On Being the Right Size, Revisited

Theoretical Underpinnings of Molecular Biology Summer School

Rijeka, 18th July 2019

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An Institute for the Advanced Study of Natural Complex Systems

KLI

Structure of Talk

1. Looking Back at Haldane's 'On Being the Right Size' (1926)
2. Why Cell Biology Needs to be Reminded of Haldane's Argument
3. How Size Conditions Protein Structure and Function
4. How Size Determines the Operation of Molecular Motors
5. How Size Complicates the Predictability of Cellular Behaviour
6. How Did Molecular Biology Come to Neglect the Impact of Scale?

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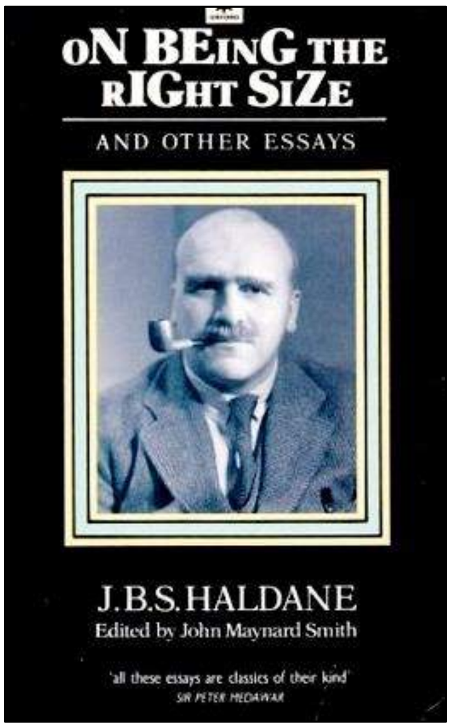
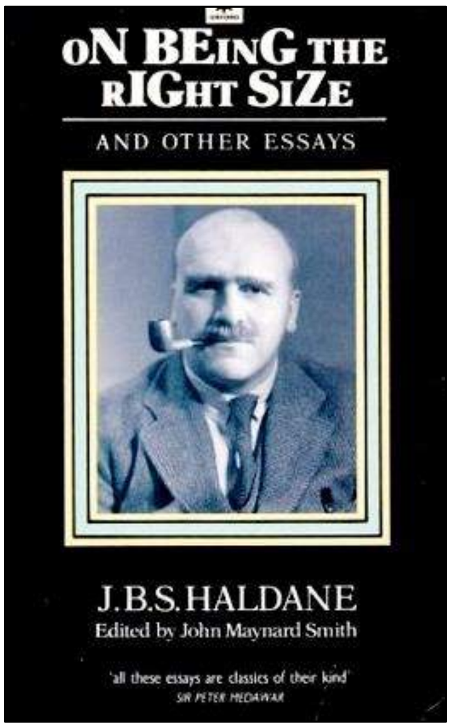
Looking Back at Haldane's 'On Being the Right Size' (1926)



- The size of an organism shapes its particular way of life
- Size imposes constraints on what physical structure an organism can assume, as well as its behaviour
- The functional capacities of organisms are determined by the physical forces that have the greatest effect at the scale in which they exist

Looking Back at Haldane's 'On Being the Right Size' (1926)



- **Gravity**:

  "If you drop a mouse down a 100-metre hole, on reaching the bottom, it gets a slight shock and walks away. A rat is killed, a man is broken, a horse splashes"

- **Surface Tension**:

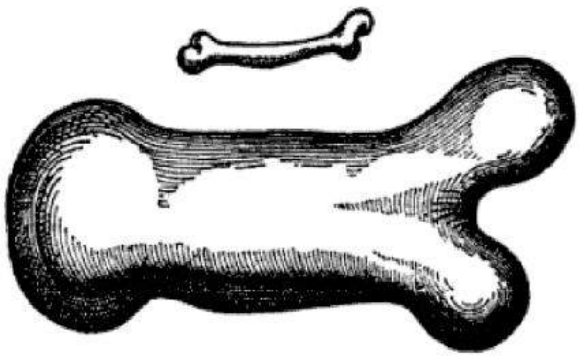
  "A man coming out of a bath carries a 500 g film of water. A wet mouse carries its own weight in water. A wet fly is likely to drown as it has to lift many times its own weight"

- **Diffusion**:

  "Insects, being so small, do not have oxygen-carrying bloodstreams. What little oxygen their cells require can be absorbed by simple diffusion of air through their bodies. But being larger means an animal must have complicated circulatory systems to reach all cells"

Looking Back at Haldane's 'On Being the Right Size' (1926)

- Many of Haldane's examples are based on the square-cube law, which states that with larger size, volume grows much faster than surface area
  - Large size of warm-blooded animals
  - Complexity of organs like lungs or intestines
  - Leaves and roots of higher plants
  - Muscles of animals capable of flight
  - Structure of bones



- Animals are not isometric. Large animals do not look like small animals scaled up in size

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Why Cell Biology Needs to be Reminded of Haldane's Argument

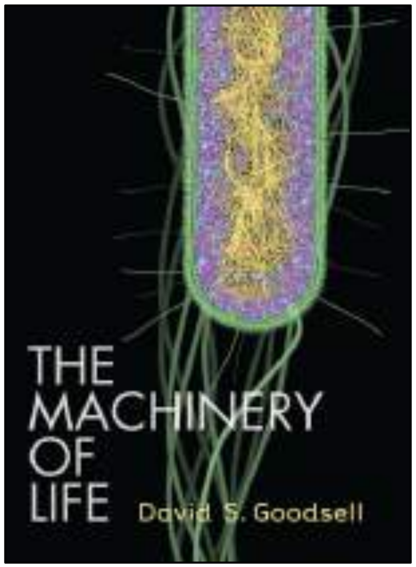
- Differences of size and in the physical scale in which organisms live are of overwhelming importance in determining their structure and behaviour
- By virtue of their microscopic size, cells and their molecular components are subject to very different physical forces than macroscopic organisms
- Microscopic and macroscopic organisms inhabit different 'worlds'. Whereas the macroscopic world is ruled by gravity and inertia, the microscopic world is dominated by Brownian motion and diffusion
- Our intuition, based as it is on our experience of the macroscopic world, fails when judging the adaptive problems that cells have to overcome

Why Cell Biology Needs to be Reminded of Haldane's Argument

- Thus, we should be extremely skeptical of analogies that seek to explain microscopic entities by appealing to the properties of macroscopic ones
- This is *precisely* what the pervasive and consistent metaphorical appeals to machines in the explanation of cellular phenomena attempt to do
- We draw on machines to explain features of cells because they are familiar and intuitively intelligible macroscopic objects of our everyday experience

But if machines *were* the size of molecules they would not be able to function, as their physical environment would be entirely different. We should avoid distorting the reality of cells by conceiving them as machines

Why Cell Biology Needs to be Reminded of Haldane's Argument



Cell, Vol. 92, 291–294, February 6, 1998, Copyright ©1998 by Cell Press

The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists

Biological machines: from mills to molecules

Marco Piccolino

Cell, Vol. 68, 415–420, February 7, 1992, Copyright © 1992 by Cell Press

Unscrambling the Puzzle of Biological Machines: The Importance of the Details

Structural biology of cellular machines

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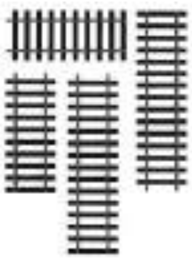
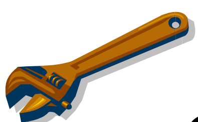
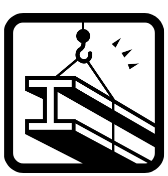
[2]Department of Biochemistry, Albert Einstein College of Medicine, Bronx, NY 10461, USA

Why Cell Biology Needs to be Reminded of Haldane's Argument

"All living things are made from cells, the chemical factories of life. Cells act as chemical factories, taking in materials from the environment, processing them, and producing "finished goods" to be used for the cell's own maintenance and for that of the larger organism of which they may be part. In a complex cell, materials are taken in through specialized receptors ("loading docks"), processed by chemical reactions governed by a central information system ("the front office"), carried around to various locations ("assembly lines") as the work progresses, and finally sent back via those same receptors into the larger organism. Far from being a shapeless blob of protoplasm,the cell is a highly organized, busy place, whose many different parts must work together to keep the whole functioning"

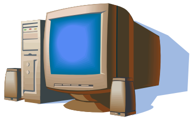
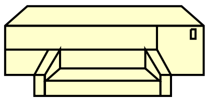
Why Cell Biology Needs to be Reminded of Haldane's Argument


Maromolecular Assembly	Machine Analogy
Cilium / Flagellum	Propeller
ATP synthase	Generator
Ribosome	Factory assembly line
Ion channel / Nuclear pore	Gate / key / pass
Actin filament network	Train tracks
Polymerase	Copy machine
Ligase	Chain coupler
Polymerase	Copy machine
Spliceosome	Film editing machine
Protein sorting mechanism	Mail sorting machine
Protease / proteasome	Bulldozer / destroyer
Magnetosome	Compass





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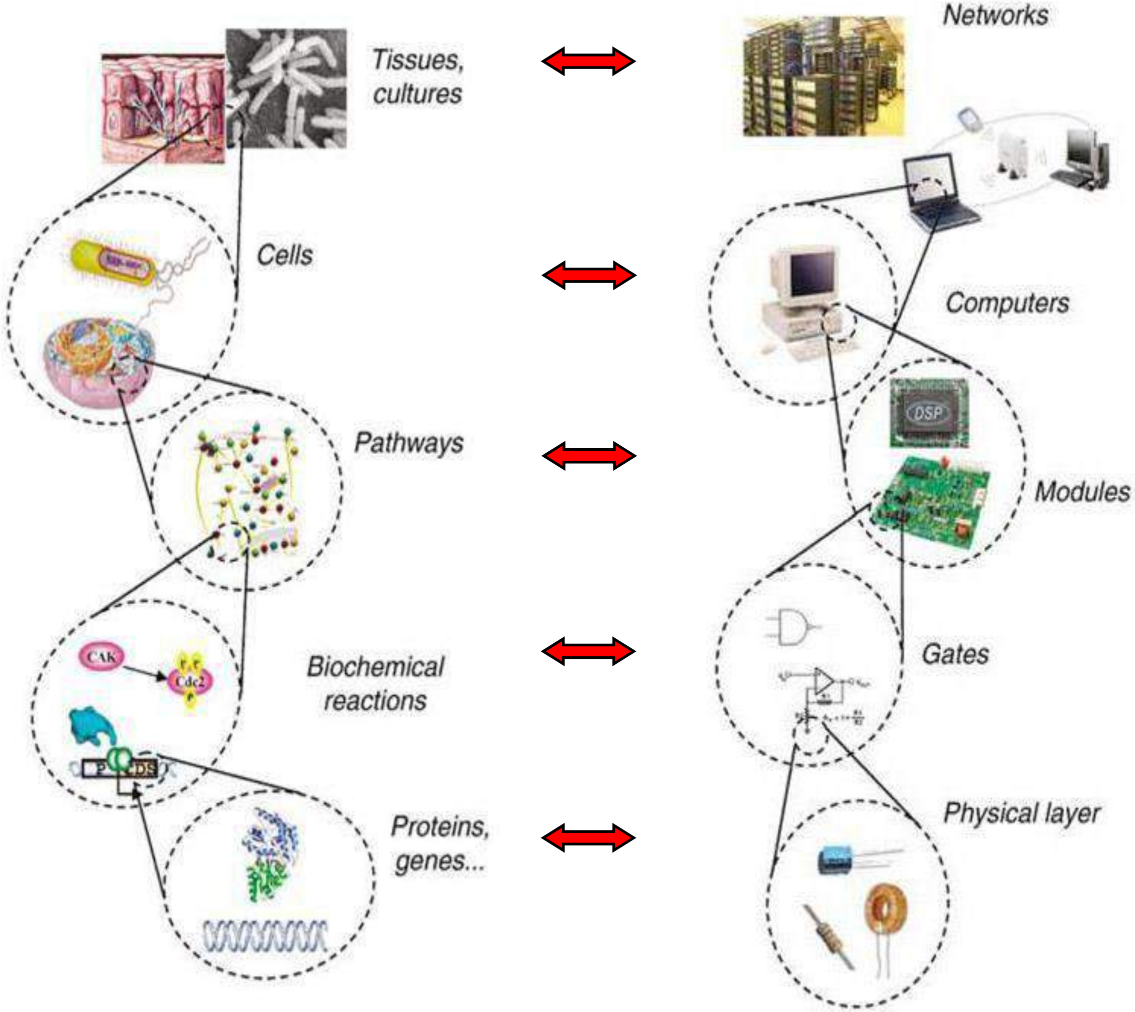

Nicholson – 'On Being the Right Size, Revisited'

Structure of Talk

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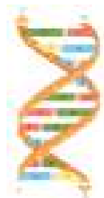
How Size Conditions Protein Structure and Function

- Understanding proteins as machines unifies two schools of molecular biology:

Structural school (STRUCTURE)



Informational school (SPECIFICITY)

As means of ascertaining function

Central dogma of structural biology

Highly coordinated operation

Context-independent activity

"Why do we call large protein assemblies underlying cell function protein *machines*? Precisely because, like the machines invented by humans to deal efficiently with the macroscopic world, these protein assemblies contain highly coordinated moving parts. Within each protein assembly intermolecular collisions are not only restricted to a small set of possibilities, but reaction C depends on reaction B, which in turn depends on reaction A – just as it would in a machine"

How Size Conditions Protein Structure and Function

Cell, Vol. 92, 291–294, February 6, 1998, Copyright ©1998 by Cell Press

The Cell as a Collection of Protein Machines: Preparing the Next Generation of Molecular Biologists

Bruce Alberts
President, National Academy of Sciences
2101 Constitution Avenue NW
Washington, D.C. 20418
Professor, Department of Biochemistry and Biophysics
University of California, San Francisco
San Francisco, California 94143

Subcellular assembly	Sample of 'molecular machine' language	Source reference
Ribosome	"probably the most sophisticated machine ever made"	Garrett 1999
Proteasome	"a molecular machine designed for controlled proteolysis"	Voges et al. 1999
Bacteriorhodopsin	"a deceptively simple molecular machine"	Kühlbrandt 2000
Apoptosome	"a seven-spoked death machine"	Salvesen et al. 2002
Glideosome	"a molecular machine powering motility"	Keeley et al. 2003
Spliceosome	"the most complex macromolecular machine known"	Nilsen 2003
Blood clotting system	"a typical example of a molecular machine"	Spronk et al. 2003
Condensin	"the key molecular machine of chromosome condensation"	Strunnikov 2003
Photosynthetic system	"the most elaborate nanoscale biological machine in nature"	Imahori 2004
Bacterial flagellum	"an exquisitely engineered chemi-osmotic nanomachine"	Pallen et al. 2005
Myosin filament	"a complicated machine of many moving parts"	Ohki et al. 2006
RNA degradasome	"a supramolecular machine dedicated to RNA processing"	Marcaida et al. 2006
Cyclosome	"a machine designed to destroy"	Peters 2006
RNA Polymerase	"a multifunctional molecular machine"	Haag et al. 2007

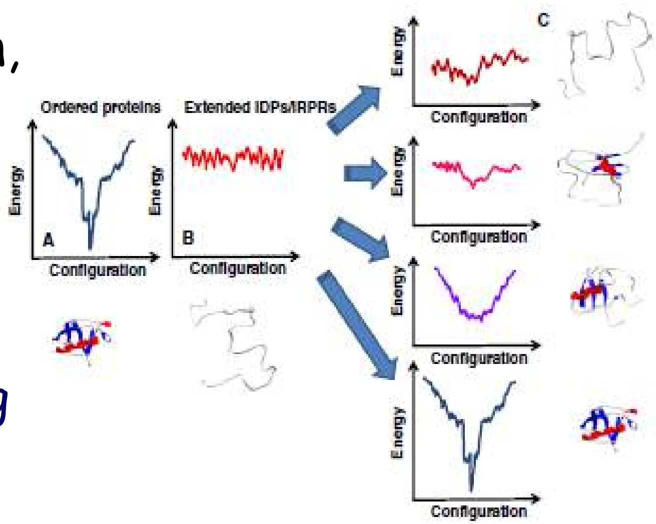
How Size Conditions Protein Structure and Function

- By virtue of their size, proteins cannot exhibit the sort of rigidity and specificity that characterizes the structure and operation of machines

- In vivo, proteins behave more like liquids than solids; they are 'dense liquids' or 'melted solids' with a 'near-solid interior' and a 'full-liquid exterior'

- Most proteins do not have a single conformation, but rather stochastically sample a suite of possible configurations depending on context

- Some proteins lack an ordered structure altogether (IDPs), forming fluid, ever-flickering 'fuzzy' complexes



How Size Conditions Protein Structure and Function

- Due to their tiny size, macromolecular assemblies cannot possibly operate in the orderly, reproducible manner that is characteristic of machines
- In a machine, the motions of the various parts are perfectly orchestrated. For example, when a gear rotates, the shaft to which it is connected rotates in synchrony, a spring is compressed, a latch is released, etc.
- These movements are purposeful, predictable, and are always precisely executed in exactly the same temporal sequence
- In contrast, macromolecules are subject to continuous Brownian motion, which means that the majority of conformational changes they undergo are the result of 'random walks' that have nothing to do with their function

How Size Conditions Protein Structure and Function

How Should We Think About the Ribosome?

Peter B. Moore

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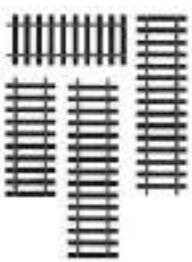
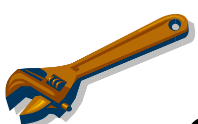
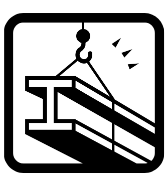
email: peter.moore@yale.edu

"Structure-based movies actively invite viewers to think that the ribosome works the same way as a clock, or a machine for making candy bars. It is no help that macromolecules are commonly called molecular machines. The use of the word 'machine' in this context is pernicious because of its implication that the functional properties of macromolecules can be explained mechanically, *which is simply not true*."

How Size Conditions Protein Structure and Function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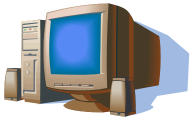
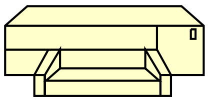



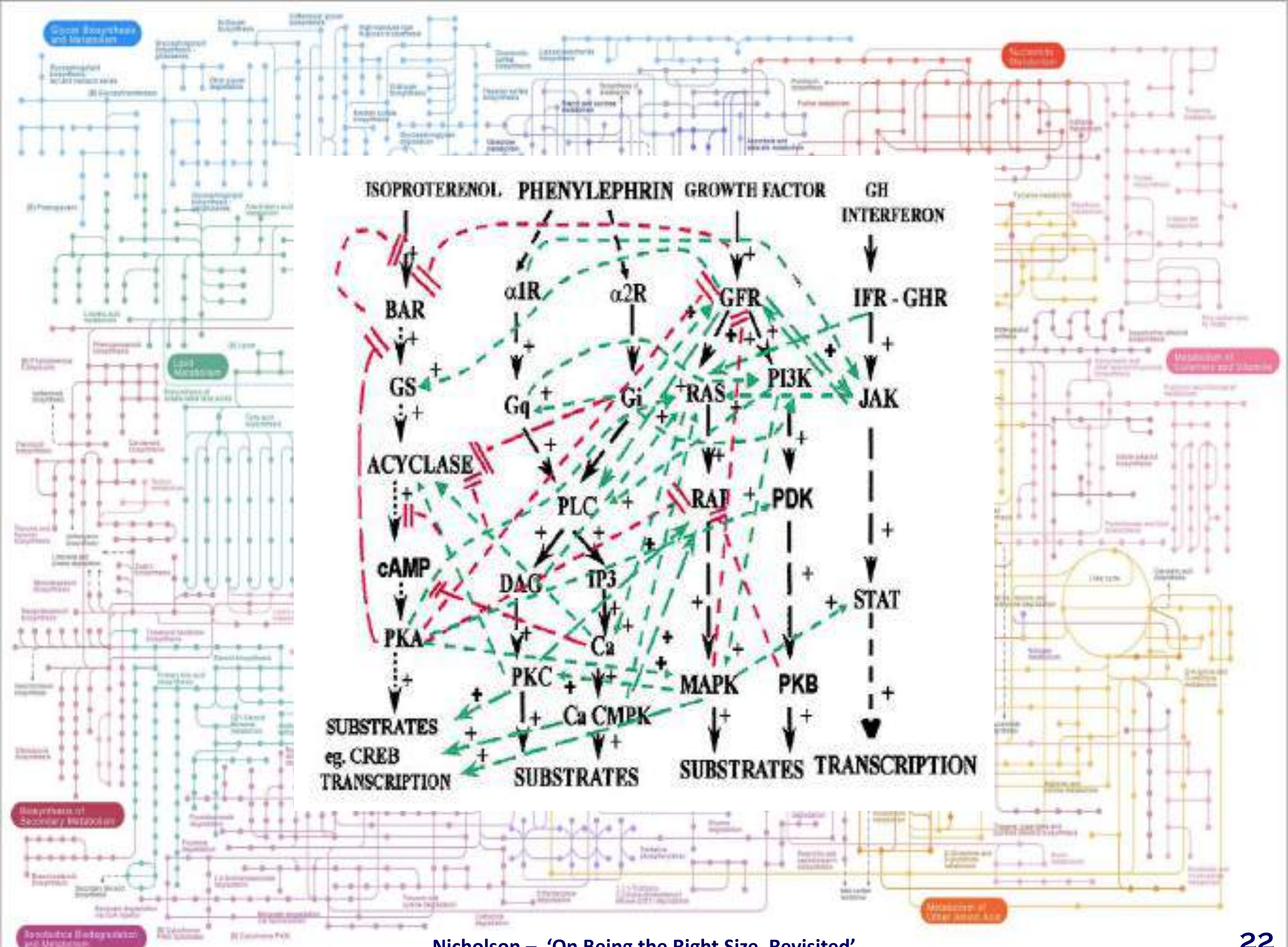
How Size Conditions Protein Structure and Function

- Structure does not unilaterally determine function. Proteins exhibit behavioural repertoires, 'moonlighting' according to the needs of the cell

- Most protein-protein interactions are non-specific. Interactions are short and caused by stochastic events. Associations are transitory & contingent

- Proteins exist in a dynamic environment and rely on probabilistic collision events with appropriate partners to reliably perform cellular functions

- This is all a consequence of the fact that, by virtue of being so small, most proteins are constantly being knocked about by stochastic forces


ISOPROTERENOL
PHENYLEPHRIN
GROWTH FACTOR
GH
INTERFERON
BAR
α1R
α2R
GFR
IFR - GHR
GS
Gq
Gi
RAS
PI3K
JAK
ACYCLASE
PLC
RAF
PDK
cAMP
DAG
IP3
STAT
PKA
Ca
PKC
MAPK
PKB
Ca CMPK
SUBSTRATES
eg. CREB
TRANSCRIPTION
SUBSTRATES
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TRANSCRIPTION

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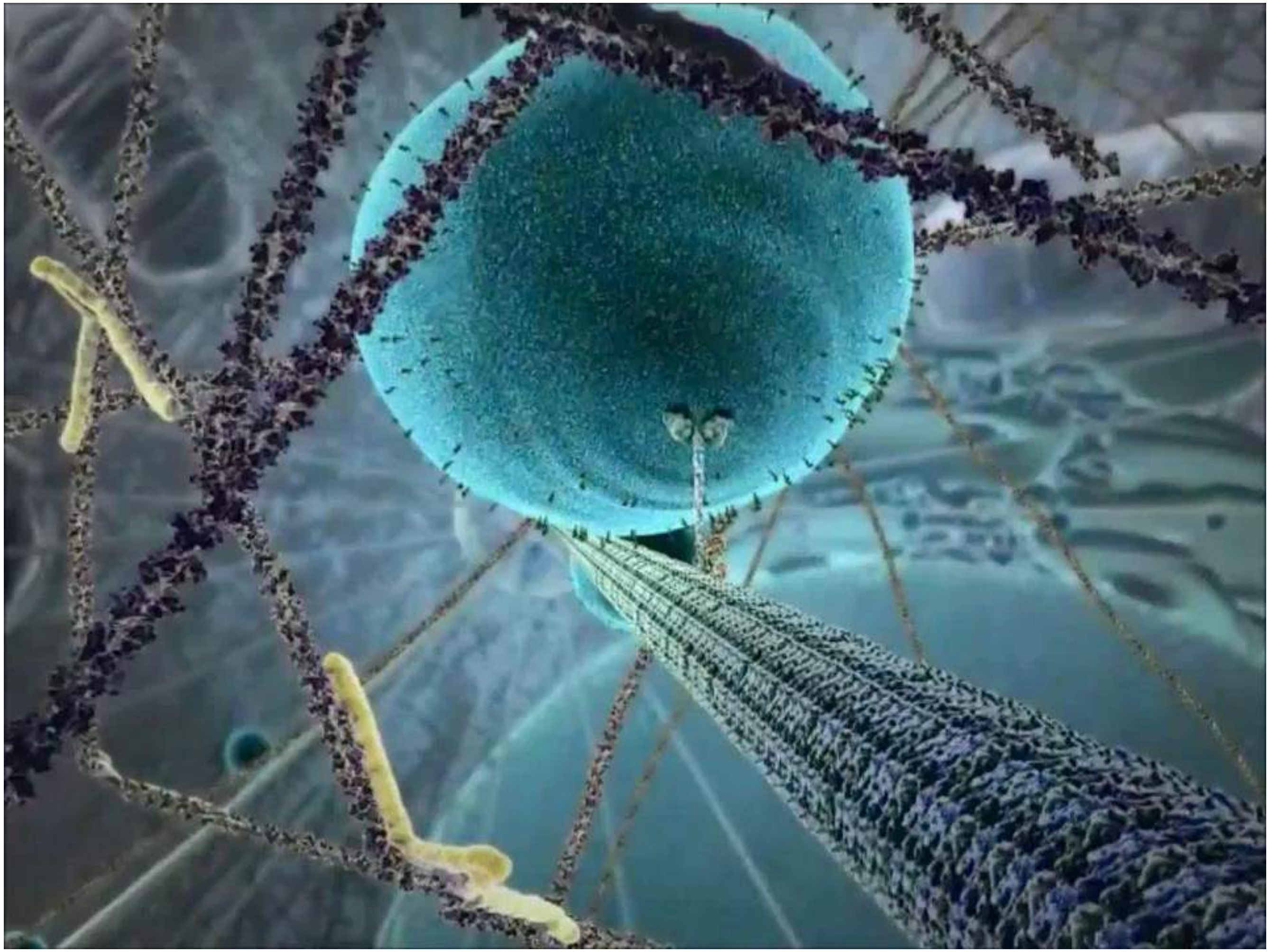
How Size Determines the Operation of Molecular Motors

- Molecular motors (e.g. kinesin, myosin) are viewed as "ingenious" nano-machines, efficiently converting chemical energy into mechanical work
- The repetitive power strokes that move the motor result from periodic conformational rearrangements driven by cycles of ATP hydrolysis
- Molecular motors move by "walking" along the cytoskeleton. The "walking" is enabled by the formation of dimers between motor domains (the "feet") and microtubules or actin filaments ("tracks")

How Size Determines the Operation of Molecular Motors



How Size Determines the Operation of Molecular Motors

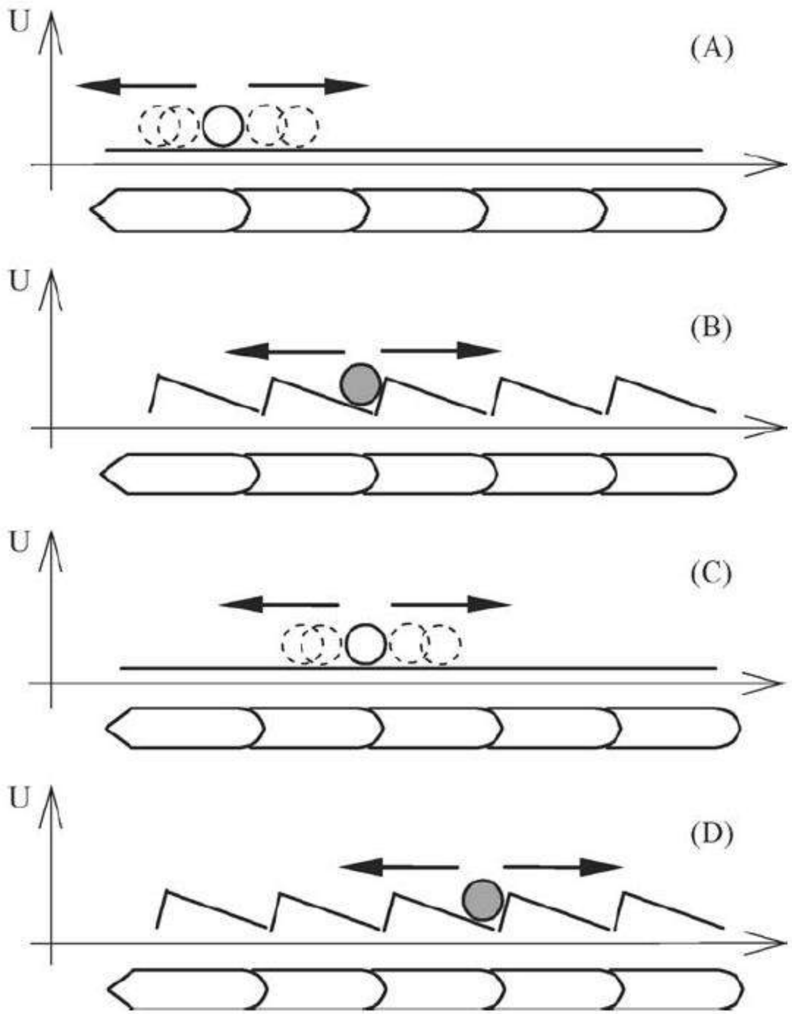
"For molecules, moving deterministically is like trying to walk in a hurricane: the forces propelling it along the desired path are puny compared to the random forces exerted by its environment"

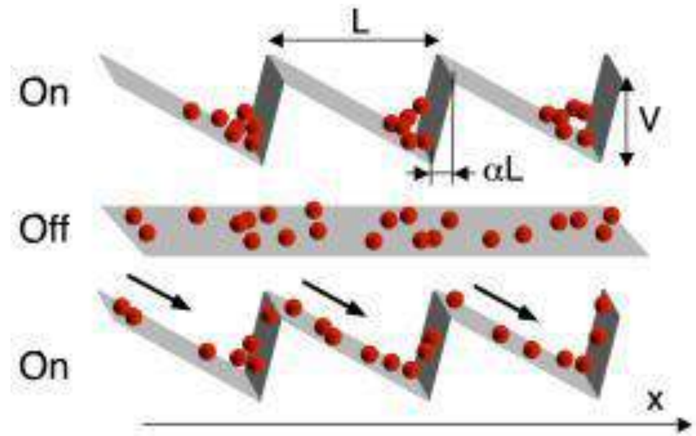
"When considering the operation of a molecular motor, the forces that control the movement of macroscopic objects—in particular gravity—have little relevance. In the molecular world, Brownian storms rage relentlessly"

"A macroscopic motor must either work with Brownian motion or fight against it, and the former seems far more preferable"

"Molecular motors are tiny and operate on a physical scale that makes them very different from the man-made, macroscopic objects we normally imagine when we hear the word "machine"

How Size Determines the Operation of Molecular Motors



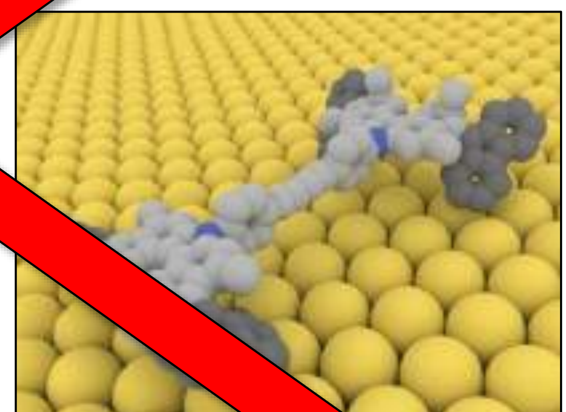


How Size Determines the Operation of Molecular Motors

Macroscopic (Power-Stroke) Motor	Molecular (Brownian) Motor
Continuous Forward Movement	Discontinuous Forward Movement
Energy Input Generates Motion	Energy Input Rectifies Motion
Gravity and Inertia are Fundamental	Gravity and Inertia are Negligible
Brownian Motion is Negligible	Brownian Motion is Fundamental
Thermal	Isothermal
Low Efficiency (Intermediates & Heat)	High Efficiency
Coordinated Motor Movements	No Coordinated Motor Movements

How Size Determines the Operation of Molecular Motors

"Motor proteins are like tiny nanomachines that work in many ways just like an automobile on the highway"



How Size Determines the Operation of Molecular Motors

- We tend to assume that motors constitute a class of machine, but maybe we should reverse this relation and consider machines to be a class of motor

Oxford English Dictionary

motor, *n.* and *a.*

('məʊtə(r))

Also 7 **moter**.

[a. L. *mōtor* (whence F. *moteur*, Sp., Pg. *motor*, It. *motore*), agent-n. from L. *movēre* to MOVE.]

A. *n.*

1. One who or something which imparts motion. **a.** ***first*** or ***prime motor*** [= med.L. *primus motor*]: †(*a*) in mediæval astronomy, = PRIMUM MOBILE 1; (*b*) applied (allusively) to God, as the cause of the motion of the heavens; †(*c*) the first instigator, or the chief director, e.g. of a plot, a political organization, etc.; †(*d*) the part that initiates motion in a piece of mechanism. (Cf. *prime mover*.)

- As machines are not the only entities capable of imparting motion, it follows that not all motors are machines
- 'Machine' carries a number of additional connotations, such as a pre-existing design, a tightly constrained operation, and a deterministic outcome
- Motor proteins are indeed motors, even though they are not machines

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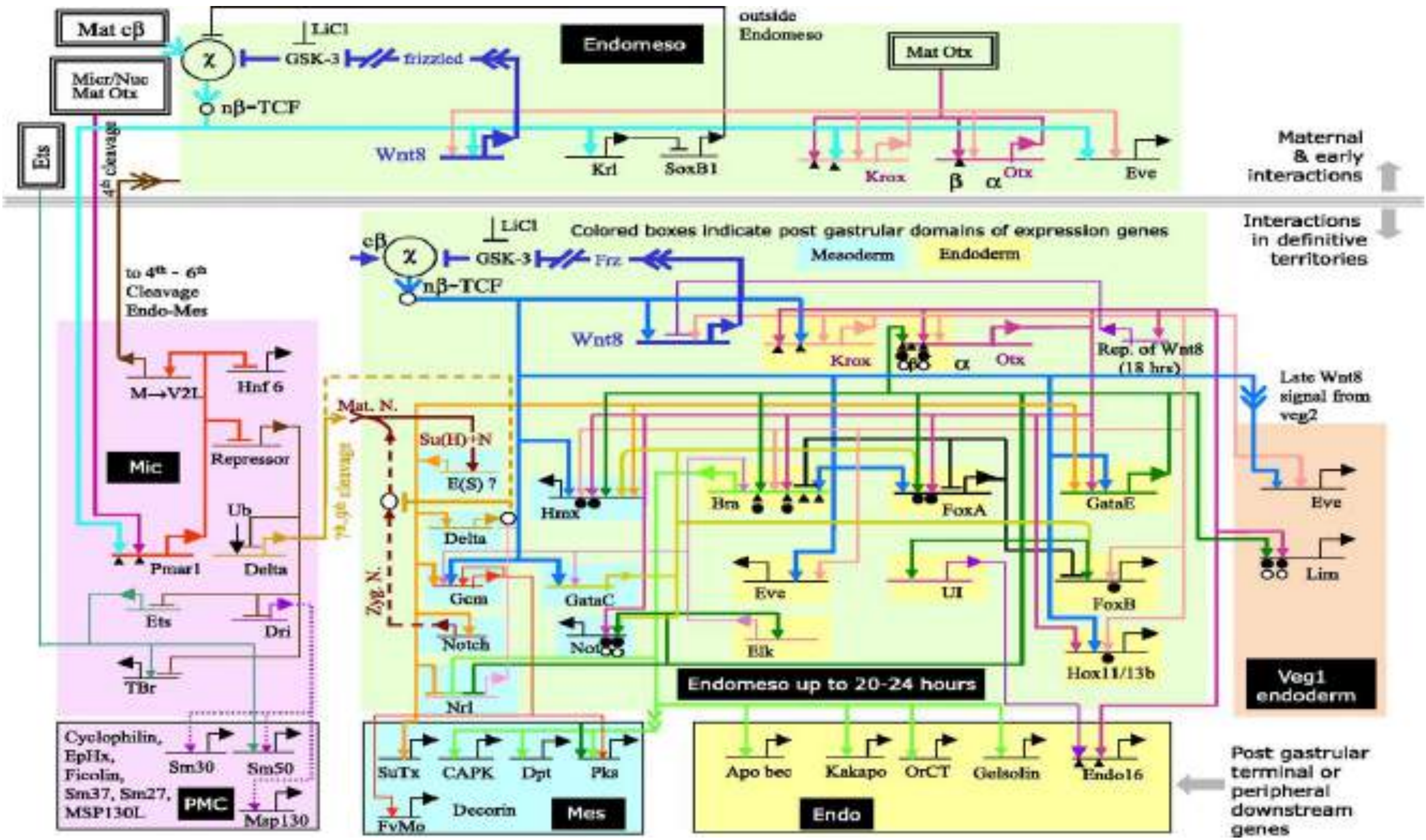
How Size Complicates the Predictability of Cellular Behaviour

- The machine conception of the cell has led to the view that its behavioural patterns are predictable because they result from the execution of set programs of gene expression or signal transduction, in which a precise sequence of steps is followed and a pre-determined response is produced

- GRNs are "modular subcircuits and their interconnections" where each subcircuit is "an information processing unit" that produces a discrete developmental output that can be described in terms of Boolean operators

How Size Complicates the Predictability of Cellular Behaviour



How Size Complicates the Predictability of Cellular Behaviour

- In actual fact, most response patterns of individual cells are unpredictable. The variation in cellular responses results from random fluctuations in the probability of occurrence of a critical, all-or-nothing step in the cell that requires the action of individual molecules subject to stochastic effects.

- The predictability of the overall process of development overall arises because millions of molecules have an extremely low probability of all "misbehaving" together. But the smaller the number of molecules involved in a process, the harder it becomes to predict its behaviour

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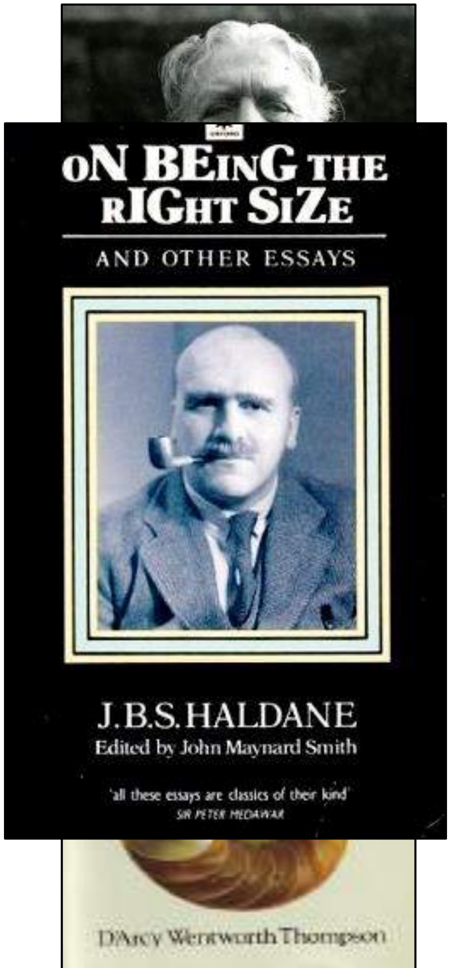

"The world where bacillus lives, gravitation is forgotten, and the viscosity of the liquid, the resistance defined by Stokes' law, the molecular shocks of the Brownian movement, doubtless also the electric charges of the ionized medium, make up the physical environment and have their potent and immediate influence on the organism. The predominant factors are no longer those of our scale; we have come to the edge of a world of which we have no experience, and where all our preconceptions must be recast" (1917)

How Did Molecular Biology Come to Neglect the Impact of Scale?

How Did Molecular Biology Come to Neglect the Impact of Scale?