The ubiquitin system

ATP + Ub → DUB
E1 → Ub
E2 → Ub
E3

substrate

degradation of substrate to short peptides

26S proteasome

THE KING FAISAL MEMORIAL ARTICLES IN MEDICINE AND SCIENCE XII
THE 2012 KING FAISAL INTERNATIONAL PRIZE
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THE 2012 KING FAISAL INTERNATIONAL PRIZE

The King Faisal International Prize
Post Box 22476, Riyadh 11495
Saudi Arabia
Custodian of the Two Holy Mosques
King Abd Allah Ibn Abdul Aziz Al-Saud
Patron of the King Faisal Foundation
HRH Prince Nayef Ibn Abd Al-Aziz Al-Saud
Crown Prince, Deputy Premier
and
Minister of Interior
Contents

Introduction ................................................................. 12

2012 King Faisal International Prize Award in Medicine ....15

Fetal and Neonatal Alloimmune Thrombocytopenia
By Professor James B. Bussel &
Professor Richard L. Berkowitz ..................................... 17

2012 King Faisal International Prize Award in Science....... 29

Recent Discoveries About the Ubiquitin System and the N-End Rule Pathway
By Professor Alexander Varshavsky .................................. 31
INTRODUCTION

The King Faisal Foundation continues the traditions of Arabic and Islamic philanthropy, as they were revitalized in modern times by King Faisal. The life and work of the late King Faisal bin Abdul Aziz, son of Saudi Arabia’s founder and the Kingdom’s third monarch, were commemorated by his eight sons through the establishment of the Foundation in 1976, the year following his death. Of the many philanthropic activities of the Foundation, the inception of King Faisal International Prizes for Medicine in 1981 and for Science in 1982 will be of particular interest to the reader of this book. These prizes were modeled on prizes for Service to Islam, Islamic Studies and Arabic Literature which were established in 1977. At present, the Prize in each of the five categories consists of a certificate summarizing the laureate’s work that is hand-written in Diwani calligraphy; a commemorative 24-carat, 200 gram gold medal, uniquely cast for each Prize and bearing the likeness of the late King Faisal; and a cash endowment of SR750,000 (US$200,000). Co-winners in any category share the monetary award. The Prizes are awarded during a ceremony in Riyadh, Saudi Arabia, under the auspices of the Custodian of the Two Holy Mosques, the King of Saudi Arabia.

Nominations for the Prizes are accepted from academic institutions, research centers, professional organizations and other learned circles worldwide, as well as from previous laureates. After preselection by expert reviewers, the short-listed works are submitted for further, detailed evaluation by carefully selected international referees. Autonomous, international specialist selection committees are then convened at the headquarters of the King Faisal Foundation in Riyadh each year in January to make the final decisions. The selections are based solely on merit, earning the King Faisal International Prize the distinction of being among the most prestigious of international awards to physicians and scientists who have made exceptionally outstanding advances which benefit all of humanity.

(Excerpt from Introduction to ‘Articles in Medicine and Science I” by H.R.H. Khaled Al Faisal, Chairman of the Prize Board and Director General of King Faisal Foundation)
Khalid Al-Faisal
Chairman of the Board of
King Faisal International Prize
WINNERS OF THE 2012
KING FAISAL INTERNATIONAL PRIZE
FOR MEDICINE

The King Faisal International Prize for Medicine (Minimal Invasive Fetal Management) for the year 1433H - 2012G has been awarded jointly to: Professor Richard Berkowitz (USA) and Professor James Bussel (USA).

Professors Berkowitz and Bussel worked together for more than two decades to study the natural history, optimal diagnostic criteria, and management of pregnant women having infants affected with Alloimmune thrombocytopenia. This disease causes intracranial hemorrhage either in-utero or during neonatal period, causing death or substantial disability in 10% of untreated cases.

Professor Bussel has provided expertise in the diagnosis and medical management of these patients through safe administration of intravenous gamma-globulins, while Professor Berkowitz has provided expertise in obstetrical management of these patients. Both professors developed the study protocols, analyzed the data, interpreted the results and wrote the reports for publications. In addition they have served as consultants for clinicians from all over the world seeking guidance for the clinical management of these patients. The worldwide ongoing treatment for this disorder is largely based on their work.
Fetal and Neonatal Alloimmune Thrombocytopenia

Professor James B. Bussel

Professor Richard Berkowitz
Fetal and Neonatal-Maternal alloimmune thrombocytopenia (AIT) due to incompatibility between the mother and her fetus for the HPA-1a antigen was initially described in 1959 by van Loghem. The first series involving sensitization to multiple other platelet antigens and confirmation of the antibody mediated nature of the disease was described in 1964 by Shulman and colleagues. A small number of reference laboratories were subsequently established in North America and Europe, and pediatric hematologists gradually became familiar with the condition as an important cause of unexplained severe neonatal thrombocytopenia. At that time, it was just becoming known that some of the intracranial hemorrhages (ICH) caused by this disorder occurred in utero, i.e. prior to delivery of the neonate.

Since 1983, Dr. James Bussel working at the Weill Medical College of Cornell University and Dr. Richard Berkowitz working initially at the Mt. Sinai School of Medicine, and then at the Columbia University Medical Center have collaborated in a series of studies devoted to understanding the natural history, optimal diagnostic criteria, and, most importantly, the management of pregnant women having infants affected with alloimmune thrombocytopenia. Throughout this period Dr. Bussel provided expertise in the diagnosis and medical management of these patients, with special emphasis on confirming the diagnosis, the safe administration of intravenous gammaglobulin (IVIG) and oversight of the data collection, while Dr. Berkowitz provided expertise in the obstetrical management of these patients as well as performing many of the fetal blood sampling procedures and analyzing issues related to complications of that procedure when done by participating physicians around the United States. Both of us developed the study protocols, analyzed the data, interpreted the results and wrote the reports for publication in peer-reviewed journals.

AIT is the platelet equivalent of Rh incompatibility, and affects at risk couples all over the world. The disease affects approximately one
in one thousand newborns,³ and causes intracranial hemorrhage either in utero or during the neonatal period, causing death or substantial disability in as many as 10% of the untreated cases.⁴ AIT is almost always unheralded because there is currently no population screening for it. In the absence of a family history, patients are not known to be at risk for this disorder until it presents with either an ICH or substantial thrombocytopenia detected in a neonate shortly after birth. Like Rh disease, it tends to worsen over time because of heightened maternal sensitization to the offending antigen in subsequent pregnancies. This means that patients whose fetuses or neonates have suffered an ICH in one pregnancy are almost certain to have that recur in affected fetuses in future pregnancies if the disease is not effectively treated, and an ICH can complicate a second pregnancy even if it did not occur during the first.

Because of the relative rarity of this disorder, it is not possible to perform epidemiologically meaningful prospective studies in any one medical center, even in as large a metropolitan area as New York City. Therefore, through the years we have spearheaded the organization of a series of national studies in order to obtain enough patients for meaningful analyses. The studies we organized to determine optimal therapeutic intervention for various subgroups of women with this disorder were all performed with very limited external funding. The design of each study, recruitment of patients throughout the United States, initiation of IRB approval at every participating site, randomization of subjects, analysis of data and preparation of the reports was all done by the two of us as principal investigators, working with our co-investigators at peripheral sites throughout North America, on a voluntary basis.

1. The first case.

In 1983, Dr. Bussel saw a patient in consultation whose first baby had suffered an ICH in utero at 32 weeks, and died one day after
having been delivered at 38 weeks with a platelet count of 3,000/ml³. The autopsy and head CT scan both showed that the ICH had been initiated in utero at approximately 32 weeks of gestation. For the subsequent pregnancy the standard therapy at that time was elective delivery at 38 weeks. Because of the high likelihood that the fetus would develop an intracranial hemorrhage prior to that time, Dr Bussel chose to treat her in utero with IVIG 1 gm/kg/wk and dexamethasone 5 mg/day. The doses were chosen empirically, but the baby did not suffer an ICH, was born with a platelet count of 30,000/ml³ and is currently a thriving young woman.

2. The first series.⁵,⁶

IVIG is extremely expensive and oral steroids can have significant maternal side effects so we began our collaboration later in 1983 by attempting to determine the types and optimal doses of the medications to be administered. The first 7 cases were reported in 1988, and a larger series of 18 patients was published 4 years later. Because oligohydramnios was evident in four of the first five cases treated with a combination of 3 or 5mg dexamethasone/day and IVIG, the remainder of the patients in these early studies were treated with IVIG alone. With the latter regimen the amniotic fluid volume remained within the normal range, but the fetuses whose mothers had also received steroids appeared to have higher platelet counts than those receiving IVIG alone.

3. The first randomized study: IVIG alone compared to IVIG + 1.5 mg of dexamethasone.⁷

In this first randomized study, all patients underwent fetal blood sampling prior to being randomly assigned to receive IVIG 1 gm/kg/week alone or IVIG at that dose plus dexamethasone 1.5mg/day, a dose that did not cause oligohydramnios. They then had a second fetal blood sampling procedure 4-6 weeks after starting therapy, and prednisone 60mg/day was added to their regimen (salvage therapy) if
they were not found to have had an adequate response to initial therapy. Fifty five patients were enrolled in this study. Approximately 70% had an adequate birth platelet response to their original therapy, while an additional 10% had a therapeutic platelet response to salvage therapy. There were no intracranial hemorrhages in the series. Finally, the addition of low dose dexamethasone did not improve the fetal platelet counts compared to using IVIG alone, and salvage therapy with daily prednisone did not cause oligohydramnios.

4. Recognition of the potential danger of fetal blood sampling.\(^8\)

We became aware of some fetuses dying in utero when fetal blood sampling was performed at institutions around the United States prior to the institution of therapy, which was an inclusion criterion for the study described above. In 1995 we published a small series comparing several of those cases to matched controls and concluded that the losses were due to exsanguination in fetuses with extremely low platelet counts. As a result we recommended having matched platelets available for transfusion whenever fetal blood sampling was done in this disorder, and described how the transfusions should be performed. This approach has become the standard of care whenever fetal blood sampling is performed in cases of AIT. At that time weekly fetal platelet transfusions were widely used to treat this disorder in Europe because many clinicians were not convinced of the effectiveness of medical therapy and were unaware of the potential risks of in utero blood sampling in severely thrombocytopenic fetuses.

5. Description of the Natural History of the Disease.\(^9\)

An analysis of our first 107 cases showed that 50% of affected fetuses already had platelet counts < 20,000/ml\(^3\) by 24 weeks of gestation. In 40% of cases, the initial fetal platelet count was lower than the birth platelet count of the previous sibling, confirming that this disease worsens in subsequent pregnancies. Furthermore, the only
predictor of severe fetal thrombocytopenia was an ICH in the previous sibling.

6. The second randomized prospective study: parallel trials according to risk.\textsuperscript{10}

This was a study of 79 patients with AIT that were prospectively segregated into two categories based on their history and initial fetal platelet counts. The high risk group either had an affected sibling with a peripartum ICH or a pre-treatment fetal platelet count <20,000/ml\textsuperscript{3}, while the standard risk group did not have a sibling with an ICH and the initial platelet count was >20,000/ml\textsuperscript{3}. Patients in the high risk group were randomized to receive IVIG 1 gm/kg/wk alone or in combination with prednisone 1 mg/kg/day. The patients who received the combination therapy did considerably better than those receiving IVIG alone – 82% of those receiving combination therapy in the subset of women with initial fetal platelet counts less than 10,000/ml\textsuperscript{3} had a satisfactory response to their regimen compared to only 18% in the group treated with IVIG alone. Patients in the standard risk group, however, were randomized to either receive IVIG 1 gm/kg/wk or prednisone 0.5 mg/kg/day, and both of those regimens were found to be efficacious and comparable.

7. The third randomized prospective study: IVIG x 2/wk vs. IVIG x 1/wk + prednisone 0.5mg/kg/day \textsuperscript{11}

This was a prospective multicenter study of 73 women with documented AIT that had not had a prior child with an ICH. It was based on the previous study which showed that IVIG 1gm/kg/wk alone was not sufficiently effective in women whose fetal platelet count was < 20,000/ml\textsuperscript{3}. These women were randomized to receive either IVIG 2 gm/kg/wk (Group A) or IVIG 1 gm/kg/wk plus prednisone 0.5mg/kg/day (Group B) starting at 20 weeks gestation. Fetal blood sampling was
not performed until approximately 32 weeks, and those who were found to have platelet counts <30,000/ml³ were given salvage therapy at that time. The outcomes of both treatment groups were found to be excellent and comparable, but 22% of the patients required salvage therapy. Only 1 neonate in each group had a birth platelet count <30,000/ml³, one of whom was not fully compliant with the treatment schedule. There were 4 complications after 79 fetal blood sampling procedures (5.1%) leading to cesarean deliveries between 32 and 37 weeks. There was a higher incidence of gestational diabetes and a tendency to more fluid retention, mood swings, insomnia and jitteriness in patients taking prednisone, and more moderate to severe fatigue in those on high-dose IVIG alone. This study, published in 2007, demonstrated that either regimen initiated at 20 weeks in women with no history of an ICH in a prior pregnancy was highly effective in most, but not all cases. An ongoing continuation of this study currently includes 95 patients who have delivered. In this expanded group empirically adding salvage therapy at 32 weeks, rather than performing fetal blood sampling at that time, has been an effective alternative for many patients.

8. The management of AIT in pregnancies with an ICH in a prior sibling.¹²

This report, published in 2010, describes the management of 37 pregnancies in 33 women compiled over a period of almost 12 years. These pregnancies were stratified according to the timing of the previous child’s ICH. The extremely high risk group had 8 patients with an ICH occurring at <28 weeks, the very high risk group had 17 with an ICH between 28-36 weeks and the high risk group had 12 with an ICH which occurred during the perinatal period. Treatment was initiated at 12 weeks with IVIG at 1 or 2 gm/kg/wk. If the platelet count determined by fetal blood sampling at approximately 22 weeks was found to be <30,000/ml³, prednisone and/or more IVIG was added. Five of the fetuses suffered ICHs, when 34 (90%) would have been expected
in the absence of therapy. Two of the five cases occurred in infants with platelet counts >100,000/ml³ and one other was a grade I bleed of no clinical consequence; however, 2 were unequivocal treatment failures. As a result of the latter two cases, who presented in special circumstances, the treatment regimens were altered and to date there have been no further cases of ICH reported in patients being treated according to the revised recommendations.¹³

Summary

We believe that our serial analyses of studies in which a variety of methods was used to treat this disorder has led to a rational, minimally invasive approach to its management in utero. This approach depends upon the stratification of patients based on their history. These efforts have eliminated the need for fetal blood sampling in almost all patients, minimized the amount of expensive and potentially toxic medication administered to the mothers, and maximized the effectiveness of therapy for the fetuses at highest risk of ICH that need it most. As a result it is now possible to offer women who are at risk to have a fetus affected with this disorder the extremely high likelihood that they will be able to safely deliver a completely normal child with no central nervous system damage caused by a devastating intracranial hemorrhage, and at very little risk to their own well-being during the pregnancy.

We are enormously grateful to the hundreds of families who have participated in our studies, and to their physicians throughout North America who entered them into our research protocols. The fact remains, however, that one cannot treat this condition without knowing that it exists. Unfortunately, the disorder is rare and the, screening technology is expensive, although the latter will undoubtedly become less costly over time. Currently, it is almost always the case that at risk couples are not discovered until they have already delivered an unexpectedly affected child. It is our fervent hope that the next step forward will
be the development of an affordable, non-invasive population based screening paradigm for the detection of couples who are not already known to be at risk to have a fetus affected by this disease, along with a biomarker of potential severity to further guide their management. A large study in Norway\textsuperscript{14} and two smaller ones in the United Kingdom\textsuperscript{3,15} have suggested that screening of all pregnancies may be feasible, but the optimal approach, even if affordable, is far from clear at this time.

References


The King Faisal International Prize for Science (Biology) for the year 1433H - 2012G has been awarded to: Professor Alexander Varshavsky (USA).

Professor Varshavsky has made ground-breaking discoveries into how the living cell works. He elucidated how cell functions are regulated by protein degradation. Proteins are essential parts of organisms and participate in virtually every process within the cell. Cells continuously produce and destroy proteins to ensure optimal function.

Varshavsky’s work led to the unraveling of the cellular mechanisms that determine how cellular proteins are being selected for destruction. He also discovered how proteins are marked for rapid degradation. These advances have created a new realm of biology and have been essential for progress in research on human cancer, neurodegeneration, immune responses and other fundamental biological processes. This may lead to clinically useful therapies.
Discovery of the Biology of the Ubiquitin System

Professor Alexander Varshavsky
What follows (after a brief introduction) is a summary of contributions by our laboratory to the emergence of the field of ubiquitin and regulated protein degradation. Today, this field is one of the largest biomedical realms. That wasn’t so in the early 1980s, when few laboratories were interested in ubiquitin.

Among the functions of intracellular proteolysis (protein degradation) are the elimination of misfolded or otherwise abnormal proteins; the maintenance of amino acid pools in cells affected by stresses, such as starvation; and the generation of protein fragments that act as hormones, antigens, or other effectors. Many intracellular proteins are either conditionally or constitutively short-lived, with in vivo half-lives that can be as brief as a few minutes. In some cases, a proteolytic pathway targets and destroys a protein cotranslationally\(^1\),\(^2\), i.e., an emerging polypeptide chain can be degraded while it is still a ribosome-associated peptidyl-tRNA. The regulated and processive degradation of intracellular proteins is carried out largely by the ubiquitin system, in conjunction with molecular chaperones, autophagy, and lysosomal proteolysis. Other mediators of intracellular protein degradation include proteases such as, for example, caspases, calpains, and separases. These and other nonprocessive proteases can function as “upstream” components of the ubiquitin system, generating protein fragments that are targeted and degraded to short peptides by ubiquitin-mediated pathways. Proteins that are damaged, misfolded, or otherwise abnormal are often recognized as such and selectively destroyed by the ubiquitin system. Physiologically important exceptions include conformationally perturbed proteins and/or their aggregates that are harmful but cannot be efficaciously repaired or removed. The resulting proteotoxicity underlies both aging and specific diseases, including neurodegeneration.

One major role of the ubiquitin system is the regulation of proteins whose concentrations must vary with time and alterations in the state of a cell. Short in vivo half-lives of such proteins provide a way to generate their spatial gradients and to rapidly adjust their concentration or subunit composition through changes in the rate of their degradation. In addition, a short half-life of a protein would lead to a rapid decrease in its concentration upon cessation of its synthesis.
This way, transcriptional or translational regulation of specific proteins can acquire switch-like properties, because a short-lived protein that is no longer made would not persist in a cell, in contrast to a metabolically stable protein. Proteolysis can also serve to activate or otherwise modulate protein molecules and specific circuits, e.g., by cleaving off and destroying an autoinhibitory domain of a protein or by selectively eliminating an inhibitory subunit of a protein complex. These and other properties of the ubiquitin system make it, among other things, a major regulator of gene expression. For example, most transcriptional activators and repressors in eukaryotes are conditionally short-lived proteins that are destroyed by the ubiquitin system at spatiotemporally regulated rates that underlie the finely tuned physiological functions of these proteins.

Ubiquitin is a small (76-residue) protein that is highly conserved in evolution. For example, yeast ubiquitin and human ubiquitin are essentially identical proteins. Ubiquitin mediates proteolysis through its enzymatic conjugation to proteins that contain primary degradation signals, called degrons. A primary degron is a feature of a protein (a region of its amino acid sequence and/or a conformational determinant) that makes the protein metabolically unstable. Ubiquitin-protein conjugation marks proteins for their recognition and degradation by the 26S proteasome, a processive, ATP-dependent protease. Ubiquitin is conjugated to proteins either as a single moiety or as a polyubiquitin chain that is linked (in most cases) to the ε-amino group of an internal Lys residue in a substrate protein. Ubiquitin is a "secondary" degron, in that ubiquitin is conjugated to proteins because they contain primary degradation signals. Ubiquitin has nonproteolytic functions as well. The design of the ubiquitin system is summarized in Figure 1.

The field of ubiquitin and regulated protein degradation was created in the 1980s, largely through the complementary discoveries by the laboratory of Avram Hershko and by my laboratory. In 1978-1985, the elegant biochemical insights by Hershko and coworkers produced the initial understanding of ubiquitin-mediated protein degradation in cell extracts, including the isolation of enzymes that mediate ubiquitin conjugation. In 1984-1990, these mechanistic (enzymological) advances with cell-free systems were complemented by our genetic and biochemical
discoveries with mammalian cells and the yeast Saccharomyces
cerevisiae. These discoveries revealed the singularly important biology
of the ubiquitin system, including the first demonstration that the bulk
of protein degradation in a living cell requires ubiquitin conjugation,
and the identification of the first ubiquitin-conjugating (E2) enzymes
with specific physiological functions, in the cell cycle (CDC34 E2) and
DNA repair (RAD6 E2). These advances initiated the understanding
of the massive, multilevel involvement of the ubiquitin system in
the regulation of the cell cycle and DNA damage responses. We also
discovered the critical roles of the ubiquitin system in stress resistance,
protein synthesis, and transcriptional regulation. In 1990, we identified
and cloned an E3 ligase termed UBR1, the first molecularly cloned and
analyzed E3 ubiquitin ligase. Together with the RAD6 E2 and CDC34
E2 results, the cloning and characterization of the UBR1 E3 opened up
a particularly large field, because we now know that the mammalian
genome encodes at least 1,000 distinct E3s. The targeting of many
different degrons in cellular proteins by this astounding diversity of
specific E3 ubiquitin ligases underlies the unprecedented functional
reach of the ubiquitin system. In 1986, we discovered the first primary
degradation signals in short-lived proteins. (Specific signals that mark
proteins for conjugation to ubiquitin were presumed to exist, but their
nature was a mystery.) These signals included degrons that give rise
to the N-end rule, which relates the in vivo half-life of a protein to the
identity of its N-terminal residue (Figure 2). The N-end rule pathway
(which gives rise to the N-end rule) was the first specific pathway of
the ubiquitin system. Other discoveries by our laboratory in the 1980s
included the first polyubiquitin chains, their specific topology, and their
necessity for proteolysis; the subunit selectivity of protein degradation
(this fundamental capability of the ubiquitin system underlies most of
its functions, as it makes possible the subunit-specific remodeling of
oligomeric proteins); the first physiological substrate of the ubiquitin
system (the MATalpha2 transcriptional repressor; before this advance,
the ubiquitin system was examined using artificial substrates); and the
first genes that encode ubiquitin precursors (a linear polyubiquitin chain
and ubiquitin fusions to specific ribosomal proteins). For accounts of
the early history of the Ub field, see refs. 4-6.
By the end of the 1980s, our studies had revealed the major biological functions of the ubiquitin system as well as the basis for its specificity, i.e. the first degradation signals in short-lived proteins. The resulting discovery of physiological regulation by intracellular protein degradation has transformed the understanding of biological circuits, as it became clear that control through regulated protein degradation rivals, and often surpasses in significance the classical regulation through transcription and translation. Just how strikingly broad and elaborate ubiquitin functions are was understood more systematically and in great detail over the next two decades, through studies by many laboratories that began entering this field in the 1990s, an expansion that continues to the present day.

The ubiquitin system is of major importance in medicine, given its immense functional range and the multitude of ways in which this system can malfunction in diseases, from cancer and neurodegenerative syndromes to perturbations of immunity and many other illnesses, including birth defects. Both academic laboratories and pharmaceutical companies are developing compounds that target specific components of the ubiquitin system. The fruits of their labors have already become, or will soon become, clinically useful drugs. Work in this arena may produce not only "conventional" inhibitors or activators of specific enzymes but also drugs that would direct the ubiquitin system to target, destroy, and thereby downregulate any specific protein. After three decades of ever-expanding studies in this vast biomedical realm, new directions of inquiry, new problems, and new applications of fundamental discoveries continue unabated. Advances in the understanding of the ubiquitin and ubiquitin-like systems are being published at a clip that exceeds anyone's ability to follow these studies in their entirety, a state of affairs that is frustrating and exhilarating at the same time. It is a safe bet that new and major breakthroughs in this arena will continue to occur and will be accompanied by momentous advances in the application of accumulated fundamental understanding to problems in clinical medicine. I feel privileged having been able to contribute to the birth of this field and to partake in its later development.
References

Figure 1. The ubiquitin system. This diagram illustrates the fundamental design of ubiquitin-mediated processes. The conjugation of ubiquitin (Ub) to other proteins involves a preliminary ATP-dependent step in which the last residue of Ub is joined, via a thioester bond, to a Cys residue of the E1 (Ub-activating) enzyme. The “activated” ubiquitin moiety is transferred to a Cys residue in one of several ubiquitin-conjugating (E2) enzymes and, from there, through an isopeptide bond to a Lys residue of an ultimate acceptor protein (“substrate”). E2 enzymes function as subunits of E2-E3 ubiquitin ligase holoenzymes that can produce substrate-linked polyubiquitin chains. Such chains mediate either the processive degradation of a substrate by the 26S proteasome (an ATP-dependent, multisubunit protease) or other metabolic fates of chain-linked substrates. Monoubiquitylation of specific proteins also occurs and has specific functions. An individual mammalian genome encodes at least 1,000 distinct E3s. One role of E3 is the initial recognition of a substrate’s degradation signal (degron). Ubiquitin has nonproteolytic functions as well. For accounts of the early history of the ubiquitin field, see refs. 4-6.
Figure 2. The mammalian Arg/N-end rule pathway\(^1\). This pathway had a shorter name, the "N-end rule pathway", before the 2010 discovery of the pathway’s another branch, termed the Ac/N-end rule pathway\(^1\). N-terminal residues are indicated by single-letter abbreviations. Yellow ovals denote the rest of a protein substrate. N-terminal C* denotes oxidized cysteine (Cys), i.e. Cys-sulfinate or Cys-sulfonate, generated in vivo through reactions that involve nitric oxide and oxygen. The "primary" destabilizing N-terminal residues Arg, Lys, His, Leu, Phe, Tyr, Trp and Ile are directly recognized by N-recognin E3 ubiquitin ligases (UBR1 and other UBR-type E3s). In contrast, the N-terminal residues Asp, Glu, Asn and Gln can be targeted by an N-recognin only after their Nt-arginylation by the ATE1 R-transferase. With N-terminal Asn and Gln, this step is preceded by Nt-deamidation, which is catalyzed by the NTAN1 and NTAQ1 Nt-deamidases\(^1\).