NOR Lateral Asymmetry and Its Effect on Satellite Association in BrdU-Labeled Human Lymphocyte Cultures*

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Summary. Second generation BrdU-labeled acrocentric chromosomes exhibit NOR lateral asymmetry (NLA) in metaphases that have been sequentially stained with silver and the Hoechst-Giemsa sister chromatid differential (SCD) technique. The NLA presumably results from suppression of NOR activity in the doubly-substituted chromatid. Examination of single chromatid (NOR) associations in pairs of acrocentrics reveals that light chromatids associate less frequently than dark chromatids and that the frequency distribution of dark and light alignment configurations can be explained by this differential tendency to associate. Thus, it appears that a hypothesis of non-random chromatid segregation as an explanation for non-random chromatid alignments in associating acrocentric chromosomes is unwarranted.

Introduction

In a recent study Bobrow and Heritage (1980) suggested that segregation of the chromatids of nucleolar organizing chromosomes at mitosis may be non-random. They examined acrocentric chromosomes in satellite association from human lymphocytes treated with 5-bromodeoxyuridine (BrdU). BrdU is incorporated into the DNA in place of thymidine and after 2 doublings, the chromatids of mitotic chromosomes are differentially substituted with the base analogue. After sister chromatid differential staining (SCD) the singly-substituted chromatids stain darker than the doubly-substituted chromatids. The authors found that in head-to-head associations involving 2 acrocentrics, dark-to-dark chromatid pairing was significantly more frequent than dark-to-light pairing and that the tendency for concordant alignment could also be observed in third generation cells. The tendency for similarly substituted chromatids to associate was interpreted as arising from non-random segregation at mitosis mediated by the formation of DNA connections between the chromosomes. These connections, which are maintained from one division to the next, hold the chromatids in the same orientation. The associating acrocentrics are therefore functioning as bi-armed chromosomes and discordant dark-to-light alignments arise from breakage in the presumed connections.

This interpretation assumes that BrdU has no effect on the functioning of the nucleolus organizing regions (NORs). Indirect evidence for an alteration in NOR activity by BrdU has been observed in silver stained chromosome preparations. When second generation BrdU-substituted chromosomes were subjected to the SCD procedure, destained, and then stained by the Ag-NOR technique, a difference in silver staining intensity was observed between sister NORs (Lau and Arrighi 1977). The chromatid that had been lightly stained showed little or no silver deposit, while the dark chromatid exhibited normal amounts of silver. Similar observations were made by Sigmund et al. (1979) and Vogel et al. (1978) on human and Chinese hamster chromosomes. Although the mechanism for the reduction in silver staining is unknown, it is possible that BrdU reduces NOR activity, resulting in less silver-stainable protein on the doubly-substituted NOR (Vogel et al. 1978). It has been demonstrated that the amount of silver staining is correlated with the frequency of satellite association in human chromosomes (Miller et al. 1977). Similarly, the dark chromatids in BrdU-treated chromosomes may associate more frequently because of their larger silver-positive-NORs, which would result in a preference for the concordant dark-to-dark pairing just described. Thus, segregation of mitotic chromosomes may indeed be random and an explanation for the non-random pairing observed by Bobrow and Heritage may involve the differential effects of BrdU on singly- and doubly-substituted NORs.

In the present study we have investigated the phenomenon of NOR asymmetry and its effects on satellite association by first subjecting BrdU-labeled chromosomes to the Ag-NOR procedure and then to SCD staining on the same metaphase plates. Our data indicate that: 1) BrdU induces NOR lateral asymmetry in human acrocentric chromosomes and that this is not an artifact of the sequential staining and destaining procedures previously used and 2) associations involving only a single chromatid (NOR) from each of 2 acrocentrics exhibit frequencies which can be explained by a differential tendency for dark and light chromatids to associate.

Materials and Methods

In Vitro BrdU Labeling of Lymphocytes

Lymphocyte cultures were set up from 2 karyotypically normal males, 26 and 32 years of age. BrdU (10.0μg/ml) was added to
half of the culture tubes at 24 h after culture initiation. At 72 h colchicine (0.4 μg/ml) was added to each tube for 30 min and the cultures harvested. The cell suspension, after KCl hypotonic treatment, was fixed in acetic-methanol mixture (1:3) and dropped on wet slides and air-dried. Control cultures were treated in the same way except that BrdU was not added to the medium.

Staining for Sister Chromatid Differentiation and Ag-NOR
BrdU-labeled cells were stained in two ways: (1) The silver staining for NOR was performed first, following the 1-step method of Howell and Black (1980). The same slides were then treated for SCD induction using the Hoechst 33258 and Giemsa-staining procedure (Wolff and Perry 1974) subsequent to satisfactory silver staining. (2) In the second set, the slides were subjected to Ag-NOR staining only. The control and experimental slides were coded, and scored for NOR-lateral asymmetry in a blind manner.

Definition of NOR-lateral Asymmetry (NLA)
An asymmetry was subjectively defined as the complete absence of silver grain on one sister NOR or a noticeable difference in size or staining intensity between two silver positive sister NORs. Any questionable asymmetry was scored as “no difference.” Chromosomes with ambiguous silver positive NORs, i.e., those with a large silver mass, those with more than 2 discrete silver-stained regions, or those sharing silver-stained material, were scored separately and listed in a “cannot determine” category.

Scoring Satellite Associations
To test the hypothesis that there exists a differential tendency for dark and light chromatids to associate, only those associations involving one chromatid of each acrocentric were scored. The associated chromatids were a chromatid-width apart or less and usually, but not always, shared silver-stained material. The unassociated chromatids were greater than a chromatid-width apart and in no case were their NORs connected with silver. Ambiguous associations, such as those which appeared to involve one chromatid of one chromosome and both of another, were not scored.

Results
Analysis of NLA
An obvious degree of NLA in a BrdU-labeled and silver-stained metaphase is shown in Fig. 1. The possibility that the SCD procedure might alter the staining of the NORs was eliminated by comparing the same silver stained chromosomes before and after SCD treatment in approximately a dozen cells. A total of 50 metaphase cells from one subject were scored for NLA (Table 1). All cultures had grown for two cell cycles in the presence of BrdU as shown by their SCD patterns. Out of 467 silver positive chromosomes, 246 had two distinct NORs of which 40.2% showed no obvious difference in NOR size or staining intensity. The remaining 59.8% exhibited NLA with the overwhelming majority (93.2%) having the association of light (doubly substituted) chromatid with the smaller NOR.

Table 1. Frequency of NLA in second generation BrdU-labeled chromosomes and untreated controls

<table>
<thead>
<tr>
<th></th>
<th>NLA present</th>
<th>NLA absent</th>
<th>Cannot determine</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Light chromatid/</td>
<td>137 (29.4)</td>
<td>10 (2.1)</td>
<td>99 (21.1)</td>
<td>220 (47.2)</td>
</tr>
<tr>
<td>small NOR</td>
<td>(55.7)</td>
<td>(4.1)</td>
<td>(40.2)</td>
<td>466</td>
</tr>
<tr>
<td>Light chromatid/</td>
<td>103 (23.1)</td>
<td>116 (26.1)</td>
<td>226 (50.8)</td>
<td>445</td>
</tr>
<tr>
<td>large NOR</td>
<td>(47.0)</td>
<td>(53.0)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* % of total Ag-positive
b % of total unambiguously scorable chromosomes

Data from 50 control cells show that only 16.6% of the unambiguously scorable chromosomes had NLA. The BrdU was therefore responsible for a 3.5 fold increase in NOR asymmetry over controls, at least in these samples.

Because of the inherent bias in scoring known treated and known control cells for NLA, a blind analysis of cells without SCD staining was performed on a second set of blood cultures (Table 2). There is good agreement between the controls in this and the previous analysis regarding frequency of asymmetry (20.2% and 16.6%, respectively), although a bias toward underestimating this measure when the cells are known controls can be seen. In the treated sample, 47% of the chromosomes exhibited NLA, compared to the nearly 60% frequency in the SCD sample. This difference is not unexpected since without SCD staining no differentiation can be made between cells grown for two cycles and those grown for only one cycle in BrdU. Nonetheless, the treated sample demonstrated a more than two-fold increase in NLA over the control. The reduced number of silver positive chromosomes in the treated sample in this comparison is unexpected but probably results from technical variation in the harvesting and staining procedure between the two sets of cultures.

Analysis of Satellite Associations
A total of 225 associations were examined in over 450 second-generation BrdU-labeled cells from both subjects. Three classes of single chromatid associations are possible: 1) concordant dark-to-dark pairing; 2) discordant dark-to-light pairing; and 3) concordant light-to-light pairing. As can be seen in Fig. 2, the dark chromatids are involved in more associations overall than are the light chromatids. It is possible to calculate a coefficient