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Role of Nuclear Size in Cell Growth Initiation

Abstract. *Swiss 3T3 cells arrested in G_0 (quiescent state) by reducing serum content of the medium all contain the same amount of DNA but vary in nuclear volume over approximately a twofold range. By use of flow microfluorimetry, scatterplots of nuclear volume versus DNA content were obtained in intervals after serum stimulation. The earliest cells to enter DNA synthesis were those with the largest nuclei, whereas cells with the smallest nuclei were among the latest. Regulation of cellular transit from G_0 to the S phase was therefore, at least in part, deterministic, since all G_0 cells did not have equal probabilities of entry into S at a given moment. All cells having the same nuclear volume did not initiate DNA synthesis at the same moment; therefore, factors other than nuclear volume must also influence this timing. Nuclear volume correlated with the maximum rate at which cells could enter S. The kinetic model of the cell cycle postulating a probabilistic event as solely responsible for entry into S thus appears too simple.*

Why individual cells within a culture required widely different time intervals to traverse their own cycle is not known. This variability has been observed with bacteria (*1*), algae (*2*), yeasts (*3*), and animal cells in culture (*4*-*7*). For example, in cultures of Chinese hamster ovary cells, the majority of cells had cycle durations varying from 14 to 21 hours (*4*), which is probably not due to genetic differences since it occurs in freshly cloned populations (*5*). Most variability occurs in the G_1 phase of the cell cycle (*6*-*8*), as does the major regulatory event (*7*, *9*); G_1 variability and cell-cycle control are probably related.

Searches for inherent differences between cells which are responsible for different times of initiating DNA synthesis have focused mainly on cell size. For lymphocytes (*8*) and fibroblasts (*10*), smaller cells require longer to transit G_1 than do larger cells. Similarly, both fission yeast (*3*) and budding yeast (*11*) cells that are below a critical size must grow before initiating their cycle, hence taking longer to complete a cycle than larger cells. Above a minimal size, their cycle time is size-independent. Bacteria also show a dependence of DNA initiation upon cell mass (*12*). These results indicate that the duration of cell-cycle traverse is not purely probabilistic (*7*) but rather is dependent on properties of individual cells.

In this report we examine the relation between *nuclear* size and transit time from the quiescent state to the S phase in contrast to earlier investigators who emphasized *cell* size. Also, we used a different technique. Flow microfluorimetry permits simultaneous determinations of light scattering by nuclei (dependent on nuclear diameter) and DNA content. The results are displayed on a two-dimensional scatterplot of these parameters, in which each point represents an observed cell (see Fig. 1). We can thus observe directly whether cells initially aligned in G_0 enter DNA synthesis at the same time regardless of their nuclear size. In this case, the scatterplot would move parallel to itself to the right. Alternatively, dependence of the timing of DNA initiation on nuclear size would be directly observed, as an earlier movement of larger nuclei, for instance.

Swiss 3T3 cells were grown at 37°C in the Dulbecco modification of Eagle's medium (DME). To align the cells in G_0, an exponential population growing in DME plus 10 percent calf serum was shifted to DME plus 0.5 percent calf serum and left for 36 or 48 hours. The resulting (G_0) population consisted primarily (approximately 95 percent) of cells having a DNA content characteristic of the G_1 phase (Fig. 1a). Determinations of nuclear diameter with a Coulter counter (Fig. 2) showed that all volumes vary over a twofold range. The initial (G_0) population's scatterplot obtained by flow microfluorimetry (Fig. 1b) showed this nuclear volume heterogeneity in contrast to the uniform DNA content of most of the cells. Growth was initiated by replacing the medium with DME plus 10 percent calf serum. At subsequent times nuclei of replicate cultures were analyzed by flow microfluorimetry (*13*, *14*) to obtain both DNA histograms and scatterplots of nuclear size (diameter) versus DNA content. The nuclear sizes are on an arbitrary scale.

After addition of complete medium to G_0 cells, significant numbers of cells had entered the S phase by 18 hours, some of which reached the G_2 phase (Fig. 1c). These 3T3 cells have a minimal G_0-to-S interval of approximately 11 hours and an S period of 8.5 hours (*14*). The scatterplot (Fig. 1d) of this 18-hour population shows that most cells of the smallest nuclear size had not yet increased their DNA content. Among cells of progressively greater nuclear size, progressively greater fractions had entered farther into S. Primarily, only the cells with the largest nuclei had acquired G_2 DNA by 18 hours.

Scatterplots made at other times reinforced the conclusions from the 18-hour data. The scatterplot made at 14 hours had a similar but narrower wedge shape than shown in Fig. 1d. After serum stimulation for 20 hours (and 22 hours, not shown), more cells with smaller nuclei acquired a G_2 DNA content (Fig. 1, e and f). Since cells with the smallest nuclei eventually synthesized DNA, nuclear size was not a necessary constraint on entry into S. The size of the arrested cells' nuclei thus correlated positively with the maximum rate at which the cells responded to serum stimulation.

An important feature of these data is that cells of a given nuclear size did not all transit to S in the same time. Had they done so, one would have seen a narrow

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scatterplot at all times, the top of which moved to the right more rapidly than did the bottom. Instead, one observed (Fig. 1) a broad distribution with time. Although larger nuclear size may permit earlier initiation of S, nuclear size alone is not a sufficient criterion; other factors must also influence the times of initiation of DNA synthesis. The kinetic heterogeneity within a nuclear size class must depend on other heterogeneities within that class. In view of the data cited earlier, cytoplasmic volume could contribute such an influence.

One possible source of heterogeneity in cell and nuclear size and also of the kinetics is genetic heterogeneity in the population (*5*). To test this possibility, clonal derivatives of the parent culture were obtained by culturing samples of the original population that initially contained one cell. Several clonal lines were tested in the same way as the original population. Their histograms and scatterplots (Fig. 3, a and b) did not differ significantly from Fig. 1. Heterogeneity of size and variability of kinetics in the original population is therefore not due to stable genetic diversity, but arises as a natural consequence of proliferation of the cells.

In these experiments the transit time between G_0 and S was investigated. This event is at least as variable as the cycle length of growing cells (*15–17*) and is of equal interest. One cannot be certain that nuclear size is a factor in determining the length of G_1 without doing experiments on cycling cells similar to these. This is because the starvation procedure used to put cells into G_0 might itself have introduced heterogeneity into the population. However, the duration of starvation was not a sensitive parameter since cells starved for 36 rather than 48 hours gave a similar scatterplot (Fig. 3, a and b).

How does this result—that the timing

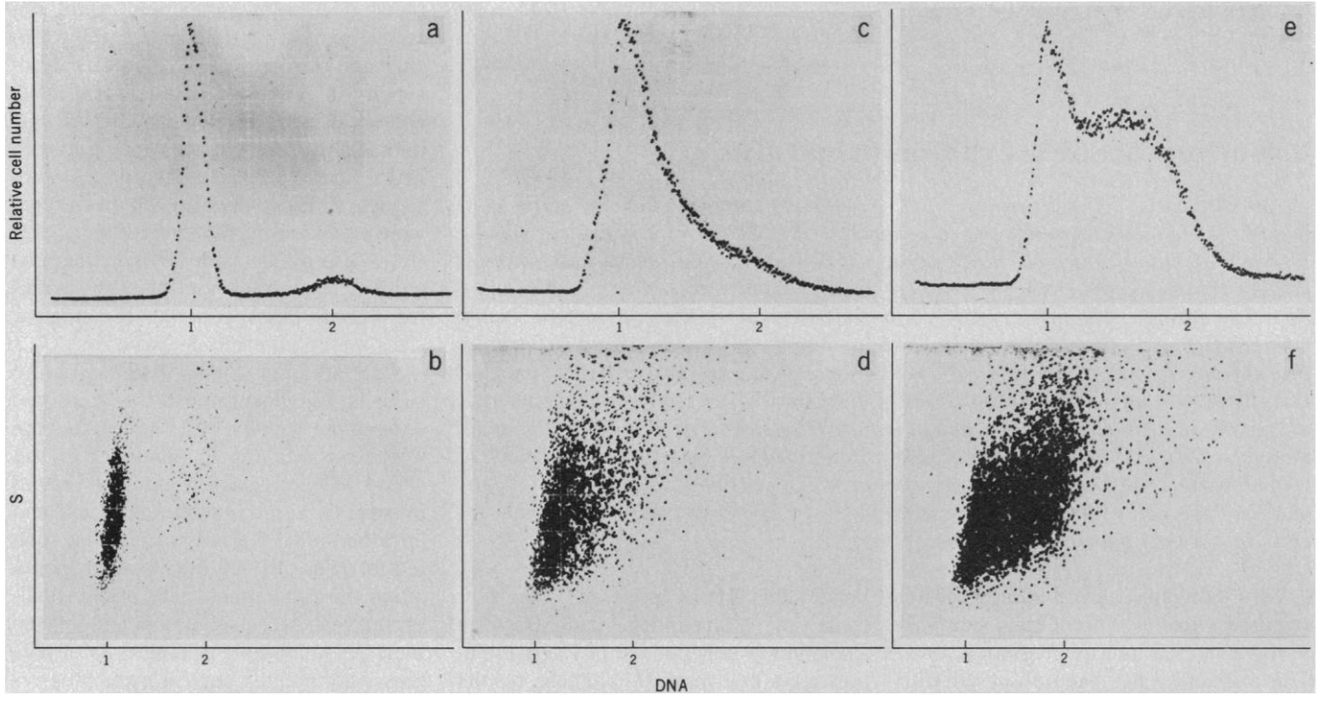

Fig. 1. (a) Histogram of DNA content for a population (G_0) arrested by exposure to medium with a low serum content for 48 hours. Abscissa: cellular DNA content, multiples of G_1 DNA. Ordinate: relative cell number. (b) Scatterplot of nuclear size (diameter), arbitrary units measured by light scattering (ordinate) versus DNA content (abscissa) for the (G_0) population of (a). (c) Histogram of DNA content for the population 18 hours after addition of complete medium with a high serum content. Abscissa and ordinate as in (a). (d) Scatterplot of nuclear size (diameter) versus cellular DNA content corresponding to (c). (e) Histogram as in (c), at 20 hours. (f) Scatterplot as in (d), at 20 hours.

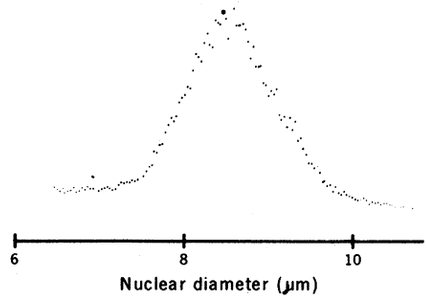

Fig. 2 (left). Histogram of nuclear diameter (in micrometers) distribution of a G_0 population. Calibration was done with latex microspheres for which the observed light scattering and size were proportional, as described previously (*18*).

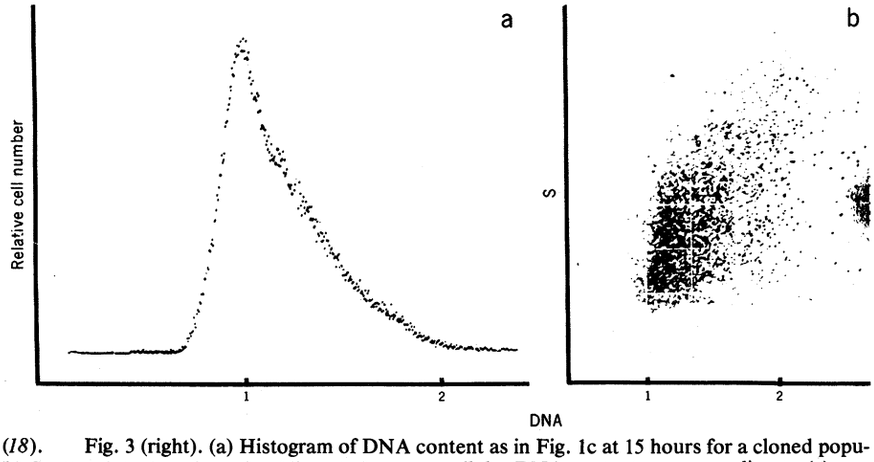

Fig. 3 (right). (a) Histogram of DNA content as in Fig. 1c at 15 hours for a cloned population of cells arrested for 36 hours originally. (b) Scatterplot of nuclear size (diameter) versus cellular DNA content corresponding to (a).

of transit from G_0 to S depends on nuclear size—relate to current models? There are two major kinds of models, deterministic and probabilistic. Kinetics do not always permit a choice between them (*16*). The present result is deterministic; an individual cell's physical characteristics affect its cycle kinetics.

According to the transition probability model (*7*), G_0 cells are not distinct from cycling cells; they simply have a longer average A phase, defined as a "waiting" phase in G_1, from which cells move to S with a constant probability per unit time (under a given set of conditions). This model has been applied to kinetics of escape from the serum-derived quiescent state (*17*). Hence the present experiments are relevant to assessing this model's validity.

The transition probability model asserts that it is not possible to determine a priori which G_1 cells will first enter S phase. Our data are contrary to this model. Cells were observed to be unequal in their capability to traverse the cell cycle. The data must invalidate at least one of the specified properties of the transition probability model; either the transition probability or the duration of the B phase (the proposed invariant portion of the cell cycle) depends on nuclear size and is not the same for all cells, as the model postulates. Moreover, if nuclear size affects the duration of the B phase, then a very significant amount of variability in cell-cycle durations is incurred in B as well as in A.

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Linkage of Loci Controlling Alloantigens on Red Blood Cells and Lymphocytes in the Horse

Abstract. *A system of equine lymphocyte alloantigens designated ELA, is identified, and it is shown that the locus or loci controlling these markers must be closely linked to the locus controlling markers in the A system of horse blood groups. Among 29 offspring in two stallion families there was evidence for one recombinant. Lod scores for linkage between the A and ELA loci in the two families were 3.61 and 3.33, respectively, for θ equal to 0.*

Although previous reports (*1*) have indicated that alloantigenic markers on horse lymphocytes are inherited as dominant traits and are not expressed on red blood cells, little information concerning their potential allelic relationships has been published. We now have evidence that four such markers, designated E1, E2, E3, and E4, are probably coded for by a single series of allelic genes, and that the locus bearing these alleles is closely linked to the A locus of horse blood groups. The A locus is known to bear at least eight alleles (*2*). The evidence for the segregation of E1, E2, E3, and E4 as the products of allelic genes and of their linkage to markers in the A system of horse blood groups is based on two sire families. One family consisted of a Shetland Pony stallion, 10 mares, and their 17 offspring. The other consisted of an Appaloosa stallion, 12 mares, and their 12 offspring.

Blood samples collected in 10 ml acid-

Table 1. Inheritance of erythrocyte antigens A_1 and Z and lymphocyte antigens E1, E2, E3, and E4 in a Shetland Pony family. The presence of the antigen is signified by +; the absence by −. An allele which may also have been contributed by the dam is shown in parentheses. Abbreviation: ND, not determined.

Horse identification	Sex	A_1	Z	E1	E2	E3	E4	Probable sire contribution
Sire		+	+	+	+	−	−	
Dam Py13		−	+	−	−	−	ND	
Offspring Py64	♀	+	−	+	−	−	−	A_1, E1
Offspring Py73	♀	−	+	−	+	−	−	(Z), E2
Dam Py16		−	−	−	−	ND	ND	
Offspring Py56	♂	+	−	+	−	+	−	A_1, E1
Offspring Py63	♂	+	−	+	−	+	−	A_1, E1
Dam Py19		−	−	−	−	ND	ND	
Offspring Py65	♀	−	+	−	+	−	−	Z, E2
Dam Py26		+	−	−	−	−	−	
Offspring Py59	♀	+	+	−	+	−	−	Z, E2
Offspring Py76	♂	+	+	−	+	−	−	Z, E2
Dam Py33		−	−	−	ND	ND	ND	
Offspring Py60	♂	+	−	+	−	+	−	A_1, E1
Dam Py35		−	−	−	−	ND	ND	
Offspring Py70	♀	+	−	+	−	−	−	A_1, E1
Offspring Py75	♀	+	−	+	−	−	−	A_1, E1
Dam Py38		+	−	+	−	−	−	
Offspring Py68	♂	−	+	−	+	−	−	Z, E2
Dam Py42		−	−	+	−	ND	ND	
Offspring Py61	♀	+	−	+	−	−	−	A_1, (E1)
Offspring Py67	♀	−	+	+	+	−	−	Z, E2
Offspring Py74	♂	+	−	+	−	−	−	A_1, (E1)
Dam Py44		−	−	−	−	−	−	
Offspring Py62	♂	+	−	+	−	−	−	A_1, E1
Offspring Py69	♀	+	−	+	−	−	−	A_1, E1
Dam Py49		−	−	−	−	+	−	
Offspring Py78	♀	+	−	+	−	−	−	A_1, E1

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