

Computing Life; A Test with Carbon Metabolism in *Neurospora crassa*.

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ABSTRACT

Large amounts of sequence data are being generated by various genome projects. These data- driven efforts will require hypothesis- driven programs to extract biologically useful information.

Neurospora crassa provides a well studied eukaryote for correlating biochemical pathways with the underlying protein and transcriptional profiles. The functioning of the *qa* cluster in *N. crassa* was modeled by a chemical network model within a computer program called Kin. An initial set of input parameters yields a linear increase in protocatechuic acid (PCA) following a lag period. Other parameters in this initial set were changed, some yielding unstable behavior. A parameter set was found which allows predictions to be compared to the *in vivo* system.

INTRODUCTION

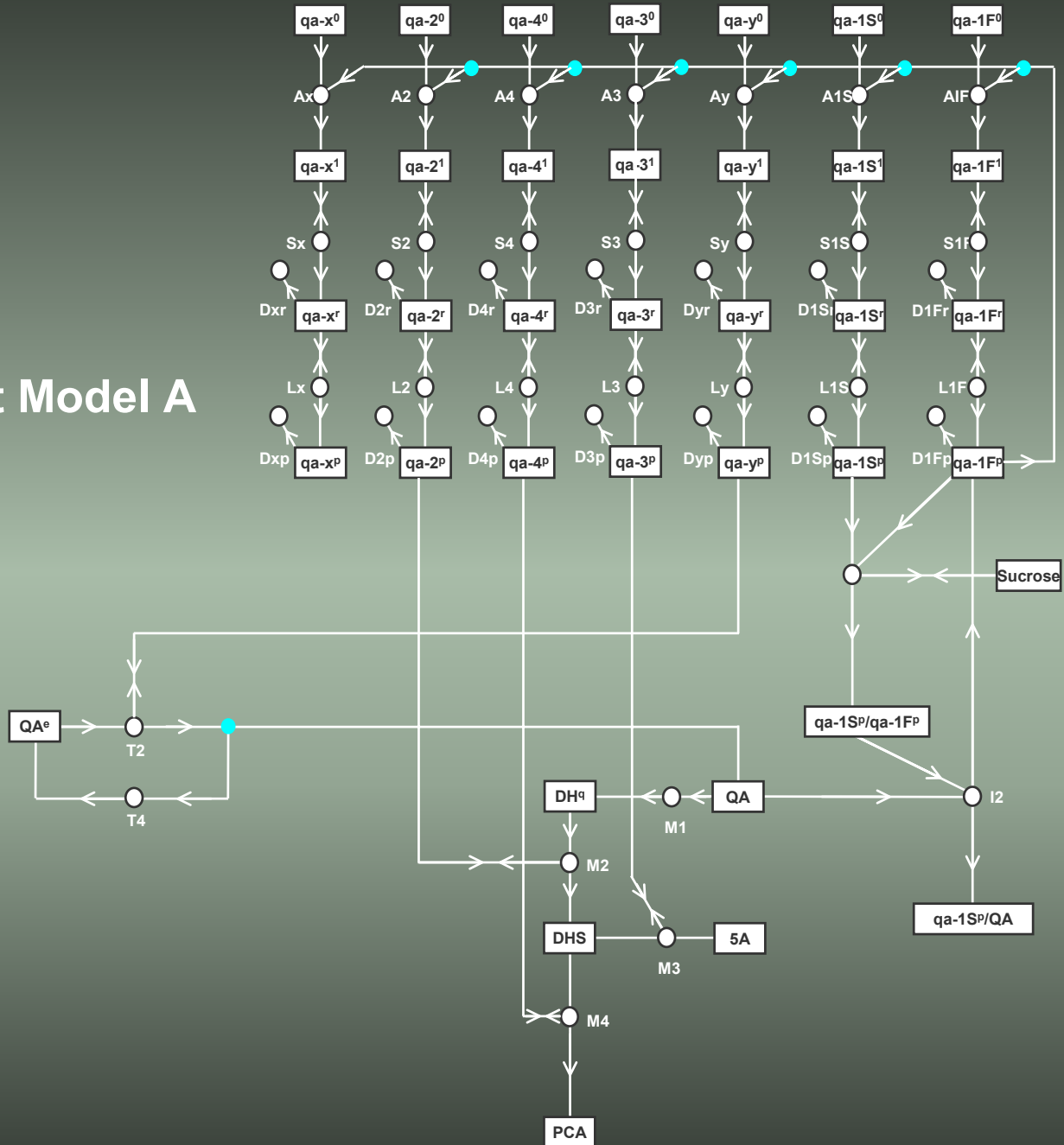
Computers have become essential tools in biological research from controlling robotic laboratory machines to modeling and predicting complex biological phenomena. Genome projects require the assistance of computers to prepare samples, collect data, and to analyze and to analyze the results in human-readable form. The data from genome projects can be utilized to develop mathematical models of the biological functioning of biochemical pathways. This requires determining kinetic and regulatory parameters of the underlying enzymes and regulators by experiment or estimation. The computer model can then be used to predict how the organism will react *in vivo* to a change in conditions. Comparing predicted to actual behavior tests the mathematical model and may suggest changes are needed in the model and models can assist gene product discovery in agriculture, medicine, and industry.

THE MODEL

The mathematical model of the qa cluster in *N.crassa* was outlined by constructing a schematic based on early work by (Geever, et al 1989). The schematic (figure 1) summarizes the flow of substrates to product, and details the regulation of the pathway. The quantity of substrates, enzymes and regulators is modeled by differential equations that are solved over a time interval. The equations are coupled to other differential equations, allowing for possible complex behavior of the pathway. Numerical approximation to the solution of the differential equations is by the program kin.

Figure 1

Transport Model A

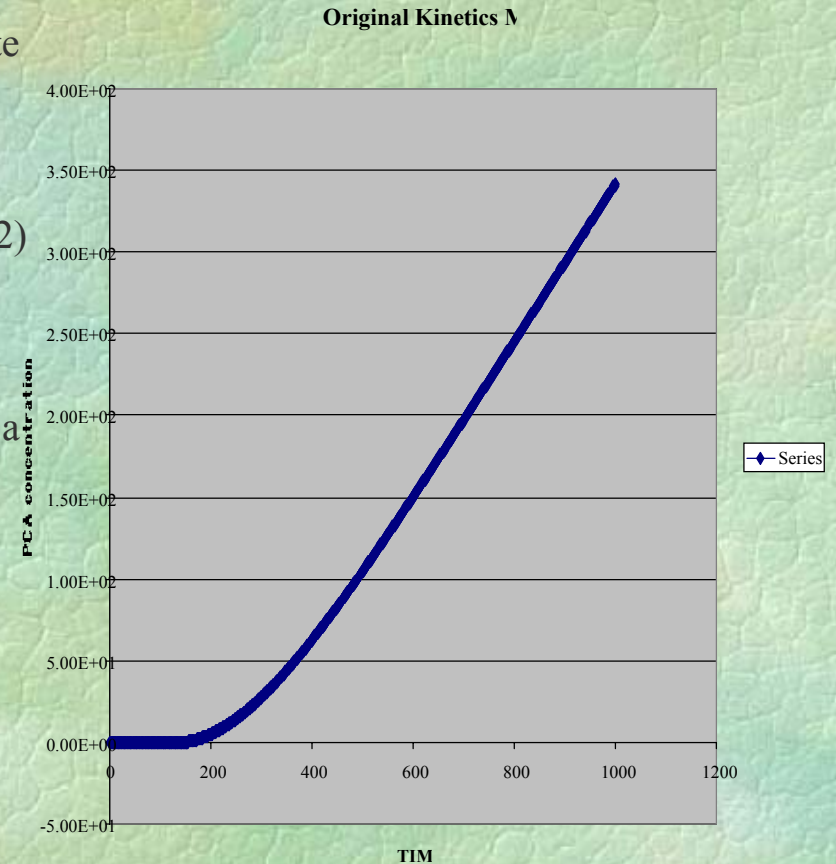


Materials and Methods

A numerical differential equation solver program (kin) was written in FORTRAN and compiled to run on the origin 2000 UNIX computer by Berndt Schuttler in the Department of Physics, University of Georgia (gene-genetics.uga.edu). An input file is required to specify the initial concentrations of all substrates in the pathway. In addition, forward and backward reaction rates constants for each biochemical reaction are required for the input file. Output files are generated recording the concentration of all molecular species over a specified time interval. Experiments involved changing one parameter value and plotting the level of PCA Vs time. The results of changing some parameter was related to the biology of the system, or resulted in a change in the model to better reflect real behavior.

Results and Discussion

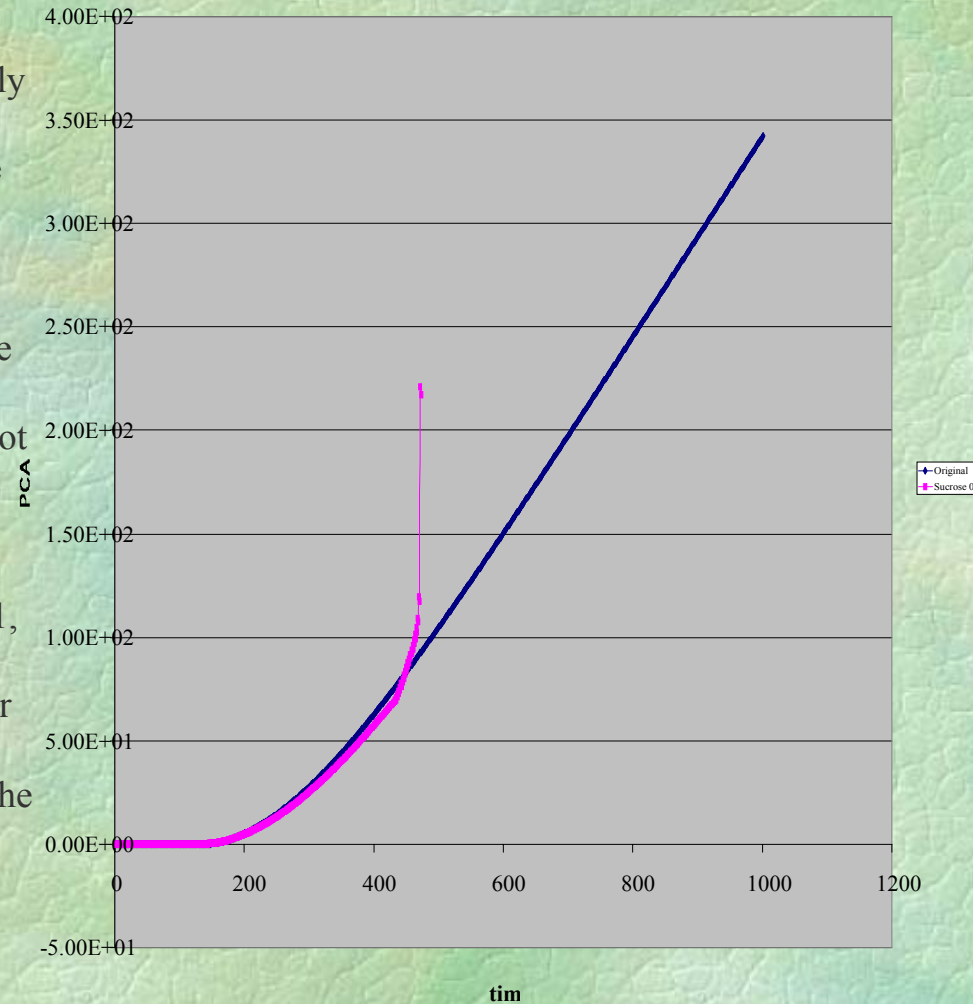
The behavior of the mathematical model of the *qa* cluster in *N.crassa* needed validation. An input file of initial substrate concentrations and reaction rates was constructed and labeled "kin.i1 version 1.1". This input file yielded (figure 2) when PCA is plotted as a function of time. The output of PCA is increasing linearly over the time interval following a short lag period. This is an expected outcome since the *qa* cluster genes need to be induced before the pathway would become active. The constant increase in PCA is due to the input file specification that the external quinic acid (QA) concentration does not change.



Five components of the *qa* cluster model were selected to determine the effects on PCA concentrations. The parameters $qa-2^0$ (concentration of the transcriptionally non-active *qa-2* gene), $qa-1s^1$ (transcriptionally active *qa-1s* gene), and $qa-3^P$ (*qa-3* protein product) were replaced by non-zero values from the original input file that yielded figure 2. These changes in the input did not influence the output of PCA different from figure 2. When the sucrose parameter was changed from 0 to 0.1, the output of PCA increased exponentially shortly after the lag period (figure 3). Sucrose is known to inhibit the QA pathway. This output behavior in the model necessitated modification of the interaction of sucrose with the *qa* cluster genes. A possible modification is to model sucrose to interact strongly only with *qa-1F*.

Figure 3

Kinetics Model



Changing the parameter qa^{-4} from 0 to 0.1 also yielded exponential increases in PCA (figure 4). This prompted an examination of the time scale parameter (ntime). Changing the time scale parameter from 1,000 to 10,000 caused PCA increase (but not a blow up as with $ntime=1000$, figure 5). Therefore it appears that this step should be evaluated with values above 10,000.

Figure 4

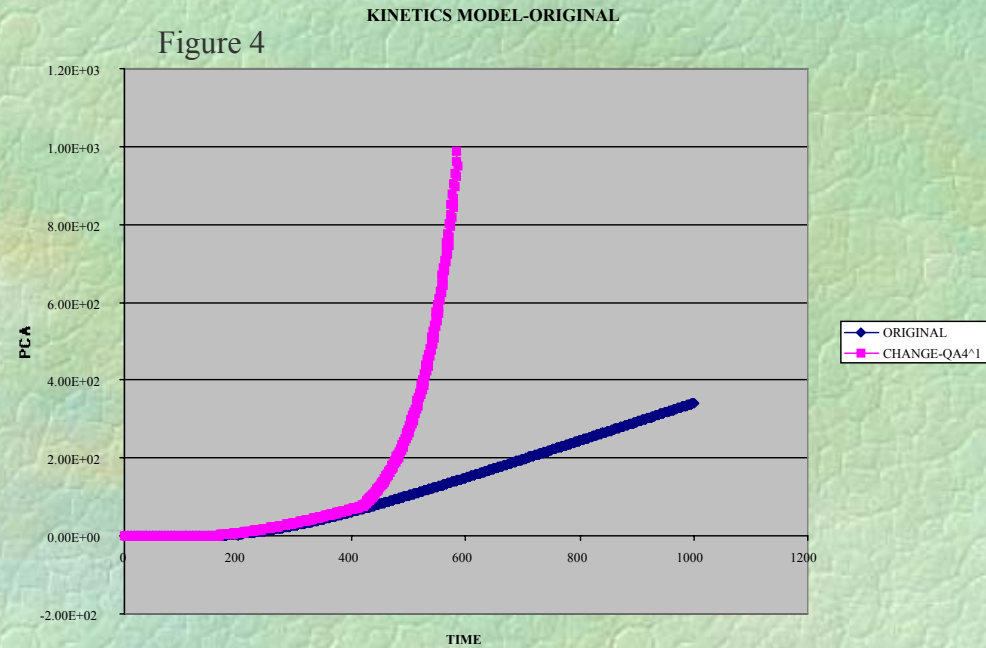


Figure 5

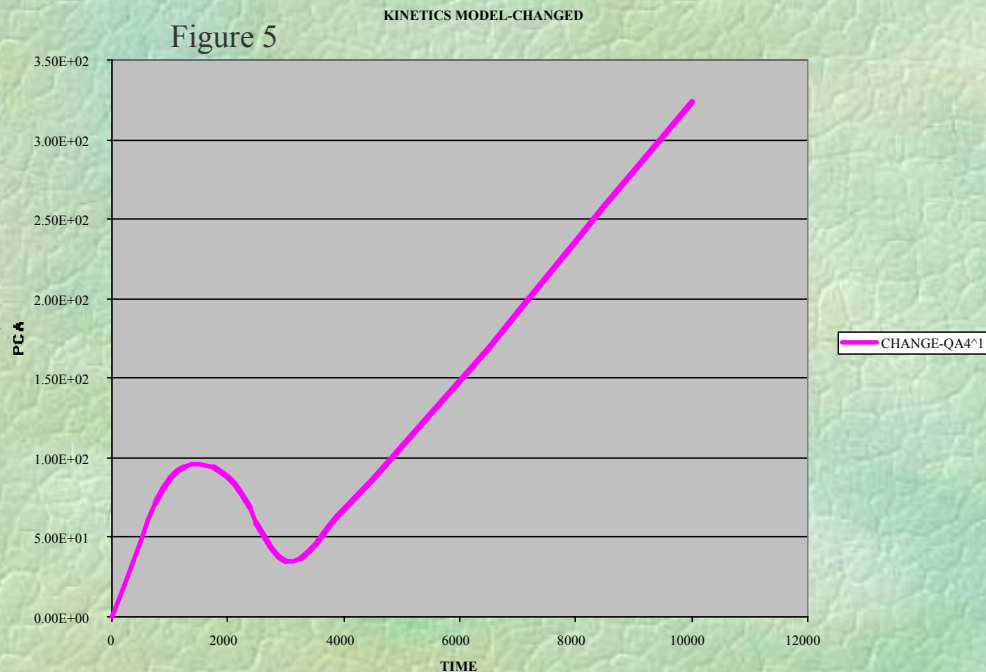
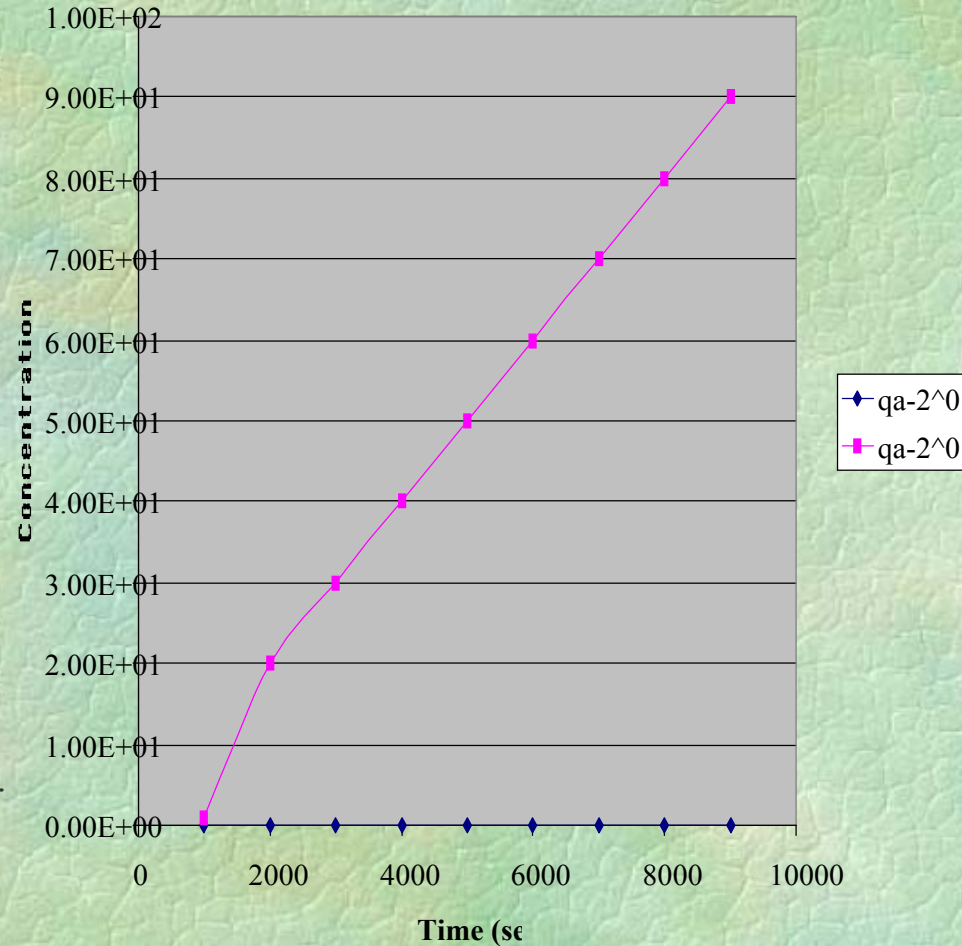


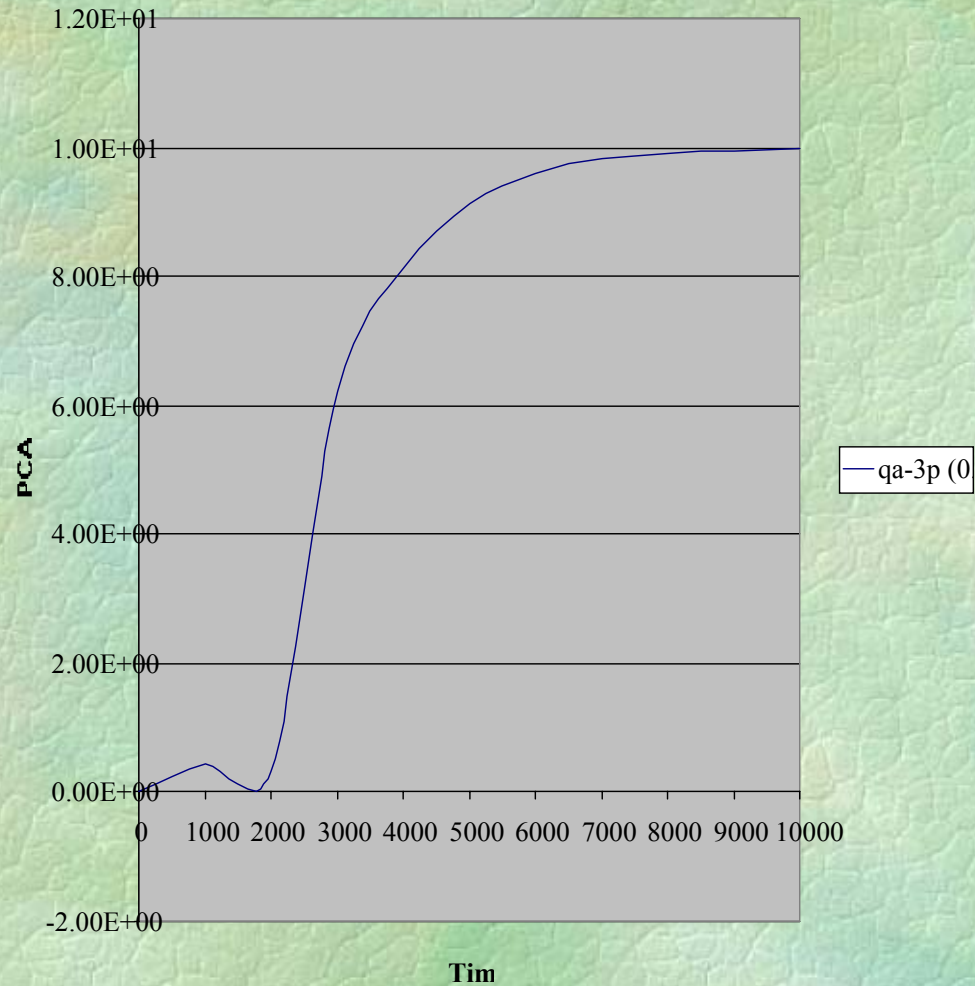
Figure 6



As mentioned above, changing the value of $qa-2^0$ (inactive form of $qa-2$ gene) did not alter the production of PCA with $n_{time}=1,000$. Yet with $n_{time}=10,000$, a difference becomes apparent (figure 6). This parameter represents the number of gene copies of $qa-2$. Hence the action of increasing the copy level of $qa-2$ results in enhanced production of PCA as shown in figure 6.

Kinetics Mc

Figure 7



Other changes in parameters yielded complex patterns. Changing qa-3^P with ntime=10,000 yield figure 7, showing several inflections in the output of PCA.

These results are our initial examination of the QA cluster model. Changes have been made in the input parameters to more accurately reflect expected behavior.

References

Geezer RF, Huiet L, Baun JA, Tyler BM, Patel VB, Rutledge BJ, Case ME, and Giles NH, 1989.
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