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increase in nuclear delivery of plasmids still complexed with the vector. Above the threshold unpackaging rate constant, NLS addition should indeed yield a substantial increase in nuclear plasmid delivery and protein production. The threshold unpackaging rate constant seems to represent a lower limit value for polyplex gene delivery such that adding a NLS will not have a significant affect on protein production. That is, if the vector can unpackage from the plasmid only very slowly, the DNA is not accessible for transcription even if there is an increase in nuclear delivery of the complex facilitated by the NLS.

Therefore, this model simulation represents the experimental addition of NLSs of varying affinities to a polymer vector while simultaneously determining the optimal chain length. Intuitively, addition of a NLS should increase nuclear uptake of delivered plasmids and should therefore cause a corresponding increase in gene expression. However, the model predicted a significant increase in gene expression only above a threshold unpackaging rate of 10^{-3} min^{-1}. Upon reconsideration, such a phenomenon makes sense; if the vector does not unpackage sufficiently, thereby inhibiting transcription initiation by protecting the DNA from the transcription machinery, no amount of nuclear plasmid delivery augmentation will increase gene expression. Model analysis reveals that, for example, at the threshold unpackaging rate constant (k_{unpack}) value of 10^{-3} min^{-1}, conjugation of a NLS onto the vector—effectively increasing the import protein binding rate constant (k_{bind}) from 10^{-3} min^{-1} to 10^{-2} min^{-1}—is predicted to increase normalized protein production from approximately 8% to 18% of the maximal value, whereas the same NLS conjugation to a vector with the optimal unpackaging rate constant of 10^{-2} min^{-1} is predicted to increase normalized protein production from approximately 14% to 100% of the maximum value. Yet, with the vast number of potential parameter combinations, many of which are not so intuitive, it is easy to see how the myriad of design characteristics available for synthetic vector development can be incorrectly applied or left unoptimized.

Although the developed model successfully predicts the *in vitro* uptake and trafficking of liposomal and polylysine delivered plasmids, it is insufficient to correlate to *in vivo* work. However, *in vivo* correlation may result from further development of the mathematical model to include equations and parameters describing events and processes involved with complex administration to the animal including, but certainly not limited to, delivery of complexes directly to tissues or intravenously, blood transport and component interaction, transport to organs, systemic clearance, diffusion through tissues, and cell binding. Such further model developments may be instrumental in characterizing gene delivery treatment behavior and perhaps in reducing or refining *in vivo* expermentation.

Further model validation will come from its application to other cell types and vector systems, viral and nonviral, as the processes occurring for synthetic vectors must also occur for their viral counterparts. Such application of the model will require quantitative, dynamic experimental data on cell and vector properties.

MATERIALS AND METHODS

Construction of computational model. A first-order mass action kinetic model was constructed for gene delivery (Fig. 1). Included in this model are the processes of vector/cell binding and internalization, endosomal escape, vector unpackaging, plasmid degradation in the cytoplasm, nuclear import protein binding, nuclear pore complex association and import, import protein dissociation, and protein production. This essentially represents the simplest computational model capable of accounting for the key processes previously reported to significantly influence transgene delivery and expression via synthetic vectors [1]. Increased complexity can be incorporated by including nonlinearities in a number of the rate processes, if appropriate quantitative experimental information is available.

Model kinetic parameters (Table 1) and species (Table 2) were combined to derive the following ordinary differential equations (ODEs) and solved simultaneously using a stiff ODE solver in MATLAB (The MathWorks, Inc., Natick, MA), where each equation is the differential mass balance for a particular species. The initial conditions for all species were set at zero plasmids; the cells were assumed to be at a steady-state in the absence of any plasmid, vector, or complex before the addition of transfection complexes.

$$d(Complex_{Internal})/dt = Complex_{total} * e^{(-t)} - k_{escape} * Complex_{Internal} \quad (1)$$

$$d(Complex_{Cytoplasmic})/dt = k_{escape} * Complex_{Internal} - k_{bind} * Complex_{Cytoplasmic} - k_{unpack} * Complex_{Cytoplasmic} \quad (2)$$

$$d(ComplexBound_{Cytoplasmic})/dt = k_{bind} * Complex_{Cytoplasmic} - k_{NPC} * ComplexBound_{Cytoplasmic} \quad (3)$$

$$d(ComplexBound_{NPC})/dt = k_{NPC} * ComplexBound_{Cytoplasmic} - k_{In} * ComplexBound_{NPC} \quad (4)$$

$$d(ComplexBound_{Nuclear})/dt = k_{In} * ComplexBound_{NPC} - k_{dissociation} * ComplexBound_{Nuclear} \quad (5)$$

$$d(Complex_{Nuclear})/dt = k_{dissociation} * ComplexBound_{Nuclear} - k_{unpack} * Complex_{Nuclear} \quad (6)$$

$$d(Vector_{Nuclear})/dt = k_{unpack} * Complex_{Nuclear} + k_{dissociation} * VectorBound_{Nuclear} \quad (7)$$

$$d(Plasmid_{Nuclear})/dt = k_{unpack} * Complex_{Nuclear} + k_{dissociation} * PlasmidBound_{Nuclear} \quad (8)$$

$$d(Vector_{Cytoplasmic})/dt = k_{unpack} * Complex_{Cytoplasmic} - k_{bind} * Vector_{Cytoplasmic} \quad (9)$$

$$d(Plasmid_{Cytoplasmic})/dt = k_{unpack} * Complex_{Cytoplasmic} - k_{bind} * Plasmid_{Cytoplasmic} - k_{degradation} * Plasmid_{Cytoplasmic} \quad (10)$$

$$d(PlasmidBound_{Cytoplasmic})/dt = k_{bind} * Plasmid_{Cytoplasmic} - k_{NPC} * PlasmidBound_{Cytoplasmic} \quad (11)$$

$$d(PlasmidBound_{NPC})/dt = k_{NPC} * PlasmidBound_{Cytoplasmic} - k_{In} * PlasmidBound_{NPC} \quad (12)$$

$$d(PlasmidBound_{Nuclear})/dt = k_{In} * PlasmidBound_{NPC} - k_{dissociation} * PlasmidBound_{Nuclear} \quad (13)$$

$$d(X\_Plasmid_{Cytoplasmic})/dt = k_{degradation} * Plasmid_{Cytoplasmic} - k_{bind} * X\_Plasmid_{Cytoplasmic} \quad (14)$$

$$d(Protein)/dt = k_{protein} * Plasmid_{Nuclear} \quad (15)$$

Equation (1), the mass ODE for the number of internalized complexes ($Complex_{Internal}$), was derived using the parameter $Complex_{total}$, the total number of plasmids delivered to a cell, fit from the experimental data to a value of 9.0×10^4 plasmids. This equation captured the saturation of uptake and escape of plasmids into the cytoplasm observed experimentally at the total delivered complex number, a process not simply described by an internalization parameter ($k_{internalization}$).

The parameter values (Table 1) are first-order rate constants representing cellular and vector processes and therefore have units of min^{-1}. When the inverse of the value is multiplied by the natural log of 2, a characteristic half-life for the process may be obtained. For example, the characteristic half-life for plasmid degradation in the cytoplasm, the natural log of 2 divided by $k_{degradation}$, yields 138 min, or approximately 2 h.