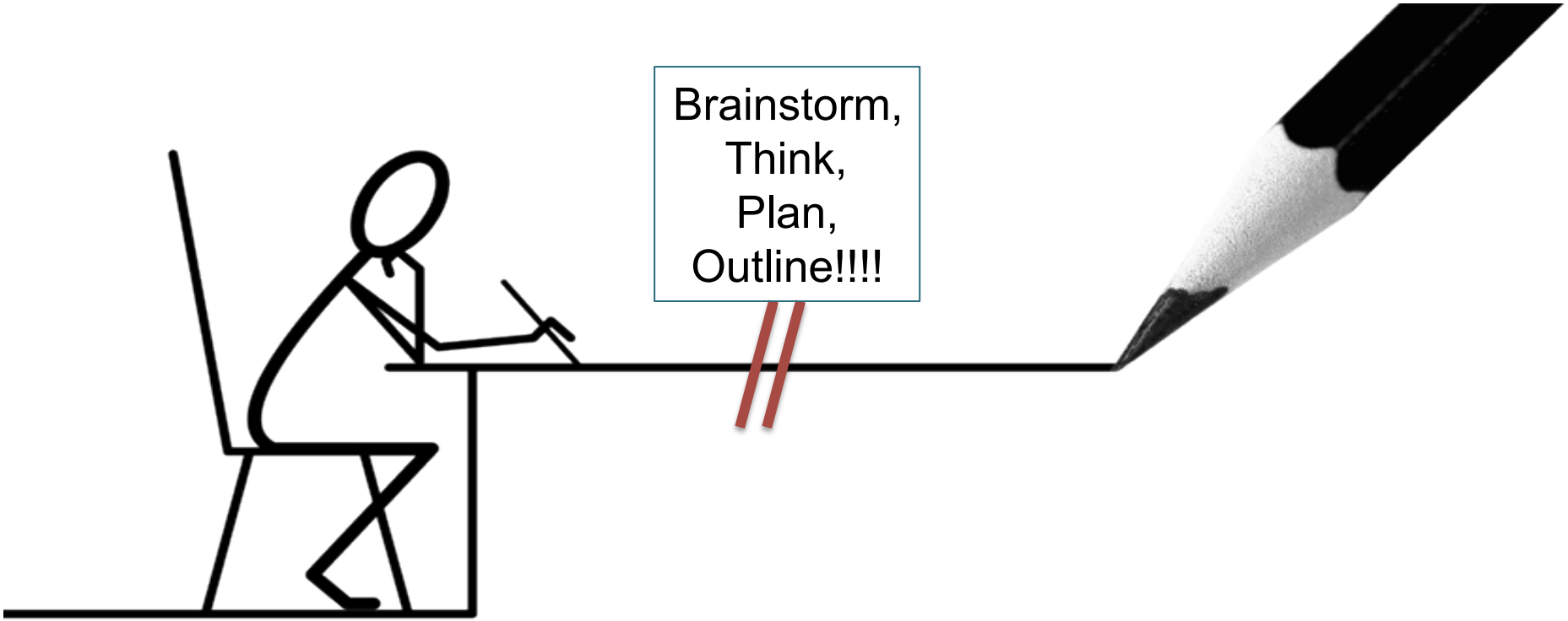
Please write your hypothesis on the board….

Scientific Writing IMB521:

Before you write



Before You Write, Understand…

The assignment

The Instructions and Format

Target Audience

The Big Picture

Brainstorm and outline…think and plan

"Chance favors the prepared (and organized) mind."
-Louis Pasteur

The Instructions

- Font
- Number of words
- Number of references
- Subsections
- Number of pages
- Figures
- Restrictions (institution, career stage, human subjects)

Read the Instructions!

NIH R01 Grant Format—1 + 12 pages

Title

Abstract (300 words)

Specific Aims (1 page)

Significance (1 pages)

Innovation (~0.5-1 page)

Approach (10-11 pages)

Literature Citations (not included in page limits)

NIH Grants

11pt Arial font (10pt font for figure legends)
0.5 in margins on all sides
250-300 word abstract
1 page specific aims
12 page research plan that includes (R21s are 6 pages)
- Significance
- Innovation
- Approach

Literature must be cited-not in page limits

NON-NEGOTIABLE!

Target Audience

Lay, no science background
Lay, science background
Scientist, general
Scientist, biologist
Scientist, biology specialist
Physicians

The big picture: what is the main point you want to make?

Big picture, big picture, big picture!

Forest for trees

There are between 1 and 200 people on the plant that will get/embrace your nitty gritty

Make sure you have your *big picture* firmly in hand

Communicate it clearly and early and often

Avoid detective stories or historical recitations

You may feel the redundancy…but it will help your reviewers

Time: Quality and Quantity

- Start early…as in now
- Leave time to put it down and come back to it with fresh eyes (2-7 days)
- Leave plenty of time to seek and use critical feedback (1 month before due date)
- Schedule a little time to write everyday and protect it
  -you can generally accomplish more writing 1-2 hours each day for a week than you can in a whole day of writing

*** The hardest and most important thing to take away ***

Think and Outline

- There is no substitute for preparation and a clear well thought out plan.
- This takes TIME!
- Dedicate time to this before reaching for the pen/keyboard

THREE WAYS to START: (use them all!)

1. Brainstorm options for questions and aims, then organize them (eliminate, combine, prioritize).
2. Outline a plan that will direct your writing
3. Decision making tree---no aim can result in a “no” answer

Beware of the blank page

- Write down the prescribed section headings
- Write down your hypothesis
- Outline
- Add your Aim subsections and aims
- Start filling in the blanks
- Try pen and paper

Generating a solid set of cohesive ideas

- First, BRAINSTORM options for questions and aims, then organize them (eliminate, combine, prioritize).
  - list
  - bubble diagrams
  - put everything down (no restrictions)

- Second, OUTLINE a *detailed* plan that will direct your writing
  - start with the subaims for each aim
  - then add the approaches/experiments for each subaim
  - add rationale for questions and approaches
  - list possible outcomes
  - list possible alternatives

- Decision making tree---no aim can result in a dead end
  - every aim must be presented in such a way that the end of the work results in some advancement
  - the answer to a question can be no so long as that is informative and allows the work to continue

Generating a solid set of cohesive ideas

Brainstorm : Get all the possibilities and ideas out….no matter how crazy

Put them all down, then begin to group and cull the ones that do not fit.

No limits! Just think!

You have discovered gene yfg encoded by merryvirus (persistent human pathogen that infects mice too) promotes cell survival and pathogenesis.
Write a grant.

Mechanisms of Human Cytomegalovirus Tropism for the Vascular Endothelium

Hypothesis: *We hypothesize that pUL135 and pUL136 manipulate host trafficking and MVB biogenesis pathways specifically for replication in ECs and that these virus-induced changes compromise EC barrier function.*

Aim 1. Determine the mechanisms by which *UL135* and *UL136* promote virus replication in ECs.

Hypothesis: Interactions between host proteins and pUL135 and pUL136 alter host trafficking pathways for virus replication in ECs

1.1 Map sequences required for interaction with cellular partners

- Mutate expression construct and test for loss of interaction
- Generate mutant viruses with disruption in both putative interaction domains
- Analyze replication of mutant viruses relative to WT

1.2 Define the significance of pUL135- and pUL136-host interactions for virus replication in ECs

- What is the effect of loss of interaction mutants on:
  - viral gene expression
  - genome replication
  - production
  - progeny maturation
  - inclusion into MVB.

Aim 2. Determine how hCMV manipulates MVB biogenesis and trafficking during infection of ECs.

UL135- and UL136-host interactions manipulate MVB biogenesis and trafficking to promote viral egress and impact the extracellular environment.

2.1 ***Define the host trafficking pathways required for the incorporation of virus products into MVB*** **.**

- shRNA knockdown of 17 targets required for MVB biogenesis
- Analyze incorporation of virus into MVB using fluorescent virus and MVB marker

2.2 ***Determine how infection alters MVB trafficking and exocytosis*** **.**

- Live cell imaging of GFP-virus and MVB in cells infected with WT or mutant viruses.
- FRAP
- Track fate of virus-containing MVB to lysosome or cell surface

2.3 Define the composition of exosomes from hCMV-infected ECs**.**

- Isolate exosomes from uninfected, WT or mutant infected cells
- Assess purity by negative staining, Nanosight and blotting
- Analyze protein content by mass spectrometry

Aim 3. Determine the impact of hCMV infection on EC function .
The integrity and function of the vascular endothelium is compromised as a consequence of viral infection.

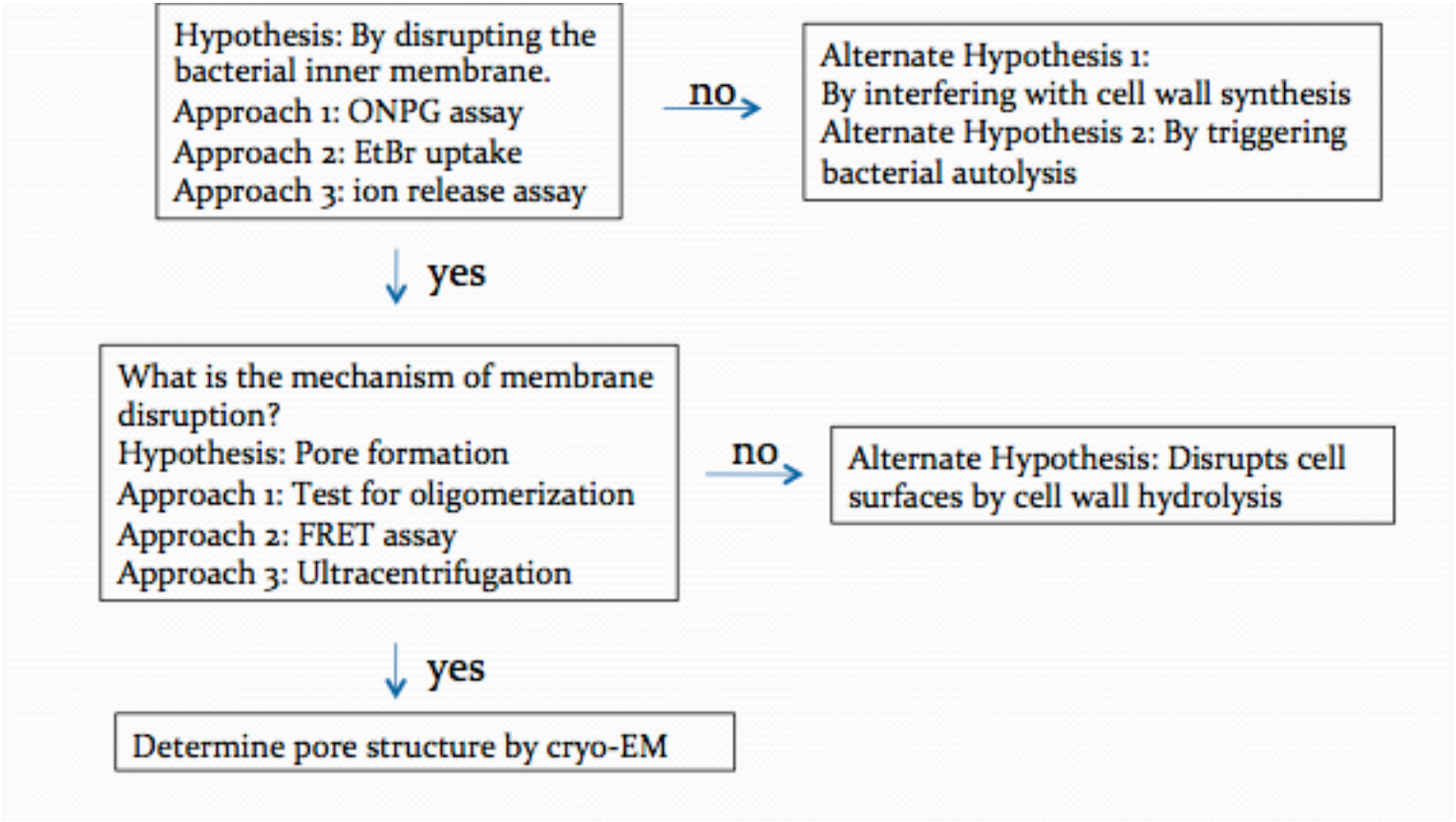
3.1 ***Determine how infection alters the integrity of intercellular junctions*** **.**
- Analyze cell-cell junctions by IF in WT or mutant infected cells
- Transendothelial resistance
- Permeability

3.2 ***Define how exosomes from infected cells impact EC infection and biology*** **.**
- Isolate exosomes from infected cells
- Analyze the impact on subsequent infection
- Analyze effect on angiogenesis (tubule formation)
- Analyze effect on wound healing

<u>COHESIVENESS/INTEGRATION</u>
- Interaction mutants from aim 1 analyzed in every aim to understand how specific UL135- and UL136-host interactions contribute to MVB biogenesis (Aim2) and EC function (Aim 3)
- Exosomes defined in Aim 2, then in Aim 3 we explore how they impact infection and EC function.
- Virology and cell biology; host interactions kept throughout.

Decision Making Tree

Aim 2B: Determine how RegIIIγ kills bacteria



White space, please

SPECIFIC AIMS

Human cytomegalovirus (HCMV), like all herpesviruses, persists indefinitely through a latent infection. HCMV persistence, reactivation from latency, and congenital infection pose significant health risks. Current antiviral strategies have limited efficacy as they fail to target latently infected cells. The long-term objective of our research is to define key virus-host interactions comprising the mechanistic basis of HCMV latency.

The objective of this application is to determine the role of newly identified viral determinants within cell types relevant to viral persistence: endothelial and $CD34^+$ hematopoietic progenitor cells (HPCs). We discovered a novel, polycistronic locus unique to clinical virus strains that *is associated with three distinct cell type-dependent phenotypes*. While the locus is dispensable for productive replication in fibroblasts, it suppresses replication for latency in $CD34^+$ HPCs and augments replication in primary endothelial cells. This locus encodes the only known HCMV latency determinant, pUL138, and three previously uncharacterized proteins: pUL133, pUL135, and pUL136. We collectively refer to this locus as the *UL133/8* locus and the proteins encoded therein as UL133/8 (Fig. 1A). Among these, pUL135 has emerged as a master regulator that dictates the outcome of infection. While the *UL133/8* locus is dispensable for replication in fibrobasts, disruption of *UL135* alone results in a virus with a severe replication defect. Importantly, disruption of *UL138* in addition to *UL135* restores replication of the *UL135*-null virus, suggesting that these genes encode opposing functions.

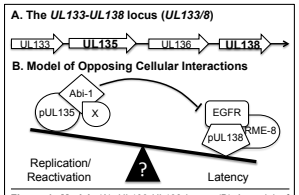


Figure 1. Model. (A) *UL133-UL138* locus. (B) A model of candidate interactions impacting the outcome of infection. pUL135 promotes replication and pUL138 promotes latency through their interactions with cellular factors. We hypothesize that pUL135:ABI-1 and yet unknown factor X negatively regulate pUL138 by targeting the EGFR network.

We propose a model whereby antagonistic proteins comprise a molecular switch that dictates the outcome of infection: replication versus latency (Fig. 1B). We hypothesize that pUL135 promotes viral replication and reactivation, while pUL138 promotes latency, both functioning through their interaction with cellular proteins. We have identified cellular interacting partners for each protein. Our preliminary data suggests that pUL135 and pUL138 might oppose one another by each targeting the cellular EGFR pathway. The following aims will test the model's core hypothesis and determine the mechanism of *UL133/8*-mediated molecular switches.

Aim 1. Define the role and regulation of pUL135 during infection. We hypothesize that pUL135 functions critically in viral replication and reactivation from latency. *UL135* expression is detected in productively infected cells, but not in latently infected cells. Given its replication-promoting role, we predict that pUL135 expression is incompatible with the establishment of latency. We will define the basis for cell type-dependent *UL135* expression and the significance of pUL135 to viral replication in fibroblasts and endothelial cells. Importantly, we will determine the role of pUL135 in latency/reactivation using our experimental CD34+ HPCs model for investigating HCMV latency *in vitro*.

Aim 2. Define pUL135 cellular interactions and their significance to pUL135 function and infection. We hypothesize that virus-host interactions involving UL133/8 proteins underlie the outcome of infection. We discovered that pUL138 interacts with RME-8, a regulator of endocytosis, and several members of the epidermal growth factor receptor (EGFR) network, including EGFR. pUL135 interacts with ABI-1, an adaptor protein that contributes importantly to the regulation of EGFR. Therefore, we propose a model whereby pUL135 and pUL138 antagonistically regulate the EGFR pathway (Fig 1B). Consistent with this model, pUL138 enhances while pUL135 decreases cell surface levels of EGFR. We will define the interaction between pUL135 and ABI-1, identify pathways bridged to pUL135 by ABI-1, and determine the significance of these interactions to the function of pUL135 in infection and the regulation of EGFR.

Aim 3. Determine the significance of pUL135 interactions to latency and reactivation *in vivo*. We hypothesize that pUL135 functions through its interactions with cellular proteins to either prevent the establishment of latency or promote reactivation from latency. We will perturb pUL135 and key pUL135 interactions in the context of infection *in vivo* using an innovative, state-of-the-art animal model. In this model, NOD/LtSz-*scidIL2R*γ_c^{null} (NSG) mice are humanized through the engraftment of human $CD34^+$ HPCs and support HCMV latency and reactivation.

IMPACT Our studies will elucidate the role of exciting and novel virus-host interactions underlying HCMV persistence using innovative and state-of-the-art model systems. The specific knowledge gained will ultimately identify new targets for antivirals aimed at latently infected cells. We are uniquely suited for the work proposed herein as we created a quantitative HCMV latency/reactivation model using $CD34^+$ HPCs, discovered the *UL133/8* locus, characterized the first viral determinant of latency, constructed the requisite tools and built the collaborations necessary to dissect the functions of UL133/8 proteins in infection and persistence.

SPECIFIC AIMS

Persistent viruses pose a present and emerging threat to human health that evades clinical control due to a deficit in our understanding of the mechanisms underlying persistence. Human cytomegalovirus (HCMV) is an endemic herpesvirus that persists through chronic and latent states of infection. Our long-term objectives are to understand the virus-host interactions underlying the molecular basis of viral latency using HCMV.

Our work is poised to define the first molecular mechanisms of HCMV latency. We have identified the *UL135* HCMV gene as an emerging master regulator of HCMV latency and reactivation. Importantly, using novel CD34+ hematopoietic progenitors cells (HPCs) and endothelial cell (ECs) systems, both of which are relevant to HCMV persistence in the host, we have defined distinct functions for pUL135 depending on the infected cell type (Fig. 1). *UL135* is expressed from the *UL133-UL138* locus, which encodes three additional proteins, pUL133, pUL136, and pUL138 in addition to pUL135. While the locus as a whole is dispensable for replication, targeted disruption of *UL135* results in a severe replication defect. This exciting result was the first to indicate that the *UL133-UL138* locus encodes antagonistic functions to both promote and suppress viral replication. Indeed, our data show that pUL135 overcomes pUL138-mediated suppression of virus replication and, therefore, is essential for reactivation from latency.

The discovery of antagonistic regulators encoded by a single locus within the viral genome presents a tractable and novel mechanism for understanding both the establishment of and reactivation from latency. The critical question now is to define how *UL135* interfaces with the host to control states of persistence. Using proteomics, we have identified several cellular proteins interacting with pUL135. Two prominent cellular adapters proteins, CIN85 and Abi-1, that function in the cell to regulate trafficking, cytoskeleton, and signaling by recruiting the Cbl E3 ubquitin (Ub) ligase to trigger the internalization of receptor tyrosine kinases such as EGFR. Intriguingly, expression of pUL135 dramatically induces the loss of EGFR from the cell surface, while pUL138 enhances surface levels of EGFR. The opposing regulation of EGFR by pUL135 and pUL138 likely underlies the antagonistic functions of these proteins in infection, where pUL138 promotes latency and pUL135 promotes reactivation. We hypothesize that UL135 co-opts Ub pathways to target a diverse array of proteins, including EGFR, to ultimately regulate productive and latent modes of infection in a variety of cell type-specific contexts of infection (Fig. 1). We will identify the cellular pathways targeted by pUL135 interactions and determine the significance of these pathways to reactivation from latency to define novel mechanisms underlying viral persistence. To this end, we propose the following aims:

Aim 1. Define the UL135-mediated Ub proteome. Ubiquitination regulates diverse degradative and non-degradative cellular pathways. *We hypothesize that pUL135 redirects Ub machinery in the cell to modulate states of latency and replication.* We will define the Ub proteome directed by pUL135 and its interactions with CIN85 and Abi-1 by comparing enriched, SILAC-labeled Ub-peptides from cells expressing wild type pUL135 or variants of the pUL135 that fail to interact with CIN85 or Abi-1. The significance of key targets will be explored by engineering variants of cellular or viral targets that cannot serve as substrates for Ub.

Aim 2. Determine the role of Ub in pUL135 function during infection. *We hypothesize that the redirection of Ub pathways underlies pUL135 activity* (Fig. 1). We will determine the role of key pUL135 targets (Aim 1), including EGFR, in the function of pUL135 to (i) promote viral replication, (ii) downregulate surface EGFR, and (iii) maintain intracellular membrane organization for virion maturation in relevant cell types. This aim will determine how the significance of specific pUL135 interactions and targets to pUL135 function.

Aim 3. Define the role of cellular pathways targeted by pUL135 in viral latency/persistence *in vivo*. *We hypothesize that pUL135 functions to promote viral reactivation from latency through the pathways it co-opts via Ub*. We will investigate the contribution of the pathways and mechanisms identified through our existing work and work proposed in Aims 1 and 2 to viral persistence in CD34+ HPCs infected *in vitro* and in humanized mice infected *in vivo*.

Mechanisms of viral persistence are among the most elusive questions in virology. Our studies will reveal fundamental virus-host interactions that modulate states of latency and reactivation. We are uniquely suited for the work proposed as we discovered and characterized the first viral determinants of HCMV latency and built the tools and models necessary to define mechanisms of viral persistence.

Mechanisms of viral persistence, and especially those of viral latency, have remained elusive despite many decades of research. Herpesviruses, through their co-speciation with us, have evolved among the most sophisticated interactions known between virus and host that ultimately result in the ability of these viruses to persist latently in the immunocompetent host. Human cytomegalovirus (HCMV), an endemic beta-herpesvirus, is known for its exquisite ability to interact with and manipulate host systems. HCMV infection is the leading cause of infectious-disease related congenital disease, infecting 1 in 100 children in utero and resulting in neurological abnormality in 1 in 1,000 children in the United States (3). Reactivation of HCMV from latency in

Visual flow

This passage contains biographical information about a NASA engineer who died.

It is taken from a NASA press release.

Owen Eugene Maynard

Owen Eugene Maynard, who died on July 15 at age 75, was an outstanding leader of the Apollo program and one of Canada's great space flight pioneers.

In 1960, Maynard was part of the small group of engineers at NASA's Space Task Group, which grew into today's Johnson Space Center, when he was assigned to a new human space flight program called Apollo that at the time had no specific goal or even authorization to proceed. Working under the direction of leading lights at NASA such as Robert Gilruth, Max Faget and Caldwell Johnson, Maynard helped sketch out the initial designs of what would become the Apollo Command and Service Modules. The following year, when President John F. Kennedy gave Apollo the goal of landing on the Moon, Maynard became involved in the debates that raged within NASA over how Apollo would fly to the Moon.

A little more than a year after Kennedy's call to land on the Moon, NASA had settled on sending astronauts to the Moon and bringing them back home by a method known as lunar orbit rendezvous or LOR. This method was championed within NASA by John Houbolt, but Maynard was among the first at the Space Task Group to see the wisdom of using LOR to fly to the Moon at a time when other methods were favored.

Another Canadian, James A. Chamberlin, had been converted to LOR and proposed landing an astronaut on the Moon using a Gemini spacecraft and a lunar "bug." Following Chamberlin's lead, Maynard began making the first serious sketches within NASA of what would become known as the Lunar Module. Maynard's conception of the LM was used by STG to help sell the idea of Lunar Orbit Rendezvous around NASA.

Apollo 11 LM (Lunar Module)

By 1963, Maynard was chief of the LM engineering office in the Apollo Program Office at the Manned Spacecraft Center in Houston. Work on building the LM was already underway at the Grumman Aircraft Engineering Corp. in New York, where Thomas J. Kelly was leading the engineering effort. Kelly, who today is

This passage contains biographical information about a NASA engineer who died. It is taken from a NASA press release.

Owen Eugene Maynard, who died on July 15 at age 75, was an outstanding leader of the Apollo program and one of Canada's great space flight pioneers.

In 1960, Maynard was part of the small group of engineers at NASA's Space Task Group, which grew into today's Johnson Space Center, when he was assigned to a new human space flight program called Apollo that at the time had no specific goal or even authorization to proceed. Working under the direction of leading lights at NASA such as Robert Gilruth, Max Faget and Caldwell Johnson, Maynard helped sketch out the initial designs of what would become the Apollo Command and Service Modules. The following year, when President John F. Kennedy gave Apollo the goal of landing on the Moon, Maynard became involved in the debates that raged within NASA over how Apollo would fly to the Moon.

A little more than a year after Kennedy's call to land on the Moon, NASA had settled on sending astronauts to the Moon and bringing them back home by a method known as lunar orbit rendezvous or LOR. This method was championed within NASA by John Houbolt, but Maynard was Space Task Group using LOR to fly to other methods were favored.

Another Canadian, James A. Chamberlin, had been converted to LOR and proposed landing an astronaut on the Moon using a Gemini spacecraft and a lunar "bug." Following Chamberlin's lead, Maynard began making the first serious sketches within NASA of what would become known as the Lunar Module. Maynard's conception of the LM was used by STG to help sell the idea of Lunar Orbit Rendezvous around NASA.

By 1963, Maynard was chief of the LM engineering office in the Apollo Program Office at the Manned Spacecraft Center in Houston. Work on building the LM was already underway at the Grumman Aircraft Engineering Corp. in New York, where Thomas J. Kelly was leading the engineering effort. Kelly, who today is known as the father of the LM, acknowledges that Maynard was the person at NASA most responsible for the design of the

From: psychology.wichita.edu