receptors

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Communication

Cycle Numbers of Cell Surface Recycling Receptors

Dietmar Steverding

Bob Champion Research and Education Building, Norwich Medical School, University of East Anglia, Norwich Research Park, Rosalind Franklin Road, Norwich NR4 7UQ, UK; d.steverding@uea.ac.uk

Abstract: The cycle number (n_c) of a recycling receptor is defined as the average number of round trips (cell surface–endosome–cell surface) the receptor can make before it is degraded. This characteristic parameter of recycling receptors can be easily determined from the receptor's half-life ($t_{1/2}$, the time in which 50% of the receptor is degraded) and cycling time (T_c, the time a receptor needs to complete a round trip). Relationship analyses revealed that n_c increases linearly with increasing $t_{1/2}$ and decreases exponentially with increasing T_c. For commonly observed $t_{1/2}$ and T_c values, it was calculated that recycling receptors have n_c values of <300. In addition, it was found that recycling receptors in cancer cells have generally smaller n_c values (<100), whereas recycling receptors in normal cells have larger n_c values (>100). Based on this latter finding, the cycle number n_c may be a useful criterion for distinguishing between cancer and normal cells.

Keywords: recycling receptors; cycle number; cycling time; half-life; cancer cells

1. Introduction

Recycling receptors are cell surface proteins that are used by cells for the endocytosis of extracellular macromolecules [1,2]. In general, after binding its ligand, the receptor clusters with other receptors in clathrin-coated pits. The receptor–ligand complex is internalized in coated vesicles which fuse with the endosome. Usually, within the endosome, the low luminal pH of this compartment leads to the dissociation of the ligand from the receptor. While the ligand is transported to the lysosome, where it is degraded, the receptor returns to the cell surface to bind another ligand and initiates another cycle of endocytosis (a scheme of the recycling process of surface receptors is shown in Figure 1).

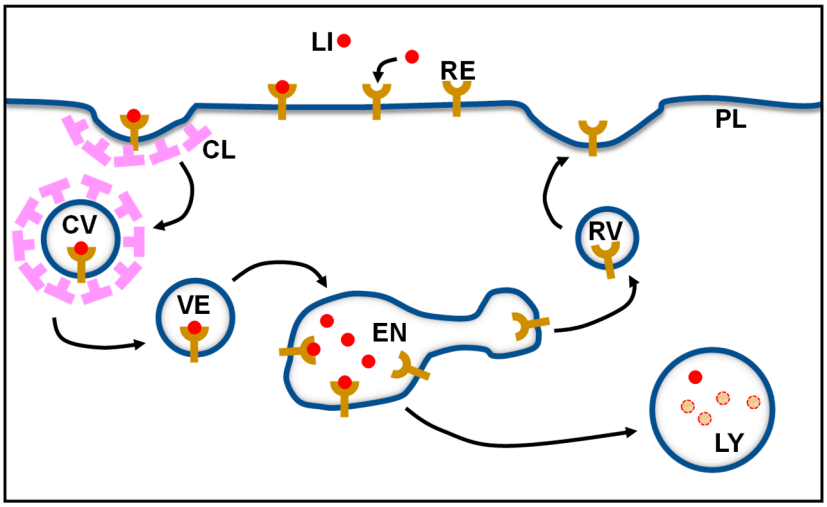

Figure 1. Schematic representation of the surface receptor recycling process. CL, clathrin; CV, clathrin-coated vesicle; EN, endosome; LI, ligand; LY, lysosome; PL, plasma membrane; RE, receptor; RV, recycling vesicle.

Citation: Steverding, D. Cycle Numbers of Cell Surface Recycling Receptors. *Receptors* **2023**, 2, 160–165. https://doi.org/10.3390/receptors2020010

Academic Editor: Stephen H. Safe

Received: 17 April 2023
Revised: 4 May 2023
Accepted: 1 June 2023
Published: 6 June 2023

Although the recycling of receptors has been studied in great detail in past decades, one parameter has not been paid much attention: the cycle number, i.e., the number of round trips a receptor undertakes before it is degraded. This may be due to the fact that the cycle number cannot be determined directly. Very few estimations of receptor cycle numbers have been published in the literature, ranging from 300 to 1000 cycles [2,3]. However, as shown herein, these values are, to some extent, hugely overestimated. Obviously, the cycle number depends on the half-life (the period of time required for half of the receptor molecules to be degraded) and on the cycling time (the time needed for the receptor to complete one round trip) of the receptor. The longer the half-life and the shorter the cycling time, the greater the cycle number is. This work analyzed the relationship between the cycle number, half-life, and cycling time of recycling receptors. In addition, the cycle numbers of different recycling receptors in normal cells and cancer cells were computed and compared.

2. Calculation of the Average Cycle Number of Recycling Receptors

The cycling time of a receptor can be easily calculated from the total number of functional receptors divided by the rate of ligand uptake [4,5]. It has been shown that this value is similar to the cycling time obtained from the sum of the individual rate constants [4,5]. The half-life of a receptor can be readily determined via radioactive metabolic labelling experiments [6].

The cycle number of a recycling receptor was calculated using a previously developed equation [7]. In brief, the average cycle number (n_c) of a receptor before it is degraded can be computed from the number of receptor molecules remaining after each cycle (N_c) divided by the number of receptor molecules (N_0) at the time t_0.

$$n_c = \frac{N_{c=1} + N_{c=2} + N_{c=3} + \cdots + N_{c=n}}{N_0} \quad (1)$$

The number of receptor molecules remaining after each cycle (N_c) is provided by:

$$N_c = N_0 \times \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c \times n}\} \quad (2)$$

where $t_{1/2}$ is the half-life, T_c is the cycling time, and n is the number of cycles of the receptor. Together, Equations (1) and (2) provide:

$$n_c = \frac{N_0 \times \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c \times 1}\} + \cdots + N_0 \times \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c \times n}\}}{N_0} \quad (3)$$

which can be simplified to:

$$n_c = \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c \times 1}\} + \cdots + \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c \times n}\} \quad (4)$$

Equation (4) equals to:

$$n_c = \sum_{n=1}^{\infty} \{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c}\}^n \quad (5)$$

The solution for Equation (5) is:

$$n_c = \frac{e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c}}{1 - e^{-[\frac{(ln2)}{t_{1/2}}]\times T_c}} \quad (6)$$

3. Relationship between Cycle Number, Half-Life, and Cycling Time

To understand how the cycle number n_c is linked with the half-life $t_{1/2}$ and the cycling time T_c of a receptor, n_c was determined as a function of $t_{1/2}$ and T_c for given T_c and $t_{1/2}$ values, respectively, using Equation (6). As depicted in Figure 2A, n_c is linearly dependent

on $t_{1/2}$ for given values of T_c. With an increase in $t_{1/2}$, n_c also increases. This is plausible as a receptor with a longer half-life can undertake more round trips. Although it appears that with increasing T_c values the increase in n_c diminishes (the slopes of the linear regressions for different T_c values decrease), the fold increase in n_c over time is the same for all T_c values. As illustrated in Figure 2B, n_c decreases exponentially with an increasing T_c for given values of $t_{1/2}$. Thus, with an increasing T_c, n_c becomes smaller and smaller and less dependent on T_c. This makes sense because with an increase in the cycling time (T_c), the number of round trips (i.e., the cycle number (n_c)) should decrease. Furthermore, when the cycling time approaches the half-life ($t_{1/2}$), the cycle number should be increasingly determined by only the half-life of the receptor. The relationship among the three variables, n_c, $t_{1/2}$, and T_c, is shown in Figure 3 in the form of a 3D surface plot. From the 3D graph, it can be seen clearly that with increasing $t_{1/2}$ values and decreasing T_c values, n_c values increase greatly.

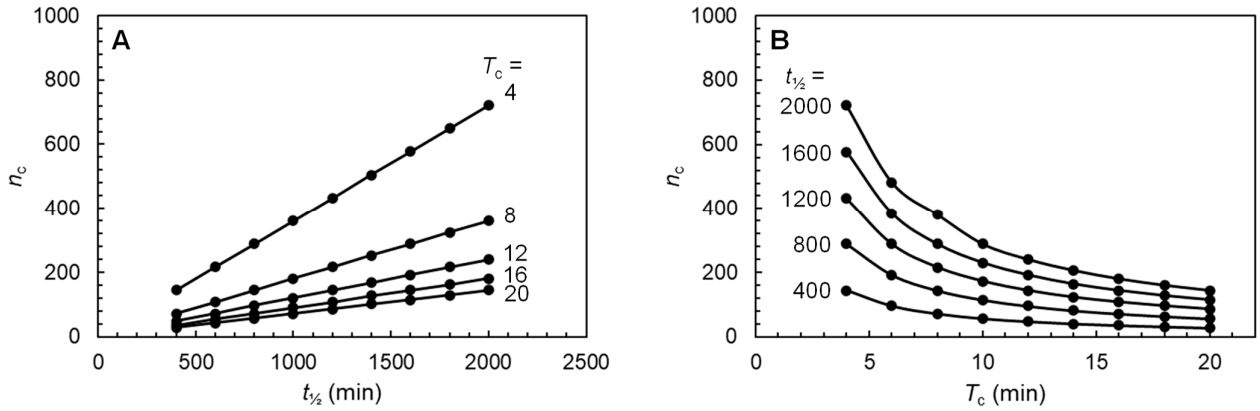

Figure 2. Plots of cycle number n_c as a function of half-life $t_{1/2}$ for given cycling time T_c (**A**) and of T_c for given half-life $t_{1/2}$ (**B**). The range of values for $t_{1/2}$ and T_c are within the ranges of half-lives and cycling times normally observed for recycling receptors. The cycle number was calculated using Equation (6).

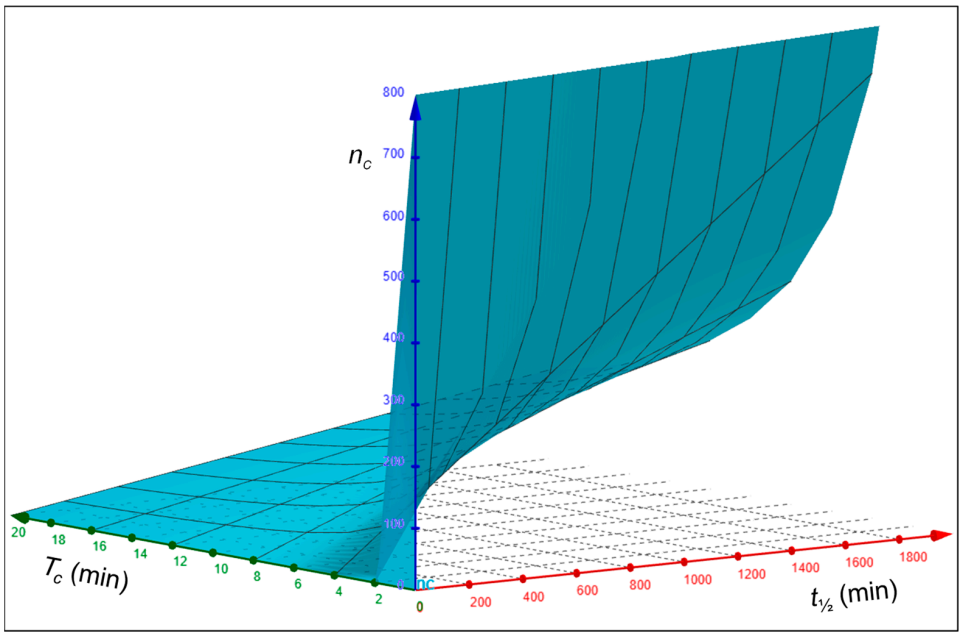

Figure 3. Three-dimensional surface plot of the relationship between the half-lives $t_{1/2}$ cycling times T_c, and cycle numbers n_c of recycling receptors. The 3D graph was created with the GeoGebra 3D Calculator [8].

Values of n_c for $t_{1/2}$ and T_c values in the ranges normally found for recycling receptors (400–2000 min and 4–20 min, respectively) are presented in Table 1. From the data, it is clear that n_c values are usually smaller than 300 and do not assume values of 300–1000, as previously estimated [2,3]. Only for $t_{1/2}$ values $\geq$1000 min in combination with T_c values $\leq$8 min does n_c assume values of >300 (see grey highlighted numbers in Table 1). Table 1 is also useful for rough estimations of n_c values.

Table 1. Values of cycle numbers (n_c) of recycling receptors for given half-lives ($t_{1/2}$) and cycling times (T_c). The cycle numbers were calculated using Equation (6).

n_c		T_c (min)								
		4	**6**	**8**	**10**	**12**	**14**	**16**	**18**	**20**
$t_{1/2}$ (min)	**400**	144	96	72	57	48	41	36	32	28
	600	216	144	108	86	72	61	54	48	43
	800	288	192	144	115	96	82	72	64	57
	1000	360	240	180	144	120	103	90	80	72
	1200	432	288	216	173	144	123	108	96	86
	1400	505	336	252	202	168	144	126	112	101
	1600	577	384	288	230	192	164	144	128	115
	1800	649	432	324	259	216	185	162	144	129
	2000	721	481	360	288	240	206	180	160	144

Cycle numbers greater than 300 are highlighted in grey.

4. Cycle Numbers of Classical Recycling Receptors

Using Equation (6), the cycle numbers n_c of the recycling receptors for asialoglycoprotein, low-density lipoprotein, mannose, and transferrin were calculated (Table 2). It was found that the n_c values for these classical recycling receptors ranged between 38 and 240. These n_c values are smaller than the previously suggested receptor cycle numbers of 300–1000 [2,3]. Moreover, the calculated n_c values correspond well with measured cycle numbers. For example, it was found that the low-density lipoprotein receptor can undergo up to 150 cycles in fibroblasts [9], which is in close agreement with the calculated n_c value of 144 (Table 2).

The n_c values determined for recycling receptors in this study, which are almost an order of magnitude smaller, are more compatible with the physiological stress a receptor experiences during the recycling process. Recycling requires that a receptor is not denatured when passing repeatedly through the acidic environment of the endosome. In the acidic compartment, a receptor must undergo substantial conformational changes to release its ligand [10] but must not become irreversibly damaged. Thus, round trips of more than 300 may harm a receptor in such a manner that it will lose its function.

Table 2. Calculated n_c values for recycling receptors. The n_c values were computed using Equation (6) and published $t_{1/2}$ and T_c values. The sources of the $t_{1/2}$ and T_c values are indicated.

Receptor	Cell Type	$t_{1/2}$(min)	T_c (min)	n_c
Asialoglycoprotein	HepG2 cells	720 [11]	15.9 [4]	65
	Rat hepatocytes	1200 [3]	7.2 [3]	240
Low-density lipoprotein	Human fibroblasts	1200 [12]	12 [12]	144
Mannose	Macrophages	1980 [13]	15 [13]	190
Transferrin	HeLa	1140 [14]	21 [15]	78
	HepG2 cells	420 [16]	15.8 [5]	38
	K562 cells	480 [17]	12.5 [17]	55
	Trypanosoma brucei	426 [7]	10.7 [7]	57

5. Cycle Numbers of Recycling Receptors Distinguish between Cancer and Normal Cells

It is interesting to note that receptors in cancer cells have smaller cycle numbers than receptors in noncancerous cells freshly prepared from tissues (Table 2). For instance, recycling receptors in human hepatocarcinoma HepG2 cells [18], human erythroleukaemia K562 cells [19], and human immortalized cancerous HeLa cells [20] have cycle numbers between 38 and 79, whereas recycling receptors in human fibroblasts (freshly prepared from the foreskin of a newborn boy [21]) and rat hepatocytes (freshly isolated from a rat liver [3]) have cycle numbers of 144 and 237, respectively. In addition, the mannose receptor in cultured macrophages has an n_c value of >100 (Table 2). The difference in the cycle numbers of recycling receptors in cancer cells and normal cells is probably associated with differences in metabolic fluxes and nutritional needs between these cells [22]. As fast-proliferating cells, cancer cells have an upregulated metabolic activity and, accordingly, a higher protein turnover than normal cells [23]. This is reflected in the shorter half-life of receptors in cancer cells compared with normal cells (Table 2). This suggestion is further supported by the finding that the transferrin receptor of the fast-proliferating protozoan parasite *Trypanosoma brucei* also has a short half-life and a small cycle number (Table 2).

The cycle number may be affected by ligand-induced signaling of the receptor. This, however, depends on the receptor-mediated endocytic pathway [9]. For receptors that are endocytosed only after they have bound a ligand, the cycle number may be lower when there is a lack of a ligand as in this case, the receptor would remain at the cell surface for a longer time. On the other hand, for receptors that are continuously internalized even in the absence of a ligand, the cycle number will be unaffected by ligand binding. Receptors for low-density lipoprotein, transferrin, and asialoglycoprotein belong to this latter group [12,24,25].

There is evidence that cancer cells show increased rates of clathrin-mediated endocytosis [26]. As clathrin-mediated endocytosis plays an important role in the process of ligand uptake by recycling receptors, it could be assumed that these receptors would be recycled faster in cancer cells than in normal cells and therefore would have smaller cycling times (T_c values). However, this is not the case. It rather seems that the recycling receptors in cancer cells tend to have longer cycling times, although no statistically significant difference between the T_c values for recycling receptors in cancer and normal cells was observed (unpaired *t*-test: $p = 0.1422$). This finding may indicate that the clathrin-mediated endocytosis rate in cancer cells is actually not different from the rate in normal cells. As the cycling times of the different recycling receptors differ only by a factor of 3, it seems that the cycle number n_c is mainly determined by the half-lives of the receptors.

6. Conclusions

This study has shown that the average number of round trips (cycle number) of a recycling receptor can be easily determined using the receptor's half-life and cycling number. The cycle numbers of classical recycling receptors range between 40 and 240. In cancer cells, the cycle numbers of receptors are <100, while in normal cells, they are >100. Thus, the cycle number of recycling receptors may be used as a characteristic to differentiate cancer cells from normal cells.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: No new data were created or analyzed in this study. Data sharing is not applicable to this article.

Conflicts of Interest: The author declares no conflict of interest.

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