Dearest Reader,

We are proud to present the spring 2008 issue of the Emory Undergraduate Research Journal! Begun only in 2005, EURJ seeks to be the logical extension of undergraduate students’ experiences at this esteemed research powerhouse. We at EURJ understand that research is not complete until it is published, and that it is only through publishing, peer review, and priority that it is authenticated.

The issue that you are holding in your hands is further groundbreaking for a variety of reasons. It is our first time publishing twice in one year, and we sincerely hope that this precedent continues for years to come. The number of submissions that we received for this issue exceeded those of all previous issues combined, and that is partly because of our significant expansion in the types of submissions we accept. For the first time in its history, EURJ accepted and published submissions that range from research proposals, short and extended abstracts, to feature-length articles and human interest stories.

We would like to invite submissions in all genres from both current undergraduates as well as recent graduates for our upcoming fall 2008 issue. Our commitment to securing a blind review of manuscripts by expert reviewers means that we are always welcoming of new faculty, post-doctorate fellows, and graduate students to help out in this capacity.

Our spring 2008 issue is bulging with articles that range from Islam in Iran to Home Gardens in Panama. We would like to thank the backing of Media Council as well as SGA for recognizing our role in the Emory research community and granting us a permanent charter at the University. We also greatly appreciate the hard work of our dedicated team of staff members and current reviewers in helping us fully engage student researchers in realizing their contribution to the academic experience.

Sincerely,
Samyukta Mullangi and Shahein Tajmir
Editors-in-Chief

Acknowledgements
Tilo Driessen for Cover Image
Having received your undergraduate degree in Russian with a minor in Soviet, Post-Soviet and East European studies, what made you decide to pursue linguistics?

Actually I was introduced to linguistics because of my Russian degree. One of my professors offered a Russian linguistics course and I realized I really, really liked that part of [language]. That’s when language kind of made sense to me - by pulling it apart and looking at it a little deeper. And I also really liked…looking at more of the social issues that are reflected with language and the role that language plays in society. After graduation, I worked in the music industry for a little while doing promotions and I was really interested in looking at youth culture and how language was a part of that as well. I realized that business was not for me and that as much as I tried, that was not what I wanted to do forever. So I sat down and tried to figure out where everything came together and between music, Russian and cultural things, it all came back to language. So that’s when I decided to go to graduate school in linguistics and try it out.

What exactly is sociolinguistics for those that are unfamiliar with the field?

Linguistics in general is just the scientific study of language. Sociolinguistics looks at how society and language reflect one another. So, how people speak differently in different places to how changing from one social situation to another social situation can change language. How you speak at an interview versus how you speak at a bar versus how you speak at the doctor’s office are going to be very different things. I also [work] with language attitudes - the perceptions and stereotypes that people associate with those differences.

What research are you currently working on?

I’m [currently] working with the medical school and I kind of fell into it. They had already worked on a project and I said, “Hi do you need help?” and they said yes and gave me all this data, which was fantastic. So what I’m looking into is the role that different dialects play in communication between physicians and patients. The [data] I have is from Grady so it’s a lot of issues of African American language and Southern American English versus more standard type dialects, like whatever the doctor brings in. And also the difference between somebody who’s a physician and high up on the prestige social ladder versus the patients. Most of the patients at Grady are working class, low income and…when you go to the doctor, you’re opening yourself up. It’s a very weird social type situation to get into. So I’m looking at the times of terminology [the doctors] use, [and] whether or not pronunciation differences matter. The other thing I’m looking at is something called health literacy, which is basically whether or not patients can maneuver through the health care system and vocabulary and those types of things.

How did you become interested in investigating this subject?

The honest answer is that I was at a party and I met somebody who said they were doing research with a physician at the med school and that she was working on health literacy…I went, “Wow that sounds interesting,” and she gave me the contact information. So I called them up and said I have never done any work in this area before but it sounds interesting. I mean I’m at Emory, I’m surrounded by medical stuff so it just seemed like something to start looking into. And as I said, the other research that I do is …attitude towards language, so it’s really closely connected because a lot of the miscommunication is people expecting different types of language situations to occur, where they may not.

Can you give an example where language and dialect differences would have an impact on health communication?

There are a couple of different scenarios. One thing that could happen is the doctors are using this highly medical vocabulary and the patients don’t know it. So what can happen is the physician can try to use more general terms as opposed to medical specific terms so that the patients can understand better. The other thing that can happen, for good or bad, is the patient trying to use more medical terminology and what happens is if they get it right, great, but if they misuse one thing, that can actually be really detrimental because the physician may think they don’t really know what they’re talking about, where they may actually know. And the other side could be that the doctor actually, instead of trying to use medical terminology, sounds like he is talking down to the patient…And whether or not you can actually understand [the doctors], there tends to be this idea that you’re not going to be able to understand this person and so that could potentially get in the way.

What is the most interesting thing you have learned from your research so far?

The broad answer is just how important this is to everybody, the fact that everybody tells me a story, the fact that medical communication is something that everybody has a stake in from both sides: physicians and patients. So what’s interesting is how important it is to people.

What, if any, difficulties or problems have run into while conducting this research?

The problems that I’ve had, since I’m working with data that’s already been collected by someone else, [are] more of I would have done it this way or I don’t have the full answer here. So that’s where some of the problems have been but I knew that going into it, which is also a learning experience so when I go to collect more data, I’ll know how to question things a little bit differently. The other problem I have is just time…actually sitting down and doing all of this research. Do you have any ideas as to what you might be interested in researching next?

I’m going to stick with this for a while since it’s brand new for me…I definitely want to pursue this and right now the decisions are what direction I want to take it in because there is so much available. Literally, being at Emory, I keep running into people that are in the med school, public health, wherever who say, “Oh you’re doing this, we should get together and talk about this project.” So I have much more to do than I have time, so it’ll keep me busy.

Do you think your research in linguistics has changed the way you think when you communicate or interact with people?

Yes, absolutely. The problem with linguistics is once you start noticing things, you can’t [stop]. And I actually tell my students at the beginning of the semester that some days [they] will curse me because [they] won’t be able to stop noticing particular things. Obviously when I go to the doctor now, I’m trying not to but I pay close attention to what’s going on and I’m probably
Attachment bonds exist in relationships across the lifespan. Adolescence may be a particularly crucial period for attachment relations. As relationships with parents shift and those with peers gain importance, patterns of attachment may change as well. There is a large gap in the attachment literature on adolescent utilization of mother and father attachment figures, specifically, how adolescents are attached to their fathers as compared to their mothers. This study explores patterns of attachment in adolescence. Twenty-four racially diverse, mostly middle class adolescents’ attachments to their mothers and fathers (grades eight and ten) were measured using the Inventory of Parent and Peer Attachment (IPPA) questionnaire. In several dimensions, overall ratings for mothers were higher than for fathers. Attachment to mother and father were highly correlated, suggesting a generalization of attachment to both parents. There were several other significant correlations, suggesting both separate dyadic relationships within the family, as well as a family “climate” as related to attachment relationships. The study suggests that adolescent relationships with both parents are important, as well as different from one another.
Adolescence is a major transitional period in a person's life. With the onset of puberty comes not only physical changes, but also many other significant changes. For example, the social world of an adolescent changes to become more peer focused than before. However, parents do not disappear from the daily life of an adolescent; the relationship of an adolescent to their parents or parents also undergoes revisions. Therefore, a major task for adolescent development is to determine which people can help satisfy what needs, and to build appropriate relationships with them. Attachment theory may be helpful in understanding some of the patterns that exist through the transition into adolescence. Specifically, this study will use attachment theory to understand the relationships that adolescents have with their mothers and fathers.

Attachment Theory

Attachment theory originated as an explanation for the bond that exists between an infant and its primary caregiver, typically the mother (Bowlby, 1988). This bond is not only important for general well being (e.g., Krepniner & Ulrich, 1998), but also functions as a template for all relationships across the lifespan (e.g., Waters, Merrick, Treboux, Crowell, & Albershein, 2000). As early as infancy, children can mentally represent their caregivers and construct ideas and expectations for relationships with both these original figures and others; Bowlby called this the internal working model of attachment.

Attachment in infancy is conceptualized as distinct behaviors, however, researchers have analyzed attachment behaviors in infancy as an organizational construct, one that relies on the quality of the primary caregiver's (typically the mother) response to the infant's behaviors, which in turn influences the infant's responses and interactions (see Sroufe & Waters, 1997). Differences in reactions and interactions lead to individual variation in the infant's sense of security. More specifically, in mother-infant interactions of this type, the infant learns that the mother is a steady and secure person to seek for comfort. In other words, the mother is perceived as a secure base for the infant. As a result of this phenomenon, it was thought that there would be differences in how infants respond to and process caregivers' alternative behaviors, and that infants would show differences in their own behaviors as well. In order to study these observations, Ainsworth developed a process to measure individual differences in a testing situation called the Strange Situation where the infant and mother interact, are separated from one another and then reunited (for more detail see Sroufe & Waters, 1997).

Stability of Attachment Over Time

Attachment researchers have examined attachment beyond infancy. Rather than study attachment behaviors, however, researchers have analyzed attachment representations. It is thought that one creates mental representations of how to interact with others, termed the internal working model of attachment, based on previous attachment related relationships and interactions. Bowlby (1988) believed that beginning in infancy, a child internalizes patterns of relating to people, generally the parents, and forms an idea of how to relate to others based on these representations. Through early interactions, children learn what represents a help or a sheltered base.

Individual Differences

Early attachment theorist Mary Ainsworth explained attachment behaviors in infancy as an organizational construct, one that relies on the quality of the primary caregiver's (typically the mother) response to the infant's behaviors, which in turn influences the infant's responses and interactions (see Sroufe & Waters, 1997). Differences in reactions and interactions lead to individual variation in the infant's sense of security. More specifically, in mother-infant interactions of this type, the infant learns that the mother is a steady and secure person to seek for comfort. In other words, the mother is perceived as a secure base for the infant. As a result of this phenomenon, it was thought that there would be differences in how infants respond to and process caregivers' alternative behaviors, and that infants would show differences in their own behaviors as well. In order to study these observations, Ainsworth developed a process to measure individual differences in a testing situation called the Strange Situation where the infant and mother interact, are separated from one another and then reunited (for more detail see Sroufe & Waters, 1997).

Stability of Attachment Across Caregivers

A related issue is the concordance of attachment, defined as the stability of attachment between attachment figures (i.e. mother, father, peer, sibling). Research on the concordance of children's attachment to mother and father focuses primarily on relationships during infancy. Such studies show mixed findings; several point toward a strong concordance between attachment to mother and father (e.g. Easterbrooks, 1989), while others suggest that the mother-infant and father-infant attachment relationships are independent (Main & Weston, 1981). Fox, Kemmerly, and Schafer (1991) found overall support for concordance of attachment to mother and father; those infants who were securely attached to their mother were more likely to be securely attached to their father (the same patterns were found for insecurity). These findings suggest that children do internalize representations of relationships and attachments, and use those to form expectations for other close relationships. On the other hand, some of the studies examined suggested a lack of concordance of attachment between caregivers (Fox, Kemmerly & Schafer, 1991). For example, Main and Weston (1981) found that mother-infant and father-infant attachment relationships were not dependent on one another. They argue that although mother-infant and father-infant relationships do interact with one another, mothers and fathers each have specific ways of raising and relating to their children. To date, concordance has only been examined in infancy and early childhood, leaving concordance of attachment in adolescence as yet unexplored. Because of the developmental changes that occur in adolescence, this period seems to be one in which it is important to look at study how attachment to each figure is related.

Adolescent Development

Adolescence is a period of significant cognitive, social and behavioral transitions. Cognitively, there are huge gains in reasoning and the ability to take the perspective of another person, as well as in the acquisition of better emotional understanding. Socially, peer relationships become much more influential than before. Physically, puberty begins, sparking hormonal and physical changes. It is during this stage that an individual develops a more mature sense of identity (Erikson, 1986) because of such advances in behavioral, social and cognitive realms (Habermas & Bluck, 2000). For example, adolescents acquire a desire for high levels of support from them (Furman & Buhrmester, 1992, Noller, 1994). Therefore, representations of interactions with parents may provide a support base for adolescents.

Adolescent Relationships with Mothers and Fathers

While attachment theory says that each person has a single style of interacting with others, it is important to note that mother-child and father-child relationships are discrete. Montemayor and Gregg (1994) speak of the identity development in adolescence and its connection to interpersonal relationships; they note that as identity develops, adolescents observe that people they relate to see them in different ways. Observing parent-infant interactions while playing, Leaper (2000) found that play with mother and father are different and that each parent understands the self in adolescence are seen through independence, autonomy, and detachment from caregivers (Erikson, 1968). Ryan and Lynch (1989) found that individuals strive for more autonomy and individuation from parents in adolescence than in any earlier period of development, and there is a higher level of detachment from parents in this period. Collins and Repinski (1994) note that the amount of physical time that parents and their children spend together decreases during adolescence as well. Although there is obvious physical distancing from parents, adolescents still show a desire for high levels of support from them (Furman & Buhrmester, 1992).
that perceived maternal availability is important across the transition from childhood to adolescence, and the quality of the mother-adolescent relationship continues to be linked strongly to attachment security (Allen et al., 2003). More recent research has shown that older children differ in their utilization of their relationships with their mother and father as well as the quality of the affect toward each parent. For example, Paterson, Field, and Pryor (1994) found that adolescents rely on mothers for support more than their fathers. This might be because of expectations about the roles of their mother and father that have become ingrained in the adolescent. Much research display the bonds that exists between mothers and adolescents; however the importance that the father plays in the child’s life should not be overlooked. Recent research suggests that fathers also are significant attachment figures for adolescents (e.g. Williams & Kelly, 2005). It has been shown that the father-adolescent relationship is related to several attachment constructs, specifically warmth, closeness and availability (Cabrera, Tamis-LeMonda, Bradley, Hofferth, & Lamb, 2000).

Father-adolescent attachment impacts adolescent adjustment in a different way than does mother-adolescent attachment. Williams and Kelly (2005) compared both mother-adolescent and father-adolescent relationships, finding that although there were more secure mother-adolescent attachment relationships, the father-adolescent attachment relationship was more related to behavior problems in adolescents. Specifically, more paternal involvement in the parenting process was related to a greater security of attachment, suggesting that more paternal involvement in the parenting process was crucial (e.g. Allen & Land, 1999). Hazan and Shaver (1990) argue that the initial mother-infant attachment is for each person to act as a “reliable haven of safety,” echoing the role of the attachment figure in times of need. In other words, the attachment figure in times of need. In other words, the attachment figure provides a “secure base” from which to explore the world, understanding of this interaction, specific hypotheses are not projected, but we expect to see some differences between attachment to mother and father. The second objective is to examine similarities within mother and adolescent ratings of one another and father and adolescent ratings of one another. Again, because this is a new topic among attachment researchers, this study will explore relationships without projecting specific hypotheses.

Method

This study is part of a larger project examining family narratives and adolescent identity. Only those methods related to the present project will be discussed.

Sample

Twenty-four families with either an 8th grade or a 10th grade adolescent participated in the study. Families were recruited through various sources such as schools and religious groups, as well as with flyers dispersed to both participating families and around a university. Most families had other children living in the household as well, typically between one and four total children. Thirteen families identified themselves as Caucasian, 10 as African American, and one as Hispanic. Nineteen families described their family structure as traditional (both biological parents living in the home) and three as blended (one biological parent and one non-biological parent living in the home). Out of the 24 families who participated, two of the adolescents were adopted, both in infancy. Ten adolescents were female (mean age 14.6) and 14 were male (mean age 14.5). At the time of the study, ten adolescents were in 8th grade (mean age 13.4) and 14 were in 10th grade (mean age 15.4). All parents and adolescents gave signed consent as approved by the Emory University Institutional Review Board. For participating in the study, families were compensated $50, and adolescents were given two movie tickets and a $25 gift certificate.

Procedure

A researcher visited the family’s home on two separate occasions. Questionnaire packets were left for both the mother, father, and adolescent to complete separately. The mother, father, and adolescent ratings of one another. Again, because this is a new topic among attachment researchers, this study will explore relationships without projecting specific hypotheses. For each parent and adolescent, the Inventory of Parent and Peer Attachment-Revised (IPPA: Armsden & Greenberg, 1987) was completed. Each adolescent was asked to complete the IPPA, a self-report questionnaire that includes 25 items that were not projected, but we expect to see some differences between attachment to mother and father. The second objective is to examine similarities within mother and adolescent ratings of one another and father and adolescent ratings of one another. Again, because this is a new topic among attachment researchers, this study will explore relationships without projecting specific hypotheses.

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designed to measure adolescent attachment to each parent (mother and father, individually) and adolescent attachment to friends (peers). The measure assesses both positive and negative affective and cognitive dimensions related to attachment. As discussed in the introduction, the three dimensions used to measure attachment are communication, trust, and alienation. The adolescent is asked to complete each set of questions in relation to their mother, their father and their peers. Each parent was also asked to complete the complementary version of the same questionnaire to allow for an exploration of the parent’s attachment to their adolescent. Questions are answered on a 5-point Likert scale, ranging from “Almost Never or Never True” to “Almost Always or Always True.”

**Results**

Results are discussed in two sections. The first section examines how male and female adolescents rate their attachment to each of their parents, as well as how mothers and fathers rate their attachment to their adolescents. The second part examines the relations among attachment ratings that address the specific objectives of the study. Only results related to the present study are discussed.

**Differences Among Attachment Ratings**

Table 1 displays the mean ratings (and standard deviations) adolescents gave on each dimension to mother and father. To look at the differences between adolescent ratings of mother and father on all dimensions, a 2 x 2 x 3 x 4 Mixed-Model Analysis of Variance (ANOVA) was conducted for the variables of grade, gender, and person (mother to adolescent to father). Only results, specifically by person (F(2, 40) = 7.56, p < .01) and by dimension (F(2, 40) = 18.59, p < .001), and the total attachment score (F(2, 40) = 475.39, p < .001). In order to examine communication, alienation and total attachment score dimensions, follow-up paired-sample t-tests comparing ratings of pairs of attachment figures (see Table 1) were conducted. On the communication dimension, there was a significant difference between mothers and fathers (t(23) = 2.27, p < .05), such that mothers were rated higher than fathers. On the alienation dimension there was a significant difference between adolescent ratings of mothers and fathers (t(23) = 2.85, p < .01), such that mothers were again rated higher than fathers. On the total attachment score, there was a significant difference between adolescent ratings of mothers and fathers (t(23) = 2.66, p < .05), such that mothers were also rated higher than fathers.

An additional question was whether adolescents as a group had higher or lower ratings on attachment dimensions than did mothers or fathers. In order to examine the consistency of ratings of attachment dimensions between adolescent-mother and adolescent-father pairs, a 2 x 2 x 2 ANOVA for the grade, gender and person variables, respectively, was conducted for each dimension. There were no significant differences on any of the dimensions of attachment between adolescent ratings of mother and mother ratings of adolescent (see Table 2). Likewise, there were no significant differences on the communication dimension between adolescent ratings of father and father ratings of adolescent (see Table 2). There was a three way interaction between person, gender and grade among the trust dimension (F(1.15) = 7.42, p < .05), alienation dimension (F(1.15) = 5.69, p < .10) and total attachment score (F(1.15) = 5.09, p < .05) dimensions between adolescent ratings of father and father ratings of adolescent (see Table 2). There was also a difference on the communication dimension (F(2, 40) = 17.10, p < .001), the alienation dimension (F(2, 40) = 18.59, p < .001), and the total attachment score (F(2, 40) = 475.39, p < .001). In order to examine communication, alienation and total attachment score dimensions, follow-up paired-sample t-tests comparing ratings of pairs of attachment figures (see Table 1) were conducted. On the communication dimension, there was a significant difference between mothers and fathers (t(23) = 2.27, p < .05), such that mothers were rated higher than fathers. On the alienation dimension there was a significant difference between adolescent ratings of mothers and fathers (t(23) = 2.85, p < .01), such that mothers were again rated higher than fathers. On the total attachment score, there was a significant difference between adolescent ratings of mothers and fathers (t(23) = 2.66, p < .05), such that mothers were also rated higher than fathers.

In order to examine individual differences among the attachment dimensions, Pearson’s r correlations were conducted. Table 3 displays the correlations among adolescent ratings of attachment to mother and adolescent ratings of attachment to father. There were significant positive relations between adolescent ratings of mother and father on the trust, communication, alienation and total attachment score dimensions.

Table 4 displays the similarity of adolescent and mother ratings of one another and father and adolescent ratings of one another. There was a significant positive correlation between mother and adolescent ratings of one another for the trust dimension as well as the total attachment score. There was a positive correlation approaching significance between adolescent and father ratings of one another for the communication dimension. All other dimensions for both pairs were not significantly related.

**Relations among attachment relationships**

Table 3. Correlations of adolescents’ ratings of attachment to mother and adolescent ratings of attachment to father by dimension of attachment.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Ratings by person</th>
<th>Mother and Father</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trust</td>
<td>0.46*</td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>0.429*</td>
<td></td>
</tr>
<tr>
<td>Alienation</td>
<td>0.50*</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.492</td>
<td></td>
</tr>
</tbody>
</table>

Table 4. Correlations of attachment ratings between mother and adolescent and father and adolescents by dimension of attachment.

<table>
<thead>
<tr>
<th>Dimensions</th>
<th>Ratings by Person</th>
<th>Mother and Adolescent</th>
<th>Father and Adolescent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trust</td>
<td>0.506*</td>
<td>0.11</td>
<td></td>
</tr>
<tr>
<td>Communication</td>
<td>0.248</td>
<td>0.349*</td>
<td></td>
</tr>
<tr>
<td>Alienation</td>
<td>0.184</td>
<td>0.261</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>0.488</td>
<td>0.379</td>
<td></td>
</tr>
</tbody>
</table>

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**Discussion**

The present study examined patterns of attachment during adolescence. Because adolescence is such a crucial point in development, it is imperative to understand how attachment relationships manifest is perceived in adolescence. Specifically, patterns in adolescent attachment to mothers and fathers were explored, as was similarity between parent and adolescent ratings of attachment to one another. Findings and interpretations for each question are discussed below, as well as the limitations of the study and implications for future research.

Adolescents showed different levels of attachment to their mothers and fathers; specifically, mothers were consistently rated higher than fathers on both the communication and alienation dimensions and on the total attachment score. Additionally, adolescents reported that, because of the level of availability, adolescents feel more comfortable and accurately. Interestingly, Laursen and Collins (2004) recently found that adolescents seek comfort from people who are most accessible. Supporting the argument by Collins and Repinsky (2004), at this point in development, mothers may be those in the family who are most easily accessible to satisfy the developmental needs of their adolescents. As previously found by Paterson, Field, and Pryor (2004), adolescents tend to rely on their mothers more than their fathers when in need of comfort and support. Therefore, present findings support the inferences that mothers may be more accessible to adolescents than fathers inside the home, an explanation for fathers being lower-rated.

Additionally, in general, mothers and adolescents rated one another similarly, whereas fathers and adolescents did not. Mothers and adolescents reported similar levels of attachment to one another, whereas fathers reported higher levels of perceived attachment to their adolescents than their adolescents did to them. This suggests that fathers perceive their relationships as stronger than their adolescents do, but that adolescents and mothers perceive the strength of their relationships more similarly and accurately. Interestingly, Laursen and Collins (2004) report that adolescents are more open to their mothers than their fathers on an emotional level. It may be that, because of the level of availability, adolescents feel more comfort and support from their mother. Mothers,
on the other hand, may be less available to satisfy their adolescents’ needs. An additional question addressed in the study was the similarity between adolescent and parental ratings of attachment to one another. Mothers and adolescents rated one another similarly on the trust dimension as well as the total attachment score, and fathers and adolescents rated one another similarly on the communication dimension. Lauren and Collins (2004) argue that more time is spent with mothers than with fathers and, more importantly, that there is more sharing of emotions with mothers as well. Other researchers have also found relations between quality of the mother-adolescent relationship and attachment security (Allen et al., 2003), and perceived maternal availability (Leiberman, Doyle, & Markiewicz, 1999). In these stronger relationships, there may also be higher levels of trust between mothers and their adolescents. Additionally, Benoit and Parker (1994) have found that secure parents tend to have secure children. It may be that mothers who are available, specifically emotionally available, are those who have children who perceive them to be this way; these children may also feel as if they are emotionally available to their mothers. 

Mothers of adolescents are consistently rated higher than fathers on both the communication and alienation dimensions and on the total attachment score. 

Whereas findings indicate that relations within the mother-infant and parent-child relationship is related to infant and child attachment security (e.g., Freitag, Belsky, Grossmann, Grossmann, & Scheuerer, 2004; Kreppner, 2002), it may be that through these interactions as a whole, as well as separate dyads, comes the creation of a household “climate.” These findings contribute to the importance of furthering research on the whole family in addition to its separate members and dyads when trying to explain how families interact and impact one another’s social and psychological development. Since there is no previous research on the topic of concordance in attachment relationships in adolescence, findings from this study are all speculative. Regarding adolescent ratings of attachment between mothers and fathers, findings suggest that there is concordance on how adolescents feel about their relationship with their mother and their father. Adolescents who give high ratings to their relationships with their mothers also rate their fathers high on trust, communication, and alienation dimensions as well as on the total attachment score. This relation between adolescent ratings of mothers and fathers may suggest that adolescents generalize across parental relationships and may view parental relationships as a “unit.” This idea supports the basic tenet of attachment theory, suggesting the development of an internal working model of attachment (Bowby, 1969). Lastly, fathers and adolescents perceived one another similarly on the communication dimension, something unexpected based on prior research. Possible reasons for this finding are not immediately apparent, particularly given the findings that fathers and adolescents, generally, did not rate their relationships similarly. Drawing upon findings by Kreppner and Ulrich (1998), suggesting that variability in communication among fathers and adolescents is related to security of the attachment relationship, it may be that communication within the father-adolescent pair helps foster a stronger relationship. Furthermore, Leaper (2000) suggests that parental involvement is related to adolescent attachment (Williams & Kelly, 2005); it may be the communication and trust in the parent-adolescent relationship that allows for adolescents to feel close to their parents and, just as importantly, to allow for parents to feel close to their adolescents. 

There was concordance between mother and father ratings of their adolescents’ relationship. The concordance between all three dyadic relationship measures (mother-adolescent, father-adolescent and mother-father) indicates that these attachment relationships may be dependent on one another. Other researchers have argued that families interact as a whole, creating a family style of interacting (Arnold, Pratt, & Hicks, 2004; Kreppner, 2002). It may be that through these interactions as a whole family, as well as separate dyads, comes the creation of a household “climate.” These findings contribute to the importance of furthering research on the whole family in addition to its separate members and dyads when trying to explain how families interact and impact one another’s social and psychological development. 


In 1989, the labor union Solidarity was poised to win an unprecedented victory against Communism in Poland. After a series of intense “roundtable talks,” Solidarity convinced the Soviet-backed government to allow semi-free elections in the summer of 1989. However, the United States became worried that a total electoral defeat of the Communists would invite a violent crackdown that would inhibit further democratization. According to declassified communications sent between the U.S. embassy in Warsaw and Washington in 1989, America began attempting to slow the revolution that it had originally encouraged. However, even though Solidarity and the United States almost found themselves at cross-purposes, the democratization of Poland represents a dramatic victory for American diplomacy and then-U.S. Ambassador to Poland John Davis.

When Promoting Democracy is Not the Best Way to Promote Democracy

Nathan Vigil is a doctoral candidate and Instructor in the History Department at Emory University. He received his bachelor's degree from the University of California, Berkeley and his master's degree from Emory University, both in American History. Nathan has published with Cambridge University Press and Routledge Press, received funding from Princeton University and the US Department of Education, and presented papers at Oxford University and the annual meetings of the American Historical Association and the Society for Historians of American Foreign Relations. This semester, he is a Dean's Teaching Fellow at Emory and is teaching a new course on the International History of the End of the Cold War.

**faculty mentor**

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**key terms**

**Solidarity**: A Polish trade union formed during a series of labor strikes in 1980. It survived almost a decade of martial law and repression and became the leading political party after the 1989 elections.

**John Davis**: The American ambassador stationed in Poland from 1983 to 1990. A popular and successful diplomat, Davis oversaw a series of negotiations that brought about democratic reform in Poland. Włodzich Jaruzelski: Polish general and leader of the Communist Party in Poland. He instituted martial law in 1981 in hopes of crushing Solidarity and was president during the 1989 elections that brought Solidarity to power.

**Czeslaw Kiszczak**: Polish general who became the country’s last Communist prime minister in the late 1980s.

**Tadeusz Mazowiecki**: Polish politician who became the first non-Communist prime minister in 1989.

**Lech Walesa**: A dock worker and labor union leader who helped form Solidarity in 1980. He was elected president of Poland in 1990.
When George H. W. Bush became president in 1988, he claimed that the Soviets’ response to self-determination efforts in Eastern Europe would be the central test of Mikhail Gorbachev’s “new thinking” about foreign policy. Unfortunately, the push for democracy by the non-communist trade union, Solidarity, was going well in Poland — too well. American policymakers in Warsaw and Washington feared that rapid democratization and a total electoral defeat of the Communists in Poland would lead to a violent crackdown by the Soviets. In that sense, a democratic victory would actually inhibit political liberalization. According to declassified communications sent between the U.S. embassy in Warsaw and Washington in 1989, America began attempting to slow the revolution that it had originally encouraged. However, even though Solidarity and the United States almost found themselves at cross-purposes, the democratization of Poland represents a dramatic victory for American diplomacy and then-U.S. Ambassador to Poland John Davis.

By the beginning of 1989, America’s three major diplomatic objectives in Poland had been accomplished. Martial law was lifted, political prisoners were released and a dialogue was held between Solidarity, the Communist Party and the Catholic Church. According to a July 7, 2000 interview with Assistant Secretary of State Thomas W. Simons, the situation was progressing better than expected. At the beginning of 1989, Poland began to prepare for its first democratic elections after Communist rule. These elections were expected to be a huge victory for Solidarity. In a cable dated April 19, Ambassador Davis called 1989 “The Year of Solidarity.”

However, elections appeared to be coming too quickly to maintain stability. Ambassador John Davis titled his June 2 cable “Solidarity’s Coming Victory: Big or Too Big?” In the cable he wrote, “The stakes have become enormous, perhaps greater than most perceive.” American analysts did not view Solidarity’s unstoppable victory as altogether legitimate, since it was a “shaper[ed]n sharp defensive reaction from the regime.” It was a “spector of utter catastrophe.” Davis speculated that a Solidarity victory could create chaos within the Communist Party, leading to a heavy-handed crackdown from the Soviets, or at the very least chaos within Poland.

Legislative elections conformed to predictions of a total rout by Solidarity. According to a June 19, 1989, New York Times article, on June 4 Solidarity won 160 of a possible 161 seats in the Sejm (the lower of the two legislative bodies) and 92 out of 100 available seats in the Senate. The elections passed without incident, and Davis wrote in a June 6 cable that Solidarity is “making every effort to soften this blow.” However, the party was becoming bolder (Davis wrote that “warnings about excessive success...fell on deaf ears outside Solidarity’s inner circle”), setting the stage for showdowns over the presidential election and the creation of a new government.

Driven by these fears, American analysts began to ponder ways to keep in power Communist leader Wojciech Jaruzelski, then the Chairman of the Council of State. Davis ominously opened his June 23 cable by writing, “Most Solidarity leaders are apparently convinced that Jaruzelski must be elected president if the country is to avoid civil war.” According to Davis, there was a spoken agreement during the Round Table Talks to allow Jaruzelski to serve as president for the next six years as long as free elections are held for all legislative positions. However, as it gained more power, Solidarity became reluctant to cast votes for Jaruzelski. Since at that point Polish presidents were chosen by the legislature, Davis worked with Solidarity to devise a plan where most party members would skip the voting, leaving people from other parties to make quorum while not forcing Solidarity to vote for Jaruzelski. The plan worked, and he was elected by a one-vote margin on July 19.

However, American fears were further stoked by Davis’s August 11 meeting with General Czestaw Kiszczak, then the Polish prime minister. Kiszczak warned that Solidarity’s plan to form a government with the Democratic and Peasant parties (previously allies of the Communist Party) was “unacceptable to the senior officials of the army and police and to the Czechs, East Germans and Soviets.”

The meeting worried Davis, especially in light of the Chinese Army’s crackdown in Tiananmen Square just two months earlier. The bloody results of the protests in China had created concern for similar democratic movements elsewhere in the world. Wrote Davis, “A repetition of the Chinese events would be a true disaster in Poland.” Also, even if no military action was taken, Soviet economic sanctions could have a disastrous affect on Poland. According to Soviet Deputy Foreign Minister Ivan Abromov, Poland imported 85 percent of its oil, 65 percent of its iron ore and 50 percent of its cotton from the Soviet Union. In addition, Kiszczak estimated that only seven percent of the Polish economy was actually competitive on a global level at that time. The final move — a dialogue between Davis and Kiszczak — led to a consensus in which Gorbachev could be deposed as general secretary, leading to the suspension of his more liberal policies regarding democratization in Eastern Europe and the installation of more hostile Communist leadership. Kiszczak warned that this was a possibility because the economic situation in the Soviet Union was deteriorating, which caused some Soviets to call Gorbachev’s reforms a failure. Worried that the Polish political system could devolve, Washington responded to Davis’s cable the following day, urging him to encourage dialogue between Kiszczak and Solidarity leader Lech Walesa.

However, some of these fears appeared to have been overstated in Davis’s cables, and the problems he predicted never materialized. On August 16, the U.S. Ambassador to Moscow, Jack Matlock, wrote that the Soviets “have been restrained in response to democratization in Poland. The Soviet press alternated between harsh criticism, factual reporting and even ‘moderate praise’ when discussing Solidarity’s progress.”

In addition, journalists in the region believed “that the Soviets will acquiesce to a Solidarity-led government.” Matlock ended his cable by writing that “although Solidarity may be a bitter pill to swallow, our best guess is that the Soviets will do so, if it comes to that, after much gagging and gulping.” The Soviets’ real goal in Poland was not to maintain a Communist government, but to keep the country a member of the Warsaw Pact, something Solidarity pledged to do. Therefore, Matlock concluded that a Solidarity government would damage the Politburo’s pride but ultimately satisfy its needs.

Solidarity moved forward with its plan to form a government. According to Davis’s August 19 cable, prominent Polish politician Bronislaw Geremek told him that Solidarity member Tadeusz Mazowiecki was set to become prime minister. Davis wrote to Matlock that there would be at least five Communists chosen as ministers. Davis and Geremek agreed that they could not allow a situation like Afghanistan, where attempts to remove all members of the former regime resulted in bloody infighting.

The legislature chose Mazowiecki as prime minister on August 24, 1989. On that day, Davis wrote that his objectives had been completed and he needed fewer instructions. According to the Secretary of State’s response, the “next task [was] to promote and ensure the realization of economic prosperity in Poland, to include stable growth, full employment, low inflation, high productivity and a Mercedes (or equivalent) in every garage.” The response reflected both a major shift in 11 John Matlock, “If Solidarity Takes Charge, What Will the Soviets Do?” August 16, 1989. Accessed online on February 12, 2008 at http://www.gwu.edu/~nsarchiv/NSAEBB/NSAEBB42
American policy and the difficulties that Poland would face as it adapted to a new government and economy.

The most noteworthy aspect of Davis’ cables is that they reflect a wide-ranging engagement with the Polish people. Davis met with all of the political parties in Poland, both formally and informally. It appears that all the major players in Poland genuinely trusted Davis and saw him as a resource and an ally. A Dec. 21, 1989 article in *The New York Times* supports this thesis. Although Communist officials were ordered not to meet with him when he arrived in Poland in 1983, Davis earned the respect of both hardliners and reformers in Poland. “Not flamboyant, but a communicator by smiles and nods who asks more questions than he gives answers. Mr. Davis has gained unusual access to all sides in the Polish conflict,” reporter John Tagliabue wrote in the article. Tagliabue also quotes Solidarity spokesperson Janusz Onyszkiewicz as saying, “He somehow managed to reconcile two things that sometimes appear irreconcilable; to keep the eye of the outside observer but also to think the way Poles think.”

Davis’ cables also present a remarkable window into a time period where America was respected as a source of political and cultural inspiration. On June 27, 1989 Davis wrote that when George Bush arrives for his first presidential visit, he will “find himself in the center of the world’s most pro-American country.” Davis goes on to detail how the Poles identify with U.S. values regarding democracy and individual freedom. American policy in Poland was effective because it was able to capitalize on this organic relationship between the two countries. The United States did not have to impose its will on Poland. Rather, it was able to latch onto a domestic movement that also served its international interests.

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In 1989 the United States almost found itself at odds with the revolution it was originally supporting. The passionate Solidarity opposition movement came close to undermining Polish democracy by pushing for the most power it could obtain, inviting a Soviet crackdown. However, careful diplomacy and compromises were successful in remedying the situation. Davis himself played a crucial role at the 11th hour by helping get Jaruzelski elected president on July 19. And most of all, the United States and Solidarity were simply fortunate enough to find itself on the right side of history. The Soviet Union had finally reached a point where it could no longer restrain democratic movements in Eastern Europe, and both Solidarity and the United States were able to exploit the opportunity. 17

Tagliabue, “Upheaval in the East; U.S. Ambassador No Longer Keeps His Poles Apart.”

This study will determine the effects of receptive and active music therapy as a potential long-term rehabilitation method through promoting neural plasticity in frontal and emotional areas of the brain that are impaired by schizophrenia. This will be studied via a randomized clinical trial in which up to 120 in-patients with schizophrenia will be selected for the study. These patients will be separated into four different treatment groups: 1) no additional therapy, 2) active music therapy, 3) receptive music therapy, and 4) active and receptive music therapy. The results of this proposed study would enable a more long-term study to determine the effects of music therapy on permanently rewiring the brain. This study would be a jumping off point for developing a more effective path of treatment for schizophrenia.

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Neuroplasticity has been considered one of the “most extraordinary discoveries of the twentieth century” [1]. It is the ability of the brain to rewire its functional and structural organization in response to experience and stimulation. Contrary to beliefs held several decades ago, the process of neuroplasticity occurs throughout a lifetime along with limited neurogenesis, which is the formation of new neurons. The primary mechanism of brain plasticity is the strengthening of synaptic connections in certain circuits while gradually eliminating weaker and less frequently used neurons; this is known as synaptic pruning.

Researchers have traditionally defined four main conditions which promote brain plasticity: developmental, activity-dependent, learning and memory, and injury-induced [2]. Developmental plasticity takes place during the first few years of life: the brain undergoes extensive synaptic pruning in order to adjust to processing external sensory information. As changes occur in the input of sensory information, the neural circuits will adjust correspondingly with activity-dependent plasticity. The processes of learning and memory also induce significant brain plasticity by altering behavioral patterns. Finally, after brain damage, injury-induced plasticity occurs as the brain’s natural mechanism to repair itself. Much research is currently being carried out in a variety of neurorehabilitation techniques to promote cortical plasticity, which will also be a focus of this study [3].

A common example of a rehabilitation mechanism is repetition of motor actions to improve function in patients who have suffered a stroke or a spinal cord injury. Remarkably, it has been found that over a long period of time, concentrated thought can also affect this property of the brain. Frequent use of the certain areas of the brain results in neuroplasticity as neural circuit synapses are strengthened, leading to changes in physical brain structure. An oft-cited study revealed that Buddhist monks who had undergone intensive meditation training showed stronger connections between the frontal and emotional regions of the brain.

Synapses are strengthened, leading to changes in physical property of the brain. Frequent use of the certain areas of the brain produces sustained gamma wave activity and showed higher activity levels in the right insula and caudate as well as stronger connections between the frontal and emotional regions [4]. Gamma waves are a pattern of brain waves (approximately 30-80 Hz) associated with higher cognitive functions, such as perception and recognition of new insights [5].

Results of recent studies also support the role of music in promoting gamma wave activity. A frequently studied phenomenon is the Mozart effect. Research participants listened to Mozart pieces and then performed spatial rotational tasks. The participants who had listened to Mozart had higher scores than the control group participants, who did not listen to music [6]. This is related to the theory that classical music, including Mozart’s music, contains frequencies that activate areas of the brain related to higher cognitive function. If music can promote gamma wave activity in a manner similar to the Buddhist monks’ meditation techniques, it is likely that music can affect neuroplasticity. This idea is supported by studies which have found that children who begin music training at a young age have stronger cortical connections than peers with limited musical background as measured by magnetoencephalography (MEG); this effect persisted as their brains matured [7].

Music is processed by the brain in milliseconds. When a person hears music, the sounds are broken down into various frequencies, which are processed by different areas of the brain, primarily but not limited to the auditory cortex, which is part of the temporal lobe [8]. The frontal and parietal lobes are also involved in the processing of sound. Impairments of the primary auditory cortex can lead to difficulties in processing melodies, harmonic chords, and speech. The auditory cortex is strongly linked to the frontal cortex, which processes higher cognitive functions, and parts of the brain that are involved in emotion, including the amygdala. Different tones have the ability to activate areas; for example, “pleasant sounds” such as a perfect fifth interval of Middle C and G minor key sounds activate areas, while “dissonant sounds” such as a perfect fifth interval of Middle C and A sharp sound activate the reward system [9]. Dissonant chords activate another area: the right parahippocampal gyrus.

Interestingly, music leads to coupling between the auditory and the premotor cortices [10]. The ratio of right to left activity is correlated with the extent to which the subjects were producing music or listening to music. When listening to music, the level of activity in the auditory cortex was slightly higher than the level of activity during music production. However, the level of activity in the premotor cortex was significantly higher during music production compared to music perception [10]. Much is still unknown about the specifics of how music produces music, but through imaging techniques such as positron emission tomography (PET), scientists have made great advancements in research.

However, it is understood that some of the areas affected by music tend to be abnormal in schizophrenic patients. Under PET imaging, the prefrontal cortex shows decreased function; this circuit includes the orbitofrontal area mentioned previously [11]. Schizophrenia is a psychiatric disorder with unknown causes; it is characterized by impairment in the perception of reality, auditory hallucinations, disorganized speech and thinking, and reduced social cognition, including self-expression abilities [12]. Neurological symptoms include increased dopamine activity, loss of grey matter, and impairment of cognition in frontal lobes [12]. Structurally, the hippocampus, amygdala, and Heschl’s Gyrus, containing the primary auditory cortex, are diminished. Standard treatment for schizophrenia is antipsychotic drugs, which maintain neurotransmitter levels, and general psychotherapy.

Recently, two teams of scientists have found positive effects of expressive music therapy on schizophrenic patients. Expressive therapy, known as creative arts therapy, includes art, dance, drama, music, writing, and play. Music therapy is loosely defined as any form of psychotherapy that includes music, including the creation of music to improve motor functions, and list listening, which is part of the research in Alzheimer’s patients, and to reduce stress in patients with acute and chronic pain. In one study, in addition to general care, schizophrenic patients who were enrolled in a twelve-week study attended sessions with certified music therapists to co-create music that corresponded to the patients’ emotional states. The study found a trend towards decreased general symptoms of schizophrenia [13]. The second study found similar results; the researchers noted positive effects on general mental state and social functioning, but they also pointed out that the results depended on the number of sessions. The author concluded that additional research should be done to determine the “dose-effect relationship” and the long-term effects [14]. Both of these studies focused heavily on the cognitive effects of music therapy; they were involved in the production of music, including improvisation and reproduction. In contrast, receptive music therapy focuses primarily on listening to pieces selected or played by the therapist [14]. Because the perception and the production of music activates areas of the brain to slightly different levels, the effects of receptive and active therapy will not be the same. Thus, the brain opens up to many possibilities for advancements in rehabilitation. Referring back to the studies of schizophrenic patients, there is much research to be done, including investigating the long-term effects of receptive music therapy.

Question and Hypothesis

Previous studies have confirmed the potential of the Mozart effect, the effects of music on the brain activity, the neurological differences between the production and perception of music, the adaptive ability of the brain through neuroplasticity, and the effects of active music therapy as a short-term treatment method. The objective is now to determine the effects of receptive and active music therapy as a potential long-term rehabilitation method through promoting neural plasticity in frontal and emotional areas of the brain that are impaired by schizophrenia. The anticipated result is that these areas will be strengthened after intensive and ongoing therapy. I hypothesize that a mixture of active and receptive music therapy will be more effective than active or receptive therapy alone. The null hypothesis is that the varying types of music therapy will produce similar results. The control for this study will be traditional schizophrenia treatments of psychotherapy and antipsychotic medication.

Research Design

The basis of this experiment is a controlled and randomized clinical trial. Up to 120 in-patients with schizophrenia will be selected as participants. In-patients are preferable for this study because it will allow for the researchers to minimize variation in their daily activities. The patients must meet the following criteria: over age 18, have a primary diagnosis of schizophrenia, have a capable grasp of English, and not be inhibited by dementia in daily activities [15]. At the beginning of the research, the patients will undergo a T1-weighted, proton density, and T2-weighted magnetic resonance imaging (MRI) procedure as well as being assessed with the Positive And Negative Syndrome Scale (PANSS), which is the standard medical assessment tool used by psychotherapists.

Patients will then be randomly assigned to one of four groups (Fig 1). All groups will receive standard care which includes nursing and access to social and recreational activities. Due to ethical reasons, patients will continue with traditional therapy; this refers to medication and psychotherapy sessions that were previously prescribed. The primary control group, which includes music therapy, any psychotherapy which includes music, including the creation of music and listening to familiar songs to trigger memories in Alzheimer’s patients.
which will be referred to as Group 1, will not receive any additional therapy over this trial. The other three groups will receive some form of music therapy. Over a course of twelve weeks, these therapy sessions will take place twice a week for up to 45 minutes per session. All sessions will be digitally recorded. Group 2 will receive active music therapy. In these sessions, patients will be provided with musical instruments and encouraged to express themselves according to their mood [13]. Therapists will listen to the music and accompany the patient as they see fit. The focus of these sessions will be on self-expression, creation, and innovation. Song-writing and lyric-writing can be incorporated at the discretion of the therapist. All music involved with this patient group will be created or used by therapists in this group will be previously created sounds. Depending on the piece of music, patients will be compared with previous studies.

At the end of the study, randomly selected recordings will be assessed to ensure the quality of the sessions and adherence to the assigned treatment style. All patients will be assessed again using PANSS. Increases and decreases in symptoms will be highly significant. Another MRI scan will be employed to determine if any changes in brain functionality are present. Particular attention will be paid to the MRI scans of patients who had notable increases or decreases in symptoms based on the PANSS.

Potential Pitfalls and Conclusions

As with most research endeavors, the structure of the study is limited by time and funding availability. Other concerns include the length of the study, access to patients, and availability of qualified music therapists. Some previous studies have shown application of the results with the PANSS scan in a twelve-week study, this length still may not be long enough for the purposes of this study because the goal is to distinguish between the subtle differences in the effects of variations of music therapy. Twelve weeks, which includes up to twenty-four sessions of music therapy, may not be enough time to affect noticeable change in brain functionality and activity as measured by the MRI scans. A certain amount of variation is always expected with working with patients, especially patients with a psychiatric disorder such as schizophrenia. The symptoms and the degree of severity of schizophrenia are unique to each patient. A large number of patients must be enrolled into the study to minimize statistical variation and standardize groups. However, it may be difficult to find enough consenting patients to meet the number specified by the study (120 patients). There may also be variation in the techniques employed by the music therapists (e.g., individual therapists may focus on some topics more than others). In addition, many music therapists are not accustomed to working with schizophrenic patients. In order to reduce these sources of variation, music therapists will undergo training before the start of the trials to normalize their techniques and initial approaches to the sessions. In order to become aware of the nuances of this disorder, therapists will also preliminarily consult with other mental health specialists who have extensive experience with schizophrenic patients. Access to these specialists will also be granted as needed throughout the duration of the trial.

The results of this study will only be a stepping stone to developing a more effective path of treatment for schizophrenia. Each patient will undoubtedly respond differently and to varying levels of therapy. Should one treatment path be remarkably more effective at reducing symptoms than others, the ethical option of “cutting the blind” is available to the researchers to ensure that all patients receive the best possible care. This option terminates the study before schedule and allows all patients in the study to receive the most effective treatment method. After the study is concluded and results are analyzed, a more long-term study should be employed to determine the effects of music therapy on permanently rewiring the brain, rather than a short-term way of promoting brain activity in limited areas. The effects of gamma wave activity due to music have much research potential and this study could provide a small amount of additional understanding for future researchers to build upon. From a medical perspective, the implications of this study could lead to an improved quality of life for many schizophrenic patients by increasing their abilities of self-expression and communication with their community.

References


Identity is as an individually constructed concept that is defined in relation to one’s society (Hogg 259). Contemporary Muslims in Iran struggle in defining identity because the Iranian society is a mix of incompatible Western ideas of modernity and traditional Islamic worldviews. Stories of Iranian individuals reveal their struggles to understand religion in the context of contemporary society. Some Muslims have rejected Western ideals for the Islamic religion; others attempt to reconcile the two; and still others choose Western ideas of modernity over religion. In the mélange of past traditions and new worldviews, Iranian Muslims must decide what to accept, what to borrow, and what to reject in the formation of their own, unique Muslim identity.

Globalization challenges the prescribed societal roles in previously Islamic countries of the Middle East, and citizens must think about what it means to be a contemporary Muslim.

“an increasingly strong interdependence between large parts of the world” (Meuleman 2). Globalization affects Islam by increasing the interaction between previously isolated Islamic communities and contributing to the development of a universal Islam (2). It results in a Muslim attempt to define and achieve a global Islamic community in the context of a secularized, modern-day civilization.

Globalization is largely affected by Western ideas of modernity, or what the west—which has more force and reach a wider audience than those from other areas of the world—defines as “modern” in the 21st century (Ahmed 3). Western concepts of modernity permeate every area of life, from technology to fashion, from business practices to notions of freedom and justice. These Western ideas disseminated through globalization pose a problem in countries where historically, “Islam provide[s] a divinely sanctioned political and social order and a coherent worldview, which [gives] direction and meaning to Muslims both as individuals and as a community” (Donohue 5). Globalization challenges the prescribed societal roles in previously Islamic countries of the Middle East, and citizens must think about what it means to be a contemporary Muslim as they construct and reconstruct their identities.

Methodology

In my analysis of Muslims and their quest for identity, I have chosen to focus on the people of Iran. Iran transformed from a monarchy to a republic in 1979. This “overthrow of the monarchy in Iran was not merely a revolution in name, …[but] was designed to achieve a complete revolution in all spheres of life” (Menashri 1). “The revolution was intended to provide a fundamental cure, based on Islamic doctrine and revolutionary politics, for the ideological, social, and economic malaise that has plagued Iranian society in modern times” (1-2). In the years following the Revolution, Iran’s domestic and foreign policy was dominated by the war with Iraq. In the 1990’s, major political unrest continually plagued Iran. The newly established Iranian government struggled between the acceptance of Western ideals and the rejection of them in an attempt to create a separate, Islamic nation free of foreign influence. The country also faced economic instability and class stratification that has caused discontent and rioting among the Iranian people. Iran today is described as a place of shifting lines: “the lines may shift in different circumstances at different times of the day or year,” and such instability forces Iranians to make day-to-day decisions based on the political and economic situation at a particular moment in time (Sciolino 32). This forces Iranians to keep their country’s present situation at the forefront of their minds. Moreover, Iran is a Republic, the “world’s only modern theocracy,” and a place were much intellectual exchange about the relationship between religion and politics occurs (5).

Iran’s economic and political instability creates an atmosphere for intellectually stimulating dialogue. The theocratic government necessitates discussion about the role of religion in politics. Iran’s political atmosphere allows for relative freedom in discourse. For these reasons, I will be focusing on the Iranian peoples in my exploration of Muslim identity.

Addressing the question of Muslim identity

Contemporary Muslims are faced with the problem of how to be a faithful Muslim in today’s society. Attempts to address this problem begin with Muslim leaders and scholars. A group of right-winged, conservative scholars feel that today’s Muslims aren’t as good as past Muslims: “How absurd it would be for the present-day Muslims to desire that they should get for the 5% work they do, the same reward as their illustrious forbearers who performed their 100% work…” (Donohue 45).
They argue for a return to original Islamic doctrine and associate Islamic civilization's previous success with religious piety and hard work. Here, the success of the Islamic civilization refers to a period of about thirteen centuries in which Islam saw a period of growth and expansion through the Abbasid dynasty (750–945 AD): “Emerging from a remote frontier province, a coalition of the empire's underdogs, Arabs and non-Arabs [who are] marginalized and discriminated against…managed to overwhelm the imperial armed forces and…unset the long-reigning dynasty” (Menashi 43). These glorious conquests were followed by a period of Islamization, during which conquered peoples were “transformed in a steady process of ethnic and cultural assimilation from an Arab into a true Muslim entity” (Menashi 43). The Abbasid caliphate witnessed the high-water mark of the Islamic civilization, which flowered in almost every sphere or human endeavor – in politics and economics; in the sciences and the arts; in jurisprudence, philosophy and theology” (Ozigboh xiv). Islam was the adhesive that held the empire together and “provided the universality of political and social structure” (Menashi 43-4). For early Muslims, “self-understanding is based upon the Quran and the early, glorious history” (Donohue 2). Right-winged scholars argue that a modern Islamic society should be created through upholding a strict version of Islam, rejecting all Western ideals, and society should be created through analyzing religious texts and traditions using Western ways of thought. These two diverse approaches to interpreting Islam in the face of modernity illustrate the Iranian situation. Iran “is still engaged in a battle over interpretations of Islam” and “Iran’s leaders haven’t figured out what they want to be” (Menashi 39). This fluid interpretation of Islam in—in addition to the general political and economic instability within Iran—makes it difficult for Iranian Muslims to establish their identity. This difficulty is made the more excruciating because it is not always explicitly acknowledged by them” (Milani 58-9). When Abbas thinks of religion, he envisions mourning and fear; when he remembers religion was a part of his childhood, he recounts the stories of the cult of martyrs and the month of mourning. Even his best memories of Ramadan are plagued with memories of taziya, a Shia practice of mourning. He remembers men whipping their bare backs with a chain and chanting elegies to Hossein. He has vivid memories of a progression of “fifty men draped in white-stained shrouds, mace-wielding in hand, the blood-gushing foreheads” (64). These memories of Iran stay with Abbas as he leaves his country: his struggle with identity continues as he travels, and in his young age, he tries to reconcile what he has been taught during his youth with new ideas of the Western world. Abbas recounts a time when he was “painfully conflicted” during a massage because of the “puritanical and prudish implications about the body” that are a part of his past (68). Specifically, Abbas refers to how he was taught to guard the body from contamination from the naje, who are the unclean, or everyone other than Shiites (68). Abbas experienced firsthand the forces of globalization when he crossed national and cultural borders, and in America, he was overwhelmed by foreign thoughts and ideas. “This fluid interpretation can be seen through personal accounts of their lives.”

### Muslims and their Conflict of Identity

Identity construction is further compounded by a problem of self-deception: Bilgrami suggests the problem of identity “within the hearts of moderate Muslims is made the more excruciating because it is not always explicitly acknowledged by them” (Bilgrami 824). The following examples of individual Iranians’ struggles reveal the underlying identity crisis that exists regardless of whether they acknowledge its existence. Taghi Aghaei is a businessman in Iran. During his youth, he fought in the Iran-Iraq war and lost his right hand in battle (Sciolino 186). Taghi demonstrated a strong faith in religion while he was in the army. He says, “As a believer in God, when I was in the call to sacrifice, he taught himself to write with his left hand and reinvented. He wrote war propaganda pamphlets and taught recruits about the Koran” (187). After the war, Taghi studied hard and was accepted into a University, where he learned English (187). He excelled in class and was chosen to become a first-class tour guide, eventually rising in rank through multiple promotions and gaining rapid success (187). Today, Taghi is “a forward-thinking businessman” who has chosen to embrace new opportunities that have come with globalization (187). However, even as Taghi embraces Western ideals of modernity, he struggles with his Muslim identity: for example, at the time of the interview, Taghi’s tourism business suffered, and his struggle to reconcile the two for forging his identity as a contemporary Muslim. Abbas Milani’s life is very different from that of Taghi. Abbas lived in Iran until his late teens and subsequently immigrated to the States to study. When Abbas recalls his childhood, he remembers that “Shiism sacramentalizes all aspects of daily life.” Abbas reflects that “life in those years seemed like walking on egg shells beneath which gap the abyss of eternal suffering” (Milani 58-9). When Abbas thinks of religion, he envisions mourning and fear; when he remembers religion was a part of his childhood, he recounts the stories of the cult of martyrs and the month of mourning. Even his best memories of Ramadan are plagued with memories of tazia, a Shia practice of mourning. He remembers men whipping their bare backs with a chain and chanting elegies to Hossein. He has vivid memories of a progression of “fifty men draped in white-stained shrouds, mace-wielding in hand, the blood-gushing foreheads” (64). These memories of Iran stay with Abbas as he leaves his country: his struggle with identity continues as he travels, and in his young age, he tries to reconcile what he has been taught during his youth with new ideas of the Western world. Abbas recounts a time when he was “painfully conflicted” during a massage because of the “puritanical and prudish implications about the body” that are a part of his past (68). Specifically, Abbas refers to how he was taught to guard the body from contamination from the naje, who are the unclean, or everyone other than Shiites (68). Abbas experienced firsthand the forces of globalization when he crossed national and cultural borders, and in America, he was overwhelmed by foreign thoughts and ideas. “This fluid interpretation can be seen through personal accounts of their lives.”

### Muslims’ Attempts to Reconcile Religion and the Products of Globalization

In the midst of this confusion, Muslims have created different ways to piece together religion and the ideas of the modern world. Some have integrated Islamic doctrine and practices with the forces of globalization by applying religion to Western ideals; others have adopted Western ideals and cloaked it in Islamic doctrine. Some appear religious in their public life and embrace secular ideas in their private life. Some reject western ideas of modernity, and others reject religion. It is in these “answers” that people find peace and a way to deal with the clashing ideas in their society. They have found creative ways to integrate religion and Western ideas of economy—namely capitalism—in creative ways. In Iran, globalization of the economy puts Iranians in a very difficult situation: they must deal with “tension between the need for capitalist growth and the drive for piety” (Sciolino 324). One Muslim response is to integrate religion with capitalist ideas and allow the “drive for piety” to contribute to the prosperity of the country (324). A billboard advertisement for French perfume with the slogan, “Rush quickly to your prayers,” captures this attempt (324). This advertisement attempts to use religion to attract
people to their product. In another example, shops selling knockoffs of American jeans are located right down the street from one of Iran's holiest sites (44). In a similar way, the shops are taking advantage of religiosity and the large traffic flow around a shrine to sustain their business. In another situation, Faezeh, the daughter of one of Iran's most powerful clerics, tries to use the framework of Islam to keep women healthy (119). She advocates for women in sports with the argument that women are depressed without exercise, which leads to increased suicide rates, and depression and suicide are against Islamic beliefs (119). Like the previous examples, Faezeh uses religion to attract people to her capitalist ambitions. These examples demonstrate that one way Muslims have integrated their religious faith with modernization is by using religion to support their own capitalist ambitions.

There have been other attempts to reconcile religion with Western ideas of sexual relations. One Western practice the media presents is sex as a source of enjoyment that should be free of restrictions, with the underlying idea that promiscuity and sex before marriage are acceptable. This idea clashes with Islam's conservative religious beliefs. One Muslim response to promiscuity is to invent legitimate traditional marriage without any intention of a sigheh, or temporary marriage (Sciolino 126). The sigheh originated during the lifetime of the Prophet Mohammed as a way to give names to children born outside of marriage (126-7). During Mohammed's time, sigheh was practiced among pilgrims and others who need to plan ahead (127). Today, the sigheh ritual allows a man and a woman to get married “for as little as a few minutes or as long as ninety-nine years” and thus legalizes extramarital sex (127). The practice of sigheh is one example of how Muslims legitimize Western ideals by “wrapping it in an Islamic cloak” (128). A different example involves a forty-year-old woman who had similar ideas: she undertook “a fun project that would help other women and not violate the Islamic codes” (Sciolino 99). I saw some eyebrows rising as I stated this agonistic [and] I cannot understand the reasons [for] this childish strife… but I can understand the pains of one who tries hard to reject Western modernity and uphold fundamental ideas of his Muslim faith. He retains his religious fervor by watching films about soldiers preparing for battle and reminds himself daily of the gloriousness of martyrdom by working at the martyr's cemetery. Hamid strengthens his identity as a Muslim through such reminders of the past and memories of the glorious ambitions of Islam. Hamid's Muslim identity is thus forged through embracing Islam and rejecting the products of globalization.

Conclusion

For many centuries, Islam and its teachings formed the core of law and doctrine, setting the standard for codes of behavior in Muslim civilizations. During centuries in isolation, Islamic civilizations saw brief but glorious periods of success; however, globalization dramatically shifted the rules by introducing new worldviews that conflict with these original Islamic codes of law. This new flow of Western thought and practice challenges Muslims to re-evaluate their religion. In the past few decades, Iran has reacted in that manner. New regimes have come into power, but each regime advocated a different way interpreting religion in the context of globalization. This has resulted in constant political unrest and economic instability. Individual Muslims in Iran seek to reconcile religion and the products of globalization. Struggles in identity construction in the face of general confusion are reflected in the intimate stories of Iran's lives discussed above.

A Muslim's story reflects the struggle in identity construction in a variety of ways and to varying degrees. Muslims respond to conflicting ideas of modernity and tradition by rejecting one for the other, integrating the two in creative ways, or portraying religiosity in traditional settings. Many Muslims seek to understand how religion integrates with modernity, and depression and suicide are against Islamic beliefs (128). A different example involves a forty-year-old woman who had similar ideas: she undertook “a fun project that would help other women and not violate the Islamic codes” (Sciolino 99). I saw some eyebrows rising as I stated this agonistic [and] I cannot understand the reasons [for] this childish strife… but I can understand the pains

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Argentina has the highest life expectancy of all Latin American countries and other countries of similar per capita income. Only 17 countries spend more on healthcare than Argentina, but its healthcare system has been ranked 75th overall [1]. Only after understanding the actual state of the entire system is it possible to form solutions to problems. Thus this study can be used as a basis through which to guide reforms in public policy.

Introduction

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faculty mentor

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Public Sector

The public sector is a network of free hospitals and health clinics that offer services to everyone. The national Minister of Health oversees the entire system, but the provincial ministers run most operations. The provinces and Federal District account for 74.2% of government health spending (Figure 1) [2].

Figure 1: Public Sector Spending by Jurisdiction (1995)

<table>
<thead>
<tr>
<th>Jurisdiction</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>National</td>
<td>35.6%</td>
</tr>
<tr>
<td>Provincial (including Federal District)</td>
<td>47.3%</td>
</tr>
<tr>
<td>Municipal</td>
<td>17.1%</td>
</tr>
</tbody>
</table>

Belmartino

Argentina has high-quality emergency services with all ambulances containing doctors. Still the public hospitals are greatly under-funded. There is a plethora of well-trained doctors, but the equipment they use is old and scarce, and they lack beds, medication and supplies. The public sector is designed mostly for those without medical insurance, yet it also treats many insured patients.

The public sector has more providers with better services) than others. Obras sociales have more money (and some insurance groups for all private sector workers who receive the necessary medical coverage.

Obras Sociales:

Obras sociales are the heart of the Argentine healthcare system and require payments from workers and employers. 50.2% of Argentines have medical insurance through obras sociales (Figure 2). Workers and employers pay a certain percentage of the workers' salary to their respective obra social which then pays a middle group of managers who pay providers. Each obra social has a group of specific healthcare providers who its members may see for medical care [2].

![Hospital 'Dr. Csene Argerich' in Buenos Aires](image)

The obras sociales began with Eva Perón between 1945 and 1955. In 1970 they became obligatory contributions by workers and employers which divided workers' insurance by their occupation [1]. Therefore all the taxi drivers pay to the same obra social and receive that coverage, while teachers, bankers, hospital workers and so on would all have their own obra social. Some obras sociales have more money (and have more providers with better services) than others. This discrepancy creates another injustice in the system. Since the 1990s this problem has been reduced with deregulation of obras sociales [2]. Now many workers are no longer limited to their syndicate's obra social and can choose between other occupations' obras sociales. Nevertheless, some unions' obras sociales remain exclusive and provincial public workers are limited to their provincial obra social.

There are over 300 obras sociales that are regulated nationally by the Superintendence of Heath Insurance which focus on specific programs (i.e. – the Program for Mothers-Infants and Nutrition or the Program against STDs and AIDS) [3]. The public sector also trains doctors, medical professionals and performs biomedical research [2].

![Figure 2: Distribution of Private Medical Companies (1998)](image)

The National Obras Sociales comprise the medical insurance groups for all private sector workers who represent 42.2% of obras sociales beneficiaries (Figure 3). National Obras Sociales require a 3% income contribution by workers and a 5% contribution by employers [2]. This division of obras sociales has undergone deregulation, so beneficiaries can choose their insurance provider between most obras sociales. The resulting freedom in choosing an obra social is important in creating equality.

Most government employees contribute to their Provincial Obras Social, and this branch holds 29.4% of obras sociales beneficiaries (Figure 3). Each of the 21 provinces has its own Provincial Obras Social which requires a 3-5% contribution from workers and a 4-6% payment by employers [2]. The Provincial Obras Sociales are not deregulated, because there is only one payment per province. Provincial workers must therefore pay to their respective Provincial Obras Social and cannot receive the same benefits as other obras sociales. If desired and financially viable, these workers are allowed to become a double affiliate with private insurance [1].

The third branch, most commonly referred to as PAMI (Program of Integral Medical Assistance), serves as a huge obra social for all people 65 or older. PAMI was created in 1971 with the intention of providing healthcare to the entire retired population, and it has fulfilled with goal. PAMI covers basically every medical need for its beneficiaries. This obra social contains 22.4% of obras sociales beneficiaries or 11.6% of the overall population (Figure 3). 2% personal contributions and 3% employer contributions by all workers fund this obra social [2]. Additional government subsidies are common for emergency costs. Deregulation of obras sociales has given retirees the freedom to choose whatever obra social they want, but most of them still elect to work with PAMI. Many PAMI beneficiaries have an additional insurance through either the private sector or another obra social.

PAMI and all other obras sociales create a huge inefficiency in spending by having a third party of managers who pay and organize the providers and get paid by the obras sociales. Ideally the managers should create more efficiency by limiting costs and obtaining the best deals and packages from providers, but their existence does the opposite. The managers are businessmen. They work to make money, not to improve the healthcare of workers. They receive more money than is deserved, taking away from the funds contributed to providers [2]. Although PAMI is financially inefficient, it still saves people's lives by covering all medical needs.

Private Sector

The private sector contains a large and diverse group of insurance plans with ranges of services and prices. Private care is offered by many different companies with the goal of profit. It is optional and serves mostly the upper and upper-middle classes. Private medical care has new technology and comfortable accommodations.

Although it may provide its clients with a secure environment when the treatment is not complex, private insurance companies find loopholes in contracts to avoid paying for expensive procedures. In these cases, the patient must use an obra social or the public sector to receive the necessary medical coverage.

Private insurance in Argentina became popular in the 1990s. In 1999, 12.1% of Argentines held private insurance, and this industry has been growing (Figure 2).

![Greater Buenos Aires Area](image)

Belmartino
The lack of resources in the public system hinders this primary and emergency care to everyone. Unfortunately, the Argentine system attempts to provide optimal care to public patients. Resources per patient in the private sector versus that for the public system’s spending and owns 55.2% of Argentine medical institutions. Although the same doctors work in both sectors, the public hospitals lack funds to maintain and improve technology, to hire enough health professionals besides doctors (techs, nurses and other personnel), and to pay for beds, supplies and medications. For example, although the private sector only serves 11.2% of the population, it accounts for 41.5% of the entire system’s spending and owns 55.2% of Argentine medical establishments and 45.2% of the beds (Figures 5 & 6) [2, 4]. There is an incredible discrepancy between the private and public sectors, between different obras sociales, and between poor and wealthy regions of the country exhibit the government’s lack of fulfilling its duty of “promoting the well-being” for all people. The main problem is the lack of budgeted funds for public hospitals and clinics.

Despite these issues, the Argentine system provides a high level of care to many people. No one is excluded from the system which has knowledgeable doctors, relatively complex services and excellent emergency care.

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S since its inception in 1990, Summer Undergraduate Research at Emory (SURE) has served as an inspiration for hundreds of young people around the country. Funded principally by the Howard Hughes Medical Institute and run by Emory’s Center for Science Education, the program provides a ten-week opportunity for college students to work with research faculty at Emory on a dedicated project of their choice. For some students, this is an introduction to the wide world of research. Others incorporate SURE into several years of undergraduate research experience in the same lab. Regardless of their background, previous training, or future plans, students find the SURE experience to enhance their college experience by including hands-on training and real-life applications for subjects about which they had only ever dreamed or heard.

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Why do research?

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The thrill of discovery

Michael Kaufman, an Emory NBB major, likes research because every day is different. “Research is like a puzzle,” he says, “which sometimes has surprising results.” Sujith Baliga, also an Emory NBB major, concurs. “It’s fun to tackle a problem that no one thought could be solved.” An Emory biology and Spanish major, Evan Calabrese points out that getting results always takes time but only makes the results more exciting. Louie Hendricks, a biology major at Morehouse College, values being able to ask questions. “In school,” he says, “we study other people’s ideas; here we have our own ideas.”

For Dorothy Chyung, an Emory biology and anthropology major, it’s exciting to have the opportunity to participate in research, to do something with a purpose, and “to contribute to the world of science. There’s still so much we don’t know,” she says, “so it gives me confidence to be able to do something that can help.”

Who would have thought that turmeric, a common spice in southeastern Asian foods, could have medicinal effects on cancer or inflammation? Emory student Sravan Dhulipala is synthesizing a molecule that resembles the active molecule in turmeric in an attempt to exploit its chemical strengths. Though research may be slow, Dhulipala demonstrates that results can be powerful as he proudly shows visitors a chemical that he was the first to create. Dhulipala finds research worthwhile because he gets to sample his dream position in pharmacology where he will be at the forefront of discovery.

Entertaining

For some SURE students, the work itself is entertaining. Kevin Lee, an Emory NBB major, works with human subjects in a Neurology lab. “Every volunteer is different,” he says. “It’s fun to see their reactions to various stimuli.” Bentley Gibson, a child psychology major at Spelman College, uses puppets to communicate with children at the Language and Learning Lab. “It’s entertaining.” Gibson asserts. “I even came on weekends to work with the children. It’s almost like playing with the four year olds.”

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Mentors and Colleagues

James Eagan, an Emory chemistry major, argues that great mentors make for a great research experience. He eagerly tells of his welcoming lab: a group of dedicated researchers who were willing to answer questions and work with a young freshman. Like anyone entering a new environment, Eagan did not know what to expect when he began, but the other lab members were very encouraging and supportive even when things went wrong. He recalls once breaking something expensive; they told him not to feel bad but “just don’t do it again.” Without doubt, this experience has been rewarding. “There’s definitely a nerdy thrill to being the first to do something,” Eagan says.

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As the summer of 2007 opened, the campus seemed strikingly empty compared to the thriving campus of the SURE program, the Coca-Cola Commons. Even in such a short time span, the students were able not only to complete their research projects but also to make friends with their mentors, fellow lab members, and each other.

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Inspired by Tragedy
When tragedy occurs near to us, how many of us would just hide from any semblance of it in our grief and anguish? When a severe illness affected a young sibling, two SURE students instead found research as a means to honor their siblings and as another way to cope with reality. Currently a biochemistry major at the University of Maryland, Baltimore County, Elizabeth Kuhn was only fourteen when her eight-year old sister succumbed to a tumor on her brain stem. “She fell into a coma suddenly one weekend,” she recalls. “I always assumed that working towards the ‘cure to cancer’ was beyond me. But when cancer came close to home, I realized I have what it takes!” Following her SURE experience, Kuhn wants to remain involved in such research, planning to earn an advanced degree in either biochemistry or science journalism.

A biology major at Spelman College, Chino Aneke was inspired to do research when her brother was born with cerebral palsy three years ago. The SURE program matched Aneke with a research neurosurgeon, Dr. Claire-Anne Gutekunst, allowing her to catch a glimpse into the life of an MD/PhD with a clinical practice and a research lab. Aneke plans to follow Dr. Gutekunst’s footsteps and use the perspective of a surgeon doing research to develop surgery techniques to intervene against congenital diseases like her brother’s while the child is still an infant. “The key is to find something that will be less risky,” Aneke says, “so that it will be a viable alternative for the parents to consider.”

Unexpected Results
As with any science experiment, there is always something that fails to turn out quite as expected. Sean Knudson, a biomedical science major at Texas A&M, discovered that research is simply too tedious for him. “I just don’t have the curiosity to do this,” he says. “I’m so impatient; perhaps that’s why I’m an EMT.” Now with some research experience, Knudson is ready to become a physician, possibly in the emergency room, in cardiology or neurology, or even as an anesthesiologist. Whatever it is, it will have action for sure.

An Emory chemistry and Latin major, Sonya Delwadia had the opposite experience. She did not expect that she would like research and planned to become a physician. Once in the lab, however, all that changed. “I [was] placed in a very helpful lab,” Delwadia reminisces. “The more experienced lab members passed on their knowledge to the four undergraduates there.” She realized that she liked the hands-on approach and is planning to work in the same lab through the coming year, now that she’s interested in the research side of the medical field.

Making research a career
Many SURE students plan to continue on to medical school after they finish their undergraduate degrees. However, some are choosing different routes. After two years of research at the Winship Cancer Institute, Emory biology major Tiji Philip is considering graduate school over medical school. “I think there have been a lot of breakthroughs in medical research,” he says. “People ought to realize how important research really is to medicine.” Philip finds it fulfilling that a small project can affect something big, perhaps even bridging the gap between life and death. “That’s something I want to be a part of.”

The Big Picture
For ten weeks in this summer undergraduate research program, these sixty students have been part of the broad and complex world of research. Whatever their future plans, they have participated in an innovative and valuable part of science and seized the opportunity to share the exhilaration of learning and discovery with their peers. For those eager to explore this additional dimension of science and education, the SURE program provides an excellent chance to discover new meaning in science and realize greater potential: the perfect complement to a college education.
Isolation and identification of proteins interacting with KLF5 transcription factor complex in human epithelial cancer

**abstract**

Identity is as an individually constructed concept that is defined in relation one's society (Hogg 259). In Iranian Muslims, identity is shaped by their religious beliefs and practices, as well as by interactions with the wider world. Muslim individuals must decide what to accept, what to borrow, and what to reject in the formation of their own, unique Muslim identity.

**faculty mentor:**

David Lynn obtained his BA in Chemistry from UNC-Chapel Hill, his Ph.D. in Organic and Biochemistry, and was a post-doctoral fellow at Columbia University. Currently a professor in the general areas of molecular recognition, biorganic chemistry and chemical biology, his lab is studies the structures and forces that enable supramolecular self-assembly, how chemical information can be stored and translated into new molecular entities, and how the forces of evolution can be harnessed in new structures with new function. Dr. Lynn is the chair of the chemistry department at Emory University, as well as a Howard Hughes Medical Institute Professor, and the Asa Griggs Candler Professor of Chemistry and Biology.

**acknowledgements**

Special thanks to Dr. Rachelle Spell, the director of undergraduate research (Biology 499R) and Dr. Jin-Tang Dong from Emory School of Medicine’s Winship Cancer Institute for making this project possible. I would also like to thank my research mentor, Dr. Ke-wen Zhao, for her unfailing guidance on all aspects of my project, and visiting research Hai-Yang Yu for his advice and help in numerous experiments.

**Background**

Cancer is a genetic disease that develops when the activity of critical genes are in some way changed or mutated. Every individual has two copies, or two alleles, of each gene – one maternal copy and one paternal copy. Unlike many other genetic diseases, cancer will only arise if both copies of a gene, both alleles that an individual has, are mutated (1). Consequently, inheriting two germ-line mutations, acquiring two somatic mutations, or accumulating one of each type of mutation will result in cancer. This criterion, that two inactivating mutations are needed for the development of cancer, is known as the Knudsen “two-hit hypothesis” (1). Essentially, this means that not one, but two mutated or defective alleles are required for an individual to develop cancer.

The mutations that cause cancer can affect two different types of genes: proto-oncogenes or tumor suppressor genes. Proto-oncogenes are activated by gain-of-function mutations to become oncogenes, or genes that are always in the “on” position. Tumor suppressor genes, on the other hand, are inactivated by loss-of-function mutations to become genes that are always in the “off” position (1) (Fig. 1).

There are three mechanisms by which chromosomal deletion can inactivate tumor suppressor genes (2). The first of these mechanisms is gene mutation through loss of heterozygosity (LOH), or the Knudsen two-hit hypothesis. The second mechanism is known as hemizygous deletion and occurs when one copy of a gene is inactivated by mutation, resulting in loss of expression. Hemizygous deletion causes haplo-insufficiency, a state in which a single functional copy of a gene is not sufficient to produce normal amounts of gene product or protein (3). Thirdly, inactivation of tumor suppressor genes can occur via homologous deletion, or loss of both copies of a gene, which results in truncation of the genome.

**Significance and primary aims**

We are interested in KLF5 (Krüppel-like factor 5) because it is a putative tumor suppressor gene and transcription factor that plays a role in regulation of the cell cycle, cell proliferation and differentiation, apoptosis, and angiogenesis (4). In various cancers, KLF5 expression is down-regulated or reduced due to chromosomal deletion (3) (Fig. 2). Prior research has shown that loss of KLF5 through genetic deletion or transcriptional inactivation occurs frequently in human prostate and breast cancer. As a result, it is hypothesized that KLF5 may be involved in the suppression of tumor cell growth (5,6).

Other research has shown, however, that KLF5 is pro-proliferative, or induces cell proliferation rather than inhibiting it. KLF5 expression directly correlates with cancer cell proliferation, and results from clinical studies show that breast cancer patients with higher KLF5 expression have lower survival rates than patients with lower KLF5 expression (7). From this evidence, it seems that KLF5 has potentially important but conflicting functions in regulating cancer. One explanation for this is that KLF5 functions in a multimeric protein complex, i.e., a complex consisting of multiple other proteins. As a result, the activity and expression of the proteins interacting in the KLF5 complex may affect or modify the activity and expression of KLF5.

Normally, a yeast-two-hybrid system is utilized to identify proteins that interact with a protein of interest, but only binary (one-on-one) interactions can be found using this method. In order to further elucidate the role of KLF5 in various human epithelial cancers, it is necessary to isolate and purify KLF5 from the complex. In addition, by identifying the interacting partners of KLF5, we can better understand how KLF5 functions and affects cellular behavior. Our aim, therefore, is to identify and characterize the multiple proteins that interact with endogenous KLF5 by purifying native protein complexes from cells through novel methods of immunoaffinity purification (8).

**Materials and Methods**

Cloning of FLAG-tagged KLF5 and generation of recombinant adenoviruses

In order to effectively identify and characterize KLF5-binding proteins, we planned to express recombinant FLAG-tagged KLF5 protein intracellulary (in vivo). To achieve this, KLF5 coding sequence with the FLAG tag was first amplified by polymerase chain reaction (PCR) using appropriate synthetic oligonucleotide primers that contained the KpnI restriction sequence. The DNA product was then gel extracted and purified, restricted with KpnI, and ligated with KpnI-digested pAdTrack-CMV, which is an adenovirus-based plasmid vector (9) (Fig. 3). In our experimental system, pAdTrack-CMV serves as a shuttle vector for gene of interest, KLF5. High-competence bacterial (E. coli) cells were used for efficient transformation of the ligated DNA. Transformated bacteria were selected for kanamycin resistance (kanR) conferred by the vector. Plasmids were isolated from a few colonies using the miniprep procedure from the QIAprep Spin Miniprep Kit from Qiagen (Valencia, CA, USA) and further characterized to confirm the presence of the KLF5.

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gene. The resultant clone, pAdTrack-CMV-KLF5, will be linearized by digestion with the restriction endonuclease Pmel and then cotransformed into competent E. coli cells with pAdEasy-1, which is an adenoviral backbone plasmid (Fig. 3). The cotransformed bacteria will be selected for KanR conferred by the pAdEasy-1 vector and recombination will be confirmed by multiple restriction endonuclease analyses. The linearized recombinant plasmid will then be transfected into 911 or 293 cells, which are adenovirus packaging cell lines (Fig. 3) and recombinant adenoviruses will be generated after 7-10 days of growth.

Expression of FLAG-KLF5 and isolation of KLF5 complexes

HeLa-S3 cells will be grown in monolayer cell culture using standard growth media and these cells will then be infected with various amounts of pAd-KLF5 DNA using electroporation reagent from Invitrogen (Carlsbad, CA, USA). At 48h post-infection, cells will be lysed by RIPA buffer, which breaks the cell membrane without compromising the integrity of biological complexes. The putative complexes containing FLAG-KLF5 will then be precipitated using anti-FLAG antibody and protein A-coupled agarose beads in a technique known as immunoprecipitation. Protein A possesses a natural affinity for immunoglobulins, and therefore, binds the FLAG antibody that is bound to FLAG-KLF5, which in turn is associated with its partner proteins (Fig. 4). When centrifuged, the large agarose beads pellet down the associated complex. Thus, all KLF5-bound proteins will be separated out using the FLAG epitope as an indicator. Once the multiprotein KLF5 complexes are isolated, they will be analyzed by SDS-PAGE to resolve the proteins and individual proteins will be identified through proteomic analysis such as mass-spec. Expected subunits or proteins known to interact with KLF5, such as TGF-β, will be detected by available antibodies, while novel subunits will be identified by mass-spec analysis (10) (Fig. 5).

Anticipated Results and Discussion

By finding and identifying the proteins involved in the KLF5 complex, our laboratory will be able to more accurately characterize the functions of KLF5 in human epithelial cancers. If we can better categorize the role of KLF5 and analyze how its activity varies based on interactions with specific factors such as TGF-β more targeted therapies to treat cancer can be created in the future.

![Figure 1: Tumor suppressor gene inactivation or deletion](image1.png)

"Lack of a functional tumor suppressor gene leads to tumor development."

Mutation or deletion of tumor suppressor genes is believed to initiate many forms of cancer. For tumors to develop, both alleles of the tumor suppressor gene must be inactivated. In familial cancer syndromes, a mutant allele of a tumor suppressor gene is inherited and is present in every cell (e.g. p53 in Li Fraumeni). However, tumorigenesis is not initiated until the second allele is inactivated in a somatic cell. In non-familial cases, inactivation of both alleles occurs via somatic mutation or deletion. The end result is the same in both cases, the lack of a functional tumor suppressor gene leads to tumor development.

![Figure 2: Reduced expression of KLF5 but not other genes from a previously mapped common region of deletion in prostate cancer.](image2.png)

A: Expression of KLF5 and four surrounding genes in a normal prostate tissue and in six cancer cell lines/xenografts as detected by RT-PCR assay. B: Verification of reduced KLF5 expression in cancer cells by northern blot hybridization analysis. PZ-HPV-7, PWR-IE, and RWPE-1 are non-neoplastic prostatic epithelial cell lines. The northern data is consistent with that of real-time PCR.

(From Chen C, Bhalala HV, Vessella RL, Dong JT. 2003. “KLF5 is frequently deleted and down-regulated but rarely mutated in prostate cancer.” The Prostate 55: 81-88.)

![Figure 3: Schematic outline of the AdEasy system.](image3.png)

Figure 4: Schematic outline for isolation of proteins from the KLF5 complex.

Figure 5: Proteomic analysis of target protein complex (KLF5).

"Mass spectrometry based methods are used to characterize protein interaction, modification and expression changes."


Home Grown: Indigenous Ngöbe Home Gardens in a Modernizing Panama

Gillian Locascio

abstract

Home gardens, highly diverse plots near the house in which plants are cultivated on a small scale, are becoming increasingly popular in Panama. They are a stable and invaluable source of nutrition. Parts of the indigenous state, however, are changing rapidly as government roads, schools, programs, and non-governmental organizations extend their influence throughout the area. This study explores how Ngöbe communities are adjusting to these changes in access to space, the national market, and government and non-governmental services.

key terms

Home gardens, highly diverse plots near the house in which plants are cultivated on a small scale. Home gardens are highly diverse plots near the house in which plants are cultivated on a small scale.

acknowledgements

The author would like to thank Dr. Scott Lacy for all of his inspiration and guidance setting up this project, Dr. Tracy Yandle for her help navigating the IRB process, the Institute for Developing Nations for funding her work, and her host families and assistants in Panama without whose friendship, education, and support this research would not have been possible.

H
ome gardens are highly diverse plots near the house in which plants are cultivated on a small scale, usually with a combination of herbs, bushes, and trees that create a multi-storied structure. Especially important in traditional, tropical societies, home gardens have been widely recognized in the last three decades for their role in food security, culture, and environmental conservation. Despite recent efforts to study and conserve these important agroecological systems, their complexity has been declining in many parts of Latin America, due in part to increased involvement by new generations in a cash economy, institutionalized educational systems, and work in urban centers (Steinberg 1998; Howard 2006).

The Ngöbe, an indigenous group in Western Panama, have a rich heritage of home gardening as part of a traditional subsistence slash-and-burn agricultural system. Multiple stories of vegetation and the use of perennial plants, common amongst tropical home gardens, help retain water and reduce erosion (Orlove and Brush 1996; Samaniego Peña 1997; Steinberg 1998). High diversity of plant species and varieties helps maintain stable production in the face of disease, pest outbreaks, and harsh weather conditions (Cleveland and Soleri 1994). The Ngöbe agricultural system is also closely linked with social structure, and remained largely unchanged between the 16th century, when the arrival of the Spanish pushed many Ngöbe into the mountains, and the 1970s, when the Panamanian government launched a campaign to incorporate indigenous regions into the rest of the country (Young 1971 cited in linare 1980, linares 1987 cited in Samaniego pena). The last three decades, however, have seen a development explosion with the building of roads, followed by government schools, political representation, and the arrival of governmental and nongovernmental organizations (Bort and Young 2001).

The 1900s saw a dramatic population increase in the Ngöbe areas, from 16,161 in 1930 to approximately 121,679 persons in 1990 (Bort and Young 2001). The ensuing land shortages, combined with the building of roads for transportation and market access, lead to massive temporary migration to other parts of the country to work (Behmel and Palacio 1996). The migrations, which continue today, are overwhelmingly male; women are left to care for the house, children, home garden, and fields with the remittances which they send home. The Ngöbe population still suffers the highest levels of poverty in the country, with 72% of the population malnourished (Ministerio de Economía y Finanzas: Dirección de Políticas Sociales 2004). One 1997 study found that, although only an average of 7% of a family’s land constituted the home garden, this area produced two to three times as much per hectare as any other agricultural use (Samaniego Peña 1997). Given the high level of malnutrition and the frequent absences of men to earn a salary on distant plantations, home gardens are especially important sources of food and nutrition year round and, in some cases, sources of income.

This study investigates the new challenges and opportunities created by urbanization, increased access to national markets, and access to governmental and nongovernmental organizations, and in what ways locals are adapting their home gardens in two Ngöbe communities, “Close” and “Distant.” “Close” is a new town on the highway at the entrance of the indigenous state, with a primary school, small university, numerous family-owned kiosks stocked in a nearby city, and the main office for numerous regional governmental and non-governmental organizations. Trucks pass daily, and the nearest city is fifteen minutes driving, at one dollar round trip. “Distant,” on the other hand, is an hour away from “Near” by foot but considered by many inhabitants as a “forgotten community” by the government and organizations. A few scattered kiosks, a kindergarden, and a subcenter for one non-governmental organization...
provide services to the scattered residents. By comparing
the structure, diversity, and use of Ngöbe home gardens
in the two communities, the study forms a preliminary
picture of the cultural and environmental context
of these gardens.

Methodology:
A review of published research, as well as government
and non-governmental organization reports, provided
the base information on home gardens, Ngöbe history
and agriculture, the current environmental and social
situation in the study area, and past agricultural projects
carried out by organizations. Field research took place
in two communities, which I will call “Close” and
“Distant,” and involved informal interviews, semi-
structured interviews, tours, mapping, and participant-
observation.

I lived with families in both communities for two
weeks, conducting numerous informal interviews
and occasionally accompanying families in their daily
activities as a participant-observer. To better understand
the layout of these two communities I walked and
mapped the locations of buildings, streets, and various
garden types, then compared my maps to maps made by
one woman from each community.

In each community I conducted semi-structured
interviews with thirteen households, defined here as a
group of persons that works and eats together. Households
were chosen by snowball sampling. I was accompanied
nearly every day by a well-respected local woman familiar
with the areas and the families. My assistants presented
me to each household and explained my purpose, and
translated between Spanish and the local language if there
was any confusion. When unaccompanied, I spoke with
persons whom I had already met and who were fluent
in Spanish. Interviews covered 1) the use of the garden,
including for food, sale, recreation, and status, 2) the
household’s food sources and consumption patterns, 3)
the presence and use of medicinal plants and the health
clinic, 4) what other lands the family worked and other
forms of income, and 5) which, if any, organizations
they had worked with, especially in agricultural projects.

During household interviews I also walked through
each home garden with the caretaker, asking about the
garden, species present, their uses, and related subjects.
I noted the name given for each species, as well as my
observations on the zonation (horizontal arrangement of
plants) and stratification (vertical arrangement of plants).
I also conducted semi-structured interviews with the
local directors of three organizations that had worked
or currently worked on agricultural projects. With each
of these organizations I participated in and observed
a meeting or event, in order to gain their confidence and
in order to better understand how the local members relate to
and work with these organizations. The fourth organization,
which was no longer in existence, I learned of through
interviews of past participants and through a review of
their reports.

The Gardens: Continuity Continues
Home gardens in these two communities serve
as a mix of play area, aesthetic setting, and productive
earth. A patchwork of different types and structures of
vegetation, they provide numerous micro-climates
in a small space. Around the house there is always a “clean”
space, in which most vegetation is cut short and
occasionally burned and where one can walk and play
without danger of bites or stings. This space, referred to
as the “patio,” is often shaded by large trees and is one of
the most important spaces to families. Ornamental plants
are often found in the “patio” and along footpaths. There
are often a number of small open areas used to cultivate
useful plants and shrubs that require more sunlight. In
“Distant,” these areas are also sometimes used to plant a
patch of sun-loving staple foods, such as yucca or bush
beans. Areas with a mix of different-height species, such
as tall timber trees, shorter fruit and palm trees, banana
plants, and, in the shade, coffee plants or tubers such
as oote and iampi. Nearly every garden has its oranges,
bananas, avocados, and the palm fruit piblue.

Residents of both communities take advantage of
a variety of sources for their plants. Not all of the plants
in the gardens are planted from seed. When a useful or
pretty plant germinates in a home garden, the caretakers
will generally allow it to remain. Other times, household
members collect wild plants from the sides of paths, such
as orchids, begonias, or medicinal plants, and transplant
them to their home gardens. Still, garden caretakers
plant the vast majority of their garden from their own
seed, unique seeds given as a gift from neighbors, and
seeds bought and brought back by workers returning
from their travels. For example, home gardens in these
two communities sported flowers from the vegetable
producing region, cherry trees and cool-weather avocados
from the coffee-producing highlands, and Cavendish
bananas from the plantations.

Despite the sudden changes that the area has seen, the
garden caretakers indicated 176 species total, with
126 and 117 species found in “Close” and “Distant,”
respectively. The communities showed similar variance
between top and bottom gardens as well, with between 49
and 52 species found in a single garden in “Close” and
between 2 and 62 found in a single garden in “Distant.”
(See Figure B) The large range of diversity within each
community was striking. There were a few species for
which the garden caretakers did not know the name, and
which I could not identify, especially for ornamental
flowers and medicinal plants. Also, the plants which local
residents deemed “weeds,” suitable only to be cut down
the next time they cleared the garden, were not counted.
Nonetheless, the similarity in diversity and complex
structure suggests that, thus far, there has been little
cultural erosion of home gardens.

There were, however, some notable differences in the
prevailing of various classes of plants in each community.
“Distant” had 21 species of timber, for example, while
“Close” had only 15. Moreover, timber was more common in gardens in “Distant,” with an average of 5
species of timber in a single garden, as compared to an
average of 2 in “Close.” The tubers, yucca, oote, iampi,
and iampi, which provide an important base in the
region’s diet, are almost nonexistent in “Close,” while
they are found in nearly every garden in “Distant.”
“Close,” however, boasted a number of laying hens, a
breed purchased from outside the indigenous state that
required special care. Such differences reflect the distinct
demographics, and the challenges, that changes in access
to resources, traditional markets, and services is providing
in these communities.

Ac cess to Space: the Challenge of
Urbanization
Clearly, living in an urbanizing area means living
ever-closer to other households. In “Close,” this effect
is particularly clear—houses are much more densely
packed, especially along the highway, and only two of the
thirteen families had their farm fields adjacent to their
home gardens. Five families did not even have access
to family farms, having moved to “Close” so that their
children would not have such dangerous walks to school
or, in one case, because of fragmentation of the family
lands. In another case, the land was so densely packed,
each family had its own home garden, usually with its
bananas and fruit trees and carefully demarcated by a live fence. Those families without access to farm fields but with manpower
would occasionally rent land in order to cultivate staples
such as rice and corn, but these lands were not always
available and often renters were not allowed to sow yucca
or beans.

Such tight quarters lead to serious problems with free-
ranging domestic animals. The most destructive by far
is the pig, which has made it all but impossible to cultivate
tubers in “Close.” Especially for landless families, the loss
of the ability to grow these staples in their home gardens is a serious blow to food security. Only the two families that lived farther from the center of town even attempted to plant root crops, and nine of the thirteen families interviewed in "Close" mentioned problems with damage by domestic animals that entered their home gardens. Despite a legal system which allows a person, if he or she catches the offending animals, to exact a fine from their owner, damages continue. Some families have given up raising pigs, which are an important source of cash for emergencies or schooling, because of the fines, but they still cannot plant tubers because other neighbors’ pigs wander free. Anger toward pigs runs so high that one resident explained that, if his pig was not tied up, it ran the risk of being hit or even killed by a neighbor.

In "Distant," on the other hand, houses are generally found in family groups, each household with its own garden, various fields, small pieces of forest or fallows, and in one case even the family cemetery. Even with more space, though, fields to work are limited; three of the thirteen households had not been permitted to work land that year by the "boss of the land," usually an elder family member. The other ten households had access to family lands but struggled with plant diseases, lack of seed, and lack of manpower. "Distant" residents can compensate in their home gardens, however, by rotating small parcels of crops such as corn, yucca, or guaná in the open areas and by planting shade-loving tubers such as aoto, ñamí, and ñampí beneath the trees and banana plants. Such compensation is not enough, and many households are turning to growing opportunities to sell products of their home gardens.

**Acess to Markets: Obstacle and Opportunity**

Given that very few of the families in either community are food independent, access to the national market is an enormous round-trip food supply. Lack of access to land in "Close" and problems with plagues and pests in "Distant" necessitate even the purchase of staples such as rice during parts of the year. The farther the community is from the national market, however, the more expensive these products become. In "Distant," families that had to buy especially large amounts of food would sometimes spend an entire day and a few dollars to travel into a nearby city and shop for provisions. At the same time, communities that are farther from the national market earn less from any sales. To make up for this, families will travel to nearby cities when they can afford passage in order to sell their goods for more. Middle men travel during the season of various fruits buying surplus fruit from families and selling them on the national market.

While it is common for families to sell surplus fruits from their home gardens, very few families plant anything with the sole aim of selling it. In fact, planting in the home gardens patches of food to sell was exclusively practiced by single and aging women struggling to support young children. Planting for the market is uncommon due in part to a strong sentiment that the home garden and the fruits of the home garden are meant to support the family or provide gifts to friends. In this way, the social function of the garden overrides its potential economic value on the national market. Selling only surplus fruits seems to be seen as converting what would go to waste (during a month’s time, for example, a single tree might produce 250 avocados) into cash, and thus not a break with the value of home gardens producing for the family.

Travelling to buy or sell, when possible, is one way that families mitigate the higher costs of food and earn more for their products. For example, in "Distant" many families take advantage of the tuber shortage in "Close," and earn extra money by planting their own tubers. "In Close," some families have purchased special chicken feed and a new breed of laying hens from the nearby city. While a chicken-breeding endeavor requires regular visits to the city for these supplies, this more fragile breed of chicken brings in greater profits both in egg sales and in chicken sales. Access to national markets can also reduce the need for certain plants; families in "Close," which can purchase cinder block and cement for construction, plant significantly less timber than families in "Distant," where it much more difficult to transport building materials. Locals are constantly searching for new ways to take advantage of the opportunities provided by market access, and sales from the home garden often provides an important supplemental income.

**Acess to Services: The Limited Effects of Organizations and Agricultural Projects**

Government services such as schools or health posts, agricultural projects, and non-governmental organizations have a much shorter reach than the market, and are barely present in "Distant." Of the four organizations and schools that have worked on or currently work with home gardens, only one has a presence in "Distant," with a subcenter and demonstration garden. The primary school in "Close" also serves the children of "Distant," although they have to walk over an hour in the dark to and from school each day. The organizations have focused on improving the environmental sustainability, productivity, and nutritional content of home gardens, while a government pilot program at the school teaches children basic agriculture and husbandry skills while providing food for school lunches.

Where these organizations and programs exist, there is a high level of participation within the community. Despite this participation, however, none of the techniques and very few of the new plants and plant varieties can be found in the area’s home gardens. This is due to several factors. The proposed techniques often include weeded and watered rows in sunny patches of well-tilled soil, the application of compost, and more productive varieties of plants purchased from the national market. Such a garden does not fit the cultural space of the Ngöbe home garden, which requires a clear area and shady spaces beneath fruit trees for recreation and relaxation. These programs rarely include the food crops that figure most prominently in Ngöbe diets, although a few successful cases in one organization suggest that techniques involving such crops will be more readily adopted. Compost and seeds are expensive that cash-strapped families cannot afford, especially when programs are using plants such as cucumbers which do not produce viable seeds. Finally, such garden plots require significantly more labor. In an area with high levels of seasonal migration, these communities suffer quite literally, from a lack of manpower.

**Conclusion**

The cultural spaces provided by home gardens are their most abiding aspect. Despite rapid changes in "Close," the diversity and structure of these gardens remains as high as "Distant." Organizations suggested that the unwillingness of locals, who volunteer in the demonstration gardens, to adopt the techniques or plant varieties proposed by these organizations is caused by unwillingness to change. Nonetheless, locals are always making small changes, bringing home new plant varieties and occasionally new income-producing ideas from trips they travel through the country seeking work. Already, people in both communities have begun adapting to the new forces that are so rapidly changing the context of their work.
Demonstration Home Gardens and Agricultural Projects

Nonetheless, if programs wish to support these communities in their quest for food security, environmental sustainability, and economic security, these programs need to take into account the cultural aspects of Ngöbe home gardens and the real restraints faced in these areas. Well managed microfinance, accompanied by economic and business management support, could help locals obtain the seed money necessary to start small income-making endeavors, such as chicken breeding, and continue adapting to new opportunities. Expansion of school programs, by complying with the requests of both communities to extend the kindergarten in “Distant” through third grade and to add a secondary school (seventh through twelfth grade) in “Close,” would improve the reach of these programs and also provide the opportunity for locally-led agricultural innovation. Finally, the integration of local persons into the planning level of programs, not just the implementation level, will ensure that the programs fit the cultural and economic realities of these communities. With its rich, well-conserved, and dynamic heritage of home gardening, there is great potential for these Ngöbe communities to improve home gardens while maintaining the cultural values, spaces, and the unique plant varieties that are so interwoven with daily life.

Works Cited

Water from a Stone: Engineering Colocasia esculenta to Produce Beta-Carotene

**abstract**

Taro root is a staple in the diet of South India, but the people there are not getting enough Vitamin A. Vitamin A is produced in the body after an intake of β-carotene, so the taro root should be able to be genetically engineered to produce β-carotene which would reduce the Vitamin A deficiency. Genes which code enzymes that create β-carotene will be over-expressed in order to increase the amount in the taro root. Numerous generations will be cultivated in order to determine if the taro root has actually been made to produce β-carotene.

**faculty mentor**

David Lynn.

**acknowledgements**

The author would like to thank Dr. David Lynn and Dean Preetha Ram for all of their assistance in ORDER and the INSPIRE program. She would also like to thank all of the graduate students who taught her section of ORPR for reviewing this proposal constantly and helping to improve it.
Taro root (Colocasia esculenta) is a staple in the diets of southern Indian peoples. In fact, owing to its rich vitamin content and its very starchy consistency, it seems to be a very good choice for the central base food in many Indian and southern Asian diets. However, although taro root contains many essential vitamins necessary to sustain life, such as vitamins B6, C, niacin, and potassium, it is lacking in the incredibly important vitamin A [1].

According to the World Health Organization (WHO), Vitamin A deficiency is a global epidemic affecting roughly 10 million individuals worldwide [2]. Not only is vitamin A deficiency a leading cause of blindness, but it is also important in the production of human growth hormone and the maintenance of a healthy immune system, particularly in young children [2]. Without vitamin A, humans are unable to sustain a high quality of life for any length of time, and typically require huge donations of vitamin A eye drops, which are administered by aid workers to needy populations to quell the potential for medical complications [2]. It is estimated by the WHO that, in addition to quelling the epidemic of blindness, there could be a 25% reduction in infant mortality through improved vitamin A intake [2].

Unlike vitamin D, which humans can synthesize from cholesterol and sunlight, retinoids (or vitamin A molecules) cannot be made without external sources—mainly C40 carotenoids [3]. The major source of these C40 carotenoids is β-carotene [7]. β-Carotene is naturally found in carrots, sweet potatoes, and cassava (3). Unfortunately for the millions of people worldwide who rely on taro root as a dietary staple, it is particularly lacking in β-carotene, which gives it no natural potential to provide the building blocks for vitamin A synthesis in humans, as β-carotene is a well-known precursor to vitamin A production [1,3].

In 1930, Thomas Moore determined the mechanism for the conversion of carotene to vitamin A in animals [4]. According to Moore’s research, in animals, vitamin A is synthesized in the small intestine as a result of β-carotene intake. The biosynthetic process involves the use of a central cleavage mechanism at the 15, 15’ double bond of β-carotene, yielding two molecules of vitamin A aldehydes (also known as retinol) [3] (Figure 1). This cleavage is catalyzed by an enzyme known as β-β-carotene 15,15’-oxygenase [3].

Though scientists have discovered the gene on a bacterial plasmid for the creation of β-carotene and the enzymes that are involved [3] (Figure 2), it has yet to be isolated from the plasmid and prepared for insertion into another genome.

In 2005, Centre de Coopération Internationale en Recherche Agronomique pour le Développement (CIRAD) scientists working in Vanuatu, in the South Pacific, and others announced their recent completion of mapping the genome of taro root at a meeting of the International Society for Tropical Root Crops held in Kerala, India [5]. This gives taro a huge potential to be engineered—with a partially published, searchable genome available to the public, bioengineering of taro is made infinitely more possible [6].

By making use of the searchable genome, already mentioned above, taro root should then be capable of β-carotene production, as it contains many of the natural precursors to the molecule and is evolutionarily very similar to the sweet potato, an organism that contains many of the same genes and enzymes and is able to produce β-carotene [7].

This β-carotene from taro root would subsequently be converted to vitamin A in humans without additional assistance and allow for populations that count taro root as a staple of their diet to enjoy the benefits of vitamin A production—lower risk of blindness, some cancers (namely gastrointestinal and esophageal), and cardiovascular disease [8].

Hypotheses and Research Design

Past experiments involving the genetic engineering of crop plants and carotenoids has been mostly limited to work with “Golden Rice,” a project in which scientists worked to redefine the metabolic pathway of India Rice in order to provide additional vitamin A to consumers [9]. Through the extensive research performed on India Rice in this regard, many helpful tips can be learned and applied to future work with taro root.

Years of research into designing the carotenoid-portion of the India Rice genome lead researchers to learn that not only are vitamin-heavy seedlings more viable and stable in their growing environments than their un-engineered equals, but they also assumed a very noticeable yellow colour, likely indicative of their increased β-carotene production [9]. β-carotene absorbs light with a wavelength of 450-500nm [10]. This is the “green-blue” part of the spectrum, which means that the “red-yellow” part is reflected back, creating the characteristic orange colour of the compound (Figure 3).

In addition, the initial method of introducing new genes into India Rice involved using a recombinant daffodil phytoene synthase (Psy) cDNA, however, in additional trials, with Golden Rice, this method was found to yield negligible levels of vitamin A in golden rice [9]. In place of the daffodil cDNA, tests with additional plant sources “indicated that the best strategy was probably to use the phytoene synthase from maize” [9]. This is likely because both maize and rice are monocots, meaning that they share many similar characteristics, such as similar embryos, stem vascular bundle and root growth patterns, and the absence of secondary growth [11]. Taro root is also a monocot, making it equally as good of a host to maize DNA as monocot rice. By combining this gene sequence with the sequence that codes for carotene desaturase (Zds) in Erwinia uredovora, a common soil bacterium, under the control of an endosperm-specific promoter, scientists were able to produce viable Golden Rice samples that provided a 23-fold increase in digestible β-carotene in comparison with their earlier trials with Golden Rice [12]. This increase allowed one serving of Golden Rice’s worth of β-carotene to provide enough vitamin A, once processed in the intestinal tract, to satisfy the daily requirement of vitamin A needed by humans [12].

All of these steps are illustrated in Figure 2. According to the rice strain being studied contained the necessary enzymes and geranylgeranyl-PP (GGPP), the protein precursor to β-carotene, needed for carotenoid synthesis, but lacked two important enzymes (shown on the figure as Psy and Zds). After the genes for production of these enzymes were engineered into the rice genome, the so-called “golden rice” was then able to produce β-carotene on its own [9].

In the proposed experiment involving taro root, I plan to draw heavily from the recognized effective procedure used to genetically engineer carotenoid synthesis in Golden Rice. As the genome for taro root has been made public in the last few years, it will be...
possible to search it for the coding sequences for one or more of the necessary enzymes needed for synthesis of β-carotene. Although the genome is public domain, it has not been made fully available online. Since at the time of this proposal the genetic maps for the enzymes of interest are not yet available, it is necessary that multiple hypotheses and corresponding experiments are proposed in order to circumvent potential pitfalls with any particular hypothesis.

First, and most likely, I propose that taro root contains geranylgeranyl-PP, the direct molecular precursor to β-carotene, and is lacking one or more enzymes needed to complete the steps of the reaction and synthesize β-carotene. This is very likely, as geranylgeranyl-PP is a precursor protein for the synthesis of a number of terpenoid compounds synthesized in plants, most notably gibberellins, a phytohormone with important roles in plant growth [12]. Without gibberellins, plant growth would be incredibly dwarfed. In order to determine the genes that code for the production of GGPP in the taro root genome, the sequence of the plant must be searched for segments known to code for GGPP. This cannot be done until the entirety of the genome is made public, at which point it will be simple to search the amino acid sequences.

In keeping with this hypothesis, it will be necessary to cut, using restriction enzymes, the cDNA of maize in order to isolate the phytoene synthase (psy). In addition to isolating these enzyme coding regions from maize, Erwinia uredovora, will be used in order to obtain, through similar methods, the coding regions for carotene deatrasynase enzymes. The combination of these two enzymatic coding regions of DNA once inserted into the genome of β-carotene pre-seedling cells by way of a vector (agrobacteria, a natural plant bacteria) will effectively reformat the taro root cells to produce β-carotene of their own accord, thanks to new genetic coding regions in their DNA.

Conversely, if taro root is found to contain neither the genes that code for GGPP, nor the requisite β-carotene synthase enzymes, then alternative methods must be taken in order to insert the genes for the synthesis of these necessary elements into taro root cells. It is highly unlikely that taro root would not contain any GGPP, as it is a molecular precursor to the production of gibberellins. Should taro root have no GGPP available for conversion into β-carotene, it is highly unlikely that this type plant would grow much more than a very dwarfed version, as without the necessary amount of gibberellins, plant growth is severely stunted [12].

or process β-carotene (such as B-LYC or β-carotene hydrolase). Each of these genetic “tweakings” would result in the production of more β-carotene, and making the choice between them depends upon whether taro root is found to be able to synthesize β-carotene and contains one of the genes above, producing proteins that either inhibit or are slow to process β-carotene.

In studies done with Solanum lycopersicum, over-expression of B-LYC and β-carotene hydrolase was found to increase the β-carotene levels in fruit 7-fold [12]. In Solanum tuberosum, however, inhibition of E-lycopene cyclase resulted in a 14-fold increase in β-carotene levels [12]. Therefore, both of these methods have proven effective in the past, although they are typically employed in different plants, as inhibiting enzymatic production might be the more useful tactic in amplifying β-carotene production in one type of organism as opposed to another.

Research Interpretation

Each of the experiments explained above are merely steps in a potentially longer experiment to refine the β-carotene production in taro root, however, there will be no way to be certain each experiment that will be necessary to determine its effectiveness. First, cells that effectively take up the DNA cut from restriction sites in various organisms (maize and E. uredovora, for example) with the assistance of DNA ligase enzymes in order to assure that the DNA is reconnected to the genetic sequence in taro root chromosomes will not be viewable until the F1 generation, or the first generation of progeny. As a result of the technique that is commonly used for genetic transformation of plants known as floral dipping, in which the floral buds of the taro root plant are dipped into an aqueous solution of agrobacteria (a vector), the seeds that are produced by the plant will then need to be planted and the results from those seeds screened for increased β-Carotene content.

A final process of floral dipping is very straightforward, and involves both botanical and microbiological steps. First, a wild type crop of plants (in this case, taro root) must be grown to adulthood. This can take anywhere from a few weeks to 1 or 2 months. Next, the agrobacteria, which will be used as a natural vector in order to carry the desired genetic material that we want into the plant and incorporate it into the plant’s DNA, will need to be made. This gene will need to be placed within the agrobacteria and expressed in taro root cells, normally through the assistance of DNA ligase enzymes in order to assure that the DNA is reconnected to the genetic sequence in taro root chromosomes. In order to test the effects of the agrobacteria, the agrobacteria will need to be planted, expecting that they will have incorporated the new genetic information into their DNA. If the floral dip technique is effective, it is likely that a yellow tint will begin to be observable in the F1 generation of plants (assuming the genetic mutation coding for enzyme production is not recessive), as production of β-carotene leads to a buildup of yellowish pigments. In addition to a yellow color, we hope that these cells will continue dividing on their own, eventually forming a taro root embryo (typically contained in a seed-coat) and then growing into a plant. In this way, the production of β-carotene will be completely contained within the taro root genome and will not require outside assistance of additional factors necessary for synthesis. If this genetic mutation is a recessive trait, the F1 generation cannot be expected to show any phenotypic differences, but also recognize that because taro root is a unique organism, each of its seedlings have the potential to act differently, even in controlled situations. It is very possible that the first two embryos grown with enhanced β-carotene content will die immediately after accepting these plasmids, and agrobacteria are put en masse into the mixture. These agrobacteria can be expected to take up one or more plasmids through their pilli. Finally, these newly-transformed bacteria are put into aqueous solution and the flowering tips of taro root plants are dipped into the solution. These plants are allowed to continue growing, and after they generate seeds, these seeds should be planted, expecting that they will have incorporated the new genetic information into their DNA.
the genes necessary for the synthesis of β-carotene, while the third and fourth trial may very well grow and flourish in the nutrient-rich environment.

Conclusions

Taro root (Colocasia esculenta) is a staple starchy food source for a huge portion of the world’s populations, as it is of huge dietary importance in India, Sri Lanka, parts of Africa, the West Indies, and the entirety of the Pacific [1]. Nutritionally, taro root is a good choice for the base of a diet, as it is a good source of protein, carbohydrates, and potassium, as well as having more Vitamin B than a glass of whole milk [1]. Unfortunately, however, taro root is a notoriously poor source of β-carotene, a metabolic precursor to vitamin A. Vitamin A is an excellent choice, of all the vitamins that these individuals all had to get infected in some manner, which putatively involves the CD4 glycoprotein receptor. This receptor is found in variety of immune cells and dendritic cells. The particular structural motif at that location is hypothesized to play a role in the process for infection. As such, a potential vaccine may need to account for any potential conformational changes that occur upon the contact of HIV and human cells. Through an examination of the membrane-spanning domain of gp41, this proposal intends to test the transmembrane portion of both normal gp41 and mutant gp41 where the transmembrane portion has been replaced with that of other genes.

References


Determining Potential Roles of the Membrane Spanning Domain in Producing Competent Envelope Trimmers in HIV-1

abstract

Though 215,653 are estimated to have HIV infection, nearly double that number live with AIDS. These individuals all had to get infected in some manner, which putatively involves the CD4 glycoprotein receptor. This receptor is found in variety of immune cells and dendritic cells. The particular structural motif at that location is hypothesized to play a role in the process for infection. As such, a potential vaccine may need to account for any potential conformational changes that occur upon the contact of HIV and human cells. Through an examination of the membrane-spanning domain of gp41, this proposal intends to test the transmembrane portion of both normal gp41 and mutant gp41 where the transmembrane portion has been replaced with that of other genes.

faculty mentor

David Lynn obtained his BA in Chemistry from UNC-Chapel Hill, his Ph.D. in Organic and Biochemistry, and was a post-doctoral fellow at Columbia University. Currently a professor in the general areas of molecular recognition, bioorganic chemistry and chemical biology, his lab is studies the structures and forces that enable supramolecular self-assembly, how chemical information can be stored and translated into new molecular entities, and how the forces of evolution can be harnessed in new structures with new function. Dr. Lynn is the chair of the chemistry department at Emory University, as well as a Howard Hughes Medical Institute Professor, and the Asa Griggs Candler Professor of Chemistry and Biology.

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The author would like to thank Dean Preetha Ram and the INSPIRE program for giving her great opportunities. She would also like to thank all of the teacher-scholars who taught her section of ORDER for providing helpful insight along the way and assisting in making this proposal a success.

ORDER Proposal

Human Immunodeficiency Virus (HIV) - Retrovirus that can lead to AIDS

Acquired Immune Deficiency Syndrome (AIDS) - A result of HIV where the immune system begins to fail and opportunistic infections can cause death

CD4 glycoprotein receptor - expressed on the surface of many immune system cells including T-helper and regulatory T cells

gp41 - glycoprotein which provides the second step by which HIV enters the cell by assisting in fusion with the host cell
HIV commonly uses the phenylalanine-43 of CD4 fills the deeper cavity [5]. The gp120 serves as the CD4 binding site, and two separate regions, which fold together into a gp120 core [5]. Two of which fold into large loop structures due to characterized by five variable regions (V1-V5), the first terminal sequences of gp120 [5]. gp120 sequences are extracellular portion of gp41 and the NH2- and COOH- terminus of gp120 known as the “fusion peptide” is inserted into the target cell membrane [5]. It has been proposed that during viral fusion to the target cell, a region on the hydrophobic NH2-terminus of gp41 known as the “fusion peptide” is has the potential to connect the viral cell membrane with that of the target cell [5]. It has been proposed that during viral fusion to the target cell, a region on the hydrophobic NH2-terminus of gp41 known as the “fusion peptide” is inserted into the target cell membrane [5]. A simplified of some of the functional areas of gp41 is shown in Figure 1 [7].

The determination of the structures of these biomolecules as well as the conformational changes that occur when they interact with each other is critical to the development of a vaccine against HIV. Clearly, the HIV virion’s persistence in vivo despite the exposure of envelope glycoproteins that would seemingly act as effective vaccine components is the result of the production of fusion-competent trimers. Several previous investigators have replaced the entire MSD of gp41 with comparable transmembrane portions of other glycoproteins such as that of influenza hemagglutinin and cellular glycoprotein CD22; the resulting envelope glycoproteins have had varying degrees of success ranging from virally infectious ability in the influenza hemagglutinin (influenza HA) replacement to full function and infectivity comparable to wild type controls in the CD22 replacement [9,10]. Differences in the length of the respective MSDs of gp41, influenza hemagglutinin, and CD22 may account for these discrepancies in virulence. Another notable difference is the presence or absence of the GXXXG motif; while glycity residues are present in the MSDs of influenza hemagglutinin and CD22, they are not present in the GXXXG motif as they are in gp41 [9,10]. Although replacing these glycity residues with alanine residues in the GXXXG motif in the MSD of gp41 did not significantly affect the functionality of the envelope trimer, this could have been due to the minimal size difference between glycine and alanine residues [11]. Thus, the GXXXG motif might also be a key component in the formation of functional envelope trimers.

Research Design and Interpretation
Testing the Effects of Length of the MSD
To determine whether or not the length in the MSD in gp41 is critical to the formation of functional envelope trimers, the MSD of influenza hemagglutinin (HA) previously used for study will be utilized. The MSD of gp41 is 22 amino acids long while the MSD of influenza HA is 25 amino acids long; the sequences of both MSDs are shown in Figure 2 [9]. The MSD of influenza HA will therefore be shortened to 22 amino acid in length by designing and incorporating it into experimental viral membranes. Expression of Mutant Envelope Trimers
To create hybrid gp41 glycoproteins in which the MSD is replaced by the shortened version of the MSD of influenza HA, cloning sites for SpeI and HpaI will be generated on the 5’ and 3’ ends of the pSVIIIenvΔ726-856 plasmid, which expresses a truncated glycoprotein, via the megaprimer PCR-directed mutagenesis method [12]. Synthetic complementary oligonucleotides containing

Complete Influenza HA MSD-5’-CTTAGTATCTTT GCAATTATGCTACAGTACGAGATCCTGTC ACTGCGAATGATGCGGTGCCATCTTTC TGGATGT-3’

Modified Influenza HA MSD-5’-CTGATATCCTT GCATTATGCTACATCAGCAGATCCTGTC ACTGCGAATGATGCGGTGCCATCTTTC TGGATGT-3’

Figure 3 - Base-pair sequences of complete influenza HA MSD and a modified influenza HA MSD. The underlined sequences in the two sequences are not part of the MSD sequence; rather, they represent the SpeI/HpaI cloning sites. The bolded portion of the complete influenza HA MSD sequence is the portion missing in the modified sequence [9].

There are several enzymes that are present in the plasma and would be able to hydrolyze any glycosylated residues present in the viral envelope glycoproteins such as that of influenza HA. It has been proposed that during viral fusion to the target cell, a region on the hydrophobic NH2-terminus of gp41 known as the “fusion peptide” is inserted into the target cell membrane [5]. A simplified representation of some of the functional areas of gp41 is shown in Figure 1 [7].

Recognize most carbohydrate groups as markers of “self” or non-foreign cells; minimizing the potential for such glycoproteins to act as effective vaccine components [5]. Thus, other vaccine components must be explored. Elucidating the conformational changes associated with the fusion of the envelope glycoprotein with the target cell membrane will provide new information about opportunities for vaccine components to interact with HIV virions and infected cells. Question and Hypothesis
I propose to study the role of the membrane-spanning domain (MSD) of gp41, the transmembrane portion of the glycoprotein, in producing competent and functional envelope glycoprotein trimers. The null hypothesis in this case is simply that the MSD of gp41 plays no role in the formation of functional envelope trimers. As alternative hypotheses, I propose that either the length of the MSD or the presence of glycine residues in a GXXXG amino acid sequence motif are critical components of the MSD in terms of the production of fusion-competent trimers.

Figure 2 - Amino acid sequences and hydropathy plot of influenza HA MSD and gp41 MSD. Amino acids are numbered according to their positions in gp160. The underlined sequence is the GlycyxG motif. In the hydrophathy plot, a higher hydrophathy value corresponds to a more hydrophobic residue [9].

As of June 2007, an estimated 215,653 Americans are living with HIV infection, and an additional 453,760 are living with AIDS [1]. Over 17,000 AIDS related deaths have occurred each year since 2001 in the United States alone. HIV is the causative agent of the AIDS epidemic there have been 21.8 million cumulative AIDS deaths worldwide [1,2]. HIV infection spreads primarily through sexual contact and needle sharing with infected persons, with a small number of reported cases due to the transfusion of infected blood or blood clotting factors [3]. The mature HIV virion is encased in a lipid bilayer envelope studded with glycoproteins [4]. A major role of the viral envelope receptors expressed on the surface of specific cells, particularly the CD4 glycoprotein receptor. The CD4 receptor is found on the membranes of T lymphocytes and monocytes as well as dendritic cells and brain microglia [5]. Dendritic cells, which line peripheral mucosal sites, are often the first cells to encounter invading HIV virions and engulf them in order to transport them to the major histocompatibility complex (MHC) class II [6]. The MHC in turn presents antigens to CD4+ T cells in lymphoid organs to initiate an immune response. It is within these very T cells that the HIV virions replicate and promote their spread [6].

Amino Acid Sequences and Hydropathy

Figure 1: Functional regions of the HIV-1 envelope glycoprotein gp41. This simplified representation of gp41 shows the fusion peptide region (fp), the ectodomain including an area of prominent hydrophobic repeats and a disulfide bridge (vs), the transmembrane portion (tm), and the cytoplasmic domain. Amino acid residues are numbered based on positions in gp160 [7].

The structures of the chemokine receptors CCR5 and CXCR4 to gain entry to target cells [5]. Several gp120 structures, particularly the third variable loop (V3) and a highly conserved structure adjacent to the V3 loop, appear to be involved in CCR5 and other chemokine receptor binding [5]. Both the formation of the deeper gp120 cavity upon its binding to CD4 as well as the interaction of the gp120-CD4 interface with chemokine receptors such as CCR5 and CXCR4 likely contribute to conformational changes within the envelope glycoprotein. More specifically, the transmembrane portion of the glycoprotein, gp41, is thought to undergo conformational changes upon binding to the target cell receptors [5]. Studies of the extracellular portion of gp41 revealed a trimeric coiled coil structure that has the potential to connect the viral cell membrane with that of the target cell [5]. It has been proposed that during viral fusion to the target cell, a region on the hydrophobic NH2-terminus of gp41 known as the “fusion peptide” is inserted into the target cell membrane [5]. A simplified representation of some of the functional areas of gp41 is shown in Figure 1 [7].

Influenza HA and gp41 MSD amino acid sequences

<table>
<thead>
<tr>
<th>HA and gp41 MSD amino acid sequences</th>
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<tbody>
<tr>
<td>HA (n=25):</td>
</tr>
<tr>
<td>CTGGCAATCATGATGGCTGGGATCTCTCTGGAAT</td>
</tr>
<tr>
<td>GCTCCTGTCAGTCATGACGAGATCCTGTCACTGCG</td>
</tr>
<tr>
<td>AATGATGCGGTGCCATCTTTC TGGATGT-3’</td>
</tr>
<tr>
<td>gp41 (n=22):</td>
</tr>
<tr>
<td>CTGGCAATCATGATGGCTGGGATCTCTCTGGAAT</td>
</tr>
<tr>
<td>GCTCCTGTCAGTCATGACGAGATCCTGTCACTGCG</td>
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<tr>
<td>AATGATGCGGTGCCATCTTTC TGGATGT-3’</td>
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Figure 1: Amino acid sequences and hydropathy plot of influenza HA and gp41. Amino acids are numbered according to their positions in gp160. The underlined sequence is the GlycyxG motif. In the hydrophathy plot, a higher hydrophathy value corresponds to a more hydrophobic residue [9].

The determination of the structures of these biomolecules as well as the conformational changes that occur when they interact with each other is critical to the development of a vaccine against HIV. Clearly, the HIV virion’s persistence in vivo despite the exposure of envelope glycoproteins that would seemingly act as excellent targets for neutralizing antibodies indicates that these envelope glycoproteins are on their own, relatively ineffective immunogens and antigens [5,8]. Heavy glycosylation surrounding the gp120 core and the large variable loops near the chemokine-receptor binding sites sterically inhibit these sites from acting as possible antigenic targets [5]. The immune system also tends to
the desired portion of the influenza HA MSD sequence will then be created. Note that the complete influenza HA MSD sequence is 79 base pairs long so as to code for 25 amino acids, while the desired portion of the sequence consists only of the first 66 base pairs to code for the first 22 amino acids [9]. These numbers do not include the base pairs involved in the SpeI and HpaI cloning sites, which will flank the sequence of the influenza HA MSD. The nucleotide sequence of the full influenza HA MSD and the shortened version to be used in the investigation are shown in Figure 3. The two synthetic complementary strands will then be hybridized to each other to create a fragment of double-stranded DNA, which will be cloned into the pSVII-env plasmid. All the resulting mutations will be confirmed via automated DNA sequencing.

Cells from a human cell line will be used to test the virulence of the mutated gp41 envelope trimers. Prior to exposing cells, however, a different cell line will be used to ensure that the mutant gp41 trimers are expressed in viral particles by co-transfecting the cells with the pSVII-env plasmid expressing the mutant trimer and an HIV-1 envelope-negative proviral construct, HXBH10 env. The cells to be exposed to the newly-formed viral particles will be infected with the mutated viral vectors using the calcium phosphate co-precipitation procedure, in which a calcium phosphate-viral vector precipitate is formed as a layer over the cells, which the cells then take up via phagocytosis. For control purposes, the pSVII-env plasmid will be used to express the wild type gp41 envelope trimer as well, and the plasmid will also be co-transfected with HXBH10 env in human cells to ensure the wild type gp41 envelope trimer is expressed in comparable viral particles.

Processing and Infectivity of Mutant Envelope Trimers

A series of analyses will be performed to determine whether or not the mutant gp41 envelope trimers can successfully infect human cells. In order to determine whether or not the wild type and mutant gp41 envelope trimers are appropriately processed, the cells in which the virus particles were transfected will be lysed and the lysates analyzed by Western blot. If the mutant gp41 envelope trimers are processed normally, the Western blot will only show bands corresponding to gp160, gp120, and envelope trimers. Whether or not the mutant gp41 trimers retain this ability will be determined by adding brefeldin A (BFA) during labeling to prevent the transport of the protein from the endoplasmic reticulum to the Golgi apparatus and then using coprecipitation techniques to determine whether or not the mutant gp41 trimers can be co-precipitated with anti-CD4 or anti-gp41 mAbs to ensure that the mutant gp41 trimers are expressed in viral particles by co-transfecting the cells with the pSVII-env plasmid expressing the mutant trimer and an HIV-1 envelope-negative proviral construct, HXBH10 env. The cells to be exposed to the newly-formed viral particles will be infected with the mutated viral vectors using the calcium phosphate co-precipitation procedure, in which a calcium phosphate-viral vector precipitate is formed as a layer over the cells, which the cells then take up via phagocytosis. For control purposes, the pSVII-env plasmid will be used to express the wild type gp41 envelope trimer as well, and the plasmid will also be co-transfected with HXBH10 env in human cells to ensure the wild type gp41 envelope trimer is expressed in comparable viral particles.

Figure 5. Analysis of radiolabeled proteins associated with virus particles. Immunoprecipitation with an HIV-1 positive human serum was used to determine whether or not the wild type and mutant gp41 envelope trimers were incorporated into virus particles. Lane 1 contains a proviral construct lacking envelope trimers, lane 2 contains the proviral construct with the envelope trimer, lane 3 contains the proviral construct with wild type envelope trimers, lane 4 contains a mutant proviral construct that is unable to incorporate envelope glycoproteins, and lane 5 contains virus particles expressing the mutant gp41 envelope trimer in which the MSD was replaced by the complete MSD of influenza HA. Comparison between lanes 3 and 5 reveals that the mutant gp41 envelope trimer in lane 5 was successfully incorporated into viral particles [9].
the lysates analyzed using immunoblotting techniques. HIV-1 positive human serum will once again be used to detect structural viral proteins. If the mutant gp41 envelope trimers are expressed and incorporated into viral particles, bands in the immunoblotting analysis will appear for both gp160 and gp120 just as they do in the wild type control, as shown in Figure 9. To determine the degree to which the mutant gp41 envelope trimers successfully fused with target cell membranes, the mutant virus particles will be transfected into MAGI (multinuclear activation of a galactosidase inhibitor) cell assays. If the virus particles are successful in mediating membrane fusion, multinucleated regions known as syncytia will be observed. Numerical data concerning the number of syncytia and the number of nuclei in each syncytium will be utilized to calculate a fusion index that can be plotted for better comparison against the wild type fusion ability (Figure 10).

Conclusions

By determining whether or not the length of the MSD of gp41 or the presence of glycine residues in a GXXXG motif play necessary and indispensable roles in the formation of fusion-competent envelope trimers, future research endeavors can continue to focus their inquiry on the most involved key structures in the HIV-1 infection process. A lack of understanding regarding the molecular HIV virion infection mechanism has proved to be one of the major stumbling blocks in the production of an effective vaccine against HIV/AIDS. Thus, either identifying the length of the MSD of gp41 or the presence of glycine residues in a GXXXG motif as key elements in the formation of functional envelope trimers or recognizing that they play secondary roles as a result of some other critical process will help continuing research converge on the most crucial structures and processes in HIV-1 infection. Ultimately, uncovering these mechanisms could direct researchers to a vulnerable structure or step that, if exploited properly, could hold the key to an effective vaccine against HIV/AIDS.

VI. References


