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On the Orders of Magnitude of Epigenic Dynamics and Monoclonal Antibody Production

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On the orders of magnitude of epigenic dynamics and monoclonal antibody production

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Abstract. The hybridoma cell's maximum capacity for monoclonal antibody *(MAb)* production is estimated to be 2300-8000 *MAb* molecules/cell/s, using measured rates of transcription and translation, and the limitations imposed by the size of the polymerase molecule and the ribosome. Nearly all the production rates reported in the literature fall into or below this range of production rates. Data from batch cultures of hybridomas demonstrate a constant specific rate of *MAb* production until the time integral of the viable cell concentration reaches about 108 cells·h/cm3. At this point, some essential nutrients from the standard media are depleted, causing *MAb* production to decline.

1 Introduction

Rapidly growing markets for monoclonal antibodies *(MAbs)* have brought about the large-scale cultivation of hybridomas and the development of optimal processing schemes. The preliminary analysis needed for process design requires us to seek order of magnitudes of key rates, capacities, and other necessary data for orientation and identification of factors that need quantitative analysis. Herein we attempt to establish how basic biochemical and cellular determinants set the order of magnitude of the rate of monoclonal antibody production. Our order of magnitude estimate is consistent with experimental observations.

2 Dynamics of antibody synthesis

Antibody synthesis in hybridoma cells proceeds in a sequence of several well-known steps, shown in Fig. 1. These steps are transcription, processing of the heteronuclear RNA *(hnRNA),* translation, assembly of polypeptides and their glycosylation, and secretion of the antibody. Antibody accounts for a large fraction of protein synthesized in *Ab*-producing cells. For instance, in rapidly growing **MOPC** 21 and MPC 11 myeloma cells, a single gene for each of the heavy *(H)* and light *(L)* chains may provide enough *mRNA* that leads to about 20-30% of the protein synthesized [1].

We will now estimate the theoretical maximum rate of antibody synthesis, which is the *steady state flux* through the *Ab* production system.[1] These order of magnitude calculations will give an upper bound on the specific productivity of hybridoma cells, and are based on the cell's capacity for transcription and translation.[2] The maximum capacity for the other stages of *Ab* synthesis, such as glycosylation and secretion, are more difficult to evaluate at present. However, these steps would only serve to reduce our estimate of the maximum *Ab* synthesis rate, ifthey would prove to be capacity limiting. As will be shown below, the estimate based on the processes of transcription and translation agrees with experimental data.

A dynamic mass balance on the cytoplasmic *Ab mRNA* concentration is:

$$\frac{d[mRNA]}{dt} = \frac{v_c}{s_c} D \cdot E - k \cdot [mRNA] - \mu \cdot [mRNA], \qquad (1)$$

where *[mRNA]* is the number of *mRNA* per cell in the cytoplasm, *D* is the gene dosage, which for hybridomas is equal to one gene per cell; *E* is the fraction of nuclear *mRNA* which reaches the cytoplasm; v_c is the velocity of the polymerase II molecule in nucleotides/s/gene; s_c is the spacing of nucleotides between polymerase molecules on the **DNA,** with units nucleotides/polymerase; k is the degradative rate constant related to the half life of *mRNA,* in s^{-1}; μ is the growth rate, in s^{-1}.

Assuming that in balanced growth, the amount of *mRNA* per cell is in a quasi-steady state, we obtain

$$[mRNA]_{ss} = \frac{v_c \cdot D \cdot E}{s_c \cdot k} \cdot \frac{1}{1 + \frac{\mu}{k}} \qquad (2)$$

1 Such an estimate should not be confused with the characteristic response times associated with the individual steps in the process. For example, the response time, defined as the approximate length of time for the process to occur, is 20-150 min for the glycosylation/secretion step [2, 3], and 40 min for nuclear processing [4]. Although these may be the steps in the *Ab* synthesis pathway with the slowest response times, they do not necessarily limit the cell's capacity for *Ab* synthesis.

2 Similar calculations have been used to estimate the rate of zymogen synthesis in cocoonase zymogen cells [5].

Fig. 1. Pathway of antibody synthesis

The various parameters appearing in this expression may be estimated from the available literature. Reported values for v_c are 83 nucleotides/s for HeLa ribosomal RNA [6], 67 in poliovirus [7], and 45 in E. coli [8]. The reported values for s_e are 75 nucleotides/polymerase in E. coli [9], and 79-92 in Triturus viridescens [10]. Based on the physical size of the polymerase II molecule, the minimum spacing is about 38 nucleotides [10], although it is unlikely that this spacing can be physically achieved. Approximately 70-90% of the *hnRNA* produced in the nucleus is transported to the cytoplasm [11], so $\boldsymbol{E}$ falls in the range of 0.7-0.9. The average half-life of *Ab mRNA* has been measured to be 12 h [12], corresponding to a k value of $1.61 \cdot 10^{-5}\ s^{-1}$. Minimum doubling times for hybridomas are on the order of 12-14 h and μ is therefore less than or equal to k Consequently, the factor $1 - \frac{\mu}{k}$ falls in the range 0.5-1.0. For doubling times of 14-20 h, this factor takes values in the range of 0.54-0.63. Using the parameter values most appropriate for myeloma cells, we obtain the estimated range for $[mRNA]_{ss}$:

$$[hnRNA] = \frac{(83 \text{ nucleotides/s}) \cdot (0.7 \text{ to } 0.9)}{(80 \text{ nucleotIdes/polymerase})\ (1.61 \cdot 10^{-5}\ s^{-1})} \cdot (0.5 \text{to} 1.0)$$

$$= 23{,}000 \text{ to } 58{,}000 \text{ H or } L \text{ } mRNA \text{ molecules/cell.}$$

The reported estimates of 54,000 *LmRNA/cell* [11], and 40,000 *LmRNA* and 30,000 *H mRNA* [13] fall within this range. The upper limit for the $[mRNA]_{ss}$ can be extended to 122,000 *mRNA* molecules/cell if the minimum polymerase spacing of 38 nucleotides/polymerase is used.

The equation for translation of *Ab mRNA*, assuming that translation is a first order process [14], is:

$$v_{Ab} = \frac{1}{2} \cdot \frac{v_s}{s_s} \cdot [mRNA]_{ss}. \quad (3)$$

We assume that the ribosome concentration is not limiting and, therefore, the translation rate is independent of ribosome concentration. Reported values for the ribosome velocity, v_s, are 20 nucleotides/s in myeloma [1], 25 in reticulocytes [15], and 48-60 in Salmonella typhinurium [16]. The number of nucleotides between ribosomes, s_s, is reported to be 90-100 in myeloma [1] and reticulocytes [15], and 88-99 in somatotropes and mammotropes [17]. The factor of 0.5 occurs because two $\boldsymbol{H}$ and two L polypeptide chains are needed for the assembly of one complete *Ab* molecule. To estimate the maximum *Ab* production rate, we have neglected the turnover of either the $\boldsymbol{H}$ or $\boldsymbol{L}$ chains. The $\boldsymbol{H}$ chains are believed to be relatively stable, while the L chains can exhibit significant turnover - L chains are reported to be produced in excess of $\boldsymbol{H}$ chains [3]. The rate of $\boldsymbol{H}$ chain synthesis thus forms a suitable upper bound on the *MAb* excretion rate.

The range of the maximum *Ab* synthesis rate can then be calculated using Eq. (3) with the parameter values appropriate for the myeloma cell, and the *mRNA* concentrations that were calculated previously:

$$v_{Ab} = \frac{1}{2} \cdot \frac{20 \text{ to } 25 \text{ nucleotides/s}}{90 \text{ to } 100 \text{ nucleotides/ribosome}} \cdot (23{,}000 \text{ to } 58{,}000 \text{ } mRNA\text{/cell}) = 2300 \text{ to } 8000 \text{ Ab/cell/s.}$$

Therefore, based on the physical constraints enumerated above (the observed polymerase and ribosome packing density, and transcription and translation velocities), the theoretical maximum output of antibody from a myeloma cell is on the order of 2300-8000 *Ab* molecules/cell/s. If the maximum estimated value of 122,000 *mRNA/cell* is used, the upper limit for the *Ab* production rate becomes 17,000 *Ab* molecules/cell/s.

3 Discussion

The reported values of *MAb* production range from 17 to 8000 Ab/cell/s for various hybridoma cell lines (Table 1), with most values falling in the range of 500-3000. Our estimate of the maximum rate seems, therefore, reasonable. Fig. 2 shows the normalized *MAb* concentration plotted against the time integral of the viable cell concentration, for several sets ofbatch data reported in the literature. The slope of this curve, which gives the specific productivity, is nearly linear up to the point around 10^8 cells $\cdot$h/cm^3 for all the data used. At this point, one or more critical components seem to be depleted from the standard media, causing the *MAb* production to decline [18]. It has been shown that when the essential amino acids were added to the media at this point, the cells continued to synthesize antibody at the same rate [18]. Figure 2 indicates that the specific productivity of *MAb* is nearly constant at the individual cell line's characteristic rate, as long as the necessary nutrients are available. All these data sets corne from experiments with 5-10% serum in the media. Data from our laboratory show that the specific *MAb* production rate is serum independent [19], except under long term adaptation to very low serum levels (unpublished results).

The high MAb-producing hybridoma cell lines, shown in Table 1, secrete *MAb* at rates that are close to our estimated capacity of the epigenic system. If nutritional conditions are

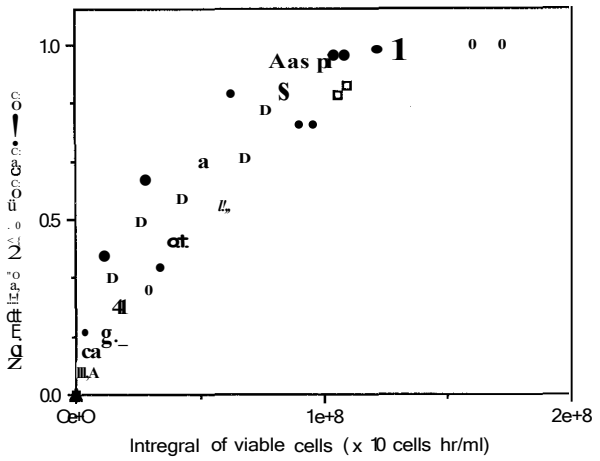

Fig. 2. Antibody concentration plotted against the time integral of the cell concentration, for cells grown in batch cultures. The antibody concentrations are normalized against the maximum antibody concentration for that cell line. Sources of the data are: Ill [20]; e, 0 [21]; A [22]; • [23]; Il [24]; .:. [25]

Table 1. Reported antibody production rates

Cell line	Ab production rate molecules/cell/s	Reference
Batch		
mouse HB8178, IgG	1200-2700	[26]
mouse VII H-8, IgG2a	580	[27]
mouse VII H-8, IgG2a	530-4650	[28]
mouse 65/26, IgG2b/x	480	[29]
mouse 455, IgG2a	17-33	[30]
mouse B103, IgG	6000	[31]
mouse MRC Ox-19, IgG	470-790	[32]
mouse N-527 IgG	8000	[33]
mouse J4C2 IgG1	830	[20]
mouse HB 32 IgG2aK	355-470	[18]
mouse 9.2.27 IgG2a	2230	[22]
mouse IgG	970	[25]
mouse S3H5/ybA2, IgG2bA	210	[24]
Semi-continuous		
mouse VII H-8, IgG2a	490-750	[28]
human HF10B4, IgM	62	[34]
Continuous		
mouse VII H-8, IgG2a	460-1400	[28]
mouse 65/26, IgG2b/x	80-2550	[29]
mouse N-146, IgG	5800	[33]
mouse N-226, IgG	615	[33]
mouse N-527, IgG	7640	[33]
mouse S-6, IgG	1210	[33]
mouse S-84, IgM	380	[33]
rat R33-18-12	2460	[33]
rat R33-24-12	110	[33]
rat R33-60	675	[33]
rat IM/41	3400	[33]
rat C2-23	2330	[33]
mouse 143, IgG2a	330-670	[35]
mouse NB1, IgM	60	[36]

not limiting, improvements in the specific *MAb* production rate will not be achieved through improved processing technology but rather through modification of cellular components. At present it is uncertain how successfully we can manipulate the cell's protein synthesis machinery.

Our results, therefore, support the view that economically successful large-scale cultivation of hybridomas is likely to depend primarily on the selection of: *1)* a high producer, and 2) a processing scheme that supports high cell densities.

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