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Combined multicolor-FISH and immunostaining

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Abstract. The combination of multicolor-FISH and immunostaining produces a powerful visual method to analyze in situ DNA-protein interactions and dynamics. Representing one of the major technical improvements of FISH technology, this method has been used extensively in the field of chromosome and genome research, as well as in clinical studies, and serves as an important tool to bridge molecular analysis and cytological description. In this short review, the development and significance of this method will be briefly summarized using a limited number of examples to illustrate the large body of literature. In addition to descriptions of technical considerations, future applications and perspectives have also been discussed focusing specifically on the areas of genome organization, gene expression and medical research. We anticipate that this versatile method will play an important role in the study of the structure and function of the dynamic genome and for the development of potential applications for medical research.

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The development of various FISH technologies has revolutionized the field of chromosomal and genome research (Heng et al., 1997; Bayani and Squire, 2002; Beatty et al., 2002; Liehr and Claussen, 2002; Beatty and Heng, 2004; Speicher and Carter, 2005). Among the many significant technical improvements that are currently in use are the following: suppression hybridization (Cremer et al., 1988; Lichter et al., 1988; Pinkel et al., 1988), high resolution fiber FISH (Heng et al., 1992; Parra and Windle, 1993), simultaneous multicolor-FISH including spectral karyotyping (SKY) and multiplex-FISH (Ried et al., 1992; Schrock et al., 1996; Speicher et al., 1996), comparative genomic hybridization (CGH) and array CGH (Kallioniemi et al., 1992; Solinas-Toldo et al., 1997; Pinkel et al., 1998), and multicolor DNA-protein in situ co-detection (Heng et al., 2001a; Ye et al., 2001), all of which represent major advances of the past two decades. In this short review, combined multicolor-FISH and immunostaining, which is one form of DNA-protein in situ co-detection, will be discussed briefly with the emphasis on its applications and perspectives.

Methodology development

Early applications of DNA-protein simultaneous detection can be classified into basic and clinical research categories. For chromosome structure studies, in situ DNA-protein co-detection was limited to the co-localization of centromer-

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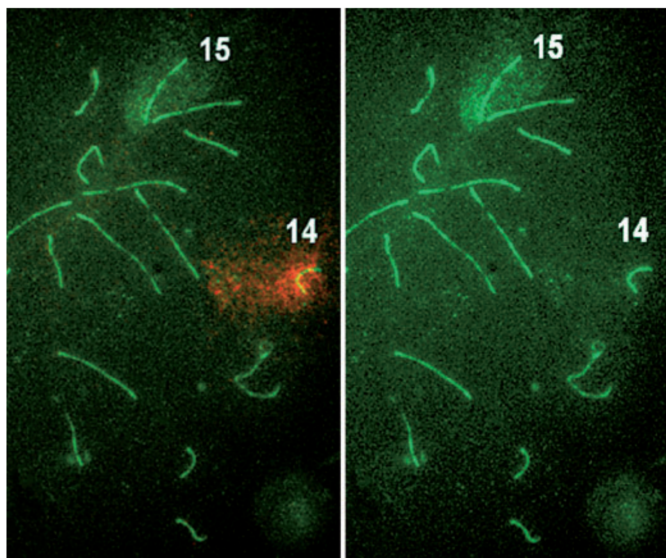

Fig. 1. Example of two-color FISH and immunostaining on mouse meiotic chromosomes. Left panel: chromosomes 14 and 15 were detected as red (rhodamine) and green (FITC) by direct labeled chromosome-specific paints. The synaptonemal complex (SC) is highlighted by the antibody for SC-specific proteins and detected by FITC. This image was captured using a dual-color filter. Right panel: represents the same image captured using the FITC filter. With the identification of chromosomes 14 and 15, the length of the SCs of these two chromosomes can be precisely compared. To generate this image, chromosomal spreads were first immunostained with the SC antibody then FITC-detected slides were subjected to two-color FISH detection with direct labeled chromosomal paints.

ic DNA sequences and the signals of anti-centromere sera (CREST) along chromosomes (Masumoto et al., 1989; Haaf et al., 1992; Page et al., 1995). For clinical related studies, simultaneous detection was achieved by co-detection of cell membrane antigens and specific DNA targets (Van den Berg et al., 1991), and was termed FICTION (fluorescence immunophenotyping and interphase cytogenetics as a tool for the investigation of neoplasmas) referring to the specific application of this technology (Baurmann et al., 1993; Weber-Matthiesen et al., 1993). Since then, the combination of FISH and immunostaining has emerged as a main experimental approach for studying the structure and function of chromosomes, particularly meiotic chromosomes (Fig. 1) (Heng et al., 1994). In these studies specific DNA fragments painted by FISH signals were detected along the protein core of mouse meiotic prophase chromosomes that were highlighted by immunostaining of anti-core antibodies. By comparing the structural features of different types of inserted foreign and endogenous sequences, the characteristic patterns of meiotic loops have been comparatively analyzed, revealing that meiotic chromatin loops appear to be associated with 'anchor sequences' that function in the formation of chromatin loops. In a similar manner, the interactions of satellite DNA arrays and centromeric protein were visualized, demonstrating that the differential binding of centromeric protein to similar sequences of satellite DNA arrays were related to functional requirements based on the binding pattern (Haaf and Ward, 1994). This straightforward but powerful approach was also used by a number of research groups (Hunt et al., 1995; Barlow and Hulten, 1996; Scherthan et al., 1996; Moens et al., 1997; Fukagawa et al., 1999; Heng et al., 2000; Hiatt et al., 2002; Craig et al., 2003; Hudson et al., 2003; Trelles-Sticken et al., 2003; Hassold et al., 2004; Kolas et al., 2004).

To date, these analyses have generated significant data sets that have elucidated a variety of phenomena including the demonstration that the size of meiotic chromatin loops is related to their position along the chromosomal core (Heng et al., 1996, 1997), that a discrete X-recognition element can distinguish the X chromosome from autosomes to recruit the dosage compensation complex (Csankovszki et al., 2004), and that active genes can share sites of ongoing transcription (Osborne et al., 2004). However, most studies used single color FISH applied with protein co-detection, which does not differentiate between chromosomes or chromosomal regions.

The first multicolor-FISH and immunostaining methodologies were introduced in 2001 by the combination of SKY and synaptonemal complex (SC) protein detection (Fig. 2) (Heng et al., 2001a; Ye et al., 2001). This technology was developed to precisely identify each meiotic prophase chromosome so that its SC could be measured precisely. SC proteins were detected by anti-SYCP1 and/or SYCP3 primary antibodies tagged with either FITC, rhodamine or gold particle-conjugated secondary antibodies following SKY detection. In addition to demonstrating the usefulness of this method in examining the various stages of meiosis, this study unexpectedly revealed an inconsistency in the size of mitotic and meiotic chromosome lengths that allowed for a systematic analysis of the relationship between the length of meiotic chromosomes, GC content and the genetic recombination rate (Heng et al., 2001a; Heng et al., in preparation). A few other groups soon used either SKY or M-FISH to examine human and/or mouse meiotic chromosomes (Froenicke et al., 2002; Lynn et al., 2002; Tease et al., 2002; Hassold et al., 2004; Sun et al., 2004). In clinical research, multicolor interphase detection coupled with immunophenotyping was also used (Martin-Subero et al., 2002). This wave of studies has established the covariation of the synaptonemal complex length and the mammalian meiotic recombination rate, and has finally settled the long debated issue of the inconsistencies between mitotic and meiotic length of chromosomes. Other modifications to this line of research combined multicolor subcentromere-specific probes with SC or SC-associated proteins (Codina-Pascual et al., 2004).

This short review will cite data based on various species rather than only focus on humans due to the fact that this methodology has been successfully applied to a number of species, and there is no significant technical difference among the research subjects. This will provide a comprehensive view of this methodology and its potential applications for human cytogenetics.

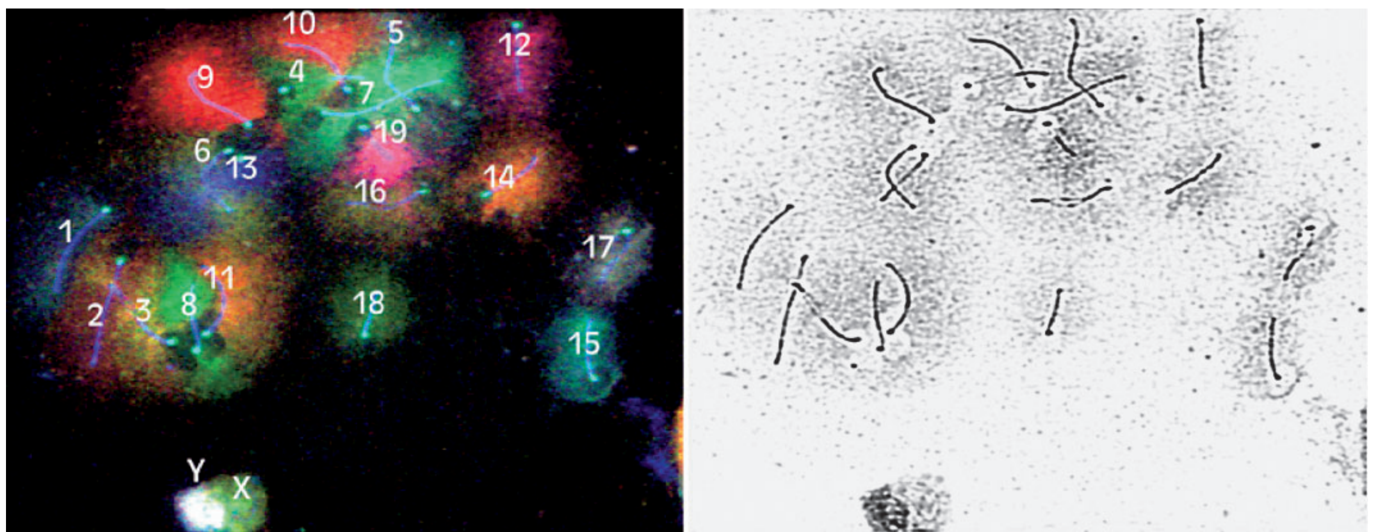

Fig. 2. Example of combined multicolor-FISH and immunostaining on mouse meiotic chromosomes. Left panel: the multicolor was achieved by using spectral karyotyping, a form of multicolor-FISH that assigns each chromosome a unique color. The chromosomal identification as indicated by numbers was achieved by spectral karyotyping analysis using SKY imaging software. Both SCs and centromeric regions were highlighted by antibodies specific to the SC (blue color) and the centromere (bright green color), respectively. Right panel: the image of SCs of the same meiotic figures taken with the DAPI filter. To generate this image, chromosomal spreads were first immunostained with antibodies for SCs and centromeres, and then subjected to SKY detection.

The significance of using DNA-protein in situ co-detection

The combination of multicolor-FISH and immunostaining represents an advanced form of DNA-protein in situ co-visualization. In general, the approach of in situ visualization is important for the following major reasons:

This combined approach fills the gap between molecular analysis and cytological description. The advantage of this technique is that it provides an understanding of timing and cytological location of an event and can answer questions of 'when' and 'where', in addition to molecular analyses that provide an understanding of specific molecular mechanisms that answers the questions of 'what' and 'how'. To date, most of the molecular analyses are based on in vitro isolation and manipulation, and it is fundamentally important to re-examine these non-cell- or non-tissue-based analyses at the tissue or cellular level. In particular, more and more researchers have discovered the complexity of multiple pathways in biological processes, the chromosomal territory (Cremer and Cremer, 2001), and the dynamic nature of chromosome and chromatin behavior (Heng et al., 2001b, 2004a; Claussen, 2005). Obviously, since conditions in vivo are not necessarily the same as those in vitro, direct examination of the structures and the processes in situ rather than in the extracted form is essential.

The combination of multicolor-FISH and immunocytology provides information at the level of the individual cell as well as at the tissue level. Such data is key in addressing the issue of genetic and physiological heterogeneity within a population of cells. In contrast, typical biochemical methodologies are based on conventional DNA, RNA or protein extraction methods and produce information on the 'average' profiles of cell populations. Heterogeneity can be detected at multiple levels including the different genomic compositions of each cell, variations within the cell cycle, different degrees of stochastic gene regulation and differential responses to the same environmental challenges (Becskei et al., 2005). To truly analyze the profile of a particular cell population, one must collect data from individual cells as well as from large numbers of cells that will accurately reflect patterns inherent to the population. Such data can easily be acquired from in situ visualization. Such analyses circumvent the problem of 'not seeing the forest for the trees' and individual cell and population patterns can be correctly integrated.

Recently, our laboratory has demonstrated that large-scale stochastic genomic aberrations (such as non-clonal chromosome aberrations or NCCAs) can only be detected by monitoring individual cells in a cell population during the process of cancer initiation and progression. Stochastic aberrations occur during the very early stages of cancer progression and it is these types of aberrations that have escaped conventional detection. As a result, the importance of NCCAs has been largely ignored. We have traced the cancer evolutionary pattern using individual cell karyotypes from representative populations and recently demonstrated that these previously disregarded aberrations are the key to understanding the heterogeneity that underlies cancer initiation and progression (Heng et al., 2004b, 2006). Our findings have demonstrated the importance of individual, cell-based analysis as a key consideration for the use of in situ visualization methodologies.

The benefits of using combined multicolor-FISH and immunostaining is also profiled in recent meiotic chromosomal research. Until now, a major challenge has been to precisely identify individual chromosomes during meiosis, particularly those of similar size and arm ratios. Thus only a limited number of cells have been examined using the classic section-

ing and reconstruction method to identify individual chromosomes (Holm and Rasmussen, 1977). By using multicolor chromatin painting to identify chromosomes, the length of both mouse and human meiotic prophase chromosomes was systematically measured based on the length of the SC detected with fluorocore-conjugated SC antibody (Fig. 1) (Heng et al., 2001a; Froenicke et al., 2002; Lynn et al., 2002; Tease et al., 2002). Furthermore, the co-detection of recombination proteins such as MLH1 served as markers that allowed scoring of recombination events. Thus, the frequency and distribution patterns of recombination proteins have been used to construct cytological recombination maps (Froenicke et al., 2002; Sun et al., 2004).

With this approach providing a higher precision to chromosome identification, analysis of the variation of MLH1 foci number along each chromosome and among different individuals revealed remarkable variation in recombination rates within and among individuals (Lynn et al., 2002). DNA-protein co-detection has been used to illustrate the structural basis of these variations, and in particular was used to analyze the mechanism of inconsistency between mitotic length (physical length defined by the number of nucleotides) and meiotic length (defined by recombination rates). The method has also been used to analyze the meiotic chromatin loop size from representative regions of the mouse genome, revealing that loop size is linked to the SC length, GC content and to the recombination frequency (Heng et al., 2004c).

In addition to the study of the cytological recombination maps, combined FISH and immunostaining can be effectively used to study the chromosomal pairing process. Using the ATM(–/–) mouse model, the homologous alignment and synapsis of mouse meiotic chromosomes were analyzed. As expected, in normal mice, homologous chromosomes align with each other and form an SC. Surprisingly, in ATM(–/–) mice, painting with chromosome-specific paints indicates that the overall chromatin alignment appears to be normal. However, when smaller chromosomal regions were targeted rather than the entire chromosome, the meiotic chromosome profile of an ATM(–/–) mouse had a significantly different pattern from the normal mouse showing that there is no precise homologous sequence alignment in ATM(–/–) nuclei. This suggested that chromosomal alignment and sequence-specific alignment may be separate events (Heng et al., 1998). Similar observations in SYCP3(–/–) mice suggests that the overall alignment is not a function of core components but might be mediated by chromatin-chromatin interactions (Kolas et al., 2004). We anticipate that sequence-specific alignment requires a functional chromosome core.

Technical considerations

Reliable protocols for FISH detection are well established and easy to follow as are the protocols for protein immunostaining (Lichter et al., 1990; Heng and Tsui, 1993; Haaf, 1995; Weise et al., 2003; Beatty and Heng, 2004; Gutierrez-Mateo et al., 2005). For simultaneous co-detection of DNA and protein, the quality of antibodies and the size of both the DNA and protein targets become factors of concern. In general, larger DNA and protein complexes make the co-detection easier. For this reason, abundant centromeric proteins and SC proteins are most often used in DNA-protein in situ co-detection. Since optimal conditions for producing the strongest signals of FISH and immunostaining sometimes conflict with each other, some technical compromise is often needed to obtain good signals of both DNA and protein (Heng et al., 2000). For example, considerations that depend on what will be emphasized are: the choice of fixation, the order of detection (detect DNA first or protein first), the choice of fluorocores (assign a stronger color to a weaker partner in a given detection system), and the choice of signal amplification (to strengthen signals of a weaker partner or both partners).

Fixation: In DNA-protein co-detection, paraformaldehyde fixation is often used in order to maintain the natural configurations for protein detection. On the other hand, the 3:1 fixation is more suitable for FISH detection as it generates the chromosomal morphology that results in strong FISH signals.

Order of detection: Since the antigen-antibody complex is much more stable than the hybridization complex, the antibody detection of protein should be performed prior to FISH detection that requires a denaturation step (Figs. 1 and 2). In addition, it is always preferential to detect proteins before the denaturation step in order to maintain the specific configuration required for antibody detection (Heng et al., 2001a; Ye et al., 2001). However, the order of detection can be reversed if it is necessary to enhance a weaker partner.

Intensity of antibody detection for proteins: For SKY-protein co-detection, optimal results are obtained for both DNA and protein when the signals of protein detection are not too strong to interfere with the color of SKY chromosomal identification. When the protein color is too strong, regardless of whether it is green or red, the strong color causes a color shift in the SKY detection. This problem can be resolved if multiple filters are used to record the signals for protein and DNA separately.

By using combinations of various fluorocores and antibodies generated in different host species (rabbits, mouse, etc.), multiple color detection of various targets can easily be achieved. In particular, simultaneous detection of multiple DNA targets with different color combinations can be used, provided direct labeling and detection is utilized. Re-hybridization with probes tagged by new fluorocores can be easily done, as direct labeling and visualization of DNA probes without the antibody is easily removed by the denaturation procedure and does not destroy the signals of the protein complex highlighted by antibodies prior to FISH detection (Heng et al., 2003). Thus, DNA detection can be performed multiple times by changing the probes for each additional hybridization.

The limitation of this method is the sensitivity of both protein and DNA detection determined by the size of the targets and the quality of antibodies and DNA probes. In general, if both FISH and immunostaining work well separately, then the combination should also work well. Thus, a pre-test of both FISH probes and protein antibody, respectively, is recommended for any new combination of detection that is

considered. If they work separately, then adjusting the factors that we mentioned previously should result in successful co-detection.

Applications and perspectives

The methodology of multicolor-FISH and immunostaining is relatively simple and easy to perform when both the DNA and protein targets are detectable in separate experiments. The beauty of this methodology lies in its versatility to analyze very difficult biological problems not amenable to traditional molecular analysis. Many examples can be found in the literature although only a limited number of color applications have been utilized to date. Due to the great potential of this technology, we would like to highlight some research areas that could benefit greatly from the application of this technology.

Genome organization: The different levels of genome organization can be analyzed by a variety of visualization technologies (Heng et al., 2004b). The dynamics of proteins and specific DNA/chromosomal regions can be studied from the macro-molecular DNA-protein complex level to the chromatin loop domain, and from the specific chromosomal territory to the entire genome. The interaction of DNA-protein can be visualized by focusing on specific structural sequences – the loop anchor, imprinting areas, or duplicated regions. It has been demonstrated that various regions of the chromosome can be 'bar-coded' with different colors (Lengauer et al., 1993; Muller et al., 1997). A similar approach could thus highlight smaller chromosomal regions with less condensed chromosomes or chromatin fibers (Heng et al., 1992), particularly when these are co-detected with specific protein complexes. Currently, up to 48-colors have been reported for differential painting of chromosomal arms (Wiegant et al., 2000; Karhu et al., 2001). It has been further suggested that unlimited colors can be applied in chromosome painting (Muller et al., 2002). Rather than push for the development of unlimited color applications for DNA detection, it may be more important to develop more color applications for the detection of proteins. The ability to simultaneously detect a greater number of proteins with various fluorocores would certainly be a significant improvement for multiple-color DNA-protein co-detection. Such assignation of different colors in protein detection should be achievable with the current SKY or M-FISH platforms.

One level of genome organization is the condensed chromosome. It is known that condensins and other associated proteins are responsible for chromosomal condensation (Hirano, 2005). Recently, a phenotype of condensation defects has been renamed as defective mitotic figures or DMFs (Heng et al., 2004b, 2006). The co-existence of condensed chromosomes and uncondensed chromatin fibers in DMFs offers an opportunity to dissect the condensation process and to identify the dynamic nature of condensation proteins. Interestingly, elevated frequencies of DMFs have been detected in many types of cancers both from cell lines and primary tumors. With the use of SKY analysis, our preliminary data indicates that there is an order to chromosomal packaging and it appears that different cancer cell lines may display different chronological orders. We anticipate that many questions can be addressed with the use of multiple-color DNA-protein co-visualization to focus on the transitional regions of DMFs during the condensation process. Such studies may identify the proteins involved in packaging-specific regions of the genome, as well as the order of protein binding on DNA or chromatin that initiates the formation of the condensation complex.

A related topic is the study of patterns of viral integration. From an evolutionary point of view, many human sequences are the result of foreign viral genome integration. For example, many human repeat-sequences are derived from retroviruses. Viral vectors have also been extensively used as tools in gene therapy. It would be interesting to study the patterns of integration, particularly in the context of the integration process and associated proteins. The chromosomal integration and association patterns of various types of viruses have been reported (Hilger-Eversheim and Doerfler, 1997; Cotter and Robertson, 1999; Omori et al., 1999). Traditionally, the focus of analysis of the integration pattern has been at the chromosomal level. Recently, we have studied the dynamics of viral infection and integration using FISH detection of virus DNA. Surprisingly, a high degree of heterogeneity has been observed among cells that are defined as a positively infected cell population. Under the same conditions of infection, the majority of cells were virus free while a minority contained large numbers of virus. Clearly not every cell is equal when facing a viral infection (Heng et al., unpublished observation). Further, viral association and integration can be detected in specific regions of the genome. For example, following extraction of non-matrix nuclear proteins, the nuclear matrix and released chromatin loops can be visualized. Interestingly the integration patterns of a number of viruses differed in location. While some can be detected in both the nuclear matrix and loop areas, others are detected only in the nuclear matrix but not the loop portion in DNA Halo preparations (D'Ugo et al., 1998; Heng et al., unpublished observation). Our data suggests that not every portion of the genome is equal with respect to integration. In depth studies are needed to follow up these interesting observations and to define the involvement of the protein complex in this process. The multiple-color DNA-protein co-detection method can be used to monitor the entire process of viral infection, host nuclear association and DNA integration.

Gene expression: Extensive research has been done on gene expression. To date, most approaches have been biochemical analyses based on the average profile of a mixed cell population rather than specific individual cells. With the development of in vivo visualization technologies, a new trend is to focus on individual cells in a given cell population and to analyze them in vivo or in situ. Reliable methods to trace both DNA and protein simultaneously are in great demand. By using the DNA-protein co-visualization method, it has been shown that DNA sequences containing the Gypsy insulator overlap with nuclear sites where insulator proteins aggregate to form insulator bodies. In addition, the DNA sequence normally located inside the nucleus has been shown to have

moved to the periphery when the Gypsy insulator was placed within these sequences. This study suggests that global changes in transcription are accompanied by alterations in the distribution of insulator proteins and DNA (Gerasimova et al., 2000).

Similarly, the correlation between the nuclear distribution of specific genes and their expression status has been illustrated using DNA halo preparations. Further, the co-localization of the Myc gene and SATB1 protein (a cell type-specific nuclear protein that recruits chromatin-remodeling factors and regulates many genes during thymocyte differentiation) showed that the Myc upstream site was anchored to the SATB1 network, indicating that the SAT1 network contributes to gene expression-related chromatin folding (Cai et al., 2003). By tracing specific DNA fragments, it has recently been demonstrated that loop dynamics are an important feature that correlates with gene regulation (Heng et al., 2004a). A model of S/MARs-mediated loop dynamics and gene expression regulation has been proposed focusing on the chromatin loop domain. According to this model, transcription machinery can be shared by many genes and is mediated by the S/MAR sequences (Bode et al., 2003; Heng et al., 2004a). Follow-up studies are needed to directly test this model, particularly by incorporating information from the protein complex that forms the transcription machinery. Interestingly, DNA-protein co-detection has been used to co-localize different genes of interest and RNAPII. Various transcribed genes often occupy the same transcription factory. Combined with other types of elegant analysis including RNA-FISH, issues such as the dynamic nature of genes, shared nuclear sub-compartments and the status of transcription have been analyzed. This study suggested that active genes migrate to preassembled transcription factories rather than recruiting and assembling transcription complexes (Osborne et al., 2004).

In the near future, it may be possible to visualize DNA-protein dynamics at the promoter region, the enhancer region, and different portions or orientations of the loop domains, the chromosomal duplication units, and the entire imprinting area. To dissect this complicated process, co-visualization can be achieved by using different levels of analysis, such as live images of cells (Zink et al., 2003), three-dimensional localization within the nucleus (Osborne et al., 2004), released chromatin loops (Heng et al., 2001b) and released chromatin fibers (Heng et al., 1992).

In addition to direct DNA-protein co-detection focusing on targets within the nuclei, other sets of protein markers can be used to classify cells of interest. These cell or nuclear markers can be simultaneously used as markers of gene expression, or markers that define the stages of the cell cycle. With such information, the dynamics of nuclear DNA-protein interactions can be much more informative.

Medical research: Most of the DNA-protein co-detection methods used in clinically related research involve the detection of membrane antigens and specific DNA targets. An earlier example is the simultaneous detection of Y chromosomes from mononuclear cells detected by antigens (van den Berg et al., 1991). This method can be used effectively to determine the chimerism within different subpopulations of unseparated mononuclear cells after sex-mismatched bone marrow transplantation. Similar approaches are commonly used as an important part of transplantation studies (van Tol et al., 1998).

DNA/protein co-detection has also been commonly used in clinical cancer research. The co-detection of lineage or tumor-specific antigens and chromosomal aberrations is of importance in linking the type of chromosome aberrations to the stages of cancer progression. This is particularly useful for studying the hemologic cancers where both the karyotypic evolution pattern and the lineage-specific antibodies can be traced (Weber-Matthiesen et al., 1996). Examples can be found in the characterization of monosomy-7-associated myeloid disorders. Based on the findings that monosomy 7 was found in all myelomonocytic and erythroid cells, in a part of CD34-positive cells, but not in lymphocytes, it has been concluded that monosomy 7 in these patients is restricted to an early committed progenitor cell that is capable of erythroid and myelomonocytic differentiation (Baurmann et al., 1993). Clearly, the ability to trace the cellular origin of tumor cells and simultaneously examine karyotype aberrations is essential to future cancer research, particularly when studying tumor stem cells. For solid tumor studies, tracing the stages of cancer progression by karyotypic patterns or cell surface markers has proven to be a great challenge. Typically, the amplification of specific oncogenes is monitored among cells identified by protein markers. The correlation between genomic change and expression profiles can be compared as in the following examples: the correlation between *c-myc* gene amplification and oncoprotein expression (Selim et al., 2002) and the correlation between the *HER-2* gene amplification and protein expression in invasive breast cancer cases, demonstrating significant intratumoral heterogeneity that correlates with the level of amplification and expression (Lewis et al., 2005). Many reports have been published based on similar ideas using a protein marker to identify gene expression, types of cells, or the stages of cancer development (Lottner et al., 2005). This information permits precise comparisons of genomic changes. We suggest that, in addition to gene amplification, a greater focus should be on the correlation of the stage of cancer development and the level of overall genomic change, for example by monitoring the frequency of NCCAs (Heng et al., 2006).

Our recent studies have demonstrated that stochastic genomic changes are significant to the initiation and progression of cancer (Heng et al., submitted). Stochastic genomic changes can be monitored using nonclonal chromosomal aberrations or NCCAs (Heng et al., 2004b, 2006). In a given population, these stochastic changes must be analyzed by profiling individual cells. It would be extremely interesting to correlate the changed genomic signatures using specific protein markers to analyze the tumorigenic stages of the cell as well as their expression profiles. In addition, we have shown that the entire genome can be monitored by SKY while specific loci can be simultaneously monitored by FISH probes (Ye et al., 2001). Therefore, detailed comparisons of specific loci can be done between cells to display overall genomic changes and cells with

normal karyotypes. Combinational detection of other protein markers and/or nuclear protein complexes can be used to determine relationships and systematically analyze a variety of genetic loci, biological pathways, overall genomic instability, and stages of cancer progression.

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