Lecture 14: Cell Communication I: Cell Signaling and Signal Transduction

10801040 莊博恩、10801128 陳俊鴻

1. Brief Intro
2. 細胞溝通(cell communication)：包括生物與環境、不同生物間(單與多細胞)，同一生物內不同細胞間(多細胞)，需要receptor(有特別的Specificity, sensitivity, location, under control)接受，轉化成細胞可以理解的訊號(signal interpretation)
3. Signal interpretation：包括這個signal怎麼被amplify、diversify(同時control很多pathway)、intergration(不同訊號間進行整合)，細胞接著根據signal做出不同response(不同cell對同一個訊號可能會有不同反應)，而從發出訊號到接收訊號的cell有幾種不同pathway(內分泌、旁分泌、神經元、contact-dependent)。
4. 關於glycogen phosphoryase的cell communication(用了很多方法推出了完整的pathway，我將最有收穫的一個寫下)：為什麼hormone和細胞表面的receptor結合會去活化AC？genetic approach發現有一種動物cell表面具有Epinephrine receptor，給他Epinephrine後，AC被活化，然後細胞死了→去找沒有死的細胞，可能是這條路上有東西壞掉了→有一個mutant的receptor是好的，AC也是好的(透過NaF驗證)，但receptor和hormone結合以後，AC卻沒有被活化，代表這兩者中間可能有東西壞掉→丟cell membrane裡的蛋白看能不能使AC活化，最後找到了G protein，證明receptor要活化AC，要先和G protein結合，再讓活化的G protein去執行。
5. Desensitization：細胞為了保護自己，不希望任何一個signaling持續不斷在cell裡維持很高濃度(例如hormone還在，AC活性卻下降)，機制為receptor和hormone結合後，receptor會被磷酸化並吸引一個蛋白過來結合，最後雖然有hormone在receptor上，但receptor不再去活化AC。
6. Q&A

Question 1:

細胞分化時，有一部分的細胞會分化為表皮細胞，有的分化為神經細胞，有的分化為結締組織等，是受什麼機制控制？老師有提到一個細胞會透過傳達訊息給周圍的細胞讓其他細胞不要分化，自己分化為某種細胞，然而我還是有疑惑，細胞怎麼知道自己要分化成哪一種細胞？(表皮、神經…)另外，個體要如何 讓各種細胞維持一定的比例？(甚麼機制讓所有細胞不會都分化為同一種細胞、或某一種細胞特別多的情況？)

Answer 1:

後來我查到了morphogen，在細胞尚未分化時，morphogen會由source cell製造出來，擴散到鄰近的組織，形成morphogen的濃度梯度，而不同濃度的morphogen會刺激一致的、尚未分化的細胞分化出不同的細胞種類，將一大群細胞依據morphogen的濃度梯度分開，最後形成身體不同的組織或器官(我有想過source cell又是怎麼分化來的？但我查了很多資料，沒有找到理想的答案，我猜想source cell可能在細胞早期，很多細胞尚未分化時就已形成，然後分泌morphogen來促進其餘細胞開始分化為各種組織)

During the course of development, cells of many tissues differentiate according to the positional information that is set by the concentration gradients of morphogens. Morphogens are signaling molecules that emanate from a restricted region of a tissue and spread away from their source to form a concentration gradient. As the fate of each cell in the field depends on the concentration of the morphogen signal, the gradient prefigures the pattern of development.

為什麼不同濃度的morphogen可以傳遞訊息、促進尚未分化的細胞分化成不同組織？因為每個細胞中都存在會被morphogen的刺激的target genes，但要使這些target genes表現的morphogen濃度門檻不同，當細胞離source cell很近，接收到濃度較高的morphogen， low- and high-threshold target genes都會被活化，但若細胞離source cell較遠，接收到的濃度較低，將只能活化low-threshold target genes，而不同基因的活化組合將會分化成不同種類的細胞。

Cells close to the source of morphogen will receive high levels of morphogen and will express both low- and high-threshold target genes. In contrast, cells far away from the source of morphogen receive low levels of morphogen and express only low-threshold target genes. The identity of particular body regions emerges as a consequence of the different combinations of target gene expression.

([https://books.google.com.tw/books?id=PtdKDgAAQBAJ&printsec=frontcover&hl=zh-TW#v=onepage&q&f=false](https://books.google.com.tw/books?id=PtdKDgAAQBAJ&printsec=frontcover&hl=zh-TW" \l "v=onepage&q&f=false))

而morphogens不只一種，包括retinoic acid, sonic hedgehog (SHH), transforming growth factor beta (TGF-β)/bone morphogenic protein (BMP), and Wnt/beta-catenin.

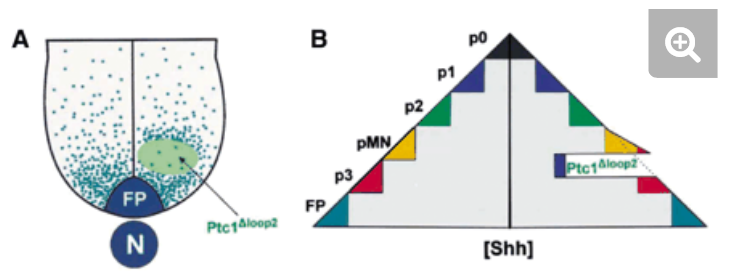
每種morphogens會負責生物體的某一部分的分化，例如Shh 促進neural tube development、BMP負責formation of bone and cartilage等

舉例：Shh (促進neural tube development)

During development of the chick neural tube, Shh does function as a morphogen. Shh emanates from the notochord to induce formation of the floor plate. Subsequent *Shh* expression in the floor plate generates a ventral-dorsal activity gradient of Shh that promotes the specification of a series of ventral cell types.

The activity gradient of Shh promotes the specification of a series of ventral cell types: p0, p1, p2, pMN and p3, which are progenitor domains from which distinct V0 neurons, V1 neurons, V2 neurons, motoneurons and V3 neurons are generated respectively. Production of a mutated form of the Shh receptor Ptc (Ptc1Δloop2; A; right half; light green), which does not bind Shh but antagonizes its signaling, causes cell-autonomous abnormal dorsal spread of Shh and (B; right half) ventral-todorsal switches in neural progenitor identity. Modified with permission from Briscoe et al.

在neural tube中，notochord會先分泌Shh，刺激floor plate的形成，接著floor plate中的Shh分泌，產生腹-背向的Shh梯度濃度(如下圖A)，進而促使細胞分化為一系列不同種類的p3, pMN , p2, p1,p0(依濃度遞減，如下圖B)。

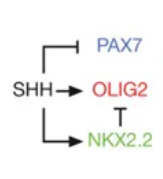


想到這裡，我轉而想到了另一個問題，如果濃度越高，代表morphogen要傳遞的訊號越強烈，使high-threshold target genes的基因能夠表現，那這個訊號是能夠隨時間累積的嗎？也就是說，morphogen若持續越久，就會使促進target genes表現的信號不斷累積增加；低濃度的morphogen持續時間夠久，也能夠使high-threshold target genes表現，還是這個門檻只限於監測濃度，不管時間過了多久都不影響？

**Interpretation of the sonic hedgehog morphogen gradient by a temporal adaptation mechanism**

<https://www.nature.com/articles/nature06347#MOESM245>

在實驗中研究了三個transcription factors的基因，分別是OLIG2, NKX2.2,和PAX7(這三個基因後來我去查了一下(註一)，似乎都是可以促進特定細胞分化的基因，我認為這應該就是上敘中需要濃度達門檻才能被表現、會被morphogen刺激，使細胞分化的基因)

We focused on three transcription factors that respond to differential SHH signalling in progenitors of the neural tube. OLIG2 and NKX2.2, expressed in the ventral neural tube of chick, depend on SHH signalling for their expression[4](https://www.nature.com/articles/nature06347" \l "ref-CR4" \o "Briscoe, J. & Ericson, J. Specification of neuronal fates in the ventral neural tube. Curr. Opin. Neurobiol. 11, 43–49 (2001)),[11](https://www.nature.com/articles/nature06347" \l "ref-CR11" \o "Lei, Q. et al. Wnt signaling inhibitors regulate the transcriptional response to morphogenetic Shh-Gli signaling in the neural tube. Dev. Cell 11, 325–337 (2006)),[12](https://www.nature.com/articles/nature06347" \l "ref-CR12" \o "Novitch, B. G., Chen, A. I. & Jessell, T. M. Coordinate regulation of motor neuron subtype identity and pan-neuronal properties by the bHLH repressor Olig2. Neuron 31, 773–789 (2001)). In contrast, PAX7 expression is repressed by SHH signaling

(註一：僅簡單舉其中一個例子OLIG2)

OLIG2 is well known for determining [motor neuron](https://en.wikipedia.org/wiki/Motor_neuron" \o "Motor neuron) and [oligodendrocyte](https://en.wikipedia.org/wiki/Oligodendrocyte" \o "Oligodendrocyte) differentiation

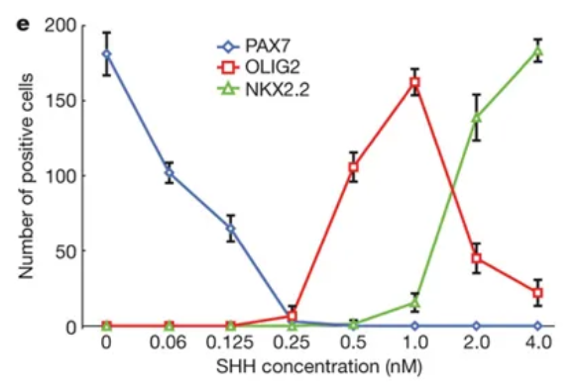
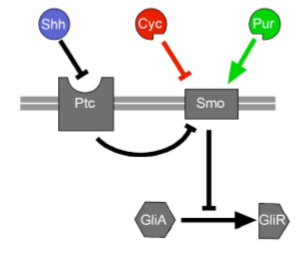
During embryogenesis, OLIG2 first directs motor neuron fate by establishing a ventral domain of motor neuron progenitors and promoting neuronal differentiation.

OLIG2 then switches to promoting the formation of oligodendrocyte precursors and oligodendrocyte differentiation at later stages of development.

在實驗中，用不同濃度的SHH (morphogen)可以使三個基因有不同的表現(符合前面提到的想法)，另外，除了產生SHH，用不同濃度的GLI或是調控SMO的活性也可以產生和SHH相似的效果(基因因為GLI的濃度不同或SMO的活性大小不同而有不同的反應)，而這個可以從SHH作用機制的關係圖解釋，GLI是SHH的transcriptional effector(也就是SHH會透過產生GLI來調控target genes(註二)，我認為GLI有點像SHH產生的、使細胞分化的signal)，而SMO位於SHH產生GLI的途徑中，因此直接調控SMO的活性也會影響基因的表現

註二：Activated GLI accumulates in the nucleus (Process "6") and controls the transcription of hedgehog target genes

We confirmed that these proteins respond to different levels of SHH signalling using an *ex vivo* assay of intermediate region naive neural plate explants[14](https://www.nature.com/articles/nature06347" \l "ref-CR14" \o "Yamada, T., Pfaff, S. L., Edlund, T. & Jessell, T. M. Control of cell pattern in the neural tube: motor neuron induction by diffusible factors from notochord and floor plate. Cell 73, 673–686 (1993)) ([Fig. 1b–d](https://www.nature.com/articles/nature06347" \l "Fig1)). In agreement with previous studies[13](https://www.nature.com/articles/nature06347" \l "ref-CR13" \o "Ericson, J. et al. Pax6 controls progenitor cell identity and neuronal fate in response to graded Shh signaling. Cell 90, 169–180 (1997)),[15](https://www.nature.com/articles/nature06347" \l "ref-CR15" \o "Ericson, J., Morton, S., Kawakami, A., Roelink, H. & Jessell, T. M. Two critical periods of Sonic Hedgehog signaling required for the specification of motor neuron identity. Cell 87, 661–673 (1996)), changes in SHH concentration controlled the expression of these genes in a manner corresponding to their *in vivo* expression patterns ([Fig. 1e](https://www.nature.com/articles/nature06347" \l "Fig1)). Similar gene expression responses were obtained by generating, *in vivo*, a gradient of GLI transcriptional activity[16](https://www.nature.com/articles/nature06347" \l "ref-CR16" \o "Lei, Q., Zelman, A. K., Kuang, E., Li, S. & Matise, M. P. Transduction of graded Hedgehog signaling by a combination of Gli2 and Gli3 activator functions in the developing spinal cord. Development 131, 3593–3604 (2004)),[17](https://www.nature.com/articles/nature06347" \l "ref-CR17" \o "Stamataki, D., Ulloa, F., Tsoni, S. V., Mynett, A. & Briscoe, J. A gradient of Gli activity mediates graded Sonic Hedgehog signaling in the neural tube. Genes Dev. 19, 626–641 (2005)), the transcriptional effectors of SHH signalling. Furthermore, manipulation of the activity of the transmembrane protein smoothened (SMO), which transduces SHH signalling intracellularly[18](https://www.nature.com/articles/nature06347" \l "ref-CR18" \o "Lum, L. & Beachy, P. A. The Hedgehog response network: sensors, switches, and routers. Science 304, 1755–1759 (2004)), was also sufficient to confer graded responses to neural cells



首先實驗中發現

1. NKX2.2比OLIG2晚開始表現(NKX2.2超過12小時才開始表現，OLIG2在六小時前即開始)
2. 能使NKX2.2表現的濃度(SHH濃度通常要大於2 nM才能使NKX2.2表現)會造成OLIG2 transient expression(我查不到這個單字的意思，我猜測是在很短時間內OLIG2即大量表現)，所以在前12小時，SHH ≥1 nM 的OLIG2的濃度都差不多(SHH濃度造成的影響小，因為OLIG2大量表現，NKX2.2又尚未出現來抑制OLIG2)

這兩個觀察指出了OLIG2和NKX2.2的sequential onset(先產生OLIG2)，另外，Genetic lineage tracing 也證明了NKX2.2要被先前已經活化OLIG2的細胞誘發，說明NKX2.2需要比OLIG2高的SHH濃度及與SHH比較長的接觸時間

We asked how the response of cells to SHH develops over time. We assayed intermediate region neural plate explants exposed to SHH for 6 h to 24 h ([Fig. 1f, g](https://www.nature.com/articles/nature06347#Fig1) and [Supplementary Table 1](https://www.nature.com/articles/nature06347#MOESM245)). NKX2.2 induction was delayed compared to OLIG2, taking >12 h compared to ∼6 h for OLIG2. Moreover, NKX2.2-inducing concentrations of SHH (≥2 nM) produced a transient expression of OLIG2 ([Fig. 1f, g](https://www.nature.com/articles/nature06347#Fig1)). Thus, during the first 12 h, ≥1 nM SHH generated similar amounts of OLIG2 induction ([Supplementary Table 1](https://www.nature.com/articles/nature06347#MOESM245)). Only after 12 h were distinct responses apparent. By 18 h, OLIG2 and NKX2.2 co-expression was evident in some progenitors treated with ≥2 nM SHH ([Fig. 1f](https://www.nature.com/articles/nature06347#Fig1)). These results are consistent with the sequential onset of OLIG2 and NKX2.2 expression *in vivo*[10](https://www.nature.com/articles/nature06347#ref-CR10),[17](https://www.nature.com/articles/nature06347#ref-CR17). Furthermore, the explant data predict that, *in vivo*, NKX2.2 should be induced in cells that previously expressed OLIG2. Genetic lineage tracing in mice harbouring an *Olig2* allele engineered to encode Cre recombinase confirmed this ([Fig. 1h](https://www.nature.com/articles/nature06347#Fig1)). Thus, compared to OLIG2, induction of NKX2.2 requires a higher concentration and longer duration of SHH exposure.

因為NKX2.2需要較長時間和較高濃度，說明時間和SHH濃度這兩項參數是很有可能會影響使細胞分化的signal，故接下來就看SHH的signal(GLI)到底會怎麼改變

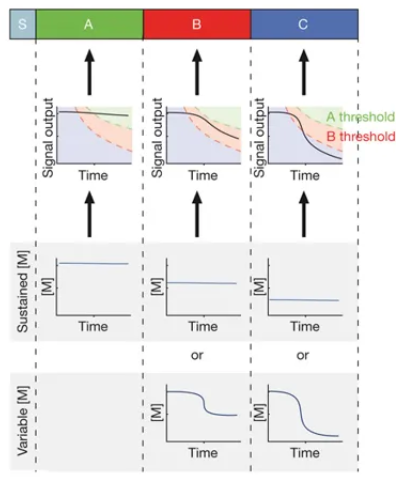
實驗發現

1. 除了最高濃度的SHH，GLI的活性(由luciferase濃度測得)會隨時間下降，且下降的速率和SHH濃度成反比
2. 1 nM和4 nM SHH一開始會產生出活性差不多的GLI，後來GLI活性開始有差異的時間點，恰巧是target gene表現有較大差異的時間點(說明不同的signal造成target gene有不同的反應)

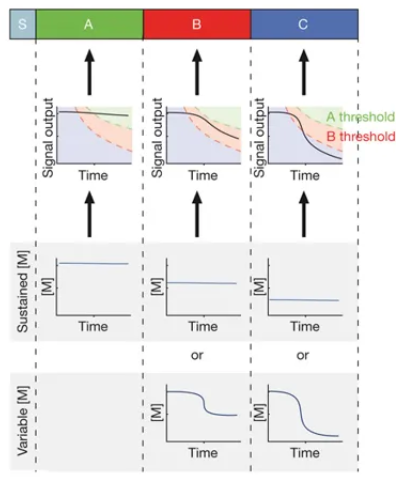
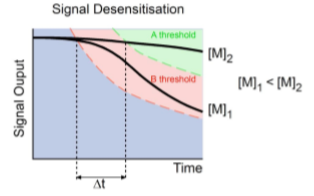
推測：細胞對持續的SHH產生了desensitized，GLI活性因而下降(這好像就是老師影片提到的temporal adaptation)，也就是SHH的作用轉為一段有時間限制的signal(GLI)，持續時間即和SHH的濃度有成正比，而這個signal將會影響基因的表現

To investigate the reason for the temporal and concentration dependence of the response, we analysed the output of the SHH signal transduction pathway. We assayed GLI activity using a reporter plasmid[19](https://www.nature.com/articles/nature06347#ref-CR19) (GBS-Luc; see [Fig. 2a](https://www.nature.com/articles/nature06347#Fig2) and Methods). Taking advantage of the short half-life of luciferase[20](https://www.nature.com/articles/nature06347#ref-CR20) (∼3 h), we measured GBS-Luc activity every 6 h. For concentrations of SHH ≥1 nM, GLI activity was similar during the first 12 h ([Fig. 2a](https://www.nature.com/articles/nature06347#Fig2)), the period when these concentrations induce OLIG2. Then, with the exception of the highest SHH concentration, GLI activity decreased over time, with a rate inversely proportional to SHH concentration ([Fig. 2a](https://www.nature.com/articles/nature06347#Fig2)). Notably, the time at which 1 nM and 4 nM SHH produced differences in the level of GLI activity corresponded to the detection of differences in gene expression ([Fig. 1f, g](https://www.nature.com/articles/nature06347#Fig1) and [Supplementary Table 1](https://www.nature.com/articles/nature06347#MOESM245)). The data indicate, therefore, that cells become progressively desensitized to ongoing SHH signalling ([Supplementary Fig. 1](https://www.nature.com/articles/nature06347#MOESM245)). Initially 1 nM and 4 nM SHH produce similar levels of GLI activity, then the level of GLI activity begins to fall, with a higher rate of decrease in cells exposed to lower concentrations. This suggests a mechanism for gradient sensing in which ‘temporal adaptation’ to the ligand transforms the extracellular concentration of morphogen into a time-limited period of signal transduction, such that the duration of signalling is proportional to ligand concentration.

現在知道SHH signal的持續時間會受morphogen濃度的影響，也知道morphogen濃度會透過signal影響到基因表現，那是否可推測signal的持續時間會影響到基因的表現？因此作了以下的實驗，在NVF explants(包含神經管的ventral regions以及製造SHH的notochord＆floor plate)中，OLIG2和NKX2.2分別會在第12和18小時的時候被活化，而在這期間GLI活性幾乎沒有變動，說明signal的大小沒有改變時，基因能仍在這期間活化，更代表可能和signal持續時間有關，因此又增加了一個實驗，將NVF explants培養12個小時後移至有cyclopamine(SHH signalling的抑制物)的media內，並在18小時的時候監測，發現若cyclopamine的含量為200，GLI活性會降至一半，將無法表現NKX2.2，若含量為400，GLI活性會降至與原來的背景相同，將無法表現OLIG2和NKX2.2，說明GLI活性的持續時間對基因表現是很重要的(明顯的只讓GLI作用12小時將無法表現OLIG2和NKX2.2)，另外，為了加強驗證，實驗將細胞和SHH接觸的時間延長至48小時，觀察達到表現OLIG2和NKX2.2的高峰時的SHH濃度，接觸48小時的SHH濃度低於接觸32小時的濃度(這符合猜測，因為和SHH接觸48個小時就如同下圖的sustain morphogen，會和剛開始濃度較高，接觸時間卻較少(32小時)的variable morphogen相同：雖然接觸32小時的初始morphogen較高(signal desensitized慢)，卻因為接觸時間不足，使得時間後期的morphogen濃度反而較接觸48小時的morphogen低，讓signal desensitized的速率較快，因此兩者發出的signal相當)



This model predicts that the response of cells relies not only on the level but also on the duration of intracellular signal transduction. To test this, we compared GLI activity and gene expression in neural cells containing an endogenous source of SHH. In explants consisting of SHH-producing notochord and floor plate together with ventral regions of the neural tube (hereafter called NVF explants), the expression of OLIG2 and NKX2.2 was induced sequentially, 12 h and 18 h after the start of culture, respectively ([Fig. 2f–h](https://www.nature.com/articles/nature06347#Fig2)). During this period the level of GLI activity remained approximately constant, confirming that the switch to NKX2.2 expression was not associated with an increase in GLI activity ([Fig. 2b, f–h](https://www.nature.com/articles/nature06347#Fig2)). To examine whether the maintenance of an OLIG2-expressing state depends on the downregulation of GLI activity, NVF explants were cultured for 12 h and then transferred to media containing cyclopamine, a small-molecule antagonist of SHH signalling ([Supplementary Fig. 2](https://www.nature.com/articles/nature06347#MOESM245)). GLI activity and gene expression were monitored at 18 h ([Fig. 2c–e, i](https://www.nature.com/articles/nature06347#Fig2)). Addition of 200 nM cyclopamine at 12 h resulted in a twofold decrease in GLI activity and a failure to induce NKX2.2 expression ([Fig. 2c, e, i](https://www.nature.com/articles/nature06347#Fig2)) without inhibiting OLIG2 expression. Furthermore, addition of 400 nM cyclopamine inhibited GLI activity to background levels, leading to a complete loss of NKX2.2 expression and a decrease in the number of OLIG2-expressing cells ([Fig. 2c, e](https://www.nature.com/articles/nature06347#Fig2), and data not shown). These data indicate that the duration of GLI activity is crucial for determining the cellular response to SHH. To test the converse prediction of the model, we assayed the effect of extending the period of SHH signalling ([Fig. 2j](https://www.nature.com/articles/nature06347#Fig2)). Consistent with the model, prolonging SHH signalling resulted in peak OLIG2 and NKX2.2 induction at lower SHH concentrations after 48 h of exposure compared to 24 h and 36 h ([Fig. 2j](https://www.nature.com/articles/nature06347#Fig2)).

總結：morphogen的濃度和持續時間都會影響基因表現，細胞將morphogen的轉成一段有時間限制的signal(GLI)(初始signal的level都差不多)，這個signal會以和morphogen濃度成反比的速率desensitized，故morphogen的濃度越高、持續時間越長都能視為提供一個不易desensitized的signal(右下圖)，而signal不易desensitized(持續時間越長)，就有更大的機率能達到表現基因的門檻(如右上圖)，也因為不同基因的門檻高低不同，細胞總是會先活化門檻較低(B threshold)的基因再活化門檻較高的基因(A threshold)，解釋了為何OLIG2總是比NKX2.2早開始表現，這個signal transduction機制使的細胞可以融合morphogen的濃度和持續時間參數，轉化成signal後活化促進細胞分化的基因，不同基因的組合可以使細胞分化成不同的細胞，這就是morphogen促進細胞分化的機制。我認為這個機制解決了我提出的問題，細胞透過morphogen知道自己會分化成哪種細胞，而不同濃度和持續時間的morphogen，決定了不同種類的細胞，使各種細胞都能維持一定的比例，以維持正常的生理功能及生物體的運作。

Question 2:

在體內常有同樣的signaling molecule卻給身體不同組織帶來各異的功能(比方說parathyroid hormone在骨骼中誘導osteoblast在細胞膜表現RANKL使osteoclast的RANK偵測並刺激osteoclast對骨骼的破壞，在腎臟卻是誘導distal tubules和renal collecting ducts打開L-type鈣離子通道以增加鈣離子通透性)，為何？

Answer 2:

我原先是假設由於分化時的epigenetic modulation導致在不同的細胞對於同一個receptor被活化時能反應的signal transduction不同(由於特定的gene expression受到限制)，但後來才發現其實並非如此單純，而是充滿可能性的。不僅可能會有相同的ligand接到同一個receptor卻引發不同的反應(PTH對於骨骼與腎, Figure1. )，也可能會有相同的ligand接到不同的receptor理所當然地引發不同的反應( acetylcholine對於橫紋肌細胞與胰腺腺泡細胞, Figure2, 3. )，甚至還可能是不同的ligand接到不同的receptor卻引發一樣的反應(epinephrine與glucagon對於肝細胞)。

然而，這並沒有解決我原先的問題，為何在不同組織對於同種signaling molecule會有不同反應。我認為這必須要從不同組織間細胞根本上的差異探討，我們知道在個體由胚胎形成成熟個體時，體內的各個細胞經歷了各式各樣的分化過程，而這些分化過程的差異則是在於source cells給予的morphogen在種類、濃度、時間上的不同，因此原先的問題可能要轉變為分化時morphogen究竟對細胞造成什麼樣的改變導致明明具有相同基因體的細胞卻對同種刺激產生不同反應。我認為最有可能的機制是epigenetic approach，因為顯然地它決定gene expression，這就決定了cytoplasm可以存在何種protein，一旦不同組織間某些gene表現，某些不表現，就會出現各異的signal transduction。然而，經過我一番查詢後發現目前尚無明確的機制，尤其是我以morphogen, differentiation, 和epigenetic為關鍵字查詢，Wikipedia甚至直接告訴我分化時morphogen對epigene的影響尚未有完整的發現，但不同組織有不同反應相當確定是因為epigenetic regulation。

A picture containing text, map

Description automatically generatedFigure 1. PTH在kidney和bone的signal transduction

A picture containing text, map

Description automatically generated

Figure 2. Acetylcholine在骨骼肌的signal transduction

A picture containing text, map

Description automatically generated

Figure 3. Acetylcholine在胰腺腺泡細胞的signal transduction