PREFACE

The book describes our important findings that when higher eukaryotic cells encounter uncomfortable intra- and/or extra-cellular environment change, in order to adapt for and/or eliminate it, they possess ability to gain new cell function through irreversible creation of chromatin structure plasticity with epigenetic modifications via a lot of generations. Outline of our results is concretely as follows.

In the chicken DT40 cell line, HDAC2 indirectly regulates transcriptions of IgM H- and L-chain genes through opposite regulations of gene expressions of Pax5, Aiolos, EBF1, OBF1, and Ikaros plus E2A. The HDAC2-deficiency in DT40 cells induces dramatic accumulations of mRNAs and proteins of IgM H- and L-chains. Thereafter these accumulated immunoglobulin mRNAs and proteins are gradually reduced in almost similar changing pattern during cultivation in all clones of HDAC2(-/-) DT40 mutants. By contrast, gene expressions of Pax5, Aiolos, EBF1 and OBF1 showed remarkably distinct changing patterns during cultivation in individual clones of HDAC2(-/-) DT40 mutants. At the late stage of cultivation, there exist at least three distinct manners for gene expressions of IgM H- and L-chains, i.e., OBF1-dependent, Pax5- and Aiolos-dependent, and Pax5-, Aiolos- and EBF1-dependent types. These distinct alterations in transcriptions of Pax5, Aiolos, EBF1 and OBF1 genes in individual clones of HDAC2(-/-) DT40 mutants should be originated from diversity of chromatin structure plasticity surrounding their proximal 5'-upstream regions. The chromatin structure plasticity should be irreversibly created through successive chromatin conformation (structure) changes based on varied changes in acetylation levels of particular Lys residues of histone H3 during cultivation in individual mutant clones. Based on these results, we clarified manners to diminish accumulated IgM H- and L-chains through irreversible creation of varied chromatin structure plasticity of particular transcription factor genes with acetylation and deacetylation during cultivation in individual clones of HDAC2(-/-) DT40 mutants.

Furthermore, we proposed a universal concept, which we named the chromatin conformation change code (4C) theory, for the bio-system to gain new cell function through irreversible creation of chromatin structure plasticity with epigenetic modifications via a lot of generations in higher eukaryotes. Outline of the 4C theory is concretely as follows. Somatic cells of higher eukaryotes are pluri-potent, elastic and flexible for gain of new cell function(s) through irreversible creation of chromatin structure plasticity, in order to adapt themselves for intra- and/or extra-cellular environment change. The pluri-potency, elasticity and flexibility of somatic cells for ability to gain new cell function(s) are fundamentally originated from those of chromatin structure. Plasticity of chromatin structure (loose or tight form) surrounding proximal 5'-upstream region of each of particular genes is created through irreversible conformation change with epigenetic modifications via a lot of generations (cell divisions). Chromatin structure of proximal 5'-upstream region, as just dynamic and changeable three-dimensional

conformation, possesses two fundamental abilities, i.e., to receive signal on environment change and to direct the switch (on or off) for latent transcription ability of the corresponding gene. Variety in irreversible creation of chromatin structure plasticity among individual cells should be triggered by the initial spontaneous unbalanced response to environment change and accomplished by the successive convergence of the response via numerous cell divisions. Thus, individual somatic cells can gain the same or different functions in distinct manners based on varied chromatin structure plasticity, even though they are the same cell type.

The 4C theory, which should open the door for gain of new cell function(s) of higher eukaryotes and innovate the general concept concerning somatic cells, is the typical fruit of my small serendipity and also the outcome of some 10 years of a small research group (of T. N. and M. N.). We had undertaken the research on the 4C theory, which made my heart beat fast with joy, under the worst research conditions, i.e., without any grants, in aperture of our other projects and by only a womanpower. While I was in active service, to my regret, all of data on the 4C theory could not been published as papers in journals and presentations in meetings. On the occasion of my thorough retirement, I had seriously started writing of rough drafts and manuscripts for this Book on the 4C theory in the form of original papers series. Therefore, I could not obtain additional data on the 4C theory any more. Anyhow, I have a tiny credit for the 4C theory, which is a creative concept on ability of higher living things, because all of the studies on it were originally started and achieved only by my small group. Finally, I earnestly crave that somebody should be interested in and take over the research on the 4C theory.

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Postscript:

The revision of the retirement commemorative monograph will be published and uploaded in 2018.

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Contents

Chapter 8. Personal closing remarks on the occasion of retirement: The real course and history of our researches prior to the 4C theory, personal interpretation on life phenomena, and experiences and opinions as a life scientist

by Tatsuo Nakayama

ABBREVIATIONS

Amp: ampicillin

ASF1: anti-silencing function 1

Blimp1: B lymphocyte-induced maturation protein-1

BSA: bovine serum albumin

CAF-1: chromatin assembly factor-1

4C machinery: chromatin conformation change complex machinery

4C theory: chromatin conformation change code theory

ChIP: chromatin immuno-precipitation

DAB: 3', 3'-diaminobenzidine

E2A: E box binding protein 2A

E, F, L or M: early, first, late or middle (stage of cultivation)

EBF1: early B cell factor 1

ECRR/ECRS: environment change recognition receptor/site

EDTA: ethylenediaminetetraacetic acid

GAPDH: glyceraldehyde 3-phosphate dehydrogenase

GCN5: general control non-depressible 5

HIRA: a homolog of S. cerevisiae transcriptional corepressors Hir1p and Hir2p

HAT: histone acetyltransferase

HDAC: histone deacetylase

HRP: horseradish peroxidase

Hyg: hygromycin

IgM Hc, Hm or Hs: whole, membrane-bound or secreted form of IgM H-chain

K9/H3, K14/H3, K18/H3, K23/H3 or K27/H3: Lys-9, Lys-14, Lys-18, Lys-23 or Lys-27 residue of histone H3

NotchIP: neighboring overlapping tiling chromatin immuno-precipitation

NHS: normal horse serum

OBF-1: origin binding factor-1

Pax5: paired box gene 5

PB: phosphate buffer

PBS: phosphate buffered saline

PCAF: p300/CBP-associated factor

PCR: polymerase chain reaction

PMSF: phenylmethylsulfonyl fluoride

PU.1: purine box factor 1

RT-PCR: reverse transcription-polymerase chain reaction

SDS: sodium dodecyl sulfate

SDS-PAGE: SDS-polyacrylamide gel electrophoresis

TB: Tris-HCl buffer (pH 7.4)

TCA: trichloroacetic acid

TE: 10 mM Tris-HCl, 1 mM EDTA (pH 8.0)

Tet: tetracycline

TFC machinery: transcription factor complex machinery

2D-PAGE: two-dimensional polyacrylamide gel electrophoresis

W: wild-type (DT40 cells)

XBP-1: X-box binding protein-1