CHAPTER-IV

Retention and transmission of active transcription memory from progenitor to progeny cells via ligand-modulated transcription factors: elucidation of a concept by a hypothetical model
INTRODUCTION

A characteristic gene expression profile creates and sustains cellular traits and cell types. In this context, the presence of a combination of transcription factors, with each one modulating hundreds of responsive genes, are crucial determinants in maintaining cell-specific proteome. Amongst the transcription factors, the Nuclear Receptor (NR) super-family is a large group of ligand-modulated transcription factors with 48 members presently identified in human genome. These transcription factors are implicated in numerous physiological processes, and have been prospective therapeutic targets for treatment of many critical diseases including hormone-related cancers (Evans, 1998; Escriva et al., 2004; Shank and Paschal, 2005; Kumar et al., 2006; McEwan, 2009). In view of the fact that ligand-induced movements and localization of transcription factors is one of the major phenomena for regulating the transcriptional activity, fluorescent protein (FP)-tagged NR chimera have been consistently used for studying their dynamic behavior in living cells (Shank and Paschal, 2005; Kumar et al., 2006; Griekspoor et al., 2007).

In principle, all intracellular NRs when bound to their agonist are nuclear and transcriptionally active. Results obtained from FP-tagged receptors expressed and imaged in live cells have shown that agonist-bound receptors reorganize in the nucleoplasm into hundreds of discrete fluorescent speckles commonly referred to as ‘nuclear foci’ (Stenoien et al., 2000; Tyagi et al., 2000; Baumann et al., 2001; Tomura et al., 2001; Karvonen et al., 2002; Saitoh et al., 2002; Black et al., 2004; Black and Paschal, 2004; Song and Gelmann, 2005; Kumar et al., 2006; Amazit et al., 2007; Arnett-Mansfield et al., 2007; Griekspoor et al., 2007; Klokk et al., 2007). However, receptors bound to pure antagonists are also mostly nuclear but remain homogeneously distributed in the nucleoplasm and are neither transcriptionally active nor form such nuclear foci. A typical example of a nuclear receptor exhibiting this ligand-mediated distribution profile is evident in Figure 38. NR-mediated gene expression is modulated by a multitude of accessory proteins that facilitate the
assembly of a functional transcription pre-initiation complex. These include p160 family of coactivators, which interact selectively with the agonist-bound form of NR. In turn, the p160 family members act as molecular scaffolds that attract the enzymes and factors necessary for chromatin modification and remodeling (Stenoien et al., 2000; Baumann et al., 2001; Karvonen et al., 2002; Black et al., 2004; Black and Paschal, 2004; Song and Gelmann, 2005; Amazit et al., 2007; Arnett-Mansfield et al., 2007; Klokk et al., 2007). Notable among the modifying enzymes are the histone acetyltransferases like cAMP response element binding protein (CREB)-binding protein (CBP) / p300 and a few more like SRC-1. Subsequent co-expression studies of NRs with the p160 family members, p300 and some of the co-activators or basal transcription factors like steroid receptor coactivators-1 (SRC-1), glucocorticoid receptor interacting protein-1 (GRIP1 / TIF2 / SRC-2), SRC-3 and CBP, etc. have shown that these factors co-localize in ‘nuclear foci’ with agonist-activated NRs (Stenoien et al., 2000; Baumann et al., 2001; Tomura et al., 2001; Karvonen et al., 2002; Saitoh et al., 2002; Black et al., 2004; Black and Paschal, 2004; Song and Gelmann, 2005; Kumar et al., 2006; Amazit et al., 2007; Arnett-Mansfield et al., 2007; Klokk et al., 2007). Thus, agonist-generated ‘nuclear foci’ in interphase cells have been suggestive of multi-protein complexes that are required for modulation of expression of ligand-responsive genes. Furthermore, emerging evidences are indicating that some of the major cellular proteins having direct or indirect roles in gene transcription regulation remain associated with condensed chromatin during mitosis (Mo and Beck, 1999; Tang and Lane, 1999; Dey et al., 2000; Chen et al., 2002; Christova and Oelgeschläger, 2002; Dey et al., 2003; Pallier et al., 2003; Harrer et al., 2004; Burke et al., 2005; Saradhi et al., 2005; Xing et al., 2005; Das et al., 2006; Velasco et al., 2006; Kobayashi et al., 2006; Yan et al., 2006; Carrierre et al., 2007; Young et al., 2007; Cherukuri et al., 2008; Blobel et al., 2009; Verdeguer et al., 2010). These include CCAAT/enhancer-binding protein, topoisomerase II, TATA binding proteins (TBP), transcription factor IID (TFIID), mitotic chromosome-associated protein
Figure 38: Typical intracellular dynamics of androgen receptor in living cells during interphase and mitosis. Mammalian cells (COS-1) were transiently transfected with GFP-AR and cultured in steroid-free medium for 24 hours. Following receptor expression the cells were treated with DMSO:ethanol (vehicle) for control cells or with 5α-dihydrotestosterone (DHT) or with pure anti-androgen. In interphase cells, unliganded GFP-AR remains partitioned in the cytoplasmic compartment. Upon binding with androgen or agonist GFP-AR enters nucleus and forms ‘nuclear foci’ and also associates with mitotic chromosomes during mitosis. However, when bound to pure antagonists the receptor is homogeneously distributed in the nucleoplasm, marked by the absence of ‘nuclear foci’ and, fails to associate with the mitotic chromosomes during cell division. Receptor localization in nucleus with formation of ‘nuclear foci’ indicates transcriptionally active state of receptor and homogenous localization in nucleus implies transcriptionally inactive state of receptor. Mitotic cells (in metaphase) shown here are merged images of GFP-AR and Hoechst-DNA with green and blue fluorescence respectively.

(MCAP), chromosome-associated protein (MCAP), bromodomain protein; high mobility-group protein (HMGB1, HMGB2, HMGA1a, HMGN), heat
shock transcription factor 2 (HSF2), upstream binding factor (UBF), RNA polymerase I, insulator protein CTCF, coactivator PC4, transcription factors FoxI1, Runx2, Hepatocyte nuclear factor-1β (HNF-1β), Mixed lineage leukemia (MLL), interlukin-33 (IL-33), BS69, calreticulin, etc. Concurrently, docking of some NR members (androgen, estrogen, pregnane and xenobiotic receptors) onto the condensed chromosomes during mitotic stages is also reported (Saradhi et al., 2005; Kumar et al., 2008). More interestingly, results from live cell imaging revealed that agonist-induced 'nuclear foci' formed during interphase co-migrate with condensing chromatin and are clearly visible during early stages of mitosis. However, after metaphase the ‘nuclear foci’ engulfed during chromatin condensation are not resolved as discretely probably due to extreme compaction of chromatin (Kumar et al., 2008). Though fewer, there are evidences to suggest that even during chromatin condensation, some target gene promoters remain exposed and accessible to interacting proteins (Christova and Oelgeschläger, 2002; Burke et al., 2005; Chen et al., 2005; Xing et al., 2005; Yan et al., 2006; Young et al., 2007; Blobel et al., 2009; Verdeguer et al., 2010). Conceivably, presence of promoter encompassing ‘pits’ within condensed mitotic chromatin and dynamic docking of transcription factors into these pits should have important physiological ramification.

**Biopit versus Epigenetics: two sides of the same coin**

In contemporary biology, epigenetic regulatory processes govern chromatin structure and gene expression through covalent modification of DNA, RNA and histone substrates via methylation, acetylation, phosphorylation or ubiquitination processes that are collectively termed as ‘epigenetic markings’ (Goldberg et al., 2007; Kouzarides, 2007). In simple analogy to these epigenetic regulatory processes, ‘biomolecular markings’ by ligand-activated transcription factors can also be broadly defined as an alternate mechanism utilized by proliferating cells in transmitting the pattern of active gene transcription from interphase nucleus, via mitosis, to the emerging progeny cells. However, cell
transverse mitosis in apparently transcriptionally silenced state. Ensuing events appear to imply that cells inherit only a biomolecular blueprint of active transcription status with transcription factors associated with mitotic chromatin while some modulatory factors (GRIP-1, SRC-3 etc.) aborting the transcription complex (Kumar et al., 2008). Operation of such a mechanism, therefore, is expected to ensure ideal transmission of an exclusive blueprint of active transcription status over to next generations that help in immediate initiation of an identical gene expression profile for sustenance of characteristic cellular proteome.

With respect to ligand-modulated transcription factors, this feedforward transmission over the generations, by receptors themselves, may also encounter active transcription pattern modifications depending on appearance and disappearance of physiological ligands during developmental stages or ageing processes. However, under normal cell proliferation conditions retention and transmission of biomolecular imprints of active genes from the progenitor to progeny is expected to help in maintenance of the inherent cellular characteristics and cell phenotype. For example, when working with cultured cell lines, experience has shown that a population of breast/prostate cancer line even after passaging for a long time will always emerge as itself rather than any other cell or tissue type. To distinguish from ‘epigenetic marking’ the act of ‘Biomolecular Imprints Offered to Progeny for Inheritance of Traits’ via transcription factors themselves can be explained by ‘Biopit Model’ or by their ‘act of BIOPIT-ing’ (Figure 39 and 40). The ‘Biopit Model’ is demonstrated in Figure 39A by citing an example of an NR represented by unliganded and ligand-activated androgen receptor in live cells. Subsequently, the phenomenon is vividly explained by a schematic cartoon in Figure 39B. Support to the model is apparent from the observation made with some of the major NRs (AR, ERα, PXR) (Saradhi et al., 2005; Kumar et al., 2008) and other transcription factors belonging outside the NR super-family (Chen et al., 2002; Christova and Oelgeschläger, 2002; Burke et al., 2005; Yan et al., 2006; Young et al., 2007).
Figure 39: Retention and transmission of active transcription memory from progenitor to progeny cells via ligand-modulated transcription factors. (A) Images show agonist-mediated generation of 'nuclear foci' and their migration with the condensed chromosomes during all the stages of mitosis. COS-1 cells were transiently transfected with GFP-AR and incubated in steroid-free medium for 24 h. Following receptor
expression the cells were treated with either DMSO:ethanol (vehicle) for control cells or with natural ligand 5α-dihydrotestosterone (DHT). After cultured period, live cells expressing GFP-AR were recorded by fluorescence microscopy. Hoechst (DNA stain) was used as a fluorescent dye to visualize corresponding nuclei/condensed chromosomes. Upper row (-H) shows unliganded AR that does not associate with the condensed chromosomes during mitosis. Middle row (+H) shows DHT-activated AR that binds to the condensed chromosomes during all the stages of mitosis. The figure also indicates the migration of ‘nuclear foci’ from interphase to mitotic stages exemplifying the retention and transmission of active cellular memory from progenitor to progeny cells during cell division by DHT-bound AR. The lower row (+H') shows a typical distribution of the magnified ‘nuclear foci’ formed by DHT-bound GFP-AR in interphase and on mitotic chromatin. The interphase cells and the mitotic stages of the naturally dividing cells are indicated. ‘H’ indicates natural ligand 5α-dihydrotestosterone (DHT). Figure 39B is the portrayal of ‘Biopit model’ to elucidate the retention and transmission of ‘nuclear foci’ as transcription memory packets formed by ligand-modulated transcription factors. In interphase nucleus, agonist-activated NR (green dots) interacting with co-activators (yellow dots) give rise to transcriptionally competent ‘nuclear foci’. With the onset of mitosis, interaction of NR with specific co-activators in ‘nuclear foci’ begins to diminish with co-activators leaving the transcription complex while the NR remaining associated with the condensing chromatin from prophase to cytokinesis. Post-mitosis, with the emergence of daughter cells, the NRs pre-occupying the chromatin can promptly recruit the dislodged components of transcription machinery again and return to the inherited active gene transcription status. Association of NRs with condensed mitotic chromatin acts as ‘biopit’ to execute the retention and transmission of transcription memory. Furthermore, such association provides a platform for timely recruitment of the components of transcription machinery and co-regulatory factors to reestablish progenitor’s active transcription memory. Thus, association-dissociation and re-association of accessory components of transcription machinery helps in retention and transmission of active transcription status from progenitor to progeny. For the purpose of clarity in depiction of mitotic stages, some of the finer details and number of chromosomes have been abridged.

The prediction at molecular level for Biopit model is presented in Figure 40. We have tried to make prediction how this might be occurring at the molecular level. In the light of our findings, it is reasonable to hypothesize that AR reside on androgen response elements (ARE) of AR target genes. The model is not meant to imply that no nucleosomes are present on the promoters in mitotic cells, only that these regions are less compacted than most of the genomic DNA at this stage of the cell cycle.
Figure 40: A hypothetical 'biopit model' depicting the retention and transmission of transcription memory from progenitor to progeny. Epigenetic marks (Me, Ac etc.) and biopit marks (by NRs) may be transmitted simultaneously for programming the cell traits and fate.
Implications of eradication of ‘Biopit Markings’

Based on the concept of ‘Biopit Model’ we can explain how therapeutic drugs and endocrine disruptors that target NRs may actually alter the inheritance of cell’s transcription memory leading to unwarranted physiological consequences. Conceivably, during anti-hormone therapy (as in case of breast/prostate cancer) or consistent exposure to hormone mimicking endocrine disruptors can alter the natural Biopit-ing process over many generations that may inflict upon target cells to undertake alternative survival strategies (Tyagi, 2003; Kumar et al., 2008). Such consistent chemical pressures may lead to emergence of a new genre of cells that will trounce the inflicted chemical stress and thereafter proliferate uninterruptedly. In this perspective, recurrence of advanced stages of cancers from hormone-dependent to hormone-independent phase when undergoing anti-hormone therapy may be a consequence of inflicted turbulences in natural Biopit-ing process. In brief, the present ‘Biopit Model’ is expected to offer a novel paradigm in explaining the concerted action and consequences of ligand-receptor interactions in the perspective of normally sustained physiology and clinically aberrant situations.

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