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CHROMOSOME HETEROMORPHISMS IN THE JAPANESE

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日本人における染色体の異形性

## Ⅱ. D及びG群にみられた変異染色体の仁形成部位

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**SUMMARY**

The nucleolus organizer regions (NORs) of variant chromosomes in D and G groups which are characterized by an enlargement of the short arm region (including the short arm, secondary constriction, and/or satellites), were examined using the silver staining method. Of a total of nine variants examined, four were found to have double satellites, since there were two Ag-stained NORs in the enlarged short arm regions; two were designated as 22pss, and one each as 13pss and 14pss. Four of the other variants had only one Ag-stained NOR. Judging from the position of the NOR, three of them were determined to have enlarged satellites (two with 15s+ and one with 22s+), and the other an enlarged short arm (15p+). In the remaining variant, no Ag-stained material was noted in the short arm region, so it could not be determined whether this variant chromosome was derived from the enlargement of the short arm and/or satellites. Based on the position of the Ag-stained NOR and staining intensity of the Q- and C-methods in the short arm region, mechanisms of producing the enlarged short arm regions of D and G chromosomes are discussed.

## INTRODUCTION

Enlarged short arms and satellites of D and G chromosomes in man are known to be heteromorphic variants. Recently developed banding techniques have made it possible to identify chromosomes involving these variants, and several specific banding patterns have been found

**要 約**

D及びG群における短腕部位（短腕，二次狭窄，付随体を含む）の増大した変異染色体の仁形成体部位（NOR）について銀染色法を用いて調べた．合計9例の染色体変異のうち，4例は重複付随体をもつもので，銀染色されるNORが2か所に認められた．これらは22pssが2例と，13pssと14pssが1例ずつであった．他の4例の変異体では1か所にのみ銀染色されるNORが認められた．NORの位置から，これらのうち3例は付随体の増大（15s＋が2例と22s＋が1例）であり，他の1例は短腕の増大（15p＋）であることが判明した．残りの1例では短腕部位に全く銀染色される部位がみられず，この変異染色体が短腕，付随体，あるいはその両者の増大のいずれであるかは識別できなかった．短腕部位の銀染色されるNORの位置とQ-及びC-法の染色性を基にして，D及びG群染色体における短腕部位の増大の生成機序について考察した．

## 緒 言

ヒトのD及びG染色体の短腕部や付随体の増大は異形性変異として知られている．近年発達した分染法によってこれらの変異を含む個々の染色体の識別が可能となり，短腕部位や付随体の増大部位にいくつ

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to occur in such enlarged short arms and satellites.[1-4] It is also known that the nucleolus organizer sites are located on the short arm of D and G chromosomes. New techniques specifically staining the NORs have recently been developed,[5,6] and offer an opportunity to study the NORs in these variant chromosomes.

In the present study, nine chromosome variants confined to D and G chromosomes, which had already been detected by the conventional, Q-, or C-staining methods, were reexamined using the NOR staining method. Size and position of the NORs in these variant chromosomes are described, and mechanisms of origin of enlarged short arms and satellites of D and G chromosomes are discussed.

## MATERIALS AND METHODS

Nine chromosome variants, characterized by the enlargement of the short arm region (including short arm, secondary constriction, and/or satellites) of one of D or G chromosomes, which had already been detected by the conventional, Q-, or C-staining methods, were reexamined using the NOR staining method. These variants were found in the RERF $F_1$ Mortality Study sample[7] in Hiroshima, consisting of the children born to parents one or both of whom received atomic bomb exposure, and appropriate controls. No clinical examination was performed for these children, unless requested; however, in none of the nine individuals studied here was an obviously unusual phenotype noted by well-trained nurses during a questionnaire-interview dealing with family and health histories. Since family studies have not yet been performed, there is no evidence to indicate whether these variant chromosomes were inherited from their parents or were produced in a parental gamete during meiosis.

Chromosome preparations were made from whole blood cultures by the routine air-dry method. The NOR staining was carried out according to the Ag-I method of Bloom and Goodpasture.[6] Slides were flooded with a 50% silver nitrate ($AgNO_3$) solution in deionized water, covered with a cover glass, and incubated in moisture-tight chambers in which deionized water was added to the bottom of the chamber to maintain high humidity, for about 18 hours at 37°C. Slides were then rinsed with deionized water and stained for 15 minutes with Giemsa

かの特異的な分染パターンのあることが判明している.[1-4] また，仁形成部位がD及びG染色体短腕に存在することも知られている．NORを特異的に染色する新しい技術が最近開発され,[5,6] 変異染色体におけるNORについて研究することが可能になった.

本研究においては，既に通常染色法，Q-又はC-分染法によって識別されたD及びG染色体変異9例について，NOR染色法を用いて再分析した．これら変異染色体におけるNORの大きさや位置を述べるとともに，D及びG染色体における短腕並びに付随体の増大の生成機序について考察した.

## 材料及び方法

通常染色法，Q-またはC-分染法によって既に識別されたD及びG染色体の短腕部位（短腕，二次狭窄部位及び付随体）の増大を示す9例の染色体変異についてNOR染色法を用いて再分析した．これらの変異例は広島における放影研$F_1$死亡率調査集団[7]にみられたもので，両親又はいずれか一方の親を原爆被爆者として生まれてきた子供，及びその対照者に属するものである．特に希望がないかぎり，これらの対象者に対する臨床診察は行わなかったが，看護婦が家族や健康歴についての質問を行った際に，これら染色体変異9例に表現型異常を認めていない．家族調査は行われてはいないので，これらの変異染色体が親から遺伝したものか，あるいは配偶子形成時の減数分裂に際して生じたものかについては明らかにできなかった.

染色体標本は全血培養後，通常の空気乾燥法によって作成した．NOR染色はBloomとGoodpasture[6]によるAg-I法に従った．スライドに脱イオン水による50%硝酸銀（$AgNO_3$）溶液をのせ，カバーグラスをかけたのちに，高湿容器（容器の底に脱イオン水をいれて高湿を保つ）に入れて37℃18時間処理した．スライドを脱イオン水で洗ったのちに，ギムザ液

(0.3ml to 60ml of pH 6.8 phosphate buffer). Chromosome preparations from all individuals were examined independently by the conventional, Q-, C-, and NOR staining methods. The Q-banding patterns were obtained by a slightly modified technique of Caspersson et al,[8] and C-staining analysis was made following the method of Sumner.[9]

In addition, successive stainings were carried out on the same metaphases in combination by either NOR and conventional methods, or Q- and NOR methods. In the former combination, the NORs of variant chromosomes were obtained by the Ag-I method, and then silver grains were removed using 1:1 potassium ferricyanide and sodium thiosulfate, and subsequently restained with Giemsa for observation of morphological appearance of short arms and satellites. In the latter, the chromosome banding was obtained by the Q-method for the identification of variant chromosomes, and the same preparations were then destained with 1:3 acetic alcohol, air-dried and restained by the Ag-I method.

The approximate size of the Ag-stained NORs was classified into the following four groups; 3-large, 2-intermediate, 1-small, 0-very small or almost no NOR.

## RESULTS

By the Ag-I method, the NORs appeared as black dots on the short arms of acrocentric chromosomes (Figure 1). As previously reported for cells of normal individuals, black dots were located selectively on the secondary constrictions of the short arms, but not on the satellites.[10,11] The number of acrocentric chromosomes with Ag-stained NOR was found to be relatively constant in each individual, and the chromosomes which were Ag-stained did not occur randomly. Generally, there was a consistent individual pattern of the inter-chromosome distribution of Ag-stained material.[12,13] The patterns of Ag-positive NORs in normal acrocentrics will be reported elsewhere; this report describes Ag-staining patterns of variant acrocentric chromosomes.

Out of nine chromosome variants studied here, four (Cases 1 to 4) were found to have double Ag-stained NORs in their short arm regions. The most typical pattern of the double NORs was found in Case 1 (MF[redacted]), in which a unique

(0.3ml の原液をpH 6.8燐酸緩衝液60ml で薄めたもの)で15分間染色した．全例とも，通常染色法，Q-法，C-法及びNOR 法による観察は全く別々に行った．Q-分染パターンは Caspersson ら[8] の方法をやや変えたものであり，C-分染による分析は Sumner[9] の方法に従った．

更に，同一中期分裂像を用いて，NOR 法と通常染色法，又はQ-法とNOR 法による連続染色を行った．前者では，変異染色体のNOR を Ag-I 法によって得たのちに，フェリシアン化カリウムとチオ硫酸ナトリウム1：1によって銀粒子を落とし，ギムザによる再染色によって短腕や付随体の形態の観察を行った．後者では，Q-法による分染バンドで変異染色体の識別を行ったのちに，同一標本を1：3酢酸アルコールによって脱染，空気乾燥後，Ag-I 法で再染色した．

銀染色法によるNOR のおおよその大きさは次の4群，つまり3－大，2－中間，1－小，0－極小又はNOR がほとんどない，に分類した．

## 結　果

Ag-I 法により NOR は端部着糸型染色体短腕上に黒点状にみられる(図1)．正常個体の細胞については既に報告されているように，黒点は短腕の二次狭窄上に選択的に位置しているが，付随体上にはない．[10,11] 銀染色性 NOR をもつ端部着糸型染色体の数は各個体ごとに一定であり，銀染色される染色体は特定の染色体であることが知られている．一般的には，銀染色性物質の染色体間分布については各個体において一定のパターンがある．[12,13] 正常の端部着糸型染色体の銀染色性 NOR パターンについては別に報告するが，本報告では変異染色体の銀染色性パターンについて記述する．

本報告における染色体変異9例のうち，4例(例1－4)には短腕部位に重複した銀染色性NOR が観察された．重複NOR の典型的なパターンは例1(MF[redacted])にみられるもので，No. 14 染色体の1個に極めて

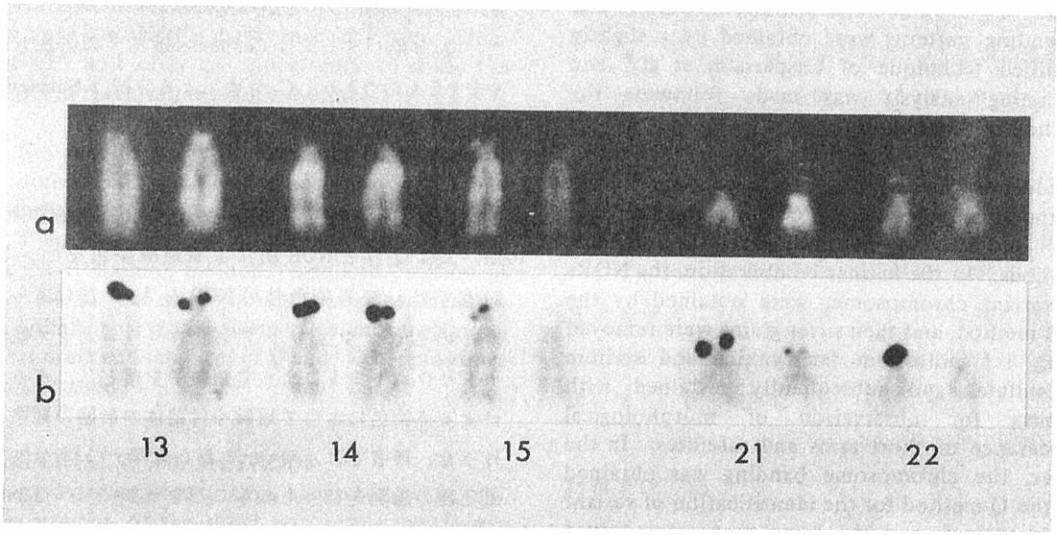


*Figure 1. Partial karyotype of normal D and G chromosomes. a: Q-band chromosomes stained with quinacrine mustard. b: Same metaphase successively treated with Ag-I method.*

図1　正常のD及びG染色体の部分核型．a: キナクリン・マスタード染色によるQ-バンド染色体．b: Ag-I 法処理による同一中期分裂像．

morphological appearance was observed in one of the No. 14 chromosomes. This chromosome was characterized by extremely large satellites and a distinct secondary constriction at a distance two fifths of the arm's length from the centromere (Figure 2a). Q-staining analysis showed the short arm and distal portion of the enlarged satellites to have pale fluorescence, whereas most of the enlarged satellites showed almost no fluorescence (Figure 2b). Though the secondary constriction had negative fluorescence, the middle region of the enlarged satellites fluoresced extremely dimly, and negative fluorescence was observed in another small area adjacent to the pale distal region. Most of the satellite which fluoresced extremely dimly, was stained darkly by the C-method, but not as dark as the C-band (Figure 2c). Both the secondary constriction and the distal region fluorescing negatively by the Q-method were also not stained by the C-method. Staining by the Ag-I method distinguished two large black dots of silver grains in the short arm region of the variant chromosome in almost all cells examined. One was situated on the secondary constriction, while the

特異的な形態が観察された．この染色体は付随体が非常に大きいのが特徴的であり，かつ動原体から約5分の2の位置に明確な二次狭窄がみられる（図2a）．Q-分染法による分析では，短腕と増大した付随体の端部は薄い蛍光性を示すが，増大した付随体の大部分は蛍光性を示さない（図2b）．二次狭窄部位は蛍光性が全くないが，増大した付随体の中央部は極めて弱いが蛍光性があり，薄い蛍光性を示す端部に隣接して蛍光性のみられない小さな部位が観察された．極めて薄い蛍光性を示す付随体の大部分はC-分染法では濃染されたが，C-バンドほど濃くはない（図2c）．Q-法による蛍光性を示さない二次狭窄部と端部もC-分染法によって濃染されない．Ag-I 法によると，ほぼすべての観察細胞において変異染色体の短腕部には銀粒子による二つの大きな黒点がみられる．一つは二次狭窄上であり，他はQ-法で蛍光性

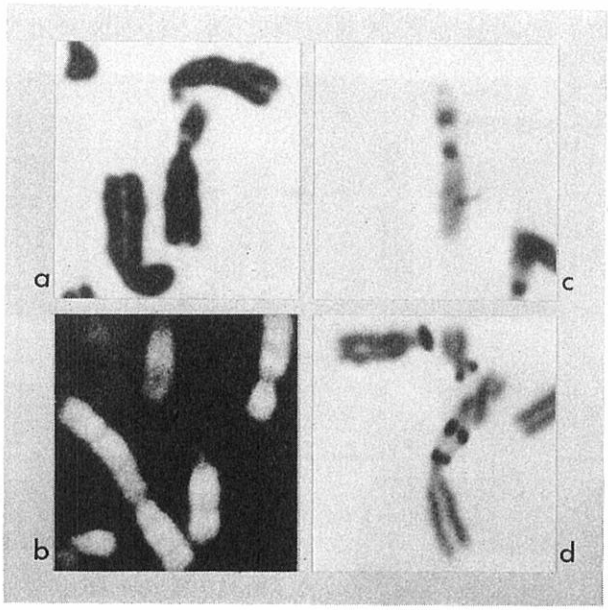


*Figure 2. Four representative variant chromosomes from Case 1 (MF[redacted]). a: Chromosome obtained from conventional Giemsa stain. b: Q-banded chromosome stained with quinacrine mustard. c: C-banded chromosome treated with barium hydroxide. d: Silver stained chromosome by Ag-I method.*

図2　例1（MF[redacted]）の代表的な4個の変異染色体．a：通常ギムザ法による染色体．b：キナクリン・マスタード染色によるQ-バンド染色体．c：水酸化バリウム処理によるC-バンド染色体．d：Ag-I 法による銀染染色体．

other was on the distal part where the Q-method showed negative fluorescence (Figures 2d, 3a). Because of the presence of two distinct Ag-stained NORs situated on the proximal and distal parts, this variant was tentatively designated as a double satellite chromosome, or 14pss.

An almost identical pattern of NORs was also observed in one of the No. 22 chromosomes from Case 2 (MF[redacted]: two intermediate Ag-stained NORs were located on two areas not stained by the Q-method; one was adjacent to the pale short arm and the other to the brilliant satellites (Figure 3b). In one No. 13 chromosome from Case 3 (MF[redacted]), a large number of silver grains was consistently observed in the distal region, whereas a small amount of silver grains was occasionally observed in the non-fluorescent region adjacent to the brilliantly fluorescent short arm (Figure 3c). In Case 4 (MF[redacted], silver grains were also found in two different regions in a No. 22 chromosome, but cells showing two Ag-stained NORs in this variant chromosome were observed less frequently than in those of Case 2. These three variants

のない端部である（図2d，3a）．二つの明確に銀染色されるNORが基部と端部に存在するために，この変異を重複付随体染色体，又は14pss と暫定的に名付けた．

ほぼ同様のNORパターンは例2（MF[redacted]）におけるNo.22染色体の1個にもみられた．二つの中間の銀染色性NORがQ-法で染色されない二つの部位にみられた．一つは薄い蛍光性の短腕隣接部位に，他は強い蛍光性の付随体隣接部位である（図3b）．例3（MF[redacted]）におけるNo.13染色体の1個では，多量の銀粒子が端部に常に観察され，一方，少量の銀粒子が強い蛍光性の短腕に隣接した非蛍光性部位に時折みられた（図3c）．例4（MF[redacted]）ではNo.22染色体の1個において2個所の異なる部位に銀粒子が認められたが，この変異染色体上の2個の銀染色性NORを示す細胞は例2の場合よりも低い頻度で観察された．これら3変異体を重複付随体染色体と呼ぶ

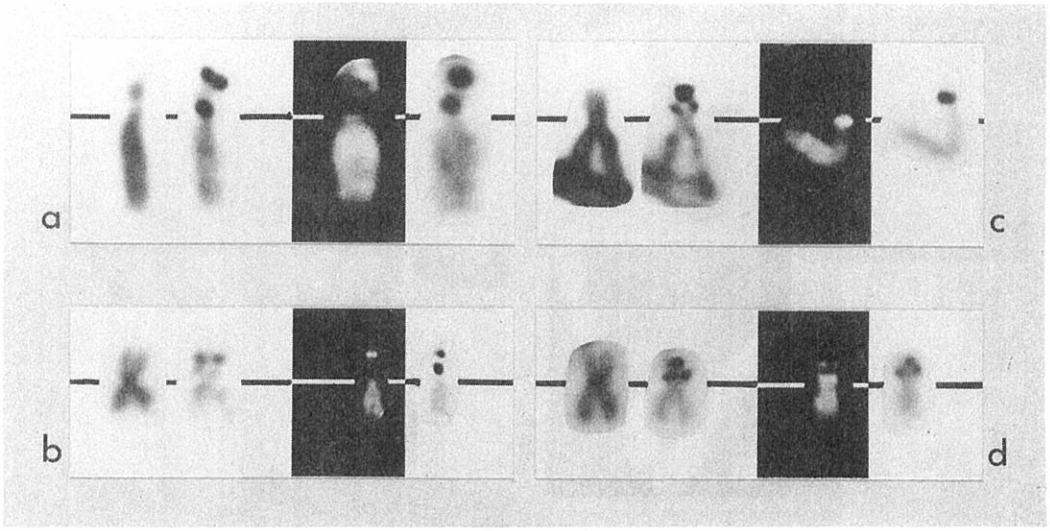


*Figure 3. Variant chromosomes from four cases, prepared by successive staining of same metaphase by conventional and Ag-I (left), and Q- and Ag-I methods (right). a: Same individual shown in Figure 2., b: Case 2 (MF[redacted]), c: Case 3 (MF[redacted]), d: Case 4 (MF[redacted]).*

図3　同一中期分裂像の連続染色法による4例の変異染色体．通常染色法とAg-I（左），Q-とAg-I法（右）．a：図2に示した例．b：例2（MF[redacted]）．c：例3（MF[redacted]）．d：例4（MF[redacted]）．

were also referred to as double satellite chromosomes (i.e., 13pss in Case 3, and 22pss in Cases 2 and 4).

Only one Ag-stained NOR was observed in four variants (Cases 5 to 8). In a No. 15 chromosome from Case 5 (MF[redacted]) and a No. 22 chromosome from Case 6 (MF[redacted]), the short arm region exhibited brilliant fluorescence by the Q-method. In both variants, an Ag-positive NOR was seen in the proximal negative area adjacent to the brilliantly fluorescing region (Figures 4a, 4b). Judging from the position of the NOR, the enlarged area was in the distal region beyond the NOR, that is, on the satellite region rather than the short arm. Thus, these two variants should be regarded as 15s+ and 22s+, respectively.

In Case 7 (MF[redacted]), the Ag-stained material was located in the proximal non-Q staining region in one of the No. 15 chromosomes, in which the short arm region was characterized by intermediate staining intensity by the Q-method (Figure 4c). Since the position of the NOR indicated that the enlarged region belonged

ことにした（例3は13pss，例2及び例4は22pss）．

単一の銀染色性NORは変異体4例に観察された（例5から8）．例5（MF[redacted]）のNo.15染色体の1個と，例6（MF[redacted]）のNo.22染色体1個において，短腕はQ-分染法で強い蛍光性を示した．両変異体とも銀染色性のNORは強い蛍光性部位に隣接した非蛍光性基部にみられた（図4a，4b）．NORの位置からみて，増大部位はNORよりも更に端部，つまり，短腕というよりは付随体部位である．したがって，これら2変異体をそれぞれ15s＋と22s＋とした．

例7（MF[redacted]）では，銀染色性物質がNo.15染色体の1個の短腕基部にあるQ-法で非染色性部位にみられた．この染色体では短腕がQ-法で中間の染色性を示す特性があった（図4c）．NORの位置は，増大部位が付随体に属することを示唆していることから，

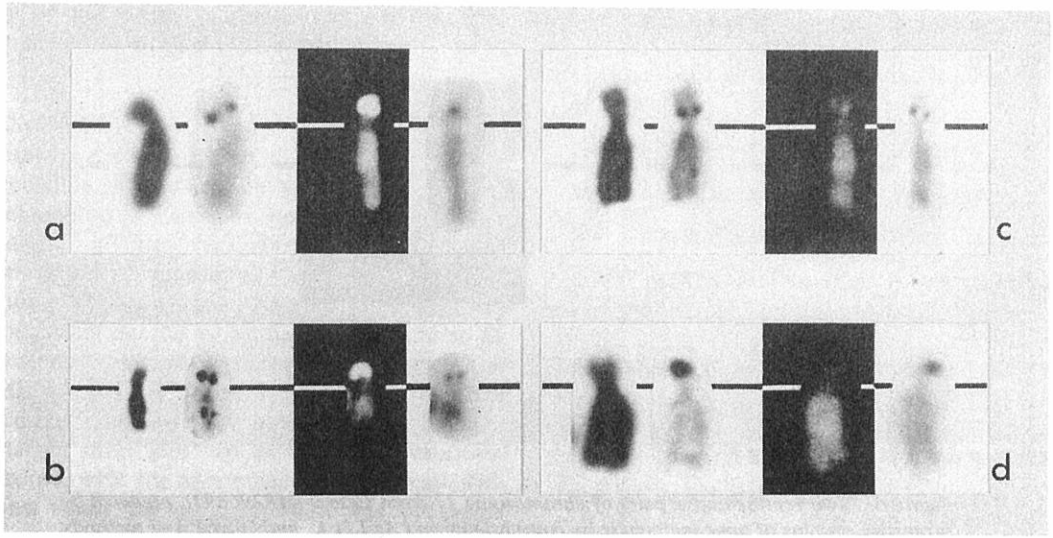


*Figure 4. Variant chromosomes from four cases, prepared by successive staining of same metaphase by conventional and Ag-I (left), and Q- and Ag-I methods (right). a: Case 5 (MF[redacted]. b: Case 6 (MF[redacted]. c: Case 7 (MF[redacted]. d: Case 8 (MF[redacted]).*

図4　同一中期分裂像の連続染色法による4例の変異染色体．通常染色法とAg-I法(左)．Q-とAg-I法(右)．a：例5（MF[redacted]）．b：例6（MF[redacted]）．c：例7（MF[redacted]）　d：例8（MF[redacted]）．

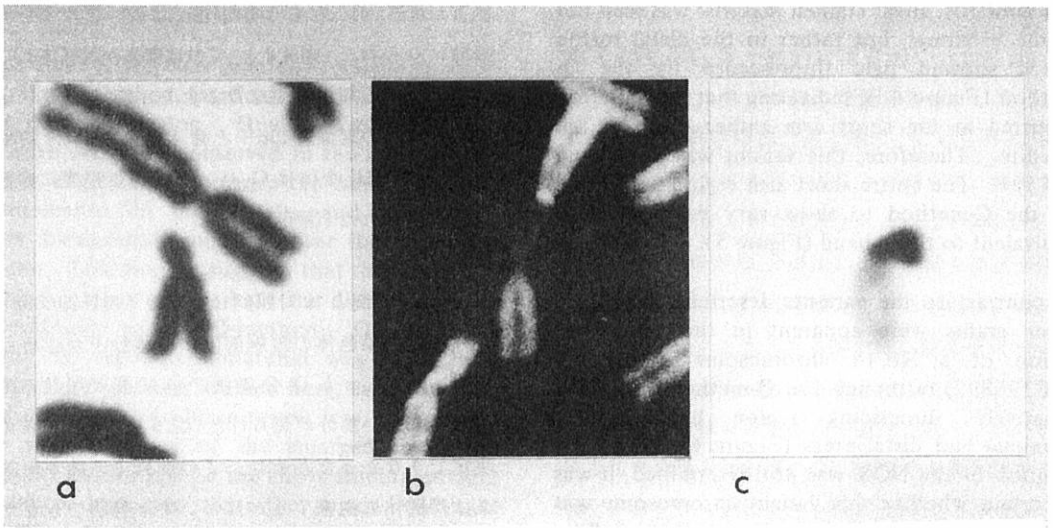


*Figure 5. Three representative variant chromosomes from Case 8 (MF[redacted]. a: Chromosome obtained from conventional method. b: Q-banded chromosome stained with quinacrine mustard. c: C-banded chromosome treated with barium hydroxide.*

図5　例8（MF[redacted]）の三つの代表的な変異染色体．a：通常染色法による染色体．b：キナクリン・マスタード染色によるQ-バンド染色体．c：水酸化バリウム処理によるC-バンド染色体．

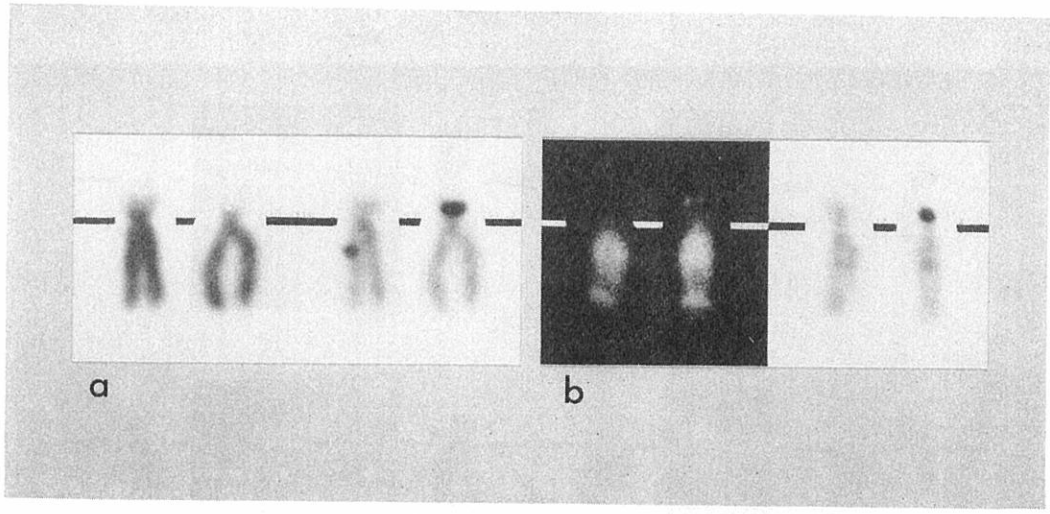


*Figure 6. Two representative pairs of chromosome 14 from Case 9 (MF[redacted]), prepared by successive staining of same metaphase by conventional and Ag-I (a), and Q- and Ag-I methods (b). The first of each is the variant chromosome.*

図6　同一中期分裂像の連続染色による例9（MF[redacted]）の代表的なNo.14染色体対．通常法とAg-I 法（a）．Q-と Ag-I 法（b）．最初の染色体が変異体である．

to the satellite area, this variant was also referred to as 15s+.

In one of the No.15 chromosomes from Case 8 (MF[redacted]), silver stained material was seen not in the proximal, but rather in the distal region which showed pale fluorescence by the Q-method (Figure 4d), indicating that the variation occurred at the short arm rather than on the satellite. Therefore, this variant was designated as 15p+. The entire short arm region was found by the C-method to show very dark intensity equivalent to the C-band (Figure 5).

In contrast to the variants described above, no silver grains were apparent in the short arm region of a No.14 chromosome in Case 9 (MF[redacted], although the Q-method showed a negatively fluorescing region between the proximal and distal areas (Figure 6). Since the position of the NOR was not determined, it was uncertain whether this variant chromosome was derived from the short arm or the satellite. However, the Q-banding pattern of the enlarged short arm region was almost identical to that of cases with double satellites, suggesting that this variant may also be referred to as double satellite, that is, a probable 14pss.

この変異体もまた15s＋と表される．

例8（MF[redacted]）のNo.15染色体の1個に銀染色性物質が基部ではなくむしろQ-法では薄い蛍光性を示す端部にみられた（図4d）．これは変異が付随体というよりはむしろ短腕に生じたことを示している．したがって，この変異を15p＋と命名した．C-分染法によると短腕部位全体はC-バンドと同じ程度に非常に濃く染色された（図5）．

上述の変異例とは対照的に，例9（MF[redacted]）におけるNo.14染色体の短腕部位には銀粒子が認められなかった．しかしQ-分染法では基部と端部の間に蛍光性のない部分が見られた（図6）．NORの位置を決定できなかったために，この変異染色体が短腕か又は付随体に由来するかについては判明できなかった．しかしながら，増大した短腕部位のQ-バンドパターンによれば，重複付随体例とほぼ同様であった．これは，この変異が重複付随体，つまり14pss，と命名し得ることを示唆した．

According to the C-method, no positively stained chromosome segments, aside from the C-bands adjacent to the centromere, were observed in any of the variant chromosomes studied, with the exception of Case 8 described above.

## DISCUSSION

Recently developed staining methods have made it possible to visualize the NORs in chromosome preparations,[5,6] and to demonstrate selective staining of the secondary constrictions of acrocentric chromosomes.[10,11] In the present study, at least one Ag-positive NOR was recognized by the silver staining method in all acrocentric variants examined, except for a case with a probable 14pss. However, no distinct secondary constriction was observed in their enlarged short arm regions by the conventional method, with the exception of another case with 14pss where the proximal NOR was located on the secondary constriction. In the distal Ag-stained NOR of Case 1, a distinct negative stain was either almost unrecognizable or occasionally stained faintly by the conventional method. In spite of the difference in staining between the proximal and distal NORs by the conventional method, the amounts of silver grains in both NORs were fairly consistent, suggesting that the size of the Ag-positive NOR may not always correlate with the size of the area negatively stained by the conventional method.

Ag-stained NORs were generally stained negatively by the Q-method, particularly those located at the proximal region. However, when the Ag-positive NOR was observed in the distal region, it was difficult to recognize the distinct negative fluorescence on these NORs, and silver grains were frequently found to cover the pale distal region. This finding suggests that the Ag-stained NORs may not always have the distinct negative fluorescence of the Q-method. On the other hand, no Ag-stained material was found in a probable 14pss case studied here, even though a distinct negative fluorescence was observed in the middle region of the enlarged short arm region. In contrast to the above finding, present evidence indicates that the region with the distinct negative fluorescence may not always correspond to the Ag-stained NOR.

Of a total of nine variants examined in the present study, four had double Ag-positive NORs, or "double satellites", consisting of two 22pss,

C-分染法によれば，動原体に近接するC-バンドを除けば，濃染した染色体部分は，上述の例8以外のいずれの変異染色体にも観察されなかった．

## 考　察

近年開発された染色法によって染色体標本にNORを観察することが可能となり，[5,6] また端部着糸型染色体の二次狭窄が選択的に染色されることも証明された．[10,11] 本研究において，14pssと推定した例を除けば，すべての端部着糸型染色体変異にすくなくとも1個の銀染色性NORを認めることができた．しかしながら，基部NORが二次狭窄に位置しているもう一つの14pssの例を除いては，増大した短腕部位に明確な二次狭窄が通常染色法では観察されなかった．例1にみられる端部銀染色性NORは，通常染色法により明らかな非染色性が認められず，時折薄く染色されることがあるにすぎない．端部と基部のNORの間には通常染色法の染色性に差異が認められるのに対し，両方のNORとも銀粒子の量はほぼ一定であり，銀染色性NORの大きさが通常染色法による非染色部位の大きさと必ずしも相関しないことを示唆している．

銀染色性NORは一般的にはQ-法では染色されず，基部に位置するものは特に染色されない．しかし，銀染色性NORが端部にある時は，これらのNORには明確な非蛍光性を認めることができず，銀粒子は淡染の端部を覆う形で観察される．この観察結果は，銀染色性NORがQ-分染法による明確な非蛍光性を必ずしも示さないことを示唆している．他方，本研究において14pssと思われる例では，銀染色性物質が認められなかったにもかかわらず，増大した短腕部の中央部に明確な非蛍光性が観察された．この事実は上述の結果とは対照的に，明確な非蛍光性を示す部位が必ずしも銀染色性NORとは対応し得ないことを示している．

本研究における9例の変異体のうち，4例は重複した銀染色性NOR，又は"重複付随体"を有していた．

one 13pss, and one 14pss. Archidiacono et al[14] reported that two cases with double satellites had double Ag-stained NORs, but the chromosomes involved in these two cases differed from the present variants, being a No. 15 and a No. 21 chromosome.

A variant chromosome similar to the 14pss described in the present study was reported in seven individuals from three generations of one family.[15] The very long short arm of a No. 14 chromosome was darkly stained by the conventional stain but palely stained by the G-, Q-, and C-staining methods, and two Ag-positive NORs were present on an unstained secondary constriction close to the distal end of the short arm and a narrow unstained region closer to the centromere. Recently, three Ag-positive NORs were demonstrated in three secondary constrictions of the giant short arm of a No. 14 chromosome,[16] and this 14p+ arm was also palely stained by Q-, C-, and G-staining methods with an almost homogeneous texture.

In the cases with double Ag-positive NORs, in which one NOR was located in the distal region and the other was in the proximal region, it is unknown which NOR is the original one; if it is the proximal NOR, then the enlargement would have occurred at the distal region beyond the proximal NOR. Since staining intensities between the original and enlarged area by the Q- and C-methods showed a lack of uniformity, it may be presumed that the enlargement is caused by a translocation of an NOR and satellite from some other acrocentric chromosome donor rather than duplication of the original NOR and satellites.

Alternatively, it is conceivable that the distal NOR is the original one and the proximal NOR including satellites is newly added, these additional materials having been derived by insertion of an NOR and satellite from an acrocentric chromosome donor. Judging from the staining intensity of the double satellites by the Q-method, the latter hypothesis is, at present, more acceptable than the former. Accordingly, the origins of the double satellites studied here are tentatively assumed to have arisen by insertion of an NOR and satellite from another acrocentric.

In the variants with one Ag-positive NOR, the staining intensity of the Q- and C-methods was

その内訳は22pss が２例，13pss が１例，及び14pss が１例であった．Archidiacono ら[14]の報告では，重複付随体を示す２例に重複した銀染色性 NOR が認められている．しかし，これら２例に関与している染色体は本研究の変異例とは異なっており，No.15 及びNo.21染色体である．

本報告に記載されている14pss と同様の変異染色体が１家系内の３世代７例に報告されている．[15] No.14染色体の非常に大きな短腕は通常染色法では濃染されるが，G-，Q-，及びC-分染法では淡染される．そして２個の銀染色性 NOR が短腕の末端部に近い非染色性二次狭窄と動原体近くの狭い非染色部位に存在した．最近，３個の銀染色性 NOR がNo.14染色体の巨大な短腕の３個の二次狭窄に認められており，[16] この14p＋の短腕は Q-，C-，及び G-分染法では淡染される均質の構造を示した．

重複した銀染色性 NOR の例では，一方は末端部に，また他方は基部に位置しているが，どちらが元来の NOR かは明らかでない．もしそれが基部の NOR とするならば，増大は基部 NOR よりも，より末端部において生じたと考えられている．Q-及び C-分染法による元と増大部位の染色性には一様性がみられないことから，増大部位は元来の NOR と付随体の重複によるというよりは，むしろ他の端部着糸型染色体に由来する NOR と付随体との転座によって形成されたものと推察される．

別の見方をすれば，端部 NOR が元来のものであり，付随体を含む基部 NOR が新しく加わったものであり，これらの付加部分は他の端部着糸型染色体に由来する NOR と付随体の挿入によるものと推定し得る．Q-法による重複付随体の染色性から判定して，後者の仮説が現状においては前者よりも許容し得る．したがって，本研究でみられた重複付随体の起原は他の端部着糸型染色体からの NOR と付随体の挿入によって生じたものと暫定的に推察される．

１個の銀染色性 NOR をもつ変異体においては，Q-及び C-分染法による染色性は，各例別により個々

uniform through the enlarged area, though their individual intensities were different among the various cases. This finding suggests that the enlarged area may be derived from duplication of the original satellites or short arm rather than by translocation from another satellite or short arm.

In one of our chromosome variants, there was no Ag-stained material in the enlarged short arm region. De Capoa et al[17] observed two cases with 15p+, in which variant chromosomes had no morphologically recognizable satellite and secondary constriction, and were not stained by the N-banding technique. They concluded that these variant chromosomes did not possess the nucleolus organizer. The present chromosome variant in which no Ag-stained material was observed, also may not have had the nucleolus organizer.

It has been reported in a study of mouse-human somatic cell hybrids that only Ag-stained NORs produce rRNA and have functional activity as the nucleolus organizer in the preceding interphase.[18,19] It is thus conceivable that the variant chromosome without Ag-stained material described here may have Ag-negative NOR which lacks the functional activity of the nucleolus organizer. It seems likely that this variant chromosome may originally have had no Ag-positive NOR but had instead Ag-negative NOR. The banding patterns of the Q- and C-methods in the enlarged area were almost identical to those of the cases with double satellites, suggesting that this variant chromosome may also have been derived from the insertion of an Ag-negative NOR and satellite from some other acrocentric chromosome donor.

According to our tentative hypothesis concerning the origin of the enlargement of the short arm region, the nine variants studied here are classified into two groups: those in one group are assumed to arise by duplication and those in the other by insertion (Table 1). Of the four duplication types, only one is due to duplication of the short arm, while three are from duplication of satellites. The remaining five variants are assumed to be caused by insertion: four with double Ag-stained NORs, may have arisen by insertion of Ag-positive NOR and satellites from an acrocentric donor, and the fifth, characterized by the absence of Ag-positive NOR, may also have arisen from the insertion of satellites and Ag-negative NOR.

の染色性に違いがみられるが，増大部位にわたって一様であった．この知見は増大部位が他の付随体又は短腕からの転座というよりは，むしろ元来の付随体又は短腕の重複に由来することを示唆している．

本観察例中の１例には，増大短腕部位に銀染色性物質がなかった．De Capoa ら[17]は15p＋の２例において，変異染色体上に形態的に確認し得る付随体と二次狭窄がみられず，N-バンド法でも染色されないことを観察している．彼らはこれら変異染色体は仁形成体を有しないものと結論した．本研究において観察された銀染色性物質が見られない染色体変異もまた仁形成体を有しない可能性が考えられる．

マウス－ヒト体細胞雑種に関する研究から銀染色性NORのみがrRNAを産生すること，及び分裂期に先立つ間期において仁形成体としての機能をもつことが報告されている．[18,19] したがって，本報告に述べているような銀染色性物質を有しない変異染色体は非銀染色性NORを持っているため仁形成体としての機能が欠けている可能性があると考えられる．この変異染色体は初めから銀染色性NORを持っておらず，かわりに非銀染色性NORを持っていたということはあり得る．増大部位に対するQ-及びC-法による分染パターンは重複付随体を有する例とほぼ同一であり，他の端部着糸型染色体に由来する非銀染色性NORと付随体の挿入によって変異染色体が形成されたことを示唆している．

短腕部位増大の起原に関する我々の暫定的な仮説によれば，本報告中の９例は２群，つまり，前者は重複，後者は挿入によって生じたものに分類される（表１）．４例の重複型のうち，ただ１例が短腕の重複であり，他の３例は付随体の重複である．残りの５例は挿入に基づくものと想定されるが，４例の銀染色性NORの重複例では，他の端部着糸型染色体由来の銀染色性NORと付随体の挿入によって生じたものと思われ，最後の１例においてもまた，銀染色性NORがみられないという事実から，付随体と非銀染色性NORの挿入によって生じたものと思われる．

TABLE 1 SUMMARY OF THE RESULTS OBTAINED FROM NINE CHROMOSOME VARIANTS EXAMINED BY NOR STAINING METHOD

表 1　NOR 分染法による 9 例の染色体変異に関する要約

| Case | MF No. | Sex | Age | Involved chromosome No. | Size of NOR* | | Designation of variant | Origin** | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Proximal | Distal | | Type | Region |
| 1 | [redacted] | M | 28 | 14 | 3 | 3 | 14pss | ins | p12,p13 |
| 2 | | F | 29 | 22 | 2 | 2 | 22pss | ins | p12,p13 |
| 3 | | F | 28 | 13 | 1 | 3 | 13pss | ins | p12,p13 |
| 4 | | M | 22 | 22 | 1 | 1 | 22pss | ins | p12,p13 |
| 5 | | M | 29 | 15 | 2 | 0 | 15s+ | dup | p13 |
| 6 | | M | 26 | 22 | 2 | 0 | 22s+ | dup | p13 |
| 7 | | F | 20 | 15 | 2 | 0 | 15s+ | dup | p13 |
| 8 | | F | 29 | 15 | 0 | 3 | 15p+ | dup | p11 |
| 9 | | M | 26 | 14 | 0 | 0 | ?(14pss) | ?(ins) | ?(p12,p13) |

*3- large, 2- intermediate, 1- small, 0- very small or almost no NOR.
3－大，2－中間，1－小，0－極小又は NOR がほとんどない．

**Presumed origin and region of the enlargement of the short arm region.
ins- insertion, dup- duplication
短腕の増大部位及びその想定される起原．insertion ＝挿入　duplication ＝重複

In situ hybridization and filter hybridization studies have shown that the secondary constriction regions of acrocentric chromosomes contain DNA (rDNA) coding for 18S and 28S rRNA, and that the number of genes for rRNA on a given acrocentric is variable.[20-22] Further, it has been demonstrated that the regions stained by the silver staining method are the same as those identified by in situ hybridization with rRNA.[23,24] Recently, a 14p+ chromosome was examined by in situ hybridization experiments with radioactive 18S and 28S rRNA,[15] and the 14p+ chromosome was shown to have a large number of rRNA genes compared with the other acrocentric chromosomes. However, the results obtained using Ag-staining and association frequency indicate that this 14p+ chromosome had no greater nucleolus organizer activity than did the other acrocentrics, suggesting that not all the rRNA genes on the 14p+ chromosome are active.

It is worth noting that Lau et al[16] observed difference between N-bands and Ag-NORs on a 14p+ chromosome; N-banding stained the entire short arm of the 14p+, whereas $AgNO_3$ stained only the areas of secondary constriction. These authors suggest that N-banding and Ag-NOR staining are equally sensitive in the detection of NOR, but may stain different proteins of the NOR, and that if silver stains NORs which contain active ribosomal genes from the preceding

生体内雑種法及び沪過雑種法による研究から，端部着糸型染色体の二次狭窄は 18S 及び 28S rRNA をコードする rDNA を有すること，並びに端部着糸型染色体の rRNA 合成に関する遺伝子の数には変異のあることが示されている．[20-22] 更に，銀染色法で染色される部位は rRNA による生体内雑種法によって識別される部位と一致することが証明されている．[23,24] 最近，14p＋染色体について放射性 18S と 28S rRNA による生体内雑種法[15] によって研究が進められており，14p＋染色体が他の端部着糸型染色体に比べて多数の rRNA 遺伝子を有することが示されている．しかしながら，銀染色法と付随体連合の頻度から得た結果によると，この 14p＋染色体は他の端部着糸型染色体に比べて仁形成体活性が大きくないことが判明し，14p＋染色体上に存在する rRNA 遺伝子のすべてが必ずしも活性を有しているのではないことを示している．

Lau ら[16] の観察した 14p＋染色体の N-バンドと銀染色性 NOR との間のちがいは注目に値いする．N-分染法によれば 14p＋の短腕部位がすべて染め出されるのに対し，硝酸銀により二次狭窄部位のみが染色される．彼らは，N-分染法も銀染色法も，ともに NOR の識別には同様の感度を示すが，NOR の異なる蛋白質をそれぞれ染め出しているため，銀染色法が，分裂に先立つ細胞周期からの活性リボソーム遺伝子を含む NOR を染め出し，N-バンドは活性並

cell cycle, then N-bands represent both active and inactive regions of the NORs. The above reports suggest that variable staining patterns of the short arm regions of acrocentric chromosomes demonstrated by various banding methods may reflect not only variation in the amount of rDNA but also the functional state of the nucleolus organizer.

びに不活性NORの両方を示している可能性が考えられる．これらの報告例から考察すれば，各種の分染法による端部着糸型染色体の短腕部位における染色パターンの多様性はrDNAの量の変化のみならず，仁形成体の機能状態を反映していることを示唆するものである．

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