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Concluding Remarks

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Concluding remarks

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Professor Darlington opened the meeting by challenging us with the view that chromosomes made the laws of heredity, rather than heredity fashioning the organization of chromosomes. To keep this wheel of logic spinning, it may be said that chromosomes also made the process of meiosis and thus determined the laws of meiotic exchange. I choose this gambit because our discussions lent considerable emphasis to the view that chromosome complexity compels its own sets of distinctive, and perhaps varied, mechanisms to effect the ultimate event of molecular recombination. The complexity that leads molecular recombination to operate in elaborate meiotic moulds is not, it should be emphasized, base sequence complexity. On the contrary, sequence repeats and genetic homoeologies, though adding disproportionately little to the base sequence complexity of a genome, adds considerably to the complexity of effecting chromosome alignment and crossing over. How chromosomes of diverse genetic content manage that complexity and in the process mould the characteristics of meiotic behaviour has been the primary target of our deliberations. That no single pattern of meiotic conduct was perceived in consequence of the discussions, is to be expected. To the extent that genomes differ in various aspects of chromosome organization – and that they do is patent – the particulars of meiotic organization might also differ. Although a strong sentiment was occasionally expressed for a single universal process of meiosis, it is my opinion that sameness and universality may be mistakenly treated as synonyms. Universals provide for diversity; they do not impose sameness. The task of identifying universal threads among different meiotic fabrics is not a straightforward one. The ultimate act of genetic recombination offers no detailed guide to the routes by which it may be achieved. Indeed, it is the structure of the chromosome that dictates the route; recombination only signals the direction.

An important but infrequently discussed property of meiosis is the mechanism whereby chromosomes avoid entanglements in the course of achieving synapsis. Bivalent interlocking is a rare event and chromosomes must therefore be so positioned as to circumvent it. One solution to the problem was proposed in the course of this meeting. Professor Maguire and Dr Dover each presented evidence for homologue alignment during the interval of premeiotic mitosis. Professor Callan, on the other hand, described observations which demonstrated the absence of any kind of homologue positioning before synapsis itself. Some organized three-dimensional distribution of chromosomes must nevertheless exist to facilitate compaction free of entanglements, and premeiotic homologue positioning might be required for some but not all karyotypes. In light of the evidence that interlocking within a particular chromosome set is most likely to involve the longest chromosomes, it would be of interest to know whether a relationship does exist between premeiotic homologue alignment and the size and number of chromosomes within a genome. Prealignment might represent a particular accommodation to the special needs of certain genomes rather than a universal event in meiocytes. Indeed, it is difficult to see how pre-alignment could be universal given the facts about zygotic meioses in haplontic organisms.

The special issue of interlocking flows into the more general issue of chromosome synapsis which commands a broad spectrum of views. Common to most, if not all, of these views is a recognition that chromosomes undergoing synapsis are appreciably compacted and contain considerable amounts of protein. Matching cannot be effected by the simple device of juxtapositioning totally extended DNA strands. Compaction of chromosomes must therefore be so regulated as to provide for an array of sites that would enable homologues to recognize one another. Since compaction effectively excludes most of the DNA in a chromosome from interacting with DNA in the homologue, the exposed sites involved in zygotene pairing must reflect overall gene order, at least to the extent of compensating for segments that are heterozygous for inversions, deletions, insertions, and translocations. The sensitivity of compensation, i.e. the length of segment that can be so affected without altering the normal pattern of synapsis, is an issue that was not addressed during the meeting. It is nevertheless understood that the minimal size of such a segment is considerably larger by several orders of magnitude than the heteroduplex configuration discussed by Dr Holliday in relation to crossing over. The nature of the matching sites remains an open question, and various models have been proposed as answers. Professor Riley dwelt on the potential of periodicity in segments of repeated sequences for effectively aligning homologues in wheat. Compared to most, if not all, animal species, the problem of synapsis in wheat is unusual inasmuch as the mechanisms required for preventing homoeologous pairing in the hexaploid may be more demanding than those required solely for justapositioning homologues in a diploid. The whole question of homoeologous versus homologous pairing takes on a broad dimension in the plant world where allopolyploidy is fairly common.

A particularly interesting aspect of meiosis in polyploids was touched upon by Dr Bennett, who presented an impressive array of data bearing on the relations between duration of meiosis and genome size. One relation, the proportionality between meiotic duration and haploid DNA amount, is conceptually welcome although one might also expect chromosome length to influence duration. The evidence that polyploidization decreases rather than increases the duration of meiosis is, however, both surprising and perplexing. Hexaploid wheat, for example, has a shorter meiosis than the diploid, *Triticum monococcum*. It appears as though increasing the number of homologues or homoeologues within a genome decreases the time required for each chromosome member to proceed through the successive stages of meiosis. Presumably, meiosis in any species would be extended if non-homologous chromosomes could be artifically introduced into the genome. If so, diploid hybrids with low homology between parental chromosome sets should have longer meioses than the parent species, assuming that chiasma frequency itself does not influence duration. The principle that sets of four homologous chromosomes require less time to undergo meiosis than those of two, if correct, has major implications for the physiology of meiosis and deserves considerable attention. Perhaps, the relation reflects a mechanism evolved to cope with polyploidy in which some of the processes that normally occur during meiosis now precede it, the factor of duration thus being more apparent than real. This possibility is suggested by the timing of three characteristic events in microsporogenesis – callose formation, nucleolar attachment to the nuclear membrane, and colchicine sensitivity – as discussed by Dr Dover. Whereas in wheat these events occur at or soon after premeiotic mitosis, in *Lilium* they occur at the beginning or during the prophase of meiosis. It would be most helpful to know whether these three events occur at corresponding times in hexaploid and diploid forms of wheat.

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The relation between premeiotic alignment and homologue synapsis, however significant to meiosis in certain groups of organisms, does not necessarily reflect fundamental features of synapsis. The sequence of meiotic events in the haplont, *Neotiella*, as described by Professor Wettstein, provides sufficient proof that synapsis does occur without the benefit of any significant interval for prealignment. Nevertheless, the regulation of homologous versus homoeologous pairing, which must be a key factor in the successful evolution of allopolyploids, certainly has deep-seated implications for the mechanisms governing synapsis. Superficially, at least, it appears as though such regulation is effected by a set of factors that control the stringency of chromosome matching, the more stringent the conditions for matching, the more tightly are homoeologues excluded from pairing with one another. There is no direct evidence for the existence of a stringency mechanism, let alone for the conditions that enter into it. Representing the phenomenon in this way is, nevertheless, attractive because it provides for different degrees of homoeologous pairing and also offers the analogy with *in vitro* reassociation of single-stranded DNA, a form of molecular matching whose stringency can be controlled by manipulating conditions. If homologous pairing is regulated by stringency of matching conditions, it is clear that those conditions can be altered to a very significant degree either by a particular chromosomal locus (as in the $5B^{L}$ of wheat) or by accessory B-chromosomes which may not even be an integral component of the genome in which they are effective.

Professor Maguire boldly but reasonably challenged the tacit assumption that synapsis is a precondition for crossing over. The thrust of her argument is that only at the incipient stages of synapsis is there any immediate relation between the two events, subsequent synapsis filling in the gaps between the actual or predetermined sites of exchange. Taken at its face value, the evidence in support of the argument is highly persuasive. Synapsis in regions heterozygous for an inversion or translocation is not regular, as is made evident by cytological observation. However, Professor Maguire reported that the frequency of crossovers between homologous segments in such regions equalled the frequency of pairing. Were pairing purely a precondition for crossing over, then the frequency of crossovers in the inverted or translocated region should have corresponded to the genetic length of the segment thus involved. If so, the proportion of meiocytes in which the affected regions paired should have exceeded the proportion of meiocytes having exchanges within that region. Since this was not the case, Professor Maguire infers that either the commitment of a particular site to crossing over, or the event itself, must have occurred before pairing of the entire chromosome. She adds strength to her interpretation by pointing to data of Gillies showing that the number of synaptic initiation sites in corn is approximately equal to the number of crossovers. The unrehearsed model of Professor Maguire and the well-rehearsed model of Dr Holliday provide compatible ingredients for yet another model in which the Maguire sites of crossover commitment at the initiation of synapsis use up all of the limited Holliday protein that is essential to effect exchange. Thus viewed, interference would already have occurred when synapsis had just begun and, presumably, any pair of homologues that is late in initiating synapsis will have been rendered achiasmatic. It would be most interesting to know whether the recessive mutation in *Hypochoeris* leading to a failure of exchange in one particular pair of homologues is associated with a retardation of synapsis in that particular pair.

Models are of course highly seductive, especially where the realities of events are poorly known and it might seem easiest to remove the temptation by embracing the model. The realities, however, once unfolded, might provoke regrets. Other possibilities need to be

considered. The implication of Professor Maguire's interpretation is that inversion loops would not be formed if they were not preceded by a crossover. Stated in more general terms, crossing over or its equivalent at incipient synapsis secures the pairing arrangement. Imposing so broad a generalization on Professor Maguire's interpretation is unjust to the presentation, but since pairing arrangements can be and are secured even in non-homologous segments, the generalization serves to indicate that other aspects of the relation need to be taken into account. The sequence of events postulated in the classical view of meiosis in which synapsis precedes crossing over had considerable support from contemporary electron microscope studies as described by Professor Wettstein. In his interpretation, crossing over follows synapsis, a sequence that is compatible with the course of appearance of Carpenter's *Recombination nodules* as described by Professor Lindsley. The sequence is also compatible with the course of biochemical events in *Lilium*. The genetic analyses of meiosis in *Drosophila melanogaster* presented by Professor Lindsley further support the scheme. The principal message of these studies is that mutants affecting chromosome synapsis to the point of its virtual elimination, have no crossing over, whereas mutants that are indifferent to synapsis have a range of effects on the frequency of recombination. A considerable segment of the machinery for effecting crossovers is thus separate from the machinery for effecting synapsis.

To dwell further on the relative merits of different models would be unproductive. The paucity of experimental data on the details of pairing and crossing over precludes a meaningful comparison in detailed terms. We are still cutting broad swaths into the problem, and the effectiveness of our analyses is limited by both conceptual and methodological advances in related areas. Biochemical data may indicate a temporal regulation of the machinery essential to crossing over, but we can neither translate such regulation in terms of individual chromosome segments nor even specify the components so involved. We are still in need of knowing whether homologous DNA strands are present within the synaptonemal complex and, if so, what their distribution might be. We are unable to make a distinction, if indeed one can be made, between a synaptonemal complex with normal morphology and function, and one seemingly normal but defective in function. The broad variations in structural organization discussed by Professor LaCour and the scope of lateral element involvement in recombination as discussed by Professor Moens, testify to the need for sorting out the necessary from the contingent in order to resolve the meiotic activities within a bivalent.

A broad and fundamental issue that underlies most considerations of meiosis is that of interference. The problem was explicitly raised by Dr Holliday, but it was implicit in most presentations. Professor Pontecorvo pointed out a number of years ago that in a relative scale for crossover frequency per base pair, bacteriophage would have 1000, bacteria – 100, a fungus (*Aspergillus*) – 10, and a mammal would rate 0.1. The extreme reduction in frequency of crossing over as one ascends the evolutionary ladder is not at all matched by a reduction in regularity of occurrence. Indeed, so deep-seated is this characteristic that the whole phenomenon of meiosis could be articulated from the standpoint of positive interference. The problem is not so much one of assuring regularity in the face of low frequency, as it is of maintaining low frequency in the face of regularity. Exclusion is the principal characteristic of positive interference and the mechanism of its operation must surely extend into the grosser aspects of non-randomness in crossing over. Rhoades once pointed out that some substance essential to crossing over may be limiting in each meiocyte and Dr Holliday has given this possibility a more concrete image by assigning the task to a protein within the synaptonemal complex. Professor Callan

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stressed the localization of chiasmata within the axial regions of lampbrush chromosomes, and attributed such localization to the exclusion of chiasmata from regions that are active in transcription. It is not clear, as some of the discussion indicated, whether the ultimate position of a chiasma at diplotene reflects the original position of a crossover or whether chiasmata are established following crossing over, perhaps by a process akin to strand isomerization. It is intriguing to speculate that the materials of the recombination nodule include the hypothetical limiting substance, but we have yet to learn whether the nodule is precursor or product of recombination.

Since Dr Holliday launched a most vigorous molecular assault on the very broad issues of meiosis, it is appropriate to make his assault the last victim of this summary, and in so doing satisfy Professor Pontecorvo's wish for a clarification of concepts. A major item in Dr Holliday's molecular assault is his model for synapsis. He provides Professor Riley's interspersed repeats with a protein that has a special affinity for them, and he provides the protein molecules with an affinity for one another by equipping them with the equivalents of hooks. The key to homologous pairing is the distinctive spacing of a limited number of classes of repeats (such as those analysed by Dr Flavell and associates in wheat) for each homologue pair. The protein translates the spacings into synapsis by a hookup of protein molecules once the repeats are in register. Direct interaction between DNA strands is thus obviated, an important consideration in view of the distance separating synapsed homologues. The scheme is elegant in its simplicity and I can only counter it with complexity. Dr Holliday's scheme makes matching and stabilization of matching a single process; I prefer to keep them separate. I like breathing DNA duplexes carrying specific sequences that are triggered to open up, perhaps in coincidence with replication, on initiation of pairing. Given a suitable intranuclear framework (a lipoprotein surface ?), homologous DNA strands, thus exposed, might sort each other out for matching by the conventional alignment mechanism. The matching is transiently stabilized by the action of a Holliday-like protein followed by the formation of a coherent synaptonemal complex structure that bridges the homologues. Complex formation is separate from matching and need not be restricted to matched segments, as is sometimes the case.

These and other model schemes neither exhaust the possibilities nor determine the probabilities. It is nevertheless significant to point out that we are better able to formulate the problems of meiosis in mechanical and molecular terms than we were ten years ago. There is now enough of a beginning in the biochemistry of the process to provide a realistic framework into which molecular models based on microbial studies can be tested. The page of prophase metabolism is no longer the blank it was ten years back; there is at least a skeleton correspondence between cytological and biochemical sequences. The opening up of the fine features of base sequence organization in chromosomes to experimental analysis is providing fresh and fundamental criteria by which to assess the role of chromosome organization in different meiotic functions. The introduction of serial sectioning into electron microscope studies of meiotic nuclei may have deprived the synaptonemal complex of its hallowed status as the embodiment of all that is essential to the achievement of recombination and disjunction, but it is now being subjected to more critical scrutiny. The recent use of spreading techniques in examining the fine structure of meiocyte nuclei will undoubtedly add to the rigour with which fine structural features of meiosis can be evaluated.

If advances in cytogenetic analyses (e.g. *Ph* locus in wheat; meiotic mutants in *Drosophila* and *Saccharomyces*; B-chromosome regulation of pairing) are added to the above list, the

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summation points to substantial progress in meiotic analysis. Such progress is gratifying, but I believe that there is a more profound significance to the deliberations of this meeting than is evident in the summary. It is not only that the fruits of recent approaches to meiosis are coming into view and are falling into logical place; it is the potential for future advance that needs to be considered. The various approaches, old and new, are now beginning to act synergistically. The combination of fine structure, genetics, and biochemistry has considerably more potential for discovery than each of these pursuits in isolation. This meeting has clearly recorded that the newer approaches have matured sufficiently to make direct combinations between them operationally meaningful. If subsequent progress in the field runs true to scientific form, the next general meeting will be dominated by concerns that are much narrower and more detailed. The dismemberment of meiosis will appear less sweeping but it will also be more realistic and hence more profound. Every good meeting provides the philosophical reminder that protagonists best be correct in their facts in order to be secure in their doctrines. This meeting will surely bring about reluctantly corrected claims and ambitiously altered doctrines.

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