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Genetics of mate choice

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Darwin proposed the idea of sexual selection to explain the evolution of elaborate weapons and ornaments which seemed to him to be detrimental to survival. He had in mind characters such as the antlers of male deer and the bright plumage of many male birds. Darwin suggested that these male sex-limited characters had evolved because they gave their bearers an advantage when competing for matings with females; weaponry would be used in direct combat between males while elaborate plumage would attract females to the ornamented males.

Two features of Darwin's theory raised more questions than they answered. First, it was not evident why males should compete for access to females rather than vice versa. This problem was first partially solved by Bateman[2], who pointed out that, because females are defined as such by their large and individually costly gametes, in many species a female's reproductive success will be limited by her own ability to produce eggs and not by the number of her mates. Sperm, in contrast, are small and therefore individually cheaper to produce, so a male will be potentially capable of fertilizing the entire reproductive output of more than one female. Male reproductive success is therefore likely to be limited by the number of mates obtained, so males compete for access to females.

The second problem with Darwin's theory caused it to be much criticized. It was not at all clear why females should have mate preferences at all, and in particular why they should prefer elaborate ornamentation. This seemed to imply previously unsuspected aesthetic senses in animals, and for a time this aspect of sexual selection went into eclipse. Evidence in favour of female preferences has indeed been very slow in accumulating, but there are now several good cases. Perhaps the most dramatic evidence has come from field experiments with the long-tailed widow bird[3]. Males of this species have extremely long tails which are displayed conspicuously during flights over defended breeding territories. In a series of elegant experiments, Andersson altered the tail lengths of males, shortening them by removal of a central section of the feathers which could then be used to lengthen the tails of other males by insertion. Two control groups were either caught and released, or had the tail cut and restuck to give the original length. Andersson could demonstrate a significant increase in the number of nests made by females in the territories of the males with experimentally lengthened tails, supporting the idea that females have a preference for males with long tails.

How could such preferences have evolved? One possibility is that no particular evolutionary explanation is required because earlier-evolved features of the female's perceptual system could make certain stimuli inherently more conspicuous to her. If this explanation were correct, one might expect related species to show similar patterns of mate choice. The extreme species-specificity of many male ornaments and displays therefore makes this explanation unlikely to be the whole story, so that some sort of special evolutionary process is required. The earliest and probably basically correct explanation came from Fisher[4], who envisaged an initially advantageous male character such as a long tail appearing and starting to spread in the male population by natural selection. Another mutant then appears, causing females to prefer long-tailed males as mates, so that non-random mating of these females with long-tailed males then occurs. Sons from these matings carry both paternal tail length and maternal preference genes and, because of the former, have higher survival. The female preference gene therefore increases in frequency as a result of its high rate of association with tail-length genes in males produced by non-random mating. At a certain threshold frequency of female preference, a new process takes over, causing what Fisher called runaway evolution. Females that mate with males with long tails have many grandchildren, because the sons of the mating are attractive to the females with the preference. The tail-length and preference genes now rapidly evolve together.

Fisher's argument was purely verbal, but a series of more recent genetic models have confirmed most aspects of his theory. These models were pioneered by O'Donald[5,6] who used a two-locus system for preferences and preferred traits, and could demonstrate the evolution of female preferences but not the runaway processes. A more recent polygenic model[7] and a modified two-locus model[8] could produce all features of Fisher's model, and also implied that the male trait need not have an initial advantage under natural selection. These different models show clearly that the exact genetic basis and biological effects of traits and preferences can have important consequences for the evolutionary process, so that genetic analysis has become essential.

Some fascinating recent work with ladybirds[9,10] has examined the genetics of female preference for dark coloured melanic males as opposed to the typical red morph. By artificial selection for or against female preference for melanic males, O'Donald and his coworkers were able to produce 'high' lines that showed a very strong preference for melanic males or 'low' lines that mated at random with the two male morphs. Detailed analysis of the response to selection strongly implied that variation in female preference was a consequence of genetic variation at a single locus. Thus it may well be that two-locus models are of genuine biological relevance to understanding the evolution of female preferences, and future work on ladybirds and other systems should produce evidence about the selective forces at work.

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Growth factors, receptors and oncogenes

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Few things are as satisfying as when an aspect of a science, for long considered a backwater of the esoteric, suddenly emerges as part of the main stream. At a recent meeting in Heidelberg* the well funded and confident community of scientists studying growth control and oncogenesis was forced to come to terms with many disciplines with which they may last have had contact as undergraduates. For if molecular biologists are uneasy with proteins, many would confess a complete ignorance of the intricacies of lipid biochemistry and enzyme kinetics. The title of the meeting was 'Growth factors, Receptors and Oncogenes', a juxtaposition of words which would have had little significance only only a few years ago. Astonishingly, however, there are now hints that an understanding of the complex processes governing normal and tumour cell growth may be on the distant horizon. Because the signals inducing a cell to divide are usually external and in the form of the binding of growth factors, the big problem is, therefore, no longer in simply trying to identify the genes involved in cell growth. Rather, interest is now centred on understanding the mechanisms by which an external mitogenic signal is transduced to its final sites of action and how that transduction might be modulated.

An insight into the genetic control of the cell cycle was provided by the first speaker, Paul Nurse (ICRF, London), who described studies on the cell cycle regulation of the yeast *Saccharomyces pombe*. The *cdc2* gene of *S. pombe* is involved in the control of the commitment to proceed through the cell cycle at two points, one in late G_1 and the second in G_2. The *cdc2* gene, related to the *cdc28* gene of *S. cerevisiae*, shares homologies with the ATP-binding and phosphorylation sites of both the *src* and cAMP-dependent protein kinases. Anti-peptide sera have identified the *cdc2* product as a 35 kDa protein kinase whose level of phosphorylation falls abruptly when cells enter the stationary phase, though the absolute amounts of the cdc2 protein itself are invariant. Clearly, the importance of protein phosphorylation in growth control of eukaryotic cells had been underscored before the meeting was one hour old. It was a theme destined to reappear often.

In higher eukaryotes attention has centred on genes which are rapidly induced when resting cells are stimulated to divide by serum or purified growth factors. It is now clear that a fair number of genes, perhaps 20–100, are rapidly induced within 20–60 min of growth stimulation. Yet more genes are induced somewhat later, within 12 h or so. A number of genes from both of these categories has been isolated from cDNA libraries of stimulated 3T3 cells. Dan Nathans (Johns Hopkins, USA) described in detail a gene induced some 3 h after stimulation and encoding a 25 kDa protein christened 'proliferin'. Proliferin shares sequence homology with preprolactin and is probably a growth factor with secondary autocrine or paracrine effects. The more familiar genes to be induced by serum stimulation of resting cells are the proto-oncogenes c-*myc*, c-*fos* and, in lymphocytes, c-*myb* (Kelly, NIH). Expression of these genes is not alone sufficient, however, to allow cells to progress through the cell cycle. Such progression requires a further mitogenic signal. In lymphocytes, this signal is the binding of interleukin 2 (IL-2). In non-lymphoid cells, the secondary mitogenic signals are the binding of growth factors such as epidermal growth factor (EGF) or the somatomedins. The mechanism of control of expression of one 'early' proto-oncogene was addressed by Richard Treisman (MRC, Cambridge) who has mapped a regulatory sequence conferring growth factor inducibility to a site some 300 bp 5′ to the c-*fos* mRNA CAP site. This region is highly conserved between mouse and man and has sequence homologies with several known enhancer elements. This is not the whole story, however, because other sequences 3′ to the c-*fos* coding sequence are also needed for correct control of expression. These 3′ sequences have not yet been defined.

The mitogen platelet-derived growth factor (PDGF) by itself induces a family of genes which, following expression, allow a cell to proceed through the cell cycle. These were christened 'competence genes' by C. Stiles (Harvard), a term which occasioned a sometimes fractious debate. Nonetheless, Stiles demonstrated that PDGF-induced genes in 3T3 cells include not only c-*myc* and c-*fos* but also another *fos* related gene, R-*fos*, and a gene *JE* which shares low homology with β-interferon. This competence family is also induced by double-stranded RNA. Both PDGF and double-stranded RNA also induce the later expression of β-fibroblast interferon and 2′–5′ oligoadenylate synthetase, both of which might act to regulate the initial growth response to PDGF. Expression of at least some 'early' genes (e.g. c-*myc*) is partially regulated at a post-transcriptional level, probably by differential mRNA destabilization. Stiles provided evidence for the existence of a short-lived RNase in cells which might mediate a rapid breakdown of certain mRNAs and is perhaps targeted to the extensive 5′ untranslated regions of some competence gene messages.

It remains to be seen how many of the 'competence' genes of Stiles are the same as the 'early' genes of Nathans, whether any possess transforming activities and what their various functions may be.

The role of growth factors and their receptors in the initiation of proliferative responses was the subject of extensive discussion. Roberts (NCI) and Moses (Vanderbilt) both described the properties of transforming growth factor β (TGFβ). TGFβ is distinct from TGFα in that its binding to cells is not competed by EGF, PDGF, or insulin. TGFβ is a homodimer of 112 amino acid subunits containing multiple cysteine-rich domains. It is ubiquitous and active in both normal and transformed cells. It stimulates DNA synthesis only after long latent periods suggesting its effect to be indirect and via the induction of other growth factors. That TGFβ may have an important regulatory role in the control of cell growth is suggested by its growth inhibitory properties on certain cells. TGFβ is able to slow the mitogenic response to growth factors such as EGF and insulin and it may thus have an important role in modulating the effects of other mitogens.

Since the identity of the β chain of PDGF with the product of the *sis* oncogene was first established, PDGF has been the focus of intense study. PDGF is now known to be one of a family of PDGF-like factors, termed PDGFc. The PDGF receptor is a 185 kDa transmembrane protein with an internal domain which is autophosphorylated and possesses a tyrosine kinase activity. These are properties shared with the EGF receptor (EGF-R), a 170 kDa transmembrane protein which is sufficiently evolutionarily conserved to be recognizable in *Drosophila* (Schlesinger, Rehovot). The intriguing structure of EGF-R, with its conserved extracellular domain made up of repeated cysteine-rich units (Ullrich, Genentech) is shared with several other receptor molecules, notably the LDL receptor.

The identity of the intracellular domain of EGF-R with the product of the transforming gene *erb*-B is now well established. However, the mechanism by which the *erb*-B protein, essentially a truncated form of EGF-R lacking an extracellular domain, is a transforming protein is not clear. Hayman (ICRF, London) provided evidence that, unlike EGF-R, the protein kinase activity of the *erb*-B protein is constitutively active and thus presumably provides a permanent proliferative signal. Evidence also suggests, however, that to achieve its transforming

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effect the *erb*-B protein must also be inserted in the plasma membrane, i.e. the normal site for activated EGF-R. Thus, not only the type, but also the source of the signal is important in the transduction of mitogenic stimuli.

The latest link between growth factor receptors and oncogenes came about by the fortuitous collaboration of C. Stiles and R. Stanley who described data showing the identity of the receptor for the macrophage mitogen colony stimulating factor 1 (CSF1-R) with the product of the proto-oncogene c-*fms*. The CSF1-R/c-*fms* protein is a 170 kDa transmembrane protein with an intracellular protein kinase domain which is autophosphorylated in response to binding of CSF1 to its extracellular binding domain.

One major message becomes clear from all these data; namely that the inappropriate activation of otherwise normal receptors for mitogenic growth factors is sufficient, at least in certain cell types, to cause or predispose a cell to transformation.

The intracellular targets for membrane associated protein kinases are, naturally, of considerable interest. In many cases the internal domain of a receptor is, itself, a substrate for its own or other protein kinases. In many cases, phosphorylation of the internal domain of a receptor modulates its ability to bind ligand, a process that can occur in several ways. For example, the receptor for insulin-like growth factor II (IGF-II-R) is specific for IGF-II, binding IGF-I poorly and insulin not at all. Nonetheless, the binding of insulin to a cell can have dramatic effects on the activity of IGF-II receptors in that same cell, apparently by increasing their availability for ligand binding. Czech proposed a mechanism by which this may occur, whereby insulin causes a decrease in phosphorylation of IGF-II-R. IGF-II-R is constantly cycling between the surface and the internal cell membranes. Hypophosphorylation of the IGF-II receptor slows receptor internalization so increasing the steady state level of receptor at the cell surface. In other cases, phosphorylation may instead lead to a decrease of cell surface receptor, as is the case with the progesterone receptor (Fox, UCLA). Modulation of receptor binding by phosphorylation need not occur only by the alteration of the absolute numbers of receptors available at the cell surface. The phosphorylation of the internal domain of EGF-R results in a decrease in affinity for EGF of the external domain by an, as yet, undefined mechanism. Moreover, the actions of protein kinases at the cell surface are not necessarily limited to other receptors. Protein kinase C (PKC), for example, phosphorylates not only EGF-R, but also the S6 subunit of ribosomes (Parker, ICRF) and it probably has a pivotal role in transduction of the mitogenic signal. Rozengurt (ICRF) described how the binding of the mitogen bombesin to mouse fibroblasts causes activation of PKC as well as a transient rise in intracellular Ca^{2+} concentrations and an increase in intracellular pH via the activation of a Na^+/H^+ antiport. E-type prostaglandin synthesis is also induced, leading to an accumulation of the secondary messenger, cAMP. Similar events to these are initiated by the binding of PDGF and FDGF. Both the transient rise in intracellular Ca^{2+} (from an intracellular store) and PKC activation occur within seconds of stimulation. However, these events are in turn initiated by earlier events, namely the activation of phospholipase C (PLC) which generates diacyl glycerol (DAG) and inositol triphosphates (IP_3). Though not proven, it is believed that PLC activation is mediated by the ras family of proteins. IP_3, of which there are two isomers (Berridge, Cambridge), seems responsible for the transiennt rise in intracellular Ca^{2+} while the short-lived DAG activates PKC.

Though admittedly full of gaps, a picture is at last emerging of a sequence of events initiated by the binding of a primary mitogen such as PDGF. Whether this sequence follows a unique pathway, and just how it is modulated by other events in the cell remain to be determined. Perhaps an even bigger problem for the future is how all this cell-surface information is communicated across the yawning gap between the cytoplasm and the cell nucleus.

With its emphasis on signal transduction, together with up-to-date accounts of the complexities of haematopoiesis and a discussion of the mechanisms of gene activation during transformation, this symposium was a timely event worth repeating. General presentation differed from that at many large USA meetings and included up-to-date reviews of a particular field as well as accounts of recent experiments. This 'unhurried' format is certainly advantageous where the boundaries between so many disciplines are being continually crossed. In his summing up, Bob Weinberg pointed out that for a long time Europe had lagged behind the USA in many of the areas covered by this symposium. Unhappily, few would be inclined to disagree with him. It is clear from this meeting, however, that this is no longer the case and that much of the leading work in the major problems of eukaryotic cell biology now happens in Europe. Let us hope things stay that way!

The cystic fibrosis gene mapped

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The gene for cystic fibrosis (CF) has now been convincingly located on the long arm of chromosome 7, an event that has generated considerable excitement among molecular and clinical geneticists, as well as those directly involved with CF research. The work involved is described in papers in *Clinical Genetics*, *Nature* and *Science*, and represents a further vindication of the strategy of gene mapping for the identification of genes for serious human disorders. It also represents the end of a frustrating period for CF research, since until the middle of 1985 there was little real progress in either the mapping of the gene or the identification of the primary gene product, and applying the techniques of molecular genetics had not yielded the same rapid results seen for X-linked disorders, like Duchenne muscular dystrophy, or autosomal dominants, like Huntington's disease.

Now everything has changed, and quick progress towards isolating the gene itself can be anticipated. The first clue came in August 1985 when the Copenhagen group of Eiberg and colleagues presented evidence suggesting linkage between CF and a polymorphic enzyme paraoxonase[1,2]. It is fair to say that the initial reaction was of excitement tempered with scepticism; the data were not conclusive, paraoxonase was one of many markers used, the polymorphism depended on a quantitative assessment, while few people at the meeting had even heard of paraoxonase and no one knew its chromosomal localization. Furthermore, this was an 'old-fashioned' protein polymorphism, not one of the new DNA polymorphisms that had been expected to solve the problem!

Molecular geneticists were not long out of the hunt, however, and by October Tsui and colleagues in Toronto, using a series of highly polymorphic probes developed by the company Collaborative Research, were able to report linkage of CF with one of them at the American Society of Human Genetics meeting in Utah, and subsequently in *Science*[3]. This probe (DOCR1-917) proved to be linked to PON and is located on chromosome 7; intensive work during the ensuing weeks by several groups has not only confirmed the CF linkage and localization, but has allowed the gene to be fitted into the already substantial framework of the chromosome 7 map[4].

The preliminary results of this work are published in a series of papers (three in *Nature*, one in *Science*) from the groups of Tsui[5], White[6] (Salt Lake City) and Williamson[7] (St. Mary's Hospital, London), and demonstrate strikingly the reactive power of the new DNA markers, which can change a topic from being a tentative hint to a conclusive and well documented linkage group of at least five DNA probes in a matter of weeks. The work also shows

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how complete the total human gene map is becoming – not one of the probes involved was isolated specifically because of its possible relation to CF. The most likely site for the CF gene now seems to be the middle part of the long arm of chromosome 7, and the data, based on families from many parts of the world, do not suggest any multilocus heterogeneity.

The original Collaborative Research probe is not particularly close to CF (around 15% recombination), but two of the others have so far shown no recombination and thus could prove to be clinically useful. For one of these (J3.11) the confidence limits for recombination are wide (0–13%) but the other, the oncogene *met*, is unlikely to be more than 5 cM distant. Two other probes in the linkage group are the gene for pro-α-2(1) collagen and the T-cell receptor B chain gene. None of the genes involved seem likely to be the CF gene itself and a close linkage can still mean a large distance in molecular terms (1% recombination is about 1 million base pairs).

Now the immediate target is to identify precisely how close and on which side of the CF gene the various markers lie and to detect further polymorphisms and closer probes that will make virtually all individuals heterozygous for 'flanking' markers. Given the pace of the past few months, along with the continuation of competition and collaboration of the groups involved, this should be achieved rapidly, leaving the isolation and cloning of the CF gene itself as a feasible goal in the next few years.

Many patients and relatives, often well informed thanks to the efforts of lay societies, will be asking whether these new developments will have any immediate practical application in therapy, carrier detection and prenatal diagnosis. The answer must be yes, but perhaps not in the most obvious ways. The really immediate need which families and their doctors must appreciate is that DNA must be isolated from blood or other suitable tissue, and stored for future use. This should be a much higher priority than rushing to use the current linkages for prediction.

Prenatal diagnosis (in the first trimester using chorion biopsy) should soon be an option for those high risk couples for whom late amniocentesis is unacceptable, but will need to prove its worth against the already available, though not perfect, combination of amniotic fluid alkaline phosphatase and sensitive ultrasound. Neither approach is likely to be helpful for the lower risk pregnancy at present.

Carrier detection has until now been totally unreliable, and linked markers should help distinguish which immediate relatives are likely to be carriers and which not, something that could be especially helpful in inbred groups. Unfortunately, the markers cannot answer the question that is really important, which is whether the unrelated spouse or other person without a family history is a carrier. In particular, population heterozygote screening will have to await a specific test for the gene or its product.

Finally, what about therapy? Until we know what the CF gene is and does, this must remain speculation, but whether we replace the gene or its product it will undoubtedly help to have the cloned gene in our hand and to be able to correlate the clinical phenotype with different types of mutational defect. This is already possible for a number of disorders where the primary product is known and is just becoming so for Duchenne muscular dystrophy. We can confidently look forward to the same becoming a reality for cystic fibrosis.

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Mapping a chromosome to find a gene

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Recombinant DNA technology is undoubtedly contributing to the accelerating pace of human gene mapping*. Of course, molecular probes yield results only when used in conjunction with established methods such as family studies, cytogenetics and somatic cell genetics. Chromosome 11, one of the most intensively studied autosomes, will serve to illustrate some problems, principles and progress.

Overlapping deletions as mapping tools

Disease entities associated with specific chromosomal rearrangements (i.e. translocations or deletions) are an invaluable resource for physical mapping. Unequivocal gene order for all available markers, including the disease locus itself, can be established from analysis of an overlapping series of deletions from several patients. Cloning of the relevant region will lead to molecular understanding of the disease.

*8th International Workshop on Human Gene Mapping, Helsinki, 4–10 August 1985.

Two, possibly related, syndromes are known to be associated with constitutional rearrangements on the short arm of chromosome 11. One is the WAGR (Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation) syndrome, in which variable-length deletions, all including band p13, are seen[1]. There is evidence that Wilms' tumour, like retinoblastoma, is recessive at the cell level[2]. This and the embryonal nature of the malignant nephroblasts suggest that the 'Wilms' gene' fulfils an important developmental function. The second disorder is Beckwith–Wiedemann syndrome (BWS), in which duplications involving p15.4-pter (Ref. 3) are characterized by fetal gigantism, hemihypertrophy and an increased risk of embryonal tumours, including Wilms' tumour. It has been suggested (Jeanpierre *et al.*, Paris) that the duplicated 11p15 region encodes a target for suppression by the product of the WAGR region at p13. According to this hypothesis, tumour development is due to imbalance between suppressor and target, as a result of genetic change at either locus.

Somatic cell hybrids

Chromosome 11p deletions were first studied in lymphoblastoid lines for the levels of expressed functions such as lactate dehydrogenase (LDHA) and catalase (CAT). The gene for CAT was assigned to 11p13 on the basis of such dosage studies[4]. Later, observations of heterozygocity for polymorphic DNA probes (β-globin complex, HBB; insulin, INS; and Harvey *ras* proto-oncogene HRAS1) placed these outside the deleted regions in several instances[5].

Because gene dosage studies either at the DNA or the expressed-function level are difficult to quantitate, somatic cell genetics has been widely used. Some WAGR-associated deletions in rodent cells have now been analysed for available 11p markers, most of which fall outside the deletions[6] (Glaser *et al.*, Boston). A consensus map of gene order has been produced (Fig. 1a). This is largely consistent with the extensive analysis (Fig. 1b) of 22 short-arm markers in a series of X-ray induced, antibody-selected deletions of chromosome 11 in a rodent–human cell hybrid (Glaser *et al.*, Boston). The position of the locus

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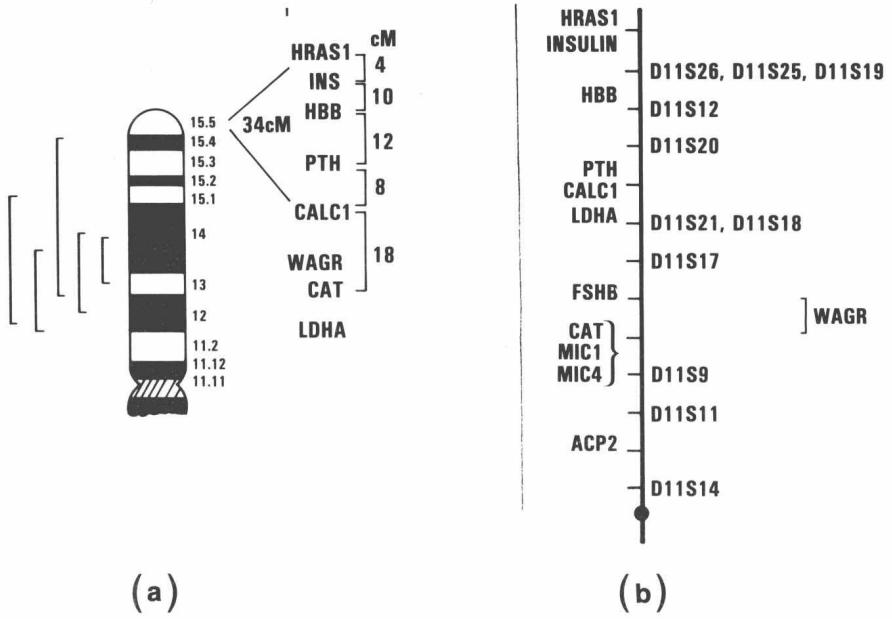

Fig. 1. (a) *The gene order on the short arm of chromosome 11 is shown with consensus recombination distances (in centimorgans) indicated for each bracketed region. The square brackets on the left indicate the extent of some WAGR-related deletions*[6]. (b) *The gene order deduced from the X-ray induced deletions of a single chromosome 11 in a hamster–human cell hybrid. There is no indication of genetic or physical distance on this map, but note the general agreement on gene order in the two types of study. D11S numbers denote chromosome 11 specific single copy random DNA probes.*
Abbreviations: CALC1, calcitonin; CAT, catalase; FSHB, β-subunit of follicle stimulating hormone; HBB, β-globin complex; HRAS1, Harvey ras *proto-oncogene; IGF2, insulin-like growth factor 2; INS, insulin; PTH, parathyroid hormone; TH, tyrosine hydroxylase; WAGR, Wilms' tumour, aniridia, genitourinary abnormalities and mental retardation; LDHA, lactate dehydrogenase A; ACP, acid phosphatase; MIC1 and MIC4, antibody-defined cell surface antigens.*

for LDHA is at variance with previously accepted assignments proximal to CAT and WAGR at p12 (Refs 1 and 5). This awaits clarification by further analysis of deletions and translocations with 11p13 breakpoints.

Genetic distance compared with physical distance

Several of the widely available gene probes on 11p exhibit high frequency restriction fragment length polymorphisms (RFLPs)[7]. Independent family studies carried out by several groups generate a map of 11p which is consistent with the illustrated physical map order (Fig. 1a). The major anomaly is the recombination distance observed between genes for parathyroid hormone (PTH) and calcitonin (CALC1), which two groups (Kittur *et al.*, Baltimore and Housman *et al.*, Boston) put at 8 cM, while a third group (Holm *et al.* Salt Lake City) failed to find any recombinants in data on 130 phase-known meioses. Again, only further work will resolve whether such between-sample variability is real or was observed by statistical chance. Assuming 8 cM between PTH and CALC1 genes, the markers from the most distal HRAS1 to CALC1 cover 34 cM. Several studies of *in situ* hybridization in metaphase chromosomes place all these markers and genes for tyrosine hydroxylase (TH) and insulin-like growth factor 2 (IGF2) at the tip of the p15 region. Somatic hybrid analysis of a large WAGR gene deletion narrows this to p15.4-pter (Ref. 6). Whilst this small region at the tip of 11p contains so many markers which recombine frequently, the CAT gene, only 18 cM from the gene for CALC1, has been assigned unequivocally to the proximal part of band p13, half a chromosome-arm length from p15.4. Relative genetic distance and cytologically observed physical distance appear to differ. Such observations may be important clues to chromosomal organization and mechanisms of recombination.

The most striking aspect of the combined data is the degree of agreement on the gene order derived by different means. It suggests that both the WAGR-associated and the experimentally induced deletions are simple interstitial breaks.

Approaching the WAGR loci

The CAT gene has been found to be deleted in almost all visible WAGR deletions and, until the Helsinki meeting, it was thought to be the closest assigned marker to the putative WAGR genes. Two deletions have now been shown unequivocally to retain CAT. One of these is deleted only for the genes for the β-subunit of follicle-stimulating hormone (FSHB) and for the cell-surface marker MIC1 (Glaser *et al.*, Boston). The other deletion retains both cell-surface antigens, but has yet to be tested for FSHB[6].

Combined data from several groups suggest the gene order: centromere–CAT–Wilms'–aniridia. The genes for FSHB and MIC1 may be on either side of the Wilms' and aniridia loci. With the help of molecular techniques, we are steadily focusing on the interesting region that must encode the WAGR genes. The race is now on to identify and isolate these genes. This is no trivial task as witnessed in the still continuing struggle for the X-linked (and therefore easier) Duchenne muscular dystrophy (DMD) gene[8] and the more comparable retinoblastoma gene. The promise of the deletion-cloning technique used by Kunkel's group[9] in studying DMD remains to be tested in studies of the more genetically complex autosomal diseases, in which separate somatic cell hybrids bearing the deleted and normal homologues must be used in a high stringency subtractive hybridization system. Imminent developments in such techniques as pulse-field electrophoresis and chromosome jumping may allow us to clone large contiguous lengths of DNA. Or perhaps the problem will be solved by the re-emerging somatic genetic method of chromosome-mediated gene transfer, followed by selective cloning of all human DNA sequences from the resulting rodent–human cell hybrids (Porteous *et al.*, Edinburgh). The observed close linkage of selectable surface antigens to the WAGR-gene region will facilitate the isolation of small chromosome fragments for this purpose.

There are two known cases of familial aniridia associated with translocation breakpoints in 11p13. Probes that exhibit altered restriction patterns in these translocation individuals may lead us directly to the aniridia gene. Again by analogy with DMD, DNA markers isolated by any of these methods may reveal cytologically undetectable deletions in some Wilms' tumour patients so bringing us nearer to the Wilms' locus. Travelling hopefully towards these loci is an exciting and instructive exercise. Perhaps it will be better still to arrive.

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Mutagenic activity of transforming genes

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One of the keys to a better knowledge of cancer physiology is to understand the relationship between gene transforming activity and mutagenesis. A relevant and good experimental system uses viral transforming functions. The mechanisms underlying malignant transformation include gene or chromosomal mutagenesis of the host cell genome. It has been demonstrated, in the case of SV40, that there is a strict relationship between induced mutagenesis and transforming activity. A temperature-sensitive mutation of SV40 (tsA) stopped the induction of both mutagenesis and malignant transformation in Chinese hamster cells at a non-permissive temperature[1]. This report indicated that the SV40 oncogene was probably responsible not only for the transforming activity but also for the mutagenic function. The mutagenic activity of SV40 was shown to have no locus specificity and to be maximal at early stages of the viral infection[1]. Since similar results were obtained with an oncogenic bovine adenovirus (BAV3), which belongs to a different systematic group than SV40, this property appears to be common to many oncogenic viruses. In BAV3, as in SV40, the maximum chromosome and gene damage was shown to be reached 12–14 h after infection[2]. Later in infection, the frequency of mutated cells is the same as that observed before the infection. This is probably due to selection against mutant cells by virtue of their lower viability compared with wild-type cells.

Synergistic mutational activity

Several drugs have been characterized as carcinogens and have been shown to modify DNA both at the gene and at the chromosomal levels[3]. It is well established that 5-bromodeoxyuridine (BrdU) increases the frequency of transformation by oncogenic viruses. In this respect, it is interesting to note that the combined mutagenic effect of BrdU and SV40, and of BrdU and BAV3, is synergistic instead of additive[2]. Thus in this case also, a correspondence between oncogenic potentiality and mutagenic effect can be observed. In a very recent paper, Lukash and colleagues[4] present results supporting the idea that the mechanisms of carcinogenesis and mutagenesis share some common elements and that this situation is not accidental. These authors show that the isolated oncogene from BAV3 is responsible for the mutagenic effect of the virus, while other parts of the BAV3 genome had no effect. It is worth recalling that the BAV3 oncogene is located in the extreme 5′ end of the viral genome, in a position similar to the transforming E1A gene in human adenoviruses[5]. These results confirm the parallel between the mutagenic and transforming activities of viral DNA and show that both properties are located in the same region of viral genome, its oncogene. As described before for increase of mutagenic activity when the action of the isolated BAV3 oncogene was combined with additional chemical products. The tumour promoter 12-*O* - tetradecanoyl - phorbol - 13 - acetate (TPA) is known to increase sharply the frequencies of both malignant transformation *in vitro* and of tumour formation. TPA has neither a mutagenic nor a transforming effect of its own, but when added to the BAV3 oncogene the frequency of induced mutants increases by a factor of 2.5–4.0. TPA induction was absent when non-oncogenic DNA was used, such as other segments of the BAV3 genome, bacterial plasmid DNA or total DNA from Syrian hamster cells. Hence mutagenic activity is inherent only in the DNA that possesses transforming activity and TPA action is restricted to the oncogene[4].

Mechanism of action of TPA

TPA itself does not cause malignant transformation and only acts as a tumour promoter when initiation due to some carcinogen has already taken place. Although the mechanism of action of TPA is unknown, there are some cases in which a direct effect on gene expression can be considered. TPA has been described as regulating the differential expression of proto-oncogenes such as c-*myc*, c-*myb* and c-*fos* (Ref. 6). In particular, while c-*myc* and c-*myb* expression are diminished during phorbol ester treatment of certain hematopoietic cells, the expression of c-*fos* is dramatically increased at least 20–30-fold within only 20 minutes of exposure to TPA (Ref. 7). These results indicate that TPA is not a general inducer and that its pleiotropic action is probably mediated by specific molecular targets. In this respect it is interesting to note that induction of c-*fos* expression is obtained using a number of mitogens, including serum, PDGF (platelet derived growth factor), EGF (epidermal growth factor), FGF (fibroblast growth factor) and the calcium ionophore A23187. Recent results indicate the presence of a transcriptional enhancer in the upstream promoter region of the c-*fos* proto-oncogene (Verma, I. M. pers. commun. and Ref. 8). Interestingly, the c-*fos* element, like other inducible promoter elements, exhibits enhancer characteristics only after exposure of the cells to the inducer (in the case of c-*fos*, the serum). This strongly suggests that the transcriptional element is the target of *trans*-acting factors which are required for enhancer function.

In conclusion, carcinogenesis and mutagenesis appear to share common physiological steps. Understanding the mechanisms by which transforming products can induce mutagenesis will greatly increase our knowledge of the metabolism of malignant cells. One of the ways in which an oncogene can increase its carcinogenic potential is its induction by mitogens. This mechanism, which is likely to include interactions between *trans*-acting factors and promoter sequences, can be studied experimentally today and is likely to be clarified in the coming years.

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