RIBOSOMAL RNA, MATERNAL AGE, AND DOWN'S SYNDROME*

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A selective loss (or a blocking) of rRNA genes in ageing oocytes, and its compensation through the retention of an acrocentric nucleolar organizer chromosome, is proposed as a possible mechanism responsible for the increased frequency of Down's syndrome with maternal age.

INTRODUCTION

In discussing the etiological factors responsible for the chromosomal nondisjunction in Down's syndrome, favored by increasing maternal age, little or no attention has been focused on the primary metabolic needs and demands proper to oocytes.

It is well know that the cytological events during meiosis differ in the two sexes. In males, all four products of each meiosis are functional gametes, while in females only one of these cells, the ovum, is a functional gamete. Besides this, the female cell is to supply very early metabolic requirements after fertilization occurs, while the male gamete does not have to cover this special task. Thus, these facts imply that the selective mechanisms which can act on the gametes are very different in the two sexes.

On the other hand, there is a remarkable correlation between increasing maternal age and Down's syndrome, which is not the case with paternal age. Furthermore, the 21-trisomy is the one that keeps in close relation with the maternal age. Hence, it is possible that the metabolic demands that the oocyte is to supply on the first stages of cleavage might suffer an alteration (" waste " or loss) with ageing, then pressing the aged ovum to take an additional chromosome involved with these metabolic demands as a compensatory mechanism.

ACROCENTRIC CHROMOSOMES

The maximum number of acrocentric chromosomes showing an association of satellited chromosomes in a cell is six (Ferguson-Smith and Handmaker 1961). This is also the maximum number of nucleoli in an interphase nucleolus at pachytene. It is generally accepted that the acrocentric chromosomes of man are the nucleolar organizer chromosomes and recently Henderson et al. (1972), using a technique of in situ hybridization, have demonstrated that the satellite regions of all acrocentrics are the sites of rRNA synthesis.

* Partially supported by a grant from the Ford Foundation.

RIBOSOMAL RNA SYNTHESIS IN OOCYTES

Studies carried out on amphibian oocytes (Brown and David 1968) have shown that these cells have an increase in synthesis of rDNA and rRNA, and that this increase goes together with supernumerary nucleoli in germ vesicle (Raven 1961). Furthermore, they have an increase in synthesis of ribosomes until ovulation occurs (Brown and Littna 1964).

The nuclei of growing oocytes are characterized by an enormous quantity of nucleolar material, and it is possible to see a single giant nucleolus, as is generally the case in invertebrates and some vertebrates, or many individual nucleoli, as in the case of amphibians (Raven 1961). Brachet (1942) was the first to show that oocytes nucleoli, like other nucleoli, contain high concentrations of RNA, and Edstrom and Gall (1963) have demonstrated that this is ribosomal RNA. A more conclusive evidence that rRNA is synthesized in nucleoli arises from the study of the anucleolate mutant of *Xenopus* (Elsdale et al. 1958). The homozygotes for this mutation have no nucleolus and are unable to synthesize rRNA; the heterozygotes have only one nucleolus instead of several, as usually observed in a normal cell. Kahn (1962) has shown that this mutant has a deletion of the chromosomal nucleolar organizer region. This region is a secondary constriction in a pair of chromosomes in *Xenopus*, it being lost by heterozygotes in one of these chromosomes, while homozygotes have lost this region in both chromosomes. Furthermore, homozygotes do not contain detectable DNA hybridizable with rRNA (Brown and Weber 1968). Thus, in this mutation, genes coding for RNA are lost.

Increase in synthesis of rRNA also occurs in oocytes of Echinoderms (Wilt 1963), birds (Levkey et al. 1963), and mammals (Mintz 1964). Thus, it is now known that the oocyte nucleoli are the sites of rRNA synthesis during oogenesis and the proliferation or enlargement of the nucleolus demonstrates the enormous accumulation of rRNA during oogenesis.

As stated by Davidson (1968): "Nucleolar ribosomal RNA synthesis is quantitatively the dominant aspect of gene activity during oogenesis." It is likely that this gene activity might be a universal phenomenon due to an extra labour of the nucleolar organizers, i.e., the acrocentric chromosomes.

AGEING

Maternal Age and Down's Syndrome

It is well known that the frequency of Down's syndrome increases with maternal age. One hundred years ago, Fraze and Mitchel (1876) called attention to the fact that children with Down's syndrome are usually born at the end of large sibships. Penrose (1933) conclusively showed that the increased incidence of Down's syndrome is related to the mother's age and not to paternal age or parity.

German (1968) proposed that the relationship of Down's syndrome to increasing maternal age might be explained by delayed fertilization among older women consequent to decreased frequency of coitus. Fialkow (1964) has proposed that this relationship is due to phenomena of autoimmunity.

Synthesis of rRNA in Ageing

Recently, Johnson and Strehler (1972) have reported a loss of genes coding for rRNA in ageing brain cells. The authors proposed a mechanism to explain the selective loss of genes. As also mentioned by the authors, there is some evidence for the occurrence of simple strand scissions in the DNA of ageing mouse neurones (Price et al. 1971), and an age-depending increase in protein cross-linking has been suggested (Von Hahn 1970). Although this selective loss of rRNA genes has been demonstrated in aged brain cells, it is not unlikely that this loss also occurs in cells as the oocytes which have a very long quiescent period.

DISCUSSION

On account of the above-mentioned facts, the hypothesis might be proposed that a selective loss of genes coding for rRNA, or their blocking in ageing oocytes, and its compensation by retaining an acrocentric nucleolar organizer chromosome, is the principal mechanism for explaining the relationship between Down's syndrome and maternal age.

Massive synthesis of rRNA being the most outstanding aspect of gene activity during oogenesis, and there being a loss of rRNA genes with ageing (Johnson and Strehler 1972), the retention in the ovum of an additional chromosome directly connected with this synthesis would be a compensatory mechanism, in order to keep up normal levels of an essential gene activity for the oogenesis to be successfully completed and an embryo be developed.

If one acrocentric D-group chromosome is added, the total genic dose would be excessive; then, a G-acrocentric chromosome is selectively preferred. In the oocyte, there might be special reasons to prefer a chromosome 21 to a 22, but, in the absence of some evidence for that, it may be supposed that a fertilized ovum with trisomy 22 is early aborted, because trisomy 22 is unfrequent when it is compared to the incidence of Down's syndrome. In this respect, it is interesting to note that, with the use of C-banding techniques (Arrighi and Hsu 1971, Salamanca and Armendares 1974), chromosome 22 appears to be more heterochromatic (inactive?) at the centromeric and proximal regions of the short arms than is chromosome 21. The latter could therefore be more efficient to supply genic loss in an aged ovum. Besides, with the use of banding techniques, an higher frequency of trisomy 22 in aborted fetuses could be demonstrated.

It should be noted that the proposed selective chromosome retention normally operates in the oocytes of those organisms in which chromosome elimination appears associated with some phase of germ line differentiation.

The germ line stem cell alone may retain the total number of chromosomes, while in other cells a portion of their chromosome set is early eliminated. In *Dysticus* (Wilson 1925), the oogonial divisions result in the appearance of 15 nurse cells and one oocyte, this being the only one cell to retain the total chromosome complement. Berry (1941) has shown that chromosome elimination also occurs early in cleavage in somatic but not in germ cells of *Sciara ocellaris*. The same is true for *Cecidomyidae* (Geyer-Duszynska 1966), in which the somatic cells retain only 6-12 chromosomes while the germ cells retain the total set of over 40 chromosomes. Thus, these facts demonstrate that the oocyte, in order to ensure a very intense gene activity, retains genetic material which is necessary in this cell and not in somatic cells. The necessity of retaining an additional acrocentric chromosome in the aged ovum may evoke this more primitive mechanism and appears directly related to the most striking meta-

bolic activity of the oocyte, rRNA synthesis. This selective retention could be favored by the phenomenon of association exhibited by human satellited chromosomes (Ferguson-Smith and Handmaker 1961).

Of course, the existence of other trisomies cannot be completely explained by the present suggestion. All three trisomies in man show a maternal age effect, but it must be noted that trisomy 21 is the one that keeps in the closest relation with maternal age. Besides, some cases of trisomy D can be due to a mistake in the selection of the additional acrocentric chromosome: one chromosome D might be retained instead of a chromosome 21. Moreover, the presence of genetic material related with the synthesis of rRNA, not located in acrocentric chromosomes but in others (18,8 or even gonosomes) cannot be ruled out.

On the other hand, the selective loss of genes coding for rRNA also evokes the already mentioned chromosome deletion described by Kahn (1962) in anucleolate mutant of *Xenopus*, and may be explained by a mechanism similar to the one suggested by Johnson and Strehler (1972).

The loss of genes would not be the only effect of ageing in the ovum. It is now well known that maternal age has other important effects on oocytes. Henderson and Edwards (1968) have shown a significant reduction in numbers of chiasmata with the increase of maternal age in mouse. Bodmer (1961) has reported lower recombination frequencies between the genes *pallid* and *fidget* with increasing age. These facts should be examined in the light of the findings of Riley and Bennett (1971) who demonstrated DNA synthesis at all stages of meiosis, and in relation with the hypothesis postulated by Whitehouse and Hastings (1965) on the polaron hydrib DNA model of genetic recombination, in which breakage is followed by enzymatically mediated strand separation and by new DNA synthesis.

Hence, ageing can alter several functions in female and not in male gametes: reduced numbers of chiasmata are noted in female but not in male meiosis. According to the present hypothesis, a decrement in rDNA, rRNA, and in the number of nucleoli in ageing oocytes, would also be expected. These parameters could be compared to those found in meiotic cells from women with Down's syndrome.

We may ask whether the aged oocytes do not resort to other mechanisms rather than retaining one acrocentric chromosome. It is likely that the cell resorts to other amplificatory mechanisms, but these ones might not be effective enough in aged oocytes. Besides, the role of mitochondrial DNA can be enquired, since there is one mitochondrial RNA polymerase. Recently, Barath and Kuntzel (1972) have shown that the structural gene for mitochondrial RNA polymerase is located on a nuclear chromosome and not in the mitochondrial DNA. We can speculate that, if such an enzyme is found in mitochondria of oocytes, its structural genes might be possibly found on an organizer nucleolar chromosome.

Selective loss of genes coding for rRNA could also be a regulatory mechanism controlling germ cell atresia and even the initiation of menopause: this loss of genes may be tolerated until a threshold level, beyond which the decreased demand of rRNA in female cells would be the alarm signal to switch the hormonal mechanism that implies the end of ovulation and the beginning of menopause.

With respect to the human Robertsonian translocation, which involve acrocentric chromosomes and loss of rDNA genes, it is interesting to note that, when a female is a carrier of a D/D Robertsonian translocation, the risk of trisomy 21 is five to ten times greater than in the general population (Dutrilleaux and Lejeune 1970). A distributive pairing, similar to the one that occurs in Drosophila, has been claimed to explain this fact (Grell 1971). But it is

possible that the higher incidence of Down's syndrome among newborns when a female carries a D/D translocation results from the need to compensate the loss of rDNA genes in the oocyte due to the Robertsonian translocation.

These suggestions do not disagree with previous hypotheses on the relationship between Down's syndrome and maternal age. Viruses (Stoller and Collmann 1965), autoantibodies (Fialkow 1964) and delayed fertilization (German 1968) could increase the loss of rRNA genes in ageing oocytes, or block these genes, or even facilitate the degradation of the synthesized RNA.

The low incidence of MZ twins with Down's syndrome has not been sufficiently explained. McDonald (1964) found a significant deficiency of MZ twins with Down's syndrome and suggested that a zygote with abnormal chromosomes might be less likely to split to form MZ twins than a normal zygote.

Keay (1958) suggested that this deficiency might result from more frequent intrauterine death of one or both members of an affected twin pair. In the light of the hypothesis herein reported, a single aged oocyte with its principal metabolic activity seriously decreased and with an additional chromosome trying to level this activity would not be able to supply the necessary amount of cytoplasmatic rRNA for a pair of MZ twins to be developed.

Finally, the present hypothesis calls the attention on the most outstanding metabolic activity of the oocyte, such as the increased synthesis of rRNA, a metabolic exigence not carried out by the male gamete, and offers a more rational explanation to the question of why maternal and not paternal age keeps in close relation with the birth of children with Down's syndrome.

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RIASSUNTO

RNA Ribosomiale, Età Materna e Sindrome di Down

Viene proposta, quale meccanismo per cui la frequenza della sindrome di Down aumenta con l'età materna, una perdita selettiva di geni di rRNA, oppure il loro blocco in oociti, compensata dal trattenimento di un cromosoma organizzatore nucleolare acrocentrico.

RÉSUMÉ

RNA Ribosomial, Age Maternel et Syndrome de Down

L'on propose, comme mécanisme par lequel la fréquence du syndrome de Down augmente avec l'âge maternel, une perte sélective de gènes de rRNA, ou bien leur blocage dans les oocytes, compensée par l'entretien d'un chromosome organisateur nucléolaire acrocentrique.

ZUSAMMENFASSUNG

Ribosomen-RNS, Alter der Mutter und Downsches Syndrom

Es wird angenommen, dass das vermehrte Auftreten des Downschen Syndroms bei zunehmendem Alter der Mütter darauf beruht, dass die rRNA-Gene entweder einen selektiven Verlust erleiden oder in Oozyten blockiert werden, wobei zum Ausgleich eines akrozentrischen Nukleolus-Organisator Chromosom festgehalten wird.

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