Cell, Vol. 76, 609–622, February 25, 1994, Copyright © 1994 by Cell Press

Metaphase Chromosome Structure: Bands Arise from a Differential Folding Path of the Highly AT-Rich Scaffold

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Summary

Using the highly AT-specific fluorchrome daunomycin, a longitudinal optical signal called AT queue, thought to arise from a line-up of the highly AT-rich scaffold-associated regions (SARs) by the scaffolding, was identified in native chromosomes. Fluorescence banding is proposed to result from a differential folding path of the AT queue during its progression from telomere to telomere. The AT queue is tightly coiled or folded in a Q band, the resulting transverse striations across the chromatid, which also represent Giemsa subbands, generating a bright AT-rich signal over the Q region. The R bands, in contrast, contain a more central (unfolded) AT queue, yielding an AT-dull signal over the R regions. The AT queue is identified by immunofluorescence against topoisomerase II (topo II) and HMG-I/Y as the scaffold of native chromosomes; the fluorescence signal from both proteins is akin to a detailed Q-type banding pattern. Native chromosomes appear assembled according to the loop-scaffold model.

Introduction

Chromosomes bands are variations in the longitudinal structure of the chromatid revealed by various staining techniques. Classically, chromosomes appear to be built of stacked disks, where each disk differs from its nearest neighbor in gene density, time of replication, base composition, repeat sequences content, and chromatin conformation (reviewed by Holmquist, 1992; Craig and Bickmore, 1993). The underlying chromosomal order responsible for the banding phenomena is not understood.

The major chromosomal bands are the following (Sumner, 1990): C bands represent constitutive centromeric heterochromatin containing tandemly repeated satellite DNA and apparently no genes. Giemsa dark (G) bands are late-replicating facultative heterochromatic bands; they appear AT-rich and, as a consequence, can also be revealed with AT-specific DNA fluorochromes such as quinacrine (Q bands), DAPI, or daunomycin. Only about 20% of the mapped human genes localize to the G/Q bands; they are essentially devoid of housekeeping genes and the facultative heterochromatin of the Q bands may serve to silence many tissue-specific genes. R bands generally replicate early in the S phase; they stain Giemsa pale or quinacrine dull (GC-rich). Virtually all the widely expressed human housekeeping genes map to R bands (Holmquist, 1992; Craig and Bickmore, 1993).

Despite the routine use of the clinically important chromosomal banding technique, no prevailing and satisfactory model exists that relates chromosome structure to the banding phenomena. Discussion concerning the banding phenomena centers around dye accessibility as modified by proteins, a long-range variation of the base pair composition along the genome, or both (Sumner, 1990). Dye accessibility appears to play a minor role in banding using DNA fluorochromes; all known DNA fluorochromes, such as ethidium bromide or propidium iodide, that bind DNA without a sequence preference stain chromosomes quite homogeneously. If dye accessibility were to play a major role, general DNA fluorochromes should have been found with banding properties.

G/Q band DNA was found to be about 3.2% richer in AT than R-band DNA (Holmquist et al., 1982). This relative minor variation in base composition along the chromosomes can not explain the good Q bands observed with daunomycin; the fluorescence emission of daunomycin is quenched almost completely upon binding to DNA having a AT-base composition between 0%–60%, implicating perhaps only the very AT-rich (>65% AT) DNA in Q banding (Comings and Drets, 1976; Johnston et al., 1978; Comings, 1978). Clearly, the alternating pattern of AT- and GC-rich bands along the chromosome is unlikely to be due to a simple linear variation of the base composition over many megabases of DNA; the banding phenomena must have a structural rationale based on chromosome organization.

The structural higher order subunit of metaphase chromosomes is a chromatin loop containing roughly 75 kb of DNA in human chromosomes; the loops are tethered, forming a network termed chromosomal scaffolding (Laemmli et al., 1977). The major scaffold protein is SC1 (Lewis and Laemmli, 1982), a protein later identified as topoisomerase II (topo II) (Earnshaw and Heck, 1985; Gasser et al., 1986). DNA elements termed scaffold-associated regions (SARs) are candidate elements for defining the base of chromatin loops. SARs are highly AT-rich (>65%) stretches of several hundred base pairs that were originally identified in Drosophila (Mirkovitch et al., 1984). Since then, SARs have been characterized in several organisms, including human and yeast (reviewed by Gasser and Laemmli, 1987; Garrard, 1990; Laemmli et al., 1992). A number of proteins bind SARs highly selectively, such as topo II, histone H1, and HMG-I/Y (Adachi et al., 1989; Sperry et al., 1989; Izaurralde et al., 1989; Zhao et al., 1993). SARs (sometimes called MARs) contain numerous A tracts (AT-rich sequences containing short homopolymeric runs of dA·dT base pairs) and the specific interaction is not mediated by a precise base sequence but by the non-B structural features of DNA, such as the narrow minor groove of the A tracts, DNA bends and/or propensity to unwind (Käs et al., 1989; Homberger, 1989; Adachi et al., 1989; Bode et al., 1992)

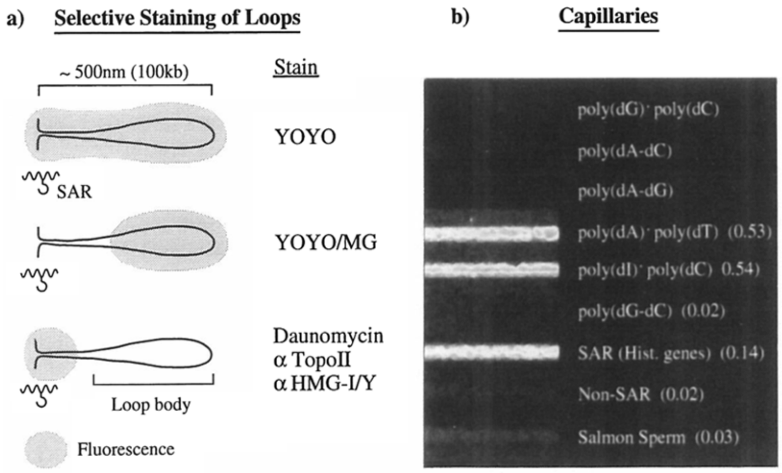

Figure 1. Selective Staining of Loops: Daunomycin and YOYO/MG

(a) Schematic of the protocols to stain selectively the base or body of the chromatin loops. Detection of the loop body is indirect; general staining of the chromatin loops with YOYO-1 is followed by quenching of the emission from the highly AT-rich SAR with MG. Daunomycin or the specific antibodies αTopo II or αHMG-I/Y are used to stain the loop base. Selective staining of the bases of the loops generates a detailed Q pattern; selective staining of the loop body (YOYO/MG) yields the complementary R pattern.

(b) Capillaries filled with a variety of daunomycin–DNA complexes (1 μg/μl) as indicated were recorded with a 1 × objective with the confocal microscope using the GHS filter. The number in parenthesis represents the residual quenched fluorescence emission completely titrated with excess DNA duplex as determined by fluorometry (see text). The residual fluorescence of the histone SAR fragment (0.14) is several fold greater then that of bulk salmon sperm DNA (0.03).

The scaffolding and loop organization of DNA was originally observed by electron microscopy in histone-depleted metaphase chromosomes as a residual metaphase-shaped structure (Paulson and Laemmli, 1977; Laemmli et al., 1977). Subsequent studies confirmed the organizational principle of loops in progressively more native unfolded chromosomes by thin sectioning (Marsden and Laemmli, 1979), spreading techniques (Earnshaw and Laemmli, 1983), and immunofluorescence with antibodies against topo II (Earnshaw and Heck, 1985; Gasser et al., 1986; Boy de la Tour and Laemmli, 1988). In chromosomes unfolded by exposure to low salt or partial extraction of histone H1, the scaffolding is observed as a central longitudinal region due to the peripheral spreading of the unraveled chromatin loops as well as the longitudinal uncoiling of the scaffolding. Possibly, as a final level of organization, the scaffolding is mirror-symmetrically helically coiled to yield compact sister chromatids (Boy de la Tour and Laemmli, 1988).

In attempts to understand the organization of the scaffolding in intact chromosomes, we discovered the structural basis for chromosome bands. They are proposed to be generated by an organized queue (or line-up) of the most highly AT-rich (>65%) DNA sequences, called the AT queue. Our experiments identify the AT queue as the scaffold that appears to mediate the line-up of the highly AT-rich SARs.

Results

Packaging of Chromosomal Loops: Feasibility and Tools

Is it reasonable to study the packaging of chromatin loops in native chromosomes by confocal microscopy? Assuming a DNA loop size of 100 kb and a length packing ratio of 36 for the 30 nm chromatin fiber, a loop would approximate a rod-shaped structure with a length of about 500 nm and a width of about 60 nm. These rods are expected to have biochemical polarity provided by the highly AT-rich (>65%) SARs and the scaffold proteins at the bases of the loops. We used the following tools to detect loop polarity.

The bases of chromatin loops can be detected using either the highly AT-specific DNA fluorochrome daunomycin or specific antisera against scaffold proteins (see Figure 1a). Daunomycin is ideally suited to "image" the highly AT-rich SARs as the daunomycin fluorescence is almost completely quenched upon binding to DNA having an A+T-base composition from 0%–60%. In contrast, this signal is only partially quenched upon binding to DNA with a very high A+T (>65%) content (Comings and Drets, 1976; Johnston et al., 1978). We have confirmed these observations quantitatively by fluorometry as described by Comings and Drets (1976) and semiquantitatively by examination of capillaries, filled with a number of different DNA–daunomycin complexes, with the help of a 1 × objective in the confocal fluorescence microscope (see Figure 1b for details). A strong residual signal (45%–55%) is obtained by fluorometry if daunomycin is titrated to completion (excess DNA) with the synthetic duplexes poly(dA-dT), poly(dA)·poly(dT) or poly(dI)·poly(dC). In contrast, the residual signal is very weak (1%–2%) upon titration with poly(dC–dG), poly (dG)·poly(dC), an isolated non-SAR (39% A+T) fragment or bulk salmon sperm DNA. Importantly, the residual emission of daunomycin titrated with the histone SAR (74% A+T) levels off at about 10%–15%, significantly above the value measured for bulk DNA. These observations are confirmed (semiquantitatively) by examination of the capillaries (Figure 1b), which also reveal the poor residual signal from the daunomycin complexes with the duplexes poly(dA–dC) and poly(dA–dG). Consequently, if SARs are organized by the scaffold into a line-up, the AT queue, then a localized signal reinforcement will result. The term AT queue is used to describe the brightest optical daunomycin signal.

We used the following indirect procedure to detect the chromatin loop body (see Figure 1a). The asymmetric cyanine dye YOYO-1 (YOYO) is a general DNA fluorochrome that stains loops (the base and the body) and chromosomes (see below) quite homogeneously. We have used the nonfluorescent dye methyl green (MG) to highlight the loop body. MG preferentially interacts with AT-rich DNA