A. What is Heritable Nongenetic Information?

Numerous studies of the tumor microenvironment, interspecies xenografting, and limb regeneration suggest the existence of a tissue spatial code (TSC) that controls tissue structure in a quasi "epigenetic" fashion. Epigenetic is an inadequate label, because this information does not act directly upon DNA molecules like methylation. A broader term is needed to capture the diversity of three-dimensional spatial codes in biology. One such term is Heritable Nongenetic Information (HNI), which encompasses the TSC. The term heritable is appropriate because this information is passed onto offspring, otherwise it would have disappeared during evolution. Another reason for the heritability of HNI is that the spatial information observed in tissues is not reducible to the laws of physics, meaning structures like epithelial tubes or neural circuits don't spontaneously form in an aqueous solution; pre-existing physiological information in the microenvironment is necessary. Section C describes molecular and subcellular examples of HNI that are independent of DNA.

The first step in cracking a code is to determine the grammatical rules of that code. For DNA, Erwin Chargoff discovered that the ratio of adenine to thymine or guanine to cytosine was similar across different species. This laid the groundwork for the eventual discovery of the double helical structure of DNA by James Watson and Francis Crick.

A tissue spatial code, however, is not semantic information, such as that of DNA sequences or the English language, and thus needs specific tools for quantifying regularities in spatial grammar. Recurring quantitative patterns in tissue regeneration or neoplasia will reveal some form of a grammar.

B. Evidence of a Tissue Spatial Code

Here is evidence that the tissue spatial code exists and is independent of the information in DNA. Note that “independent of the information of DNA” does not mean that DNA is unnecessary, because DNA mutation experiments prove that DNA is quite essential for life. The point is that distinct code systems can operate in parallel and be integrated with each other via
dynamic feedback loops to achieve the same goals. In principle, there needs to be a minimum set of genes to produce the functional protein machinery that sustains the functions of cells. However, at the tissue level, the TSC can compensate for the inadequacy of heavily damaged DNA within the cells that comprise the tissue.

**Tumor Histology Exhibits Recurring Patterns Identifiable by Pathologists**

One of the oldest lines of evidence that a TSC exists comes from the medical field of tumor pathology. While tumors exhibit a tissue architecture that seems haphazard and random compared to the tissues of their origin, the disorderly architecture does present in the form of recurring patterns. These recurring patterns of tumor architecture make it possible for a pathologist to recognize the common histological subtypes of each solid cancer. Tumors of epithelial origin, such as breast cancer (reviewed Makki, 2015) and lung cancer (reviewed in Kuhn, 2008), often exhibit features that are reminiscent of normal epithelial ducts, such as finger-like tubes referred to as a “papillary” histology when viewed in a two-dimensional slice of the tumor.

**Limb Regeneration in Salamanders**

The fact that amputated limbs regrow the same arm and hand structure without a pre-existing embryonic patterning environment is evidence of a tissue spatial code (reviewed in Simon & Tanaka, 2013; McCusker & Gardiner, 2013). Amputating an arm of the salamander results in a new arm that regrows in its place. This extra arm has the same macroscopic features as an original arm. After limb amputation, the movement of existing nerves towards the edge of the injury activates a tissue regeneration program (Endo, 2004). This limb regeneration program is activatable even without the movement of existing nerves, but via exogenous treatment with retinoic acid and growth factors (Vieira, 2019), which suggests that an inherent tissue development blueprint exists in the cells of the wound site. While DNA carries instructions for creating protein machinery, something else -- heritable nongenetic information -- is dictating tissue patterning outside of the embryonic environment.

**Regenerative Abilities of Flatworms**

Flatworms (Planarians) contain stem cells throughout their body, which allows for a small fragment of a worm to regenerate into a new worm, normally functioning worm. Cells within flatworms communicate by sending electrical signals through gap junctions that connect neighboring cells to each other. Temporarily disrupting electrical signals, by treating worms with a chemical that inhibits gap junctions, permanently changes the anatomical structure of worms to be two-headed worms instead of single-headed worms (Durant, 2017). This effect persisted indefinitely even though the genomes of the two-headed worms were identical to that of single-headed worms, because they were both clones of the original worm. This is very compelling evidence that a bioelectrical signal can control anatomical morphology without altering DNA sequences. Planarians have a physiological memory that controls anatomy independently of DNA sequence.

The relatively simpler anatomical structure of flatworms makes it an ideal experimental model by which to measure the spatial information of tissue structure, in the form of cellular shape and
arrangement. The morphology of neurons has evolved to expedite the sending and receiving of electrical signals. Why wouldn’t we expect that epithelial and mesenchymal cells that transmit electrical signals between each other won’t be shaped or arranged in a way that optimizes the transmission of these signals? If this spatial optimization is true, it can be measured histologically with the right computational tools.

**Human-Mouse Xenografting Overrides DNA Integrity or Sequence Divergence**
Injecting human B16 melanoma cells into a mouse blastocyst can result in a viable adult mouse that is a mosaic of human and mouse cells throughout its body (Mintz, 1978). This is the first example here of "species specific DNA autonomy" (SSDA). Not only are the melanoma cells non-murine, their DNA harbors many mutations and chromosomal aberrations. Humanized mouse mammary glands. There is a method to inject human mammary epithelial cells along with human mammary fibroblasts into the mammary fat pad of mice. This results in functional human mammary ducts in an immunocompromised mouse (Kuperwasser, 2004). This is another evidence of SSDA, meaning the extracellular/tissue environment can override the DNA of the xenografted cells.

**In Vitro Microenvironmental Programming of Cancer Cells**
Artificially derived extracellular matrix (matrigel) can revert the cancerous phenotype of very aggressive cancer cell lines, a mechanism mediated by Integrin β1 (Weaver, 1997). Breast cancer cell lines that have many mutations and chromosomal aberrations can be coaxed into forming organized acini in culture, just like how pre-malignant breast cell lines behave. There is also evidence that malignant breast cancer cell lines only respond to growth inhibiting molecules in a 3D environment and not in 2D cell culture despite harboring the same mutations in both contexts (Wang, 1998; Liu, 2004). This is evidence that the extracellular environment can override the instructions in damaged DNA codes.

**C. Molecular and Subcellular Evidence of Heritable Nongenetic Information**

**Natively Unfolded Proteins (NUPs) as Evidence of Heritable Nongenetic Information**
Natively unfolded proteins and protein domains that are natively unfolded, do not exhibit a regular structure until they interact with an appropriate environment (reviewed in Nishikawa, 2009). For example, a NUP may not fold into a regular 3D structure until it interacts with the hydrophobic environment of a cellular membrane. NUPs have also been reported to take on different 3D structures depending on which protein partner they bind to (reviewed in Tompa, 2005). The fact that a polypeptide chain needs spatial information that is independent of the polypeptide's own sequence suggests that the full set of instructions for protein folding -- and thus, function -- is NOT contained in DNA. This is evidence of a "molecular environment spatial code."
Specific Docking Sites for Each Chromosome on the Nuclear Lamina
Photobleaching experiments have shown that mammalian chromosomes have specific "addresses" on the inner nuclear membrane (Boyle, 2001; Essers, 2005). The lamina-associated domains (LADs) in DNA contain methylated adenines, and are responsible for chromosome regions that physically locate at the inner nuclear membrane, specifically at the nuclear lamina (reviewed in van Steensel & Belmont, 2017). LADs are ubiquitously distributed across all mammalian chromosomes, yet each chromosome has a preference for specific locations on the nuclear lamina; there must be a code in operation.

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