Cell signalling is the music of life

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ABSTRACT

Cell signalling is an immensely important topic in biological and biomedical sciences, and one which has an ever-increasing literature. As more and more is known about it, and more components are discovered, it is getting harder and harder to visualise how it all might work to create an holistic mechanism in the cell. To achieve a better understanding of a complex issue such as this, it is often useful to use an analogy which is familiar to the researcher to encourage better understanding. In this essay it is suggested that music, and the instruments used to produce it, can be used as such an analogy. Various elements and issues in cell signalling are discussed and musical comparisons are made. Clearly, the true understanding of cell signalling will come from systems biology and mathematical modelling, but it is proposed that this analogy might prove useful. The phrasing used may be considered a little loose and flamboyant for a scientific topic of such importance, but it is hoped that the discussion will not only be interesting but might also be useful in fostering debate and facilitating teaching in this area of molecular biology.

KEY WORDS: Intracellular signalling peptides and proteins.
Music.
Oscillations.
Sensory thresholds.

Introduction

Cell signalling is now one of the most important aspects of modern biology. It encompasses the production and release of signalling compounds (e.g., hormones, cytokines and pheromones) by cells and organisms, and the perception of, and responses to, these signals. Commonly, this involves a plethora of events and activities inside the cells, often culminating in a change of metabolism or an alteration to gene expression. Therefore, a good knowledge of cell signalling is useful in order to understand how organisms thrive in their niche environments, or how they might survive and even acclimatise to stress conditions. However, in cells and organisms – including humans – disease or death can often result from cell signalling dysfunction. The vast majority of drugs under development are aimed at the modulation of proteins (e.g., kinases or G protein-coupled receptors [GPCRs]) involved in cell signalling events.[1]

Conceptually, however, cell signalling is very hard to understand. It is often taught at a fairly simple level, allowing the main components and players to be discussed, and placed in neat rows, giving a misconception of its lack of simplicity. By definition, cell signalling has a need to control one of the most complex machines ever created,[2] and therefore it must be complex itself. From the moment that a new organism is conceived to the moment of death, cell signalling must control the actions of its cells and the enzymes within. As the organism develops, its environment changes, and when things go wrong, cell signalling must regain control. This is a complex challenge that involves a complicated solution. Although common principles are seen across the range of signalling found, the complexities are often ignored and skipped over.

Here, to try to delve into some of these complexities and therefore encourage thought about some of the issues, a musical analogy is suggested. Indeed, musical words or phrases (e.g., orchestrate) are often used when talking about cell signalling events.[3] So, can the analogy be taken further? Hopefully, the following discussion will convince you that it can.

Music has been seen to effect cell signalling events. Mechanical action on cells has been shown to effect cellular function,[4] while music can have effects. For example, the expression or action of some proteins has been shown to be changed if music is played.[5] However, that is not of concern here. Can we take some of the principles of music to enlighten our thought on some of the issues in cell signalling?

For example, intercellular cell signalling by pheromones could be likened to music. Both are created by one organism (song, for example, for music) and perceived and responded to by another organism. Indeed, music is often used in seduction – one of the roles of pheromones (see elsewhere for more in-depth discussion of pheromones[6,7]).

Common principles

As mentioned above, common principles are often discussed when describing cell signalling.[8–10] For example, extracellular signals are perceived by receptors, and many receptors share the same basic structure (e.g., GPCRs).[11] However, these have specific ligands and specific downstream signalling and are somewhat limited in their action in that respect. This is very much like instruments. A violin works well but has a limited range of notes. To create lower notes we need something different, but we don't need to throw out a good idea. The basic shape, structure and principle are used to make bigger versions (the viola, cello and double bass), allowing an increase in the scope of use of the 'same' instrument. Evolution has done the same with many cell signalling components, taking a unit structure and adapting it.

Many proteins used in signalling are found to have domain structures, and these, too, can be seen to be similar

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but adapted between different proteins. They might have different binding specificities or different enzymatic activities, but they often share the same basic structures and mechansims.[12]

Single instruments

Cell signalling is often depicted as a single chain of events (Fig. 1A). The perception of a hormone at the cell surface leads to the activation of one intracellular component, which passes the signal on to the next component, and then the next, until the end response is reached. Krauss[10] said: "The classical view of signalling pathways has been that of sequential transmission of signals in a linear signalling chain". This would be like listening to solo instruments, always, as the only option. However, as Krauss[10] goes on to point out, signalling is far more complicated.[13]

It is fair to say that some solo instruments are fantastic to listen to, but few people would argue that this would be a good way to listen to all music. There is no depth, and it lacks subtlety and variety. Cell signalling is more like listening to an orchestra, where hundreds of instruments are played at the same time. At times one may be prominent, but this might not last, and then others take over and merge, or indeed take the lead. However, they are all playing the same tune; they are all working towards the same goal – in the case of cell signalling, this would be the holistic and managed control of the cell.

So, Figure 1A needs to be replaced by Figure 1B. We need to consider all the players present, even if they appear to be inactive at the moment of analysis or measurement. The drums may be quiet when we look, but their moment will come, for otherwise they would not be part of the orchestra, and if we miss their contribution then we may have missed a vital passage of the music.

Furthermore, orchestras have several of the same instruments, all playing together. The same is true for cell signalling. It is often found that if a protein is knocked out, its expression or function is totally disrupted, or a signalling pathway is disrupted chemically; however, the end result for the cell is surprising, as nothing seems to happen. This is probably due to the fact that another protein, which has not been disrupted, has taken its place – a process referred to as redundancy.

What would happen in the orchestra if one of the violinists broke a string (which does happen). Clearly, he or she would stop playing, but the orchestra would carry on – the survival of the music, or the cell, is far more important than the consequences for the individual. The show must go on, otherwise the cell or the organism would die.

Different instruments

As no one would like to be restricted to solo music for ever, so no one would like to be restricted to listening to orchestras for ever, either. Therefore, music has developed into different genres. Clearly, cells are the same. Not all cells need to contain all the components ever evolved to be involved in cell signalling. This would be a waste. If a cell has no need to respond to adrenaline, why should it bother to waste precious resources synthesising an appropriate receptor. This is true of all cells. Only the genes required for the correct functioning of that cell will be expressed. The proteins in cells in this context can be thought of as ensembles, brought together so that all the components are playing from the same sheet of music. The result is harmony.

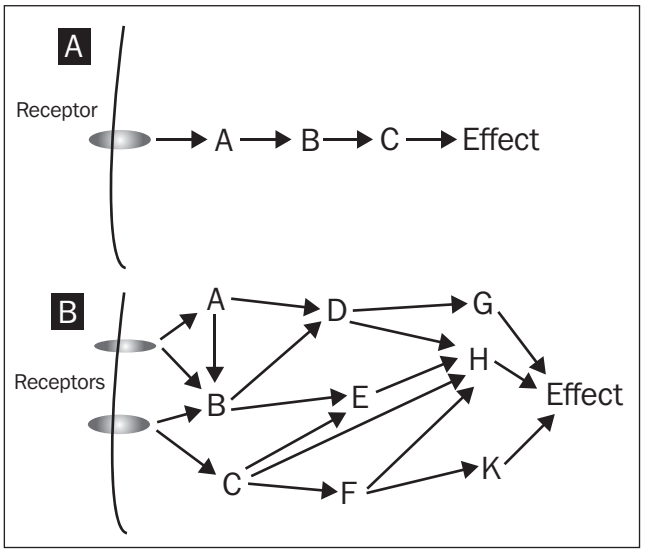

Fig. 1. Cell signalling is often misrepresented as a simple pathway. A) Signalling components are often linked in simple pathways; however, B) signalling should be thought of as a network, with lots of 'instruments' contributing to the holistic response.

Thresholds and volume

One of the main challenges of cell signalling is to understand how one or more events are coordinated when so many things are going on in the cell at the same time. It is often thought that components are either active or inactive, and the misconception is that this is an all-or-nothing response. This is true particularly in respect of the compounds referred to as second messengers (e.g., cAMP). It may be thought that these are not present in the cell, but on activation of the enzyme that makes it, in this case adenylyl cyclase, the cAMP is made and a response, usually the activation of protein kinase A (PKA), ensues.

However, an understanding of kinetics and binding constants provides a hint that this is not the case. Furthermore, when cAMP is measured in cells it is usually found at low concentration, even before an activation or modulation event. On activation, cAMP does indeed go up, and a response is seen. But how far does it need to increase before a response is 'heard'? There is probably a threshold level below which nothing happens, even if the concentration fluctuates. Above that threshold, a series of events would be initiated. This is further complicated by the location of the generation of cAMP, the location of the enzymes removing it, and the location of the PKA that responds.[14]

Using the orchestra as an example, dozens of instruments may play at the same time, but can an individual instrument be picked out from all the rest, or is it the overall effect to which we listen. If one instrument becomes prominent, as discussed above, it will be heard, it will change the overall effect, and the response will be different; however, that

individual instrument had to reach a threshold of volume before that effect was seen.

Music does not always remain at the same volume, and volume can have a great effect on the overall response to it being played – what is referred to as dynamics in music. So, can thresholds change in cell signalling? This is quite hard to envisage, as in general a protein will have a fixed binding constant, or a fixed turnover number, but this is not always the case. Receptors can be down-regulated, which might be a little like wearing ear defenders when listening to music that is not liked.

Proteins are modified constantly (i.e., phosphorylated) and this can have profound effects, not only on the activity of the protein but also on its ability to bind other proteins or ligands, so the threshold for a protein can be altered, and relatively quickly. Furthermore, many proteins are found in families in which a range of isoforms appears to do the same thing. However, binding constants, activation, phosphorylation capacities, and so on, are often subtlety different in such isoforms. Thus, over a longer timescale, a new set of isoforms may be synthesised, so changing the thresholds at which the cell has the capacity to respond. Therefore, if the overall volume of the cell decreases, a protein that has a threshold (playing volume) that usually is too low to have an effect can suddenly be heard, producing a very different effect.

Some instruments (e.g., bagpipes) have a background note that plays as a base note or tonic, usually A, over which the rest of the tune is heard. This analogy works well for an overall look at cell signalling where, as discussed, individual components need to be heard against the background levels of inevitable activity. However, perhaps the analogy works particularly well for the role of reduction oxidation potential (redox) signalling in cells.

It is now apparent that the cells are held at a very low redox mid-point potential, and oxidative stress is seen as a disruption of that potential.[15] It has been argued that oxidation inside the cells triggers a multitude of downstream signalling, with many components being identified.[16] However, it may be more profound than this. Perhaps the low redox poise is like the constant note of the bagpipes, always needing to be there, always allowing the harmony of the rest of the signalling events. Perhaps disruption of this background note is a fundamental trigger that disharmony has arrived.

Oxidation of proteins will have effects on other signalling. Removal of a thiol group by oxidation will stop its modification by nitric oxide (NO), a very important signal in its own right. Therefore, such modification, called S-nitrosylation, will not go ahead, and so the signalling will now have to be different, as the background 'note' (redox poise) has been disrupted. Other events such as phosphorylation of that same protein may also not now take place, so, as well as being a signal leading to an individual event, redox perhaps is more important in that it maintains a steady background on which other events can take place, just as the background note of the bagpipes makes the harmonies work.

Subtle changes

In a scenario in which there is a background of activity and noise, thresholds may not be the only way to change the overall effect; it may be the combination of factors that matters. Maybe cAMP has increased, slightly, and a protein has been phosphorylated and calcium ions are elevated. This will give one effect. If cAMP now drops and is replaced by cGMP, would the effect remain the same? The answer is almost certainly not. Even though two out of the three variables have not changed, the overall effect might be very different to the cell.

How can music help in this as an analogy? Imagine a person playing a C major chord; they would play C natural, E natural and G natural. It sounds happy and a person would generally respond to it in a happy frame of mind. Now, keep the C and the G the same, but replace the E natural with an E flat – a subtle change, but a very significant one. The chord is now C minor, and it sounds sad. A person listening would have a very different response.

This technique is used prodigiously by composers, an excellent example being Beethoven's Sonata Op. 27 No. 2 (Moonlight Sonata). Here, chords are played and then repeated with very subtle changes of single notes. The effect is fantastic, as the whole mood of the music changes, and quickly. Is this not how cells would respond to subtle and blended changes?

Timings and phasing

Rhythms and timings in music are crucial. Most pieces of music start with a time signature (e.g., 2/4 or 6/8) that dictates the emphasis and timings of the notes. So, do cell signalling events have timings like this? In fact they can, with calcium signalling being the most well studied (see elsewhere for examples[17]). Calcium levels oscillate, and a great example is shown in Alberts *et al*.[18] in response to vasopressin, where the concentration of hormone added alters the frequency of the oscillations seen. So, the cells are responding not to the amplitude of the change in calcium ions, but the speed at which they change and then return to baseline levels.

Reactive oxygen species (ROS) level in cells after stimulation also tends to change in phases. It goes up, drops for a while, and then rises again, only finally to drop once more.[19] Other signalling components may do the same, and could influence each other as they do so. Even extracellular signals such as insulin secretion has been found to be biphasic.[20]

Is cell signalling like a fugue in music, where a theme is played by more than one instrument, all brought together to make a whole? Perhaps it is not necessarily the exact rise and fall of levels of second messengers and events such as phosphorylation that are important, but how they fit together to make a continuous phrasing.

Dischord: setting up for the future?

In cell signalling, often there is apparent conflict and dischord in what has been measured. This is usually not understood, and it seems to go against what we understand about cell signalling. Perhaps this is the cell's way of signalling for the future.

In music, composers often use what should be a blatant dischord (e.g., F sharp and G natural played together). Often these are not the notes next to one another but ones separated by several octaves. It still sounds wrong but better

perhaps than if the notes were adjacent, but the phrase often changes quickly to a harmonious chord, thus relieving the tension built up by the dischord. The result sounds much better, perhaps better than just playing the end harmony on its own. The composer was setting the scheme, using a 'stress' to achieve the ultimate aim of a pleasing harmony.

Perhaps cells do this, too. It has been found, for example, that some stresses such as short-term low temperature allow organisms to survive subsequent stress better.[21] The short-term 'dischord' has allowed the cells to respond in a way that makes the future result better. A short-term stress has initiated cell signalling responses, often involving gene expression, to allow the cell to be prepared for changes in the future. Perhaps we should look at these times of apparent disharmony in cell signalling as positive events; ones that allow the cell to prepare for what they might have to endure in the near future, and indeed make its future more harmonious than it would otherwise have been.

Evolution and the future

It would be naïve to think that cell signalling has evolved and will now remain the same for eternity, just as it would be foolish to think the same about music and instruments. Over billions of years, cell signalling proteins have mutated and changed to give the polypeptides that we can find today, but they will continue to change, adapt and no doubt increase in number. Families of isoforms can be recognised, some with added domains, some with extra phosphorylation sites and some in truncated form. Good examples are phosphatase proteins that remove the phosphate groups from proteins.[22]

Musical instruments also evolve. Some have certainly been around for a long time, but even those that form part of current orchestrates are different from those used by great composers such as Mozart. He would never have imagined the possibility of an electric violin, but today his music is often played on such an instrument.

Music itself evolves, with successive composers building on the work of those who went before them. As the music and instruments of the future change and adapt to current tastes, so will the genes that encode proteins involved in cell signalling continue to mutate. Unfortunately, the consequences of this will be that some mutations will lead to disease for the organism in which they reside, including humans.

Conclusions

Cell signalling is a complex topic, and many of the ideas and concepts discussed here have been covered before.[10] No doubt, systems biology and mathematical modelling will go a long way to solve exactly how cell signalling works and what happens when dysfunction occurs. However, it is suggested here that the use of music as an analogy may help in the understanding of how signalling may work. It is not a single event, which takes place at a set point in time, but rather it is like a major composition such as a concerto that continues for a long period of time, changes as it goes along, comes back to its main theme, wanders off to another tune and then returns, all the while maintaining that crucial harmony. Likewise, harmony is what cell signalling strives to maintain for the survival of the cells of an organism. □

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