Research Articles

A unifying model of the cell proliferation emphasizing plasma membrane fluxes

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Summary. The regulation of cellular growth and proliferation is perhaps the most investigated and elusive problem in cell biology and seems to be possible to solve from almost any angle of study chosen. Among the non-systemic factors that have been discussed are genetic damage, genomic control, regulation by stimulatory and inhibitory peptide factors such as EGF, chalones, and fibronectin, protein kinase activation with tyrosine phosphorylation, adenylylcyclase and cAMP, cGMP, membrane perturbations and specifically in tumours the failure of the Pasteur effect in control of glycolysis, excessive membrane ATPase activity, and excessive hydrolytic and proteolytic activities at the cell surface. This article focusses on the central role of fluxes within the plasma membrane and re-examines the possibility that changes of flux of metabolites, ions, and reducing equivalents may be the common denominator regulating cellular proliferation.
Key words. Cell proliferation; cancer; plasma membrane; general metabolism; biomathematical models; biological transport.

In the preceding paper in this series, evidence was collected indicating that there is a correlation between the metabolic rate and the rate of proliferation of biological cells [1]. When the intracellular branching of metabolism into the two main categories of proliferation and maintenance of specialized function is taken into account, simple mathematical expressions can be found that describe this correlation. Metabolism can be regarded as a chain of probabilistic interactions between enzymic and genomic elements which are either stabilized by iteration, consolidating the specialized function, or broken down by end-product inhibition, induction followed by competition etc., leading to metabolic evolution towards cell division. In as much as the metabolic flux is diverted from specialized functions that comprise iterative enzymic and genomic events and all the permissive nutrients are present to sustain growth, cellular proliferation is in most instances a function of both the rate of metabolic flux and the rate of change of quality of end products. The rates of product formation are more important than the amounts because of the continuous breakdown by various hydrolytic enzymes, thermal inactivation, irreversible enzymic regulation, enzymic competition, leakage, etc.

The rate of appearance of end-product metabolites, $n_n/\Delta t$, is equal to the rate of input of metabolites, $q_n/\Delta t$, times the probabilities of interaction between the enzymic elements k and l contained in the enzymic pathway:

$$\frac{n_n}{\Delta t} = \prod p_{kl} \frac{q_n}{\Delta t} \tag{1}$$

Some of these end products can not be by-passed in the cell cycle and may correspond to the so-called genomic 'checkpoints' [2] while others are interchangeable, for example carbon source pathways or the energy metabolism supplying ATP. Therefore, the rate of evolution of the metabolic process, $q/\Delta t$, and the rate of cellular proliferation, v, are proportional both to the total rate of appearance of end-products:

$$v \propto \frac{q}{\Delta t} = K_1 \sum \frac{n_n}{\Delta t} \tag{2}$$

and to the rate of appearance of new end-products, $n/\Delta t$

$$v \propto \frac{q}{\Delta t} = K_2 \sum \frac{n}{\Delta t} \tag{3}$$

The contribution to $q/\Delta t$ in (2) can actually be divided into one fraction of metabolic flux available for iterative and specialized functions or entries into deposits, dD/dt, and the fraction in excess of the latter which is available for metabolic evolution and the cell cycle, dC/dt:

$$\frac{q}{\Delta t} = K_3 \frac{dC}{dt} + K_4 \frac{dD}{dt} \tag{4}$$

where $K_4 \to 0$. $K_1, K_2, \ldots K_4$ are constants or time-dependent parameters [1]. cAMP is a common regulator of these fluxes, inducing differentiation and synthesis of specialized products concommittantly with inhibition of cell proliferation in tumour cells [1], or inducing cell proliferation of systemic cells as part of their physiological response [3]. This is related to cGMP such that the concentrations of cAMP and cGMP often are inverse and the latter is associated with proliferating cells [4].

In most cases, the precursors necessary for cell proliferation enter the cell through the plasma membrane although notable exceptions involving deposits of nutrients, such as for example the embryogenic morula, exist.

It is known that a slight specific or unspecific perturbation of the plasma membrane or the environment of stationary mammalian cells easily results in the resumption of cell proliferation if there is an adequate supply of nutrients. The perturbations causing cell division have never been explained on the basis of a common mechanism, probably because of their great variety and the complexity of the structure of the plasma membrane. The current focus of interest in regulation of cellular proliferation is at the oncogenes, the receptor-specific growth factors, and the absent or damaged genomic elements of tumour cells, where the most recent advances have been made, so it is almost unavoidable that other approaches are left in the background. However, these and many previous models of growth control are based on the presupposition that there is a specific cause-effect relationship in regulation of cellular proliferation and defining this relationship in terms of the chemistry involved. In view of the great number of such models that have been presented through the years, many of them still unrefuted, it may be appropriate to add a phenomenological model which incorporates as many features as possible of what has been written and examined previously but with fewer assumptions regarding the specific chemistry of the control and instead relying on a more abstract principle in terms of which the chemistry can be understood.

Based on the sole principle that metabolic evolution through the genomic and morphological events of the cell cycle has been given evolutionary advantage and is the preferred metabolic state of a well-nourished eucharyotic cell [1], such a model can be constructed from what is known about the structure of the plasma membrane, its under-coating, and its flux rates of metabolites, ions and reducing equivalents. The metabolites and enzymic pathways carrying the fluxes can be identified as vertical and horizontal components of the energy metabolism occurring there. Suppressed flux rates associated with stationary cells and enhanced rates associated with controlled and uncontrolled cell proliferation can be discerned. The fluxes in plasma membranes of cancerous cells seem to be less tightly coupled than those in normal cells, reflecting an over-all randomness and escape from efficient inhibitory control of the type that is operating in normal cells.

The topography of the membrane fluxes and the structure of the plasma membrane

The topography of some of the enzymes and pathways carrying the fluxes within the plasma membrane can be deduced from studying the *ecto*-sialyltransferase, which is often elevated in proliferating cells, correlating with the increased sialic acid density of most tumour cell membranes relative to their normal counterparts [5]. In the appropriate experimental systems, the surface-associated sialoglycoproteins do not seem to be recycled to any significant extent, and the enzyme itself is accessible to exogenous modulators of its activity. Such studies show that the *ecto*-sialyltransferase, the Na^+, K^+-ATPase, and glycolysis are functionally related and that the *ecto*-sialyltransferase is metabolically coupled to membrane-compartmentalized glycolysis [6, 7]. These enzymes and the membrane-associated acto-myosin provide a common branching point via ATP of pathways related to energy metabolism (fig. 1), namely, formation of reducing equivalents (glycolysis), transport of metabolites (Na^+, K^+-ATPase) movement (actomyosin), and functions reminiscent of the sporulation and cell wall synthesis of procharyotic cells (*ecto*-sialyltransferase, glycosylation, and membrane trafficking). As illustrated in figure 1, the plasma membrane can be regarded as a functional unit made of these and other contributions.

The density and amounts of the extrinsic constituents of the so-called membrane skeleton are high enough to seriously consider the possibility that it may form a continuum in the most investigated model systems, and in general, i.e. an inner membrane or a 'cortex' [8–10], delineating a sub-plasmalemmal or intermembraneous space harbouring metabolism (fig. 2). There is also much biochem-

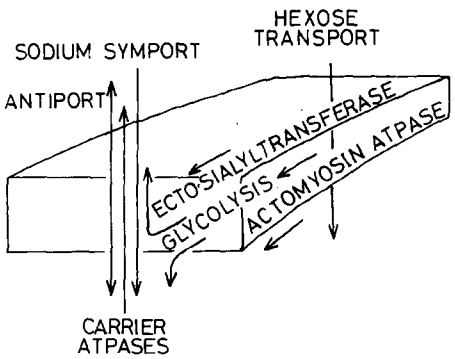

Figure 1. Illustration of vertical and horizontal components of the metabolic fluxes associated with the plasma membrane of mammalian cells, and which tend to be increased in proliferating cells relative to stationary ones. The vertical components have been detected by isotope tracers or specific inhibitors and include the second messenger Ca^{2+} (not shown). The horizontal fluxes are evident from the movement of proliferating cells, capping, patching, and translocation of components in the plane of the membrane (acto-myosin), lectin agglutinability, or can be detected using the *ecto*-sialyltransferase and exogenous CMP-sialic acid and are inferred from the intra-membraneous energy metabolism of intact cells or red blood cell ghosts (glycolysis).

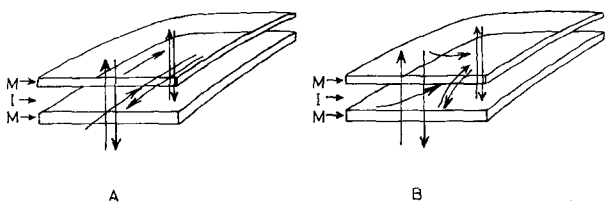

Figure 2. Schematic illustration of the plasma membrane and its undercoating (membrane skeleton, cortex, inner membrane, sub-membraneous gel) including the intermediate space and the ordered or coupled fluxes of a stationary or 'normal' cell (*A*), subject to point control at receptor sites, and the uncontrolled and uncoupled fluxes of a cancerous cell plasma membrane (*B*). In the figure, the extra-cellular space is up and the cytoplasm down, M represents the outer lipoid membrane (the lipid bilayer) or the inner membrane skeleton (cortex, plasmalemmal under-coating etc.) and 'I' represents the intermediate space.

ical evidence through the years from other than structural investigations in support of a membraneous compartment on either side of the plasma membrane proper, namely from studies of the kinetics of transport of metabolites and nerve cell depolarization [11–13], the crypticity and kinetics of membrane-associated glycolytic enzymes and nucleotide metabolism [14–16], the predominantly glycolytic enzymes fuelling the Na^+, K^+-ATPase from an intramembraneous pool of metabolites [17], and the extractable diffusion-barrier to ouabain in inside-out red blood cell vesicles [18]. The compartmentation of energy metabolism within the plasma membrane seems to be a common feature of mammalian eucharyotic cells and provides a natural framework for competition/cooperation and coupling between its energy-requiring metabolic pathways and other chemical and physical fluxes.

Experimental evidence has now accumulated in favour of restricted diffusion of membrane proteins [19, 20] and cytoskeleton-mediated long-range structure [10, 21, 22] within the plasma membrane of most mammalian model systems investigated. Then, depending on the proximity of the enzymes and pathways in the membrane, its metabolic fluxes can be either tightly or loosely coupled, two alternatives that are schematically illustrated in figure 2A and figure 2B, respectively, where the plasmalemmal under-coating also has been included. The coupling is presumably at the microscopic level but may include chemi-osmotic coupling of the type previously described in mitochondriae where the concept originated [23, 24].

A priori, tightly coupled fluxes can be interrupted more efficiently by blocking any part of them than loosely coupled fluxes can; the latter are always possible to circumvent, even if one component is blocked completely at any specific point of control, as is already known empirically from mitochondriae. In the plasma membrane, this leads to a situation where a highly structured membrane or membrane skeleton with its associated enzyme is more susceptible to inhibition of the contained metabolic fluxes than a membrane whose components are loosely put together, and as will be shown below, it is in fact possible to correlate empirically such lack of structure with cellular proliferation.

The model

In order to continue the preceding sections, there are relationships between the sum of the rates of transport of metabolites into the cell, dA/dt, and the inputs of these metabolites into various metabolic pathways such that:

$$\frac{dA}{dt} + \frac{dB}{dt} = \frac{dC}{dt} + \frac{dD}{dt} + \frac{dE}{dt} \tag{5}$$

where dB/dt is the rate of contribution of metabolites exiting from deposits of nutrients built up in the cell, dC/dt is the fraction used for metabolic evolution in the cell cycle, dD/dt is the fraction of entry into intracellular deposits or specialized functions, and dE/dt is the fraction of re-export from the cell by leakage or transport, as for example in brush border epithelium, glia cells, etc.

The relationship between the rate of exogenous supply of nutrients and the intracellular rates of metabolism at time $t = -1$ is then given by:

$$\frac{dA_{-1}}{dt} = \frac{dC_{-1}}{dt} + \frac{dD_{-1}}{dt} + \frac{dE_{-1}}{dt} - \frac{dB_{-1}}{dt} \tag{6}$$

and at time t by:

$$\frac{dA}{dt} = \frac{dC}{dt} + \frac{dD}{dt} + \frac{dE}{dt} - \left(\frac{dD_{-1}}{dt} + \frac{dB}{dt}\right) \tag{7}$$

The latter term would constitute a possibility to describe the commonly occurring metabolic oscillations in biological cells, but which is out of the scope of the present analysis.

In order to obtain a more stringent description of the metabolic coupling, one may return to the factors regulating the probabilities of interaction between two enzymic elements k and l:

$$P_{kl} = \frac{k_1 + X}{c_k + X} e^{-A\tau} \tag{8}$$

where k_1 is the number of copies of the enzyme l with which the enzyme k can interact via its products and c_k is the total number of different such enzymes. X is unity if one copy of l is added to the compartment composed of the c_k complementary enzymes, otherwise zero. τ is the time interval between one and the next interaction of the same type and the regulatory factors are accessible in the association parameter, A. If one chain of such probabilities, $\prod p_n$, corresponding to an enzymic pathway in the plasma membrane, is coupled to another, for example when a by-product and/or the flux of one pathway is necessary for the other, this can be described by:

$$C_1\prod p_1 = C_2\prod p_2 = C_n\prod p_n = \dots \tag{9}$$

where C_n are coupling factors, and when they are not coupled, no such relationship exists. In the plasma membrane, each of these products is intimately related to the parameter dA/dt of eq. (7) such that when the pathways are tightly coupled, dA/dt is proportional to the rate of one pathway:

$$\frac{dA}{dt} = C_n\prod p_n \tag{10}$$

while if they are not and in general the case, dA/dt is proportional to the sum of the rates of all the independent uncoupled pathways:

$$\frac{dA}{dt} = \sum K_n C_n \prod p_n \tag{11}$$

Each of the factors in eq. (10) and (11) are in their turn affected by the concentrations of precursors available, and the nutritive status, which mostly is plentiful within

a surviving organism, as described by:

$$\frac{dA}{dt} \propto \Sigma[C_n] \quad (12)$$

where $[C_n]$ are the concentrations of metabolites.
However, in the most extreme case of metabolic coupling, the rates are determined by one single probability of interaction, that of the lowest value in eq. (10), even if excess precursors are available. In a regulated system, this key point is subject to various control mechanisms. In eq. (8) these enter the parameter A, decreasing the effective frequency of catalysis $1/A\tau$ beyond that given by the frequency of product-substrate interaction $1/\tau$. This decreases the rates below those allowed at a given concentration of substrates. When the inhibitory control is released in a coupled system, all the rates again increase to sustain the intracellular metabolic rates, dC/dt and dD/dt given in eq. (5). On the other hand, in a system that is not metabolically coupled, each pathway is sustained directly by the systemic supply of metabolites, by-passing the membraneous level of control.

Experimental evidence included by the model

It is an established scientific method to use the simplest hypothesis out of many to explain the largest amount of data possible. Therefore, once the aforementioned principle is adopted that cellular proliferation is the metabolic state of mammalian cells that has been given evolutionary advantage, it follows naturally that the increased metabolic fluxes in the plasma membrane of proliferating cells pushing them towards division, are a biochemical marker for the proliferating state. Within the nutritious internal environment of an organism this implies that proliferation is subject to inhibitory control which would be inefficient when the structure of the membrane is perturbed or its fluxes out of control.
Much of the research in this area has focussed around the release of contact inhibition or topoinhibition of proliferation of stationary cells and the opposite phenomenon known as reverse transformation or "retro-transformation" of neoplastic cells in culture. The agents causing these phenomena are either compounds that bind to or alter the cell surface, ions, or compounds and conditions that otherwise directly or indirectly interfere with the transport of metabolites (table 1). Almost without exception, a main effect of the agents is to change flux rates of metabolites and ions across or within the plasma membrane. These metabolic fluxes comprise certain enzymatic pathways, *ecto*-enzymes, carrier molecules, and also translocation of the membrane itself known as membrane recycling, membrane trafficking, exo- and endocytosis (table 2). Particularly, proliferating cells are often characterized by increased hexose transport, glycolysis, Na^+, K^+-ATPase activity, amino acid symport and transport, nucleoside transport, *ecto*-ATPase, and *ecto*-sialyltransferase activities. The increased flux rates include the actomyosin responsible in part for the enhanced cell motility of proliferating cells, and which is associated with the membrane skeleton together with glycolytic enzymes (table 2). It is also becoming increasingly evident that fluxes of reducing equivalents play a role in proliferating cells, and that exogenously added oxidants/reductants may cause the cells to enter a proliferat-

Table 1. Activators and inhibitors of cell proliferation

A) Activators Agent	Increased fluxes documented	References
Peptide hormones and growth factors	+	29, 48, 49
Lectins	+	50, 51
Serum exchange in culture	+	52, 53
Proteolytic enzymes	+	54, 55
Agitation (increased diffusion)	+[1]	56
Phospholipids	+	57
Phosphotyrosine	+[2]	25
Vanadium ions	+	58, 59
Zinc ions	+	60
Calcium ions	+	28, 51, 61, 62
Intracellular alkalinization and sodium-proton antiport	+	63–69
Oxidants	+	70–72
B) Inhibitors **Agent**	**Decreased fluxes or metabolization documented[3]**	**References**
Cyclic AMP, dBcAMP	+	73–80
Shortage of nutrients	+[4]	81–88
Tunicamycin, ouabain, phloretin, herbimycin	+	89–95
Chalones and peptide inhibitors	x[3]	98, 99
Fibronectin	x[3]	100
Butyrate	x[3]	100, 101
Succinylated Con A	x[3]	102
Retinoids	x[3]	103, 104
Magnesium ions	x[3]	105
Potassium ions	x[3]	106
Reductants	x[3]	107, 108

[1] Implicit; [2] non-biochemical organochemical data; [3] most studies are based on measurements of nucleoside incorporation into DNA or cell count; [4] implicit.

Table 2. Enhanced trans- and intra-membraneous fluxes in the plasma membrane of proliferating cells

Substance, carrier, enzyme, pathway, or function	References
Sodium ions, potassium ions, Na^+, K^+-ATPase	109–114
Membrane depolarization	36, 115, 116
Membrane ATPases	41, 117
Protons, sodium-proton antiport, alkalinization	63–69
Sodium symport, amino acids	118–121
Nucleosides	122–125
Calcium ions	28, 61, 62, 126–129
Phosphoinositides	28, 57, 130
Glucoses, hexoses	131–135
Glycolysis (or substituting carbon sources)	136–140
Reducing equivalents (NADH-NAD^+)	141, 142
Acto-myosin (contact-inhibition of movement)	30, 31, 143–148
Lipid fluidity and agglutinability	42, 55, 149–151
Ecto-sialyltransferase	152–160

ing or stationary state respectively, indicating that even electronic currents are involved possibly via phosphotyrosine [25]. There are exceptions to the almost general rule of increased operation of these metabolic pathways, but in most such cases reported, a restricted number of pathways seems to have been investigated, while according to the model, it is the sum of the flux rates that determine whether or not the critical threshold for proliferation will be reached.

Another explanation of these exceptions may be that there is competition within the plasma membrane between the various pathways. Such competition may explain, for example, the inverse relationship between plasma membrane lateral mobility and Na^+, K^+-ATPase activity observed in one system [26]. The competition and cooperation of the membraneous pathways is also evident from the investigations of the *ecto*-sialyltransferase mentioned earlier [6, 7], and may explain an apparent lowered surface glycosyltransferase activity observed after denervation [27]. The physiological significance of these metabolic interactions within the plasma membrane is still unknown.

In contrast to the case of the cancerous plasma membrane, that of a normal stationary systemic cell is known to offer many well-defined choices of points of efficient control of proliferation such as adenylyl cyclase receptors, calcium channels, and receptors for growth factors and inhibitors which may be occupied and vacant, respectively. Of these, regulation by calcium influx correlating with phosphoinositide turnover [28] and activation of the sub-membraneous actomyosin, and the protein kinase C-activation following binding of growth factors to the membrane [29] has been given particular attention. The normal cell plasma membrane is distinguished by well-defined actin cables associated with its membrane skeleton [30, 31] while tumour cells are notoriously deficient of regulatory cytoskeletal components such as tropomyosin [32, 33] and can be mutated in the part of the genome coding for actin [34, 35]. Another concrete example of the control of flux rates in stationary cells is their often observed higher trans-membraneous potential, contrasting to proliferating cells which are depolarized [36] and transformed cell plasma membranes which are leaky to ions, metabolites, proteins, and drugs [37–40], and excel in futile carrier ATPase and ATP-regenerating cycles [41]. The irregularities of transformed cell plasma membranes are also evident in their higher phospholipid fluidity and lectin agglutinability [42], and in the abundance of hydrolytic enzymes causing proteolytic breakdown and sublethal autolysis of the cell surface [43].

Furthermore, the less specific endocytosis of some tumour cells [44] would constitute still another example of a membrane perturbation since it is known that endocytosis can be mediated by clathrin, which requires a highly ordered structure. This may be related to the abundance of microvilli on most tumour cells, contributing in some cases to their increased surface area and transport of metabolites. On the other hand, exocytosis is often favoured in transformed cells, which when involving lysosomal hydrolytic enzymes aggravates the lack of structure already there. This type of unspecifically increased flux rates can be inhibited by cAMP and antiproliferative agents [45, 46] while the specific membrane trafficking in normal cells may be stimulated by cAMP. The loss of structure of the tumour cell plasma membrane shows few exceptions and goes hand in hand with the genetic damage of such cells, which may be just another facet of their dedifferentiation.

The conclusion that membrane fluxes are suppressed in stationary cells, thereby regulating cell proliferation, also conforms with the genetic damage and the absence of certain, possibly inhibitory, genomic elements in neoplastic cells, and with the finding that viral oncogenes may have natural counterparts, proto-oncogenes, and thus may represent part of the natural sequence of events that lead to cell proliferation [47]. Even though such genomic factors are sure to be extremely important in regulating the cell cycle, it is safe to conclude that the role of the trans- and intra-membraneous fluxes and the structure of the plasma membrane should not be overlooked or forgotten.

1 Cervén, E., Experientia *43* (1987) 1094.
2 Hartwell, L. H., and Weinert, T. A., Science *246* (1989) 629.
3 Dumont, J. E., Jaumaux, J. C., and Roger, P., TIBS *14* (1989) 67.
4 Channon, M., Trivedi, A., Sridhar, R., and Tustanoff, E. R., Biochem. Int. *15* (1987) 981.
5 Yogeeswaran, G., and Salk, P. L., Science *212* (1981) 1514.
6 Cervén, E., Acta Univ. Upsal. *341* (1979) 1.
7 Cervén, E., and Ronquist, G., Naturwissenschaften *72* (1985) 86.
8 Bretscher, M. S., Science *181* (1973) 622.
9 Cervén, E., Upsal. J. med. Sci. *82* (1977) 167.
10 Bray, D., and White, J. G., Science *239* (1988) 883.
11 Regen, D. M., and Tarpley, H. L., Biochim. biophys. Acta *339* (1974) 218.
12 Masiak, S. J., and Lefevre, P. G., Biochim. biophys. Acta *465* (1977) 371.
13 Katchalsky, E., in: The Neurosciences. A Study Program, p. 326. Eds G. C. Quarton, T. Melnechuk and F. O. Schmitt. The Rockefeller University Press, New York 1967.
14 Duchon, G., and Collier, H. B., J. Membr. Biol. *6* (1971) 138.
15 Niehaus, W. G. Jr, and Hammerstedt, R. H., Biochim. biophys. Acta *443* (1976) 515.
16 Ågren, G., and Ronquist, G., Upsala J. med. Sci. *80* (1975) 1.
17 Proverbio, F., and Hoffman, J. F., J. gen. Physiol. *68* (1977) 605.
18 Zachowski, A., Lelievre, L., Aubry, J., Charlemagne, D., and Paraf, A., Proc. natl Acad. Sci. USA *74* (1977) 633.
19 Wier, M., and Edidin, M., Science *242* (1988) 412.
20 Ryan, T. A., Myers, J., Holowka, D., Baird, B., and Webb, W. W., Science *239* (1988) 61.
21 Heath, J. P., J. Cell Sci. *60* (1983) 331.
22 Elgsaeter, A., Stokke, B. T., Mikkelsen, A., and Branton, D., Science *234* (1986) 1217.
23 Mitchell, P., Biol. Rev. *41* (1966) 445.
24 Brand, M. D., and Lehninger, A. L., Proc. natl Acad. Sci. USA *74* (1977) 1955.
25 Peterson, D. A., Kelly, B., Butterfield, J., Ashley, J., Peterson, R., and Gerrard, J. M., Med. Hypotheses *26* (1988) 271.
26 van Zoelen, E. J. J., Mummery, C. L., Boonstra, J., van der Saag, P. T., and de Laat, S. W., J. cell. Biochem. *21* (1983) 77.
27 Jeffrey, P. L., Appel, S. H., and Lewis, J., Exp. Neurol. *61* (1978) 432.
28 Alkon, D., and Rasmussen, H., Science *239* (1988) 998.
29 Rozengurt, E., Science *234* (1986) 161.
30 Gabbiani, G., and Gabbiani, F., Proc. natl Acad. Sci. USA *80* (1983) 2361.

31 Ryan, M. P., and Higgins, P. J., J. cell. Physiol. *137* (1988) 25.
32 Urbancikova, M., and Grofova, M., Neoplasma *29* (1982) 655.
33 Cooper, H. L., and Bassin, R. H., Prog. Leukocyte Biol. *5* (1986) 167.
34 Leavitt, J., Goldman, D., Merril, C., and Kagunaga, T., Clin. Chem. *4* (Pt. 2) (1982) 850.
35 Antecol, M. H., Mutat. Res. *199* (1988) 293.
36 Cone, C. D., and Tongier, M., J. cell. Physiol. *82* (1973) 373.
37 Ronquist, G., Frithz, G., Ericsson, P., and Hugosson, R., The Lancet *8025* (1977) 1284.
38 Abdukarimov, A., Aripdzhanov, A. A., and Khamidov, D. Kh., Dokl. Nauk. Uzb. SSR *7* (1980) 63.
39 Killion, J. J., Biophys. J. *45* (1984) 523.
40 Kartner, N., and Ling, V., Scient. Am. *260* (3) (1989) 26.
41 Racker, E., J. cell. Physiol. *89* (1976) 697.
42 Schnitzky, M., Biochim. biophys. Acta *738* (1984) 251.
43 Bernacki, R. J., Niedbala, J. M., and Korotnyk, W., Canc. Metast. Rev. *4* (1985) 81.
44 Chu, F. F., and Doyle, D., J. biol. Chem. *260* (1985) 3097.
45 Firestone, G. L., and Heath, E. C., Archs Biochem. Biophys. *201* (1980) 453.
46 Bar-Sagi, D., McCormick, F., Milley, R. J., and Feramisco, J. R., J. cell. Physiol. Suppl. 5 (1987) 69.
47 Velu, T. J., Beguinot, L., Vass, W. C., Willingham, M. C., Merlino, G. T., Pastan, I., and Lowy, D. R., Science *238* (1987) 1408.
48 Racker, E., Resnik, R. J., and Feldman, R., Proc. natl Acad. Sci. USA *82* (1985) 3535.
49 Seo, M. K., Lynch, K. E., and Podolsky, D. K., Cancer Res. *48* (1988) 1792.
50 Powell, A. E., and Leon, M. A., Exp. Cell Res. *62* (1970) 315.
51 Whitfield, J. F., MacManus, J. P., Boyuton, A. L., Gillan, D. J., and Isaacs, R. J., J. cell. Physiol. *84* (1974) 445.
52 Griffiths, J. B., Exp. Cell Res. *75* (1972) 47.
53 de Asua, L. J., Rozengurt, E., and Dulbecco, R., Proc. natl Acad. Sci. USA *71* (1974) 96.
54 Sefton, B. M., and Rubin, H., Proc. natl Acad. Sci. USA *68* (1971) 3154.
55 Turner, R. S., and Burger, M. M., in: The Cell Surface in Cell Interactions. Reviews of Physiology, p. 121. Eds R. H. Adrian, E. Helmreich, H. Holzer, R. Jung, K. Kramer, O. Krayer, F. Lynen, P. A. Miescher, H. Rasmussen, A. E. Renold, U. Trendelenburg, K. Ullrich, W. Vogt and A. Weber. Springer Verlag, Berlin/New York 1973.
56 Froehlich, J. F., and Anastassiades, T. P., J. cell. Physiol. *86* (1975) 567.
57 Majerus, P. W., Connolly, T. M., Deckmyn, H., Ross, T. S., Bross, T. E., Ishii, H., Bansel, V. S., and Wilson, D. B., Science *234* (1986) 1519.
58 English, L. H., Macara, I. G., and Cantley, L. C., J. Cell Biol. *97* (1983) 1299.
59 Smith, J. B., Proc. natl Acad. Sci. USA *80* (1983) 6162.
60 Berger, N. A., and Skinner, S. A. M., J. cell. Biol. *61* (1974) 45.
61 Berridge, M. J., J. cycl. Nucl. Res. *1* (1975) 305.
62 Dulbecco, R., and Elkington, J., Proc. natl Acad. Sci. USA *72* (1975) 1584.
63 Burns, C. P., and Rozengurt, E., Biochem. biophys. Res. Commun. *116* (1983) 931.
64 Cassel, D., Rothenberg, P., Zhuang, T.-X., Deuel, T. F., and Glaser, L., Proc. natl Acad. Sci. USA *80* (1983) 6224.
65 Christen, R., Schackmann, R. W., Dahlquist, F. W., and Shapiro, B. M., Exp. Cell Res. *149* (1983) 289.
66 Moolenar, W. H., Tsien, R. Y., van der Saag, P. T., and de Laat, S. W., Nature *304* (1983) 645.
67 Mendoza, S. A., Acta paediatr. scand. *76* (1987) 545.
68 Brasitus, T. A., Dudeja, P. K., and Foster, E. S., Biochim. biophys. Acta *938* (1988) 483.
69 Lagarde, A. E., Franchi, A. J., Paris, S., and Pouyssegur, J. M., J. cell. Biochem. *36* (1988) 249.
70 Parker, J. W., O'Brien, R. L., Steiner, J., and Paolilli, P., Exp. Cell Res. *83* (1974) 220.
71 Liteplooand, R. G., and Kerbel, R. S., Cancer Res. *46* (1986) 577.
72 Sun, I. L., Crane, F. L., and Chou, J. Y., Biochim. biophys. Acta *886* (1986) 327.
73 Froehlich, J. F., and Rachmeler, M., J. Cell Biol. *55* (1972) 19.
74 Grimes, W. J., and Schroeder, J. L., J. Cell Biol. *56* (1973) 487.
75 Bombik, B. M., and Burger, M. M., Exp. Cell Res. *80* (1973) 88.
76 Storrie, B. J., J. Cell Biol. *59* (1973) 471.
77 Bannai, S., and Sheppard, J. R., Nature *250* (1974) 62.
78 Friedman, D. L., Handb. exp. Pharmac. *58* (1982) 151.
79 Kirsch, D., Kemmler, W., and Häring, H. U., Biochem. biophys. Res. Commun. *115* (1983) 398.
80 Tagliaferri, P., Katsaros, D., Clair, T., Neckers, L., Robins, R. K., and Cho-Chung, Y. S., J. biol. Chem. *263* (1988) 409.
81 Tobey, R. A., and Ley, K. D., Cancer Res. *31* (1971) 46.
82 Franze-Fernández, M. T., and Pogo, A. O., Proc. natl Acad. Sci. USA *68* (1971) 3040.
83 Holley, R. W., Proc. natl Acad. Sci. USA *69* (1972) 2840.
84 Griffiths, J. B., Exp. Cell Res. *75* (1972) 47.
85 Holley, R. W., Nature *258* (1975) 487.
86 Bhargava, P. M., and Chandani, S. A., J. Biosci. *8* (1985) 329.
87 Tanaka, H., Zaitsu, H., Onodera, K., and Kimura, G., J. cell. Physiol. *136* (1988) 421.
88 Saier, M. H. Jr, and Deutscher, J., Naturwissenschaften *75* (1988) 451.
89 Kimelberg, H. K., and Mayhew, E., J. biol. Chem. *250* (1975) 100.
90 Olden, K., Pratt, R. M., Jaworski, C., and Yamada, K. M., Proc. natl Acad. Sci. USA *76* (1979) 791.
91 Watanabe, K., Tamura, G., and Mitsui, H., Cell Struct. Funct. *4* (1979) 127.
92 Loh, J., and Brunson, K. W., Proc. Indiana Acad. Sci. *92* (1982) 323.
93 Shefcyk, J., Molski, T. F. P., Volpi, M., Naccache, P. H. and Sha'afi, R. I., Biochim. biophys. Acta *728* (1983) 97.
94 Leister, K. J., Wenner, C. E., and Tomei, L. D., Proc. natl Acad. Sci. USA *82* (1985) 1599.
95 El-Battari, A., Muller, J. M., Fantini, J., Bellot, F., Tirard, A., Ducret, F., and Marvaldi, J., J. Cell Sci. *80* (1986) 269.
96 Tagliaferri, P., Yanagihara, K., Fortunato, C., Talbot, N., Flatow, U., Benade, L., and Bassin, R. H., Int. J. Cancer *40* (1987) 653.
97 Murakami, Y., Mizuno, S., Hori, M., and Uehara, Y., Cancer Res. *48* (1988) 1587.
98 Nilsson, G., Biochim. biophys. Acta *418* (1976) 376.
99 Bell, G. I., Science *192* (1976) 376.
100 Hayman, E. G., Engvall, E., and Rouslahti, E., Exp. Cell Res. *127* (1980) 478.
101 Ryan, M. P., Higgins, P. J., and Paul, J., J. cell. Physiol. *137* (1988) 25.
102 Burger, M. M., and Noonan, K. D., Nature *228* (1970) 512.
103 Patt, L. M., Itaya, K., and Hakomori, S., Nature *273* (1978) 379.
104 Rapaport, E., Schroeder, E. W., and Black, P. H., J. Cell. Physiol. *110* (1982) 318.
105 Rubin, H., Cancer Res. *42* (1982) 1761.
106 Lai, C. N., Gallick, G. E., Maxwell, S. A., Brinkley, B. R., and Becker, F. F., J. cell. Physiol. *134* (1988) 445.
107 Olivotto, M., Caldini, R., Chevanne, M., and Cipolleschi, M. G., J. cell. Physiol. *116* (1983) 149.
108 Lohman, W., and Hugo, F., Naturwissenschaften *76* (1989) 72.
109 Gail, M. H., and Boone, C. H. W., Exp. Cell Res. *73* (1972) 252.
110 Smith, J. B., and Rozengurt, E., Proc. natl Acad. Sci. USA *75* (1978) 5560.
111 Adam, G., Ernst, M., and Seher, J.-P., Exp. Cell Res. *120* (1979) 127.
112 Tandon, N. N., Davidson, L. A., and Titus, E. O., J. biol. Chem. *258* (1983) 9850.
113 Tenang, E. M., and McCaldnin, B., Biochem. Med. Metab. Biol. *38* (1987) 338.
114 Vairo, G., and Hamilton, J. A., J. cell. Physiol. *134* (1988) 13.
115 Cone, C., J. theor. Biol. *30* (1971) 151.
116 Cone, C. D., and Cone, C. M., Science *192* (1976) 155.
117 Soulinna, E.-M., Lang, D. R., and Racker, E., J. natl Canc. Inst. *53* (1974) 1515.
118 van den Berg, K. J., and Betel, I., Exp. Cell Res. *84* (1974) 412.
119 Ronquist, G., Ågren, G., Pontén, J., and Westermark, B., J. cell. Physiol. *89* (1976) 433.
120 White, M. F., and Christensen, H. N., J. Biol. Chem. *257* (1982) 4450.
121 Owen, N. E., and Villereal, M. L., Am. J. Physiol. *248* (3 Pt. 1) (1985) C288.
122 de Asua, L. J., Rozengurt, E., and Dulbecco, R., Proc. natl Acad. Sci. USA *71* (1974) 96.
123 Lingwood, C. A., and Thomas, D. B., J. Cell Biol. *61* (1974) 359.
124 Roller, B. A., Hirai, K., and Defendi, V., Biochim. biophys. Acta *366* (1974) 402.
125 Uehara, Y., Hori, M., and Umezawa, H., Biochem. biophys. Res. Commun. *125* (1984) 129.
126 Gilkey, J. C., J. Cell Biol. *97* (1983) 669.
127 Ralph, R. K., FEBS Lett. *161* (1983) 1.
128 Kaplan, D. R., Whitman, M., Schaffhausen, B., Raptis, L., Garcea, R. L., Pallas, D., Roberts, T. M., and Cantley, L., Proc. natl Acad. Sci. USA *83* (1986) 3624.

129 Alonso, T., Morgan, R. O., Marvizon, J. C., Zarbl, H., and Santos, E., Proc. natl Acad. Sci. USA *85* (1988) 4271.
130 Petzelt, C., and Hafner, M., in: Calcium-Binding Proteins Health Dis., (Int. Symp.) 1986, p. 101. Eds A. W. Norman, T. C. Vanaman and A. R. Means. Academic Press, San Diego 1987.
131 Dexter, T. M., Whetton, A. D., and Bazill, G. W., Differentiation *27* (1984) 163.
132 Kitagawa, K., Nishino, H., and Iwashima, A., Biochim. biophys. Acta *821* (1985) 63.
133 Whetton, A. D., Bazill, G. W., and Dexter, T. M., J. cell. Physiol. *123* (1985) 73.
134 Hatanaka, M., Jikken Igaku *5* (1987) 1185.
135 White, M. K., and Weber, M. J., Molec. cell. Biol. *8* (1988) 138.
136 Warburg, O., Science *123* (1956) 309.
137 Burk, D., and Woods, M., Archs Geschwulstforsch. *28* (1967) 305.
138 Wallach, D. F. H., Proc. int. Congr. Chemother. 13th *18* (1983) 233/1.
139 Watanabe, A., Brain Nerve *40* (1988) 637.
140 Bosca, L., Mojena, M., Ghysdael, J., Rousseau, G. G., and Hue, L., Biochem. J. *236* (1986) 595.
141 Schwartz, J. P., Passoneau, J. V., Johnson, G. S., and Pastan, I., J. biol. Chem. *249* (1974) 4138.
142 Navas, P., Sun, I. L., Morré, D. J., and Crane, F. L., Biochem. biophys. Res. Commun. *135* (1986) 110.
143 Wickus, G., Gruenstein, E., Robbins, P. W., and Rich, A., Proc. natl Acad. Sci. USA *72* (1975) 746.
144 Pollack, R., Oxborn, M., and Weber, K., Proc. natl Acad. Sci. USA *72* (1975) 994.
145 Varani, J., Wass, J. A., and Rao, K. M. K., J. natl Canc. Inst. *70* (1983) 805.
146 Leader, W. M., Stopak, D., and Harris, A. K., J. Cell Sci. *64* (1983) 1.
147 Notter, M. F. D., and Balduzzi, P. C., Virology *136* (1984) 56.
148 Abercrombie, M., In Vitro *6* (1970) 128.
149 Inbar, M., Schnitzky, M., and Sachs, L., FEBS Lett. *38* (1974) 268.
150 Tani, E., Kochi, N., Nakano, M., Yokota, M., Sukenaga, A., Nakaya, Y., and Itagaki, T., Acta neuropath. *58* (1982) 215.
151 Blitterswijk, W. J., Physiol. Membr. Fluid. *2* (1984) 53.
152 Bosmann, H. B., Biochem. biophys. Res. Commun. *48* (1972) 523.
153 van Beek, W. P., Smets, L. A., and Emmelot, P., Cancer Res. *33* (1973) 2913.
154 Bernacki, R. J., J. cell. Physiol. *83* (1974) 457.
155 Onodera, K., Yamaguchi, N., Kuchino, T., and Yaoi, Y., Proc. natl Acad. Sci. USA *73* (1976) 4090.
156 Painter, R. G., and White, A., Proc. natl Acad. Sci. USA *73* (1976) 837.
157 Spataro, A. C., Bosmann, H. B., and Myers-Robvogel, M. W., Biochim. biophys. Acta *553* (1979) 378.
158 Yogeeswaran, G., and Tao, T.-W., Biochem. biophys. Res. Commun. *95* (1980) 1452.
159 Sasaki, R., Takaku, F., Lin, Y. L., and Bollum, F. J., Leuk. Res. *6* (1982) 197.
160 Baker, M. A., Taub, R. N., Kanani, A., Brockhausen, I., and Hindenburg, A., Blood *66* (1985) 1068.

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The hummingbird's restraint: A natural model for weight control[1]

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Summary. By day, a breeding male hummingbird gives priority to aerial performance over energy security, minimizing body weight gain to expedite flight activity. We observed behavior and monitored weight via perches on electronic balances in a territory with unlimited resources (artificial feeder). The male restricted his first feeding to less than 1/6 of crop capacity. In the ensuing 15 h, his body weight increased only 1% despite an unlimited resource of artificial nectar. This facilitated acceleration for pursuit and courtship flights and reduced energy cost. In 20 min at dusk, an intake of 34% of body weight provided adequate energy for temperature regulation all night. In July, cessation of territorial activity was accompanied by a regular upward drift of dawn weight, suggesting that weight had been regulated previously as a balance between expenditure and intake, perhaps without involving set-points.
Key words. Weight control; energetics; foraging; territory.

Causes of human weight problems and eating disorders are often unknown[2,3]. A fundamental issue is whether body weight is stabilized without reference to set-points via a simple balance between intake and expenditure, responding independently to exogenous and endogenous factors[4,5] or via servo-regulation to specific 'set-points'[6–8]. In the latter, obesity would represent an elevated set-point, anorexia a depressed one. We describe a case of natural control in terms of these models. (Although the gram is a unit of mass, we revert here to customary reference to 'weight control', appropriate in this case because flying requires lift to balance weight.)

Birds are 'useful models for studying control of appetite and obesity'[9]. This should be especially true of hummingbirds, whose quantifiable activity yields unambiguous patterns amenable to mechanistic interpretation. Small size and high energy costs of hovering [41.5 ml oxygen/(g body mass × h)] and temperature regulation[10,11] result in intense metabolism and rapid energy turnover. This would seem to necessitate a progressive day-long increase in stored energy as survival insurance.

Progressive gain is also implied in an assumption of optimal foraging theory, in which hummingbirds have been important, that reproductive fitness increases linearly