Computational Modelling Folate Metabolism and DNA Methylation: Implications for Understanding Health and Ageing

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Abstract

Dietary folates have a key role to play in health as deficiencies in the intake of these B vitamins have been implicated in a wide variety of clinical conditions. The reason for this is folates function as single carbon donors in the synthesis of methionine and nucleotides. Moreover, folates have a vital role to play in the epigenetics of mammalian cells by supplying methyl groups for DNA methylation reactions. Intriguingly, a growing body of experimental evidence suggests DNA methylation status could be a central modulator of the ageing process. This has important health implications because the methylation status of the human genome could be used to infer age-related disease risk. Thus, it is imperative we further our understanding of the processes which underpin DNA methylation and how these intersect with folate metabolism and ageing. The biochemical and molecular mechanisms which underpin these processes are complex. However, computational modelling offers an ideal framework for handling this complexity. A number of computational models have been assembled over the years, but to date no model has represented the full scope of the interaction between the folate cycle and the reactions which govern the DNA methylation cycle. In this review we will discuss several of the models which have been developed to represent these systems. In addition we will present a rationale for developing a combined model of folate metabolism and the DNA methylation cycle.

Introduction

The term 'folate' (vitamin B9) is used to denote a group of compounds which possess the same vitamin activity and includes natural folates as well as the pharmacological compounds folic acid and folinic acid [1]. The dietary importance of folate cannot be overstated. This is emphasized by the wide range of clinical disorders which correlate with low folate status [2-4]. In addition, polymorphisms in genes coding for folate dependent enzymes [5, 6] are associated with several cancers (cervical, bronchial, colon and breast) [7-10], Alzheimer's disease [11], Down syndrome [12], unexplained recurrent early pregnancy loss and pre-eclampsia [13].These associations are unsurprising when one considers that folates are involved in a ubiquitous array of cellular processes. For instance, folates are involved in the synthesis of nucleotides from purine precursors, participate indirectly in the synthesis of transfer RNA and function as single carbon donors during the re-methylation of homocysteine (Hcy) to methionine [14] (Figure 1). Folate-derived one-carbon units also play a central role in DNA methylation [15, 16]. This epigenetic process involves methyl groups covalently bonding to CpG dinucleotides to establish tissue specific methylation patterns. A CpG dinucleotide consists of a deoxycytidine followed by a deoxyguanidine, with the "p" indicating the phosphate group between these nucleotides. Covalent bonding of methyl groups occurs at the carbon-5 position of a deoxycytidines to create a methylated CpG dyad [11, 12]. Dynamic changes to methylation patterns are an important gestational phenomenon, which regulate gene expression during embryonic development [17]. However, during adult life alterations to DNA methylation patterns can have significant implications for the onset of disease [18, 19]. Specifically, advancing age is often accompanied by global hypomethylation [20-23] in conjunction with site specific hypermethylation at the promoter region of a variety of genes [24-27]. Hypermethylation, involves CpG islands (~500-2000 base pairs) within the promoter becoming excessively methylated [28, 29], and often occurs with concomitant transcriptional silencing [30, 31]. Gene promoter hypermethylation is a feature of certain diseases, the most notable being cancer [25]. For instance,

Wang *et al.* (2016) [32] recently found that DNA methylation changes during ageing were closely correlated to the occurrence of cancer. In addition to cancer aberrant DNA methylation has an emerging role to play in many age related diseases including, cardiovascular disease (CVD) [33], Alzheimer's disease [34] and osteoporosis/osteoarthritis [35]. Such findings consolidate the growing view that DNA methylation status and health-span are inexorably entwined. Moreover it has also been revealed that investigating changes to the methylation profile of the genome could help develop our understanding of ageing. This assertion is supported by the recent bioinformatics work of Horvath (2013) [36] who utilized publicly available methylation data sets to identify an "epigenetic clock " underpinned by methylation changes in 353 CpGs. Intriguingly, Horvarth postulates that "DNA methylation age" measures the cumulative effect of an epigenetic maintenance system. This finding indicates that a deeper mechanistic understanding of DNA methylation preservation could be pivotal to improving our overall understanding of ageing and health-span.

To extend our understanding of DNA methylation and its bidirectional relationship with ageing it is imperative we consider how subtle alterations to folate one carbon metabolism (FOCM) impact the regulatory processes which govern DNA methylation. Specifically, it is necessary to explore how such perturbations affect gene promoters which are susceptible to hypermethylation. In addition, it is vital we consider how other factors associated with ageing impact this relationship. However, this is not a straightforward task as both FOCM and the DNA methylation cycle are complex processes which are underpinned by a large number of biochemical and molecular reactions (Figure 1). Many of these reactions are non-linear in nature and are influenced by a variety of other B vitamins/nutrients and enzymatic cofactors [37]. Ageing adds a further degree of complexity by altering the dynamics of the reactions. For example, age-dependent decreases in the expression levels/activity of human methionine synthase (MS) and methylenetetrahydrofolate reductase (MTHFR) have been observed [38, 39]. Both enzymes are recognised as key regulators of FOCM and expression/activity changes potentially affect their reaction kinetics. Similarly, the activity of human

DNA methyltransferase 1 (Dnmt 1), a key enzyme involved in the addition of methyl groups to cytosine residues, has also been observed to decrease with age [40]. Moreover, ageing also affects the availability of cofactors such as vitamin B12 [41], which is central to FOCM, while oxidative stress, which is generally regarded as a key contributor to ageing, has been observed to effect both DNA methylation and FOCM [42, 43]. Furthermore, it is not uncommon that older people have diminished folate status as a result of a low dietary intake of the vitamin [44]. Thus, it is necessary to consider an array of factors when investigating the maintenance of DNA methylation. Fortunately, there is a growing appreciation that complex biological process can be studied in a holistic manner by adopting a systems biology approach [45]. Computational modelling resides at the centre of this paradigm shift as it provides a framework for representing and exploring the dynamics of complex systems [46-50]. In this review we discuss the role computational modelling has played in developing our understanding of FOCM and DNA methylation. In addition we propose coupling FOCM and the DNA methylation cycle into one computational model, which could be used to further explore the dynamics of their relationship.

Folate metabolism and the DNA methylation Cycle

FOCM is fundamentally important to DNA methylation. Various folates are the cofactors for the *de novo* synthesis of methyl groups from more oxidized one carbon units and 2) the methyl groups on 5-methyltetrahydrofolate are used to re-methylate hcy to methionine and to regenerate tetrahydrofolate, the metabolically active form of folate. Methionine is a precursor of S-adenosylmethionine (SAM), which has a predominant role to play in the majority of biochemical methyl donation events, including that of DNA methylation. Post replication, Dnmt1 uses SAM as a substrate to transfers methyl groups to the DNA molecule [51] this produces S-adenosylhomocysteine (SAH). SAH is then converted to Hcy permitting the continuation of the methylation cycle [52, 53] (Figure 1). In conjunction with Dnmt1 DNA methylation patterns are dynamically regulated by several other enzymes (Figure 1). As, Dnmt1 preferentially acts on

hemimethylated DNA and is potentially unable to methylate neo-synthesized DNA strands, this enzyme is generally regarded as solely a maintenance enzyme [54].Thus, other enzymes are needed to perform *de novo* DNA methylation. Dnmt3a and Dnmt3b are widely regarded as the main enzymes responsible for this role [55, 56]. These methylation reactions are offset by active and passive demethylation. Passive demethylation occurs during replication, while active methylation can involve ten eleven translocation (TET) dioxygenases, which oxidize the methyl groups of cytosine [57]. TET are thought to function by oxidizing the methyl groups of cytosine. This process eventually culminates with the reintroduction of unmethylated cytosine in the DNA molecule [57]. Thus, it would appear that the steady state levels of both site specific and global DNA methylation patterns are maintained by an antagonistic balancing act between those activities responsible for maintenance/*de novo* methylation and those reactions responsible for passive/active demethylation. Moreover, methylation fidelity studies suggest these processes are subject to inherent stochasticity. For example, Landan *et al.* (2012) [58] tracked the *in vitro* evolution of immortalized fibroblasts for >300 generations and found that changes in population-averaged methylation occur through a stochastic process. In addition, Shipony *et al.* (2014) [59] suggest the persistence nature of the somatic methylome is one factor which makes it increasingly vulnerable to noise with time. This is intriguing from the perspective of ageing as it would appear that in young somatic cells, site specific methylation density is characterised by low level noise, which maintains average methylation density. Therefore, the persistent nature of the methylome and increased stochasticity with time could contribute to the formation of aberrant DNA methylation patterns which are a hallmark of the ageing process in humans.

Computational Models of Folate Metabolism

The models described in the next two sections are summarised in Table 1 and 2 respectively. Models of FOCM have traditionally adopted a continuous deterministic approach, with the dynamics of the system being represented by ordinary differential equations (ODEs), which are underpinned by

kinetic reactions [60]. In the main reaction velocities are assumed to depend on the concentrations

of the reaction substrates (the law of mass action [61]). Rate laws for more complex reactions laws

are in general described by Michaelis–Menten kinetics [62] for one, two, or three substrates

assuming a random-order mechanism. The following mathematical expressions taken from the

recent model by Salcedo-Sora and Mc Auley (2016) [63] encapsulate the different Michaelis–Menten

based functional forms as used for one , two, and three substrates in these types of kinetic models,

where V is the velocity of the reaction, V_{max}, is the theoretical maximum velocity of the reaction, S1

to S3 are substrates and k_{m1} to k_{m3} are the substrate concentrations at which the reaction is half

maximum velocity.

$$V = \frac{V_{max} * [S]}{k_m + [S]} \quad [1]$$

$$V = \frac{V_{max} * [S_1][S_2]}{k_{m_1} k_{m_2} + k_{m_1}[S_2] + k_{m_2}[S_1]} \quad [2]$$

$$V = \frac{V_{max} * [S_1][S_2][S_3]}{k_{m_1} k_{m_2} k_{m_3} + k_{m_1}([S_2]+[S_3]) + k_{m_2}([S_1]+[S_3]) + k_{m_3}([S_1]+[S_2]) + [S_1][S_2][S_3]} \quad [3]$$

The first mathematical model to adopt this kinetic approach to represent intracellular folate

metabolism was developed in the 1970s and had a strong pharmacological theme [64]. It was used

to simulate the actions of methotrexate on DNA synthesis. The next significant computational model

of the folate cycle, was developed by Nijhout *et al.* (2004) [65]. This model is underpinned by the

enzyme kinetic data that characterises the reactions in Figure 1, and was assembled using the type

of mathematical equations outlined above. As well as being the first detailed mathematical

description of the folate cycle, model simulations were able to quantitatively reproduce the

intracellular levels of the various folate metabolites and the model was able to predict the effect of

vitamin B12 deficiency. Building on this work, Reed *et al.* (2006) [66] used this model combined with

models of methionine metabolism [67, 68] to investigate genetic polymorphisms in the folate pathway. Polymorphisms in MTHFR predicted a reduction in MTHFR activity reduces concentrations of SAM and 5-MTHR, and DNA methylation, while slightly increasing SAH and hcy concentrations and thymidine or purine synthesis. Decreased folate together with a simulated vitamin B12 deficiency resulted in decreased DNA methylation and purine and thymidine synthesis. This model was also used as a template to study the effect of intracellular folate deficiency and excess. The model suggested that the enzyme thymidylate synthesis is very sensitive to changes in epithelial intracellular folate and increased significantly under conditions of high intracellular folate [69]. This framework was further extended by Duncan *et al.* (2013) [70] to hepatic and plasma folate turnover. It was applied to a population of virtual individuals and showed that tissue and plasma folate is highly correlated, but liver and plasma folate much less so. Moreover, this model showed that oxidative stress increases the plasma S-adenosylmethionine/S-SAM/SAH ratio. The most recent ODE model of folate metabolism was developed by Salcedo-Sora and Mc Auley (2016) [63]. Our model is the first computational model of microbial folate biosynthesis and utilisation. Using the model we were able to identify specific targets within folate metabolism which synergise with current antifolates. In addition the results from our model support experimental findings which suggest the folinic acid substrate cycle is an important biochemical mechanism deployed during active cell growth. A key finding which emphasizes the utility of computational models for shedding light on this complex system.

Computational Models of the DNA Methylation Cycle

A variety of approaches have been used to model various aspects of DNA methylation [71-79]. Our focus is the precise representation of the DNA methylation cycle. To this end the DNA methylation cycle has been modelled explicitly by McGovern et al. (2012) [80]. The model centred on the activity of DNMTs and partial differential equations was used to represent methylation reactions. It was used as a predictive tool for haematological malignancies. The model was able to predict the relative

abundances of unmethylated, hemimethylated, fully methylated, and hydroxymethylated CpG dyads in the DNA of cells with fully functional methylation and TET enzymes. In addition to this deterministic framework stochastic models of the methylation cycle have also been used to study the methylation cycle. The goal has been to derive models which account for the noise associated with maintaining DNA methylation levels. For example, a reduced probabilistic mathematical representation of methylation dynamics (known as the standard/classical model) has been proposed by Pfeifer *et al.* (1990) [81], and further utilised by Riggs and Xiong (2004) [82]. This model is presented in equations 4-7.

$$\frac{dM}{dt} = E_m * M + E_d * U \quad [4]$$

$$\frac{dU}{dt} = (1 - E_d) * U + (1 - E_m) * M \quad [5]$$

$$M^\wedge = \frac{M}{Total} = \frac{M}{(M + U)} \quad [6]$$

$$M^\wedge = \frac{E_d}{(1 + E_d - E_m)} \quad [7]$$

To briefly explain ODEs 4 and 5, and equations 6 and 7, M is a CpG site, U is its unmethylated state, E_d is the efficiency of *de novo* methylation, E_m is the efficiency of maintenance methylation, M is the number of methylated sites, $M^\wedge$ is the fraction of methylation, while equation 7 represents the steady state solution to ODEs 4 and 5 when $dM^\wedge/dt=0$. This model is underpinned by the idea that site specific methylation levels are underscored by a stochastic process, where *de novo* and maintenance methylation activity are central. Recently Jeltsch and Jurkowska (2014) [83] provided an intriguing theoretical extension to this concept. Jeltsch and Jurkowska propose a stochastic model which also includes the rate of cell division together with passive and active demethylation (equation 8). θ_m^i, is the change in the fraction of methylation at any CpG site (i). This is determined by the difference between the rate of *de novo* DNA methylation (r_{met}^i) and rates of loss of

methylation by replication and active demethylation (r_{demeth}^i) at this site. D, is the rate of cell division,

f_{main}^i is the efficiency of maintenance methylation at site i.

$$\frac{d\theta_m^i}{dt} = r_{met}^i * (1 - \theta_m^i) - (\frac{1}{2} D(1 - f_{main}^i) + r_{demeth}^i) * \theta_m^i \quad [8]$$

According to the authors, equation 8 has the significant advantage over its predecessor as it includes the possibility of proofreading/repair mechanisms due to the incorporation of negative feedback. This level of regulation is attained because an increase in methylation causes a reduction in the first term of equation 8 with a subsequent increase in the second term. Consequently the methylation rate drops and demethylation increases, resulting in a stable steady-state methylation level at each site. To our knowledge this theoretical framework has yet to be applied to experimental data or simulated dynamically. A stochastic mathematical framework also inspired Haerter *et al.* (2014) [84] to create a computational model of this system. Their model differs from the classical/standard model as it included dynamic collaboration between CpGs. More recently Olariu *et al.* (2016) [85] built on the work of Haerter *et al.* (2014) to model the regulation of transcription and DNA methylation.

Rationale for Computationally Integrating FOCM and the DNA Methylation Cycle
Computational models of FOCM are not fully integrated with the DNA methylation cycle. Rather, as outlined they have been restricted to the cycle itself or its involvement with DNA synthesis and have tended not to include the DNA methylation cycle. Some have attempted to account for DNA methylation but no model to our knowledge has explicitly integrated both systems in a meaningful way. We propose computationally integrating FOCM and the methylation cycle. Our biological rationale is unpinned by the knowledge that perturbations in the folate cycle are strongly coupled with aberrant DNA methylation. This is evidenced by the association between low folate status,

global DNA hypomethylation and increased cancer risk [86]. In addition, genetic polymorphisms most notably in the MTHFR gene, which codes for MTHFR, an enzyme which catalyses the conversion of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (5-MTFHF) (Figure 1) is associated with gene promoter hypermethylation [87]. Moreover, methionine synthase A2756G (MTR A2756G) is a common polymorphism in the gene encoding MS. This enzyme catalyses the regeneration of methionine from hcy and disruptions to its enzymatic capabilities also emphasise the close link between folate metabolism and disruptions to DNA methylation. For instance, Weiner and colleagues (2014) [87] found that individuals homozygous for this mutant allele showed higher leukocyte genomic DNA methylation levels than individuals possessing the wild-type genotype (MTR 2756AA). It makes mechanistic sense that perturbations of this nature to FOCM have an impact on DNA methylation, as 5-methyltetrahydrofolate, is a cosubstrate for hcy remethylation to methionine, while MS modulates the dynamics of this reaction and perturbations to this enzyme result in a rise in plasma hcy [88], which is associated with CVD [89]. Intriguingly, a rise in plasma hcy has also been associated with gene promoter hypermethylation [90-93] and global hypomethylation [94-96] of human DNA.

In addition to perturbations due to genetic mutations it is important to recognise that FOCM compartmentalisation could have implications for DNA methylation status as compartmentalisation of FOCM has a role to play in regulating the distribution of one-carbon units within the cell. The partitioning of one-carbon units between nucleotide synthesis and hcy remethylation is the focal point of this process, where methylenetetrahydrofolate (MTHF) is at the centre of a metabolic competition [97, 98]. On the one hand MTHF is used as a cofactor during nucleotide synthesis to facilitate the conversion of dUMP to dTMP by thymidylate synthase (TYMS). However, MTHF can also be reduced and is then fully committed to the remethylation of hcy. The separation of FOCM into distinct cellular compartments is the determining factor which regulates the competition between TYMS and MTHFR for MTHF [99]. This regulation requires the trifunctional, enzyme

methylenetetrahydrofolate dehydrogenase (MTHFD1), whose activity depends on its cellular location [99]. The interdependence of this enzymes activity to its location and its involvement in regulating the competition between MTHFR and TYMS is evident, when intracellular folate levels are deficient, as insufficient levels of folate cause an accumulation of MTHFD1 in the nucleus, when compared to its cytosolic counterpart [100]. The nuclear build-up of MTHFD1 emphasises FOCM will sacrifice hcy remethylation in favour of nucleotide synthesis and underscores the regulatory role compartmentalisation plays in one-carbon metabolism. Intriguingly this aspect of FOCM could have wider implications as recently it has been shown that changes to MTHFD1 can affect the regulation of DNA methylation. Groth *et al.* (2016) [101] identified a mutant (*mthfd1-1*) in *Arabidopsis thaliana*, which carried a mutation in cytoplasmic MTHFD1. This mutant suffered from accumulation of hcy and SAM, coupled with extensive genome-wide hypomethylation. Compartmental regulation of FOCM is also highlighted by cyserine hydroxymethyltransferase (SHMT). SHMT1 catalyses the conversion of tetrahydrofolate (THF) to 5, 10-methylenetetrahydrofolate and also converts serine to glycine. It has been found that cytosine serine hydroxymethyltransferase (cSHMT1) preferentially supplies one-carbon units for thymidylate synthesis [98, 102]. Mechanistically, it is has been found this preference is mediated by small ubiquitin-like modifier (SUMO) modification. This alteration enables the translocation of SHMT to the nucleus and also modifies its catalytic capabilities [103]. Sumoylation could also be crucial to both DNA methylation and demethylation as SUMO-1 modification of DNMT1 had been shown to significantly enhance DNMT1 activity [104]. It is also possible sumoylation is capable of modulating the activity of Dnmt3a and Dnmt3b [105]. This level of crossover between FOCM and DNA methylation consolidates the view that disruptions to FOCM impact the dynamics of DNA methylation/demethylation events. However, the full extent of this relationship remains to be elucidated, hence the growing need for an integrated computational model to explore the complex interactions of these systems.

Theoretical Frameworks and Obstacles to Creating a Combined Model

To develop a combined model several theoretical approaches could be adopted. FOCM could be retained as a set of kinetic based ODEs, as is the case in the majority of models of this nature. It would then be straightforward to add the methylation cycle as ODEs also and to define the combined system as a coupled set of ODEs. Despite the obvious computational advantages of this simplification, these benefits are offset by several factors. Firstly, to assemble this deterministic model the ODEs need to be informed by kinetic data, which accounts for the behaviour of the methylation enzymes. This is not a straightforward task as parameter uncertainty is a significant issue in the field of computational systems biology. As outlined, kinetic based models of folate metabolism tend to have rate laws where the velocity of a reaction assumes mass action kinetics or is based on an enzyme kinetic law (e.g. Michaelis–Menten kinetics). Unfortunately kinetic parameters can vary significantly depending on the circumstances in which their kinetics were quantified and the biological source, with the possibility of model parameters varying by several orders of magnitude depending on the particular source [106]. Therefore, it is extremely unlikely that a mathematical model can be assembled from one biological source. This is highlighted by our recent model of microbial folate metabolism, where the parameters were derived from a number of sources [63]. It is also a feature of the mammalian computational models which have previously relied on data from rat, mice and human studies [65]. Despite such pitfalls, there is no alternative to this approach of determining model parameters. To this end, a number of databases now exist which archive kinetic data for the purpose of assembling computational models [107, 108]. We used these databases to identify a series of indicative kinetic parameters for the model outlined diagrammatically in Figure 1 and are summarised in Table 3. The identification of these parameters serve to emphasize that it is possible to assemble a combined kinetic model of folate metabolism and the DNA methylation cycle based on human data, however the data is limited. Moreover, we

were able to identify kinetic parameters for Dnmt1 and Dnmt3A [109, 110], however kinetic information for the TET demethylation enzymes could not be identified.

Although as outlined above it is clearly possible to assemble a combined deterministic kinetic model, given experimental evidence indicates DNA methylation events are underscored by stochasticity a kinetic based deterministic approach is not the correct way to model this aspect of the system. A more accurate way is to incorporate noise into the model. The standard way of representing noise within biological cells is to describe it mathematically by a master equation or to simulate molecule fluctuations by using a stochastic simulation algorithm (SSA), such as the Gillespie algorithm or one of its derivatives [111, 112]. Routinely embedded within the idea of intracellular noise is the concept of intrinsic versus extrinsic noise, as developed experimentally by Elowitz and colleagues (2002) [113]. Within this context intrinsic noise refers to the variability inherent to the system under consideration, while fluctuations in those factors classified as external to the system of interest are responsible for extrinsic noise (Figure 2). Specifically, this mathematical approach has been ubiquitously applied to the study of cell to cell variability in gene expression levels within isogenic cell populations [114-117].

Applying the above logic to our indicative model it can be argued the reactions of the methylation cycle are responsible for generating intrinsic noise e.g. the inherent statistical mechanical fluctuations in the binding and diffusion dynamics of the molecules involved in maintaining methylation levels within a CpG island/gene body. Conversely, DNA methylation/the epigenetic state of the cell is considered to be a component of extrinsic noise when variations in gene expression are modelled in this way. We suggest extrinsic noise in our integrated model arises due to biochemical fluctuations originating from the folate cycle and other cell parameters particularly those related to ageing (Figure 2). Including biochemical noise arising from FOCM is crucial especially if one considers that site specific (e.g. gene promoter) DNA methylation events interact with FOCM within a

microscopic rather than macroscopic environment. This microenvironment is characterised by low molecular populations, which react at discrete time-points, via random collisions between individual molecules. The advantage of this framework is its simplicity as a complex biological network is reduced to a much simpler abstraction. A drawback of this approach is that it reduces the methylation cycle and FOCM to two variables within a phenomenological model, which lumps all sources of extrinsic noise together. Moreover, as we are concerned with the complexities of the reactions of folate metabolism and how changes to these impact DNA methylation events, it is challenging to separate this source of noise from other extrinsic events e.g. the impact of the cell cycle. A further challenge associated with this proposal is that in order to characterise the intrinsic noise for a given gene promoter it would be necessary to experimentally characterise promoter specific cell to cell methylation variability in young disease free cells and how this relates to protein expression levels. For instance, microscopy or flow cytometry are routinely used to quantify protein expression variability in reporter genes embedded in homogenous cell populations [118, 119]. Protein count distributions then provide an overview of cell to cell fluctuations in gene expression. The stochastic profile can then be characterised as the squared coefficient of variation of the fluorescence levels [120], the coefficient of variation [113] or the Fano Factor [121]. Therefore, it would be necessary to develop experimental methods which are capable of determining the exact nature of the stochasticity of the DNA methylation process. However, given that DNA methylation levels are quantified in a totally different manner to protein fluctuations it is could be challenging to define/characterise its stochastic signature in a precise way experimentally. Next generation sequencing techniques could help to alleviate this problem. These techniques enable the determination of the methylation status at each cytosine for a specific segment of the genome. An eloquent example of this approach is the work of Hansen et al. (2011) [122], who showed stochastic methylation of cancer-specific DNA-methylated regions. This was able to distinguish cancer from normal tissue. More recently Cheow *et al.* (2016) [123] developed a method to genotype individual cells while concurrently probing gene expression and DNA methylation levels at multiple loci. Such

methods could help identify an individual stochastic signature for a particular gene promoter/gene body, which would help inform the mathematics underpinning future computational models. An alternative to the reduced stochastic model approach would be to explicitly represent each deterministic kinetic reaction within FOCM and the DNA methylation cycle as stochastic propensity functions and to simulate the integrated model with a SSA [99], as has been done with other complex biochemical systems [124]. In Figure 2 part b we have created a list of reactions which could be represented as propensity functions, where C_1- C_7 are indicative of stochastic rate constants. This is not unrealistic as mathematical approaches have been developed previously for converting deterministic systems biology models into stochastic models [125-127]. However, regardless of the approach it is our opinion that future computational models should combine these two systems together. Moreover, it is imperative that any combined model accounts for the biochemical and molecular variability which is inherent to both systems. This will improve our overall understanding of how those gene promoters which appear to be susceptible to hypermethylation interact with both folate metabolism and the ageing process.

Conclusions

Folate metabolism and the DNA methylation cycle are inexorably entwined as the folate cycle provides the methyl groups used in DNA methylation reactions. Experimental evidence suggests the dynamics of the folate cycle and the DNA methylation cycle are subject to inherent stochasticity. Investigating this behaviour is challenging, however computational modelling offers and ideal framework for exploring the interactions between these two systems. In this review we proposed building a computational model of folate metabolism coupled with the reactions of the methylation cycle. This model would help to further explore the dynamics of this relationship and could be used to investigate how disruptions to these processes result in aberrant DNA methylation status, specifically gene promoter hypermethylation.

Key Points

- Folate metabolism has a vital role to play in health and possibly intrinsic ageing

- Folates supply methyl groups for DNA methylation reactions which are central to gene regulation.

- Dynamic computational models have been successfully used to study the complexities of folate metabolism and the DNA methylation cycle respectively, but no model has fully coupled both systems.

- To combine both systems computationally it is important to account for the inherent stochasticity which influences the dynamics of both systems.

- Incorporating inherent biochemical and molecular variability will provide an improved understanding of how perturbations to these systems impact the onset of disease and will help to further elucidate the bidirectional relationship between ageing and DNA methylation.

Glossary

Deterministic model	A model which assumes variability does not impact the system of interest. The model will produce the same output given the same initial conditions and parameters.
Extrinsic noise	Fluctuations in the factors external to the biological system of interest e.g. biochemical fluctuations in the folate pathway and its impact on gene expression.
Gillespie algorithm	An algorithm used to generate stochastic models of reaction networks.
Intrinsic noise	Noise associated with a biological system of interest e.g. in the case of this review, site specific DNA methylation.
Stochastic model	A model grounded in probability theory which is used to represent the variability inherent in biological processes. In this review we suggest FOCM is combined with the DNA methylation cycle into a single stochastic model, to capture the inherent variability in body systems.

Figure1. Folate metabolism and its intersection with DNA Methylation: Ingested folates, are the cofactors for the *de novo* synthesis of methyl groups. Methyl groups on 5-methyltetrahydrofolate are used to re-methylate

homocysteine (Hcy) to methionine, with the aid of vitamin B12 and methionine synthase. This reaction regenerates tetrahydrofolate, the metabolically active form of folate. Methionine is a precursor of S-adenosylmethionine (SAM), which has a predominant role to play in the majority of biochemical methyl donation events, including that of DNA methylation. DNA methyltransferase 1 is central to maintaining genomic methylation patterns, while DNA methyltransferase 3a and 3b are both involved in *de novo* methylation reactions. Ten eleven translocation (TET) dioxygenases are actively involved in demethylating cytosine, while CH_3 groups are also lost passively. Pink circles indicate methylated CpG's.

Figure 2. Stochastic contributions to CpG island methylation: Methylation density is affected by intrinsic and extrinsic noise. On the one hand noise is necessary and evolution has finely tuned this system so that stochasticity helps to maintain average methylation density in young cells. However, the ageing process results in gradual increase in stochasticity which eventually culminates with promoter hypermethylation. During aging gene promoter methylation has been identified within a wide variety of genes involved in health-span/ageing (Refer to table 4 for full function and description). Included also are the equations which define intrinsic and extrinsic noise, which are discussed in the main text (Mathematical equations based on framework introduced by Elowitz colleagues (2002) [113]). Part B presents a summary of the combined model represented by a set of indicative reactions which could be modelled using propensity functions. Abbreviations: F, folate; THF, tetrahydrofolate; 5MTHF, 5-methyltetrahyrofolate; 510MTHF, 5,10 methylenetetrahydrofolate; Met, methionine; SAM, S-adenosylmethionine; SAH, S-adenosylhomocysteine; Hcy, homocysteine; DHF, dihydrofolate; 10Form, 10,formyl THF.

Tables 1 to 4.

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