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Spatio-temporal protein dynamics in single living cells

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The development and application of single cell optical imaging has identified dynamic and oscillatory signalling processes in individual cells. This requires single cell analyses since the processes may otherwise be masked by the population average. These oscillations range in timing from seconds/minutes (e.g. calcium) to minutes/hours (e.g. NF- κ B, Notch/Wnt and p53) and hours/days (e.g. circadian clock and cell cycle). Quantitative live cell measurement of the protein processes underlying these complex networks will allow characterisation of the core mechanisms that drive these signalling pathways and control cell function. Ultimately, such studies can be applied to develop predictive models of whole tissues and organisms.

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Introduction

Systems level approaches require that, in order to faithfully recreate a biological network *in silico*, key parameters must be measured first at the molecular level in single cells to allow accurate simulation of overall behaviour of the complete system. A model derived from population data will miss crucial dynamic and functional details. However, single cell studies unmask stochastic events that lead to cell to cell variation. Definition of the stochastic non-linear dynamics of the system allows a better understanding and appreciation of the kinetics and function of mechanisms within signalling networks.

Within all complex biological systems, cells must communicate to produce co-ordinated temporal, local or global responses. Cells regulate these responses by alterations in complex protein signalling cascades. Proteins undergo changes in their interactions, stability and location within

and between cell compartments. Dynamic modifications, such as the phosphorylation state of proteins that typically transduce a signal into a response, are usually measured using techniques that necessarily give a population average. These and other measures are often from a population of cells over time, or single cells at a given time point and any temporal heterogeneity is lost as a result. Where single cell time-lapse data are available, such as in nuclear factor kappa B (NF- κ B) signalling [1^{••}], or circadian rhythms [2], single cell heterogeneity is shown to be masked by population averaging.

Dynamic behaviour in cell signalling

Although complex when taken as a whole, intracellular networks are often made up of a common set of simple motifs (e.g. feedback loops [3[•]]), which generate a wide range of dynamic behaviours, including oscillations. The negative feedback loop (e.g. when a protein inhibits its own transcription) is one example, which can lead to oscillatory behaviour, such as cycles in protein concentration or translocation between cellular compartments. Although a single delayed negative feedback loop is all that may be required to drive oscillatory behaviour, many systems have multiple feedback loops, leading to much more complex dynamic signalling networks. The involvement of a core negative feedback loop to generate sequential cycles of nuclear to cytoplasmic (nucleo-cytoplasmic) protein translocation is well documented particularly in the NF- κ B signalling pathway [1^{••}] and thus, this provides a good model system for evaluating complex and non-linear protein dynamics.

NF- κ B: a key oscillatory system

The NF- κ B signalling pathway describes one of the most important stress response networks in mammalian cells, involving the stimulated expression of genes controlling a range of crucial activities, such as cell growth [4], inflammation [5] and apoptosis [6]. The NF- κ B pathway acts to convey an extracellular signal through the cytoplasm to the nucleus via protein interactions centred around members of the reticuloendotheliosis (Rel) and inhibitor kappa B (I κ B) families, including p65 (RelA), RelB, p50/p105, p52/p100, cRel (and its retroviral homologue vRel), I κ B α , I κ B β and I κ B ϵ . The architecture of the system involves interactions between different Rel and I κ B family members, several of which are under the transcriptional control of NF- κ B. These include I κ B α as the prototype negative regulator as well as I κ B ϵ [7] and the inflammation regulator A20 [8] (Figure 1).

Inflammatory stimuli, for example the cytokine TNF α , induce rapid phosphorylation (within five minutes),

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Figure 1

Schematic diagram of core components of the NF-κB signalling network.

ubiquitination and proteasomal degradation of IκBα [9]. IκBα removal enables free NF-κB to move to the nucleus where it can activate transcription from target genes include that for IκBα. Newly synthesised IκBα binds to nuclear NF-κB removing it from the nucleus via the IκBα nuclear export sequence (NES) to be sequestered in the cytoplasm. Although original bulk-cell studies suggested that this stimulus-induced movement of NF-κB occurred as a biphasic response [10,11], recent theoretical and experimental studies focussing on single cell protein dynamics have suggested that asynchronous nucleo-cytoplasmic oscillations occur in single cells [1,11]. These oscillations were observed to persist for several hours albeit showing dampening with time [1]. Asynchrony in the timing of the oscillations, though readily observable when considering single cell protein dynamics tracked over time in the same cells, is masked by an averaging effect when looking at the population level (Figure 2). The known complexity of the NF-κB pathway has been increased due to the identification of further negative feedback loops controlling NF-κB function. There is also significant cross-talk with a large number of other important cell-signalling systems, such

Figure 2

Sustained nucleo-cytoplasmic oscillations in NF-κB. Synchronicity is lost after initial translocation. The inserted panel of images is of an individual single representative SK-N-AS neuroblastoma cell expressing a p65-DsRed-Express fusion protein taken at the indicated time in minutes following stimulation.

as the Glucocorticoid Receptor signalling [12] and p53 pathways [13].

Oscillatory behaviour in other biological systems

As well as that described in the NF- κ B pathway, oscillations in other signalling and transcription factor pathways have been discovered using fluorescence techniques and imaging at a single cell resolution. Variation in oscillatory signalling between single cells was first characterised in acute responses such as cytosolic calcium concentration [14], where the response to stimuli was transduced by changes in the frequency of oscillations in intracellular calcium concentration that can occur from fractions of a second [15] to minutes [16]. Although changes in intracellular calcium concentration were detected many years ago with the calcium-specific photoprotein, Aequorin [17,18] the onset of highly selective fluorescent calcium indicators enabled the visualisation of small, high frequency changes in ion concentration within individual live cells [19], and thus first made the connection between oscillatory behaviour and downstream consequences [20,21,22].

Oscillatory nucleo-cytoplasmic translocations shown by the tumour suppressor protein p53 result from a feedback loop, similar in concept to that of NF- κ B, involving the relationship between p53 and its inhibitor MDM2 [23]. In contrast to the timing of calcium oscillations, these changes occur in the order of hours [23]. Regulation of MDM2-independent nuclear export depends on the exposure of the p53 NES, contained within a leucine-rich tetramerisation domain. Upon import as a monomer species, p53 tetramerises to become transcriptionally active [24], masking its intrinsic NES from exportins and maintaining its active, nuclear localisation. It is only upon fragmentation of the p53 tetramer to dimers that its NES becomes accessible and subsequently targetable for nuclear export. Not only have oscillations in p53/MDM2 been shown at the single cell level [25] but also from an MDM2-luciferase reporter *in vivo* from living irradiated transgenic mice [26]. The emergence of molecular oscillators has been documented in recent studies, for example Notch/Hes1 and Wnt [27]. These genes have been shown to have significant roles in the somitic segmentation clock, most notably in showing oscillations within cell populations in whole embryos independent of β -catenin levels [28]. Similar oscillations have also been suggested in the Smad and Stat1 [29] signalling pathways.

Molecular oscillations have also been visualised on much longer time-scales, for example in the mammalian and plant circadian clocks (reviewed in [30,31], respectively). Here, the discovery that circadian rhythms could be induced in mammalian culture cells [32] has allowed new and detailed single cell investigations of the mam-

malian circadian clock [2]. Single cell studies have also given new insights into the mammalian cell cycle oscillator. Live cell imaging has been used to show dynamic shuttling of both Cyclin E and Cyclin A (associated with G1/S and S phase progression, respectively) [33]. Recently, non-invasive 'markers' of cell cycle transition have been developed to track particular phases of specific proteolysis [34], and destabilised GFP constructs used to investigate fail-safe mechanisms to maximise cell cycle synchrony between single budding yeast [35].

Visualisation and quantification of protein dynamics

The onset of fluorescent fusion protein technology and time-lapse fluorescence microscopy has provided the ability to perform non-invasive measurement of protein dynamics in the same cell over time. Transient expression, stable integration or the generation of transgenic animals provide tools for studying the localisation, translocation and stability of specific proteins of interest at the single cell level and ultimately in living tissues.

Photobleaching techniques have been used to assess the dynamic properties of fluorescent fusion proteins in living cells. The simplest of these techniques involves bleaching the fluorescent signal from a defined area of a cell and either timing the recovery of the fluorescence in the bleached area [fluorescence recovery after photobleaching (FRAP)] or the time of fluorescence loss from a different region outside the one that was bleached [fluorescence loss in photobleaching (FLIP)] [36]. FRAP and FLIP and related techniques enable the measurement of the mobility of the fluorescent fraction of a specific protein, and can therefore be used to identify changes in mobility due to binding between the fluorescent protein of interest and unknown (or unlabelled) binding partners. Mathematical techniques are available for unveiling specific properties using these techniques [37–39].

A more thorough single molecule-based analysis of the kinetics of proteins of interest within single cells has been provided by the development of fluorescence correlation spectroscopy (FCS) (also called fluorescence fluctuation spectroscopy and fluorescence cross correlation spectroscopy (FCCS)), which can quantify protein concentration, complex formation and molecular diffusion rates [40]. These technologies involve monitoring signals emitted from single fluorescent molecules of interest within a defined confocal volume within a single cell. Crucially, diffusion rates can identify unbound and complex molecules, providing methods to assess protein–protein interactions on a molecular level.

A more common method to assess protein interactions is Förster resonance energy transfer (FRET), which involves the transfer of non-radiant energy between

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two fluorophores with overlapping donor emission and acceptor excitation spectra (typically Cyan and Yellow fluorescent proteins (ECFP and EYFP) [41]) each 'tagged' to one terminus of a protein of interest. Unfortunately, this approach is not as straightforward as might be expected due to unwanted overlaps between the spectra of the donor and acceptors. For instance, the emission spectrum of ECFP fusions has an emission peak at the same wavelength as EYFP which itself is excited with the laser line used to excite ECFP. To alleviate these problems, acceptor photobleaching is a commonly used control. Increases in the levels of donor protein fluorescence are observed if the two proteins are close enough (~ 8 nm) to cause FRET and energy is no longer transferred to the acceptor and is therefore emitted as light (at the donor emission wavelengths). A distinct FRET signature can provide evidence of interactions between two proteins. However, this approach still lacks dynamic range and real quantification. The achievement of real-time quantification of dynamic molecular processes in living cells now represents an important goal for a systems biology approach to the increased understanding of cell-signalling processes.

The systems biology approach

Advancing techniques to both measure and quantify experimental data provide an increasingly complete picture of how biological systems operate. Drawing upon quantitative data gathered from both single cell based and bulk cell based experimental work, as well as from high-throughput proteomic, transcriptomic and bioinformatic approaches, entire networks can be investigated. The main aim is to assess dynamic interactions in a holistic manner in order to develop predictive hypotheses to explain and test experimentally observed behaviour.

A key challenge in systems biology is to define the behaviour of systems across scales, ranging from single molecules to whole organisms. A classic example linking ion channel function to organ physiology comes from the model of the virtual heart [42]. Other modelling projects have encompassed biochemical-based [43[•]], cellular-based [44], organ-based [45^{••}] and population [46] level-based approaches. Cell-signalling networks are integral to organism adaptation and survival and underlie normal cell function, communication and disease. The observation that key pathways may oscillate suggests the possibility that this may be one important feature of many signalling networks. It perhaps offers an explanation for accurate signal interpretation and signalling 'pathway' interactions. That such oscillating processes may span periods of time from fractions of a second/minute (calcium) to minutes/hours (NF- κ B, p53 and segmentation clock) to hours/days (circadian clock and cell cycle) suggests the intriguing possibility that such interactions between these dynamic processes may well intermesh like the cogs in a gearbox to provide

error-free interpretation of signalling information in normal cells.

Pathologies such as cancer and immune diseases can only be truly understood through a whole systems approach [47]. Such integrated studies should assist in the understanding of the genesis, prognosis and treatment of these and other complex diseases. Towards this aim, the development of a virtual human may therefore have many important outcomes, though understanding and modelling the complexities of the brain and behaviour will undoubtedly pose a huge challenge.

Work performed on biological systems taking a cell population average rather than considering individual cells, may mask the subtleties of dynamic events to the observer. In thinking about how these dynamics are orchestrated within cells producing an average response, we can draw upon an emergent analogy with musical orchestration [48]. For example, the climax of Mozart's Requiem Mass involves upwards of 35 separate instruments and voices. The resultant crescendo whilst representative of the entire orchestra is difficult to disseminate into its individual parts, necessary subtleties may, in effect be 'lost' to the listener. However by considering the 'dynamics' of individual instruments, it is possible to intuitively reconstruct the ensemble effect, piece by piece.

The aim of assessing individual contributions whilst keeping an appreciation for the 'bigger picture' is the remit of Systems Biology and takes a step forward with advancing single cell (and single molecule) techniques working towards models which can both faithfully represent individual components on a single cell level and predict the overall population response.

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- of outstanding interest

1. Nelson DE, Ihekweba AE, Elliott M, Johnson JR, Gibney CA, Foreman BE, Nelson G, See V, Horton CA, Spiller DG *et al.*: **Oscillations in NF- κ B signaling control the dynamics of gene expression.** *Science* 2004, **306**:704-708.
- An integrated theoretical and experimental study demonstrating that asynchronous oscillations in NF- κ B protein dynamics in single live cells can control downstream gene expression.
2. Welsh DK, Yoo SH, Liu AC, Takahashi JS, Kay SA: **Bioluminescence imaging of individual fibroblasts reveals persistent, independently phased circadian rhythms of clock gene expression.** *Curr Biol* 2004, **14**:2289-2295.

3. Milo R, Shen-Orr S, Itzkovitz S, Kashtan N, Chklovskii D, Alon U:
 • **Network motifs: simple building blocks of complex networks.**
Science 2002, **298**:824-827.
 A key description of how different control systems comprising different combinations of positive and negative feedback can combine to regulate biological networks. This paper makes clear that certain network motifs are commonly used in biological systems.
4. Guttridge DC, Albanese C, Reuther JY, Pestell RG, Baldwin AS Jr:
NF-kappaB controls cell growth and differentiation through transcriptional regulation of cyclin D1. *Mol Cell Biol* 1999, **19**:5785-5799.
5. Shakhov AN, Kuprash DV, Azizov MM, Jongeneel CV, Nedospasov SA: **Structural analysis of the rabbit TNF locus, containing the genes encoding TNF-beta (lymphotoxin) and TNF-alpha (tumor necrosis factor).** *Gene* 1990, **95**:215-221.
6. Schaulvliege R, Vanrobaeys J, Schotte P, Beyaert R: **Caspase-11 gene expression in response to lipopolysaccharide and interferon-gamma requires nuclear factor-kappa B and signal transducer and activator of transcription (STAT) 1.** *J Biol Chem* 2002, **277**:41624-41630.
7. Kearns JD, Basak S, Werner SL, Huang CS, Hoffmann A:
IkappaBepsilon provides negative feedback to control NF-kappaB oscillations, signaling dynamics, and inflammatory gene expression. *J Cell Biol* 2006, **173**:659-664.
8. Lee EG, Boone DL, Chai S, Libby SL, Chien M, Lodolce JP, Ma A:
Failure to regulate TNF-induced NF-kappaB and cell death responses in A20-deficient mice. *Science* 2000, **289**:2350-2354.
9. Miyamoto S, Maki M, Schmitt MJ, Hatanaka M, Verma IM:
Tumor necrosis factor alpha-induced phosphorylation of I kappa B alpha is a signal for its degradation but not dissociation from NF-kappa B. *Proc Natl Acad Sci U S A* 1994, **91**:12740-12744.
10. Baeuerle PA, Baltimore D: **Activation of DNA-binding activity in an apparently cytoplasmic precursor of the NF-kappa B transcription factor.** *Cell* 1988, **53**:211-217.
11. Hoffmann A, Levchenko A, Scott ML, Baltimore D: **The IkappaB- NF-kappaB signaling module: temporal control and selective gene activation.** *Science* 2002, **298**:1241-1245.
 The first computational model of the NF-κB signalling system that suggested that this system had the potential to undergo nuclear to cytoplasmic oscillations of NF-κB.
12. Nelson G, Wilde GJ, Spiller DG, Kennedy SM, Ray DW, Sullivan E, Unitt JF, White MR: **NF-kappaB signalling is inhibited by glucocorticoid receptor and STAT6 via distinct mechanisms.** *J Cell Sci* 2003, **116**:2495-2503.
13. Webster GA, Perkins ND: **Transcriptional cross talk between NF-kappaB and p53.** *Mol Cell Biol* 1999, **19**:3485-3495.
14. Berridge MJ: **Calcium oscillations.** *J Biol Chem* 1990, **265**:9583-9586.
15. Nelson MT, Cheng H, Rubart M, Santana LF, Bonev AD, Knot HJ, Lederer WJ: **Relaxation of arterial smooth muscle by calcium sparks.** *Science* 1995, **270**:633-637.
16. Cuthbertson KS, Cobbold PH: **Phorbol ester and sperm activate mouse oocytes by inducing sustained oscillations in cell Ca²⁺.** *Nature* 1985, **316**:541-542.
17. Ridgway EB, Durham AC: **Oscillations of calcium ion concentrations in *Physarum polycephalum*.** *J Cell Biol* 1976, **69**:223-226.
18. Woods NM, Cuthbertson KS, Cobbold PH: **Repetitive transient rises in cytoplasmic free calcium in hormone-stimulated hepatocytes.** *Nature* 1986, **319**:600-602.
19. Grynkiewicz G, Poenie M, Tsien RY: **A new generation of Ca²⁺ indicators with greatly improved fluorescence properties.** *J Biol Chem* 1985, **260**:3440-3450.
20. Dolmetsch RE, Lewis RS, Goodnow CC, Healy JL: **Differential activation of transcription factors induced by Ca²⁺ response amplitude and duration.** *Nature* 1997, **386**:855-858.
 This paper demonstrates that the frequency of calcium oscillations can control downstream gene expression.
21. Dolmetsch RE, Xu K, Lewis RS: **Calcium oscillations increase the efficiency and specificity of gene expression.** *Nature* 1998, **392**:933-936.
22. Li W, Llopis J, Whitney M, Zlokarnik G, Tsien RY: **Cell-permeant caged InsP3 ester shows that Ca²⁺ spike frequency can optimize gene expression.** *Nature* 1998, **392**:936-941.
23. Lahav G, Rosenfeld N, Sigal A, Geva-Zatorsky N, Levine AJ, Elowitz MB, Alon U: **Dynamics of the p53-Mdm2 feedback loop in individual cells.** *Nat Genet* 2004, **36**:147-150.
 Oscillatory protein dynamics in the p53 pathway is owing to negative feedback between p53 and its prototypical inhibitor MDM2.
24. Friedman PN, Chen X, Bargonetti J, Prives C: **The p53 protein is an unusually shaped tetramer that binds directly to DNA.** *Proc Natl Acad Sci U S A* 1993, **90**:3319-3323.
25. Geva-Zatorsky N, Rosenfeld N, Itzkovitz S, Milo R, Sigal A, Dekel E, Yarnitzky T, Liron Y, Polak P, Lahav G et al.: **Oscillations and variability in the p53 system.** *Mol Syst Biol* 2006, **2**:1-13.
 Characterisation of the factors controlling the oscillatory protein dynamics in the p53 pathway is owing to negative feedback between p53 and its prototypical inhibitor MDM2.
26. Hamstra DA, Bhojani MS, Griffin LB, Laxman B, Ross BD, Rehemtulla A: **Real-time evaluation of p53 oscillatory behavior in vivo using bioluminescent imaging.** *Cancer Res* 2006, **66**:7482-7489.
 This paper demonstrates that oscillatory dynamics in p53 regulated MDM2-directed gene expression occurs *in vivo* in intact-irradiated mice.
27. Shimojo H, Ohtsuka T, Kageyama R: **Oscillations in notch signaling regulate maintenance of neural progenitors.** *Neuron* 2008, **58**:52-64.
28. Aulehla A, Wiegraebe W, Baubet V, Wahl MB, Deng C, Taketo M, Lewandoski M, Pourquie O: **A beta-catenin gradient links the clock and wavefront systems in mouse embryo segmentation.** *Nat Cell Biol* 2008, **10**:186-193.
 Fluorescence-based, real-time imaging showing clock oscillations in mouse embryos are independent of beta-catenin protein levels.
29. Yoshiura S, Ohtsuka T, Takenaka Y, Nagahara H, Yoshikawa K, Kageyama R: **Uladian oscillations of Stat, Smad, and Hes1 expression in response to serum.** *Proc Natl Acad Sci U S A* 2007, **104**:11292-11297.
30. Ko CH, Takahashi JS: **Molecular components of the mammalian circadian clock.** *Hum Mol Genet* 2006, **15**(Spec No 2):R271-R277.
31. Schultz TF, Kay SA: **Circadian clocks in daily and seasonal control of development.** *Science* 2003, **301**:326-328.
32. Balsalobre A, Damiola F, Schibler U: **A serum shock induces circadian gene expression in mammalian tissue culture cells.** *Cell* 1998, **93**:929-937.
33. Jackman M, Kubota Y, den Elzen N, Hagting A, Pines J: **Cyclin A- and cyclin E-Cdk complexes shuttle between the nucleus and the cytoplasm.** *Mol Biol Cell* 2002, **13**:1030-1045.
34. Sakaue-Sawano A, Kurokawa H, Morimura T, Hanyu A, Hama H, Osawa H, Kashiwagi S, Fukami K, Miyata T, Miyoshi H et al.: **Visualizing spatiotemporal dynamics of multicellular cell-cycle progression.** *Cell* 2008, **132**:487-498.
 Development of fluorescent markers of cell cycle phase transition. E3 ligase substrates Cdt1 (marker for G1 phase) and Gemmin (marker for S/G2 and M phases) were cultured in NMuMG cells before being injected into live mice, serving as model systems for future cell cycle studies.
35. Bean JM, Siggia ED, Cross FR: **Coherence and timing of cell cycle start examined at single-cell resolution.** *Mol Cell* 2006, **21**:3-14.
36. Axelrod D, Koppel DE, Schlessinger J, Elson E, Webb WW: **Mobility measurement by analysis of fluorescence photobleaching recovery kinetics.** *Biophys J* 1976, **16**:1055-1069.
37. Sprague BL, Pego RL, Stavreva DA, McNally JG: **Analysis of binding reactions by fluorescence recovery after photobleaching.** *Biophys J* 2004, **86**:3473-3495.

6 Systems biology

38. Phair RD, Gorski SA, Misteli T: **Measurement of dynamic protein binding to chromatin in vivo, using photobleaching microscopy.** *Methods Enzymol* 2004, **375**:393-414.
39. Zicha D, Dobbie IM, Holt MR, Monypenny J, Soong DY, Gray C, Dunn GA: **Rapid actin transport during cell protrusion.** *Science* 2003, **300**:142-145.
40. Palmer AG III, Thompson NL: **Molecular aggregation characterized by high order autocorrelation in fluorescence correlation spectroscopy.** *Biophys J* 1987, **52**:257-270.
41. Karpova TS, Baumann CT, He L, Wu X, Grammer A, Lipsky P, Hager GL, McNally JG: **Fluorescence resonance energy transfer from cyan to yellow fluorescent protein detected by acceptor photobleaching using confocal microscopy and a single laser.** *J Microsc* 2003, **209**:56-70.
42. DiFrancesco D, Noble D: **A model of cardiac electrical activity incorporating ionic pumps and concentration changes.** *Philos Trans R Soc Lond B: Biol Sci* 1985, **307**:353-398.
43. Novak B, Tyson JJ: **A model for restriction point control of the mammalian cell cycle.** *J Theor Biol* 2004, **230**:563-579.
This is a key computational model of the mammalian cell cycle.
44. Turner S, Sherratt JA: **Intercellular adhesion and cancer invasion. A discrete simulation using the extended Potts model.** *J Theor Biol* 2002, **216**:85-100.
45. Noble D: **Systems biology and the heart.** *Biosystems* 2006, **83**:75-80.
This is a description of pioneering work to develop a predictive model of heart function.
46. Spencer M, Tanner JE: **Lotka-Volterra competition models for sessile organisms.** *Ecology* 2008, **89**:1134-1143.
47. Hornberg JJ, Bruggeman FJ, Westerhoff HV, Lankelma J: **Cancer: a systems biology disease.** *Biosystems* 2006, **83**:81-90.
48. Noble D: *The Music of life: Biology beyond the Genome.* Oxford University Press, 2006.