correspond to promoter regions (*18*). The second approach used a microarray of 1500 promoter regions associated with cell cycle–regulated genes (*17*). Each experimental strategy identified groups of genes involved in cell cycle control whose promoters were bound by E2F4. One interesting outcome from both papers was that a minority of the target gene promoters did not have obvious E2F4 binding consensus sequences. Some of these targets were confirmed by conventional ChIP analysis, implying that they were not false positives. It is possible that E2F4 is recruited by other proteins, or alternatively is binding to another control region (such as an enhancer) that interacts with the promoter region being interrogated.

An alternative to ChIP has been developed by van Steensel *et al.* to examine the location of several chromatin-binding proteins (such as Sir2 and Hp1) on 500 *Drosophila* genes (*19*). This methodology, unlike the ChIP assay, does not rely on the availability of a good antibody for the protein of interest. In this assay, tethering of the *Escherichia coli* enzyme DNA adenine methylase to specific transcription factors illuminates the chromosomal locations of binding sites for these regulatory proteins (*19*). This approach does, however, require the introduction of a fusion protein that might in some cases alter the DNA binding capabilities of the protein to be tested. Obviously, these studies only searched a fraction of the genome for transcription factor binding sites, but they do demonstrate the feasibility of this approach. Developments in microarray technology will soon allow the entire human genome to be displayed on one or a small number of chips.

These are exciting times for researchers in the gene transcription field. By developing and embracing new technologies, according to the knowledge derived from the sequencing of complete genomes, researchers can now start to answer many difficult questions. These approaches will lead to enormous insights into the functional features of the genome and should prove to be a powerful tool for the discovery and mapping of global regulatory networks.

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PERSPECTIVES: IMMUNOLOGY

Lethal Weapons

Dirk Roos and Christine C. Winterbourn

The engulfing (phagocytosis) and killing of microorganisms by white blood cells called neutrophils is essential for protection against microbial infection. Neutrophils are the first cells to reach sites of pathogenic invasion. Patients who have too few neutrophils or neutrophils that are dysfunctional suffer from recurrent, often life-threatening, microbial infections.

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Neutrophils are equipped with an array of microbicidal weapons, such as proteases, enzymes that hydrolyze sugars, and proteins that disrupt membranes. This weaponry is stored in at least three different kinds of granules in the cytoplasm. In addition, these cells have a powerful system for generating large amounts of reactive oxygen species. Microorganisms are taken up into an intracellular compartment, called a phagosome, into which these cytotoxic agents are released. It is generally considered that neutrophils use a combination of oxidative and nonoxidative mechanisms to defend against the wide range of microorganisms that they encounter. The microbicidal potential of many of these components is well established (*1*, *2*). However, we really do not know how microbes are killed in the phagosomal environment, where extremely large amounts of oxidants and granule constituents are released and factors such as pH, ionic strength, and enzyme substrate availability are likely to be critical (*3*). This question is addressed by Tony Segal's group in their recent *Nature* paper (*4*), which elegantly shows how oxidative and nonoxidative mechanisms cooperate. Their findings challenge the established view that direct killing of pathogens by oxidants is the principal arm of neutrophil antimicrobial action.

There is no doubt that the generation of reactive oxygen species is essential for adequate antimicrobial defense. Neutrophils from patients with chronic granulomatous disease (CGD), who are deficient in the NADPH oxidase system responsible for oxidant generation, fail to kill many strains of bacteria, yeasts, and fungi. These same microorganisms also cause the severe infections seen in CGD patients. The NADPH oxidase generates superoxide radical (O_2^-), the one-electron adduct of molecular oxygen that by itself has limited toxicity. However, superoxide can be converted into hydrogen peroxide (H_2O_2), which can oxidize chloride ions to the extremely toxic hypochlorous acid (HOCl) in a reaction catalyzed by myeloperoxidase. Myeloperoxidase is stored in large quantities in azurophil granules and is released into the phagosome (see the figure). The formation of H_2O_2 and HOCl by neutrophils that are phagocytosing microbes has been proven, but the fraction of superoxide converted into HOCl in the phagosome is still a matter of debate (*5*). The current view is that in normal neutrophils, HOCl is primarily responsible for oxidative killing (*6*). However, deficiency of myeloperoxidase is a common condition and does not lead to obvious susceptibility to bacterial infections. So, back-up systems must exist to compensate for this deficiency. These may involve other reactive oxygen species (such as hydroxyl radicals), but solid evidence that they are produced in biologically relevant amounts is lacking.

The alternative view proposed by Segal's group arises from studies of mice engineered to lack either the NADPH oxidase or the two granule proteases, elastase and cathepsin G. These investigators found that neutrophils from both groups of mice were equally compromised in killing *Staphylococcus aureus* and *Candida albicans* in vitro, and that the animals were equally susceptible to infection with these microorganisms in vivo (*2*). This implies that the oxidants and proteases work together. Their explanation stems from an observation they made 20 years ago that NADPH oxidase activity leads to a rapid

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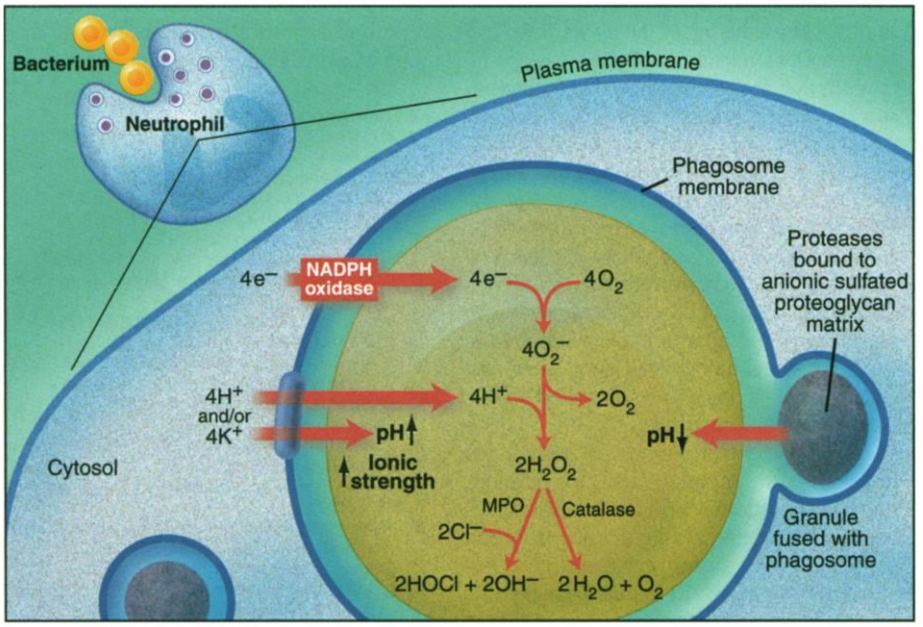

In the belly of the phagocyte. Within the small space between an ingested bacterium (shaded area) and the membrane of the phagosome, a number of chemical reactions take place. Molecular oxygen is reduced to the superoxide radical (O_2^-) by electrons pumped into the phagosome by the NADPH oxidase system. This charge transfer is compensated by the influx of protons (H^+) or other cations. The protons are used to reduce superoxide to hydrogen peroxide (H_2O_2), which can be broken down to oxygen and water in a catalase-dependent reaction. Alternatively, H_2O_2 can combine with chloride to form hypochlorous acid (HOCl) in a reaction catalyzed by myeloperoxidase (MPO). Under certain conditions, MPO may act as a catalase. If all electrons pumped into the phagosome are compensated by proton influx, the pH in the phagosome will remain neutral. However, the pH does in fact rise to about pH 8, despite the release of acid contents from granules in the cytoplasm that fuse with the phagosome. This indicates that other cations, such as potassium ions (K^+), may enter the phagosome instead of protons (*4*). If there is an influx of K^+, these cations then mediate solubilization of proteases that are tethered to the proteoglycan matrix of the granules. As a result, ingested bacteria within the phagosome are killed. Whether this is the main mechanism of bacterial killing by neutrophils, or even the only one, or whether HOCl and other reactive oxygen species are also involved is a matter of debate.

rise in intraphagosomal pH to about pH 8, despite the release of the acid contents of granules into the phagosome (*7*). This rise in pH is due to the generation of large amounts of superoxide in the phagosomes, followed by consumption of protons leading to the formation of H_2O_2 (see the figure). Charge compensation takes place by proton transfer into the phagosome, both through the NADPH oxidase itself and other proton channels (*8*, *9*), but apparently not in sufficient amounts to totally compensate for the electrogenic process of superoxide formation.

In the new work, the Segal group shows that this process is accompanied by an influx of large amounts of potassium ions into the phagosome (*4*). This influx enables the pH to remain elevated and more importantly, increases the potassium concentration to an estimated 200 to 300 mM. The high tonicity induces solubilization of the cationic proteases that are bound to a strongly anionic sulfated proteoglycan matrix in the azurophil granules. On the basis of these observations plus an elegant array of biophysical and biochemical studies, the authors propose that the elastase and cathepsin G, once liberated, attack and kill the ingested microorganisms. In their view, superoxide generation is needed only to increase the ionic strength of the phagosome, which then allows solubilization of the proteases.

So, is this the prime mechanism by which neutrophils kill microbes? Do reactive oxygen species themselves have no place in this process? In our opinion, it is premature to draw these conclusions. There is a wide body of evidence supporting the notion that oxidants are involved in killing (*6*), and many questions remain. For instance, CGD neutrophils are able to kill catalase-negative bacteria (which excrete H_2O_2) and also catalase-positive bacteria if they coingest glucose oxidase, which generates H_2O_2 (*10*–*12*). Both phenomena point to a contribution of H_2O_2, or products derived from it, in the killing process. Moreover, the killing of bacteria by H_2O_2 in a cell-free system is dramatically enhanced by myeloperoxide (*13*, *14*). Myeloperoxidase-deficient mice also succumb to bacterial challenge (*15*).

In contrast to the conventional view, the Segal group proposes that myeloperoxidase acts as a detoxicant for H_2O_2, thus protecting other microbicidal systems from inactivation. Their evidence for this notion is not convincing. The conclusion that myeloperoxidase does not enhance killing was obtained with an unrealistically high concentration of H_2O_2 (100 mM). Not only will this rapidly inactivate myeloperoxidase, but it is several orders of magnitude higher than best estimates of what could be achieved in the phagosome, when consumption by myeloperoxidase and leakage are taken into account (*3*). However, the view that myeloperoxidase is not strictly an HOCl generator should not be dismissed. The enzyme does have catalase activity, although this requires concentrations of other substrates, such as chloride, to be low (*16*). Furthermore, chlorination of bacteria after ingestion by neutrophils is surprisingly low (*5*).

Other roles for superoxide must also be considered. *S. aureus* coated with superoxide dismutase is killed less efficiently by neutrophils, but only when myeloperoxidase is active (*17*). This points to the involvement of superoxide in a myeloperoxidase-dependent process, rather than an effect on protease action. Some bacterial strains even excrete superoxide dismutase, possibly to evade killing by the myeloperoxidase system (*18*–*21*). Antibodies against superoxide dismutase enhance the killing of these strains both by isolated neutrophils and in mice (*22*, *23*). The mechanism of protease release from neutrophils into the extracellular environment (where tonicity is low) (*24*), and how neutrophils kill some bacterial strains under anaerobic conditions (*25*) also require explanation.

In spite of these uncertainties, the new mechanism of "oxidative" killing proposed by Segal and colleagues needs to be explored. It is a thought-provoking concept that may lead to a radical change in the way we view this vital area of host defense.

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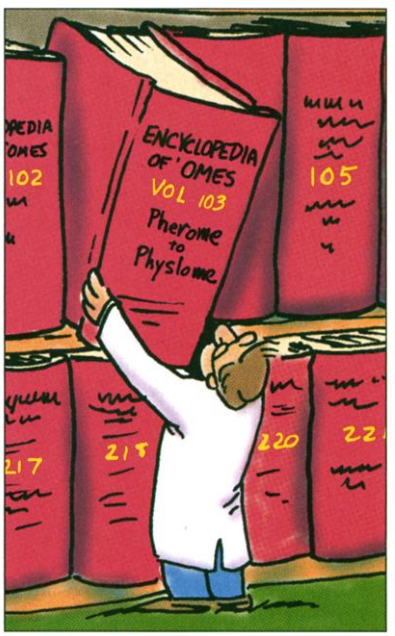
PERSPECTIVES: GENOMICS

A Crisis in Postgenomic Nomenclature

Stanley Fields and Mark Johnston

We all know what a genome is, and we think we understand the term proteome, but can anyone tell us the constituents of a functome? As the availability of complete genome sequences has spawned analyses of entire complements of proteins, RNAs, metabolites, and other cellular constituents, there has arisen a need for a terminology expansive enough to encompass the global scale of the data. A sensible suffix was appropriated for this purpose, but now is proliferating uncontrollably: genome, proteome, transcriptome, metabolome, interactome, even phenome, with many more 'omes sure to be in various stages of gestation. Perhaps it is not completely coincidental that 'ome is also the anglicized form of 'oma (*1*), commonly used to name such unwelcome intrusions as sarcoma, lipoma, and fibroma. This metastatic growth of the 'ome is spreading imprecision and confusion. Meanwhile, research summaries in the front of major scientific weeklies with titles like *'Ome Sweet 'Ome*, and *'Ome... 'Ome... 'Ome: The Genomicist's New Mantra*, and our personal favorite, *The 'Ome: A Pièce de Résistance*, only serve to confuse us further. Because a clear and widely accepted nomenclature is essential for the health of any discipline, a systematic solution to this problem is urgently needed.

It is often instructive to look to the past for guidance. A now familiar nomenclature grew up around the related suffix 'some (for which 'ome is sometimes mistaken), meaning "body," which has been used to name various intracellular particles. "Chromosome" dates back more than 100 years, "ribosome" and "lysosome" are nearly half a century old, and "nucleosome" and "replisome" originated more than a quarter century ago (*1*). Even "spliceosome" and "proteasome" are approaching two decades of service. This relatively modest growth in the application of the suffix 'some contrasts with that for 'ome. Although "genome" was coined by German scientists ("genom") in 1920 and first used in English in 1930 (*1*), none of the other 'omes can lay claim to more than a few years of history.



There are two underappreciated and so far unresolved predicaments with the 'ome terminology. First, there is a problem with its scope. Whereas the extent of the genome is clear (all the genetic material of a cell), what constitutes a transcriptome is not so obvious. Is it just the mRNAs, or does it include the transcripts produced by RNA polymerases I and III? What about transcripts that end up in the enzymes telomerase or RNaseP, or in ribonucleoprotein particles such as snRNPs? The precise constituents of an 'ome are often not well specified.

A second, and much more severe, problem is the conditional nature of some 'omes. The genome—notwithstanding the occasional hop of a transposon or rearrangement of an immunoglobulin gene—is a relatively fixed entity, and reasonable people can agree on its definition. But the proteome present in a cell at one moment will differ drastically from that in the same cell moments after it has been heated to 65°C. Or, if we define a cell's glycosylome at time zero, and seconds later the cell undergoes programmed cell death, its carbohydrate moieties are likely to give up the ghost nonuniformly, with some persisting to the last. At what point in this process do we define the glycosylome?

To circumvent these difficulties and others sure to emerge, we propose some simple rules. First, considerable precision can be gained by a more circumscribed representation of the 'ome's constituents, for example, phospholipidome rather than lipidome; inositol phospholipidome rather than phospholipidome. Of course, this has the potential to be abused and to lead to absurdly finer subdivisions. For example, do we want the transcription factorome to be subdivided into the transcriptional activatorome and the transcriptional repressorome? Does not the transcriptional activatorome then include the zincfingerome, which itself includes the Cys-His zincfingerome and the Cys-Cys zincfingerome? To avoid this pitfall, we propose that the minimum number of similar cellular constituents that constitute an 'ome be clearly defined. Seven or eight seems to us a conservative yet valuable cutoff. Thus, there can be no "nucleicacidome" (there's only DNA and RNA, after all), but there certainly is a "nucleotideome" (A, T, G, C, U, I, plus myriad modified purines and pyrimidines); no "actinome," of course (humans have only six actin isoforms), but definitely a "tubulome" (multiple α and β tubulin isotypes, not to mention γ, δ, ε, ζ, and η tubulins).

Second, it would be helpful if the state of the cells for which an 'ome is defined were apparent in the nomenclature. If initially we use basic parameters like temperature, pH, cell cycle stage, and subcellular

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