Published in final edited form as:

Adv Neurobiol. 2018 ; 21: 267–281. doi:10.1007/978-3-319-94593-4_10.

Using Systems Biology and Mathematical Modeling Approaches in the Discovery of Therapeutic Targets for Spinal Muscular Atrophy

Matthew E. R. Butchbach

Center for Applied Clinical Genomics, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA

Center for Pediatric Research, Nemours Biomedical Research, Nemours/Alfred I. duPont Hospital for Children, Wilmington, Delaware, USA

Department of Pediatrics, Thomas Jefferson University, Philadelphia, Pennsylvania, USA

Department of Biological Sciences, University of Delaware, Newark, Delaware, USA

Abstract

Systems biology uses a combination of experimental and mathematical approaches to investigate the complex and dynamic interactions with a given system or biological process. Systems biology integrates genetics, signal transduction, biochemistry and cell biology with mathematical modeling. It can be used to identify novel pathways implicated in diseases as well as to understand the mechanisms by which a specific gene is regulated. This review describes the development of mathematical models for the regulation of an endogenous modifier gene, *SMN2*, in spinal muscular atrophy—an early-onset motor neuron disease that is a leading genetic cause of infant mortality worldwide—by cAMP signaling. These mathematical models not only can aid in understanding how *SMN2* expression is regulated but they can also be used to examine the best ways to manipulate cAMP signaling to maximally increase *SMN2* expression. These models will lead to the development of therapeutic strategies for treating SMA. This systems biology approach can also be applied to other neurological diseases, particularly those in which a disease-causing gene or a modifier gene has been identified.

Keywords

spinal muscular atrophy; drug discovery; *SMN2*; ordinary differential equations; gene expression; mathematical modeling

contact information: Matthew E. R. Butchbach, Ph.D., Nemours Biomedical Research, 4462 E400 DuPont Experimental Station, 200 Powder Mill Road, Wilmington, Delaware, USA. Matthew.Butchbach@nemours.org.

Conflict of Interest

The author declares no conflict of interest.

1 Introduction

Systems biology integrates biochemical, genetic and cellular approaches to provide a more comprehensive understanding of higher level processes in living organisms. In this integrated approach, the components of a system are best understood by their relationships within the system as well as with other systems. These interconnected components are referred to as networks. Mathematical modeling of networks is an essential facet of systems biology (Dhurjati and Mahadevan 2008). When studying complex and dynamic interactions, experimental and/or mathematical approaches provide a means to explore and understand the system of networks in question (Dhurjati and Mahadevan 2008). These approaches can also highlight ways in which this system can be manipulated. Systems biology can be used to identify novel pathways implicated in different diseases and to determine the optimal ways of manipulating regulatory networks to treat these diseases.

Network analysis and pathway connectivity approaches in systems biology have provided important insights into the pathogenesis of neurodegenerative diseases (Villoslada et al. 2009). These approaches have led to the identification of novel molecular pathways affected by diseases like hereditary ataxias. Systems biology and mathematical modeling approaches can be used in the development of therapeutic strategies for neurological diseases. Neurological diseases having a clearly genetic etiology, like the pediatric-onset motor neuron disease spinal muscular atrophy (SMA), are particularly amenable to systems biology and mathematical modeling approaches. To illustrate this application, we describe below how mathematical modeling is being used to more thoroughly understand the regulation of *SMN2*, an endogenous modifier gene for SMA, expression and to develop optimal therapeutic targets for this disease.

2 Spinal Muscular Atrophy

Proximal SMA is an autosomal recessive early-onset neurodegenerative disease characterized by the loss of α -motor neurons in the anterior horn of the spinal cord which leads to muscle weakness and atrophy (Crawford and Pardo 1996; Kolb and Kissel 2015). Proximally innervated muscles are preferentially affected over distal muscles in SMA. It is a leading genetic cause of infant and early childhood mortality across the world with an incidence of 1 in ~10,000 live births (Pearn 1978; Cuscó et al. 2002; Sugarman et al. 2012). The carrier frequency for SMA ranges from 1:25 to 1:50 (Zaldívar et al. 2005; Labrum et al. 2007; Hendrickson et al. 2009; Ben-Shachar et al. 2011; Su et al. 2011; Sugarman et al. 2012; Lyahyai et al. 2012; Sangaré et al. 2014). While SMA is primarily a disorder affecting motor neurons, other cells are affected by this disease (Shababi et al. 2014). Arrhythmias and other cardiac abnormalities have been observed in mouse models for SMA (Heier et al. 2010; Shababi et al. 2010; Biondi et al. 2012; Shababi et al. 2012). SMA mice have also demonstrated abnormalities in the autonomic and enteric nervous systems (Bevan et al. 2010; Gombash et al. 2015). Loss of insulin-producing β -cells has been observed in the pancreas (Bowerman et al. 2012; Bowerman et al. 2014). While peripheral organ dysfunction in SMA has been described, it is not yet clear whether or not this dysfunction is a direct result of the disease or a consequence of motor neuron loss and muscle atrophy.

There is a high degree of phenotypic variability within the SMA population. As such, SMA is divided into 5 clinical grades based on age of onset and severity of the disease (Munsat and Davies 1992; Russman 2007). The more severe SMA (types 0 and I) patients have a short lifespan and usually die because of respiratory complications arising from weakness in the intercostals muscles. Due in part to better supportive care (Wang et al. 2007), type II SMA patients generally have a life expectancy into early adulthood. Type III SMA patients usually have a normal lifespan but have difficulty walking. Adult-onset type IV SMA patients generally have a fairly benign disease progression.

Most cases of SMA, regardless of clinical grade, result from large-scale deletions within chromosome 5q13.2 and the loss of the *Survival Motor Neuron 1* (*SMN1*) gene (Lefebvre et al. 1995). The *SMN* gene is duplicated in humans to give rise to *SMN1* and *SMN2* (Rochette et al. 2001). This duplication event is not perfect in that there are single nucleotide differences between *SMN1* and *SMN2*. The major difference between these two *SMN* genes is a translationally silent, C-to-T transition in exon 7 (*SMN2* c.850C>T) (Lorson et al. 1999; Monani et al. 1999). This position on exon 7 lies within an exonic splicing enhancer (ESS) sequence that regulated the inclusion of exon 7 in *SMN1* mRNA transcripts (Fig. 1). This ESS is disrupted in *SMN2* so that most (about 90%) of the *SMN2*-derived mRNAs lack exon 7 (SMN 7) after splicing. The resultant SMN 7 protein is unstable and not fully functional (Lorson and Androphy 2000; Burnett et al. 2009; Cho and Dreyfuss 2010). Some *SMN2* mRNAs—roughly 10%—contain exon 7 which results in the production of some full-length SMN (FL-SMN) protein from *SMN2*.

3 *SMN2* as an Endogenous Genetic Modifier of SMA Phenotype

Since the region of chromosome 5 containing the SMA locus is subject to unequal segmental duplication, *SMN1* and *SMN2* copy numbers are quite variable in the genome. Numerous studies have demonstrated an inverse relationship between *SMN2* copy number and disease severity amongst patient with SMA (reviewed in (Butchbach 2016). As a general rule, those patients with milder forms of SMA have higher *SMN2* copy numbers than severe SMA patients. There are some rare exceptions to this inverse relationship between *SMN2* copy number and disease severity in SMA. Some type II and III SMA patients have been shown to harbor only 2 copies of *SMN2* (Prior et al. 2009; Vezain et al. 2010; Bernal et al. 2010). These patients contain a rare single nucleotide variant (SNV) in exon 7 (*SMN2* c.859G>C) that regulates exon 7 inclusion. *SMN2* is a genetic modifier of disease progression in SMA patients.

The modifier effect of *SMN2* is also observed in animal models for SMA. In *zSmn* (zebrafish orthologue to *SMN1*) mutant zebrafish, *SMN2* extends the survival of mutant larvae and rescues deficits in neuromuscular junction formation in these mutant fish (Le thi Hao et al. 2011). Transgenic insertion of *SMN2* into *mSmn* (murine orthologue to *SMN1*) nullizygous mice rescues embryonic lethality (Schrank et al. 1997; Monani et al. 2000; Hsieh-Li et al. 2000; Michaud et al. 2010). *SMN2* transgene copy number dictates the severity of the SMA phenotype in these mice. In other words, SMA mice with low *SMN2* copy numbers show a severe SMA phenotype (i.e. death within 8 days after birth) while high copy *SMN2* SMA mice have no phenotype (Monani et al. 2000; Hsieh-Li et al. 2000;

Michaud et al. 2010). *SMN2* is, therefore, a major modifier of disease severity in humans as well as in animal models for SMA. These studies also show that *SMN2* is an ideal endogenous molecular target for the development of therapies for SMA.

4 Regulation of *SMN2* Expression by cAMP Signaling

Because of this phenotype modifying property, *SMN2* has been the target for numerous drug discovery strategies (Cherry et al. 2014). Targeting cyclic adenosine monophosphate (cAMP) signaling is of particular interest in developing inducers of *SMN2* expression. The cAMP signaling cascade (Fig. 2) regulates various cellular processes including gene expression, cell growth, metabolism and stress response (Kleppe et al. 2011). The *SMN2* promoter contains at least one cAMP-response element (CRE) that is able to bind to activated CRE-binding protein (phospho-CREB) (Majumder et al. 2004). The β 2-adrenergic agonist salbutamol increases the amount of FL-SMN protein in SMA fibroblasts and leukocytes of SMA patients (Angelozzi et al. 2008; Tiziano et al. 2010). Forskolin, which stimulates adenylyl cyclase (AC) catalysis to produce cAMP from ATP, increases *SMN2* promoter activity (Majumder et al. 2004). The synthetic analogue dibutyryl cAMP (dbcAMP)—which activates cyclic AMP-dependent protein kinase (PKA)—also increases *SMN2* promoter activity (Majumder et al. 2004). We have recently shown that modulators of cAMP signaling significantly increase the number of gems—subnuclear foci of SMN protein (Liu and Dreyfuss 1996)—in fibroblasts derived from a type II SMA patient (Mack et al. 2014). Taken together, these studies show that modulation of cAMP signaling can increase SMN levels from *SMN2*.

5 Mathematical Modeling of Gene Expression

Mathematical models use mathematical concepts and terminology to describe a biological network using a set of variables and equations to define the relationships between these variables. Mathematical models are initially generated using available experimental data and domain knowledge. Through a process involving multiple iterations, the model assumptions are revised and refined in order to develop improved models that better fit the biological network (Dhurjati and Mahadevan 2008). This adaptability of mathematical models also makes it possible to integrate multiple pathways into a network model.

There are two types of mathematical models, quantitative and logic (Le Novère 2015). Quantitative models are linear representations of quantitative variables over time and can be used to compute concentrations of biomolecules and genes as well as durations of biomolecular interactions and processes. Quantitative models are precise and provide a direct comparison with experimentally-derived quantitative measurements but *a priori* knowledge of initial conditions and kinetic parameters is required to generate these models. Logic models, on the other hand, use qualitative activities and define phenotypes to compute transitions between two states and stable behaviors, known as attractors. While logic models are easy to generate and to use for simulation experiments, they are not useful for making quantitative predictions and it is difficult to select between multiple attractors. Historically, mathematical models have been designed to be either quantitative or logic; however, newer

models which integrate quantitative modules with logic modules are being developed (Ryll et al. 2014).

Gene expression networks can be modeled mathematically using either thermodynamic, differential equation-based or Boolean (probabilistic) approaches (Ay and Arnosti 2011). The selection of modeling approach depends on the type of biological data available (qualitative or quantitative), the nature of the system to be modeled (static vs. dynamic), the level of detail and the scale of the model. Thermodynamic models are generated by factoring the quality and the arrangements of binding site for a biomolecule, for example, binding of transcription factors to their response elements within DNA. Thermodynamic models assume that the system is at a state of equilibrium and, hence, cannot describe the dynamic nature of the system. Differential equation models focus on regulatory interactions where time, state and space are viewed as continuous variables. As a result, differential equation models readily factor in the dynamic nature of the system in question. These models use ordinary differential equations (ODEs) if only one continuous variable, like time, is being factored or partial differential equations (PDEs) when multiple variables are being factored. Since ODEs and PDEs can be difficult to solve analytically, differential equation-based models can be hard to implement computationally, especially for larger biological networks. Boolean models represent relationships as one of two possible states, on or off, and can combine qualitative data into a logical structure. Instead of viewing variables as continuous, Boolean models consider time, state and space as discrete variables. While Boolean models are easy to analyze and implement computationally, they can be inaccurate if the system depends on fine details.

In most cases, there are unknown parameters within a mathematical model; as a result, these parameters need to be estimated so as to fit the proposed model with the experimental data. Parameter estimation begins with an initial estimate and new estimates are iteratively generated so as to minimize the error between simulated and experimental data. An objective function that measures model performance—such as the sign squared error or sum of squares of the residuals between model simulations and experimental data—is used in parameter estimation. More detailed information on parameter estimation approaches can be found elsewhere (Banga and Balsa-Canto 2008; Ay and Arnosti 2011). Parameter estimation is affected by both the structure of the model and the biological system for that model (Ay and Arnosti 2011). In order to assess how the model structure can affect parameter estimation, the effects of changes in parameter inputs on model outputs needs to be measured by process of sensitivity analysis. Local sensitivity analysis focuses on a specific set of parameter values at one point in time or space while global approaches examine the entire model over a range of parameter values. Further detailed information regarding the specifics of sensitivity analysis can be found elsewhere (Ingalls 2008). Sensitivity analysis is essential for the building and interpreting mathematical models.

6 Overall Strategy for Building Mathematical Models of Gene Expression

Mathematical modeling is an essential component of systems biology but it can appear daunting to biologists, especially those with limited experience in mathematical biology. Fig. 3 provides a generic workflow for generating and testing a mathematical model for gene

expression and cell signaling. This workflow is designed for differential equation-based models of the regulation of gene expression and cellular signaling. When beginning the process of model generation, one of the best sources of information for building mathematical models is the primary literature. There are also some recent reviews which describe the methodological details of generating a mathematical model for gene expression and cell signaling (Zi 2012; MacLean et al. 2016).

The first step in mathematical model generation is to create a comprehensive gene regulatory pathway map using existing biological information. CellDesigner is a convenient tool to graphically represent these pathway maps (Funahashi et al. 2003). CellDesigner uses standardized set of symbols known as Systems Biology Graphical Notation (SBGN; (Hucka et al. 2003) to represent components of a biological network and their relationships (Le Novère et al. 2009). Complex Pathway Simulator (COPASI) is another platform-independent biological simulator program that can be used to generate mathematical models (Hoops et al. 2006; Mendes et al. 2009). The models can then imported into the mathematical software MATLAB using the Systems Biology Toolbox (Schmidt and Jirstrand 2006).

Once the pathway map has been generated, the kinetics for each reaction in the regulatory pathway must be assigned. The two primary components of a mathematical model, the differential equations and the conservation equations, can now be generated. The differential equations, which generally take the form of ODEs, represent changes in the components of a reaction in response to stimulation. The conservation equations are meant to show the balance between active and inactive forms of a signaling intermediate. The values of all of the parameters within each reaction kinetics equation must also be set from either *a priori* knowledge or be estimated using an objective function as described in the previous section (Banga and Balsa-Canto 2008; Ay and Arnosti 2011).

Simulations for the mathematical models can be completed once the parameters have been set and the initial concentrations of signaling components are estimated or determined from the literature. The robustness of a biological model can be assessed with sensitivity analysis as described in the previous section (Ingalls 2008; MacLean et al. 2016). For a model to be considered robust, its outcomes must not be markedly affected by perturbations of the parameters or initial concentrations. With a robust model, the effects of altered expression of a component on the outcome, i.e. expression of the target gene, can be measured and future biological experiments can be designed with the assistance of mathematical models.

7 Mathematical Modeling of *SMN2* Regulation by cAMP Signaling

A systems biology approach can be used to investigate *SMN2* gene regulation. We recently developed mathematical models to characterize the regulation of *SMN2* expression by cAMP signaling (Mack et al. 2014). This approach is based on additive interactions between experimental data and mathematical models. We focused on the *SMN2* regulation by cAMP signaling because there is ample evidence in the literature showing that activation of cAMP signaling increases *SMN2* expression (Majumder et al. 2004; Angelozzi et al. 2008; Tiziano et al. 2010). The experimental data for these mathematical models were obtained from gem—a marker for SMN localization within the nucleus—assays in type II SMA fibroblasts

because the reduction in gems correlates with SMN protein expression and SMA severity (Coover et al. 1997) and this assay has been used in multiple studies identifying compounds which increase SMN expression (Andreassi et al. 2001; Sumner et al. 2003; Andreassi et al. 2004; Lunn et al. 2004; Grzeschik et al. 2005; Jarecki et al. 2005; Riessland et al. 2006; Mattis et al. 2006; Novoyatleva et al. 2008; Thurmond et al. 2008; Xiao et al. 2011). These gem inducing agents were validated by immunoblot or enzyme-linked immunosorbent assays (ELISAs).

The cAMP signaling treatment data were used to generate two distinct mathematical models, the full cAMP:*SMN2* and alternate cAMP:*SMN2* models (Fig. 4) (Mack et al. 2014). The full cAMP:*SMN2* model (Fig. 4a) factors in the effect of CREB activation on *SMN2* transcription. As some groups have suggested that cAMP signaling regulates *SMN2* expression post-transcriptionally by influencing FL-SMN protein stability (Burnett et al. 2009; Harahap et al. 2015), an alternate cAMP:*SMN2* model (Fig. 4b) was generated. Both models are extensions of a cAMP signaling mathematical model in yeast (Williamson et al. 2009). The models contain ODEs as well as conservation equations. The full cAMP:*SMN2* model contains seven ODEs and three conservation equations while the alternate cAMP:*SMN2* model contains five ODEs and two conservation equations (Mack et al. 2014). Simulated data from both models match with the experimental gem data showing that either model is valid. When these two models were combined, however, the resultant simulated data did not fit well with the experimental data suggesting that only one model correctly recapitulates the effect of cAMP signaling cascade on *SMN2* expression. Since the experimental data used to generate these models were fixed at one point in time, it is currently not possible to assess which mathematical model more accurately simulates cAMP signaling-dependent regulation of *SMN2* expression. Future studies examining various facets of *SMN2* regulation including gem formation will allow better refinement and distinction between these two models.

8 Conclusions and Future Directions

Regulation of *SMN2* expression by cAMP signaling can be modeled mathematically. The regulation of *SMN2* by cAMP signaling is complex, multi-faceted and not completely understood. SMN is directly phosphorylated by PKA *in vitro* (Burnett et al. 2009; Wu et al. 2011). The interactions between SMN and other components of the core SMN macromolecular complex may be dependent upon PKA-dependent phosphorylation of SMN. PKA phosphorylation of SMN protein could not be factored into either mathematical model because the effects of PKA phosphorylation of SMN on its function and localization are not yet known. If PKA phosphorylation impacts SMN function, then this component of cAMP signaling can be factored into refined mathematical models of cAMP signaling and *SMN2* expression.

Another facet of gene regulation is the impact of other signaling pathways on the target pathway. For example, numerous extracellular stimuli including activation of ionotropic glutamate receptors, exercise and inhibition of insulin-like growth factor I receptor (IGF1R) increases SMN expression in the spinal cord by AKT-mediated phosphorylation of CREB (Biondi et al. 2010; Branchu et al. 2013; Biondi et al. 2015). CREB is regulated by the

protein serine/threonine phosphatase 2A (PP2A) (Wadzinski et al. 1993). Protein serine/threonine phosphatase inhibitors like cantharidin and tautomycin have been shown to increase *SMN2* expression (Novoyatleva et al. 2008; Zhang et al. 2011). These natural product inhibitors may act through suppression of CREB dephosphorylation and, as a result, activation of the *SMN2* promoter. As new insights are gained as to how these other intracellular pathways affect CREB-mediated activation of *SMN2* expression, the intersection of AKT and PP2A with cAMP signaling can be integrated into current mathematical models of *SMN2* expression.

In addition to identifying the optimal component of the cAMP signaling pathway responsible for regulating *SMN2* expression, mathematical models can be used to predict the effects of drug combinations. For example, activation of AC by forskolin can act in concert with cyclic nucleotide phosphodiesterase (cnPDE) inhibition by rolipram to additively increase gem formation, as predicted mathematically (Mack et al. 2014). Once validated experimentally, mathematical modeling can be used to design combination strategies that target different parts of a signaling cascade, in this case cAMP signaling. Furthermore, drug discovery efforts have identified numerous small molecule activators of *SMN2* expression that operate either by increasing *SMN2* transcription or alternative splicing to increase the proportion of *FL-SMN* transcripts (reviewed in (Cherry et al. 2014)). As the molecular targets and signaling pathways affected by these small molecules are identified, parallel mathematical models can be generated for each pathway as it relates to *SMN2* gene regulation. These pathways can then be integrated so as to create a comprehensive mathematical model for *SMN2* gene regulation. This comprehensive model can be used to predict which pathways could be modulated synergistically in order to maximize *SMN2* upregulation which will drive the development of combination therapeutic strategies for SMA.

In conclusion, mathematical modeling is a systems biology approach that can be used to understand how gene expression can be regulated by a signaling pathway. This approach has recently been applied to the regulation of *SMN2* expression by cAMP signaling. This systems-based mathematical modeling approach can ultimately aid in the development and optimization of cAMP signaling-based therapies for SMA. A similar approach could also be used for other molecular pathways that regulate *SMN2* expression. Mathematical models of these individual pathways regulating *SMN2* expression can then be integrated to create a more comprehensive model of *SMN2* gene regulation. Furthermore, mathematical modeling can be applied to other neurogenetic diseases wherein modifier genes, like *SMN2* for SMA, have been identified.

Acknowledgments

This work was supported by an Institutional Development Award (IDeA) from the National Institute of General Medical Sciences of the National Institutes of Health (P30GM114736) and by the Nemours Foundation.

ABBREVIATIONS USED

AC	adenylate cyclase
----	-------------------

cAMP	cyclic AMP
CRE	cAMP-response element
cnPDE	cyclic nucleotide phosphodiesterase
CREB	CRE binding protein
dbcAMP	dibutyryl cAMP
ELISA	enzyme-linked immunosorbent assay
ESS	exonic splicing enhancer
FL-SMN	full length SMN
GPCR	G protein-coupled receptor
IGF1R	insulin-like growth factor 1 receptor
ODE	ordinary differential equation
PDE	partial differential equation
PKA	cAMP-dependent protein kinase
PP2A	protein phosphatase 2A
SMA	spinal muscular atrophy
SMN 7	SMN lacking exon 7
SMN1	survival motor neuron 1
SMN2	survival motor neuron 2
SNV	single nucleotide variant

References

- Andreassi C, Angelozzi C, Tiziano FD, Vitali T, De Vincenzi E, Boninsegna A, et al. Phenylbutyrate increases SMN expression in vitro: relevance for treatment of spinal muscular atrophy. *Eur J Hum Genet* 2004;12:59–65. [PubMed: 14560316]
- Andreassi C, Jarecki J, Zhou J, Coover DD, Monani UR, Chen X, et al. Aclarubicin treatment restores SMN levels to cells derived from type I spinal muscular atrophy patients. *Hum Mol Genet* 2001;10:2841–9. [PubMed: 11734549]
- Angelozzi C, Borgo F, Tiziano FD, Martella A, Neri G, Brahe C. Salbutamol increases SMN mRNA and protein levels in spinal muscular atrophy cells. *J Med Genet* 2008;45:29–31. [PubMed: 17932121]
- Ay A, Arnosti DN. Mathematical modeling of gene expression: a guide for the perplexed biologist. *Crit Rev Biochem Mol Biol* 2011;46:137–51. [PubMed: 21417596]
- Banga JR, Balsa-Canto E. Parameter estimation and optimal experimental design. *Essays Biochem* 2008;45:195. [PubMed: 18793133]
- Ben-Shachar S, Orr-Urtreger A, Bardugo E, Shomrat R, Yaron Y. Large-scale population screening for spinal muscular atrophy: clinical implications. *Genet Med* 2011;13:110–4. [PubMed: 21233719]

- Bernal S, Alías L, Barceló MJ, Also-Rallo E, Martínez-Hernández R, Gámez J, et al. The c.859G>C variant in the SMN2 gene is associated with types II and III SMA and originates from a common ancestor. *J Med Genet* 2010;47:640–2. [PubMed: 20577007]
- Bevan AK, Hutchinson KR, Foust KD, Braun L, McGovern VL, Schmelzer L, et al. Early heart failure in the SMN⁻⁷ model of spinal muscular atrophy and correction by postnatal scAAV9-SMN delivery. *Hum Mol Genet* 2010;19:3895–905. [PubMed: 20639395]
- Biondi O, Branchu J, Ben Salah A, Houdebine L, Bertin L, Chali F, et al. IGF-1R reduction triggers neuroprotective signaling pathways in spinal muscular atrophy mice. *J Neurosci* 2015;35:12063–79. [PubMed: 26311784]
- Biondi O, Branchu J, Sanchez G, Lancelin C, Deforges S, Lopes P, et al. In vivo NMDA receptor activation accelerates motor unit maturation, protects spinal motor neurons and enhances SMN2 gene expression in severe spinal muscular atrophy mice. *J Neurosci* 2010;30:11288–99. [PubMed: 20739549]
- Biondi O, Lopes P, Desseille C, Branchu J, Chali F, Ben Salah A, et al. Physical exercise reduces cardiac defects in type 2 spinal muscular atrophy-like mice. *J Physiol* 2012;590:5907–25. [PubMed: 22930275]
- Bowerman M, Michalski JP, Beauvais A, Murray LM, DeRepentigny Y, Kothary R. Defects in pancreatic development and glucose metabolism in SMN-depleted mice independent of canonical spinal muscular atrophy neuromuscular pathology. *Hum Mol Genet* 2014;23:3432–44. [PubMed: 24497575]
- Bowerman M, Swoboda KJ, Michalski JP, Wang GS, Reeks C, Beauvais A, et al. Glucose metabolism and pancreatic defects in spinal muscular atrophy. *Ann Neurol* 2012;72:256–68. [PubMed: 22926856]
- Branchu J, Biondi O, Chali F, Collin T, Leroy F, Mamchaoui K, et al. Shift from extracellular signal-related kinase to AKT/cAMP response element-binding protein pathway increases survival-motor-neuron expression in spinal-muscular-atrophy-like mice and patient cells. *J Neurosci* 2013;33:4280–94. [PubMed: 23467345]
- Burnett BG, Muñoz E, Tandon A, Kwon DY, Sumner CJ, Fischbeck KH. Regulation of SMN protein stability. *Mol Cell Biol* 2009;29:1107–15. [PubMed: 19103745]
- Butchbach MER. Copy number variations in the Survival Motor Neuron genes: implications for spinal muscular atrophy and other neurodegenerative diseases. *Front Mol Biosci* 2016;3:7. [PubMed: 27014701]
- Butchbach MER, Burghes AHM. Perspectives on models of spinal muscular atrophy for drug discovery. *Drug Disc Today Dis Models* 2004;1:151–6.
- Cherry JJ, Kobayashi DT, Lynes MM, Naryshkin NN, Tiziano FD, Zaworksi PG, et al. Assays for the identification and prioritization of drug candidates for spinal muscular atrophy. *Assay Drug Dev Technol* 2014;12:315–41. [PubMed: 25147906]
- Cho S, Dreyfuss G. A degtron created by SMN2 exon 7 skipping is a principal contributor to spinal muscular atrophy severity. *Genes Dev* 2010;24:438–42. [PubMed: 20194437]
- Coovert DD, Le TT, McAndrew PE, Strasswimmer J, Crawford TO, Mendell JR, et al. The survival motor neuron protein in spinal muscular atrophy. *Hum Mol Genet* 1997;6:1205–14. [PubMed: 9259265]
- Crawford TO, Pardo CA. The neurobiology of childhood spinal muscular atrophy. *Neurobiol Dis* 1996;3:97–110. [PubMed: 9173917]
- Cuscó I, Barceló MJ, Soler C, Parra J, Baiget M, Tizzano E. Prenatal diagnosis for risk of spinal muscular atrophy. *Br J Obstet Gynaecol* 2002;109:1244–9.
- Dhurjati P, Mahadevan R. Systems biology: the synergistics interplay between biology and mathematics. *Can J Chem Eng* 2008;86:127–41.
- Funahashi A, Morohashi M, Kitano H, Tanimura N. CellDesigner: a process diagram editor for gene-regulatory and biochemical networks. *BIOSILICO* 2003;1:159–62.
- Gombash SE, Cowley CJ, Fitzgerald JA, Iyer CC, Fried D, McGovern VL, et al. SMN deficiency disrupts gastrointestinal and enteric nervous system function in mice. *Hum Mol Genet* 2015;24:3847–60. [PubMed: 25859009]

- Grzeschik SM, Ganta M, Prior TW, Heavlin WD, Wang CH. Hydroxyurea enhances SMN2 gene expression in spinal muscular atrophy cells. *Ann Neurol* 2005;58:194–202. [PubMed: 16049920]
- Harahap NIF, Nurputra DK, Rochmah MA, Shima A, Morisada N, Takarada T, et al. Salbutamol inhibits ubiquitin-mediated survival motor neuron protein degradation in spinal muscular atrophy cells. *Biochem Biophys Rep* 2015;4:351–6. [PubMed: 29124224]
- Heier CR, Satta R, Lutz C, DiDonato CJ. Arrhythmia and cardiac defects are a feature of spinal muscular atrophy model mice. *Hum Mol Genet* 2010;19:3906–18. [PubMed: 20693262]
- Hendrickson BC, Donohoe C, Akmaev VR, Sugarman EA, Labrousse P, Boguslavskiy L, et al. Differences in SMN1 allele frequencies among ethnic groups within North America. *J Med Genet* 2009;46:641–4. [PubMed: 19625283]
- Hoops S, Sahle S, Gauges R, Lee C, Pahle J, Simus N, et al. COPASI—a COMplex PATHway SIMulator. *Bioinformatics* 2006;22:3067–74. [PubMed: 17032683]
- Hsieh-Li HM, Chang JG, Jong YJ, Wu MH, Wang NM, Tsai CH, et al. A mouse model for spinal muscular atrophy. *Nat Genet* 2000;24:66–70. [PubMed: 10615130]
- Hucka M, Finney A, Sauro HM, Bolouri H, Doyle JC, Kitano H, et al. The systems biology markup language (SBML): a medium for representation and exchange of biochemical network models. *Bioinformatics* 2003;19:524–31. [PubMed: 12611808]
- Ingalls B Sensitivity analysis: from model parameters to system behaviour. *Essays Biochem* 2008;45:177–93. [PubMed: 18793132]
- Jarecki J, Chen X, Bernardino A, Coover DD, Whitney M, Burghes AHM, et al. Diverse small-molecule modulators of SMN expression found by high-throughput compound screening: early leads towards a therapeutic for spinal muscular atrophy. *Hum Mol Genet* 2005;14:2003–18. [PubMed: 15944201]
- Kleppe R, Krakstad C, Selheim F, Kopperud R, Døskeland SO. The cAMP-dependent protein kinase pathway as therapeutic target—possibilities and pitfalls. *Curr Top Med Chem* 2011;11:1393–405. [PubMed: 21513494]
- Kolb SJ, Kissel JT. Spinal muscular atrophy. *Neurol Clin* 2015;33:831–46. [PubMed: 26515624]
- Labrum R, Rodda J, Krause A. The molecular basis of spinal muscular atrophy (SMA) in South African black patients. *Neuromuscul Disord* 2007;17:684–92. [PubMed: 17627822]
- Le Novère N Quantitative and logic modelling of molecular and gene networks. *Nat Rev Genet* 2015;16:146–58. [PubMed: 25645874]
- Le Novère N, Hucka M, Mi H, Moodie S, Schreiber F, Sorokin A, et al. The Systems Biology Graphical Notation. *Nat Biotechnol* 2009;27:735–41. [PubMed: 19668183]
- Le thi Hao, Burghes AHM, Beattie CE Generation and characterization of a genetic zebrafish model of SMA carrying the human SMN2 gene. *Mol Neurodegen* 2011;6:24.
- Lefebvre S, Bürglen L, Reboullet S, Clermont O, Burlet P, Violette L, et al. Identification and characterization of a spinal muscular atrophy-determining gene. *Cell* 1995;80:155–65. [PubMed: 7813012]
- Liu Q, Dreyfuss G. A novel nuclear structure containing the survival of motor neurons protein. *EMBO J* 1996;15:3555–65. [PubMed: 8670859]
- Lorson CL, Androphy EJ. An exonic enhancer is required for inclusion of an essential exon in the SMA-determining gene SMN. *Hum Mol Genet* 2000;9:259–65. [PubMed: 10607836]
- Lorson CL, Hahnen E, Androphy EJ, Wirth B. A single nucleotide in the SMN gene regulates splicing and is responsible for spinal muscular atrophy. *Proc Natl Acad Sci U S A* 1999;96:6307–11. [PubMed: 10339583]
- Lunn MR, Root DE, Martino AM, Flaherty SP, Kelley BP, Coover DD, et al. Indoprofen upregulates the survival motor neuron protein through a cyclooxygenase-independent mechanism. *Chem Biol* 2004;11:1489–93. [PubMed: 15555999]
- Lyahyai J, Sbiti A, Barkat A, Ratbi I, Sefiani A. Spinal muscular atrophy carrier frequency and estimated prevalence of the disease in Moroccan newborns. *Genet Test Mol Biomarkers* 2012;16:215–8. [PubMed: 21950724]
- Mack SG, Cook DJ, Dhurjati P, Butchbach MER. Systems biology investigation of cAMP modulation to increase SMN levels for treatment of spinal muscular atrophy. *PLoS ONE* 2014;9:e115473. [PubMed: 25514431]

- MacLean AL, Harrington HA, Stumpf MPH, Byrne HM. Mathematical and statistical techniques for systems medicine: the Wnt signaling pathway as a case study. *Methods Mol Biol* 2016;1386:405–39. [PubMed: 26677193]
- Majumder S, Varadharaj S, Ghoshal K, Monani U, Burghes AHM, Jacob ST. Identification of a novel cyclic AMP response element (CRE-II) and the role of CREB-1 in the cAMP-induced expression of the survival motor neuron (SMN) gene. *J Biol Chem* 2004;279:14803–11. [PubMed: 14742439]
- Mattis VB, Rai R, Wang J, Chang CWT, Coady T, Lorson CL. Novel aminoglycosides increase SMN levels in spinal muscular atrophy fibroblasts. *Hum Genet* 2006;120:589–601. [PubMed: 16951947]
- Mendes P, Hoops S, Sahle S, Gauges R, Dada J, Kummer U. Computational models of biochemical networks using COPASI. *Methods Mol Biol* 2009;500:17–59. [PubMed: 19399433]
- Michaud M, Arnoux T, Bielli S, Durand E, Rotrou Y, Jablonka S, et al. Neuromuscular defects and breathing disorders in a new mouse model of spinal muscular atrophy. *Neurobiol Dis* 2010;38:125–35. [PubMed: 20085811]
- Monani UR, Lorson CL, Parsons DW, Prior TW, Androphy EJ, Burghes AHM, et al. A single nucleotide difference that alters splicing patterns distinguishes the SMA gene SMN1 from the copy gene SMN2. *Hum Mol Genet* 1999;8:1177–83. [PubMed: 10369862]
- Monani UR, Sendtner M, Coover DD, Parsons DW, Andreassi C, Le TT, et al. The human centromeric survival motor neuron gene (SMN2) rescues embryonic lethality in *Smn*^{-/-} mice and results in a mouse with spinal muscular atrophy. *Hum Mol Genet* 2000;9:333–9. [PubMed: 10655541]
- Munsat TL, Davies KE. International SMA Consortium meeting. *Neuromuscul Disord* 1992;2:423–8. [PubMed: 1300191]
- Novoyatleva T, Heinrich B, Tang Y, Benderska N, Butchbach MER, Lorson CL, et al. Protein phosphatase 1 binds to the RNA recognition motif of several splicing factors and regulates alternative pre-mRNA processing. *Hum Mol Genet* 2008;17:52–70. [PubMed: 17913700]
- Pearn J. Incidence, prevalence and gene frequency studies of chronic childhood spinal muscular atrophy. *J Med Genet* 1978;15:409–13. [PubMed: 745211]
- Prior TW, Krainer AR, Hua Y, Swoboda KJ, Snyder PC, Bridgeman SJ, et al. A positive modifier of spinal muscular atrophy in the SMN2 gene. *Am J Hum Genet* 2009;85:408–13. [PubMed: 19716110]
- Riessland M, Brichta L, Hahnen E, Wirth B. The benzamide M344, a novel histone deacetylase inhibitor, significantly increases SMN2 RNA/protein levels in spinal muscular atrophy cells. *Hum Genet* 2006;120:101–10. [PubMed: 16724231]
- Rochette CF, Gilbert N, Simard LR. *SMN* gene duplication and emergence of the SMN2 gene occurred in distinct hominids: SMN2 is unique to *Homo sapiens*. *Hum Genet* 2001;108:255–66. [PubMed: 11354640]
- Russman BS. Spinal muscular atrophy: clinical classification and disease heterogeneity. *J Child Neurol* 2007;22:946–51. [PubMed: 17761648]
- Ryll A, Bucher J, Bonin A, Bongard S, Gonçalves E, Saez-Rodriguez J, et al. A model integration approach linking signalling and gene-regulatory logic with kinetic metabolic models. *Biosystems* 2014;124:26–38. [PubMed: 25063553]
- Sangaré M, Hendrickson B, Sango HA, Chen K, Nofziger J, Amara A, et al. Genetics of low spinal muscular atrophy carrier frequency in sub-Saharan Africa. *Ann Neurol* 2014;75:525–32. [PubMed: 24515897]
- Schmidt H, Jirstrand M. Systems Biology Toolbox for MATLAB: a computational platform for research in systems biology. *Bioinformatics* 2006;22:514–5. [PubMed: 16317076]
- Schrank B, Götz R, Gunnensen JM, Ure JM, Toyka KV, Smith AG, et al. Inactivation of the survival motor neuron gene, a candidate gene for human spinal muscular atrophy, leads to massive cell death in early mouse embryos. *Proc Natl Acad Sci U S A* 1997;94:9920–5. [PubMed: 9275227]
- Shababi M, Habibi J, Ma L, Glascock JJ, Sowers JR, Lorson CL. Partial restoration of cardio-vascular defects in a rescued severe model of spinal muscular atrophy. *J Mol Cell Cardiol* 2012;52:1074–82. [PubMed: 22285962]

- Shababi M, Habibi J, Yang HT, Vale SM, Sewell WA, Lorson CL. Cardiac defects contribute to the pathology of spinal muscular atrophy models. *Hum Mol Genet* 2010;19:4059–71. [PubMed: 20696672]
- Shababi M, Lorson CL, Rudnik-Schöneborn S. Spinal muscular atrophy: a motor neuron disorder or a multi-organ disease? *J Anat* 2014;224:15–28. [PubMed: 23876144]
- Su YN, Hung CC, Lin SY, Chen FY, Chern JPS, Tsai C, et al. Carrier screening for spinal muscular atrophy (SMA) in 107,611 pregnant women during the period 2005–2009: a prospective population-based cohort study. *PLoS ONE* 2011;6:e17067. [PubMed: 21364876]
- Sugarman EA, Nagan N, Zhu H, Akmaev VR, Zhou Z, Rohlf AM, et al. Pan-ethnic carrier screening and prenatal diagnosis for spinal muscular atrophy: clinical laboratory analysis of > 72400 specimens. *Eur J Hum Genet* 2012;20:27–32. [PubMed: 21811307]
- Summer CJ, Huynh TN, Markowitz JA, Perhac JS, Hill B, Coover DD, et al. Valproic acid increases SMN levels in spinal muscular atrophy patient cells. *Ann Neurol* 2003;54:647–54. [PubMed: 14595654]
- Thurmond J, Butchbach MER, Palomo M, Pease B, Rao M, Bedell L, et al. Synthesis and biological evaluation of novel 2,4-diaminoquinazoline derivatives as SMN2 promoter activators for the potential treatment of spinal muscular atrophy. *J Med Chem* 2008;51:449–69. [PubMed: 18205293]
- Tiziano FD, Lomastro R, Pinto AM, Messina S, D'Amico A, Fiori S, et al. Salbutamol increases survival motor neuron (SMN) transcript levels in leukocytes of spinal muscular atrophy (SMA) patients: relevance for clinical trial design. *J Med Genet* 2010;47:856–8. [PubMed: 20837492]
- Vezain M, Saukkonen AM, Goina E, Touraine R, Manel V, Toutain A, et al. A rare SMN2 variant in a previously unrecognized composite splicing regulatory element induces exon 7 inclusion and reduces the clinical severity of spinal muscular atrophy. *Hum Mutat* 2010;31:E1110–E1125. [PubMed: 19953646]
- Villoslada P, Steinman L, Baranzini SE. Systems biology and its application to the understanding of neurological diseases. *Ann Neurol* 2009;65:124–39. [PubMed: 19260029]
- Wadzinski BE, Wheat WH, Jaspers S, Peruski LF Jr, Lickteig RL, Johnson GL, et al. Nuclear protein phosphatase 2A dephosphorylates protein kinase A-phosphorylated CREB and regulates CREB transcriptional stimulation. *Mol Cell Biol* 1993;13:2822–34. [PubMed: 8386317]
- Wang CH, Finkel RS, Bertini ES, Schroth M, Simonds A, Wong B, et al. Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol* 2007;22:1027–49. [PubMed: 17761659]
- Williamson T, Schwartz JM, Kell DB, Stateva L. Deterministic mathematical models of the cAMP pathway in *Saccharomyces cerevisiae*. *BMC Syst Biol* 2009;3:70. [PubMed: 19607691]
- Wu CY, Curtis A, Choi Y, Maeda M, Xu MJ, Berg A, et al. Identification of the phosphorylation sites in the survival motor neuron protein by protein kinase A. *Biochim Biophys Acta* 2011;1814:1134–9. [PubMed: 21609790]
- Xiao J, Marugan JJ, Zheng W, Titus S, Southall N, Cherry JJ, et al. Discovery, synthesis and biological evaluation of novel SMN protein modulators. *J Med Chem* 2011;54:6215–33. [PubMed: 21819082]
- Zaldívar T, Montejo Y, Acevedo AM, Guerra R, Vargas J, Garofalo N, et al. Evidence of reduced frequency of spinal muscular atrophy type I in the Cuban population. *Neurology* 2005;65:636–8. [PubMed: 16116135]
- Zhang Z, Keleman O, Van Santen MA, Yelton SM, Wendlandt AE, Sviripa VM, et al. Synthesis and characterization of pseudocantharidins, novel phosphatase modulators that promote the inclusion of exon 7 into the SMN (survival of motoneuron) pre-mRNA. *J Biol Chem* 2011;286:10126–36. [PubMed: 21220421]
- Zi Z A tutorial on mathematical modeling of biological signaling pathways. *Methods Mol Biol* 2012;880:41–51. [PubMed: 23361980]

Fig. 1. Molecular difference between *SMN1* and *SMN2* and its effect on splicing.
This figure is adapted from (Butchbach and Burghes 2004; Butchbach 2016).

Fig. 2. Regulation of *SMN2* gene expression by the cAMP pathway.

Ligand binds to and activates its membrane-bound G protein-coupled receptor (GPCR) leading to the dissociation of the $G_{\alpha,s}$ subunit from the GPCR. $G_{\alpha,s}$ then activates adenylyl cyclase (AC) which converts intracellular ATP into cAMP. cAMP then activates cAMP-dependent protein kinase—or protein kinase A (PKA). The catalytic PKA subunit, now freed of its regulatory subunits, acts on cAMP-response element-binding (CREB) protein. Phosphorylated CREB (phospho-CREB) binds to cAMP response elements (CREs) with the promoter regions of *SMN2*. Cyclic nucleotide phosphodiesterases (cnPDEs) diminish cAMP signaling by breaking down cAMP into AMP. This figure is adapted from (Mack et al. 2014).

Fig. 3.

Strategy for generating and testing mathematical models of gene expression and cell signaling.

Fig. 4. Mathematical models for modulation of *SMN2* expression by cAMP signaling. Schematic representations of the full cAMP:*SMN2* (a) and alternate cAMP:*SMN2* (b) models for the regulation of *SMN2* expression by cAMP signaling. This figure is adapted from (Mack et al. 2014).