**Mutability of constitutive heterochromatin (C-bands)
during eukaryotic chromosomal evolution
and their cytological meaning**

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ABSTRACT

A quantitative analysis of the alterations of constitutive heterochromatin in eukaryotic chromosomal evolution was attempted using the accumulated C-banding data available for mammals, amphibians, fish, ants, grasshoppers, and plants. It was found that these eukaryotes could be classified into two types by their C-banding patterns: 1) Type I included mammals, fish, and ants, and 2) Type II included amphibians, grasshoppers, and plants. C-bands were rather scarce in Type I eukaryote chromosomes and were found around the pericentromeric region when present at all, whereas the predominance of interstitial or terminal C-bands was found in Type II eukaryote chromosomes. The Type I and II C-banding patterns can best be interpreted by assuming that in the former group of eukaryotes the saltatory increase in constitutive heterochromatin occurs preferentially at the pericentromeric regions of telocentric chromosomes induced by centric fission, with C-bands being eliminated almost completely by centric fusion and/or pericentric inversion. On the other hand, C-bands appear in the Type II eukaryotes both interstitially and in the telomeric regions of chromosomes, and there may be no effective mechanism to eliminate these bands once they are integrated.

1. INTRODUCTION

Constitutive heterochromatin, which is cytologically detectable as C-bands, is principally characterized by highly repetitive DNA sequences (satellite DNA). A number of hypotheses for functional roles of satellite DNA have been proposed in the 1960's and 1970's, for examples, resources of a new gene creation, chromosomal housekeeping, chromosome recognition in meiotic pairing, and regulation of recombination (for details see Sumner, 1990).

Since the sequence composition of satellite DNA is remarkably heterogeneous in most organisms, and since its phenotypic or evolutionary function is not yet clear, satellite DNA is often called "selfish DNA" or "parasitic DNA" (Doolittle and Sapienza, 1980; Orgel and Crick, 1980). There is, however, accumulating evidence that such DNA sometimes contains functional DNA, centromeres (it is usually localized in pericentromeric heterochromatin, the so-called alphoid DNA;

Pluta et al., 1990; Willard, 1990), telomeres (Meyne et al., 1990), or ribosomal DNA (Babu and Verma, 1987; Hadjiolov, 1985).

Constitutive heterochromatin is also mysterious at the cytological level, because C-bands are abundant and variable in amphibians and grasshoppers, but are rather scarce in mammals. The detailed review articles by John and Miklos (1979), and Verma (1988) indicate that the puzzle of constitutive heterochromatin is not yet solved.

I have taken note of a cytological characteristic of constitutive heterochromatin which is that, in spite of its substantial heterogeneity, it associates non-specifically in interphase nuclei, and have considered how such non-specific association could affect the chromosomal evolution of eukaryotes under the minimum interaction hypothesis (Imai et al., 1986).

According to this hypothesis, the centromere and the telomere provide the major frame for eukaryotic chromosomal evolution through their hammock structure (=suspension arch structure). The minimum interaction hypothesis assumes that an increase of satellite DNA should accompany the duplication of centromeres and telomeres, and that the constitutive heterochromatin in C-bands should involve multiple (inactivated) centromeres and (latent) interstitial telomeres (see Fig. 1 in Imai, 1988). As discussed in more detail later, there is some evidence for the existence of inactivated centromeres and especially for the presence of interstitial telomeres. The hypothesis also raises the question of whether constitutive heterochromatin increases infinitely without any selection pressure. However, this is unlikely since constitutive heterochromatin increases the genetic risk by increasing deleterious chromosomal interactions such as reciprocal translocation, and thus it is eliminated from the genome sooner or later by centric fusion or pericentric inversion (Imai et al., 1988a, b).

In the present study, C-banding patterns in various eukaryotes were analyzed on the basis of the karyological data accumulated during the last two decades.

2. CLASSIFICATION AND NOMENCLATURE OF C-BANDED CHROMOSOMES IN EUKARYOTES

A) Definition of basic categories (T, A, and $\overline{M}$)

In addition to several empirical groupings of chromosomal morphology (see Imai, 1973a), at least three well-defined systems have been proposed, i.e., the arm ratio system (Levan et al., 1964; Fig. 1a), the TAM system (Imai, 1978; Fig. 1b), and the $\overline{AM}$ system (Imai and Crozier, 1980; Fig. 1b). The first system is obviously arbitrary, while the others are based on the non-random localization of the centromere, which is defined by the percent length of the short arms (S) against the total chromosomal length of a haploid set (Imai, 1975, 1976). However, none of these systems is always appropriate for the description of the more complicated C-banding patterns of eukaryotic chromosomes. Accordingly, this

Fig. 1. Classification and nomenclature systems for chromosomal morphology. **a.** Levan's arm ratio (r) system. T: telocentric. A: acrocentric. ST: subtelocentric. SM: submetacentric. M: metacentric. $r = L/S$ (L and S indicate the sizes of the long and short arms). **b.** $\bar{A}\bar{M}$ and $T\bar{A}\bar{M}$ systems based on the non-random localization of the centromere ($=S$ value). $\bar{A}$: acrocentric in a broad sense ($T + A$). $\bar{M}$: metacentric in a broad sense, including ordinal M, SM, or ST chromosomes. **c.** The size of the whole chromosome ($C = S + L$). A^e : acrocentric with euchromatic short arms. A^M : pseudoacrocentric with an extraordinarily elongated heterochromatic short arm. $\bar{M}^h$ and A^h : metacentric and acrocentric with totally heterochromatic arms ($=H$ chromosomes). For details, see the text.

study used a modified $T\bar{A}\bar{M}$ system (Fig. 1c), which is explained below.

The sizes of the short arm, long arm, and whole chromosome are defined as S , L , and C , where $C = S + L$. S^e , L^e , S^h , and L^h stand for euchromatic short and long arms, and totally heterochromatic short and long arms. Chromosomes are grouped into three categories, i.e., chromosomes with (1) S^e and L^e , (2) those with S^h and L^e or S^e and L^h , and (3) those with S^h and L^h . To simplify the terminology, the euchromatic arms (S^e or L^e) and the heterochromatic arms (S^h or L^h) are abbreviated as E and H, respectively, so that the three chromosome types mentioned above can be represented as E chromosomes (Fig. 2a), EH chromo-

Fig. 2. Basic categories of chromosomal morphology. a. Chromosomes with euchromatic short and long arms (E chromosomes). b. Chromosomes with both euchromatic and heterochromatic arms (EH chromosomes). c. Chromosomes with heterochromatic short and long arms (H chromosomes). S^e and L^e : Euchromatic short and long arm. S^h and L^h : Heterochromatic short and long arm. A^e and A^M : Acrocentric with a euchromatic short arm and pseudoacrocentric. For details, see Fig. 1.

somes (Fig. 2b), and H chromosomes (Fig. 2c).

S^e describes the localization of the centromere of E chromosomes, with S^h being used for H chromosomes and S^h or L^h for EH chromosomes. T, A, and $\bar{M}$ chromosomes are defined by the terminologies E, H, S^e , and S^h as follows:

T, E chromosomes with $S^e=0$

A, EH chromosomes with $0 < S^h \leq 0.6$

$\bar{M}$, E chromosomes with $0.6 < S^e \leq C/2$

where T is an ideal product of centric fission (the details are discussed later), and A is synonymous with the $\bar{A}$ of the $\bar{A}\bar{M}$ system (compare Figs. 1b and 1c).

Besides these basic categories, the following chromosomes are special cases of the TAM system (Fig. 1c):

A^e , E chromosomes with $0 < S^e \leq 0.6$

A^M , EH chromosomes with $0.6 < S^h \leq C/2$ or $C/2 < L^h < C$

A^h , H chromosomes with $0 < S^h \leq 0.6$

$\bar{M}^h$, H chromosomes with $0.6 < S^h \leq C/2$

A^e is an acrocentric chromosome with a euchromatic short arm. A^M is the "pseudoacrocentric" introduced by Imai et al. (1988b), i.e., an acrocentric chromosome with extraordinarily elongated heterochromatic arms. A^h and M^h are often found in Y or B chromosomes.

These definitions of chromosomal morphology were found to be quite valuable for a quantitative analysis of mammalian karyotype evolution, the full details of which will be published elsewhere. It is, however, difficult to apply the TAM system directly to eukaryotes other than mammals (e.g., amphibians, fish, insects and plants), because there is no information available on the non-random localization of the centromere. To minimize such difficulties, this study used approximate definitions of A, A^M , A^e , and $\bar{M}$ for eukaryotes other than mammals, based on the width of the short (W_s) and long arms (W_l):

A, EH chromosomes with $W_s < W_l$ and S^h

A^M , EH chromosomes with $W_s = W_l$ or $W_s < W_l$ and S^e

A^e , E chromosomes with $W_s < W_l$

$\bar{M}$, E chromosomes with $W_s = W_l$

In mammals, more than 90% of the A and $\bar{M}$ chromosomes identified intuitively by cytogeneticists were respectively $S \leq 0.6$ and $S > 0.6$ (Imai, 1973a), and they were also respectively characterized by $W_s < W_1$ and $W_s = W_1$ (Imai, 1973b).

B) Terminology for C-banded chromosomes

Let c, i, and t be the C-band(s) located at the peri-centromeric, interstitial, and terminal regions of the chromosome arms (a similar idea was proposed by John et al., 1985). Using this "cit" nomenclature system, $\bar{M}$ chromosomes having C-band(s) at the pericentromeric, interstitial, and terminal regions in one arm (S or L) are represented as $\bar{M}^c$, $\bar{M}^i$, and $\bar{M}^t$, respectively. In the same way, $\bar{M}^{tt}$ means an $\bar{M}$ chromosome with C-bands at the terminals of both arms, and A^{Met} is a pseudoacrocentric chromosome (A^M) with C-bands at the pericentromeric and terminal regions. Various types of C-banded chromosomes observed in mammals and their mode of representation using this system are shown in Figure 3. To simplify descriptions, acrocentric (metacentric) chromosomes and their variants are denoted as the A group ($\bar{M}$ group). The A and $\bar{M}$ groups of mammalian chromosomes shown in Figure 3 involve 18 and 17 variant types, respectively. In the same way, A^M , A^i , A^e , $\bar{M}^i$, and $\bar{M}^t$ groups can be used if it is necessary. The A^M group would include A^M , A^{Mc} , A^{Mt} , A^{Mi} , etc.

Fig. 3. Schematic representation of C-banded chromosomes observed in mammals and their symbolic nomenclature. a. A group. b. $\bar{M}$ group. Each type of chromosome is defined by a combination of the basic categories of chromosomal morphology shown in Fig. 2 (A , A^e , A^M , A^h , $\bar{M}$, $\bar{M}^h$) and by the location of the C-bands (c; pericentromeric, i; interstitial, or t; terminal). To simplify matters, chromosomes with more than two interstitial C-bands are represented by one C-band (e.g., A^i , A^{Mi} , M^i , etc.). Chromosome types are arranged in the order of their frequency (see Table 1). For the detailed definition of each chromosome type, see the text.

3. SPECTRUM OF C-BANDING PATTERNS IN EUKARYOTIC CHROMOSOMES

A quantitative analysis of the C-banding patterns of some eukaryotes (mammals, amphibians, fish, insects such as grasshoppers and ants, and plants) was performed mainly on the basis of the C-banding data which have been accumu-

[illegible]

NB. G: Number of genera observed. Sp.: Number of species observed.

*: Unusually high frequent chromosome types.

Because of shorten the paper, full references cited in Tables 1-6 were omitted by the editor. For the full references request the author directly.

References cited. **MONOTREMATA:** Wrigley & Graves (1988). **MARSUPIALIA:** Casarelli et al. (1986), Eldridge et al. (1989), Hayman & Sharp (1981), Merry et al. (1983), Murray et al. (1980), Rofe & Hayman (1985), Schmid & Gattenbach (1988), Seluja et al. (1984), and Stock & Mengden (1982). **INSECTIVORA:** Harada & Takada (1985), Jiménez et al. (1984), Mandahl (1978), Raman & Nanda (1986), and Tada & Obara (1988). **CHIROPTERA:** Ando et al. (1980, 1983, 1987), Bickham (1979, 1987), Haiduk et al. (1981, 1983), Harada et al. (1982, 1987a, 1987b), Harada Uchida (1982), Harada & Yosida (1978), Hood & Baker (1986), Hood et al. (1988), Mortelle et al. (1988), Naidu & Primatees: Babu & Verna (1986), Barros et al. (1990), Bernstein et al. (1980), Dresser & Hamilton (1979), Friedrich (1978), Puthak et al. (1985), Schmid & Haaf (1984), Schmid et al. (1986), Schweizer et al. (1979), Shanyon et al. (1980), Tantravahi et al. (1975), and Yoshiida et al. (1983). **CETACEA:** Arnason (1980, 1987), Arnason & Widgren (1989), Arnason et al. (1980), and Stock (1981). **PERISSODACTYLA:** Graphodatsky (1988), Rong et al. (1988), Scott & Long (1980), and Trommershausen-Bowling & Millon (1988). **TUBULIDENTATA:** Pathak et al. (1982), Shi et al. (1982), Wada (1987), Ward et al. (1987), and Yoshiida et al. (1983). **ARTIODACTYLA:** Benirschke & Kumamoto (1987), Benirschke et al. (1980), Bianchi et al. (1990), Bongso & Hilmi (1982), Bruneir et al. (1974), Graphodatsky (1988), Herzog (1987), Melander & Hansen-Lemmer (1980), Pathak & Kieffer (1979), Pinheiro et al. (1980), Rubini & Fontana (1988), Schwarzacher et al. (1984), Wang & Du (1981), and Zartman et al. (1981). **RODENTIA:** Almeida & Yonemaga-Yassuda (1985), Baverstock et al. (1982), Brum-Zorrilla et al. (1986), Burgos et al. (1988a, b, c, 1989, 1990), Burton & Bickham (1989), Civitelli et al. (1989), Cothran & Honeycutt (1984), Deaven et al. (1977), Dev et al. (1977), Dowlir (1989), Elder & Lee (1985), Fredga et al. (1980), Greenbaum et al. (1986), Gropp et al. (1976), Gunn & Lee (1984), Kobayashi & Abe (1984), Kuffi et al. (1986), Lee & Elder (1980), Lee & Martin (1980), Markvong et al. (1975), Mascarello & Rogers (1988), Modi (1986, 1987), Murer-Orland & Richer (1983), Patton & Sherwood (1982), Popescu & DiPaolo (1979, 1980), Qumsiyeh (1986), Robbins & Baker (1981), Rodriguez et al. (1983), Sasaki et al. (1979), Schmid et al. (1984, 1986, 1988), Sbalquero et al. (1984), Smith et al. (1986), Souza & Yonemaga-Yassuda (1982, 1984), Stangl & Baker (1984), Traut et al. (1984), Vidal-Rioja (1985), Vidal-Rioja et al. (1988), Viegas-Pequignot et al. (1984), Vitullo et al. (1986), Wahrman et al. (1983), Walker et al. (1984), Yates et al. (1979), Yonemaga-Yassuda (1979), Yonemaga-Yassuda et al. (1985, 1988), Yong (1983), Yoshida & Kodama (1986), and Yoshiida (1980, 1985). **LAGOMORPHA:** Graphodatsky (1988), Kimura et al. (1983), Robinson (1980), Robinson et al. (1983, 1984), Ruedas et al. (1989), and Stock (1976).

Table 2. Frequency of chromosomes with various C-banding patterns in fish

A	A ^M	A ^t	A ^c	A ⁱ	A ^{Mc}	A ^{Mt}	A ^{it}	A ^{ct}	A ^{Mi}	Total
744	113	80	33	30	8	8	6	2	1	1025
39.26	5.96	4.22	1.74	1.58	0.42	0.42	0.32	0.11	0.05	54.08%

$\bar{M}$	$\bar{M}^c$	$\bar{M}^t$	$\bar{M}^{cc}$	$\bar{M}^{tt}$	$\bar{M}^i$	$\bar{M}^{ct}$	$\bar{M}^{ci}$	$\bar{M}^{it}$	$\bar{M}^{ctt}$	$\bar{M}^{ci}$	$\bar{M}^{itt}$	$\bar{M}^{cit}$	$\bar{M}^{citt}$	Total
636	82	57	33	24	17	10	2	2	1	1	1	1	1	870
33.56	4.33	1.74	3.01	1.27	0.90	0.53	0.11	0.11	0.05	0.05	0.05	0.05	0.05	45.91%

N.B. A total of 62 species including 39 genera were examined.
References cited. Almeida Toledo et al. (1988), Cau et al. (1988), Deiana et al. (1990), Feldberg et al. (1987), Foresti et al. (1989), Hafez (1981), Hartley (1989), Hartley & Horne (1984), Hong & Zhou (1985), Kitayama & Ojima (1984), Li et al. (1985), Lima & Galetti Junior (1990), Lopez et al. (1989), Majumdar & McAndrew (1986), Mufufushi et al. (1990), Ojima (1983), Ojima & Kikuno (1986), Ojima & Takai (1979), Ojima et al. (1984), Oliveira et al. (1988, 1990), Rishi & Mandhan (1990), Saitoh (1989), Sola et al. (1984), Takai et al. (1987), Takai & Ojima (1983, 1988a, b, 1990), Thode et al. (1985), Ueda & Ojima (1978, 1983a, b, 1984), Ueda et al. (1983, 1985), Uwa & Iwata (1981), Uwa & Ojima (1981), Vitturi et al. (1990), Wiberg (1983), Zan (1982), and Zenzen & Voiculescu (1975).

Table 3. Frequency of chromosomes with various C-banding patterns in ants

A	A ^M	A ^c	A ^{Mc}	A ⁱ	A ^t	A ^{Mt}	A ^{Mi}	A ^{ci}	A ^{ct}	Total
862	442	180	46	6	3	1	1	1	1	1544
32.20	16.51	6.72	1.72	0.22	0.11	0.04	0.04	0.04	0.04	57.68%

$\bar{M}$	$\bar{M}^c$	$\bar{M}^{cc}$	$\bar{M}^t$	$\bar{M}^{ctt}$	$\bar{M}^{ci}$	$\bar{M}^i$	$\bar{M}^{ct}$	$\bar{M}^{tt}$	$\bar{M}^{ci}$	$\bar{M}^{itt}$	$\bar{M}^{cit}$	$\bar{M}^{citt}$	Total
497	428	167	12	8	6	4	3	2	1	1	1	1	1133
18.57	16.99	6.24	0.45	0.30	0.22	0.15	0.11	0.07	0.04	0.04	0.04	0.04	42.33%

N.B. This table is based on the original C-banded karyotypes of 159 ant species, for which the chromosome numbers were reported in following papers; Goni et al. (1982), Imai et al. (1977, 1984a, b, 1985, 1988), Tjan et al. (1986), Imai & Taylor (1989).

Table 4. Frequency of chromosomes with various C-banding patterns in amphibians

A	A ⁱ	A ^M	A ^c	A ^{Mt}	A ^{ct}	A ^{it}	A ^t	A ^{Mi}	A ^{ci}	A ^{Mt}	A ^{Mc}	A ^{Mc}	A ^e	A ^{ct}	Total
70	27	18	10	8	7	6	5	2	2	2	1	1	1	1	161
3.62	1.40	0.93	0.52	0.41	0.36	0.31	0.26	0.10	0.10	0.10	0.05	0.05	0.05	0.05	8.32%

M	M ⁱ	M ^{it}	M ^{itt}	M ^t	M ^{cc}	M ^{tt}	M ^{ctt}	M ^c	M ^{ct}	M ^{ctt}	M ^{ct}	M ^{ci}	M ^{ctt}	M ^{cci}	M ^{ccitt}	M ^{ccit}	Total
444	394	195	193	104	85	78	51	48	45	30	29	19	16	14	5	4	1770
25.99	20.40	10.10	9.99	5.39	4.40	4.04	2.64	2.49	2.33	1.55	1.50	0.98	0.83	0.73	0.26	0.21	91.61%

N.B. A total of 130 species including 32 genera (Urodela 30 species and Anura 100 species) were examined.

References cited. **URODERA:** Baldwin & Macgregor (1985), Bucci-Innocenti et al. (1978), Herrero (1989), King (1990), Nardi et al. (1986), Ragghianti et al. (1978, 1980), Schmidt et al. (1979), Sessions (1980, 1982), Sessions & Kezer (1987), Sessions et al. (1982), Seto et al. (1986), and Sims et al. (1984). **ANURA:** Gao (1989), Green (1986, 1988), Green et al. (1987), Iizuka (1989), Iturra & Veloso (1989), King (1989, 1990), Lee & Park (1986, 1989), Li et al. (1982), Liu & Zan (1984), Matsui et al. (1985), Ruiz et al. (1981, 1982), Schmid (1978a, b, 1979, 1980a, b, 1982), Schmid & Almeida (1988), Schmid et al. (1983, 1985, 1986, 1987, 1988, 1990), Shang & Deng (1983), Veloso & Iturra (1987), and Wu et al. (1987).

Table 5. Frequency of chromosomes with various C-banding patterns in grasshoppers

A	A ^{it}	A ^t	A ⁱ	A ^c	A ^{cit}	A ^{ct}	A ^{ci}	A ^{ct}	A ^{ct}	A ^{ci}	A ^M	A ^e	A ^{Mi}	A ^{Mt}	A ^{Mt}
279	252	159	145	115	69	61	35	24	19	18	15	13	10	8	8
18.05	16.30	10.28	9.38	7.44	4.46	3.95	2.26	2.13	1.55	1.23	1.16	0.97	0.84	0.65	0.52

A ^{Mci}	A ^{Mc}	A ^h	A ^{Mct}	A ^{ect}	A ^{Mct}	A ^{eci}	A ^{ect}	A ^{ect}	A ^{ec}	Total
8	6	5	3	3	2	1	1	1	1	1286
0.52	0.39	0.32	0.19	0.19	0.13	0.06	0.06	0.06	0.06	83.15%

M ⁱ	M ^{it}	M	M ^{itt}	M ^t	M ^{ci}	M ^{ct}	M ^{ctt}	M ^c	M ^{cc}	M ^{cci}	M ^{ccit}	Total
75	61	26	25	18	15	10	9	7	5	1	1	260
4.85	3.95	1.68	1.62	1.16	0.97	0.65	0.58	0.45	0.32	0.06	0.06	16.80%

N.B. A total of 43 species including 37 genera were examined.

References cited. Arnold (1986), Arnold & Shaw (1985), Cabrero & Camacho (1985), Cabrero et al. (1985), Camacho & Cabrero (1983), Garcia de la Vega et al. (1986), Hota & Patnaik (1989), John & King (1980, 1982, 1983, 1985), John et al. (1985, 1986), King & John (1980), Sentis et al. (1986), Shaw et al. (1986), White et al. (1982), and Zhan et al. (1984).

Table 6. Frequency of chromosomes with various C-banding patterns in plants

A		A ^M	A ^{Mc}	A ⁱ	A ^{it}	A ^t	A ^{Mc}	A ^c	A ^{ci}	A ^e	A ^{ci}	Total
11	0.65	9	9	6	6	3	1	1	1	1	1	49
		0.53	0.53	0.35	0.35	0.18	0.06	0.06	0.06	0.06	0.06	2.89%

M ⁱ	M ⁱ	M ^{it}	M ^{itt}	M ^t	M ^{ci}	M ^{tt}	M ^{cc}	M ^{eci}	M ^{ecit}	M ^{cit}	M ^{ecitt}	M ^{citt}	Total
312	306	239	152	146	106	72	70	58	39	32	27	24	1647
18.40	18.04	14.09	8.96	8.61	6.25	4.25	4.13	3.42	2.30	1.89	1.59	1.42	97.12%

N.B. A total of 111 species including 34 genera were examined.
Reference cited. Aguiar-Perecin (1985), Aguiar-Perecin & Vosa (1985), Ashmore & Shapcott (1989), Azzioui et al. (1990), Bebeli & Kallsikes (1985), Cai et al. (1987), Chen & Yu (1989), Cortes et al. (1983), Deumling & Greihuber (1982), Dhaliwal et al. (1990), Dvorak (1983), Endo (1986), Evans & Fillion (1982), Fominaya et al. (1988), Friebe et al. (1987, 1990), Fujigaki & Tsuchiya (1990), Jouve et al. (1989), Gustafson & Bennet (1982), Hutchinson & Miller (1982), Jones & Flavell (1982), Kalkman (1984), Kenton & Jones (1985), Kenton et al. (1987), Kongsuwan & Smyth (1980), Konig et al. (1987), Konishi & Linde-Laursen (1988), Kota & Dvorak (1985), Lapitan et al. (1989), Li & Chen (1980), Liang (1988), Linde-Laursen (1988), Linde-Laursen & Bothmer (1986), Linde-Laursen et al. (1986a, b), Lukaszewski (1983), Mariani & Falstocco (1990), Morris & Gill (1987), Mouras et al. (1986), Nakamura et al. (1982), Noguchi (1986), Noguchi & Tanaka (1981), Owen & Larter (1988), Putter & Ban de Vooren (1988), Rousi & Arohonka (1980), Sato (1981), Sato & Yoshioka (1984), Seal (1982), Shang et al. (1989), Singh & Hymowitz (1988), Teoh & Hutchinson (1983), Teoh et al. (1983), Viinikka & Kavander (1986), Wang (1985), Wisudharom & Smyth (1985), Wu (1990), and Ziauddin & Kasha (1982).

lated during the last two decades. To minimize biases due to small sample sizes, the single best C-banded karyotype was taken as the standard of a given species group in which more than two species (or subspecies) shared an identical karyotype or very similar karyotypes. Also, only those chromosomes with a unique C-banding pattern were added to the standard karyotype as variants. This method will almost certainly lead to an overestimation of some minor variant chromosomes (A^i , A^t , $\bar{M}^i$, $\bar{M}^t$ etc.), but that is not likely to affect the major argument of the present paper. The results of the C-banding analysis are summarized in Tables 1–6, with the appropriate references cited.

The six eukaryotes mentioned above could be divided into two distinct types (I and II) by their chromosomal C-banding patterns, i.e., Type I (mammals, fish, and ants) and Type II (amphibians, grasshoppers, and plants).

Type I eukaryotes were characterized by a balanced appearance of A and $\bar{M}$ groups (56% and 44% in mammals, 54% and 46% in fish, and 58% and 42% in ants), and by the predominance of ordinal A and $\bar{M}$ chromosomes (20%–40%). On the other hand, Type II eukaryotes had remarkably unbalanced karyotypes, i.e., a metacentric-rich karyotype in amphibians ($\bar{M}$ group, 92%) and plants (97%), but an acrocentric-rich one in grasshoppers (A group, 83%). Despite such remarkable differences in their karyotypes, Type II eukaryotes were characterized by a high frequency (51%–64%) of A^i , A^t , $\bar{M}^i$, and $\bar{M}^t$ groups. In other words, constitutive heterochromatin (C-bands) was generally scarce in Type I eukaryote chromosomes and, if present, was mostly localized to the totally heterochromatic short arms of A and A^M chromosomes or the pericentromeric heterochromatin blocks of $\bar{M}$, A^c , $\bar{M}^c$, and $\bar{M}^{cc}$ chromosomes (Fig. 3). The only exceptions among Type I eukaryotes were Cetacea (Árnason, 1980), *Uromys caudimaculatus* (Rodentia, Baverstock et al., 1982), and *Callithrix emiliae* (Primates; Souza Barros et al., 1990) among the mammals. In contrast, C-bands were extremely abundant and scattered over the whole chromosome (especially at the terminals of the euchromatic arms) in Type II eukaryotes.

4. THEORETICAL ANALYSIS

A) Basic chromosomal mutations accompanying alterations of constitutive heterochromatin (C-bands)

To undertake a quantitative analysis of the highly complicated C-banding patterns found in eukaryotes, the present study adopted some new concepts regarding chromosomal rearrangements (centric fission, addition of constitutive heterochromatin, shift of centromeric activity, and telomere fusion) following the minimum interaction hypothesis (Imai et al., 1986; Imai, 1988).

Centric fission: Centric fission in the strict sense of the word was adopted only as an ideal rearrangement for changing $\bar{M}$ to T chromosomes (Fig. 4a). As is discussed shortly, centric fission was used here in the broad sense of changing $\bar{M}$

Fig. 4. Basic chromosomal rearrangements accompanying alterations of C-banding patterns. **a.** Centric fission in a strict sense (abbreviated as fis). **b.** Addition of constitutive heterochromatin (C^+). There are three categories of C^+ defined by the location of heterochromatin addition: C^{+c} ; pericentromeric (**e**), C^{+t} ; terminal (**b** and **f**), and C^{+i} ; interstitial (**g**). **c.** Shift of centromeric activity (sft) by centromeric inactivation and/or reactivation of a dormant (latent) centromere. **d.** Telomere fusion. There are three types of telomere fusion: HH fusion (so called centric fusion), EH fusion (tandem fusion), and EE fusion, where E and H are the euchromatic and heterochromatic arms. Telomere fusion induces deleterious dicentric chromosomes, but they can become stable by inactivating one of the two centromeres (sft). **h.** $\overline{A}\overline{M}$ inversion (a pericentric inversion changing A to $\overline{M}$). **i.** $\overline{M}\overline{A}$ inversion changing $\overline{M}$ to A. **j.** Paracentric inversion inducing multiple interstitial C-bands. The black parts of the chromosomes are constitutive heterochromatin and the centromeres are represented by the constrictions in the constitutive heterochromatin. For details of the chromosomal nomenclature and abbreviations, see Figures 1-3.

to A or A^M chromosomes via T chromosomes (Fig. 5). A possible molecular base of centric fission was proposed by Holmquist and Dancis (1979), and for its theoretical (speculative) background see Imai (1988). The crucial point regarding centric fission is to assume a priori the compound structure of the centromere involving multiple functional units as the spindle microtubule attachment.

The centromere has traditionally been recognized by cytogeneticists as simply the primary constriction. However, the recent accumulation of molecular data has provided us with a broader perspective on the centromere, as follows: (1) the centromere is composed of at least three structural domains (the kinetochore domain, the central domain, and the pairing domain), (2) each domain is a complex of alphoid DNA and centromere proteins (CENP-A and -C, CENP-B, and INCENP), and (3) the alphoid DNA of the central domain is a key component of

the centromere and is characterized by "tandem repeats" ($\sim 300 \rightarrow 5,000$ kb) of the 170 bp unit with a 17 bp motif, the so-called CENP-B Box (e.g., Brinkley, 1990; Masumoto et al., 1989a,b; Pluta et al., 1990; Rattner, 1987; Willard, 1990). The tandemly repeating structure of alphoid DNA is consistent with the repetitive nature of centromeres, as was suggested by Brinkley (1990) and Willard (1990). Indeed, the finding that both the deleted chromosome 17 and its derivative supernumerary fragment retained their mitotic function and possessed centromeric protein antigens strongly suggests that human centromeres are structurally and functionally repetitive (Wevrick et al., 1990).

So far, all the experimental data obtained have been quite consistent with the compound structure of the centromere given in the fission hypothesis, and an important point to note is that centric fission has actually been observed in many mammals, such as Chinese hamsters (Kato et al., 1973), humans (Sinha et al., 1972), Mauritian black rats (Yosida, 1980), Japanese raccoon dogs (Wada and Imai, 1991), zebras (Whitehouse et al., 1984), root voles (Fredga et al., 1980), donkeys (Trommershausen-Bowling and Millon, 1988), as well as in marsupials (Rofe and Hayman, 1985).

Addition of constitutive heterochromatin (C^+): The concept of "addition of constitutive heterochromatin" was previously proposed under the term "tandem growth of constitutive heterochromatin (t.g.c.h.)" (Imai, 1978), to describe the chromosomal alterations $T \rightarrow A$ or $T \rightarrow A \rightarrow A^M$ (Fig. 4b). The present paper uses the simpler term C^+ , modifying the " $C+$ " of Robbins and Baker (1981). There are three types of C^+ , which are distinguished by the locations of the C-bands incorporated into chromosomes as follows: C^{+c} (peri-centromeric), C^{+i} (interstitial), and C^{+t} (terminal). The term C^- is used for the (secondary) elimination of constitutive heterochromatin.

Concerning C^{+t} , it is noteworthy that a reasonable number of A^M chromosomes (ca. 6%–17%) were observed in mammals, fish, and ants (Tables 1–3). In these Type I eukaryotes, C^{+t} tends to occur preferentially at the pericentromeric region of T chromosomes after centric fission, and (as discussed later) it seems to be the major source for inducing A^i , $\overline{M}^i$, A^t , $\overline{M}^t$, A^c , and $\overline{M}^c$ chromosomes. On the other hand, C^{+t} occurs at euchromatic terminals in Type II eukaryotes, i.e., amphibians (King, 1990), grasshoppers (Shaw et al., 1976), and plants (Kenton and Jones, 1985), where A^t , $\overline{M}^t$ or $\overline{M}^{tt}$ chromosomes are induced directly from A or $\overline{M}$ chromosomes (Fig. 4f). This type of rearrangement is extremely rare in mammals, and *Uromys caudimaculatus* (Rodentia) is one of the few such cases (Baverstock et al., 1982). The same is true for the insertion of constitutive heterochromatin (C^{+i}) (Fig. 4g), i.e., it is rarely found in Type I eukaryotes (e.g., mice; Traut et al., 1984), but is common in Type II eukaryotes (the A^i group accounts for 38% in grasshoppers, and the $\overline{M}^i$ group for 39% in amphibians and for 58% in plants) (Tables 4–6).

The molecular mechanisms of C^+ are still virtually unknown, but there are

some possible candidates, e.g., so-called unequal crossing-over (Ohno, 1970; Smith, 1974, 1976), the two step model by gene amplification and multiplication (Walker, 1971), telomere-telomere recombination (Wang and Zakian, 1990), or the de novo synthesis of DNA from an RNA template as occurs in telomere synthesis by telomerase (Greider and Blackbaum, 1989). In any case, C^{+t} seems to be closely related to telomeric instability, as suggested by Imai et al. (1988a).

Shift of centromeric activity (sft): Sft is used as a general term indicating the inactivation and reactivation of centromeric activity. According to the fission hypothesis, the de novo appearance of telomeres and centromeres after centric fission is inevitable. For this reason, it has been proposed that the totally heterochromatic arms of A or A^M chromosomes elongated by C^+ should contain multiple "dormant telomeres and centromeres" (for details see Fig. 1 in Imai, 1988). Quite recently, this model was provided with a strong experimental basis when it was shown that the telomeric sequence $(TTAGGG)_n$ is distributed abundantly in non-telomeric sites of vertebrate chromosomes, especially in the pericentromeric heterochromatin of $\bar{M}$ chromosomes and in the totally heterochromatic short arms of A chromosomes (Meyne et al., 1990; Moyzis et al., 1988; Wurster-Hill et al., 1988).

The "centromere shift" and "de novo formation of centromeres" found in the ants *Myrmecia (pilosula) n=1* (Imai and Taylor, 1989) and *Myrmecia (piriventris) HI85-302* (Imai et al., 1988b) also suggest the presence of multiple dormant (or inactivated) centromeres in C-bands. Inactivation of centromeres after centric fusion or tandem fusion is another piece of evidence supporting the present model (Daniel, 1979; Daniel and Lam-Po-Tang, 1976; Nakagome et al., 1984). This phenomenon is highly consistent with the finding that only one kinetochore appears in dicentric or multicentric chromosomes in mouse cells (Rattner and Lin, 1985; Zinkowski et al., 1986). Thus, inactivation and reactivation of centromeres can be assumed to be one of the basic characteristics of chromosomes with localized centromeres, and to be the mechanism by which A^c or A^{Mc} chromosomes arise from A^M chromosomes (Fig. 4c).

Telomere fusion: The concept of telomere fusion was proposed by Hsu et al. (1975), and they used the terms centromere-centromere (C-C) translocation, centromere-telomere (C-T) translocation, and telomere-telomere (T-T) translocation in their report. Recently, Imai et al. (1988a) proposed a different nomenclature system for telomere fusion using the term E (euchromatic arms) and H (heterochromatic arms), so that the three types of telomere fusion mentioned above were respectively designated as HH fusion, EE fusion, and EH fusion (Fig. 4d). The present study used the EH nomenclature system, because "C" and "T" have previously been assigned to designate C-bands, the size of whole chromosomes ($C=S+L$), or telocentric chromosomes (T).

HH fusion is synonymous with so-called centric fusion and the other types correspond to the category of ordinal tandem fusion. These rearrangements have

long been considered to be unstable, because they induce dicentric chromosomes (White, 1973). Dicentric chromosomes, however, can become stable following inactivation of one of the two centromeres (sft, Fig. 4d). Indeed, there are a number of reports of stable centric fusion and tandem fusion produced by the inactivation of centromeres (e.g., Bongso and Hilmi, 1982; Cheung et al., 1990; Lau and Hsu, 1977; Murata and Orton, 1984; Nielsén, 1976; Vig, 1988; Warburton et al., 1973; Yonenaga-Yassuda, 1979).

Sherthan (1990) proposed that the occurrence of preferential tandem fusions in the Indian muntjac (ca. 20 independent events) could completely eliminate pericentromeric heterochromatin (C-bands). There is, however, evidence that most of the tandem fusions (EH fusions) observed accompany the insertion of C-band(s) as a vestige of the pericentromeric heterochromatin of one of the two chromosomes. For more details regarding tandem fusion see Imai (1988) and Imai et al. (1988a).

Inversion: Four types of pericentric inversion (AA , $A\bar{M}$, $\bar{M}A$, and $\bar{M}\bar{M}$ inversion) are theoretically possible, and these were previously designated as p.i. (AA), p.i. ($A\bar{M}$), p.i. ($\bar{M}A$), and p.i. ($\bar{M}\bar{M}$) (Imai and Maruyama, 1978). Among them, $A\bar{M}$ and $\bar{M}A$ inversion are essentially important to the present arguments. The former changes an A to an $\bar{M}^t$ chromosome with a terminal heterochromatin cap (Fig. 4h), and the latter produces an A^e from an $\bar{M}$ chromosome (Fig. 4i). The possibility of $A\bar{M}$ inversion was first pointed out in ant chromosomes by Imai et al. (1977), and supporting evidence was observed in a Japanese lung fluke (*Paragonimus ohirai*; Hirai et al., 1981). Deer mice (*Peromyscus*) will hopefully provide us with additional substantial evidence for the occurrence of $A\bar{M}$ inversion, because their karyotypes evolve mostly by pericentric inversion and because all the types of chromosomes (A , A^M , $\bar{M}^t$ and $\bar{M}$) expected to arise from $A\bar{M}$ inversion can actually be observed in these animals (Deaven et al., 1977; Greenbaum et al., 1986; Hale, 1986; Hale and Greenbaum, 1988; Robbins and Baker, 1981). In situ hybridization of the human chromosome 1 using biotin-labeled telomeric and centromeric probes may also be an attractive method of obtaining cytological evidence of $A\bar{M}$ inversion (Dekken and Bauman, 1988).

The preferential occurrence of sister chromatid exchanges (SCEs) between the euchromatin-heterochromatin junction (Kato, 1979) may be an important mechanism for eliminating the terminal heterochromatin cap of $\bar{M}^t$ chromosomes. Paracentric inversions (Fig. 4j) may also induce A^i or $\bar{M}^i$ chromosomes with complicated interstitial C-bands, such as those found in grasshoppers (John and King, 1983; Shaw et al., 1988).

B) Chromosomal alterations in Type I eukaryotes

Following the definitions of chromosomal mutations given by Imai (1988), the present study used the terms centric fission (Fig. 5a), centric fusion (Figs. 5b and 5c), and $A\bar{M}$ inversion (Figs. 5d and 5e) "in a broad sense" for combinations of the

Fig. 5. Major chromosomal mutations contributing to or referred to in the discussion of eukaryotic chromosomal evolution. **a.** Centric fission in a broad sense. **b** and **c.** Centric fusion in a broad sense (HH fusion). **d** and **e.** $\overline{A}M$ inversion in a broad sense. Elimination of constitutive heterochromatin is complete in **b** and **d**, but incomplete in **c** and **e** (i.e., accompanying incorporation of C-bands). For details of the chromosomal nomenclature and abbreviations, see Figures 1–4. Arrowheads indicate break points or exchange sites.

Fig. 6. Chromosomal alteration networks in mammals. The primary network: Bold arrows (>18%) or solid arrows (10%–17%). The secondary network: Broken arrows (1%–9%). The tertiary network: Dotted arrows (<1%). Minor chromosomal types (<1%) are represented by small alphabets. Each network was classified principally based on the frequencies of the chromosomal types involved. The primary network corresponds to the fusion-fission and fission-inversion cycles by Imai et al. (1988a, b). The chromosomal types involved in the primary and secondary networks cover more than 92% of mammalian chromosomes. For details of the chromosomal nomenclature and abbreviations, see Figures 1–5.

basic chromosomal mutations shown in Fig. 4. This section will show that these three rearrangements have essentially contributed to the karyotype evolution of Type I eukaryotes (mammals, fish, and ants).

The morphological alterations of C-banded chromosomes observed in mammals (Table 1) and the chromosomal mutations concerned are shown in Figure 6; these are designated as the chromosomal alteration network. To simplify its description, this network is subdivided into three categories principally based on the frequencies of the chromosomal types involved—the primary network represented by bold arrows (>18%) or solid arrows (10%–17%), the secondary network (broken arrows, 1%–9%), and the tertiary network (dotted arrows, <1%).

The primary network includes T, A, $\overline{M}$, and $\overline{M}^t$ chromosomes. They can transform into each other by centric fission ($\overline{M} \rightarrow T \rightarrow A$), centric fusion ($A \rightarrow \overline{M}$), or $A\overline{M}$ inversion ($A \rightarrow \overline{M}^t \rightarrow \overline{M}$), where T and $\overline{M}^t$ chromosomes are the intermediate products. The primary network is essentially identical with the fission-inversion and fission-fusion cycles that have been proposed as the major mechanisms of chromosomal evolution in mammals and ants (Imai, 1988; Imai et al., 1988a, b). The evidence that A and $\overline{M}$ chromosomes appear most frequently (37% and 29%), and that the potential inflow from other networks ($\overline{M}^c \rightarrow A + A^c$ by centric fission and $A^M \rightarrow \overline{M}^t$ by $A\overline{M}$ inversion) is at most 4%–8% indicates the overwhelming predominance of the primary network in mammalian chromosomal evolution.

The secondary network includes the chromosomes A^M , A^c , A^{Mc} , $\overline{M}^c$, $\overline{M}^{ct}$, and $\overline{M}^{cc}$. Although their interactions are rather more complicated than those of the primary network, they are mostly derived from A^M chromosomes by centric fission, centric fusion, or $A\overline{M}$ inversion. Therefore, the dynamic balance of the secondary network is principally dependent on the frequency of $A \rightarrow A^M$ alterations by C^{+t} . The frequency of A^M chromosomes is 7.7% in mammals, and that of the other types ranges between 5% and 1% (Table 1). There are some similarities in the patterns of chromosomal alteration between the primary and secondary networks. For example, the sequence $A \rightarrow \overline{M}^t \rightarrow \overline{M}$ in the primary network corresponds to $A^M \rightarrow \overline{M}^{ct} \rightarrow \overline{M}^c$ in the secondary network, except for the partial incorporation of constitutive heterochromatin in the latter process (compare Figs. 5d and 5e). Note that the chromosomal types involved in these two networks cover more than 92% of mammalian chromosomes.

The chromosomes A^i , A^t , A^{it} , A^{Mi} , A^{Mt} , A^{ci} , A^{ct} , A^{cit} , A^{Mci} , A^{Mct} , A^{Mcit} , A^e , A^{ec} , A^h , $\overline{M}^i$, $\overline{M}^t$, $\overline{M}^{it}$, $\overline{M}^{tt}$, $\overline{M}^{itt}$, $\overline{M}^{ci}$, $\overline{M}^{cit}$, $\overline{M}^{ctt}$, $\overline{M}^{cct}$, $\overline{M}^{cctt}$, $\overline{M}^{eci}$, $\overline{M}^{ecit}$, and $\overline{M}^{ecitt}$ are the components of the tertiary network. They are interpretable as occasional derivatives of the primary or secondary networks mainly by C^+ , and their frequencies are extremely low in mammals (less than 1%) (Table 1; Fig. 6). In this network, the A^e chromosome is theoretically induced from $\overline{M}$ by $\overline{M}A$ inversion (Fig. 4i), and this has been actually observed in Lagomorpha (Robinson et al., 1984). However, the extremely low frequency of A^e chromosomes (0.18%) could be taken as supporting evidence for the rare occurrence of $\overline{M}A$ inversion in mammalian

chromosomal evolution, which has been theoretically proposed by Imai and Maruyama (1978). This means in other words that pericentric inversion shows a strong directionality, with $\overline{A}\overline{M}$ inversion occurring much more frequently than $\overline{M}A$ inversion.

Chromosomes with interstitial C-bands (A^i , A^{Mi} , $\overline{M}^i$, $\overline{M}^{it}$, etc.) are shown in Figure 6 as the end products derived from A , A^M , $\overline{M}$, $\overline{M}^t$, etc. by C^{+t} , paracentric inversion, or so-called tandem fusion (EH fusion) (Fig. 4d). Since i-bearing chromosomes comprise at most 1.41% ($\overline{M}^i$) and mostly less than 1% of mammalian chromosomes, it appears that these rearrangements (especially tandem fusion) have seldom contributed to mammalian chromosomal evolution.

Fig. 7. Chromosomal alteration networks in fish.

Fig. 8. Chromosomal alteration networks in ants.

These proposals mentioned for mammals are also largely applicable to fish (Fig. 7, Table 2), and to ants (Fig. 8, Table 3) with minor modifications. Most species of fish appear to have acrocentric karyotypes ($2K=48A$) and $\overline{AM}$ inversion seems to predominate over centric fusion or centric fission, though further accumulation of C-banding data is needed to confirm this. On the other hand, the fission-inversion cycle operates more effectively than the fusion-inversion cycle in ants (Imai et al., 1988b), and the frequent appearance of A^M , A^c , and $\overline{M}^c$ chromosomes (members of the secondary network in mammals) is another characteristic in this insect.

Thus, it may be safe to conclude that centric fission, centric fusion, and $\overline{AM}$ inversion in a broad sense (Fig. 5) appear to have played an essential role in the chromosomal evolution of Type I eukaryotes. On the contrary, tandem fusion accompanying C-band insertion and $\overline{MA}$ inversion inducing A^e chromosomes may be less important rearrangements in this group than has previously been thought. The available C-banding data for birds, though further studies are needed, suggest that they are also Type I eukaryotes (Belterman and De Boer, 1984; De Boer and Sinoo, 1984; Schmid et al., 1989).

C) Chromosomal alterations in Type II eukaryotes

The chromosomal alteration networks of amphibians, grasshoppers, and plants are summarized in Figures 9–11. These networks are prominently different from the Type I eukaryote network (Figs. 6–8) with regard to two points: (1) the low level of chromosomal alterations between A and $\overline{M}$ group chromosomes (the primary network in Type I eukaryotes), and (2) the extraordinary predominance of A^i , $\overline{M}^i$, A^t , and $\overline{M}^t$ groups induced by C^{+i} or C^{+t} (Fig. 4). Such essential differences may have originated partly from karyotype conservation, in that the

Fig. 9. Chromosomal alteration networks in amphibians.

form A^t chromosomes ($A-(C^{+t})\rightarrow A^t$) (e.g., Shaw et al., 1976). A^t chromosomes are induced in Type I eukaryotes from A chromosomes by $\overline{AM}$ inversion and centric fission ($A-(\overline{AM} \text{ inv})\rightarrow \overline{M}^t-(\text{fis})\rightarrow A+A^t$). The direct elongation of terminal C-bands ($\overline{M}\rightarrow \overline{M}^t$) has been observed in some amphibians (*Litria*), where C-bands involve NORs (King, 1990). Remarkable terminal C-bands ($\overline{M}^{tt}$) are rather common in plants, e.g., *Allium cepa* (Kalkman, 1984) and *Secale cereale* (Owen and Larter, 1988), and also in some animals such as *Parascaris univalens* (Goday and Pimpinelli, 1986), spiders (*Delena cancerides*, Rowell, 1985), and reptiles (Moritz, 1984). Such terminal C-bands seem to have an important role in the construction of so-called Renner complexes in plants (Kenton et al., 1987).

D) Centric fusion and $\overline{AM}$ inversion as mechanisms eliminating constitutive heterochromatin in Type I eukaryotes

As mentioned in the previous sections, centric fission in a broad sense accompanies the addition of constitutive heterochromatin (C^+ , Fig. 5a), which is the main mechanism for increasing constitutive heterochromatin in Type I eukaryotes (especially mammals and ants). On the other hand, both centric fusion (Figs. 5b and 5c) and $\overline{AM}$ inversion (Figs. 5d and 5e) eliminate constitutive heterochromatin (C^-). These two types of rearrangements are therefore antagonistic and form a chromosomal alteration cycle, designated as the fission-fusion and fission-inversion cycles (Imai et al., 1988a,b), which correspond to the primary network in Type I eukaryotes (Fig. 6–8).

Figure 12 indicates alternative models of karyotype evolution for Type I eukaryotes. Both models start from a hypothetical haploid karyotype with $1\overline{M}$, and proceeds (as a whole) towards increasing the number of chromosomes by centric fission or $\overline{AM}$ inversion in a broad sense (Fig. 5) following the minimum interaction hypothesis, with centric fusion occurring as an occasional back eddy. These models ignore paracentric inversion, tandem fusion, and the insertion of constitutive heterochromatin (C^{+i}), because they contribute little to karyotypic evolution, as mentioned in the previous sections.

The first model assumes that constitutive heterochromatin is incorporated into chromosomes principally by C^{+t} after centric fission, and is completely eliminated by centric fusion or $\overline{AM}$ inversion. Only A or $\overline{M}$ chromosomes are expected to occur in this model (Fig. 12a). On the other hand, the second model assumes the a priori elongation of terminal C-bands in $\overline{M}$ chromosomes (i.e., $\overline{M}^{tt}$), and that constitutive heterochromatin is never eliminated once it is incorporated. In this model, all chromosomes sooner or later develop complicated C-banding patterns (e.g., A^t , A^{ct} , $\overline{M}^{tt}$, $\overline{M}^{cctt}$ etc.) (Fig. 12b).

The karyotypic evolution of Type I eukaryotes (mammals, fish, and ants) seems to be rather more consistent with the first model (Fig. 12a). Since this model corresponds to the primary network in Figures 6–8, it explains about 70% of the chromosomal alterations found in mammals (Fig. 6, Table 1) and fish (Fig.

Fig. 12. Three models for karyotypic evolution with (a and c) or without (b) the elimination of constitutive heterochromatin (C^-) according to the minimum interaction hypothesis. Each model starts from the haploid karyotype, $K=1\bar{M}$, and increases chromosome numbers and arm numbers mainly by centric fission or $A\bar{M}$ inversion, with the reduction of chromosome numbers by centric fusion (fus) occurring as a local event. **a.** Addition of constitutive heterochromatin (C^+) occurs mainly at the pericentromeric region after centric fission, and C-bands incorporated into chromosomes are completely eliminated again by centric fusion or $A\bar{M}$ inversion accompanying C^- . **b.** Elongation of C-bands occurs at euchromatic terminals as well as at the pericentromeric region, and C-bands are never eliminated after incorporation. **c.** This model is similar to "a", except that C-bands of A^M are occasionally incorporated into chromosomes as A^c , A^{ct} , $\bar{M}^c$, $\bar{M}^{ct}$ or $\bar{M}^{cc}$. The black parts of the chromosomes indicate constitutive heterochromatin (C-bands) and constrictions in the C-bands indicate the centromeres. The Type I eukaryotes (mammals, fish, and ants) fit the third model (c). On the other hand, Type II eukaryotes (amphibians, plants, and grasshoppers) do not fit any of these models, since C^+ occurs at euchromatic terminals or interstitially and C-bands once incorporated into chromosomes seem to be difficult to eliminate by centric fusion or $A\bar{M}$ inversion.

7, Table 2), but only about 50% of those noted in ants (Fig. 8, Table 3). The secondary network can also eliminate constitutive heterochromatin from A^M chromosomes by centric fusion or $A\bar{M}$ inversion (Figs. 5c and 5e), though the efficiency of elimination is lower than that of the primary network because of the formation of A^c or $\bar{M}^c$ or $\bar{M}^{ct}$ chromosomes. The secondary network affects roughly 20% of the C-banded chromosomes in mammals and 40% in ants. A modified scheme is shown in Figure 12c incorporating both the primary and secondary networks, which covers more than 90% of the C-banded chromosomes seen in mammals, fish, and ants.

These series of arguments suggest that centric fusion and $\overline{AM}$ inversion both play an important role in eliminating constitutive heterochromatin in Type I eukaryotes (a kind of "splicing" at the cytological level), whereas they operate far less effectively in Type II eukaryotes (Figs. 9–11, Tables 4–6). This persistent elimination of constitutive heterochromatin may be an evolutionary advantage, because it releases chromosomes from the struggle to inactivate excessive centromeres and interstitial telomeres integrated into their structures by C^+ after centric fission. In addition, it will reduce the risk of deleterious chromosomal interactions (reciprocal translocation) resulting from the nonspecific association of constitutive heterochromatin in interphase nuclei, as proposed by the minimum interaction hypothesis (Imai et al., 1986). This may be the answer to the question of why so many centric fusions have been realized during the karyotypic evolution of some eukaryotes, especially mammals, e.g., mice (Gropp et al., 1972; Capanna et al., 1976) and humans (see the chromosomal catalogue by Borgaonkar, 1984).

However, this conclusion does not support the revival of the radical fusion hypothesis, because centric fusion is "in the long run" a local or instant solution for eliminating C-bands in the main current (i.e., centric fission and $\overline{AM}$ inversion) to minimize the genetic risks.

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