Morphostats: A Missing Concept in Cancer Biology

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Abstract
The role of specific morphogens is well established in the determination of body plans in development. A variety of morphogens have been identified; others are suspected. Pathways have been delineated.

In complex tissues, the ability to maintain fidelity of microarchitectural structure is crucial. Microarchitecture is a consequence of relationships among cells, not a function of single cells. Epithelial layers, in particular, are able to maintain their microarchitecture with remarkable accuracy over many decades despite recurrent damage, regular cell turnover, and complexity of structure. Nonetheless, metaplasia and transdifferentiation (change in tissue structure without cell dysplasia) do occur, suggesting that there is the possibility of loss of control or change of control of the microarchitecture. A strong inference to be derived from the above is that there are control systems and molecules and that these are derived from cells that are outside, but plausibly adjacent to, the respective epithelia.

It is postulated that there are morphogen-like controller molecules with morphogen-like functions in adult epithelial tissues. These are responsible for the maintenance of normal tissue microarchitecture. Because the function of these putative molecules is maintenance of tissue structure, I have chosen to call them morphostats by analogy with morphogens. It seems plausible that morphostats and morphogens may constitute overlapping families of molecules.

Evidence for the existence of morphostats can be derived from a variety of in vivo and in vitro data and from studies of normal tissue, precancer, and cancer, including: (a) the existence but rarity of metaplasia and transdifferentiation; (b) the fact that metaplasias are multicentric and are only one step from normal but do not show any consistent epithelial mutation; (c) the genesis of animal cancers by simple transplantation of tissues into the wrong environment and the evidence that epithelial mutation is not a feature of such transplantation carcinogenesis; (d) the fact that carcinogenesis occurs frequently at the junctions of different epithelial types, e.g., squamocolumnar junctions in gastrointestinal and genital tracts; (e) the fact that cancer-associated fibroblasts can stimulate proliferation in transformed cells but not influence normal cells; and (f) the failure to grow most epithelial organs in a fully differentiated structural pattern in monolayer culture.

It is suggested that morphostats may function like morphogens inasmuch as they may act via a diffusion gradient from source mesenchymal cells and provide architectural instruction for complex adult epithelia. Morphostats may influence architecture via control of cell adhesion, apoptosis, and proliferation. Some specific predictions follow from this hypothesis, most notably, a new two-hit model of cancer: one mutation in an epithelial cell resulting in disruption of cell function and structure (e.g., dysplasia); and the other in a mesenchymal or other supporting cell resulting in disruption of tissue microarchitecture. The corollary of this is that there will be mesenchymal mutations producing microarchitectural abnormalities without epithelial dysplasia and vice versa. Disruption of the functions of morphostats may result in a variety of abnormalities. Such disruption may be a key event in carcinogenesis.

Introduction
"Taxonomies are not neutral or arbitrary hat-racks for a set of unvarying concepts; they reflect (or even create) different theories about the structures of the world." — Gould and Vrba (Ref. 1)

The limited number of cell types (i.e., neurons, specific epithelia, the spectrum of hematological cells, etc.) has been noted, and questions have been raised regarding why these cells exist and not others (2). In contrast, we have not asked why there is a limited number of tissue types. Furthermore, we have not asked what maintains fidelity within tissues and prevents, for instance, the endometrial epithelium from becoming a gastric epithelium.

It could be argued that tissue architecture is an inevitable outcome of the cross-talk between cells, and, indeed, as noted below, there are a variety of theories as to how this is accomplished. Nonetheless, metaplasia, transdifferentiation, and neoplasia can occur; therefore, cross-talk can fail as the architect of tissues. Thus, there are normal constraints on the tissue morphology; therefore, there must be specific controller pathways and molecules.

The earliest observable change in the progression toward cancer is disruption of tissue architecture. Therefore, cancer is not just a function of cells (see Ref. 3) and, despite opinions to the contrary (4), is not specifically a function of proliferation; sometimes there are excess numbers of cells, sometimes there is cell loss, and sometimes there is metaplasia, but there is always disruption of architecture.

Bissell et al. (5) have shown that cross-talk is central to the cancer process. The hypothesis advanced here is that a key element is the loss of a specific kind of instructional cross-talk,
namely, that which is responsible for maintaining tissue architecture.

Models of cancer are focused almost exclusively on cells and molecules (6). I have previously suggested, nonetheless, that it is possible to construct models of cancer at the level of population, organism, organ, cell, and molecule (7) and that these are not all reducible to the molecular level. Certainly, others have argued that cancers themselves should be regarded as complex tissues in their own right (3, 6, 8–12). Here I argue that cancer is importantly characterized not only by cell and molecular dysfunction but by loss of normal tissue microarchitecture and that this loss is a (perhaps the) fundamental step in carcinogenesis.

The Generation of Form in Tissues

Tissues are complex mixtures of cells. They are constructed via an intricate set of processes that involve, even in simple organisms, multiple signaling pathways, stepwise and integrated differentiation, proliferation, apoptosis, cell migration, etc. Most of these same processes persist in replicating postembryonic (and, in adults, largely epithelial) tissues. Blood cells also undergo controlled replication throughout life but are not organized into tissues. Cells of the immune system exist in both states.

In reference to the process of tissue construction and morphogenesis, Lawrence and Straub (13) describe the class of fundamental organizers of tissue morphology–morphogens–as follows: “a morphogen emanates from a localized source and diffuses away to make a concentration gradient.” They further note that the complete morphogen does more than just turn genes “on” or “off” at different concentrations; it orchestrates cellular behavior coherently so that its distribution prefigures the pattern. Hence, if the distribution changes, “even details of the pattern change in a predictable and coordinated way.” Perhaps the most complete understanding of the process of constructing an organism has been generated for Drosophila (14–19).

In the context of developmental biology, Ettinger and Doljanski (20) define pattern formation, morphogenesis, and differentiation. However, they do not provide a definition of the process that is involved when, in adult tissue, microarchitectural form is maintained. The process in adult tissue includes a limited form of morphogenesis as well as differentiation, and thus, many of the ideas that they explicate are appropriate in the context of adult tissues as well. For instance, they stress the importance of the ECM in molding the architecture of tissue and note that the basement membrane forms the scaffold of epithelial tissue (20, 21). They note that elimination “of the basement membrane results in loss of the tissue-specific structure” (20). They also note the role of the ECM in determining the “condensations” that are characteristic of mesenchyme that is organizing itself into an instructive unit, here in the setting of limb morphogenesis, but such condensations are a more general phenomenon in the initiation of morphogenesis of many organs (22). They note that although the mechanism underlying the condensation process remains unknown, there are a variety of possibilities, but it seems likely that important signaling pathways including those involving TGF-β and basic fibroblast growth factor and cell adhesion molecules such as cadherins are involved (20).

The primary thesis of the Ettinger and Doljanski paper (20) builds on the central idea of the work of Bissell et al. (5) regarding dynamic reciprocity between the ECM and epithelia; Bissell et al. have argued cogently that the ECM is likely to direct epithelial cell gene expression, an idea that has subsequently been shown to be the case in a variety of different systems and tissues, with evidence that ECM interactions can induce morphogenesis, differentiation (23–25), and reversion of malignant phenotype (26).

Ettinger and Doljanski (20) argue on several grounds that it is a small group of mesenchymal cells, in concert, that shape the ECM. Importantly, among these grounds is the unpredictability of the behavior of single cells (27) and the evidence from Gurdon (28) of a community effect: in early development, “the ability of a cell to respond to induction . . . is enhanced by, or even dependent on, other neighboring cells differentiating in the same way at the same time.” Ettinger and Doljanski (20) go on to suggest that “there may be no specific way by which cells proceed to form a tissue; rather there is a continuous passage from one configuration of cells and ECM to other more stable configurations. At each moment in time, the configuration reached serves as a template to increase the chance of certain configurations and reduce that of non-relevant configurations.”

Thus, morphogenesis is not controlled by a genetic program within each cell with each step obligatorily coded, rather there is regular interaction between the external environment, the programming capacity of cells outside the epithelium (mesenchyme), and the physical constraints of the ECM on the one hand and the developing epithelium on the other.

If this kind of selective stabilization (Refs. 29 and 30; see also Ref. 31) applies, mutatis mutandi, to adult tissues and to cancer tissues, there are at least two important consequences: first, studies of microarray differences between normal and cancer tissues will be seriously misleading and probably uninterpretable. It has already been noted that the patterns of expression within tissue from any one cancer, irrespective of treatment etc., are more alike than are patterns even across tumors from the same organ (32). Second, unless we look at the expression patterns of mesenchymal cells and the structure of the ECM, the essential nature of differences across adult tissues, both normal and pathological, will escape us: we will not be able to read the extraepithelial controls—in their normal or their pathological state—that define the epithelium of interest.

Adult epithelia face the same issues as embryonic tissues: how to maintain an overall form while constituent elements (cells) proliferate, move, differentiate, and die. However, adult epithelia face additional issues: the need to restore integrity after injury and the need to avoid chronic damage. Despite these challenges, most adult tissues do maintain a consistent microarchitecture over many decades. It is reasonable to ask whether the same kinds of controls and signals that build the tissues in the embryo have counterparts in the maintenance of the adult architecture. Hodges (33) notes that the stroma possesses “certain qualities that generate specific genetic expression and regional specialization from the epithelial repertoire . . . . In the absence of stroma, epithelia show, in general, a limited capacity for survival and cytodifferentiation, failure to undergo morphogenesis, and loss of histotypic organization.”

Just as the outcome of tissue development is a consequence of relations among cells and support structures (especially the ECM) and not just the differentiation of the cells themselves, it is reasonable to ask whether the maintenance of the architecture of replicating complex adult tissues is a function of relations among cells rather than a function of the individual cells themselves. This question applies to the normal

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The abbreviations used are: ECM, extracellular matrix; TGF, transforming growth factor; BE, Barrett esophagus; LOH, loss of heterozygosity.
maintenance of epithelia, the repair of epithelia in the face of acute (e.g., wounding) and chronic (e.g., ulcerative colitis) damage, and the generation of new tissues (e.g., angiogenesis). This last has been the focus of considerable attention (34–37), but adult epithelial microarchitectural integrity has been much less considered.

**Fidelity and Loss of Fidelity of Microarchitecture**

There is overwhelming empirical evidence from all living organisms that microarchitectural consistency of tissues is the norm. Disruption of tissue architecture—gross or subtle, acute or chronic—is part of the definition of many diseases, regardless of whether they are initiated by endogenous processes or exogenous agents. Apart from inflammatory/infectious destruction of tissue architecture, the most obvious forms of distorted microarchitecture are metaplasia and transdifferentiation.

Metaplasia involves loss of control of a whole differentiation sequence. Metaplasias used to be described regularly and in detail in histopathology reports (38), particularly in relation to cancer, but they seem to be much less the focus of contemporary reports. Whether this is because the existence of metaplasia is taken for granted and is not seen to need explanation or because its meaning in the context of the more significant neoplasia is not understood, remains unclear. There are some obvious exceptions, perhaps most notably BE, where the metaplastic change is an established malignant precursor, but where much of the molecular biology remains to be unraveled (see the text below).

Slack (39) points out that metaplasia represents a single step from normal, otherwise one would sometimes find examples of metaplasia within metaplasia. Slack further argues that the switch that is altered in any specific case must be a master switch that allows different pathways to differentiation in different tissues, for example, a homeobox gene. Again the key implication for the hypothesis being advanced here is that the cells of origin of this signal must be outside the metaplastic epithelium.

Although BE is the most extensively studied of the metaplasias (largely because it is a premalignant condition), there are a large number of other identified metaplasias. These include intestinalization of the gastric mucosa [also a cancer precursor (40, 41)], cystitis glandularis of the bladder (42), a variety of abnormalities of the female reproductive tract epithelium (43), and squamous metaplasia of the lung and other sites (44). The appearance of bony metaplasias in tissue displaying chronic inflammation is also well described (38).

The fact that these and other metaplasias occur repeatedly in a consistent histotypic form and the overall rarity of metaplasia in general, both argue for a system whereby tissue architecture has complex controls, inasmuch as even when the controls fail, they do so in a relatively organized fashion. The tendency of the upper gastrointestinal tract, for instance, to produce lower intestine-like features may be evidence of a default state that has lost the fine-tuned structure that matches esophageal function; alternatively, it may represent a specific switch that allows different pathways to differentiation in different tissues, for example, a homeobox gene. Again the key implication for the hypothesis being advanced here is that the cells of origin of this signal must be outside the metaplastic epithelium.

In summary, the rarity of these phenomena (metaplasia and transdifferentiation) argue that there are controls to prevent such tissue restructuring, but the fact that the phenomena occur demonstrates that these controls can be disrupted.

**Maintenance of Tissue Architecture**

There is a close relationship between the maintenance of the specific architecture of tissues and the original construction of those tissues. In morphogenesis, sets of cells are defined in the embryo by a variety of organizer genes that specify the anterior-posterior axis, the dorsoventral axis, and, in *Drosophila*, segments and parasegments, ultimately resulting in blocks of cells with specific relations to each other and reduced potential for differentiation (13, 51).

Within these blocks, further separation and differentiation occurs, finally resulting in specific functional groups of cells in clearly defined structural compartments. In specific cases, the details of the control of the latter part of this process remain to be delineated, but a key element in the process is the morphogen gradient, a specific signaling molecule that determines or influences cells’ fates by virtue of differences in its concentration, which are largely specified by the diffusion distance from its source (52–54).

This process can occur over a continuous period of time or discontinuously with intervening growth periods (e.g., in insects and amphibia). For mammals, particularly humans, there is an extensive delay between embryonic morphogenesis and pubertal morphogenesis that modifies primary and secondary sexual characteristics.

The lifelong growth, maturation, and differentiation in adult vertebrate epithelia is most like this stage of pubertal morphogenesis. The major blocks and compartments of the body design are long since established, but the final steps are a continuous task. Whether the pubertal maturation of breast, prostate, larynx, endometrium, and gonads, in part, also parallels some of the earlier morphogenetic steps, does not pose any major problems for the general argument being advanced here. It might, however, suggest that even controllers of the earlier stages of morphogenesis could be involved in the control of maturation/differentiation and thus could be potentially involved in the dysregulation (and even carcinogenesis) of these organs; that is, known morphogens may continue to exert control over the architecture of the adult tissues throughout life.

**From the Concept of Morphogenesis to the Concept of Morphostasis**

The major distinction between the way in which unicellular and multicellular organisms function as whole animals is the distribution of tasks among different units (cells). One of the consequences of this specialization is that there are specific cell layers that constitute the interfaces between the organism and the environment. Such layers line the respiratory tract, the

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digestive tract, the renal tract, and the reproductive tract as well as making up the skin.

There is a special set of problems for both developing and adult epithelia that is related to the need for regular migration from basal layer to the surface, perhaps with specific functional (e.g., secretory) intermediate stages or alternative fates. The control of this process will include variation in short-range signaling, cell adhesion, and cell-cell and cell-matrix interactions. At the DNA level, the cell will have several functional stages: (a) reproductive cell basally; (b) functional capacity in the intermediate stages; and (c) quiescent/senescent cell or cell remnant at the surface. Therefore, gene expression will vary considerably. This again suggests the need for a morphogen gradient or at least some method of relating position to function.

In long-lived animals, the steps of morphogenesis are insufficient because interfaces need, in addition to the initial construction phase, prolonged maintenance, repair, and renewal. As a clear distinction, there are about 1000 cells that have to last a lifetime of 10 days in Caenorhabditis elegans (55) compared to perhaps 10^{13} cells that persist (with replication) for 7–8 decades in a human. In lower animals, growth continues (perhaps in separate stages, as in arthropods) until death; in these animals, growth and tissue shaping are coupled. In fish and reptiles, slow overall growth persists until death, but there is little change in the relations among body parts. In birds and mammals, growth ceases at a specific time. Sexual maturation, particularly in primates and especially in humans, is dissociated in time from the initial extensive period of morphogenesis but also, as noted above, involves new morphogenetic changes. Nonetheless, in general in long-lived animals, tissue shaping and tissue maintenance are dissociated; in all of these phyla, the general problem of maintenance of the structural fidelity of replicating epithelial layers after cessation of morphogenesis needed to be solved.

The argument advanced here is that the mechanism for this process is a specialized version of tissue design machinery, the purpose of which is not to build new structures (morphogenesis) but to maintain the shape of existing structures (morphostasis). Here we advance both general and specific evidence for the existence of morphostasis and morphostats.4

General Evidence for Morphostats

The argument in its simplest form is this: fidelity of tissue microarchitecture is the norm. Metaplasia and transdifferentiation occur but are rare events. Therefore, there is a robust instructive system that normally maintains the microarchitectural form of any given tissue.

The general evidence is derived from a variety of observations and experiments.

In Vitro. The maintenance of cells in culture involves a wide variety of issues and problems. Perhaps the central issue is that growing normal cells in monolayer is difficult. Most cell lines that grow in monolayer are transformed or frankly malignant. Normal cells grow better with “conditioned” media or a “feeder” layer. The act of placing cells in culture disrupts tissue structure and will allow the emergence of cells with malignant potential (11, 56–58). Organotypic cultures, the in vitro mimicking of in vivo epithelial architecture, will grow with feeder layers, but for most epithelia, even these systems result in a caricature of the normal tissue architecture (59, 60). Nonetheless, some systems are well established and result in reliable reproduction of the organ structure (61). Among the more successful ways to grow cells in three-dimensional structures that at least approach those of normal tissue is the use of Matrigel. Matrigel is the ECM produced by EHS sarcoma cells (62). Components of the ECM including collagens and particularly EHS-derived Matrigel can readily support branching morphogenesis, for example, of breast, to the point where duct-like structures appear, and there is an appropriate response to lactogenic hormones (63–65). Some plausible, if somewhat surprising, morphogen-like molecules have been identified (66). Other morphogenetic-like behaviors are also supported by the ECM (67). Finally, in vitro carcinogenesis (perhaps particularly in fibroblasts) is much more frequent and predictable than its in vivo counterpart. The former can be accomplished readily and consistently by a variety of chemical and genetic manipulations, whereas experimental carcinogenesis in vivo requires complex, even if well studied, protocols (68).

The above data suggest that there is a set of signals that produce normal cell morphology and, more importantly for the argument being advanced here, normal tissue architecture. These signals originate from outside the epithelial cells themselves. The plausible origin of these signals (and there are other data to support this; see the text below) is the stromal fibroblasts, although other nonepithelial cells can be the source of some signals (e.g., macrophages). It is notable that normal fibroblasts are not difficult to grow in primary culture in vitro. In Vivo. Organ regeneration, especially epithelial regrowth, and in the gastrointestinal tract in particular, shows an ordered process akin to morphogenesis. Whereas limb regeneration occurs in amphibia (69), that ability does not exist in other vertebrate phyla. In very young mammals, the ability to regrow terminal phalanges of digits exists, presumably as a remnant of morphogenetic influences, but this ability is generally lost by the age of 2 years. In contrast, fingerprints, if damaged by injury, can be regenerated throughout life. Furthermore, the digestive tract (and possibly other epithelial linings) appear to retain the capacity to maintain and restructure architecture throughout life.

For example, the large-scale and small-scale structure of the intestine is markedly influenced by fasting, nutritional load, and specific nutrient mix, including quality, quantity, concentration, and variety of single nutrients. Many of the adaptational changes are rapid and specific to particular regions of the bowel (70). This pattern is further modified among animals with normal gut flora and with gnotobiotic flora and in germ-free animals (70). These specific, predictable, and sometimes localized responses speak to a marked degree of control over the proliferation/apoptosis balance and cell function. This degree of control makes considerable sense in light of regulation of energy balance: in the presence of adequate energy and nutrient availability, the energy expenditure required to produce a lush epithelial absorptive surface is a good investment; the reverse is true in a low-energy or fasting environment. To be effective, switching from one state to another requires a high degree of subtlety in the nature of the control, and strong selection pressure plausibly exists to get it right. Both endocrine and paracrine processes appear to be involved (71). Such a system needs both an afferent arm (detection of nutrient availability) and an efferent (signaling) arm. The net signal to maintain the epithelial morphology at an optimal match to the nutrient environment is an example of a morphostatic process.

It is worth noting that feast-fast environments must be the

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4 By analogy with “morphogen,” from the Greek μορφή (form) and στάσις (to stand). Hence, a morphostat is that which maintains and regulates form.
Precancer. BE, as already noted, is a metaplastic disorder where the normal squamous epithelium is replaced by columnar epithelium and crypt-like structures (72). BE is a classic metaplastic change in that it is not itself malignant; it is multicentric and patchy, suggesting that whatever its origin, a single mutation and clonal expansion cannot explain its etiology. It is the tissue, not the cellular appearance, that is abnormal; in the earliest stages of BE, the tissue shows an atypical cryptal structure that distantly resembles that of the right colon, but the cells are not dysplastic. At later stages, the cells become dysplastic, and the tissue organization becomes more markedly disrupted.

In a series of carefully integrated studies involving physical mapping, flow, and molecular approaches, Reid’s group (73–75) has shown clear evidence of LOH at 9p and 17p in particular in patients with high-grade dysplasia in BE (73). They have further shown that mutations in *p53* are common in BE. In some patients, these changes are consistent with a field effect, with single clonal expansion across wide areas of the BE epithelium; in others, the *p53* mutant clones are of different origins (74). Overall, this suggests a tissue environment in which normal maturation pathways are disrupted and that cells with insufficient cell-cycle controls are likely to be selected. An analogous situation appears to exist in ulcerative colitis (76).

Neither in BE nor in ulcerative colitis are the *p53* lesions a primary explanation of the unstable (metaplastic or ulcerating) epithelium. Indeed, Reid’s group has shown that a variety of common, but not universal, mutational and LOH events occur in BE, particularly in patients with an increased 4N fraction or aneuploidy on flow analysis; nonetheless, among the more normal diploid populations of cells, multiple and even single LOH events, thus far, are in the minority (73). Furthermore, these findings are in patients already showing high-grade dysplasia; mutational events occurring in metaplastic tissue in patients without dysplasia have not been described.

While showing that epithelial mutational events are central in progression, these findings are consistent with the interpretation that the primary controlling events in metaplasia occur outside the epithelium, plausibly in the mesenchyme. Furthermore, they suggest that the epithelial cells in that setting are receiving growth and maturation signals to which they are only partly able to respond because the metaplastic morphology is probably a compromise between the restricted-expression genome that is normal for squamous differentiation and an abnormal mesenchymal signal. Finally, these findings suggest that in such circumstances, there is a greater opportunity than usual for outgrowth of one or more clones that are less dependent on these abnormal mesenchymal organizing (morphostatic) signals.

Cancer. Experiments with teratocarcinomas go back more than four decades (10, 77–80). Teratocarcinomas contain a multiplicity of tissues with differentiated appearances along with embryonal cells derived from primordial germ cells. It is possible to generate such tumors from normal germ cells (as well as from these embryonal carcinoma cells) by transplanting them into abnormal environments. However, such cells, while expressing a malignant phenotype, are capable of differentiating into benign tissues. More crucially, they are also capable of being incorporated into the inner cell mass of an embryo essentially as totipotent cells and giving rise to normal cells in the resultant chimeric mouse (81, 82).

For some time, it was believed that this phenomenon was peculiar to germ-cell tumors. However, other tumors, including liver tumors (83), leukemias (84), and breast tumors (26), can also be induced to differentiate or return to a normal phenotype when placed in specific environments.

What these experiments suggest is that there are conditions under which abnormal, even frankly cancerous, cells can be induced to behave and differentiate normally. It seems likely that even if there are genomic abnormalities in such cells, tight morphostatic control will limit expression to that subset of genes that is needed in the normal environment via yet-to-be-determined pathways that plausibly involve histone acetylation or gene methylation. That the converse can occur, namely, that cells with entirely intact genomes (as demonstrated by their ability to give rise to normal cells in an intact animal when implanted in the inner cell mass) can give rise to a cancer-related phenotype, further argues for the importance of morphostatic controls external to the epithelium of interest.

Foreign body carcinogenesis is an uncommon event that may be largely confined to particular strains of mice (85–87). Despite its relative rarity in humans (88), (although it remains unclear whether asbestos acts in this way), any theory of carcinogenesis must encompass this manifestation. Solid sheets of inert material (glass, plastic, or metal), when transplanted in rodents, result in tumors, often sarcomas (85, 86). In contrast, the same material, as a porous sheet or completely fragmented, results in a very marked reduction in tumor yield. The only plausible explanation for this phenomenon is that the intact sheet prevents stromal-epithelial or other cell-cell communication. The molecular traffic that is thus inhibited is likely, in part, to transduce signals that maintain normal architecture. In the absence of these morphostats, either cells are more vulnerable to initiation or such an environment selects cells that are less subject to cell-cycle control (see the text below on the relationship between cell adhesion and genomic instability).

Data from both animals and humans show that carcinogenesis is more common at the junction of dissimilar epithelial morphologies [e.g., at the squamocolumnar junction of the human cervix (89) and the squamocolumnar junction between esophagus and stomach in both humans (90) and animals], regardless of whether cancer is induced in transgenics (91, 92), by wounding (93, 94), or by carcinogens (95). Although it is possible to show that the gastroesophageal junction has higher p450 nitrosamine-metabolizing activity (95), this observation does not explain the findings for transgenic and wounding-induced carcinogenesis.

A more comprehensive explanation for these findings, which follows from the morphostat thesis advanced here, is that the cells at the junction between two dissimilar epithelia will be receiving input signals from the mesenchyme that controls both epithelium A and epithelium B. A conflict of signals regarding the morphology that the junctional cells “ought” to adopt may increase the likelihood of a metastatic morphology or the
Evidence for Specific Functions

Signaling events in early development involve diffusible factors (morphogens) that influence their targets in a concentration-dependent manner (54, 97). The point in the morphogen gradient at which a cell is positioned determines gene activity and differentiation pathway. To qualify as a morphogen, two conditions must be met: (a) that cells respond directly to the signaling molecule; and (b) that the target cells display (apart from the null response) at least two qualitatively different responses, depending on signal concentration (54). Examples of molecules with this kind of morphogenetic capacity include members of the TGF-β family in Xenopus blastula models (53), MHC-peptide complexes that determine the fate of mammalian T cells (98), wingless/wnt in insect wing morphogenesis (99), and others. At least one family of relevant receptors has been identified (52, 53).

In principle, this diffusion gradient from a point or line source can provide three kinds of information (19): (a) scalar concentration provides positional information about how far a specific cell is from the top of the gradient; (b) vector information, the direction of maximal change, provides data about the orientation of the cell relative to the source; and (c) slope provides data on the size of the morphogenetic field. It seems plausible that the microarchitecture of adult epithelia is maintained in a similar manner, perhaps even by the same signaling systems.

There is evidence, at least in insects, that morphogenesis can even be determined by exogenous molecules: the caterpillar form of Nemoria arizonaria exists in two forms, which are selected for camouflage. In summer, when the tannin in its food supply (oak leaves) is high, it develops as a twig morph; in spring, when the tannin is low (catkins), it resembles a catkin. The relevant experiments demonstrated that it is the tannin that determines morphology (100). It is not known whether exogenous molecules can influence morphology in higher animals, but exogenous sources of signaling molecules [e.g., retinoids (effector molecules in a variety of growth control pathways) (101, 102)] can mimic or override their endogenous counterparts, depending on concentration. Given the role of semisynthetic retinoids in cancer chemoprevention (103), possible effects on morphostatic pathways, as postulated here, may provide guidance for selecting other testable agents, chosen not for their structural similarity to retinoids but for their comparable functional role in other signaling pathways.

The general argument advanced here is that the maturation patterns as well as the presence of cells with different functional capacities (e.g., secretory cells) within an adult epithelium may be determined in a manner analogous to the influence of a morphogen gradient. Other tissue-shaping mechanisms may also be involved (104).

At the moment, it is not possible to do much more than make hand-waving arguments about the identity of specific morphostats in specific tissues. Because the control of adult tissues has not been postulated to occur in exactly this way (although others have proposed closely related ideas), particular molecules have not been explored for their morphogen-like (morphostatic) role in adult tissues. Nonetheless, it is intriguing that known or probable morphogens are clearly involved in cancers of specific organs, for example, patched/PTC, an anti-hedgehog involved in basal cell nevus syndrome and basal cell carcinoma (105, 106); TGF-β in colon (107), ovarian (108), and other cancers (109); wingless/wnt in breast (110, 111) and colon (112) cancer; and β-catenin, which is responsible for dorsal mesoderm induction in embryogenesis (113), in colon cancer (114).

These associations may be worth exploring not just empirically, but with the specific idea that they may be instructive or permissive in the maintenance of adult epithelial microarchitecture and thus, when their signals are disrupted, are involved at an early stage of the carcinogenic process in the target epithelia.

In a review of the interface between cell adhesion and genomic instability, Tlsty (115) cites evidence that cells that are nonadherent do not go into cell-cycle arrest when exposed to radiation. Even in adherent cells that are damaged, cell-cycle arrest is abrogated if these cells are released from adherence (116). Both observations show that cells that are not in intact tissues are not subject to the cell-cycle control that prevents abnormal cells from replicating. This suggests that cell-adhesion molecules may be an important part of morphostatic control. Because these molecules are literally a glue that allows tissues to have shape, this seems to be an important inference. These observations further suggest that it may be important that tissues with cells in close contact, whether via cell-matrix or cell-cell molecules, are also at close and predictable distances so that diffusion gradients can be interpreted appropriately by the individual cells.

One intriguing source of evidence for the widespread influence of fibroblasts in controlling epithelial functions comes from the expression microarray studies of Iyer et al. (117). Using a classic serum deprivation/addition experiment on human foreskin fibroblasts, these workers showed not only the expected expression of cell-cycle genes but also a wide variety of genes that control wound healing, from clotting and clot dissolution through lymphocyte chemotaxis to angiogenesis and epithelial regrowth. They acknowledged that, although proliferative control was predicted, wound-healing control was not. Nonetheless, as they note, these findings were immediately interpretable as an obvious response of cells that see serum only after tissue damage. It seems likely, however, that even this interpretation may be too narrow and that low level maintenance of epithelial integrity and structure would also be facilitated by a more modest version of this fibroblast wound-healing repertoire—a testable hypothesis.

If mesenchymal influences on epithelial cancers are important, there should be some evidence of genetic/genomic
abnormalities in mesenchymal cells associated with cancer, with or without changes in the target epithelial cells. In several systems, it has been shown that the “expected” abnormalities are found, not in the tumor cells, but in the supportive tissues. For example, in vascular tumors associated with human herpes virus 8/Kaposi sarcoma herpes virus, the virus is found not in the tumor cells themselves but in the dendritic cells (118). In breast cancer, LOH of BRCA1 is not found in the tumor cells but in the surrounding tissue (119). In familial breast cancer patients and perhaps in their unaffected relatives, skin fibroblasts have been shown to possess a fetal-type migratory phenotype (120). In the colon, LOH in MOM1 (a modifier of the APC-associated polyposis phenotype in mice) is found in Paneth cells but not in the colonic epithelial tumors (121).

Furthermore, Tlsty’s group (122) has shown that carcinoma-associated fibroblasts stimulate the progression of initiated prostatic epithelial cells both in vivo and in vitro, whereas their same fibroblasts do not influence the growth of normal prostate epithelium. The tumor-associated fibroblasts appear to function by reducing the likelihood of apoptosis in the tumor cells (123). Indeed, the role of stromal signaling in cancer is probably best established for prostate cancer (124–126). However, there are also much earlier data to show that in the mouse mammary gland, normal stromal architecture is sufficient to prevent preneoplastic cells from expressing their phenotype (127).

There is, of course, as is obvious from the discussions above, an extensive literature on the role of stromal-epithelial interactions in cancer—a literature that goes back to Waddington. Many of the relevant ideas have been canvassed widely including: the role of signaling and disruption of signaling; the importance of stroma in cancer; and the role of nonmutagenic events in cancer. What may be unique about the present formulation is that it focuses on the microarchitecture of tissues rather than the differentiation of cells, and furthermore, it postulates that a specific class of morphogen-like molecules has a primary role, not only in functional cross-talk among heterogeneous cells, but also in the maintenance of the adult epithelial microarchitecture. It also implies that disorders other than cancer may be manifestations of the loss of such maintenance. Conceptualizing a specific group of molecules in this way allows testable predictions to be made about their likely nature and mechanisms of action (see the text above) and specifically about their role in cancer (see the text below).

Counterargument to the Importance (Existence?) of Morphostats

One of the most successful theories of cancer places mutation as the central event, even if some have challenged this theory (128, 129). It is abundantly clear that many aspects of cancer are explained by loss or gain of gene function after a mutagenic event. There is no attempt here to deny the importance of mutation in epithelial cancers; indeed, as noted above and again below, we may not have been looking in all of the right places for the mutations.

Nonetheless, two important points need to be made. The first is that mutational events can be mimicked by hypermethylation of the promoter regions of key genes (130–138) and by loss of function at the protein level via the formation of specific viral protein-tumor suppressor protein complexes (139, 140). Thus, it can be demonstrated that mutation is a special (even if it is the most common) case among the more general phenomenon of loss of function of crucial epithelial cell proteins.

The second response may be more contentious. Although it is established that mutational events are central to human carcinogenesis, the inheritance of mutant tumor suppressor or DNA repair alleles is a rare contributor, based on current evidence, to the overall population burden of cancer (141). On the other hand, environmental carcinogenesis as a consequence of exposure of somatic cells to mutagenic agents certainly contributes extensively to the population burden, with tobacco smoke, radiation, and specific industrial (and possibly food-borne) chemicals being the most obvious examples. However, even in this setting, carcinogenesis is the uncommon event: although cigarette smokers, for instance, are at markedly elevated (10–20-fold) risk of lung cancer, only about 12–15% of all smokers develop the disease. This observation argues strongly for differences in susceptibility to mutational carcinogenesis. Currently, evidence is being sought for genetically determined differences in the capacity to metabolize carcinogens, repair DNA damage, and provide substrates for that repair; such differences do indeed appear to modulate cancer risk (141).

However, given the frank rarity of cancer per cell at risk (1 clinical presentation/3 × 1014 cells over 75 years), it seems likely that there are multiple and redundant ways that cancer risk is reduced (141). One global mechanism for keeping cells behaving normally would be a position gradient morphostatic field. Cancer occurs much more frequently, as noted above, when postulated morphostatic influences fail (metaplasia) or at the junction of two different morphostatic fields. Failure of control allows a selective advantage to cells with important mutations, but, I argue here, it is the rare failure of the field that is the primary event, not the mutations.

Testable Consequences/Predictions

A variety of testable predictions follow from this hypothesis, some of which are obvious. A few will be outlined here:

(a) A new two-hit model is predicted: one hit in an epithelial cell, resulting in disruption of cell function and structure (e.g., dysplasia); and the other in a mesenchymal or other supporting cell, resulting in disruption of tissue microarchitecture. The corollary of this is that patterns of premalignant conditions will become clearer because there are mesenchymal mutations that produce tissue microarchitectural abnormalities without epithelial dysplasia and vice versa (see Table 1). This, as noted above, is testable by examining both epithelium and stroma for abnormal patterns of expression using microarray technology. The specific stromal cell abnormalities are predicted to be overexpression of instructive proliferation signals or loss of differentiation gradients.

(b) In organotypic studies of nonmalignant and premalignant tissues, such as psoriasis and BE, one coculture combination (abnormal mesenchyme plus normal epithelium) will express the phenotype whereas the reverse coculture combination will not. A similar pattern could also be true under conditions of metaplasia or overgrowth but of chronic ulceration/
inflammation, such as ulcerative colitis, and chronic gastritis and pancreatitis; it should be noted, however, that culture conditions for the latter experiments are not well established.

(c) In a variety of malignant and premalignant conditions, it should be possible to identify mutation in, abnormal proliferation of, or possibly loss of mesenchymal cells in the proximity of the epithelial condition. For example, does smoking induce mesenchymal mutations in lung? Is the fibroblast proliferation that is characteristic of colorectal tumors a causal event?

(d) If viral proteins produced by human herpes virus 8/Kaposi sarcoma herpes virus and other cancer-associated viruses act because they infect adjacent mesenchymal cells rather than epithelial cells, then a plausible mode of action of the viral proteins would be to complex with signaling proteins, probably from families of morphogens such as TGF-β, wingless/wnt, and others, rather than to interact with tumor suppressor proteins.

(e) In normal epithelia, it will be possible to identify morphostat gradients (morphogens are prime candidates, as already noted) that are associated with differences in cell fate, function, and maturation.

(f) As noted above, the success of retinoids, effector molecules in growth control pathways, in aerodigestive cancer chemoprevention may provide guidance for selecting other agents. These would be chosen, not for their structural similarity to retinoids, but for their comparable functional role in other signaling pathways. It may also be possible that specific chemopreventive agents will work only in specific tissues because of their capacity to mimic or induce morphostatic pathways that are topographically defined.

Summary
What is postulated here is that there are morphogen-like controller molecules, morphostats, with morphogen-like functions in adult epithelial tissues. These molecules are responsible for the maintenance of normal tissue microarchitecture and cell differentiation. Disruption of the functions of morphostats results in a variety of abnormalities. Such disruption may be a key event in carcinogenesis.

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References


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John D. Potter


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