ESKİŞEHİR TEKNİK ÜNİVERSİTESİ BİLİM VE TEKNOLOJİ DERGİSİ C- YAŞAM BİLİMLERİ VE BİYOTEKNOLOJİ

Eskişehir Technical University Journal of Science and Technology C- Life Sciences and Biotechnology

2022, 11(1), pp. 31-38, DOI: 10.18038/aubtdc.907029

REVIEW

WHAT TELL US THE BIONUMBERS FOR PROTEIN PHOSPHORYLATION IN PLANT DEFENSES

Berna BAŞ*

ABSTRACT

Plants to survive against to devastating impact of invasive biotic agents have to powerfully struggle in armed combat with microorganisms. Therefore they need to activate rapidly and efficiently pre-existing potential defensive chemicals. As soon as perception initial external stimuli through plant cell membrane receptors and/or cytoplasmic resistance proteins before activity of related genes, some proteins participated in plant immunity undergo alterations referred as molecular modification. Phosphorylation is one of the first steps and most important modifications in signal transduction pathways of plant immunty. While transcription/translation of the gene dependening to molecular size, organism type, ribosome number is proceed in time unit from seconds to minutes, whereas phosphorylation is occurred in the time period expressed with milliseconds/seconds. Why does protein phosphorylation in plant cells occur quickly in comparison to gene expression? In this commentary work inquired of this question, speedity of gene expression and phosphorylation processes on time profile is compared outlining with bionumbers.

Keywords: Bionumbers, Protein/amino acid phosphorylation, Time-course of phosphorylation, Plant defense

1. INTRODUCTION

Due to be continuously exposed to external stimuli in sub-optimal surroundings, plants have to speedly respond to biotic and abiotic agents. Unlike vertebrates, intermediary molecules in plant immune system do not circuit throughout plant in motion. If the subversive actions started at the point of infection by the invader cannot be defeated with versatile plant defense strategies for pathogen defense, defense genes with long-distance are prompted at whole plant level. Biotic elicitor-effector molecules as non-self or self-modified stimulus in plants are recognized by plant cell surface sensors or cytoplasmic receptors. Initial alterations upon biotic stress sensing in plants, rather genetic regulation, are molecular modifications of the molecules participated to plant immune system such as the change of self-defense molecules or receptor proteins. Molecular modifications include chemical, physical and biological construct alterations in molecules [1]. Thus, this is caused generation of new variations derived from compounds undergoing change. In medicine, molecular modification is most widely used term for chemical changing of any molecule to design drugs [2]. Also chemical modificationis the changing with reagents of structure of biological macromolecules like proteins, nucleic acids, polysaccharides (UIPAC, 1997: The International Union of Pure and Applied Chemistry, Compendium of Chemical Terminology). However, post-translational modifications (PTMs) are related to the chemical modification process of proteins with addition into or removal from target molecule of functional group, any ion, small molecules after protein biosynthesis occured, it may be considered within chemical modification. Till now more than 90,000 individual PTMs were explored [3]. Eventually these chemical reactions without altering basic scaffold of molecules vary physical structure of molecule, consequently their biological functions change as well. In plant immunity, the term "chemical modification" is not originally used in its genuine content in actually. But it is attempted to draw manipulations by chemical process ascribed to resembling to chemical modification. Plants have a wide group of chemical modifications involvement in phosphorylation, acetylation, methylation, sumoylation, proteolysis, glycosylation chemical reactions, named as PTMs

*Corresponding Author:bbas65@hotmail.com
Received:31.01.2021 Published: 19.01.2022

[4,5]. PTMs are vitally significant to modulate functions of proteins that play role in cell metabolism, in operating of intracellular signal transduction pathways, in promoting cell differentiations, division and proliferation [6,7,8,9]. Amongst PTM varieties, phosphorylation is the most studied and the best known sort of chemical modification [5,10]. Moreover, it has also the most data with proteome-wide input [11].

Since database records of phosphorylation of key molecules responsible for almost all biological regulations are registered in many various scholarly websites, they are always updated with new phosphoproteomic data input (**http**://www.p3db.org/;**http**://phosphat.mpimp-golm.mpg.de/;**http**://www.cbs.dtu.dk/services/NetPhos/; **http**://iekpd.biocuckoo.org/). For instance, by now there are records of 197348 phosphorylation regulators, 109912 protein kinases, 23294 phosphatases, 68748 phosphoprotein binding domains belonging to 164 eukaryotic species. Moreover 47 of these are in plants (http://iekpd.biocuckoo.org website). However, the reports of large-scale comparative analysis and time-course of phosphorylation corresponding plant immunity are restricted with a few model plant species. For works directly on quantity of time-course of phosphorylation about plant defense proteins, it should be solely referred to Schulze et al [12].

While protein/amino acid phosphorylation reaction which allows shifting of protein activities rapidly in a very short time [13] is occured within seconds/milliseconds, synthesizing of a new protein is taken place within minutes. Why do some cells first react to a biotic/abiotic signal by phosphorylation? In this short commentary evaluated from different perspective of this issue, time intervals of phosphorylation reaction with gene expression have been compared using bionumbers.

2. TYPES OF PTMs

Plants exposed to harsh ambient conditions have to reply rapidly and efficiently to cope with external detrimental stimuli come from biotic/abiotic variables. Althouh more than 200 different types of PTMs have been determined [14], some of the most common PTMs can be functionally classified into subgroups as shown Figure 1. Chemical modifications of proteins related to manage direction of signaling systems and metabolic changing is mainly reversible, but modifications through alterations in structure of polypeptide backbone such as deamidation, eliminylation or amino acid substitution are irreversible [5, 15]. PTMs cause to shift on enzyme activities, inter/intra-molecular conformational changes in proteins, replacement of subcellular location of proteins and variations in protein-protein and protein-other molecule interactions.

Reversible phosphorylation plays much significant role for perceiving of environmental stresses, for transduction of signals throughout membrane in the cell, to multiple intracellular signals, to express immune response, to regulate cell-cycle control mechanisms and to sense endogenous hormones [16, 17, 18]. Conventionally in chemistry, phosphorylation of a molecule is chemically addition of terminal phosphoryl (PO_3^-) group of ATP as phosphate source to an organic molecule. Also removal of phosphorus from any substrate is dephosphorylation. In addition either activities of phosphorylation reaction are catalyzed diverse kinases and phosphatases, respectively. Protein phosphorylation is occurred on most commonly specific amino acid side chains in proteins. Protein kinase enzymes facilitate to be carried phosphate groups of ATP to serine or threonine or tyrosine residue in proteins [19, 20, 21]. Proteins/amino acids/enzymes phosphorylation entailed to alterations of structural conformations of molecules allows to emerge derivative new molecule forms [22] in shortest possible time. For instance, when membrane receptor proteins are phosphorylated with inter/intra molecular interactions upon directly/indirectly attached to microbial elicitors/effectors molecules is subjected to conformational change, a plant perceives this molecular transformation as disruption of normal homeostasis. Consequently the novel derivative molecules gain the competence to stimulate specific events series deployed in plant defense system.

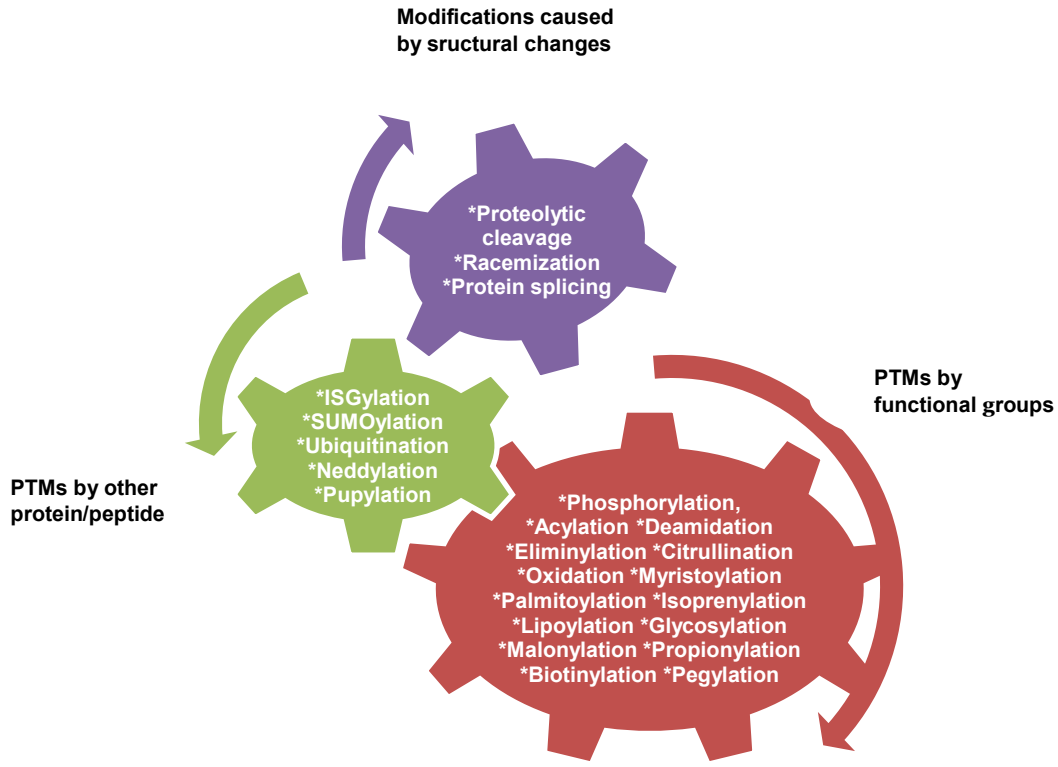

Figure 1. Post-translational modification types [3,14].

It is estimated that there exist hundreds of protein kinases and phosphatases in plants and animals [23]. While Benschop et al. [24] has determined about 1170 phosphopeptide sites on 472 phosphoproteins in Arabidopsis cell culture, Al-Momani et al. [25] has reported ~6500 phosphopeptides identified from over 3000 phosphoproteins in *Arabidopsis*. Li et al. [18] identified 1522 unique peptides, of which 1365 were phosphorylated in rice seedlings, some after infections and some associated with biological processes. Weintz et al. [26] has characterized almost close to 7000 phosphorylation sites on 1800 phosphoproteins against to lipopolysaccharide activation in animals. In actually, these bionumbers show importance and complexity of sophisticated phosphorylation network and the size of the cumulative area occupied by phosphorylation in the eukaryotic genome.

3. PHOSPHORYLATION REACTIONS DEVELOP WITHIN SECONDS

Schulze et al. [12] quantified phosphorylation rate with experimental studies in the time-course analyses of phosphorylation with *Arabidopsis thaliana* FLS2-BAK1 complex protein. Bacterial elicitor Flg22 is perceived by FLS2-BAK1 complex which is an *Arabidopsis* cell membrane receptor. *De novo* phosphorylation trials indicated which measurable phosphorylation has been detected in the 15^{th} second after treatment with flg22 of *Arabidopsis* cells. Furthermore, the reaction has been occurred just before plant immune response is aroused [12]. Also Haj Ahmed et al. [27] published results of the analysis of phosphorylation time-course works such as rapid phoshorylations of NADPH-oxidases and Ca^{+2}-ATPase enzymes, dephosphorylations of H^{+}-ATPases initiated by triggering of up-regulation of plant cell surface receptors. They applied to tomato cell culture a peptide hormone systemin as stimulant to mediate for induction of signal transduction defense pathways in injured plants by herbivory insect damage. Systemin susceptibility reaction has been quantifed from phosphorylation time-course evaluations. After systemin treatment, they assessed situations of phosphorylation, dephosphorylation, not responsive phosphopeptide from a total of 3312

phosphopeptide patterns. Accordingly, in time profile of phosphorylation on their notification, protein enhancement was not observed in the period expressed in minutes. However, incremental phosphopeptide amount (both phosphorylation and dephosphorylation) was explained as change of phosphorylation status. Likewise in an assay directed by phytochrome-mediated photoresponses using foliage protein from *Avena sativa* seedlings, it was revealed that half-time of protein phosphorylation/dephosphorylation reactions reached approximately in 2 seconds at 0℃ under their laboratory conditions [28].Whereas *in vitro* radioactive labelled phosphorus from ATP was transferred into endogenous protein kinases as intermediate from plasma membrane fractions of corn root cells in less than 30 seconds [29].

Similarly, Briskin [30] indicated quantification of enzyme phosphorylation/dephosphorylation reaction kinetic parameters by time-course ratios in red beet plasma membrane H-ATPase enzyme during *in vitro* under different conditions. According to their outcomes, in presence of $MgSO_4$ and radioactive labelled substrate (P) mixture at pH 6.5 at 10℃ phosphoenzyme formation rate $k = 3.24\ s^{-1}$ was reported, and also to the enzyme saturation with its substrate was attained in 600 milliseconds. After 20 seconds pre-incubation of enzyme-radioactive labelled substrate subsequently addition of $MgSO_4$, phosphoenzyme formation rate, $k = 7.46\ s^{-1}$ was calculated. Although existence of magnesium reduced the binding speedity of phosphorus to enzyme, both of two cases [γ-32P]-ATP phosphorus group was carried to the enzyme within milliseconds.

One of the earliest works in this field was published in 1976 [31]. According to Rose and Dube [31]'s results, phosphorus incorporation from phosphoenzyme into phosphoglycerate took places in 38 seconds at 4℃. But this kinetic parameter was too fast to measure at 25℃ and finally reaction was completed in as little as 22 milliseconds. Although phosphorylation reaction is strikingly occurred quickly within milliseconds, it should be regarded this result was obtained from *in vitro* laboratory conditions. Also this data might be possibly variable in living plant cells when environmental factors are changed, nevertheless it should not be expected unusual time variation as more as minutes or hours for the time intervals of phosphorus transference.

The rate of a protein synthesis or transcription process in eukaryotic organisms in comparison with length of time of phosphorylation duration from reports explained above is rather slow. The studies particularly relevant with the time course of phosphorylation of the plant immune molecules is fairly former and a few and also insufficient for the specific issue addressed herein. Tang et al. [32], Park et al. [33], Minkoffet al. [34], Yin et al. [35] have reported qualitative results that phosphorylation of plant immune molecules (proteins) is rapidly occurred without defined exact time intervals. This may be due to the fact that protein phosphorylation is a fast and transient dynamic period and makes difficult to quick measurement of phosphorylation ratio in small area occupied by phosphorus with currently application techniques. Besides the concentration of signal molecules in cell is at low level and experimental models/strategies with range of analytic measurement techniques in use has the limitated capacity, so the stoichiometry of phosphorylation of protein/amino acids is at low level [36]. Nevertheless this preliminary informative inferences display that plants may readily adapt with quick phosphorylation responses to biological systems which are rearranged by environmental suppressions against disturbance caused by external biotic factors in the plant cells for evolution of the long range plant defense signaling.

4. THE RATE OF TRANSCRIPTION AND TRANSLATION

The rate of transcription and translation alters depending on the identification techniques, the cell and organism types, and ribosome number. Studies on the kinetics of gene expression in mammal cells show that the rate of transcription is 1000 nucleotide/minute, this means it is generated 1 kb/minute mRNA [37]. According to Lewin [38], protein synthesis ratio (translation rate) is 140 amino acids/minute, the synthesis of protein molecule in the chain length about of 1000 amino acids residue

drives almost 10 minutes. According to Rawn [39] and Alberts et al. [40], while RNA elongation in mammals and bacteria are 30 nucleotide/second and 30-85 nucleotid/second, respectively, protein synthesis also is 10 amino acid sequences in a second and 50 nucleotide/second = 18 amino acid/second.

Transcription and translation in mammals may take minutes depending on the protein property. When the ratio measurements of phosphorus transfer by Rose and Dube [31] were simply calculated according to results of Hargrove et al [37], the translation of the phosphoenzyme is expected to complete in 131.5 seconds. Based on results of Hargrove et al [37] it might be considered molecular weight of each subunit as approximately ~29.000 da in size containing of ~263 amino acid residues (*****). However by Rawn [39] and Alberts et al. [40], the generation of the same phosphoenzyme from gene level takes 26 seconds.***** *In general biochemistry, one amino acid is considered an average of 110 daltons*

There is a large rate difference between times of protein phosphorylation and translation. While the time length of protein/amino acid phosphorylation is stated as seconds/milliseconds, time span of translation is stated as minutes. Why do some cells first react to a biotic/abiotic signal by phosphorylation? These bionumbers may be attributed to, the cell spends more time to make new proteins by regulation through turning genes on/off than creation new derivative molecules with post-transcriptional modifications. Priority for the plant cells have to adapt as possible quickly to micro vicinity that is re-decorated at molecular level within seconds/milliseconds to self-protect from bio-detrimental impact of surroundings along evolutionary process. So the result is inevitable that plants should be primarily carried out protein phosphorylation, subsequently attempted to molecule synthesis by promoting gene induction in plant immune systems.

5. CONCLUSION

Protein phosphorylation has crucial roles for plant immunity. Quantitative analysis experiments with the time-course of immune protein phosphorylation are usually limited. Due to researches related on time length of protein/amino acid expression are standardized in chronobiology and also difficulty of the stoichiometric measurement of protein phosphorylation they are not intensely focused nowadays. Hence the expansion of analytical studies on quantitative determination level of plant immune protein phosphorylation is necessary. With advances in innovative technologies, the discovery of novel phosphoproteins/amino acids or uncovering of currently existing ones in plant defense pathways may contribute to ponder layout behind mechanism of cellular communication network which is very complex. Endeavors in this area might be the boost to foster for designing new paths of alternative fundamental plant protection strategies.

CONFLICT OF INTEREST

The authors stated that there are no conflicts of interest regarding the publication of this article.

REFERENCES

[1] Li S, Xiong Q, Lai X, Li X, Wan M, Zhang J, Yan Y, Cao M, Lu L, Guan J, et al. Molecular Modification of Polysaccharides and Resulting Bioactivities. Compr Rev Food Sci F, 2016;15:237-250.

[2] Pandeya SN, Dimmock JR. An Introduction to Drug Design. New Age International Publishers, New Delhi, 1997.

[3] Audagnotto M, Dal Peraro M. Protein post-translational modifications: *In silico* prediction tools and molecular modeling. Comput Struct Biotechnol, J 2017;15:307-319.

[4] di Pietro M, Vialaret J, Li GW, Hem S, Prado K, Rossignol M, Maurel C, Santoni V, et al. Coordinated post-translational responses of aquaporins to abiotic and nutritional stimuli in *Arabidopsis* roots. Mol Cell Proteomics, 2013;12:3886-3897.

[5] Spoel SH. Orchestrating the proteome with post-translational modifications. J Exp Bot, 2018;69:4499-4503.

[6] Krause C, Richte, S, Knöll C, Jürgens G. Plant secretome: from cellular process to biological activity. Biochim Biophys Acta, 2013;183:2429-2441.

[7] Matsubayashi Y. Posttranslationally modified small-peptide signals in plants. Annu Rev Plant Biol, 2014;65:385-413.

[8] Hashiguchi A, Komatsu S. Posttranslational Modifications and Plant-Environment Interaction. Methods Enzymol, 2017;586:97-113.

[9] Yalçın A. Posttranslasyonel Modifikasyon ve Protein Fonksiyonu. Uludağ Üniversitesi Veteriner Fakültesi Dergisi, 2012;31:29-38.

[10] Kadota Y, Macho AP, Zipfel C. Immunoprecipitation of plasma membrane receptor-like kinases for identification of phosphorylation sites and associated proteins. Methods Mol Biol, 2016;1363:133-144.

[11] Lemeer S, Heck AJ. The phosphoproteomics data explosion. Curr Opin Chem Biol, 2009;13:414-420.

[12] Schulze B, Mentzel T, Jehle AK, Mueller K, Beeler S, Boller T, Felix G, Chinchilla D. Rapid heteromerization and phosphorylation of ligand-activated plant transmembrane receptors and their associated kinase BAK1. J Biol Chem,2010;285:9444-9451.

[13] Stram AR, Payne RM. Post-translational modifications in mitochondria: protein signaling in the powerhouse. Cell Mol Life Sci, 2016;73:4063-4073.

[14] Virág D, Dalmadi-Kiss B, Vékey K, Drahos L, Klebovich I, Antal I, Ludányi K. Current trends in the analysis of post-translational Modifications. Chromatographia, 2020;83:1-10.

[15] Martin BL. Regulation by Covalent Modification. In eLS, John Wiley and Sons Ltd (Ed.), Chichester, 2014.

[16] Osakabe Y, Yamaguchi-Shinozaki K, Shinozaki K, Tran LS. Sensing the environment: key roles of membrane-localized kinases in plant perception and response to abiotic stress. J Exp Bot, 2013;64:445-458.

[17] Yu Q, An L, Li W. The CBL-CIPK network mediates different signaling pathways in plants. Plant Cell Rep, 2014;33:203-214.

[18] Li J, Silva-Sanchez C, Zhang T, Chen S, Li H. Phosphoproteomics technologies and applications in plant biology research. Front Plant Sci, 2015;6:430.

[19] Krebs EG. The enzymology of control by phosphorylation. In:Boyer PD, Krebs EG, editors. The Enzymes. New York: Academic Press, 1986;17:pp.3-20.

[20] Olsen JV, Blagoev B, Gnad F, Macek B, Kumar C, Mortensen P, Mann M. Global, in-vivo, and site-specific phosphorylation dynamics in signaling networks. Cell, 2006;127:635-648.

[21] Thingholm TE, Jensen ON, Larsen MR. Analytical strategies for phosphoproteomics. Proteomics, 2009;9:1451–1468.

[22] Ha JH, Loh SN.Protein conformational switches: from nature to design. Chemistry, 2012;18:7984-7999.

[23] Xu S, Xiao J, Yin F, Guo X, Xing L, Xu Y, Chong K. The Protein Modifications of *O*-GlcNAcylation and Phosphorylation Mediate Vernalization Response for Flowering in Winter Wheat. Plant Physiol, 2019;180:1436-1449.

[24] Benschop JJ, Mohammed S, O'Flaherty M, Heck AJ, Slijper M, Menke FL. Quantitative phosphoproteomics of early elicitor signaling in *Arabidopsis*. Mol Cell Proteom, 2007;6:1198-1214.

[25] Al-Momani S, Qi D, Ren Z, Jones AR. Comparative qualitative phosphoproteomics analysis identifies shared phosphorylation motifs and associated biological processes in evolutionary divergent plants. J Proteom, 2018;181:152-159.

[26] Weintz G, Olsen JV, Frühauf K, Niedzielska M, Amit I, Jantsch J,Mages J, Frech C, Dölken L, Mann M, et al. The phosphoproteome of toll-like receptor-activated macrophages. Mol Syst Biol, 2010;6:371.

[27] Haj Ahmad F, Wu XN, Stintzi A, Schaller A, Schulze WX. The Systemin Signaling Cascade As Derived from Time Course Analyses of the Systemin-Responsive Phosphoproteome. Mol Cell Proteom, 2019;18:1526-1542.

[28] Otto V, Schäfer E. Rapid Phytochrome-Controlled Protein Phosphorylation and Dephosphorylation in *Avena sativa* L. Plant Cell Physiol, 1988;29:1115-1121.

[29] Briskin DP, Leonard RT. Phosphorylation of the adenosine triphosphatase in a deoxycholate-treated plasma membrane fraction from corn roots. Plant Physiol, 1982;70:1459-1464.

[30] Briskin DP. Phosphorylation and dephosphorylation reactions of the red beet plasma membrane ATPase studied in the transient state. Plant Physiol, 1988;88:84-91.

[31] Rose ZB, Dube S. Rates of phosphorylation and dephosphorylation of phosphoglycerate mutase and biphosphoglycerate synthase. J Biol Chem, 1976;251:4817-4822.

[32] Tang W, Yuan M, Wang R, Yang Y, Wang C, Oses-Prieto JA, Kim TW, Zhou HW, Deng Z, Gampala SS, et al. PP2A activates brassinosteroid-responsive gene expression and plant growth by dephosphorylating BZR1. Nat Cell Biol, 2011;13:124-131.

[33] Park CJ, Caddell DF, Ronald PC. Protein phosphorylation in plant immunity: insights into the regulation of pattern recognition receptor-mediated signaling. Front Plant Sci, 2012;3:177.

[34] Minkoff BB, Stecker KE, Sussman MR. Rapid Phosphoproteomic Effects of Abscisic Acid (ABA) on Wild-Type and ABA Receptor-Deficient *A. thaliana* Mutants. Mol Cell Proteom, 2015;14:1169-1182.

[35] Yin J, Yi H, Chen X, Wang J. Post-Translational Modifications of Proteins Have Versatile Roles in Regulating Plant Immune Responses. Int J Mol Sci, 2019;20:2807.

[36] Delom F, Chevet E. Phosphoprotein analysis: from proteins to proteomes. Proteome Sci, 2006;4:15.

[37] Hargrove JL, Hulsey MG, Beale EG. The kinetics of mammalian gene expression. Bioessays, 1991;13:667-674.

[38] Lewin B. Genes VI. Oxford University Press; New Ed Edition, Oxford, 1997.

[39] Rawn JD. Biochemistry, Neil Patterson Publishers, Burlington, USA, 1989.

[40] Alberts B, Bray D, Lewis J, Raff M, Roberts K, Walter P. Molecular Biology of the Cell. 3rd ed. New York: Garland Science, 1994.