Ericson (Oosterveen et al., 2012, 2013) and McMahon laboratories (Peterson et al., 2012), which collectively arrived at conclusions we believe to be conceptually indistinguishable from those of Cohen et al. In these studies, functional analyses of endogenous CRMs firmly establish that transcriptional interpretation of graded Shh signaling and Gli TFs is critically reliant on the cooperative activity of uniformly expressed SoxB1 TFs. Oosterveen et al. (2012) find that SoxB1 and Gli TFs operate synergistically at CRMs, rendering Gli-mediated gene activation a largely concentration-independent event. In a subsequent paper (Oosterveen et al., 2013), it was shown that the activity of SoxB1 can be extended and applied to morphogen signals other than Shh, as well as that morphogen-regulated transcriptional networks underlying neural patterning are functionally recapitulated in limb bud tissue in response to forced SoxB1 expression and morphogen signaling. Moreover, the Oosterveen studies provide direct evidence that morphogen-regulated activator and repressor inputs directly influence the output of Shh-regulated CRMs, and this is also supported by studies from the Matise laboratory (Lei et al., 2006; Wang et al., 2011). Furthermore, a relationship between GBS affinity properties and regional expression of Shh target genes was reported by the Oosterveen et al. (2012) and Peterson et al. (2012) studies.

Cohen et al. (2014) cite the Oosterveen and Peterson studies, but none of the conceptual conclusions presented in those papers, as briefly outlined above, are properly introduced, acknowledged or discussed at any point in their text. Instead, when the concepts of GBS affinity, multiple transcriptional inputs or their integration into Shh-regulated CRMs are being introduced, non-vertebrate model systems are typically discussed and the aforementioned studies either disregarded or misrepresented. Given that the study by Cohen et al. (2014) explicitly models the transcriptional interpretation of Shh signaling in the vertebrate neural tube, we believe that the failure to accredit previously established concepts in the system actually being modeled and only referring to related concepts defined in unrelated morphogen systems is completely inappropriate. This is particularly important, as the conclusions drawn by Cohen et al. (2014) show a high degree of overlap with previously established models of Shh interpretation.

With respect to the role of GBS affinity properties, the authors predict a theoretical 'neutral point' around the boundary of *Nkx2.2* and *Olig2* expression, which results in opposite effects on the range of expression for target genes expressed below or above this point in response to alterations of GBS affinity. This is strikingly reminiscent of the 'mechanistic differences between local and long-range interpretation of Shh' outlined by Oosterveen et al. (2012). In that study, by interfering in an unbiased manner with the ability of both GliA and GliR to bind their DNA-binding sites, it was empirically established that genes expressed with a dorsal limit above or below the *Nkx2.2-Olig2* boundary interpret Gli input differently. Consequently, this position was defined as discriminating between local and long-range interpretation of Shh signaling (see figs 4 and 7 in Oosterveen et al., 2012). Based on these data, GBS-swapping experiments and other functional analyses of CRMs, it was proposed that genes induced at long range require GliR, together with CRM-specific repressive input, to prevent default (permissive) activation by GliA and SoxB1 proteins at ectopic positions (see figs 3-5,7 in Oosterveen et al., 2012). Accordingly, the Oosterveen study established that genes activated at long range (i.e. above the neutral point) become derepressed when GBS affinity (or GliR) is lowered, in agreement with the Cohen computational model.

By contrast, for local genes, Gli interference experiments resulted in a notable ventral retraction of expression of *Nkx2.2*, demonstrating that locally restricted Shh target genes critically require instructive input by GliA to overcome default repression (see fig. 4 in Oosterveen et al., 2012). It was further shown that the induction of local genes (*Nkx2.2*) is less dependent on CRM contextual co-activator input (compared with long-range genes; see fig. 7 in Oosterveen et al., 2012) and that high-affinity GBSs examined in isolation were able to largely recapitulate the expression of endogenous local CRMs *in vivo* (see fig. 3E in Oosterveen et al., 2012). Based on these and other data, it was proposed that local gene activation is centered around individual high-affinity GBSs associated with these genes, and that the role of GliR is to restrict gene activation by GliA at these sites (see fig. 2B and fig. 3 in Oosterveen et al., 2012). These empirically based conclusions are in line with the effect of reducing Gli affinity for genes below the neutral point, as subsequently outlined by Cohen et al. (2014). Thus, the differential responses of local and long-range genes to Gli proteins and alterations of Gli affinity defined by Oosterveen et al. correspond precisely to the predicted output of

Development (2016) 143, 1638-1643

genes expressed below and above the neutral point of Cohen et al. Importantly, Cohen and colleagues define the neutral point as being 'determined only by the concentrations of GliA and GliR and the strength of their cooperative binding with polymerase (Eqn 6) and is independent of the basal level of gene expression'. It is notable that the term 'basal level of expression' corresponds to the 'CRM-specific contextual [non-Gli] input' described and functionally examined in Oosterveen et al. (2012). In light of this, it seems obvious that the Oosterveen model outlining mechanistic differences regarding short- and long-range interpretation of Shh signaling bears strong conceptual resemblance to most aspects of Cohen's theoretical model, albeit employing different terminology.

Oosterveen et al. (2012) reported a broadly inverse correlation between the range of gene expression and GBS affinity (see fig. 3 in Oosterveen et al., 2012). That said, Oosterveen et al. (2012) did not identify the positive correlation between GBS affinity and regional expression pattern for genes expressed below the neutral point, but this was clearly outlined and empirically validated by a number of criteria, including GBS affinity-swapping experiments in CRMs for *Nkx2.2* and *Foxa2*, in Peterson et al. (2012) (see fig. 5D-G and Discussion of that study). However, when Cohen and colleagues address these studies in their introduction, they incorrectly state '…analysis of GBSs within enhancers of Shh target genes failed to find a positive correlation between binding site affinity and range of gene induction (Oosterveen et al., 2012; Peterson et al., 2012)'. This could easily be interpreted to mean that no correlation has been identified, let alone the positive correlation of the Peterson study. Inconsistent with this introductory statement, however, they later affirm that the Oosterveen and Peterson studies describe correlations between GBS affinity and expression.

It is notable that, a year and a half before the present study, Cohen and Briscoe extensively reviewed the findings of the Oosterveen and Peterson studies (Cohen et al., 2013). In this review, they presented a schematic summary of the Gli-affinity data in the Oosterveen and Peterson studies that is remarkably reminiscent of Cohen et al.'s present theoretical model, including the positioning of a presumptive 'neutral point' close to the *Nkx2.2/Olig2* boundary – apparently without any need for computational modeling (see fig. 1 in Cohen et al., 2013, reproduced as Fig. 1 here). We have not found any reference that the input parameters for the model in Cohen et al. (2014) are based on empirical observations, and these instead appear to have been arbitrarily selected. Thus, given that their modeling so comprehensively matches the existing empirical models that they reviewed in Cohen et al. (2013) (see Fig. 1), we find it astonishing that they consider it irrelevant to discuss or even mention these similarities in their more recent paper (Cohen et al., 2014).

When introducing the concept of multiple transcriptional inputs in CRMs, the authors neglect the rather extensive body of data provided by the Oosterveen and Peterson studies, and instead cite different non-vertebrate morphogen systems, only vaguely stating later in their introduction: 'The pan-neuronal transcriptional activator Sox2 provides neural specificity to these Shh target genes (Bailey et al., 2006; Graham et al., 2003; Oosterveen et al., 2013)'. By handling previously published work in this manner, the reader is left with the impression that very little is known about the transcriptional interpretation of Shh signaling at the CRM level. Subsequently, without having clearly introduced previously established Shh interpretation models, the authors propose at the end of the introduction, citing only their own work: 'An alternative is that the dynamics of the transcriptional network, which is composed of Gli proteins, uniformly expressed TFs and TFs downstream of Shh signalling, explains the spatial pattern of gene

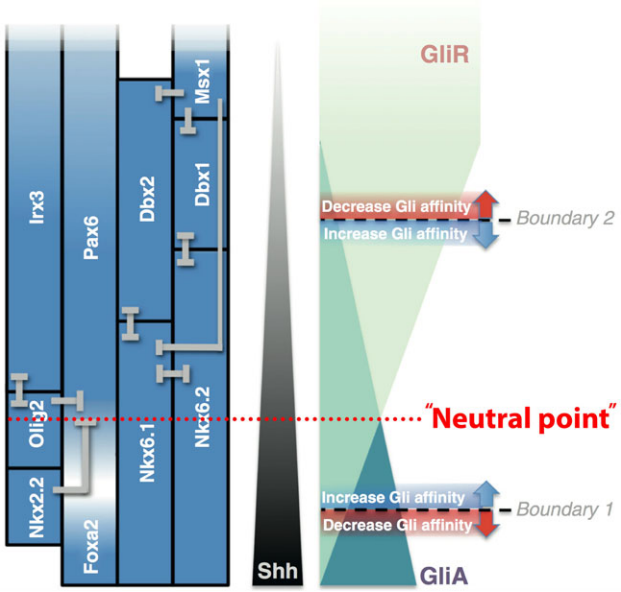

Fig. 1. Modified reproduction of fig. 1B,C from Cohen et al. (2013). In their review from 2013, Cohen and Briscoe summarize Gli-affinity data in the Oosterveen and Peterson studies in fig. 1. This figure illustrates that the expression boundary of genes regulated by Shh at long range will expand if the affinity property of GBS is lowered (Boundary 2) while expression instead will retract for locally induced genes if GBS affinity is decreased (Boundary 1). A presumptive 'neutral point' positioned dorsal to domain of *Nkx2.2* expression but ventral to the dorsal boundary of *Olig2* expression is evident in this figure (delineated by us as a red dotted line in the reprinted figure). Selected quote from fig. 1 in Cohen et al. (2013): 'A ventral-to-dorsal gradient of Shh is converted into opposing gradients of GliA and GliR. In different regions of the gradient, where Gli acts predominantly as either a transcriptional activator or repressor, gene expression boundaries (boundaries 1 and 2) respond differently to changes in Gli binding affinity. Blue and red arrows indicate the direction of the shift in gene expression domains after increasing or decreasing Gli affinity, respectively'. Reproduced with permission from Elsevier.

expression in the neural tube (Balaskas et al., 2012)'. It is noteworthy that the Balaskas study did not include any CRM analyses nor examine uniformly expressed TFs.

Throughout the paper, we noted a tendency for the authors to cite their own work while overlooking contributions by others when outlining network dynamics or well-established repressive interactions amongst morphogen-regulated TFs. One such example relates to the phenomenon of hysteresis, or cellular memory of Shh morphogen exposure, on which both we and the Briscoe laboratory have presented related but distinct models (Lek et al., 2010 and Balaskas et al., 2012, respectively). In Cohen et al. (as well as other publications from the Briscoe laboratory), only the Balaskas study is cited, despite the fact that both describe mechanisms of adaptation in which a cell, once exposed to Shh, will not respond in the same manner to subsequent exposure. Whereas to our knowledge no studies have been published that would disqualify the earlier model by Lek and colleagues, both mechanisms are compatible with recent quantitative analyses by Junker et al. (2014).

In their Discussion, Cohen et al. conclude, 'Three distinct classes of inputs can be defined: the MR-TF, the activity of which is determined by the distribution of the morphogen in the tissue; uniformly expressed TFs that are active throughout the tissue; and morphogen-controlled target genes that are dynamically regulated downstream of the morphogen. Each of these inputs can comprise multiple individual TFs with either inhibitor or activator function'. This bears strong resemblance to conclusions made in Oosterveen

et al. (2013): 'SoxB1 and Gli proteins therefore appear to define the central node of a neural-specific GRN required to translate graded Shh signaling into regional gene expression patterns…Although SoxB1 and Gli proteins are sufficient to trigger activation of this network…many genes cooperatively activated by SoxB1 and Gli proteins…encode transcriptional activators or repressors that themselves are integral components of the network…Such proteins are likely to act in a more CRM context-dependent manner to influence the regional expression pattern of Shh-regulated genes within the neural tube'. Considering the similarity between these conclusions, we find it remarkable that the Oosterveen and Peterson studies are not cited on a single occasion in their Discussion.

The conceptual conclusions of the Oosterveen studies regarding Shh-regulated transcriptional inputs are partly based on analyses of CRMs of genes that are not directly modelled in Cohen et al. However, we would argue that this does not justify ignoring them, given that Cohen et al. draw conceptually equivalent conclusions and likewise stress the general applicability of their model. In our opinion, the failure to properly accredit work by others risks leaving an impression that the model described in the Cohen study is entirely novel, despite the fact that all of its major conclusions primarily confirm published models of Shh interpretation. By publishing this Correspondence (and the associated response) we hope to protect the integrity of the scientific record by bringing these issues to light. Nevertheless, while we have strived to be factual in our criticism, we obviously cannot be considered to be unbiased and would therefore recommend that engaged readers establish their own opinion on this subject.

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Mathematical models help explain experimental data. Response to 'Transcriptional interpretation of Shh morphogen signaling: computational modeling validates empirically established models'

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In our paper, 'A theoretical framework for the regulation of Shh morphogen-controlled gene expression' by Cohen et al. (2014) we formulate a mathematical model of gene regulation by morphogen signalling that brings together empirical findings from several sources including Balaskas et al. (2012), Oosterveen et al. (2012, 2013) and Peterson et al. (2012). We use an approach based on statistical thermodynamic ensemble models of gene regulation and Approximate Bayesian Computation. We argue that the mathematical model provides a single coherent framework that explains experimental observations and that the approach can be applied to similar morphogen systems.

Uhde and Ericson (2016) do not dispute our mathematical model. Instead they claim: (1) our study 'overlooks previous experimental work' and we have not 'acknowledged or discussed…conceptual conclusions' of the Ericson lab; and (2) our conclusions are 'conceptually indistinguishable' from Oosterveen et al. (2013, 2012) and Peterson et al. (2012), and our 'major conclusions primarily confirm published models of Shh interpretation'.

We disagree. Here, we clarify the issues that appear to have caused these misunderstandings.

The contribution of the Ericson and McMahon laboratories to understanding the roles and dissecting the architecture of key patterning genes in the neural tube is indisputable. Far from overlooking their studies, we use these explicitly as motivation for the mathematical model and we cite the papers extensively throughout the manuscript (we cite the Peterson study seven times

and the Oosterveen papers nine times). We do not think readers could fail to notice our references to these papers or easily miss our use of their work to support the model. Moreover, as Uhde and Ericson (2016) point out, we also reviewed and cited their work extensively in a separate publication (Cohen et al., 2013).

Nevertheless, the description of morphogen-controlled gene expression is the product of a large number of studies that go well beyond the neural tube field (Briscoe and Small, 2015). The model of gene regulation we construct contains three types of input: (1) uniformly expressed transcription factors (TFs). The role of these in morphogen interpretation became obvious from work on Zelda and Stat92E in *Drosophila* (e.g.Kanodia et al., 2012; Nien et al., 2011). (2) A transcriptional effector of the morphogen for which there is a lack of correlation between binding affinity and the position of target gene activation. This has been extensively documented for Bicoid in the Gap gene system (Ochoa-Espinosa et al., 2005). (3) A set of morphogen-regulated TFs that form a transcriptional network. The Gap genes also provide a well-established example of the importance of transcriptional network dynamics in morphogen pattern formation (e.g. Jaeger et al., 2004; Manu et al., 2009).

Each of these elements was described in *Drosophila* prior to the work of Oosterveen et al. (2012, 2013) and Peterson et al. (2012). Furthermore, the idea that *cis*-regulatory modules combine multiple inputs to 'compute' an output is at the heart of the gene regulatory network framework developed by Davidson and colleagues (reviewed in Davidson, 2010). Thus the precedents for the broad conceptual conclusions to which Uhde and Ericson (2016) refer arose from studies of non-vertebrate systems that predate the work of Oosterveen et al. (2012, 2013) and Peterson et al. (2012).

Our intention by citing the non-vertebrate studies was not to diminish the contribution of the Ericson and McMahon labs, but to provide a broader context and the appropriate background. For example, in the section dealing with the function of uniformly expressed TFs we write: '…previous studies have demonstrated how the levels of binding of a spatially uniform factor to target genes in a morphogen patterning system can significantly influence their expression profiles (Kanodia et al., 2012). In the neural tube, the TF Sox2 has been suggested to provide a spatially uniform activation input into neurally expressed genes (Bailey et al., 2006; Oosterveen et al., 2012; Peterson et al., 2012)'. We believe that these comparisons are highly relevant and emphasize the importance of Oosterveen et al. (2012, 2013) and Peterson et al. (2012). Taken together, the studies suggest common principles underpin the transcriptional interpretation of morphogen signalling in several tissues.

More importantly, the suggestion that our conclusions are 'conceptually indistinguishable' and that our 'major conclusions primarily confirm published models of Shh interpretation' misses the key point of our paper. Cohen et al. (2014) describe and analyse a mathematical model. The Oosterveen and Peterson studies do not contain mathematical models, neither does Cohen et al. (2013). Moreover, the interpretation that Oosterveen et al. (2012) offer of their data is not equivalent to the mathematical model in Cohen et al. (2014).

We believe that the explanatory and predictive power of mathematical models is of most value when firmly rooted in experimental observations. The empirical observations we use to construct the model are based on the studies of Oosterveen et al. (2013, 2012), Peterson et al. (2012), among many other studies, and we cite these papers accordingly. These observations are the basis for the model not, as Uhde and Ericson (2016) seem to suggest, the 'conclusions' of the model. In our view, the analytical framework in Cohen et al. (2014) helps to rigorously establish the relationships between different pieces of empirical evidence and formulates a mechanistic, predictive model of gene regulation.

Moreover, mathematical models, particularly of non-linear dynamical systems such as transcriptional networks, often provide insight into complex behaviours that are difficult to discern from experiment alone. We think this is the case here. As a consequence, there are several differences that distinguish the model proposed in Cohen et al. (2014) from Oosterveen et al. (2012, 2013).

(1) In the Cohen et al. (2014) model there are no inherent differences between how target genes interpret GliA and GliR gradient. By contrast, the study by Oosterveen et al. (2012) proposes two classes of genes: 'local' genes that interpret 'the balance between GliA and GliR', and 'long-range' genes that only interpret GliR and have more dorsally positioned boundaries. They label this a 'GliR gradient interpretation model' and comment: 'This GliR-gradient interpretation model differs significantly from prevailing models suggesting…that cells strictly measure the balance between GliA and GliR'. This leads Oosterveen et al. (2012) to their principal conclusion that the interpretation of Shh signalling involves 'mechanistic differences' between the 'local' and 'long range'.

In the model proposed in Cohen et al. (2014), the expression of both short and long-range Gli-regulated genes depend on the concentration and strength of binding of both GliR and GliA (the binding affinity of both is the same – parameterized by a single value). This could be summarized as 'genes measure the balance between GliA and GliR'. Thus, Cohen et al. (2014) do not invoke two classes of genes nor mechanistic differences between short and long-range target genes.

The model proposed in Cohen et al. (2014) also reveals that differences in GBS affinity between target genes are not necessary to explain the observed spatial-temporal dynamics of target gene expression. This appears at odds with the proposal of 'mechanistic differences' in the transcriptional regulation of 'local' and 'long-range' target genes (Oosterveen et al., 2012).

(2) Uhde and Ericson (2016) state that their data indicate that 'Gli-mediated gene activation [is] a largely concentration-independent event'. This is not the case in the mathematical model described in Cohen et al. (2014) in which the response of target genes is dependent on the concentrations of GliA and GliR. The way a specific target gene responds to alterations in activator and repressor levels depends not only on the Gli input but also its other inputs. An important consequence of this is that changes in the Gli binding affinity of a target gene can have opposite effects on the range of activation of different genes. We believe that this represents a good example of how the mathematical model helps explain the experimental data. We make this point in our manuscript by citing data in Oosterveen et al. (2012) and Peterson et al. (2012).

(3) As Uhde and Ericson (2016) indicate, our model describes a 'neutral point'. However, our definition of the neutral point appears to differ conceptually from their interpretation. In their correspondence, Uhde and Ericson imply that the neutral point is a specific location in the tissue between the *Nkx2.2* and *Olig2* boundaries. Furthermore, they suggest that genes on either side of this point have different Gli binding affinities. In the mathematical model this is not the case. In Cohen et al. (2014), we define the neutral point for a gene as the point in the GliA/GliR gradient at which altering the GBS affinity does not alter the probability of gene expression (see eqn 5). We show how this point depends on the concentration of GliA/GliR and the basal levels of expression of each gene (see eqn 6 and eqn S4). Hence the neutral point is

Development (2016) 143, 1638-1643

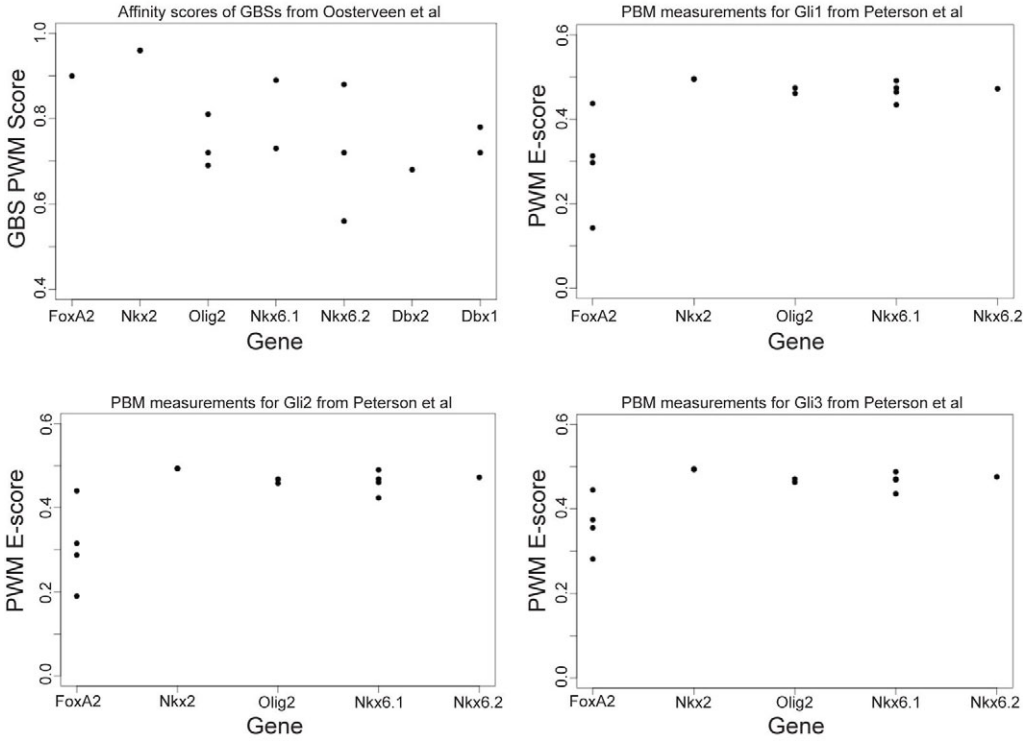

Fig. 1. Scatter plots of the predicted binding affinities of the putative gli binding sites (GBSs) associated with the indicated genes as reported in Oosterveen et al. (2012) and Peterson et al. (2012). Data were extracted from fig. 3A of Oosterveen et al. (2012) and from table 1 of Peterson et al. (2012).

independent of the binding affinity for Gli TFs (parameter K) and is not a single position in the tissue – for each target gene it depends on the non-Gli regulatory input (eqn S4). Importantly, our analysis indicates that the observation that changes in GBS affinity result in opposite shifts of gene expression boundaries on either side of the neutral point is an emergent property of the model.

We agree with Uhde and Ericson that this behaviour is evident in the Oosterveen and Peterson experimental data, but we could not find the idea of a 'neutral point' proposed in Oosterveen et al. (2012, 2013). Instead Oosterveen et al. (2012) use the data to propose 'mechanistic differences' between 'local' and 'long-range' interpretations of Shh signalling and that 'Gli activators have a noninstructive role'. In Cohen et al. (2014), we highlight and cite the experimental evidence of the neutral point revealed by their experimental data and show how a mathematical model suggests a single mechanism to explain the experimental observations. We think this represents one of the successes of the mathematical model and it illustrates how such models provide new insight into experimental data.

(4) Oosterveen et al. (2012) suggest that gene regulation involves 'cooperative' interactions between Gli and HD proteins and between Gli and SoxB1. The model we formulate does not contain these cooperative interactions. This does not rule out cooperative interactions *in vivo*. However, these are not required in the mathematical model.

(5) Uhde and Ericson (2016) say that we 'incorrectly state "…analysis of GBSs within enhancers of Shh target genes failed to find a positive correlation between binding site affinity and range of gene induction (Oosterveen et al., 2012; Peterson et al., 2012)" '. We are confused by this statement. The data reported in fig. 3A of Oosterveen et al. (2012) and table 1 of Peterson et al. (2012) lack a positive correlation between binding site affinity and range of gene induction – at best there might be a weak negative correlation in the Oosterveen data, which is not evident in the Peterson analysis (see Fig. 1). In Oosterveen et al. (2012) two classes of genes, 'local' and 'long range' are defined, but even within each of these two classes Oosterveen et al. (2012) conclude 'there is no predictive correlation between gene expression pattern and affinity score or number of GBSs' (p. 1009). This appears to be in line with our statement. We note that the P19 data in Oosterveen et al. (2012) also support this conclusion – fig. 3D in Oosterveen et al. (2012) shows no difference between the Nkx2.2 and FoxA2 activity and the statistical significance of the other differences is unclear.

While not a point of distinction between the conclusions of Cohen et al. and the earlier studies, Uhde and Ericson (2016) raise concerns about our discussion of hysteresis. The term 'hysteresis' was originally coined to describe the behaviour of magnetic materials and is widely used in dynamical systems theory to indicate that the output of a system depends not only on its current input, but also on past inputs. We show that the mathematical model developed in Cohen et al. (2014) displays similar hysteresis to the simpler dynamical system described in Balaskas et al. (2012). This is a validation of the model formulism and it links the Cohen et al. model to the experiments performed in Balaskas et al. (2012). Hysteresis suggests an explanation for the maintenance of gene expression in cells in which the levels of Gli activity decrease over time; an observation described in Balaskas et al. (2012) and recently independently observed in the elegant quantitative approach taken by Junker et al. (2014). This mechanism is distinct from that proposed in Lek et al. (2010), which is why we did not cite that work.

In summary, there are several features that distinguish the mathematical model described in Cohen et al. (2014) from previous interpretations. The Cohen et al. (2014) model demonstrates that neither differences in the binding affinity of GliA and GliR, nor differences in GBS affinity between target genes are required to explain the patterns of gene expression. Contrary to the conclusions of Oosterveen et al. (2012), the model in Cohen et al. (2014)

proposes that all target genes respond to the ratio of GliA and GliR and the mathematical model does not impose mechanistic differences between local and long-range targets. Together, the Cohen et al. model provides a distinct mechanistic explanation for the experimentally observed position-dependent shifts in gene expression upon perturbations of binding site affinity.

We note that the section entitled 'Discussion' in Cohen et al. (2014) was originally titled 'Conclusion' to signify it as a short summary and the section entitled 'Results' was originally titled 'Results and Discussion'. These headings were changed in response to an editorial request, after acceptance, to match Development's house style and we regret any confusion this caused.

We are saddened that Uhde and Ericson believe we undervalue their work. The intention of Cohen et al. (2014) was not to diminish or undermine studies to which they and others have contributed. Rather, we believe that accommodating the empirical data of Oosterveen et al. (2012, 2013) and Peterson et al. (2012) in a single theoretical framework and reconciling this with other studies in the field emphasizes the importance and success of their work. We think this exchange of correspondence also highlights the benefits of mathematical models: they provide formal, precise and transparent descriptions of ideas that are not subject to the ambiguities or differences in interpretations of narrative accounts or informal 'cartoon' models. Moreover, mathematical models make clear predictions that can be tested experimentally and we look forward to working with the Ericson lab and others in the field to revise, extend or refute current models.

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