DIRECTIONAL EVOLUTION: A NEW HYPOTHESIS

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ABSTRACT: The genome is considered as a teleological system capable of adaptive response to environmental stimuli which induce differential underreplication or amplification of genes. The underreplication of repetitive DNA is functional in controlling the onset of cell differentiation and the timing of gene activities. Ageing is conceived as an outcome of underreplication of repetitive genes interfering with the chromosome behavior, competitive exculsion of recessive alleles reducing homeostasis, and heterochromatization affecting chromosome replication pattern and pairing. The limitation of reproductive age is related to an age-associated decrease of recombinational repair and rectification of repetitive genes and also to distortion of the sex ratio in the offspring. At least intermittent sexual reproduction is needed to eliminate the destabilizing affect of allelic competition in ageing clones. The amplification of repetitive genes may promote autonomous proliferation of tumor cells. The amplified repeats induce breakage, provide insertion sites for viruses and act as episome-like elements when excised. Environmentally induced changes of the repetitive DNA content result in heterochronies which give rise to major evolutionary novelties. This process affects also the developmental succession of isozymes, causing retention of juvenile isozyme patterns in adults. Amplification of preferentially active genes explains lasting modifications and orthogenesis.

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INTRODUCTION

In the current evolutionary paradigm, a population of organisms is recognized as a teleological system (i.e., a system capable of adaptive response to environmental signals). Among subordinate systems, an organism has transient individual adaptability (modification) which is not transmitted to its offspring, while the genome possesses no teleological qualities at all and mutates occasionally. However there are no philosophical grounds for the belief that teleology should arise at the population level only. In individual development, the genotype behaves as a teleological system. Historically, the molecules of genetic material might have originated as protoorganismic aggregates, independent of their current function. They have subsequently developed intricate superstructures - phenotypes - to increase their adaptability in unpredictable environments. Davis (1978) has shown that Fisher's theorem of natural selection is applicable to heterogenous populations of replicating polymers. It is reasonable to assume at least residual adaptability at the genotype level, manifested through individual development as well as from generation to generation. This assumption is not Lamarckian, because Lamarckists claimed external, not internal teleology (there was also a most odous power-seeking group using pseudo-Lamarckian language and therefore labelled neo-Lamarkian, though they had nothing in common with either Larmarck or science in general). In fact, the concept of the genotype as a teleological system, propounded by Weismann and lately by Williams (1966), can be viewed as an extension of the Darwinian principle.

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The genotype can be envisaged as a population of various cistrons performing their metabolic and reproductive functions - transcription and replication. The environmental stimuli affecting transcription cause adaptive shifts in the differential reproduction of genes. Because survival of genes depends on the fitness of their carriers, the genomic adaptations are usually advantageous for the phenotypes. Sometimes they interfere with phenotypic viability or fitness. There is where conventional natural selection intervenes.

The unorthodox concept of directional evolution of the genome, supported by some recent achievements of molecular biology, encourages the causal analysis of many hitherto largely unexplained phenomena, such as the regulation of gene activity in development, ageing, carcinogenesis, and origin of the evolutionary novelty. This concept allows also a revaluation of many morphological, embryological, and paleontological observations linking developmental and evolutionary processes. These observations have found no place in the synthetic evolutionary theory and have been put aside without much indication of future study.

GENETIC CHANGES DURING DEVELOPMENT

According to the text-book notion, every somatic cell carries the same full complement of chromosomes and genes. The cloning technique based on this assumption is partially successful in producing embryos from transplanted nuclei of somatic cells. However the genome is largely inactive at the onset of embryogenesis. The genes controlling early differentiation are most essential and evolutionary conservative. Their change during development is unlikely, especially in somatic cell genomes which have passed through only a few mitotic cycles. The notion that a full-grown individual can be produced from a nucleus of each somatic cell is implausible, because it is well known that cells become less pluripotent with age (see Clowes,1961) The organs which are shed and established anew from the same meristematic tissues, such as leaves, antlers, or the exoskeletons of crustaceans, show directional morphological changes (Severtsov, 1939).

There is a growing body of studies suggesting directed genetic changes during development. The genome of higher organisms consists of unique and repeated sequences. The latter form more or less highly repetitive DNA consisting of the genes for ribosomal RNA, transfer RNA, 5S RNA, and histones, as well as the essentially non-transcribed redundant or satellite sequences localized in the pericentromeric, telomeric, and intercalary heterochromatin regions, and the intermediate repetitive DNA, which is mostly interspersed between unique sequences. The repetitive DNA, rich in adenine and thymine, is mainly slow-replicating. In the human genome there is about 6 per cent of the fast-replicating intermediate DNA and 30 per cent of slow repeated sequences finely interspersed with non-repeated units (Cinelli and Corneo, 1976). The slow-replicating DNA is potentially liable to under-replication. The most evident changes during development affect the reiterated sequences. Heterochromatin is drastically under-replicated in polytene nuclei (Rudkin, 1965). There are two types of chromocentric heterochromatin - alpha and beta, distinguished as relatively dense and diffuse areas. While the alpha type, forming centromeric and pericentric heterochromatic regions, is transcriptionally and replicatively inactive, the beta type is transcribed and replicated to the same extent as the euchromatin (Lakhotia, 1974). It is suggested that the polytene nuclei do not require a kinetochore because they never re-enter the mototic phase. The intercalary heterochromatin is also under-replicated during polytenization (see Barr and Ellison, 1972). According to Leibovitch (1977), even the unique sequences which are transcriptionally inactive in a given cell type or tissue are underrepresented in the polytene chromosomes. This situation shows the interdependence of transcription and replication.

The ribosomal cistrons (rDNA) located within the alpha heterochromatin behave differently from it. They replicate during polytenization, though to a lesser extent than euchromatin. Underreplication of rDNA is a widespread phenomenon,

resulting in delayed development and an inviable phenotype (reduced bristle or bobbed phenotype in Drosophila).

Detectable changes during development occur in the immunoglobulin genes. The light and heavy chains of immunoglobulin molecules consist of variable (V) and constant (C) regions. In embryonic cells, the V and C genes are separated by intercalary sequences while in lymphocytes they are joined. Hozumi and Tonegawa (1976) have supported the excision model of DNA change during differentiation of lymphocytes. Alternatively, the underreplication of intercalary repetitive DNA can be responsible for the joining of V and C genes and simultaneous activation of V. Only one allele is expressed in any given lymphocyte. Hozumi and Tonegawa have suggested the loss of one chromosome followed by reduplication of its homologue. The segregation of alleles in lymphocytes can be related also to the conversion or competitive exclusion of alleles (see below).

The relative amount of repetitive DNA undergoes more or less evident changes in embryogenesis of many organisms (e.g., in Cyclops: Beerman, 1977; for reviews see also Yunis and Yasmineh, 1971; Tobler et al., 1972). Heterochromatic chromosomes or segments can be lost, as in Ascaris megacephala. Euchromatization is a characteristic feature of early embryogenesis. According to Nur (1967 and subsequent studies), euchromatization is best manifested in male mealy bugs (Homoptera)

Woodcock and Sabatani (1975) have found underreplication of intermediate repeats and possibly also adjacent unique sequences in larval Drosophila DNA and supported earlier suggestion of Kaufmann (1946) that the intercalary heterochromatin represents regions of underreplication in the chromosome arms. They cited many cases of DNA loss during development (in chironomids, Ascaris, etc) as well as of DNA increase in specific chromosome bands in Sciara and Rhynchosciara. At the same time they acknowledged only one well-established case of specific gene amplification, the rDNA magnification associated with the bobbed phenotype, while no amplification of the structural genes for globin or ovalbumin has been shown in the tissues producing large amount of these proteins. However, the experiments of Strom et al. (1978) have shown tissue-specific amplification of regulatory and structural genes during differentiation of chicken cartilage and neural retina. Alt et al. (1978, cited in Strom et al., 1978) have revealed amplification of structural genes in cultured mouse cells. Perlman et al. (1976) showed differences in the DNA sequences of various cells in Xenopus. These studies are consistent with earlier cytological observations of variable amount and appearance of heterochromatin in different adult tissues.

The functional role of underreplication of repetitive DNA during development can be many-sided. The deletions of the ribosomal RNA and transfer RNA cistrons cause a decreased number of cells per organ, delayed development, and retention of fetal features (see Yunis and Yasmineh, 1971). It is conceivable that underreplication of these cistrons affects the repression of mitotic spindle formation and the onset of cell differentiation.

The underreplication of the histone genes may be essential in regulating transcription rates at many loci. The redundant sequences may function in (1) chromosome behaviour during cell cycles, and (2) timing of successional gene activities.

Barr and Ellison (1972) reviewed earlier works on ectopic pairing (that is, pairing between non-homologous positions along the chromosomes) and confirmed that AT-rich bands were involved. Conventional pairing of mitotic and meiotic chromosomes depends on both the large blocks of heterochromatin and the interspersed repetitive sequences. Heterochromatin segments can cause aggregation of chromosomes, forming chromocentres. According to Yunis and Yasmineh (1971), the pairing through heterochromatin is an important initial step in synapsis. It was suggested by Stern and Hotta (1969) and other authors reviewed in Burnham et al. (1972) that synaptic pairing, instead of following the classical "zipper" model, occurs at selected sites specified as the short late-replicating sequences distributed along the chromosome. Elastic ligatures between homologous regions,

suggested by Maguire (1974), may also be related to the interspersed repeats.

Chiasmic pairing is rare or absent in the heterochromatic regions (John and Lewis, 1965; see also Macgregor and Andrews, 1977; Jones, 1978). This is considered to be a device for protecting the rDNA and other reiterated genes from crossing over (Yunis and Yasmineh, 1971). However, the differences between replication rates may cause breaks and pairing of non-sister chromatids at the junctions of fast and slow replicating segments (see Subrahmanyam, 1977; Doyle, 1978). Yamamoto and Miklos (1978) have shown that the amount and position of recombination on each heterochromatically deleted X chromosome is substantially different from that of a normal X. Further evidence of the effect of heterochromatin on recombination came from the studies of supernumerary chromosomes, or B chromosomes, consisting mainly of heterochromatin. It is known that B's enhance recombination of normal or A, chromosomes in maize, rye, wheat, etc. There is also a dosage effect, for three B's cause more enhancement than one. Ward (1973) has confirmed experimentally that only heterochromatic segments of B's affect recombination. Rhoades and Dempsey (1972) have suggested that B's cause longer meiotic cycles with therefore more time being available for recombination. However, the enhancing effect of B's is clearly related to their interaction with heterochromatic regions of A chromosomes, which often lose their heterochromatic knobs by the latter sticking to the heterochromatin of B's. An abnormal A chromosome 10 (K10), with additional heterochromatic segment in the long arm, can also enhance recombination by interaction with a knob on chromosome 9 (Nel, 1973).

To summarize, the process of recombination depends on the alternation of segments with different replication rates along the chromosome. It is affected by centromeric heterochromatin either through interaction with interspersed repetitive sequences or through interstitial translocations of the kind described by Hoehn and Martin (1973). Changes of chiasma frequencies would then be associated with parallel changes in the amount of satellite DNA. An age-associated decrease of chiasma frequencies in mice was first described by Crew and Koller (1932) and confirmed by subsequent observations reviewed in Luthardt et al. (1973), wallace and MacSwiney (1976), and King and Hayman (1978). Henderson and Edwards (1968) have shown that the changes in chiasma frequency are due not to terminalization (i.e., loss of once-formed chiasmata) but rather to genuine decrease along the "production line" of oocytes. Speed (1977) has suggested that chiasma frequencies are responsive to nutrient gradients. King and Hayman (1978) observed seasonal variation of chiasma frequency in reptiles. They argued that environmental factors, especially temperature, were more important than putative internal control. However, the temperature and other environmental factors may act through their effect on internal control, that is, on repetitive DNA. Rees and Naylor (1960) and Couzin and Fox (1974) revealed developmental variation of chiasma frequency in anthers of rye and tulip. Meiosis in the pollen-mother cells proceeds in a wave away from the vascular bundle, and chiasma frequencies increase in the same direction. In the development of tulip anthers, this process is associated with an increase of total chromosome lengths. It appears that in this case the chiasma frequencies are directly related to amplification of (presumably repetitive) DNA.

The role of interspersed repetitive DNA in regulation of transcription has been discussed by Britten and Davidson (1969), Georgiev et al. (1974), Holliday and Pugh (1975), Korochkin (1977), and other authors. It was suggested that regulation is achieved by simultaneous formation of loops containing identical repeats or by a suppressive effect of the short-lived RNA transcribed from intermediate repeats. Kaufmann and Iddles (1963) and later Zuckerkandl (1976) have postulated a controlling function of AT-rich bands in gene activity, expressed cytologically by puffing (see also Dickson et al., 1975). My suggestion is that the interspersed repeats suppress transcription of adjacent structural genes or batteries of structural genes (depending on the organization of genome), possible by attracting histone particles. Because these interspersed units are slow-replicating, they are systematically underreplicated in the course of successive mitoses and their effect on adjacent structural genes decreases (presumably due to release of histones and repatterning of nucleosomal structure). After a number of mitoses, at a certain stage of

underreplication of repeats, a structural gene becomes accessible to a transcribing enzyme. In this way the interspersed repeats might regulate the succession of gene action by counting the preceding mitoses. In the case of immunoglobulin, the underreplication of repeats between V and C genes might turn on V. Another consequence of underreplication is the creation of a new gene by joining two non-repetitive units.

Position effect in relation to larger blocks of heterochromatin is expressed in sequential gene activity (e.g., in Saccharomyces: Tauro et al., 1968; Mortimer and Hawthorne, 1973) and the modified activity of genes causing variegation (see review by Quirós, 1976). The effect of heterochromatin is felt also in the diachronous DNA synthesis in heteromorphic chromosome pairs observed by Latt (1975), Lau et al. (1977), and other authors.

According to the chromosome field theory (lima-de-Faria, 1976 and earlier work), the positions of genes are specified with respect to the prime organizers - kinetochore and telomere. Amplification of telomere heterochromatin causes displacement of genes maintaining constant relation to the prime organizers. The kinetochore serves as a reference point in the chromomere-size and replication-gradient fields. The increase of satellite DNA is usually conceived as occasional (amplification in a single chromosome during meiosis and subsequent spread to others). However, in the light of the chromosome field theory, parallel amplification in both homologues appears more probable, the more so in that recombination in heterochromatic regions is arrested.

Directed genetic change during development comprises also such processes as gene conversion, magnification, dosage compensation, and segregation of alleles. Gene conversion, or the change of a recessive allele to its dominant alternative during development, is regulated by transposible elements. The models of conversion are reviewed in Brink (1973), Birky and Skavaril (1976), and Kitani (1978). The regulatory elements have been compared to bacterial episomes, but there are important differences between them (see Doerschug, 1976). These elements appear rather like excised segments of repetitive DNA affecting gene expression and mitotic recombination at the points of their reintegration into the chromosome.

In the case of DNA deficiency, normal synthesis of rRNA can be restored by the process called magnification and ascribed to unequal sister-chromatid exchange (Ritossa, 1968, 1973; Graziani et al., 1973; Tartof, 1975). Because in Drosophila melanogaster sister-chromatid exchanges do not occur in the heterochromatic regions (Dolfini, 1978), it seems more plausible that magnification is due to parallel amplification of ribosomal cistrons in both sister chromatids (see Meyer and Hennig, 1974; Tartof and Dawid, 1976).

A regulatory compensation of rRNA synthesis also occurs, evidently on the same basis as the dosage compensation of sex-linked genes in males. Khesin and Leibovitch (1974) have used the single X chromosome from male larvae of Drosophila melanogaster as a template for RNA synthesis by Escherichia coli polymerase. The effect of dosage compensation in this heterologous system is the same as in the intact cells. Thus regulatory genes in the autosomes and Y chromosome are not involved in the dosage-compensation increase of RNA synthesis, which can rather be related to competition of genes for transcribing enzyme. The competition of homologous genes in the two X chromosomes of females might result in a decreased level of RNA synthesis in each of them and also in more exact tuning of gene activities in general - increased developmental homeostasis of females. Schwartz (1971) has developed a model for regulation of gene activities by competition of alleles. A number of studies (reviewed in Eanes, 1978) confirmed an effect of heterozygosity on developmental homeostasis. Competition in a heterozygous system would result in less active transcription and underreplication of a recessive allele and the dosage-compensatory activation of a dominant allele. One can assume competitive exclusion of recessive alleles through a number of mitoses.

In the case of sequential segregation of alleles (e.g., during differentiation of lymphocytes) and isozyme transition the outcome of competitive interaction between alleles evidently depends on intercellular environments. The ontogeny of

lactate dehydrogenase is a paradigm example of directional shift in the isozyme pattern induced by the oxygen supply, the state of cell differentiation, and the physiological function of muscular contriction (Market, 1965; Morris et al., 1976; Kolombet, 1977; Frenkel and Hart, 1977).

The segregation of alleles and the homogenizing processes of conversion and competitive exclusion of recessive alleles may be responsible for disruption of developmental homeostasis in ageing organisms and the fading of heterosis in the course of vegetative propagation. Darlington (1958) has observed variation among vegetative propagules persisting over subsequent cycles of vegetative propagation. Breese et al. (1965) as well as Shimamoto and Hayward (1975) have shown that heritable variation among vegative propagules is due to genetic segregation which is age-dependent: only young clones segregated, while the old ones, maintained by vegetative propagation, did not. The decline of synthetic varieties was related to segregation. These studies show actual significance of sex in maintaining developmental homeostasis.

AGEING AND CARCINOGENESIS

Senescence is often attributed to an accumulation of defects (somatic mutations) disrupting the genetic program. The accumulation of defects, instead of being erratic, can be related to the directional developmental DNA changes outlined above, i.e., the underreplication of repetitive DNA, heterochromatization of inactivated structural genes, and competitive interaction of alleles. Individual life histories can be conceived as cyclical, beginning with euchromatization due to heterochromatin losses and activation of structural genes and ending in heterochromatization associated with decreased activity.

The occurrence of similar ageing of paramecia and human cells having different genome organizations (Rodermel and Smith-Sonneborn, 1977) suggests general regularities of senescence. Though ageing in multicellular organisms may not be reducible to local failures (Rosen, 1978), it is undoubtedly associated with an increasing number of non-functional cells. It was shown by Hayflick (1965), Holliday and Tarrant (1972) and Orgel (1972) that human somatic cells have a limited *in vitro* lifespan, inversely related to the age of the donor. Schneider and Mitsui (1976) reported a statistically significant decrease in the onset of cell-culture senescence, *in vitro* lifespan, and cell population replication rates of cultures derived from old donors.

A theory of ageing as a programmed process has been expounded by Krooth (1974), Berdyshev (1977), and other authors. The appearance of enzymes with altered properties in ageing cells both in vitro and in vivo (reviewed in Krooth, 1974) has led to assumption that ageing is related to isozymic shifts during development. As suggested above, developmental homeostasis can be disrupted by the homogenizing effect of allelic competition. The isozymic shifts can also be related to allelic competition in the changing genetic environment. It is affected by transition from proliferation to differentiation of cells, as exemplified by the LDH isozymes (Frenkel and Hart, 1977). According to Wada (1974), the RNA released from nucleoli has a critical importance for spindle formation and cell division. He pointed to degeneration of nucleoli in ageing cells. Nucleolar volumes are known to be proportional to the number of rDNA copies per nucleolar-organizer region (Miller and Knowland, 1970). It is conceivable that the underreplication of rDNA during successive mitoses can suppress proliferation of cells. Vilenchik (1970) postulated a decrease of rRNA synthesis with age, and Muradian (1977) observed 3-or 4-fold decrease of the rRNA-to-mRNA ratio in post-natal rat liver cells. However in old rats this ratio suddenly increased (due to malignant transformation of cells?) Lezhava (1977) reported a decrease of RNA synthesis in the centromeric region of A_1 in humans over eighty. In this age category he established also considerable change of intercalary heterochromatin due to heterochromatization. The age-associated heterochromatization and increase of histone H1 content was shown also by Khilobok et al. (1977). Indirect evidence of the functional role of highly repetitive DNA in promoting cell prolifera-

tion is provided by a significant correlation between longevity and Y-chromosome lengths (see Cherkasskaya, 1977).

Cell senescence is associated with aneuploidy (Luthard et al., 1973; Jacobs et al., 1976), single-strand breaks (Price et al., 1971; Chetsanga et al., 1977) and decreasing repair capacity (Little, 1976). All these phenomena can be explained by satellite-DNA losses and/or heterochromatization. Satellite DNA ensures proper behaviour of chromosomes in mitosis and meiosis. The spindle-fiber proteins are attached at the centromere and at heterochromatic knobs. The firmness of attachment and the centromere strength depends on the amount of satellite DNA (Walker, 1971). Therefore underreplication of satellite DNA would affect centromere potency and the separation of homologues, causing non-disjunction and aneuploidy. Other sources of aneuploidy are related to the role of the centromeric heterochromatin and interspersed repetitive sequences in aggregation of chromosomes, synapsis, and chiasmic pairing (see above). Underreplication interferes with these processes, predisposing chromosome losses. Heterochromatization can also add to aneuploidy through its effect on the replication rates. A mechanism of chromosome losses in this case can be perceived by analogy with aneuploidy in Triticale. In this hybird plant (wheat x rye), the rye chromosomes often form univalents and contribute to aneuploidy because they have more telomeric heterochromatin than the wheat chromosomes and fail to accomplish replication before the prophase of meiosis (Thomas and Kaltsikes, 1974).

Luthard et al. (1973) have discussed the relation of aneuploidy to the age-associated decrease of chiasma frequency. The decrease of chiasmata is accompanied by a significant increase of univalents, preferentially among small chromosomes, which have fewer chiasma. Though aneuploidy does not inevitably follow, at least some of the univalents may be lost. The effect of maternal and paternal age in chromosome anomalies is discussed by Penrose (1933), Polani (1969), Zeuten et al. (1973 and other authors. The increase of aneuploidy in women 45-64 years old due to sex-chromosome loss is associated with decreased sexual activity (Jacobs et al., 1976). These observations probably reflect more general association of aneuploidy with inactivation and concomitant heterochromatization.

Other genetic events associated with senescence - chromosome breaks and decreasing repair capacity - can also be related to heterochromatization. Since heterochromation affects replication rates, heterochromatization would disrupt the replication patterns, causing breaks. This suggestion is supported by association of extra heterochromatin with multiple breaks in Drosophila (Baimai, 1975, 1977). Hatch et al. (1976) related the high proportion of metacentrics in kangaroo rats to extra heterochromatin. They concluded that chromosome rearrangements were controlled by satellite DNA. Recently Yoon and Richardson (1978) have observed clusters of breaks at intercalary heterochromatin sites, producing chromosome rearrangements, in Hawaiian species of Drosophila.

The decrease of repair activity in ageing cells indicates that DNA damage becomes less accessible to repair enzymes. The access of repair nucleases to damaged sites depends on the nucleosomal structure of chromatin: excision repair is rapid in the linker DNA regions while in the core particles, or nucleosomes, it is hampered by histones. Repair is most active in proliferating cells (Cleaver, 1978). The heterochromatization process conceivably affects repair by altering the replication rates and/or because of the DNA condensation in the linker filaments. The effect of repetitive DNA interspersion on repair was discussed by Kram et al. (1972).

Ageing affects the genotype of progeny primarily by increasing chromosome anomalies. Decreased recombination diminishes genetic variability. Less obvious effects are related to recombinational repair and rectification of repetitive genes. Bernstein (1977) has suggested that meiotic recombination is essentially a repair process conserving the germ line. Amaldi et al. (1973) have postulated that all reiterated gene families are established anew at each meiosis: each of the repetitive segments is excised in the form of a circular monogenic DNA molecule, which replicates and then reintegrates into the chromosome. It is known also that ageing has some effect

on the sex ratio of offspring. The following mechanism can be suggested. A sperm with more heterochromatin in its genome is more fit in the sense that it preferentially fertilizes the egg. In maize, a sperm carrying supernumerary B chromosomes usually fertilizes the egg, while its sister sperm without B's unites with the polar nuclei (Roman, 1947, 1948; Carlson, 1969, 1973). In man, a sperm carrying a Y chromosome is evidently more fit but as the underreplication of heterochromatin progresses, its fitness decreases. It follows that limitation of reproductive age may have an even more important stabilizing effect than is usually conceived.

Carcinogenesis contrasts with ageing in its vigorous cell poliferation, but there are also many points of resemblance. Both are associated with aneuploidy, chromosome breakage, rearrangements, and decreased repair activity (Tiepolo and Zuffardi, 1973; Berghe et al., 1977; Marx, 1978). These similarities suggest similar causes, that is, changes of repetitive DNA. According to the above hypothesis, the different classes of repetitive DNA control the onset of cell differentiation and the timing of structural gene activities. Wada (1974) has argued that viruses and other exogenous agents causing cell lesions are responsible for preconditions of cancer only, while carcinogenesis itself depends on continuous mitotic spindle formation as a prerequisite of cell division. He postulated two conventional categories of genes - the spindle-formation inducers (SFI) and repressors (SFR). SFR deficiency in stem cells causes unrestricted proliferation without entering the differentiated state. It seems likely that spindle-fiber formation is induced by rRNA, and possibly also by other non-translated RNA's transcribed from the repetitive DNA, which can function as SFI or SFR depending on the number of repeated copies

Renaturation kinetic studies show that repetitive DNA is sensitive to various agencies, such as growth media, temperature, viruses and increased activity (see Romanov and Vanyushin, 1977). Sudden changes of DNA redundancy in certain cell lineages may disrupt the timing of cell differentiation and give rise to autonomous tumor growth. The amplication of interspersed repeats would alter nucleosomal structure, resulting in a restriction of both transcription (see Flickenger et al., 1973) and repair (Marx, 1978; Cayama et al., 1978). The amplified slow-replicating DNA would affect the pattern of chromosome replication, causing breaks and also providing the insertion sites for viruses which induce additional breaks (see Yunis and Yasmineh, 1971). If excised, the amplified repeats may act as episome-like transposible elements involved in malignization.

At lease some characteristics of carcinogenesis support these suggestions. Mutagenic and paramutagenic effects of variations in the redundancy of repetitive DNA have been demonstrated in plants and animals (see Brink, 1973; Phillips et al., 1974). Atkin and Baker (1977) have related the pericentric inversions associated with cancer to heterochromatin changes.

Sachs (1978) ascribed reversions of malignancy of leukaemic cells to transposible elements. Anderses (1978) has shown that neoblastomas in fishes are caused by a gene (Tu) present on different chromosomes in several copies. Tu is located terminally on sex chromosomes and can be dose-compensated by the autosomal Tu when deleted. These features are suggestive of amplified repetitive-DNA segments. Tu possesses also some virus-like properties. According to Gateff (1978), the mutant genes causing tumors in Drosophila are distributed over the entire genome and involve developmental genes which control crucial events during differentiation of cell types or tissues. Many virus-like particles occur in the nuclei of all neoplastic cells. She concludes that "tumor genes in Drosophila represent nothing more than fundamental developmental genes which, during normal development, affect the proliferation rates and the differention of cells" (l.c., p.1458).

These considerations suggest the normalizing of repetitive DNA content as a prerequisite of cancer therapy. It is known that in maize the B chromosomes are more effective when in odd numbers, while they neutralize each other when in pairs. The amplification of satellite DNA may also be neutralized by injections of complementary amounts of the repetitive DNA fractions.

EVOLUTION

Developmental and phylogenetic aspects were intimately related in early evolutionary concepts such as the "laws" of K. von Baer, E. Haeckel, and E. Cope. It was assumed that the heterochronies - acceleration, retardation, telescoping, and repatterning of developmental stages - are responsible for evolutionary novelties. According to the orthogenesis concept of Th. Eimer (1898) the differences between closely related species were analogous to those between successive developmental stages. A theory of evolution affecting allometric growth rates (with paedogenic mutations as an extreme case) as developed by Garstang (1928), Bolk (1926), Goldschmidt (1940), De Beer (1951), and later by Romer (1972), Takhtajan (1972), Gould (1977), and other authors.

It was hypothesized above that changes in the repetitive DNA content affect the timing of cell differentiation, causing heterochronies. Increase or decrease of the intermediate repetitive units seems to be related also to homoeotic mutations, the shift of certain cells into different developmental pathways (see Ouweneel, 1976). The analogous environmentally induced shifts - homoeotic phenocopies may have a similar cause.

These suggestions are supported by (1) experimental induction of repetitive DNA changes, (2) variability of DNA redundancy in natural populations, associated with variation in developmental rates and morphology and (3) differences in amount of repetitive DNA between races and closely related species.

Durrant (1962) discovered environmental induction of heritable changes in flax (Linum usitatissimum), variety Stramont Cirrus. The application of nitrogen and phosphorus to plants grown in a heated greenhouse caused 3- to 6-fold changes in the plant weight. The large (L) and small (S) forms have been maintained for many generations, showing stable inheritance irrespective of the subsequently applied soil nutrients. It was found also that after 5 weeks of induction L and S diverged in nuclear DNA content, L having 16 per cent more DNA than S (Evans et al., 1966). Under low temperatures the DNA content gradually reverted toward that of the original form. This process could be stopped at any selected level of the DNA content by heating in a greenhouse (Joarder et al., 1975). Callis (1975) and Timmis and Ingle (1973, 1974) have shown that the intermediate repeats were preferentially affected, though the amplification of some unique sequences was also suspected.

A mechanism comparable to the environmental induction in flax can be assumed in the case of temperature-induced modifications in Drosophila melanogaster. Cavicchi et al., (1978) have described modifications of wings due to rearrangements of developmental patterns. These modifications are heritable and progressive over several generations. Svetlov and Korsakova (1974) have shown heritable adaptive response of the temperature-sensitive forked mutants to repeated heating.

Processes similar to the saltational creation of genetically distinct forms of flax, though possibly longer than in five weeks, may occur in nature. Considerable variation in the number of rRNA genes and other repetitive gene families has geen observed in plants (Flavell and Mohan, 1973; Maher and Fox, 1973), Drosophila (Meyers and Henning, 1974), amphibians (Miller and Brown, 1969), mice (Henderson et al., 1976), and man (Evans et al., 1971). Forejt (1973) described a polymorphism of centromeric heterochromatin in demes of the house mouse. He suggested that in this case the polymorphism was maintained either because of neutrality of occasional repetitive DNA amplifications or because of a high frequency of amplification events. The constancy of centromeric heterochromatin in laboratory inbred strains is rather against the hypothesis of occasional mutations. There is ample evidence of environmental induction of repetitive DNA changes, which are by no means neutral, though the phenotypic expression varies. Schroeter and Hewitt (1974) discussed the adaptive effect of additional heterochromatin causing earlier emergence in grasshoppers and faster see germination in Allium. Considerable divergence in the emergence time, growth rates, metamorphosis, germination, anthesis, and other developmental events has been observed among local populations of various organisms. Such variation associated with changes in repetitive DNA can be seen as incipient speciation.

No allozyme differences were found among island populations of Anolis lizards diverging in body size and coloration (Gorman and Kim, 1975). These and similar observations agree with the conclusions of Bernardi (1976), Mukai and Cockerham (1977), and other authors that fast adaptive shifts are due to repatterning of the repetitive DNA. Differences in DNA redundancy have been described between races of grasshoppers, rodents, and man (John and King, 1977; Baverstock et al., 1976; Lubs et al., 1977) and among closely related species in plants (Maher and Fox, 1973), Drosophila (Laird and McCarthy, 1969), fishes (Narayan and Rees, 1977; Vladychenskaya and Kedrova, 1977), Xenopus (Buongiorno-Nardelli et al., 1977), salamanders (Macgregor and Jones, 1977), and rodents (Ginatulin et al., 1977).

The relative amount of repetitive DNA diminishes in polyploids and unisexual forms (Maher and Fox, 1973, Cimino, 1974). In transspecific evolution, decrease of DNA redundancy in both plants and animals is associated with specialization trends (see El-Lakany and Dugle, 1972; Pathak and Worster-Hill, 1977) and with a transition from long-period to short-period interspersion (Crain et al., 1976), which can be related to more elaborate regulation of developmental processes.

The acceleration or retardation of ontogenic processes caused by the changes of repetitive DNA may affect also the developmental succession of isozymes, resulting in retention of juvenile isozyme patterns in adults. At least some genetic polymorphisms revealed by the electrophoretic technique may be due to paedogenic fixation of one or another successional isozyme in different populations (see Nair et al., 1977). Isozyme spectra can be affected by competitive exclusion of recessive alleles during individual development (see above). Gene conversion is another hitherto underestimated factor in population genetics (Gutz and Leslie, 1976). These homogenizing processes oppose heterotic selection.

The increased activity of a structural gene due to ecological shifts may have important evolutionary consequences. The experiments with behaviorally "enriched" rats (see Wallace, 1974; Kazakhashvili, 1974) have shown that transcriptional activity of certain genes, as measured by the RNA-to-DNA ratio in the brain cells, is sensitive to environmental factors and behaviour. Because replication is related to transcription, one can assume that less actively transcribed genes are systematically underreplicated and eventually become extinct, while the replication of more active genes is accelerated The environmental stimuli affecting transcription of certain genes would induce also adaptive changes in replication and amount of genetic material. In microorganisms, the use of new substrates is usually achieved by constitutive synthesis of inducible enzymes, which requires joined mutations of structural and regulatory loci (see review in Clarke, 1974). The frequently observed coincidence of such mutations shows that they are related to altered activity of these loci. Inderlied and Mortlock (1977) have shown that in Klebsiella aerogenes xylotol stimulates the selective duplication of a gene coding for ribitol dehydrogenase, which promotes faster growth on the substrate. Perlman and Strickgold (1977) have described the induction of selective amplification of genes for sesistance to antibiotics on Proteus mirabilis. These and other analogous observations suggest that genetic constitution can be altered not only by stochastic events and selection of phenotypes, but also by the directional evolution of genes.

In the case of polygenic characters, the effect of each pair of genes can easily be modified by environment. If modification is maintained by constant application of certain environmental agents, the preferentially activated genes would undergo amplification, making the modification irreversible or at least lasting a number of generations. This mechanism would explain some of the "Dauermodificationen", first described by Jollos (1913), as well as the genetic change in the direction of environmentally induced modification (the mysterious "Baldwin effect" of early genetic writings). Orthogenetic trends described by paleontologists (e.g., Plate, 1920) possibly belong in the same category of evolutionary phenomena. They can also be related to orthogenetic changes of chromosome morphologies (Lima-de-Faria, 1976; Imai, 1976) and parallel evolution of families of repetitive gene (Tartof and Dawid, 1976; Buongiorno-Nardelli et al., 1977).

CONCLUSIONS

The genome is envisaged as a population of nucletide sequences capable of direct adaptive response to environmental stimuli in the form of differential reproduction - underreplication or amplification - of genes. The genomic adaptations are usually advantageous not only for the genes, but also for their carriers, and play an important role both in ontogeny and evolution.

Underreplication and amplification of both regulatory and structural genes has been observed during polytenization and for ribosomal and immunoglobulin genes, embryogenic DNA losses, and cell differentiation in vivo and in vitro.

The underreplication of repeated cistrons may be functional in controlling the onset of cell differentiation and the rates of transcription at many loci. Redundant DNA is essential for the formation of chromocentres and for ectopic, synaptic, and chiasmic pairing, which depends on the alternation of segments with different replication rates along the chromosome. The centromeric heterochromatin enhances breakage and chiasmic pairing by interaction with interspersed repetitive sequences. The decrease in chiasma frequency with age may reflect progressive underreplication of satellite DNA.

The interspersed repetitive sequences may suppress transcription of adjacent genes, possibly by attracting the histone particles. These sequences are systematically underreplicated in the course of successive mitoses until the structural genes are turned on. Thus the timing of gene activities is regulated by counting the preceding mitoses.

The underreplication of interspersed repeats may result also in the creation of a new gene by joining the unique sequences initially separated by these repeats. The excised repetitive segments may act as transposible elements and cause gene conversion.

The dosage compensation of underreplicated cistrons is related to competitive interaction of alleles. The competition of alleles for transcribing enzyme is essential for adjusting gene activities. It is assumed that the competitive exclusion of a recessive allele and the compensatory activation of a dominant one interfere with developmental homeostasis in ageing organisms and clones. At least intermittent sexual reproduction is needed for maintaining developmental homeostasis.

Ageing is conceived as an outcome of (1) the underreplication of repetitive DNA repressing cell division and weakening centromere potency, (2) the competitive exclusion of recessive alleles interfering with homeostasis, and (3) heterochromatization of inactive genes affecting chromosome replication pattern and pairing. These processes result in aneuploidy and increased repair failures and breakage. The decrease of chiasma frequency with age affects recombinational repair and the rectification of reiterated genes. The sex ratio in the progeny of ageing parents can be distorted by decreasing fitness of sperms carrying heterochromatic chromosomes. The limitation of reproductive age has an important stabilizing effect.

Carcinogenesis resembles ageing in important ways. The amplified repetitive genes may act as spindle-formation inducers, encouraging autonomous proliferation of tumor cells. The amplification of interspersed repeats affects both transcription and replication, causing breaks and/or providing the insertion sites for viruses. The excised amplified repeats act as episome-like elements. Normalizing of the repetitive DNA content is suggested as a prerequisite of cancer therapy.

Major evolutionary novelties are due to heterochronies caused by changes of the repetitive DNA content. The latter are induced by environmental factors both experimentally and in nature. The saltational creation of morphologically and genetically distinct forms of flax can be seen as incipient speciation, and many natural populations and species differing in developmental rates and adaptive morphological traits show high polymorphism of the redundant DNA.

Heterochronies affect developmental succession of isozymes. The retention of juvenile isozyme patterns in adults adds to the isozyme polymorphisms, which are modified also by gene conversion and the competitive exclusion of recessive alleles. Because replication is related to transcription, the increased transcriptional activity of certain genes may induce their amplification. This mechanism explains Dauermodifikationen, the "Baldwin effect" and orthogenesis.

These suggestions, admittedly speculative and oversimplified, are intended to trace the approaches to predictive evolutionary theory.

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