

Know Your Chromosomes

4. The Paths to Disorder are Many

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Vani Brahmachari is at the Developmental Biology and Genetics Laboratory at Indian Institute of Science. She is interested in understanding factors other than DNA sequence *per se*, that seem to influence genetic inheritance. She utilizes human genetic disorders and genetically weird insect systems to understand this phenomenon.

Mutations are vital in deciphering the regulated expression of genes in all organisms. In humans, study of mutant traits has helped in the management of genetic disorders. This article focuses on the nature of mutations that are encountered in general and the possible ways in which these mutations are produced.

From the gene mapping strategies discussed in the earlier articles of this series, you know that the trail of the gene is initially detected at the level of a disorder or an abnormality that runs in families. What I hope to do in this article is to give you a glimpse of the varied nature of the defects (mutations) that may ultimately result in the shutdown of an essential function of a cell, a tissue and therefore an organism and the probable ways in which these mutations are produced.

What Can Go Wrong With Genes?

The task at each cell division is that a 2 metre long DNA (or 6 billion units) has to be copied faithfully in every dividing cell in a limited time. The accuracy of the process is remarkable, otherwise genetic abnormalities and disorders would be more common than they are now. The reason behind this is that the system is able to handle errors, in a variety of ways. For example certain mutations being lethal lead to a reduction in the fertility of those carrying these mutations and the abnormal embryos get aborted in early stages of development.

The first step at which errors can arise as the fertilized egg begins to divide is a possible mispairing of bases during DNA



synthesis. This results in mismatches, with an adenine (A) pairing with some base other than a Thymine (T) and so on. The potential error rate can be as high as 1 to 10% per nucleotide, if we take into account the difference in free energy of pairs of complementary bases as against noncomplementary bases. But the observed frequency of mutation is much less, of the order of 1 in a million (10^{-6}) nucleotides. This high degree of fidelity of DNA replication is achieved by different components of the replication machinery, which can recognise and repair errors in replication.

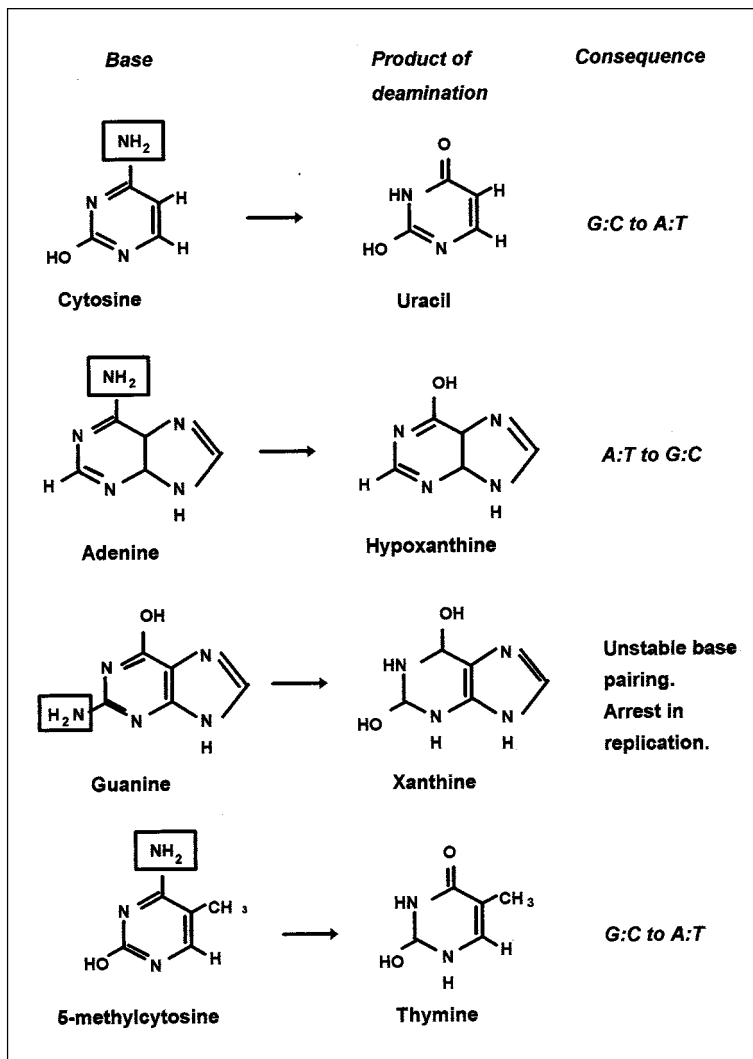
Another source of change in a base pair is the deamination of bases. For instance, if an amine group is removed from cytosine, it forms uracil, which can now pair with adenine during replication. Thus a C:G base pair is converted into a T:A base pair eventually, bringing about a mutation (*Figure 1*). Deamination of bases is known to occur spontaneously during DNA metabolism and is influenced by pH and temperature. The basic change is an alteration in one base pair, a C:G to T:A or vice-versa. A mutation of this sort is called a *point mutation*. The effect of a point mutation depends on where it occurs within the gene. It can be entirely without effect, in which case one may not even know that a change has taken place. Alternatively, a mutation can result in the protein coded by the gene becoming inactive or being unable to carry out its normal function.

There are instances where an additional length of DNA is inserted into the normal sequence. This may be due to the duplication of an existing sequence. Such an intrusion could scramble the normal message and therefore the protein that is made from that message. On the other hand insertion of a small number of bases into a coding region can lead to a different kind of disorder. If this number is neither three nor a multiple of three, the triplet code in the messenger RNA will be disturbed and the reading frame changed. This would result in the synthesis of protein with an aminoacid sequence different from

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Figure 1 Products of deamination of bases commonly present in DNA and their consequences.



the wildtype protein, or the protein may terminate prematurely. This type of mutation is called a *Frame Shift* mutation. The removal of bases can also shift the codon reading frame. The reaction of free radicals with DNA can be a source of such errors (*Box 1*). There are several ways in which free radicals are generated in living systems. They interact with DNA and cause strand breakage and discontinuity.

All these types of mutations are seen in human genes. The probable mechanisms of mutations have been understood



Box 1

Free Radicals and DNA

1. *What are free radicals?*

Free radicals are chemical species that contain one or more unpaired electrons, are capable of independent existence and are highly reactive. Example: hydroxyl free radical represented as OH[·], superoxide radical (O₂^{·-}).

2. *How are free radicals produced in living organisms?*

Exposure of organisms to ionizing radiation leads to fission of O-H bonds in water resulting in OH[·]. Oxidative stress due to several factors including cigarette smoking is believed to result in production of superoxide species, O₂^{·-}.

3. *Why are free radicals harmful?*

Free radicals are highly reactive, they react with any molecule in their vicinity – proteins, lipids, carbohydrates and DNA. In DNA they lead to base modifications resulting in change of base pairs. They also react with sugar moieties which results in the deletion of a base and therefore leads to frameshift mutations. However not all free radicals are harmful. An oxide of nitrogen, nitric oxide NO[·] is a vasodilator and possibly an important neurotransmitter.

4. *How do living systems handle free radicals?*

Living systems have antioxidant defenses to remove O₂^{·-}. Enzymes like superoxide dismutase convert O₂^{·-} into hydrogen peroxide (H₂O₂) and another enzyme, catalase converts H₂O₂ into water and molecular oxygen. It is interesting to note that a gene for superoxide dismutase is localized to chromosome 21, trisomy of which causes Down syndrome.

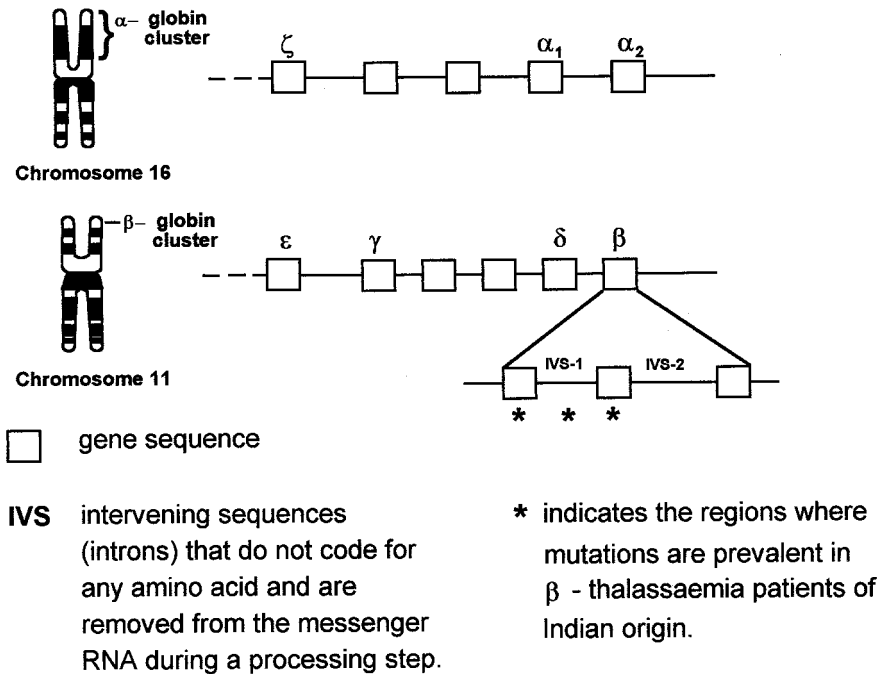
primarily by the study of bacteria, fungi and insects in which they can be induced by chemicals and radiation. One instance wherein different kinds of mutations occur and lead to similar disease states is haemoglobinopathies, the disorders due to defects in the protein globin in haemoglobin. The major groups of disorders are known as the thalassemys (α or β -thalassemia) (*Box 2 and Table 1*) — derived from the greek word *thalassa*, which means Mediterranean (from where many of these variations of globin gene originated). In humans, haemoglobin is made up of four protein chains of two different varieties α and β , and is represented as $\alpha_2\beta_2$. The gene coding for the α chain is on the short arm of chromosome 16, and that for the β chain is on the



Box 2

Mutations leading to Thalassemias

Haemoglobin in humans is made up of four polypeptide chains designated as $\alpha_2\beta_2$. Mutations result in either β -thalassaemia or α -thalassaemia depending on whether the mutation is in the gene coding for β -globin chain or α -globin chain respectively. There are different types of haemoglobin at different stages of development, each adapted to oxygen requirement at these stages. The difference arises in the nature of polypeptide chains expressed from the α -globin gene cluster from chromosome-16 and β -globin gene cluster from chromosome-11. They differ in nucleotide sequence and are designated as ζ , α_1 , α_2 , ϵ , γ , δ , and β . There are several kinds of mutations that ultimately result in either reduced levels of haemoglobin or its total absence. Asian Indian, Chinese and African are the major ethnic groups at risk for thalassaemias.



short arm of chromosome 11. Defects in either of the two globin genes can result in reduced levels of haemoglobin or even in its total absence. Persons whose globin genes contain mutations of the kind described above are known to exist. The mutations affect the steps required to make a functional globin protein from the appropriate genes.

Table 1 Population studies have shown that within each ethnic group a certain mutation is more prevalent than others.

Type of mutation	Effect of mutation	Ethnic group where prevalent
Point mutation	Messenger not made	American black
	Defect in mRNA processing	Asiatic Indians & Chinese
	Incomplete protein made	Mediterraneans
Deletion	619 base pairs deleted, incomplete gene.	Asiatic Indians
	25 base pairs deleted; defect in mRNA processing	Data not available
Frameshift	Deletion of 1, 2 or 4 base pairs	Chinese

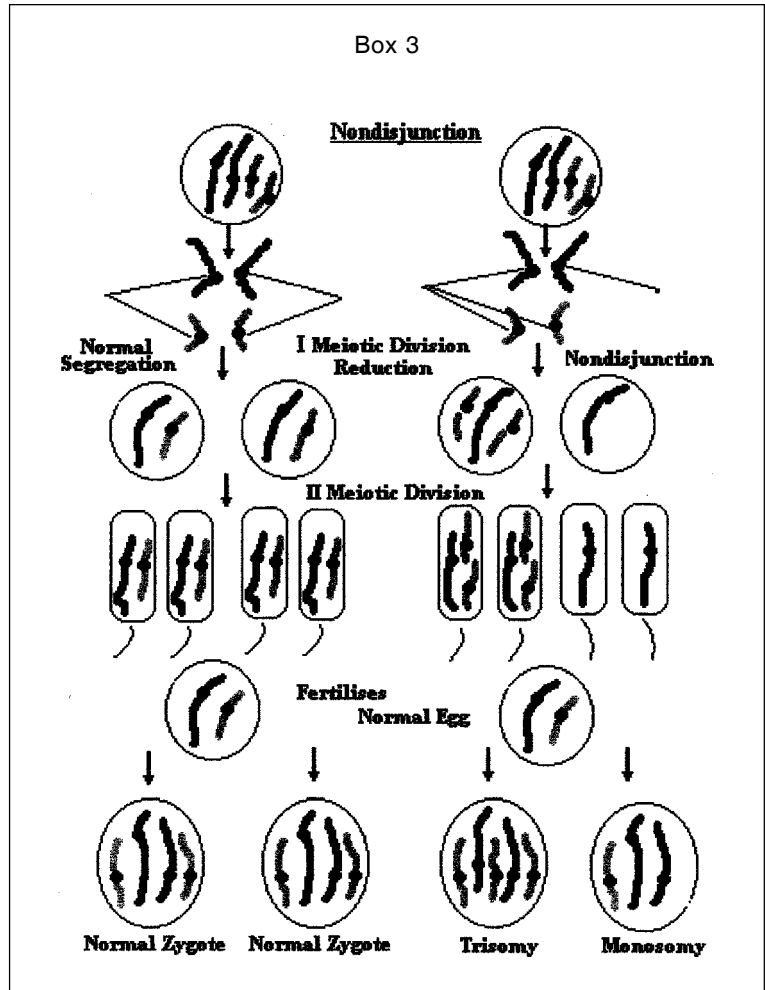
Data of Chakravarty, Purandare, Jaiswal and Gagati taken from the 7th International Conference on early prenatal diagnosis of genetic diseases (1994) and from *Essential Medical Genetics* by J M Connor and M A Ferguson-Smith.

Good Genes Needed But in the Right Number

The first chromosomal disorder to be recognised in humans was *Down's Syndrome*. This was earlier called *Mongolism*. Here chromosome 21, with its full complement of wild type or normal genes, is present in 3 copies instead of 2. Chromosome 21 belongs to the G group of chromosomes and is smaller than most other human chromosomes. The presence of abnormal chromosomal number described in general as aneuploidy, here trisomy, is observed in certain other syndromes too. Trisomies of chromosome 18, 13, 22, 8, 9 and X are known. Children with these 'numerical' anomalies have severe and complex malformations. Mental retardation is seen in all cases except in the trisomy of the X-chromosome. Anomalies in other chromo-



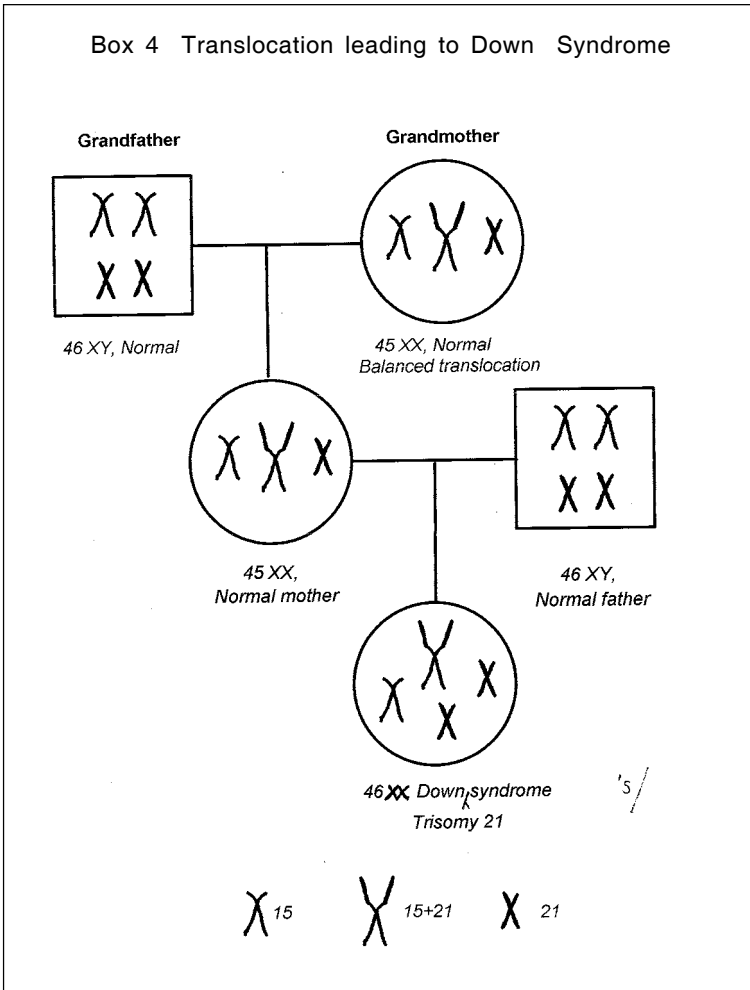
For simplicity diploid number is taken as two. Nondisjunction (ND) can result in trisomy or monosomy for a chromosome. The diagram here depicts nondisjunction at first meiotic division. Readers are encouraged to work out the consequence of ND at II meiotic division and ND for sex chromosomes at the I and II meiotic division.



some are rarely seen in newborns, but are more frequent in natural abortions. This suggests that a deviation in the number of chromosomes disturbs normal embryonic development so much that the foetus is naturally aborted. In fact, a large fraction of natural abortions have chromosomal anomalies.

Similarly, a decrease in chromosome number from the diploid state can also lead to malformations. This is monosomy, there being only one chromosome instead of two. Monosomy for an X chromosome in the absence of a Y chromosome leads to female development but with malformations and mental retardation, a

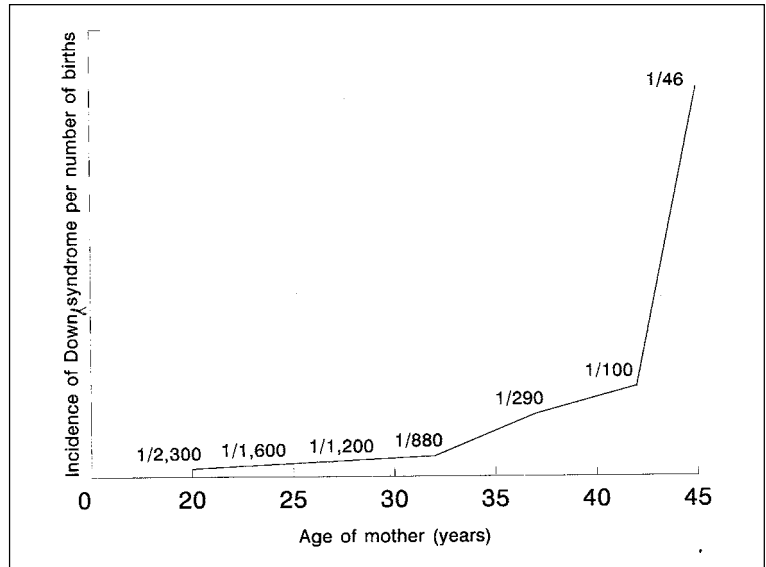




situation known as *Turner syndrome*. The chromosome constitution of a Turner patient is 45 XO. The loss of the diploid state can also be caused by a deletion or removal of a gene or a part of the chromosome, in any one of two homologous chromosomes. Deletions of the short arm of the chromosome 18 and chromosome 5 are known to occur. The deletion of the short arm of chromosome 5 leads to the *Cri du Chat Syndrome* (cat cry syndrome). Children with this syndrome are not particularly malformed in their external features but have a striking cry resembling that of a cat and exhibit mental retardation.



Figure 2 The chances of birth of a Down syndrome child increase with age of the mother. (The figure is redrawn from *An Introduction to Genetic Analysis* by A J F Griffiths et al (1996) Sixth edition, W H Freeman and Company, New York).



Variations from the normal chromosome number can occur due to inappropriate separation during meiosis, which takes place before sperm and egg formation. Technically this is called *nondisjunction*. Similar segregation defects can lead to monosomy (Box 3). Sometimes chromosomes remain in the middle after metaphase and during anaphase (*Resonance* January 1996) and fail to segregate to the poles and enter the daughter nuclei. This is described as *anaphase lagging*. It is important to note that these are only terminologies that describe the phenomena but do not give any indication of mechanisms. We do not know how and why such disturbances in segregation are caused. In the case of trisomy 21 (Down syndrome) there is strong correlation between the age of the mother and the frequency of birth of a Down child (*Figure 2*), though there are cases where the extra chromosome 21 is contributed by the sperm. Therefore both an increased frequency of nondisjunction during meiosis in the mother and a decreased ability to reject abnormal embryos may contribute to the increased frequency of birth of Down children to older mothers. Thus perfectly normal parents can have normal children as well as a Down syndrome child. But when more than one Down child is born to young parents in a family, the clinician



would suspect reasons other than nondisjunction during egg or sperm formation. One such instance was described in 1960. The mother in this family had only 45 chromosomes but one of her two chromosome 15 was longer than the other. By banding, it was identified that a major part of chromosome 21 was attached to chromosome 15. She was normal as she had the correct complement of all chromosomes but one chromosome 21 was not free but attached to chromosome 15. The woman had inherited this from her mother. Her child had received one normal chromosome 21 from each of her parents along with the unusual chromosome (15 + 21) from her mother. Thus she had three copies of chromosome 21 instead of two and hence exhibited Down syndrome (*Box 4*). This brings us to yet another aberration in chromosomes namely *translocation* – basically meaning that parts of chromosomes change their location and move over to other chromosomes.

Abnormal Alliance of Chromosomal Regions Can be Unpleasant

Translocation of chromosomes can be harmless as in the mother and grandmother in the above example. These are described as *balanced translocation*. There are cases where two nonhomologous chromosomes exchange their parts but still maintain normal status with respect to their chromosome complement. This is described as a *balanced reciprocal translocation*. However when such chromosomes are passed on to the child along with a normal complement from the other parent it will result in aneuploidy (monosomy or trisomy) and lead to symptoms like developmental delay, malformations, mental retardation and congenital heart diseases.

These aberrations occur because of gaps and breaks within chromosomes which probably are caused by environmental factors like radiation and chemicals. The broken chromosomes can attach to their original counterparts but occasionally may join or

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get ligated to other nonhomologous chromosomes. Fusion of regions from different chromosomes may be brought about by recombination – like processes that take place during sperm and egg formation (*Resonance* March 1996).

The Philadelphia Chromosome

There are examples where a chromosomal translocation affects only certain cell types even though it is present in all the cells of the organism. In several patients with a type of leukemia called *chronic myelogenous leukemia* (CML), a balanced chromosomal translocation has been observed between chromosomes 9 and 22. This has apparently no effect in most of the tissues except in those of the circulatory system in which it induces leukemia. It was discovered by David Hungerford at the Fox Chase Institute in Philadelphia, U.S.A, based on careful cytology. The molecular basis for this has been traced to the activation of an oncogenic or cancer-inducing protein. Because of the translocation, 5 million base pairs of DNA originating from the end of chromosome 9 and carrying the cellular complement of an oncogene called *c-abl*, are translocated into a region of the long arm of chromosome 22. This results in expressing a protein which is a combination of the *c-abl* protein and the resident protein at this position on chromosome 22. The change activates the *c-abl* oncogene and leads to CML. This cancer is truly genetic, that is children of a CML patient have a finite chance (50%) of getting CML. But the question often asked is, are cancers genetic? Cancers are due to mutations at the DNA level, thus they are passed on from one cell to another, but this would not imply that the child of a cancer patient will also get cancer. For transmission from parent to child the mutation has to be in the germline of the parent, that is in the sperm or the egg cell (germline mutation). The other class of mutations which occur for instance in a liver cell of an individual leading to a malignant tumor is likely to be due to environmental abuses and is called *somatic mutation*. Therefore mutations in germ cells will not affect the parent but can affect



the children whereas somatic mutations can affect the individual but not the children.

Thus the right sequence of the gene, in the right dose and in its right neighbourhood are all essential for normal development and well being. Considering the number of ways in which the system can be derailed it is surprising that so many of us are considered 'normal'!

Note From the Author

The two previous two articles in the series had a list of genes on chromosomes 1 to 4. I plan to produce a poster containing the gene map of all human chromosomes later this year. Therefore the listing of genes and their functions is discontinued.

Acknowledgement

The author would like to thank Milind Kolatkar for all the illustrations.

Suggested Reading

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