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The intestinal epithelium and its neoplasms: genetic, cellular and tissue interactions

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The Min (multiple intestinal neoplasia) strain of the laboratory mouse and its derivatives permit the fundamental study of factors that regulate the transition between normal and neoplastic growth. A gene of central importance in mediating these alternative patterns of growth is *Apc*, the mouse homologue of the human adenomatous polyposis coli (*APC*) gene. When adenomas form in the Min mouse, both copies of the *Apc* gene must be inactivated. One copy is mutated by the nonsense *Apc* allele carried in heterozygous form in this strain. The other copy can be silenced by any of several mechanisms. These range from loss of the homologue bearing the wild-type *Apc* allele; to interstitial deletions surrounding the wild-type allele; to intragenic mutation, including nonsense alleles; and finally, to a reduction in expression of the locus, perhaps owing to mutation in a regulatory locus. Each of these proposed mechanisms may constitute a two-hit genetic process as initially posited by Knudson; however, apparently the two hits could involve either a single locus or two loci. The kinetic order for the transition to adenoma may be still higher than two, if polyclonal adenomas require stronger interactions than passive fusion.

The severity of the intestinal neoplastic phenotype of the Min mouse is strongly dependent upon loci other than *Apc*. One of these, *Mom1*, has now been rigorously identified at the molecular level as encoding an active resistance conferred by a secretory phospholipase. *Mom1* acts locally within a crypt lineage, not systemically. Within the crypt lineage, however, its action seems to be non-autonomous: when tumours arise in *Mom1* heterozygotes, the active resistance allele is maintained in the tumour (MOH or maintenance of heterozygosity). Indeed, the secretory phospholipase is synthesized by post-mitotic Paneth cells, not by the proliferative cells that presumably generate the tumour. An analysis of autonomy of modifier gene action in chimeric mice deserves detailed attention both to the number of genetic factors for which an animal is chimeric and to the clonal structure of the tissue in question.

Beyond *Mom1*, other loci can strongly modify the severity of the Min phenotype. An emergent challenge is to find ways to identify the full set of genes that interact with the intestinal cancer predisposition of the Min mouse strain. With such a set, one can then work, using contemporary mouse genetics, to identify the molecular, cellular and organismal strategies that integrate their functions. Finally, with appropriately phenotyped human families, one can investigate by a candidate approach which modifying factors influence the epidemiology of human colon cancer. Even if a candidate modifier does not explain any of the genetic epidemiology of colon cancer in human populations, modifier activities discovered by mouse genetics provide candidates for chemopreventive and/or therapeutic modalities in the human.

Keywords: Min mouse; *Mom1* modifier locus; *APC*/*Apc*; Knudson hypothesis; clonality; cancer genetics

1. INTRODUCTION

As a dynamically self-renewing tissue, the intestinal epithelium must maintain homeostasis by possessing both positive regulators driving cell proliferation and negative regulators mediating cell loss. The complexity of homeostatic regulation can be inferred from the fact that neoplasms of the intestinal epithelium, although among the most common of human cancers (Cutler *et al.* 1974), do not afflict every individual in spite of the risk attendant to vast numbers of mitotic events per day within the intestinal epithelium. Indeed, the age dependence for the onset of clinically diagnosed colon cancer has been modelled mathematically as reflecting a six-hit process (Peto *et al.* 1975).

Descriptive analysis introduces members of the molecular and cellular casts of characters found in three biological dramas: normal, self-renewing growth; neoplastic growth; and regenerative growth after damage from ionizing radiation. Some of these characters can be shown to act as causative protagonists or antagonists within one or more of these dramas. A causative role is inferred by the discovery of a mutation affecting the molecular or cellular character and simultaneously affecting the biological process. The genes for which such mutational evidence has been available involve human

families with strong predispositions to cancer. These highly penetrant, dominant mutations have enabled the molecular cloning of the causative molecules by analysis of DNA samples from affected and unaffected members of these families. However, the full dramas of normal self-renewing growth and its deranged neoplastic counterpart involve far more than the highly penetrant protagonists and antagonists. How can investigators identify more fully the set of molecular and cellular interactions that can influence the outcome of the play? And once any one interaction has been identified on an abstract, formal basis, how can one analyse its mechanics?

These questions can best be addressed in an experimental organism, preferably a mammal. The laboratory mouse has been highly developed for controlled studies of cancer genetics and cancer biology. The production of genetic models for familial human cancers has not been routinely successful (Jacks 1996). However, the Min (multiple intestinal neoplasia) mouse strain, heterozygous for a nonsense mutation in the mouse adenomatous polyposis coli (*Apc*) gene (Su *et al.* 1992), does show at least the early stages of the familial cancer syndrome (Moser *et al.* 1990). A number of investigators are studying how well the Min mouse can provide an experimental stage on which to identify functional interactions in the normal and neoplastic growth of the intestinal epithelium (see Dove *et al.* 1995; Shoemaker *et al.* 1997*a*). The biology and genetics of the laboratory mouse is used in these studies to analyse *in vivo* the mechanism of interaction, through classical genetic pathway analysis and several modes of chimerism and mosaicism. It remains to be seen how accurately these *in vivo* analyses can be carried over to *in vitro* culture.

The final goal of the investigators working with the Min mouse strain is to determine whether mechanisms found to be important for the intestinal epithelium and its neoplasms in the laboratory mouse are also relevant to the human. No one presumes identity either in the biology or the genetic epidemiology between the mouse and the human species. But all must respect the depth of understanding that can be created by controlled experimentation. Given this starting point, it is necessary to make an appropriate interspecies translation from mouse to human.

2. THE CENTRALITY OF THE *Apc* REGULATOR

It is useful to view the 'tumour suppressor gene' *Apc* as a regulator rather than an engine of cell behaviour: tumour cells and trophoblastic giant cells within embryos (Moser *et al.* 1995) can thrive in the absence of *Apc* function. Several cell processes are known to be directly or indirectly regulated by *Apc*. These include transcription, adherens junction formation, the microtubular cytoskeleton, calcium-dependent cell adhesion, and cell migration (see Polakis 1995).

As expected for a negative regulator, intestinal tumour formation commonly requires the loss of function of both alleles at the *Apc* locus (Knudson 1971; Luongo *et al.* 1994) (figure 1). For the $Apc^{Min}/+$ mouse on the tumour-sensitive C57BL/6 genetic background, this process is completed by loss of the chromosome carrying the wild-type *Apc* allele. Interstitial deletions induced by somatic gamma irradiation

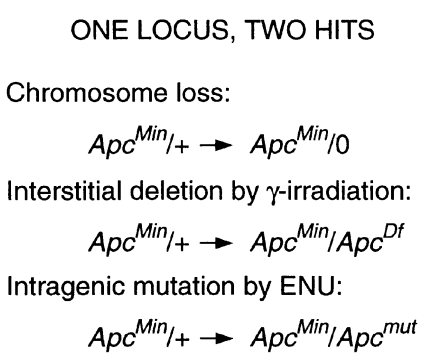

Figure 1. Adenoma formation in $Apc^{Min}/+$ mice. Under sensitive conditions of genetic background and/or exposure of animals to ethylnitrosourea (ENU), adenomas form by a canonical Knudson (1971) process involving genetic inactivation of both alleles at the *Apc* locus.

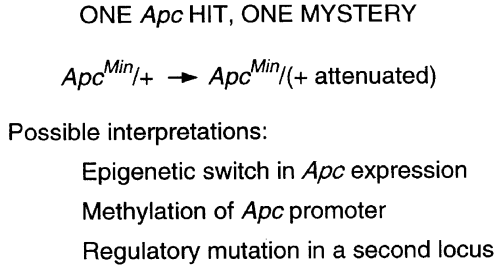

Figure 2. Adenoma formation in $Apc^{Min}/+$ mice on the resistant genetic background of AKR. When the canonical Knudson (1971) process (figure 1) is less probable, a distinct route for silencing the wild-type *Apc* allele can be inferred (see text).

delimit the necessary region of genetic loss to the *Apc* locus (Luongo & Dove 1996). Somatic treatment with ethylnitrosourea (ENU) induces point mutations in *Apc*, most frequently detected as chain terminations of the Apc polypeptide (Shoemaker *et al.* 1997*b*).

When the *Min* mutation is placed on the highly tumour-resistant AKR genetic background, the chromosomal loss pathway for removing wild-type *Apc* function is less prevalent (A. R. Shoemaker, unpublished data). Interestingly, F1 hybrids between the sensitive C57BL/6 strain and the resistant AKR strain show the chromosomal *Apc*-loss pattern of C57BL/6 (Luongo *et al.* 1994; Luongo & Dove 1996). This observation is consistent with dominant determination of chromosomal instability by an allele or alleles carried by C57BL/6 (see Lengauer *et al.* 1997). Evidence for germline factors influencing karyotypic stability has now been published (Cahill *et al.* 1998).

When the chromosome loss pathway is less frequent, as with strain AKR·Min, a new scenario comes to light (figure 2). Tumours form at very low multiplicity, owing not only to the attenuation of the chromosome loss pathway but also to the action of resistance alleles of polymorphic modifier loci such as *Mom1* (to be discussed below). When tumours form while retaining the chromosome carrying the wild-type *Apc* allele, the expression of the Apc polypeptide is attenuated (figure 3). This loss of expression is not accompanied by any known mutation of the *Apc* gene: the wild-type DNA sequence at the *Min* site is retained, and no chain-terminating mutations of *Apc* are detected (A. R. Shoemaker, unpublished data). This

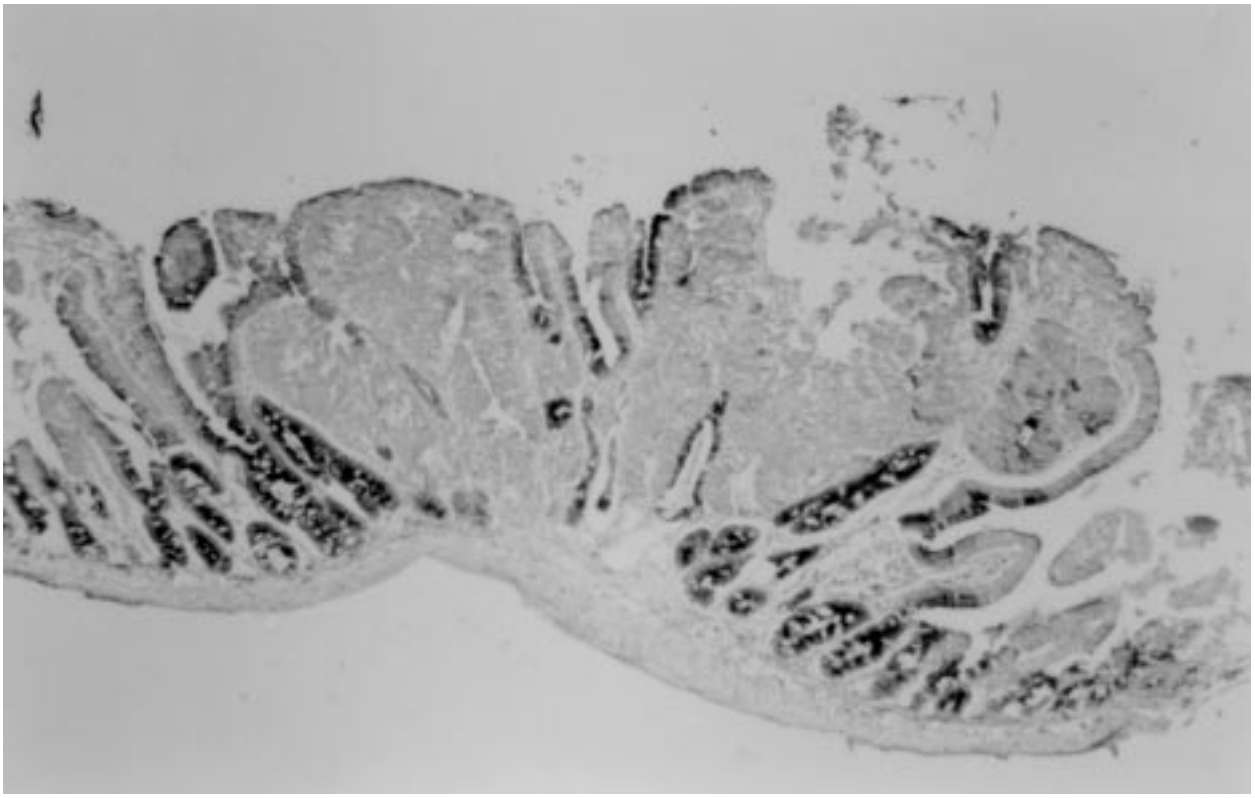

Figure 3. Silencing of Apc^{+} expression. Antibody 3122 to Apc (Midgley *et al*. 1997) was used for the detection of wild-type Apc antigen by immunoperoxidase (A. R. Shoemaker and C. Midgley, unpublished data). Normal crypts display abundant antigen, but adenomatous tissue is negative in tumours that form in AKR·Min animals after neonatal exposure to ENU.

scenario is currently mysterious, with several conceivable explanations: (i) the wild-type allele might be silenced by a stable epigenetic change, along lines initially suggested by Solomon, Bodmer and their colleagues (Solomon *et al*. 1987); (ii) a specific epigenetic mechanism, hypermethylation of the *Apc* promoter, might attenuate *Apc* expression (Hiltunen *et al*. 1997); or (iii) a second locus that regulates the expression of *Apc* might be somatically mutated (Haber *et al*. 1990). Consistent with this last possibility, the incidence of tumours retaining but not expressing an apparently normal *Apc* allele is increased by appropriately timed ENU treatment of AKR·Min mice (A. R. Shoemaker, unpublished data).

Note that adenoma formation by this alternative pathway remains dependent on the germline Apc^{Min} allele: no intestinal adenomas have been found in control AKR animals. Evidently, the mechanism of attenuation, whether purely epigenetic (model (i) or (ii)) or downstream of a regulatory mutation (model (iii)), is not sufficiently strong to silence successfully both wild-type copies of *Apc* in an $Apc^{+/+}$ animal.

The presentation of an alternative molecular genetic route to *Min*-dependent adenoma formation in strain AKR raises the possibility that there can be more than one stem cell origin for such tumours. The study of this possibility would be greatly enhanced if a rich set of diagnostic molecular markers became available for the stem cells of the intestinal epithelium and its neoplasms. At present, only the *lacZ* insertion ROSA11 provides such a marker (Gould & Dove 1996).

Altogether, one feature is shared by the several specific mechanisms of adenoma formation in the Min mouse: Apc function must be strongly reduced or lost. This function must play a central role in protecting the normal intestinal epithelium against the dysplastic and neoplastic derangements that lead to adenoma and beyond. A central protective function such as this has been designated a 'gatekeeper' by Kinzler & Vogelstein (1996). If *Apc* acts as a 'gatekeeper', is loss of its function sufficient to generate the intestinal adenoma?

The issue of sufficiency is being pursued along three lines of inquiry: the search for developmental stages at which adenoma formation is enhanced; the analysis of cellular interactions between *Apc*-negative clones in adenoma formation; and the identification of loci other than *Apc* in which mutations enhance or suppress the probability of *Min*-induced adenoma formation. The developmental profile for adenoma formation was studied by timed somatic treatment of Min mice with ENU. It was found that neonates up to 14 days of age were particularly susceptible to stimulation of adenoma formation (Shoemaker *et al*. 1995).

3. THE POLYCLONAL STRUCTURE OF EARLY ADENOMAS IN Min MICE

The number of genetic events involved in tumorigenesis is increased if tumours are polyclonal (Novelli *et al*. 1996). Whether this increase is obligatory depends upon whether the polyclonality is necessary. This issue requires deeper experimental study and has been started by analysing the clonal structure of early adenomas in the Min mouse (Merritt *et al*. 1997). The analysis has employed a cell lineage marker, ROSA26, that expresses

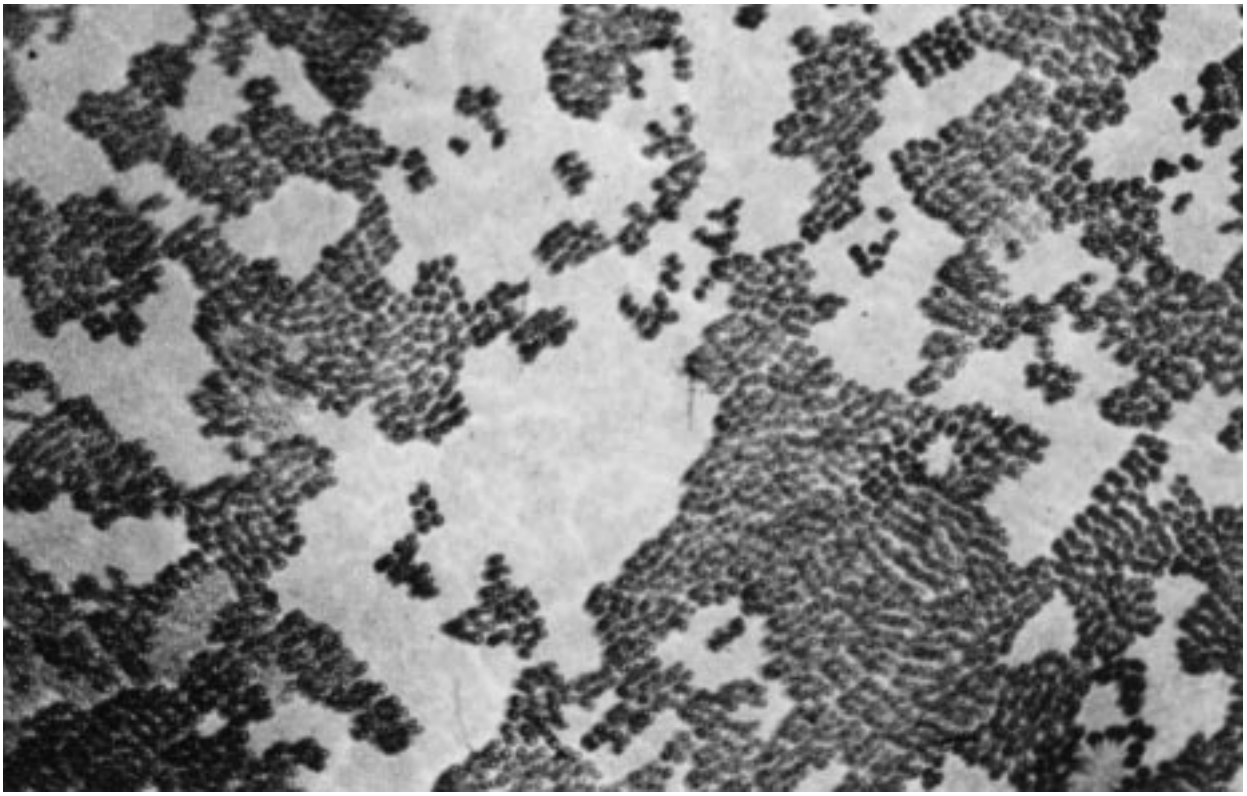

Figure 4. Chimerism for *ROSA26* (*lacZ*). Whole-mount colonic tissue from a morula fusion chimera C57BL/6 ↔ C57BL/6·*ROSA26*/+ was histochemically stained by XGal. Control non-chimeric ROSA26/+ animals display uniform staining in all cell types of the intestinal epithelium. Negative controls (C57BL/6) show no staining.

the β-galactosidase of *E. coli* in all cell types of the intestinal epithelium or its neoplasms (Wong *et al.* 1996; Zambrowicz *et al.* 1997; Gould & Dove 1997). Whole-mount tissue stained with the substrate X-Gal can be visualized as blue before or after sectioning. To avoid complications of genetic modifiers of the tumour phenotype, ROSA26 was introgressed onto the standard sensitive genetic background, C57BL/6. Perhaps owing to the isogenicity of the two components of the aggregation chimeras, the intestinal epithelium shows very fine-grained chimerism for the two participating genetic lineages (figure 4). The average patch diameter is only six crypts. By fusion of morulae emerging from appropriate crosses, two informative chimeric animals were generated, each with the composition

ROSA26/+ *Min*/+ ↔ +/+ *Min*/+.

Analysing tumours from these chimeras, Ch112 and Ch113, established that both the white and blue components carried the *Min* allele.

Each chimera was asymmetric, with a minority ROSA26/+ (blue) component. The best estimator of the proportion of adenomas that are polyclonal in structure can be made from this minority component, as the probability that a polyclonal tumour will be covert (blue × blue) is small. Of a total of 260 adenomas from the entire intestinal tracts of the two informative chimeras, 28 adenomas were observed with at least some blue contribution. Of these, 22 were mixed with white tumour tissue. Thus, the proportion of polyclonal adenomas can be estimated to be at least 79%.

These observations are consistent with the study of polyclonality in a human familial adenomatous polyposis (FAP) patient reported by Novelli *et al.* (1996). Importantly, the chimeric analysis that is permitted by the *ROSA26* cell lineage marker is not open to the alternative interpretation offered by Novelli and his colleagues for their material: that the mixed XY/XO karyotype of the human FAP adenomas had been created after the initiation of the tumour by loss of the dicentric Y chromosome within the tumour. However, neither set of observations demonstrates that the adenoma is polyclonal when first formed. Alternative models will be discussed below, wherein the polyclonality is acquired, not *de novo*.

Bjerknes *et al.* (1997) have reported adenomas in FAP patients in which only a subset of the dysplastic crypts have lost the expression of APC. Do these phenotypically mixed FAP human adenomas correspond to the polyclonal Min mouse adenomas documented by lineage marking? Gould & Dove (1997) observed that adenomas in chimeric mice in which one component was $Apc^{+/+}$ were derived only from the *Min*/+ component. In microdissected blue and white sectors of mixed adenomas from Ch112 and Ch113, the *Apc* genotype has been analysed. In most cases, each sector is Apc^{Min}/0 (Merritt *et al.* 1997).

Remarkably, at least two rare somatic events have contributed to the polyclonal adenoma, in distinction to the observation of assimilative dysplasia by Bjerknes *et al.* (1997). How can one understand the coincident occurrence of at least two somatic non-disjunction events within a few crypt diameters of one another? Perhaps the events are not independent. Can mitotic non-disjunction be induced by a

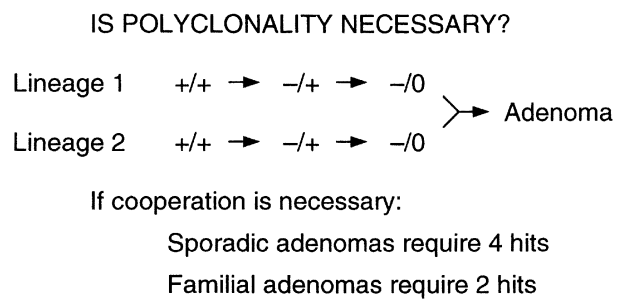

Figure 5. Is polyclonality of intestinal adenomas necessary? The extreme model is presented in which the formation or survival of adenomas requires clonal cooperation ('polyclonal origin').

neighbouring *Apc*-negative clone? Do clastogenic 'storms' pass over the developing gut? As noted above, the neonatal crypt seems to be a favoured precursor for the adenoma; neonatal crypts have an intrinsic polyclonal architecture (Schmidt *et al.* 1988).

The polyclonal structure of most, if not all, early familial adenomas can reflect any of a broad range of mechanisms for clonal interaction. Strong interaction models posit that cellular interactions are necessary for the initiation of adenomas (figure 5). These models resemble the formalism of the 'community effect' in determinative changes of embryonic potential (Gardner 1993; Gurdon *et al.* 1993), or polyclonal transdetermination within imaginal disks of *Drosophila* (Gehring 1967), but require that the cooperating cells be *Apc*-negative somatic variants. Intermediate models involve quantitative rather than qualitative requirements for cellular interaction. For example, the ability of a nascent adenoma to grow to detectable size or to survive any purging process of the host may be enhanced by fusion with a neighbouring adenoma. Finally, passive interaction models propose that only a limited region within the intestinal epithelium of an animal can develop adenomas, enhancing the probability of fusion (see Novelli *et al.* 1996, footnote 25). A simple mathematical representation of the spatially random fusion model posits that pre-adenomas form by a homogeneous Poisson process and fuse if they are sufficiently close (M. A. Newton, unpublished data). Approximations derived from this model lead to a likelihood function for a parameter governing the expected number of preadenomas in a particular region, involving only the counts of pure and mixed tumours. Linking this information with the dimensions of the intestinal regions, it is calculated that the mixed tumour counts in the small intestine of chimeras Ch112 and Ch113 do not rule out random fusion. However, for the colons of these two chimeric Min mice, the fusion model would require a spatial restriction of colonic adenomas to 20% of the available tissue. It remains unstated what biological factor would spatially restrict adenoma formation in this passive model.

Depending upon the strength of the clonal interaction that explains the polyclonal structures of most, if not all, familial intestinal adenomas, we must re-evaluate the process of sporadic cancer in this tissue. If adenoma formation absolutely requires cooperation between a pair of initiated clones, then two initiation hits are required in the familial and four in the sporadic case (cf. Knudson 1971). In this cooperation model, it seems plausible that promoting agents would provide the necessary community of initiated cells within a single lineage through formation of an initiated microclone (Rous & Kidd 1941). Indeed, Ponder & Wilkinson (1986) and Griffiths *et al.* (1989) have provided strong evidence for monoclonality in chemically induced intestinal tumours in mice. The latter report is particularly persuasive in that very small patch sizes were achieved, owing to the isogenicity afforded by X-inactivation mosaicism. Covert polyclonality would be rare in these experiments.

Daunting experimental challenges now emerge: to determine the nature and strength of the cellular interactions that explain the polyclonal structure of early familial adenomas; and to extrapolate an understanding from the familial case to the far more prevalent sporadic case. Because chemical carcinogenesis may involve tissue damage and regeneration, we cannot reliably equate that process to sporadic neoplasia.

4. MODIFIERS OF THE MIN PHENOTYPE

A number of molecules are already known to interact directly with the APC/Apc polypeptide, and a number of molecular species are known to be induced in intestinal adenomas. In principle, mutations that modify the Min phenotype can occur in molecules that interact with APC/Apc either directly or indirectly. The search for these interactors began with the finding that F1 hybrids generated by mating the sensitive B6-Min strain to certain other inbred mouse strains show tumour multiplicities reduced by up to an order of magnitude (Moser *et al.* 1992). By synergizing with the emergent dense genetic map of the mouse organized in the Genome Center at the Massachusetts Institute of Technology (MIT), it was possible to map a polymorphic modifying locus, *Mom1* (modifier of Min), to a 15-centimorgan (cM) interval on mouse chromosome 4 (Dietrich *et al.* 1993). To study *Mom1* in the absence of other polymorphic modifier alleles carried by *Min*-resistant inbred strains, a congenic derivative of C57BL/6 was established, heterozygous for a 30-cM region containing the resistance allele of strain AKR (Gould *et al.* 1996*a*).

With this 'purified' genetic material, it was possible to obtain contemporaneous parallel sets of progeny each carrying the Apc^{Min} allele but differing in the *Mom1* region. The alleles at the *Mom1* locus control the net growth rate of *Min*-induced adenomas semidominantly. Tumour multiplicity also shows a semidominant response to the alleles of *Mom1*. The semidominance prevented knowing which *Mom1* allele is active: resistance, sensitivity, or both.

A further refinement of the map location of *Mom1* was carried out by identifying animals carrying chromosomes recombinant within the 30-cM congenic substitution and then phenotyping a set of testcross progeny carrying the recombinant chromosome. This demanding process delimited the *Mom1* locus to a 3-cM interval (Gould *et al.* 1996*b*).

It is almost prohibitive to identify at molecular resolution a locus whose phenotype is only quantitative (Falconer & Mackay 1996). Recombinants must be phenotyped by establishing populations of animals. The difficulty is compounded for polymorphic determinants,

owing to the background level of polymorphic nucleotide substitution of at least one substitution per 1000 base pairs (see Dove 1987). One can, however, explicitly test candidate genes that map within the 3-cM region to which the quantitative determinant can be feasibly mapped. One candidate, a secretory phospholipase (encoded by *Pla2g2a*), was suggested by MacPhee *et al.* (1995), survived the 3-cM delimitation process (Gould *et al.* 1996*b*), and therefore deserved an explicit test. A cosmid transgene carrying the entire genomic sequence for *Pla2g2a* was isolated by our collaborators Karen Hong and Eric Lander (MIT) and transferred into B6-Min mice (Cormier *et al.* 1997). Western blots and immunohistochemistry for the secretory phospholipase indicated that the transgene was expressed throughout the small and large intestine, although the levels were much higher and the distribution more patchy in comparison with the antigen encoded by the AKR allele of *Mom1*.

The transgene confers a twofold reduction in tumour multiplicity on B6-Min mice, establishing that the secretory phospholipase can provide for active resistance (Cormier *et al.* 1997). It is premature, however, to conclude that *Pla2g2a* accounts fully for *Mom1*. The transgene, although overexpressed, does not achieve the same level of resistance as found with two copies of the AKR allele of *Mom1*. Furthermore, the fine-structure analysis of Gould *et al.* (1996*b*) indicated that the *Mom1* locus could be subdivided into more than one factor. The full story of the effects of *Mom1* on the net growth rate and multiplicity of *Min*-induced intestinal adenomas remains to be learned.

If the effect of *Mom1* involves active resistance mediated by a secretory phospholipase, does it act as a cell-autonomous negative regulator of the intestinal adenoma, that is, as a classical 'tumour suppressor gene'?

Two tests for cell-autonomous action of the resistance allele of *Mom1* have been carried out: chimeras generated by grafting foetal intestine from *Mom1*-sensitive donors to *Mom1*-resistant hosts (Gould & Dove 1996); and chimeras generated by morula fusion, in which the *Mom1*-resistance allele is introduced either in the same lineage as the *Min* mutation, or in a separate lineage (Gould & Dove 1997). In each case, the Min phenotype matched that of the $Apc^{Min}/+$ component of the chimera; no effect of the *Mom1*-resistance allele could be detected when that allele was carried by the *Apc* wild-type component of the chimera. Note that this test for cell autonomy cannot detect intercellular action between different cells lying within the same crypt lineage. Because the adult crypt is monoclonal, all its derivatives belong to the same lineage. The secretory phospholipase seems to be expressed at least in the Paneth cell compartment of the small intestinal crypts and could diffuse from that source to affect the behaviour of proliferative cells within the same crypt.

Two strategic points need to be recognized in these chimeric analyses of cell-autonomous versus intercellular gene action. First, the interpretation of these experiments becomes ambiguous if the components of the chimera differ by more than one gene affecting the phenotype. In plausible situations, autonomy for one factor will obscure non-autonomy for a second (figure 6).

For example, consider an autonomous positive regulator, A, and a diffusible positive regulator, D. If the active wild-type alleles of both A and D lie in one component of a

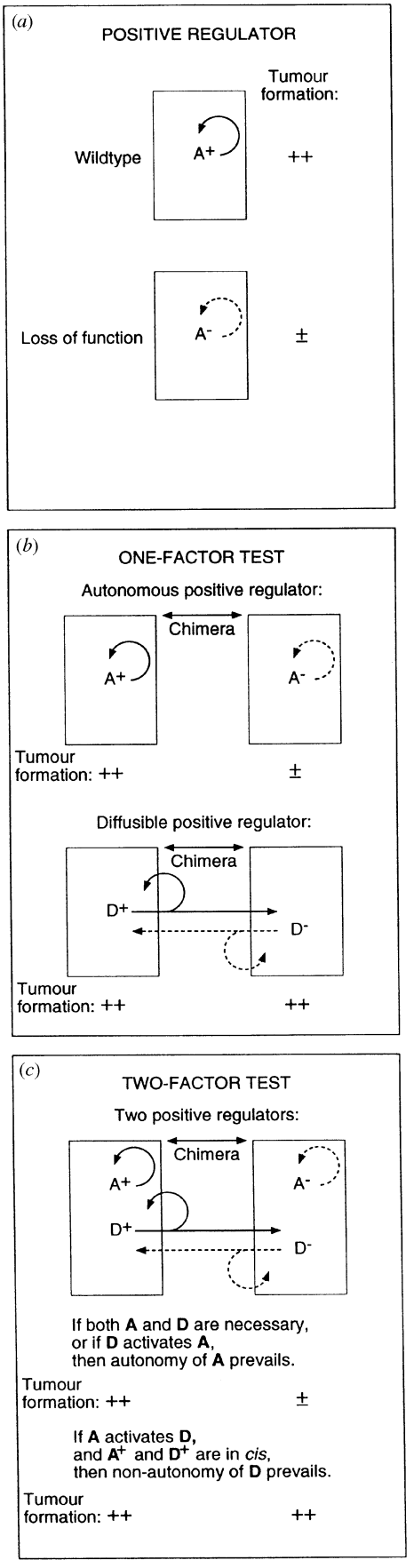

Figure 6. Tests by chimerism for autonomy of gene action. Ambiguity of interpretation arises if more than one genetic factor controlling the phenotype differs between the two components of a chimera (see text).

chimera and the inactive mutant alleles in the other, the autonomy of A will prevail if both A and D are necessary or if D operates by activating A. By contrast, if A operates by activating D, then the non-autonomy of D will prevail.

A second strategic consideration, raised above in respect to the Paneth cell, indicates that the resolution to which cell autonomy can be assessed depends upon the clonal architecture of the tissue in question. In an extreme situation in which an entire tissue emerges from one somatic clone, one clearly cannot judge whether there is non-autonomous gene action between different cells within that tissue.

Mom1 fails to show one hallmark of a cell-autonomous negative regulator of neoplasia: loss of heterozygosity within tumours. *Min*-induced tumours in *Mom1* heterozygotes remain heterozygous over the *Mom1* region (Dove *et al.* 1994) and in particular for *Pla2g2a* (Gould *et al.* 1996*b*). This genetic behaviour of a non-autonomous negative regulator of neoplasia can be classified as maintenance of heterozygosity (MOH). This observation is consistent with expression of the secretory phospholipase from the Paneth cell (Gould & Dove 1997).

Finding that a secretory phospholipase can act as an active resistance factor, controlling the size and multiplicity of *Min*-induced adenomas, indicates that in some way lipid signalling positively regulates a biological process that limits the adenoma. Conceivable candidate biological processes include migration from the proliferative niche of the tumour, differentiation into post-mitotic derivatives, or position-dependent apoptosis. Superficially at least, these possibilities are distinct from those that underlie the effects of mutations in the inducible cyclooxygenase gene *Cox2* (Oshima *et al.* 1996). Here, a loss of function results in a strong reduction in adenoma size and multiplicity, as if *Cox2* were a positive growth regulator involved in the metabolic maintenance of the adenoma through prostaglandin biosynthesis. Thus, two contrasting roles can be imagined for the effects of lipid metabolism in general on adenoma formation or maintenance. Perhaps, the secretory phospholipase does not operate through prostaglandin biosynthesis.

Does the study of *Mom1* and the secretory phospholipase in the Min mouse bear on human colon cancer, familial or sporadic? Several investigators have failed to find an association of the allelic status of the human homologue *PLA2G2A* with the severity of familial colon cancer or the risk for sporadic colon cancer. Furthermore, somatic mutations in this gene have not yet been found in human colon cancers (Riggins *et al.* 1995; Spirio *et al.* 1996; Tomlinson *et al.* 1996*a*,*b*). The failure to find mutations in tumour material is explained if the effects of the secretory phospholipase are non-autonomous to the tumour. The failure to find epidemiological associations between familial disease severity and the allelic status of *PLA2G2A* may reflect the fact that severely affected mutant alleles of this gene have not yet been found in the human population, in contrast to the frameshift allele of the sensitive C57BL/6 mouse strain. Thus, the relevance of the secretory phospholipase to human colon cancer is unresolved.

As a general proposition, the difficulties in identifying quantitative modifier loci in the human may remain largely insuperable if one works solely with human family data and performs genomic scanning for association of genotype and phenotype. Crucially, one is also not confident *a priori* that the phenotyping of the available human families has the same objective quality as that of a closed population of heterozygous congenic mice segregating for a single genetic factor. The clinical diagnosis may emphasize the status of the most advanced tumour in an individual rather than the overall adenoma phenotype (see Tomlinson *et al.* 1996*a*). Further, the contribution of environmental variation to the phenotypic variation in human populations may overwhelm subtle quantitative effects of modifying alleles. Thus, in a genomic scan for modifying alleles, the statistical power to map a genetic modifier may be inadequate to overcome the environmental variation and the imprecision of the phenotyping. It would seem that the best available strategy is to test specific candidate loci for phenotypic effect within the available human families. One source for candidate loci is the set of human homologues of any modifier loci identified for the cognate process in the mouse. However, one is not assured that equally informative alleles are available in the two species. Tomlinson *et al.* (1996*a*,*b*) have found an association of an index of disease severity in FAP families with the 1p35–36 region in the human genome surrounding the *PLA2G2A* locus, although, ironically, not with alleles of the secretory phospholipase.

5. CONCLUDING REMARKS

An entrée into the study of the intestinal epithelium is provided by the Min strain of the mouse, a familiar intestinal cancer model. Beyond identifying the highly penetrant germline lesion that leads to this cancer, we have been able to identify a modifying locus, *Mom1*, that alters the expressivity of the *Min* mutation. The strain AKR contains alleles that modify the phenotype even further, yet retain the common theme: Apc function must be attenuated or lost in intestinal adenomas. The *Mom1* locus constitutes the beginning of an exploration of the genetic network that impinges on *Apc* and intestinal cancer. The general use of the *ROSA26* cell lineage marker permits analysis of the clonality of the neoplastic transitions and the range of action of any genetic modifier.

The cellular interactions within epithelia reflect a network of genetic interactions. The cellular/genetic interactions and the overall dynamics of the network can best be approached by finding single genetic differences that affect the system, identifying the molecule involved and then analysing the mode of action by chimerism and by cellular biochemistry. The analysis of an entire system will bring human genetics into a new level of dialogue with the biology of the human, moving beyond the search for 'disease-causing genes'.

The geneticist's response to the challenge lodged by Garrod in his valedictory 1927 lecture (table 1) is to start with a population of individuals at high risk to a disease and to find mutant alleles that either suppress or enhance that disease. We believe that *Mom1* has no strongly detrimental effects of its own, because the C57BL/6 mouse is healthy although homozygous for a frameshift mutation in the secretory phospholipase. In the presence of the *Min* mutation, however, the status of the *Mom1* locus makes a significant difference, apparently by conferring active resistance. Modifying activity on a

Table 1. *Protective alleles*

Difficult as it is to detect an error of the body chemistry by its evil effects, which may be long postponed, it [is] more difficult to detect those which are harmless or have only good effects.
(Garrod 1927)

. . . my colleagues and I foresee decades in which mouse strains that closely simulate human disease predispositions will be used to detect protective genes. . . . It will be interesting to see what comes of this.
(Dove 1995)

Genetic mutation proves to be a blessing
These new discoveries [that a genetic mutation can protect certain individuals from the AIDS virus] are a reminder that genetic mutations, though often deemed ''defects'', can sometimes prove a blessing, conferring surprising benefits on those lucky enough to inherit them.
(New York Times 1996)

primary disease phenotype is one way to discover alleles with only favourable effects.

The *APC/Apc* gene is broadly expressed in the mammal. Indeed, both human and mouse families heterozygous for *APC/Apc* mutations develop extracolonic lesions, including desmoid fibromas. The incidence of these extracolonic lesions depends on specific modifier loci. Deficiency for *p53* greatly enhances desmoid formation in Min mice, but has little effect on the early stages of intestinal neoplasia (R. B. Halberg and W. F. Dove, unpublished data). By contrast, *Mom1* does not strongly affect desmoid neoplasia. Thus, the neoplastic phenotype of Min mice involves loci secondary to *Apc* that are specific for particular tissues.

In pursuing the whole story of intestinal cancer with the laboratory mouse, there are two major needs: (i) the expansion of the search for modifying alleles; and (ii) the development of *in vitro* systems of analysis that recapitulate aspects of the processes that are relevant *in vivo*. Meeting the first need will surely enhance meeting the second, as each *in vitro* preparation from mutant material will provide a substrate for the study of the single missing substance. The successes in developing haematopoietic cultures that show response to the *Steel* and *W* mutations have provided a strong precedent for such a general *in vivo*/*in vitro* strategy (Dexter 1986; Dexter & Allen 1992). Over the coming decade, investigations of the intestinal epithelium *in vivo* may develop a more direct line of communication with manipulations *in vitro*.

It will be interesting to see whether experimental investigations will bring forward an understanding of the apparent six-hit actuarial incidence curve for clinically diagnosed colon cancer in the human. Beyond epidemiology, experimental investigators will want to explore which fundamental biological lessons can be conveyed from the laboratory into the realms of prevention and therapy.

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