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Building a Computational Model of The Sonic Hedgehog Signalling Pathway

A six week placement within the Centre for Integrative Physiology at the University of Edinburgh.

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Abstract

Shh is an important protein involved in development of the neural tube, development of digits[1], cell proliferation[2] and cell death[3]. To date, no model exists which is useful to explore the pathway as all other computational models of the Shh pathway are very specialised to specific parts of the Shh pathway. These other models cannot investigate other parts of the Shh pathway.

The model of the Sonic Hedgehog (Shh) pathway was created using COPASI software. Data from previously published papers containing valuable information were used as source data for input values. Expressing the input values into COPASI as code allowed the model to simulate the biochemical pathway.
Examples of some of the interactions were.
Patched (Ptc1), Smoothened (Smo) and Shh.
They had a complex interactive system with each other. When Shh inhibits Ptc1, Ptc1's inhibitory effect on Smo is lost, allowing for the pathway to eventually activate the transcription factor Gli.

The model shows that when Shh levels increased, Gli would be activated instead of repressed. This activation would allow for target gene synthesis. It also showed that when Shh is not present the system represses Gli. The model also showed that the primary cilia had a large impact on the pathway and that mutations that modified the primary cilia reduced efficiency considerably.

This model is the first easily accessible comprehensive computational model of the Shh pathway released at present, and is intended to be usable by others to explore further Shh signalling dynamics.

Introduction

The pathway being investigated in this report is the Sonic Hedgehog (Shh) pathway. The hedgehog family proteins are found in almost every cell in the body. There are 3 distinct members of the Hedgehog family found in mammals: Desert, Indian and Sonic[4]. These proteins are tightly controlled and impact development and cell proliferation of different cell types. Indian Hedgehog controls cell proliferation of chondrocytes which induces bone repair and formation.[5] Desert Hedgehog plays an important part in genital development with malfunctioning DHh causing improper development of the genitals.[6] Sonic Hedgehog is responsible for the development of the neural plate in embryos and stem cell differentiation and gene proliferation and various other genetic activities.

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1 Tickle, Cheryll, and Heather Barker. "The Sonic hedgehog gradient in the developing limb." *Wiley Interdisciplinary Reviews: Developmental Biology* 2.2 (2013): 275-290.

2 Lai, Karen, et al. "Sonic hedgehog regulates adult neural progenitor proliferation in vitro and in vivo." *Nature neuroscience* 6.1 (2003): 21

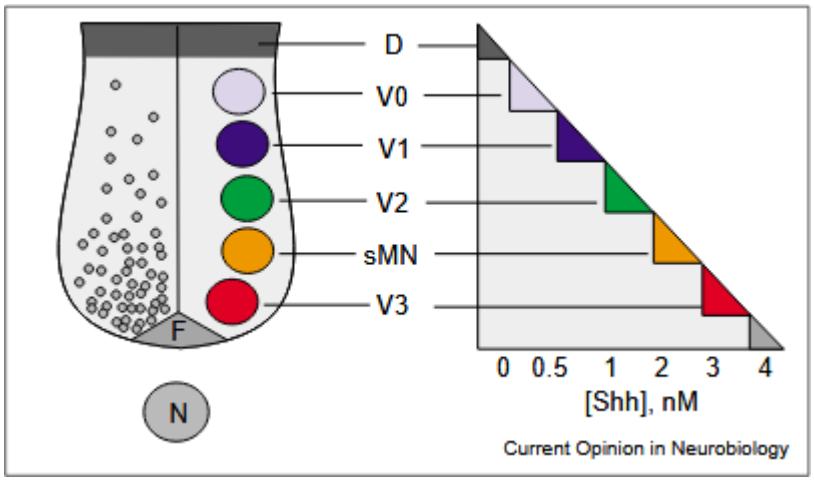
3 Bhardwaj, G., et al. "Sonic hedgehog induces the proliferation of primitive human hematopoietic cells via BMP regulation." *Nature immunology* 2.2 (2001): 172.

4 Heussler, H. S., and M. Suri. "Sonic hedgehog." *Molecular Pathology* 56.3 (2003): 129.

5 Karp, Seth J., et al. "Indian hedgehog coordinates endochondral bone growth and morphogenesis via parathyroid hormone related-protein-dependent and-independent pathways." *Development* 127.3 (2000): 543-548.

6 O'Hara, William A., et al. "Desert hedgehog is a mammal-specific gene expressed during testicular and ovarian development in a marsupial." BMC developmental biology 11.1 (2011): 72.

Shh is the most important of the Hedgehogs though all are vital to development. Shh uses transcription factors, usually the Gli family, to activate proteins for cell proliferation and growth[7]. In development there is a gradient slope across the neural tube which determines the neuron type each cell differentiates into. This concentration-based specialisation of Shh only occurs in embryos. In adult cells the canonical pathway of Shh is very tightly controlled and is highly conserved. Diagram[8]



Shh is responsible for neural differentiation[9] in embryos and cell proliferation and apoptosis in adult cells. Shh is also found to be a cause of glioblastoma tumours[10] and various disorders. Shh is involved in multiple forms of development: throughout all cell ages Shh plays a role in cell differentiation and specialisation[11]. In early embryonic development it plays a major role in the development of the neural tube[12] and development of the earliest stages of neural growth. In the case of a malfunction of Shh expression this early usually results in holoproscencephaly[13] which is often fatal, however surviving infants will often have eyes closer together than is normally expressed in unaffected infants[14]. In very extreme cases it causes cyclopia[15] and malformation of the brain which is expressed via the two hemispheres of the brain being conjoined. However, mutations these extreme are usually fatal and rarely reach birth.[16]

Shh is also a key component of digit development of foetus's fingers and toes. Mutations cause polydactyly[17] (extra digits) and syndactyly[18] (lack of digits), usually conjoined than missing however. In adult cells Shh is

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7 Ashe, Hilary L., and James Briscoe. "The interpretation of morphogen gradients." *Development* 133.3 (2006): 385-394.

8 Briscoe, James, and Johan Ericson. "Specification of neuronal fates in the ventral neural tube." *Current opinion in neurobiology* 11.1 (2001): 43-49.

9 Dutton, Renée, et al. "Sonic hedgehog promotes neuronal differentiation of murine spinal cord precursors and collaborates with neurotrophin 3 to induce Islet-1." *Journal of Neuroscience* 19.7 (1999): 2601-2608.

10 Filbin, Mariella Gruber, et al. "Coordinate activation of Shh and PI3K signaling in PTEN-deficient glioblastoma: new therapeutic opportunities." *Nature medicine* 19.11 (2013): 1518.

11 Dutton, Renée, et al. "Sonic hedgehog promotes neuronal differentiation of murine spinal cord precursors and collaborates with neurotrophin 3 to induce Islet-1." *Journal of Neuroscience* 19.7 (1999): 2601-2608.

12 Ericson, J., et al. "Sonic hedgehog induces the differentiation of ventral forebrain neurons: a common signal for ventral patterning within the neural tube." *Cell* 81.5 (1995): 747-756.

13 Nanni, Luisa, et al. "The mutational spectrum of the sonic hedgehog gene in holoprosencephaly: SHH mutations cause a significant proportion of autosomal dominant holoprosencephaly." *Human molecular genetics* 8.13 (1999): 2479-2488.

14 American Journal of Roentgenology. 1990;154: 143-148. 10.2214/ajr.154.1.2104699

15 Roessler, Erich, et al. "Mutations in the human Sonic Hedgehog gene cause holoprosencephaly." *Nature genetics* 14.3 (1996): 357.

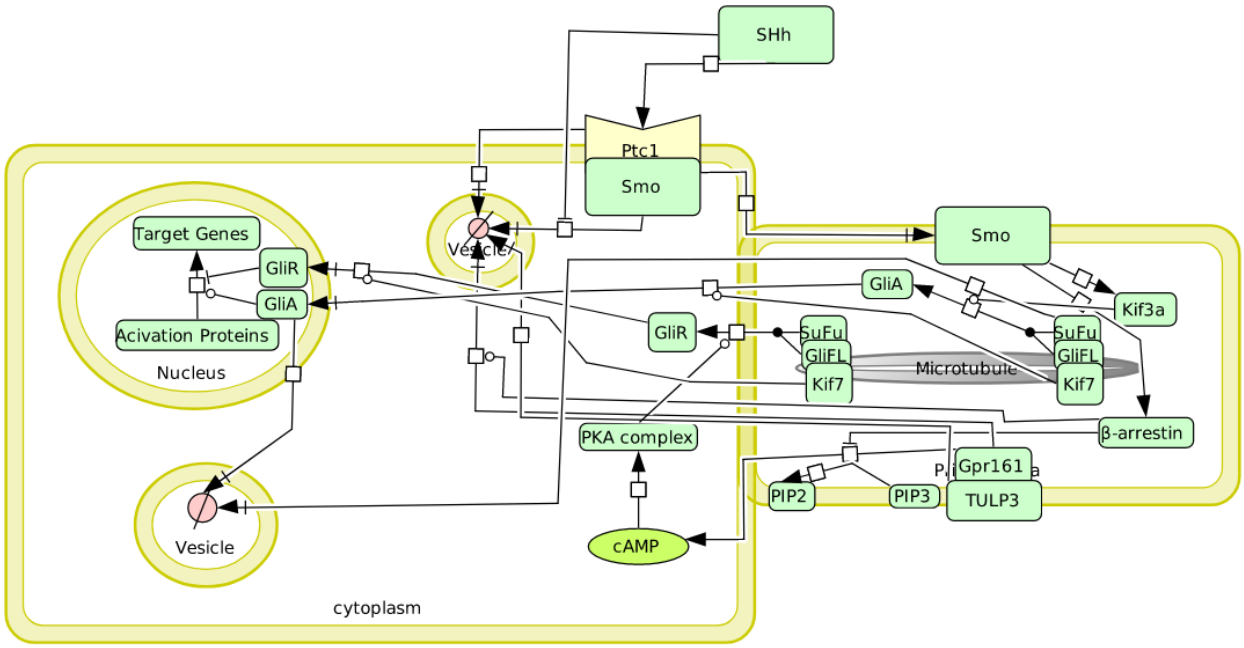
16 https://www.healthline.com/health/cyclopia

17 Anderson, Eve, et al. "Human limb abnormalities caused by disruption of hedgehog signaling." *Trends in Genetics* 28.8 (2012): 364-373.

18 Miner, Jeffrey H., Jeanette Cunningham, and Joshua R. Sanes. "Roles for laminin in embryogenesis: exencephaly, syndactyly, and placentopathy in mice lacking the laminin α5 chain." *The Journal of cell biology* 143.6 (1998): 1713-1723.

used in signalling between neurons and in stem cell proliferation. In neural signalling it is theorised to be impactful on memory recall[19] and in stem cells it is responsible for cell division and apoptosis[20]. Problems with Shh in the adult stage results in glioblastoma and cell death in stem cells and unknown problems within neurons.

The Shh Pathway



Shh is released by cells around the target cell, which then bind to the surface receptor Patched (Ptc1)[21]. After being bound the Shh pathway activates and Shh is immediately removed from the system and stored in vesicles[22].

When Shh binds to Ptc1 it inhibits it. As Ptc1 is inhibited it is no longer able to inhibit Smoothened (Smo), therefore Smo is now in the system[23]. Smo is then transported to the primary cilia[24]. Smo has many important uses in the Shh pathway, Smo inhibits protein kinase A (PKA) by dephosphorylating the complex PKA is in. Smo, Kif3a and β-arrestin are all localised to the primary cilia[25]. The primary cilia is an organelle that is found in most cells, it protrudes from the cell wall and is essential to the Shh pathway. β-arrestin inhibits GPR161 and transports it from the membrane of the primary cilia to a vesicle located outside of the primary

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19 Hung, Hui-Chi, Ya-Hsin Hsiao, and Po-Wu Gean. "Learning induces sonic hedgehog signaling in the amygdala which promotes neurogenesis and long-term memory formation." *International Journal of Neuropsychopharmacology* 18.3 (2015): pyu071.

20 Ahlgren, Sara C., and Marianne Bronner-Fraser. "Inhibition of sonic hedgehog signaling in vivo results in craniofacial neural crest cell death." *Current Biology* 9.22 (1999): 1304-1314.

21 Quijada, Luis, et al. "The patched receptor: Switching on/off the Hedgehog signalling pathway." *Hedgehog-Gli Signalling in Human Disease* (2007): 23.

22 Rohatgi, Rajat, Ljiljana Milenkovic, and Matthew P. Scott. "Patched1 regulates hedgehog signaling at the primary cilium." *Science* 317.5836 (2007): 372-376.

23 Incardona, John P., Jean Gruenberg, and Henk Roelink. "Sonic hedgehog induces the segregation of patched and smoothened in endosomes." *Current Biology* 12.12 (2002): 983-995.

24 Wu, Victoria M., et al. "Small molecule inhibitors of Smoothened ciliary localization and ciliogenesis." *Proceedings of the National Academy of Sciences*109.34 (2012): 13644-13649.

25 Kovacs, Jeffrey J., et al. "β-Arrestin–mediated localization of Smoothened to the primary cilium." *Science* 320.5884 (2008): 1777-1781.

cilia[26]. As GPR161 produces cAMP[27], cAMP is no longer in the system, as it is also an important molecule to the repressive pathway it prevents Gli from being repressed until GPR161 is reactive again.

Kif3a allows the Gli activation reaction to occur[28]. Due to the lack of cAMP, suppressor of fused (SuFu) is unable to repress Gli therefore Gli gets activated and is then transported by Kif7 into the nucleus[29]. GliA then activates the target genes which are then expressed. The target genes expressed work as a negative feedback by synthesising Ptc1 and Gli3 which switches off the active path and turns Gli into its repressive form.

The Biochemical Model

Biochemical modelling is a way to observe how reactions take place without having to physically make the reaction occur. This is done by using the parameters of the real life scenario and using them in the model as well. Compartment sizes, kinetic values, concentrations and molecule-molecule interactions all change the system and are editable variables within computational models. By inputting values for each variable and setting a few reactions with kinetic values you can observe the rate of reaction at any point and the concentrations of all molecules at any point. The concentrations and kinetic values always interact with each other in accordance of the law of mass action.

This model was created to be the first easily accessible Shh model. The software used to create this model (COPASI) is available for all widely used platforms (Windows, Mac & Linux), and is easy to use and understand[30]. This program allows anyone to experiment with models such as this one for free and easily, even with limited understanding. Other models on Shh use much more complicated systems that are not published online, which in certain circumstances is required however, for general use or experimentation to do with the system rather than exact kinetic values a model that is free and easy to use is needed. This model uses ratio values rather than exact values. This allows for a simple understanding of the system and reduces time spent finding exact concentrations and reaction speeds. This simpler version gives reliable results as to what would realistically happen in a true system however, the exact numbers would not be precise. This version is also close to all types of Shh signalling and isn't biased towards any cell type.

Other computational models have been made before however, they were too specific to be used in general research. For example, Cohen *et al.'s* (2015) model was made using complex mathematical equations rather than a simple computer program. Though their numbers are more likely to be correct, it is less intuitive to use[31]. All the Shh canonical pathways use similar systems however each system has its own specific and unique systems to reach their specific outputs. This means that having one system can allow for all pathways to be accommodated via a small modification.

The information used to create this model sourced from various online databases and journal articles. The research was almost always on 3 or fewer molecules, meaning various sources had to be used for each molecule. Sources would rarely give a large amount of useful information. This is because the Shh pathway has not been very thoroughly researched in all areas therefore it is hard to find lots of general information in a single article. Most papers focused on smaller groups of chemicals such as Pct1, Smo and Shh for example rather than a long chain of molecules. This limited research pool and small amount of usable data in each paper means a large amount of research papers have to be examined to find adequate data for the model. Though some articles provide a good framework to be built upon[32]. These more general sources provide a

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[26] Pal, Kasturi, et al. "Smoothened determines β-arrestin–mediated removal of the G protein–coupled receptor Gpr161 from the primary cilium." *J Cell Biol* 212.7 (2016): 861-875.

[27] Minton, Kirsty. "Cell signalling: Putting the brakes on sonic hedgehog." *Nature Reviews Molecular Cell Biology* 14.3 (2013): 129.

[28] Sasai, Noriaki, and James Briscoe. "Primary cilia and graded Sonic Hedgehog signalling." *Wiley Interdisciplinary Reviews: Developmental Biology*1.5 (2012): 753-772.

[29] Barakeh, Duna, et al. "The many faces of KIF7." *Human genome variation* 2 (2015): 15006.

[30] copasi.org

[31] Cohen, Michael, et al. "A theoretical framework for the regulation of Shh morphogen-controlled gene expression." *Development* 141.20 (2014): 3868-3878.

[32] https://www.genome.jp/kegg-bin/show_pathway?hsa04340

good start though are unspecific and need addition sources to back up all of the information they provide. This allows for more focused research which helps reduce the amount of time taken to find suitable articles however the time is still substantial in the scale of weeks finding specific numbers or ratios.

Aims & Hypotheses:

The aim for this model is to advance and accelerate research into the Shh canonical pathways. As all other models are not suitable for the average researcher, having a model such as this will allows research to be done in a faster pace. This new rate of research will hopefully find many new things about the Shh pathways that will have genuine useful effects. As Shh plays a large part in development it could be modified to help with embryonic developmental research. Thus creating a model suited to that pathway allowing a preliminary hypothesis to be tested computationally before being tested practically as well. Shh plays a large part in many deformities, from the aforementioned digit mutations and cancers and apoptosis in adult cells.

Hypotheses for building models are foremost that the model will function and produce outputs similar to those seen in *in vitro* work. We also set out to explore if altering the input level and pattern of Shh effected gene transcription. A final hypothesis was that changing the size of the primary cilia has significant effect on the output of Shh genes through altering the rates of certain reactions.

Method

18	Cyclic_System-1	A + B -> 2 * A + B	Mass action (irreversible)	C
19	Cyclic_System-2	A ->	Mass action (irreversible)	C
20	Cyclic_System-3	B -> 2 * B	Mass action (irreversible)	1
21	Cyclic_System-4	A + B -> A	Mass action (irreversible)	C
22	Cyclic_Release	A -> A + SHh	Mass action (irreversible)	C
23	SHh Reuptake	SHh ->	Mass action (irreversible)	C

Figure A

Fig A is an example readout of the reaction list of a model in COPASI. Reactions are written in normal chemical reaction formats, and named by the modeller. These reactions are converted into differential equations by COPASI and used to track changes to concentrations of these molecules over time.

The model was created using the COPASI[33] (COmplex PAthway SImulation) software. No safety precautions were required, as all work was carried out on a desktop computer (HP Z240 workstation), with risk assessments performed prior to work commencing.

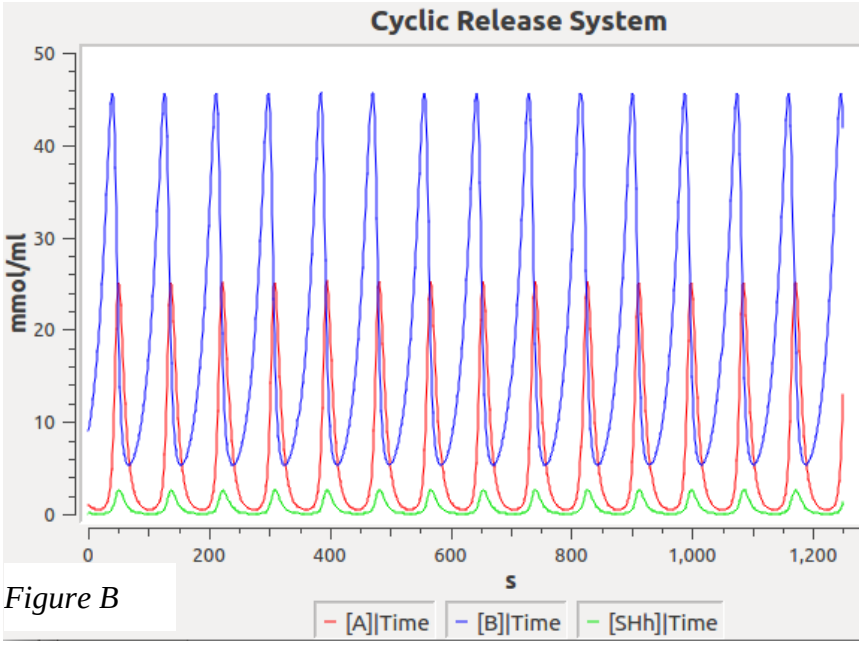

Figure B

Fig B is the graphical version of the reactions found in Fig A.

COPASI uses input values given by the user to create differential equations to find the rates of reactions and in turn find how molecules are affected. This data can be turned into graphs like Fig B.

Fig A shows how reactions are laid out in the COPASI software. Reaction 21 (A + B → A) molecule A and molecule B react together to form molecule A at the loss of one molecule B, therefore this is the degradation of molecule B. This reaction is a basic example as are all the shown reactions. However, when combined they create more a far more advanced system.

[33] Hoops, Stefan, et al. "COPASI—a complex pathway simulator." *Bioinformatics* 22.24 (2006): 3067-3074.

Fig B represents the transient concentrations of each molecule by time in the example above.
This shows how the reactions interact with each other in terms of concentrations by time.

Reactions are one of the many different variables taken into account by the COPASI program. Other controlled variables include compartment size, volumes and kinetic values of reactions. Depending on the system changing these variables will drastically change how systems act.These tables contain the exact information which was used by the master file in COPASI. By copying these values the graph will be recreated exactly alike to the master file.

Fig C is the compartments, Fig D is the reactants, Fig E is the reactions with kinetic values. Fig F is the global quantities.

Compartment Name	Volume (ml)	Type
Cytoplasm	1	Fixed
Primary Cilia	0.05	Fixed

Figure C

Species	Compartment	Initial concentration
Shh	Cytoplasm	0
Ptc1	Cytoplasm	0.75
Ptc1(I)	Cytoplasm	0
Smo	Cytoplasm	0
Smo(I)	Cytoplasm	0.35
Smo(p)	Primary Cilia	0
SuFu	Primary Cilia	0.8
SuFu(A)	Cytoplasm	0
cAMP	Cytoplasm	0
PIP3	Primary Cilia	0.2
PIP3(I)	Primary Cilia	0
PKA	Cytoplasm	1
PKA(c)	Cytoplasm	0
β-arrestin	Primary Cilia	0
Kif3a	Primary Cilia	0
Gli(FL)	Cytoplasm	1
Gli(A)	Cytoplasm	0
Gli(R)	Cytoplasm	0
Shh Target Genes	Cytoplasm	0
A	Cytoplasm	1
B	Cytoplasm	9

Figure D

Reaction Name	Reactions	Kinetic Value of Reaction
Smo Inhibition	Smo + Ptc1 -> Smo(I)	0.1
Ptc1 Inhibition	Shh + Smo(I) -> Ptc1(I) + Smo	0.1
Repression of Gli	SuFu(A) + Gli(FL) -> Gli(R) + SuFu(A)	0.1
Activation of SuFu	SuFu + PKA(c) -> SuFu(A)	0.1
Localisation of cAMP	PIP3 -> cAMP + PIP3	0.1
Localisation of β-arrestin & Kif3a	Smo(p) -> β-arrestin + Kif3a + Smo	0.1
Suppression of PIP3	β-arrestin + PIP3 -> PIP3(I)	0.1
Activation of Genes	Gli(A) -> Shh-Target-Genes	0.1
cAMP Removal/Degradation	cAMP ->	0.1
Repression of Genes	Gli(R) + Shh-Target-Genes ->	1
Activation of Gli	Gli(FL) + Kif3a -> Gli(A)	0.1
Ptc1(I) Degradation	Ptc1(I) ->	0.1
SuFu Degradation down Active Path	SuFu + Gli(A) -> Gli(A)	0.1
Transcription of protein	Shh-Target-Genes -> Gli(FL) + Ptc1	*
β-arrestin reuptake	β-arrestin ->	0.1
Repression of SuFu(A)	SuFu(A) + Smo(p) -> SuFu + Smo(p)	0.1
PIP3 reactivation	PIP3(I) -> PIP3	0.02
Cyclic_System-1	A + B -> 2 * A + B	0.008
Cyclic_System-2	A ->	0.15
Cyclic_System-3	B -> 2 * B	0.05
Cyclic_System-4	A + B -> A	0.008
Cyclic_Release	A -> A + Shh	0.19
Shh Reuptake	Shh ->	1.8
Activation of PKA	PKA + cAMP -> PKA(c)	0.35
Deactivation of the PKA complex	PKA(c) -> PKA + cAMP	0.27
Phosphorylation of Smo	PKA + Smo -> PKA + Smo(p)	0.1
Dephosphorylation of Smo	PKA + Smo(p) -> PKA + Smo	0.1
Kif3a Relocation	Kif3a ->	*

Figure E

Note that all reactions used were irreversible and so backwards reactions were written separately
* see Fig F

Global Quantity	Reaction	Input	Unit
Shh Dependant Transcription	Shh-Target-Genes -> Gli(FL) + Ptc1	if({[Shh]} < 0.2,2,0)	s^{-1}
Kif3a Degradation	Kif3a ->	if({Kif3a]} >= 5,3,0.1)	s^{-1}

Figure F

Fig C-F are all numerical values input into COPASI, Assume all values are estimates unless stated otherwise.

Results:

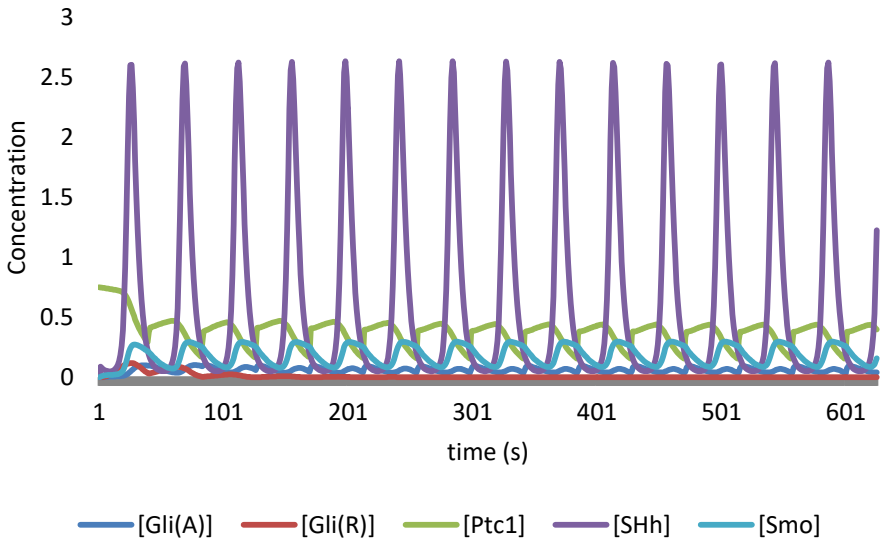

Figure G

Fig G shows the model working. It is a selection of the most vital proteins in the system and is a visual guide to how the system affects each protein. Recreating the system in COPASI will allow you to observe the true graph of all proteins in the system. The model can be modified to recreate different mutations that may occur in the body, such as, ciliopathy. To modify it this way you would change the primary cilia to have the same volume as the cytoplasm as this recreates the effect of ciliopathy. Modifications allow for the observation of different types of mutations which affect the Shh pathway or for investigating if a specific mutation would make a large impact on the signalling.

Kinetic values for enzymatic reactions for example (The affinity of bonding between molecules).
Investigations are able to be carried out easily by changing single figures and observing graphs or by using parameter scans and using information given by the scans. Parameter scans are a tool that COPASI provides, the scans replaces a variable with multiple values and creates a graph which displays the differences between each value.

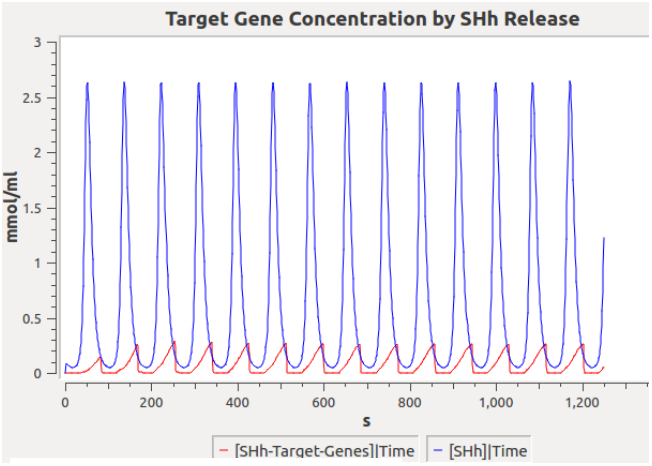

Figure H

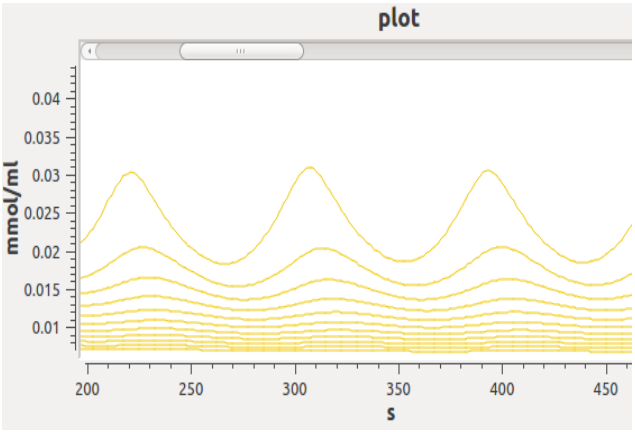

Figure I

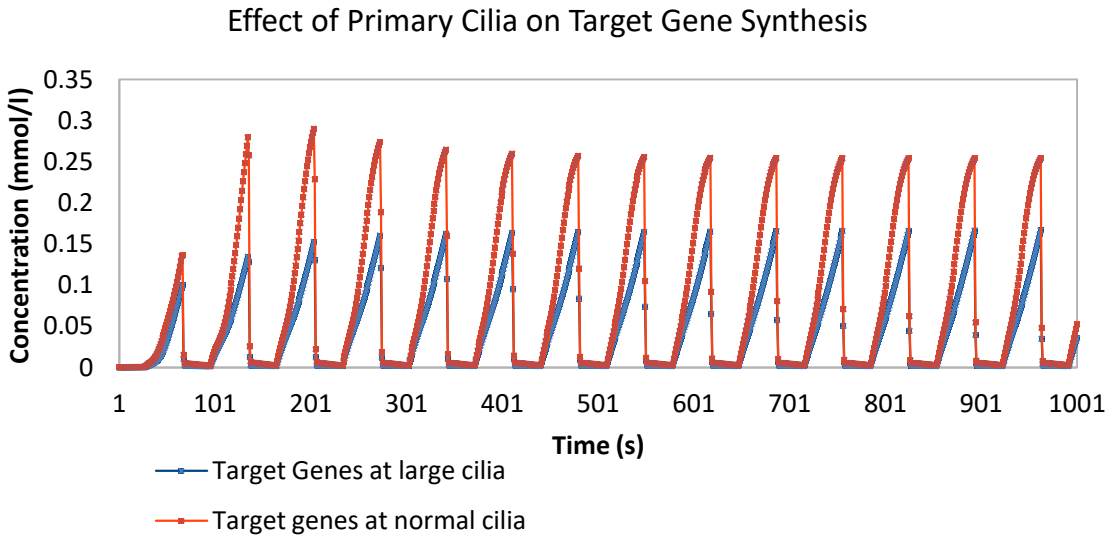

Figure J

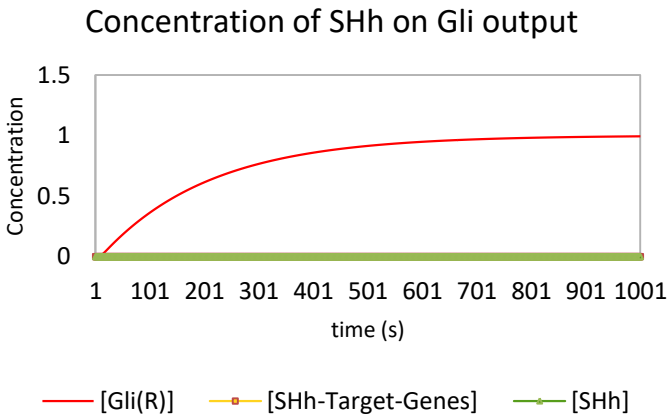

Figure K (note that Shh-Target-Genes and Shh are both = 0

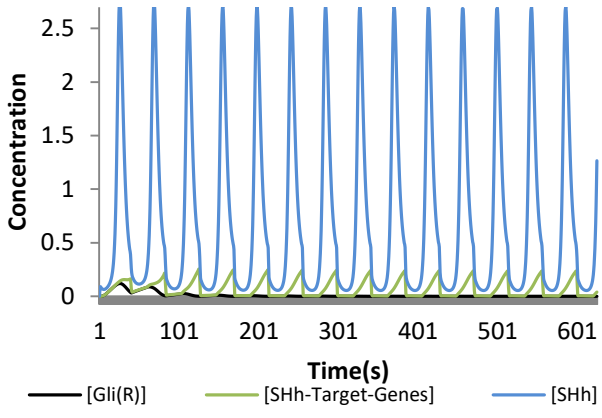

Figure L

Fig H shows clearly that as Shh levels increase the level of target genes also increases. This is the expected response of the pathway and so provides evidence for the reliability of the model for further results.

Fig I represents the concentration of cAMP in the system at different sizes of the primary cilia, the parameter scan shows the difference in concentration between 0.025 ml and 1ml. As previously mentioned cAMP is used in the repressive cycle of the Shh pathway. So by the changing the primary cilia volume we can observe if the repressive pathway activates. This corroborates with other research that claims that the Shh pathway is unable to activate without the primary cilia[34]. Note that the top lines were found in larger cilia sizes and the bottom lines were found in smaller cilia sizes.

Figure J

Fig J depicts the concentrations of Shh target gene synthesis by time at different volumes of the primary cilia (the larger cilia was large enough to be considered missing from the system). While in vivo experiments show that the larger cilia/ non-existent cilia via ciliopathy are unable to actually make it this far in the pathway, however, the proteins required to make this same effect in the model broke the system. So while it was unable to prove the inactivity of that path with ciliopathy it could prove decreased efficiency in the rate of target gene synthesis, which agrees with experimental data already released.

Fig K and Fig L demonstrate the difference in the system when Shh is introduced and missing from the system. In these two examples the pattern between Shh present and not present shows that Gli(R) is only synthesised when Shh is not present or is not at the point to affect Gli synthesis reactions. In the model it takes around 40 seconds to affect Gli synthesis. Therefore it takes 2 cycles to prevent Gli(R) from being synthesised which is shown in Fig K.

[34] Goetz, Sarah C., and Kathryn V. Anderson. "The primary cilium: a signalling centre during vertebrate development." Nature Reviews Genetics 11.5 (2010): 331.

Discussion

Fig G shows that the model works in accordance with published papers and is able to be used for specific proteins information. It follows the pathways important proteins but shows that the interactions between molecules are in place in order for this result to be reached. This shows that the model is able to recreate each separate part of the system and so is able to recreate mutations or experiments and give expected responses.

The model was able to prove that the primary cilium plays an important part of the system and that the system is Shh dependant in order to activate. This was backed up by studies and research[35]. These experiments showed that the smoothened pathway was activated by Shh. It also showed that if more Shh is in the system that more Shh-Target-Genes are also created, thus showing a possible cause of cancers or polydactyly. This would require practical experiments to confirm if Shh concentrations are higher or altered in any way in comparison to ordinary cells. This could be done using north western blots or other microscopic measurement techniques.

Fig H demonstrates that Shh concentrations has a significant effect on Shh-Target-Genes as when Shh increases there is a corresponding increase in Shh-Target-Genes and as Shh decreases the Shh-Target-Genes also decreases. The rate of synthesis of Shh-Target-Genes is not true to real time as it takes roughly 4-24 hours to begin synthesis[36]. This was accelerated to make the graph readable within the chosen timeframe despite it being arbitrary.

Fig I shows the concentrations of cAMP by differing cilia sizes in a Shh present system. With the highest levels in a ciliaopathic systems (cilia being non existant or mutated) this shows that increased amounts of cAMP are localised in the cell when cilia are affected by mutations. This shows that the signalling pathway is repressive when mutated.

Fig J shows the concentration difference between Shh-Target-Genes at differing primary cilia volumes. The enlarged cilia shows a drastic decrease in Shh-Target-Genes this illustrates how the primary cilia plays a vital role in the pathway as this change in concentration could result in under activity or a overall repression of the pathway.

Fig K and L represents the modification of Gli by the concentration of Shh. It clearly demonstrates that in a Shh present system the Gli is activated and that in a Shh non-present system it is repressed. This change is a vital step of the pathway as it dictates how Shh-Target-Genes are expressed. If Gli is repressed it prevents synthesis of target proteins (Ptc1 and Gli) and causes the pathway to stop as Gli is not produced. If Gli is activated it causes the Shh-Target-Genes to be expressed which in turn synthesis the target proteins. As the proteins are synthesised the negative feedback given off by Ptc1 will switch off the pathway.

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35 https://www.genome.jp/kegg-bin/show_pathway?hsa04340

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Future Directions

The model could be improved by adding a more complex system to do with the PIP3 and GPR161 interactions. GPR161 is a protein found in the primary cilia that recruits cAMP to the primary cilia as well. When this occurs it is part of the repressive cycle of the pathway. PIP3 is a protein that allows GPR161 to bind to the primary cilia. Therefore by inhibiting PIP3, GPR161 is removed from the primary cilia. I tried to create these interactions however my usual method of coding broke the system so I had to simplify the system to only include a fraction of the true chemicals. Using proteins such as TULP3 and the reaction of PIP3 to PIP2 which would unbind GPR161 to the primary cilia would allow the system to be more realistic though I could not find a way to code this into COPASI without making system errors.

Conclusion

I have built the canonical pathway Sonic Hedgehog signalling protein using COPASI. It is an easy to use and edit model that is general enough to be used in most general research and is able to be modified for specific scenarios. It has found that the primary cilia is vital to proper signalling and the main molecular switch in the pathway is Shh. These findings can be built upon by using practical experiments to backup the findings or finding other papers that corroborate with this information.

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