transfer rate depends on the degree of thermal motion.

Electron transfer from BPh to Q_A was found to be dominated by two constructively interfering paths, whereas the transfer from Q_A to Q_B appeared to proceed through a web of pathways with a high degree of destructive interference. The authors suggest that for the Q_A to Q_B transfer, the electron probes the different paths that arise in the course of thermal fluctuations and selects for the actual transfer event pathways that occur only for suitable conformations; the latter may deviate substantially from the crystal structure.

Electron transfer processes are ubiquitous in cellular bioenergetics and require high efficiency as well as robustness against thermal disorder. The scenario that emerged in the study of Balabin and Onuchic shows that natural systems can go beyond the ubiquitous thermal activation for barrier crossing in using thermal fluctuation to their advantage. This effect is likely to occur in instances other than the RC. An example has been found already in the light-harvesting system that fuels the RC with energy. This system contains aggregates of chlorophylls that, in the absence of thermal motion, share their excitation coherently in the form of so-called excitons but appear to revert to localized, less coherent excitations through thermal noise (*8*, *9*).

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PERSPECTIVES: CANCER

Proximity Matters

John R. K. Savage

Chromosomal aberrations, where segments of chromosomes are rearranged in various ways or even lost, are a universal result of exposure to ionizing radiation. Such changes are found in thyroid tumors from many children exposed to radiation after the Chernobyl nuclear reactor accident.

One of the earliest controversies facing the field of radiation cytogenetics was the debate about just how close chromosomes have to be for radiation-induced rearrangements to occur. Obviously, the radiation-damaged regions of separate chromosomes must "touch" at some stage of the exchange process. However, it is unclear whether these regions come into close contact after radiation damage (the "breakage first" hypothesis), or whether exchanges occur only where close contact already exists (the "contact first" hypothesis). Breakage-first has always been the dominant idea and is considered to be more consistent with quantitative data on chromosome aberrations (*1*, *2*). Even so, many experiments indicate that the radiation-induced DNA strand breaks in chromosomes cannot be very far apart for exchange to be possible (*3*).

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Enter Nikiforova *et al.* (*4*), on page 138 of this issue, with their study of two regions on chromosome 10 that are inverted in many radiation-induced thyroid tumors. One region contains the gene encoding the RET receptor tyrosine kinase, and the other region (30 Mb away) contains the *H4* gene. In many radiation-induced thyroid tumors, there is an intrachromosomal inversion resulting in the fusion of the tyrosine kinase domain of the *RET* gene with a section of the *H4* gene. The investigators show that in 35% of normal thyroid cells the *RET* and *H4* genes are actually in close proximity within the interphase nucleus, as judged by resolution of fluorescent probes with three-dimensional microscopy. They postulate that such a preformed molecular association—perhaps a normal event during thyroid cell differentiation—may favor radiation-induced "intrachange" between the regions containing these genes. An association between *RET* and *H4* was only marginally present in normal lymphocytes and was completely absent in mammary epithelial cells.

Recurrent chromosome aberrations (commonly, but not exclusively, interchanges) are an established feature of many cancers and are used for diagnosis, prognosis, and the tracking of tumor progression. Structural rearrangements at the molecular level can juxtapose segments of DNA that are not normally adjacent to one another such that genes are switched on or off, suppressor sequences are unmasked, or hybrid genes are formed that produce aberrant proteins with oncogenic activity. Frequently, these juxtapositions are very precise, with the exchange point in one participating chromosome (very occasionally both) being positioned to within a few base pairs in many cases.

Many of the arguments adopted in debates about the relative merits of the contact-first and breakage-first hypotheses depended on a traditional picture of chromosome architecture and interphase organization. The traditional view held that chromosomes had a solid backbone that was completely severed by radiation, forming open mobile ends (primary breaks) that wandered around the nucleus, rejoining with similar ends in the vicinity—like cut-up spaghetti in a plastic bag (*5*). Today we know that chromosome integrity depends on enormous lengths of DNA packaged with histone proteins into a complex tertiary structure. We also know that the principal damage inflicted by radiation is the DNA double-strand break, which (in view of the complex packaging of DNA with protein) will not produce the open-ended primary backbone breaks envisaged by the early theorists.

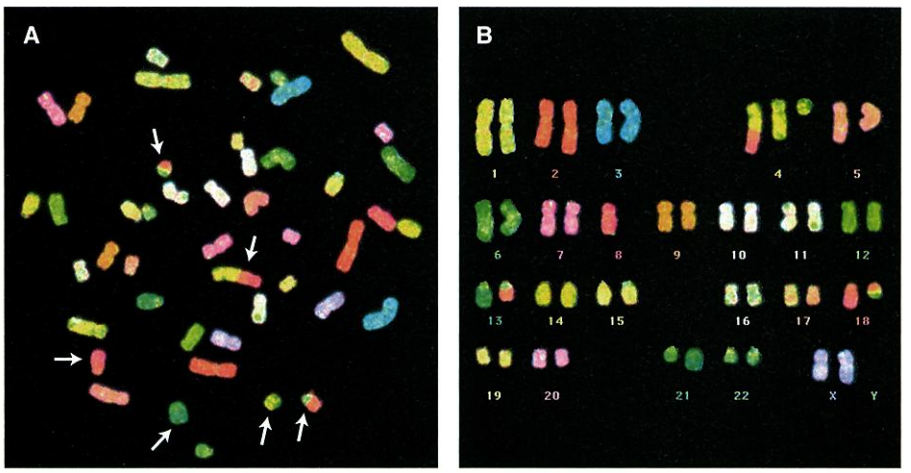
Moreover, during interphase of the cell division cycle, the two (p and q) arms of each chromosome occupy very discrete domains. Even though the two arms of a chromosome must lie fairly close together, there is no evidence for an ordered arrangement of domains relative to one another within the nucleus. Thus, there is no massive intermingling of chromatin or unrestricted movements of open broken ends. However, although the bulk of the DNA is confined to the chromosome domains, some of it is spun out into loops. Some of these loops are attached to the nuclear envelope near the pores, and others are anchored to the intranuclear matrix where many "factories" controlling cellular processes are located (see the figure, next page, lower left). The regions between the chromosome domains form an interconnected network of channels throughout the nucleus, which can be visualized as containing multistranded "cables" of extended DNA (*6*, *7*).

This situation has modified our thinking considerably about the proximity of chromosomes and the movement of radiation-induced broken ends (lesions). Much of the chromatin is so wrapped up, "splinted," and anchored that structural exchange

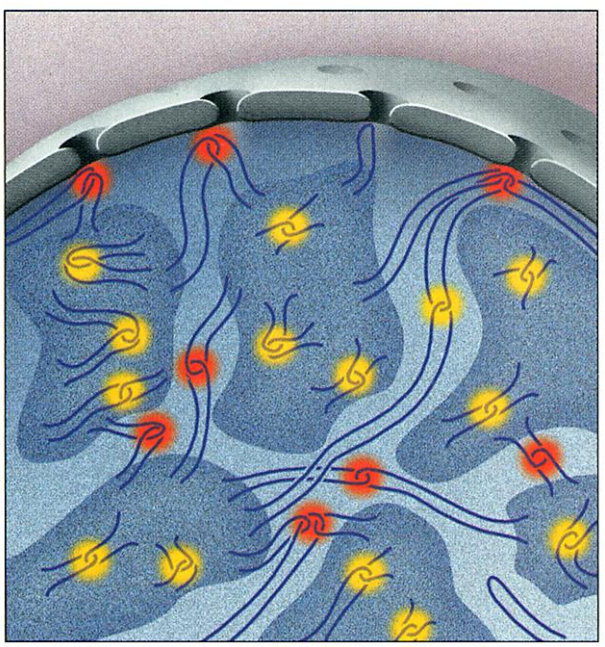
The author is at 34 City Road, Tilehurst, Reading RG31 5HB, UK.

of any sort is precluded. Most of the chromosomes are predicted to be susceptible only to exchanges within a domain (intrachange). There would be very few exchanges between chromosome domains (interchange) because most of the DNA is not available. In this compartmentalized arrangement of the nucleus, the only places where different chromosomes could touch would be in the DNA of the channel "cables" and the loops close to the nuclear pores (see the figure, below).

The picture has been further complicated by recent results from fluorescence in situ hybridization (FISH) studies of chromosome aberrations (see the figure, right). A large proportion of chromosomal exchanges, once thought to be simple two-break events, turn out to be complex (three or more lesions in two or more chromosomes) (*8*). This is particularly true of multichromosome exchanges induced by α-particle irradiation, which are common even though the actual volume of the nucleus that "sees" ionizations is minuscule (because very few α-particle tracks traverse a nucleus) (*9*). Clearly, during transit, the tracks must encounter a very large number of regions where several chromosomes are in proximity, giving the impression that despite the seemingly rigid compartmentalization, there is probably a very large number of suitable meeting places. Hence, either chromosomal broken ends move a lot or many preassociations between chromosome domains exist.



Gone fishin'. FISH "painted" human metaphase (**A**) and karyotype (**B**) chromosomes showing the characteristic and extensive chromosomal damage induced after α-particle irradiation. The chromosome exchange is very complex, involving six chromosomes (4, 8, 13, 18, 18 and 21) with a minimum of seven breaks (white arrows). Lymphocytes in G_0 of the cell cycle were exposed to 0.5 Gy of α particles from a ^{238}plutonium source (mean tracks per cell = 1).



A place for chromosomes to meet. The arrangement of chromosomes within a nucleus. During interphase, the two arms of a chromosome are confined to a discrete region called a domain (speckled blue area). Some of the DNA in this chromosome is extruded as loops that extend either within or beyond the domain. These loops can extend into channels between different domains (pale blue), sometimes reaching as far as the pores of the nuclear membrane. Extruded loops can form specific associations either with other loops from the same domain (yellow) or with loops from different chromosomes in different domains (red). Such associations could provide the proximity necessary for the intrachange (exchange within the same chromosome) or interchange (exchange between different chromosomes) induced when a cell is exposed to ionizing radiation.

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The proximity of chromosomal regions involved in the *RET–H4* inversion reported by Nikiforova and colleagues suggests that association of these regions may be important in thyroid cell differentiation. If this association is a target for radiation-induced exchange, as the authors suggest, then the precision of resultant breakpoints implies that mere proximity cannot be the sole prerequisite for exchange. Clearly, very complex molecular machinery is required to align, clamp, and execute precise chromosomal breakage and rejoining. The Nikiforova findings represent an extreme example of "contact first."

The existence in cancer cells of so many recurrent aberrations, with such precise exchange-point positioning, prompts the conclusion that functional association between remote sequences is a regular feature of the interphase nucleus, even in normal cells. In actively dividing cells, many of these associations may be transitory and therefore not readily detectable with FISH probes. In differentiated (and differentiating) cells, which in the course of their specialized duties pack away a lot of the unnecessary chromatin, chromosomal associations required for specific functions may be much longer lived and more likely to be detectable (if one selects the right kind of cells and knows the probes to use).

The sites of chromosomal association in normal cells may be the only locations where the exchange process can take place. To use an analogy, if a fishing net is irradiated, the damage will be restricted to the knots. It follows, therefore, that all observed exchanges are really mapping sites within the nucleus where preexisting functional associations are located. If so, then we might anticipate that precise exchange-point positioning is always present. However, in actively dividing cells, chromosomal associations are difficult to detect because they are transitory, altering as cells progress through their division cycle. The logical next step is to take cells that have a known chromosomal association (such as the normal thyroid cells of Nikiforova *et al.*), irradiate them, and check them for an increase in chromosomal aberrations at the loci of the association point.

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